

International Symposium on Induced Mutations in Plants



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IAEA-CN-167

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Symposium web site

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OPENING SESSION (09:00-10:15)

IAEA Boardroom C04

UNIDO Boardroom C04 (via CCTV)

Tuesday, 12 August 2008

Keynote presentation

IAEA-CN-167-349

EXPANDING THE BOUNDARIES OF GENE VARIATION FOR CROP IMPROVEMENT

R.L. PHILLIPS

Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN, USA

Email: phill005@umn.edu

The increased attention on genomics and the use of model species have greatly expanded the ability to detect and create variation. At least 23 plant species either have their genome sequenced or will soon be sequenced. Utilization of the TILLING technology in wheat has allowed the identification of more variation at the waxy locus than had been detected in the previous 25 years. Site-specific mutagenesis via oligonucleotide mismatches or zinc finger nucleases directed toward specific genes allows changes in a directed manner. The use of RNAi allows expression changes leading to useful variation such as root-knot resistance in *Arabidopsis*, reduction in cotton seed gossypol, cytoplasmic male sterility in tomato and tobacco, and delayed senescence in wheat. We have produced oat plants with individual maize chromosomes by crossing oat x maize followed by embryo rescue. Oat-maize addition lines (OMAs) are now available for all 10 maize chromosomes in various oat genetic backgrounds. OMAs also have been produced with the maize chromosomes coming from different genetic backgrounds (Seneca 60, B73 and Mo17). Monosomic OMAs have been gamma-irradiated to produce Radiation Hybrid (RH) lines with either a diminutive form of the maize chromosome addition or a translocation of a piece of the maize chromosome with an oat chromosome. Approximately 650 RH lines have been produced and defined by 40 markers for that particular chromosome. The OMA and RH lines are useful for many genetic studies, such as mapping individual sequences (polymorphisms are not required), transposable elements, and members of gene families; chromosome isolation, chromosome pairing, centromere isolation, etc. Also of interest is the evaluation of the materials for the introgression of maize characteristics into oat, such as disease resistance and C4 photosynthesis. All of these technologies expand the boundaries for more directed genomic changes enhancing the options for crop improvement.

Oral Presentation

IAEA-CN-167-409

NETWORKING AND FOSTERING OF COOPERATION IN PLANT MUTATION GENETICS AND BREEDING: ROLE OF THE JOINT FAO/IAEA PROGRAMME

P.J.L. LAGODA

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy

Agency, Vienna, Austria

Email: p.lagoda@iaea.org

The use of induced mutations has over the past 50 years played a major role in the development of superior crop varieties translating into a tremendous economic impact on agriculture and food production that is currently valued in billions of dollars and tens of millions of cultivated hectares. For the past 40 years, the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations have through the Joint FAO/IAEA Division for Nuclear Techniques in Food and Agriculture, Vienna, sponsored extensive R&D activities in their Member States on mutation induction to enhance the genetic diversity in the germplasm of food and industrial crops and these efforts have resulted in the official release to farmers of over 2700 new crop varieties in some 170 species. With increasing recognition of the roles of radiation in altering genomes and phenotypes and of isotopes as detection systems in molecular biology, demands from countries and their institutions for support in various applications of "modern biotechnology" increased dramatically over the last 20 years. Hence support for both R&D (through the IAEA Research Contract activities, CRPs) and for training and capacity building through fellowships, expert services and provision of equipment (through the IAEA Technical Cooperation Programme, TCPs) in molecular and genomic approaches to solving agricultural constraints have increasingly become part of the technological packages - combining mutation induction and efficiency enhancing bio-/molecular technologies - fostered by the Agency in recent years. The IAEA currently coordinates research networks (CRPs) and supports human and institutional capacity building TCPs for integrating plant bio-/molecular technologies and induced mutations within the framework of national plant breeding and conservation programmes to characterize plant genetic resources, widen plant genetic diversity, and identifies and introduces agronomically and commercially useful traits.

PLENARY SESSION 1 (10:15-12:45)
Induced Mutations in Food and Agriculture
IAEA Boardroom C04
UNIDO Boardroom C04 (via CCTV)

Tuesday, 12 August 2008

ROLE OF INDUCED MUTATIONS IN WORLD FOOD SECURITY

M.C. KHARKWAL

Indian Agricultural Research Institute, New Delhi, India

Email: mckharkwal@gmail.com

Ever since the epoch making discoveries made by Muller and Stadler eighty years ago, induced mutations have made significant contributions in development and release of >3000 mutant cultivars by a large number of countries in the world in more than 175 crop species. China and India are the top two countries of the world, which have released the largest number of mutant cultivars in several crop species. Gamma rays have been found to be most convenient, useful and successful in release of the largest number of mutant cultivars in the world. The success stories of commercially released mutant cultivars occupying substantial area in several countries of the world started in the fifties and sixties and continued till seventies and eighties. These four decades were ruled by some of the very widely known high yielding mutant cultivars such as Pallas, a two-row barley mutant cultivar in Sweden; Sanilac, a navy pea bean mutant cultivar; Stadler, a wheat mutant cultivar, Calrose, a rice mutant cultivar and Pennrad and Luther, two barley mutant cultivars in USA; Castelporziano, Castelfusano and Creso, the three durum wheat mutants in released in Italy; Balder, a barley mutant and stiff straw oat mutants Rythi and Puhti in Finland; Diamant, a barley mutant in Czechoslovakia; Trumpf, the best known barley mutant cultivar in Germany; Jagannath, a rice mutant cultivar, Aruna, an early maturing castor mutant, a number of Trombay Groundnut (TG) mutant cultivars and TAU-1, a blackgram mutant released in India. The last two decades have also seen release of several high yielding and biotic stress resistant mutant cultivars of rice in China and Vietnam; cotton, wheat, chickpea and mungbean mutants being cultivated in millions of hectares in Pakistan. India has successfully released the largest number of high yielding and disease resistant mutant cultivars in a number of legume crops being cultivated in large areas in the country. Although an exact estimate of the area covered by these commercially released mutant cultivars in a large number of countries is not readily available, but the limited information gathered clearly indicates that they have played a very significant role in solving food and nutritional security problems in their own countries as well as elsewhere. The advent of plant molecular biology, molecular marker assisted selection and high throughput DNA techniques, for example, TILLING have opened a new era in molecular mutation breeding technique that will overcome the disadvantages and limitations of conventional mutation breeding and play a significant role in solving world food security.

IAEA-CN-167-172

EIGHTY YEARS OF SCANDINAVIAN BARLEY MUTATION RESEARCH AND BREEDING

U. LUNDQVIST

Nordic Genetic Research Center, Alnarp, Sweden

Email: udda@nordgen.org

In 1928, the Swedish geneticists Hermann Nilsson-Ehle and Åke Gustafsson initiated experiments with induced mutations using a diploid barley species. The experiments started with X-rays and UV-irradiations, soon the first chlorophyll mutations were obtained followed by the first viable 'Erectoides' mutations. Several other valuable mutants were isolated: high-yielding, early maturity, lodging resistance and with changed ecological conditions. The X-ray experiments were then expanded with different pre- and aftertreatments, and using other types of irradiation. Neutrons, positrons etc, were used and finally chemical mutagenes, starting with mustard gas and concluding with the inorganic sodium azide. This research brought a wealth of observations of general biological importance, high increased mutation frequencies, differences in the mutation spectrum and the ability to direct mutagenesis of specific genes. This Scandinavian mutation research was non-commercial although some mutants have become agronomically valuable. Its peaks of activities were during the fifties, sixties and seventies. Barley has been the main experimental material, but other species were also included in the program. About 10 000 different mutants with a broad variation were collected and several mutant characteristics have been analyzed in more detail genetically and with regard to mutagen specificity. Among these mutant groups most effort has been concentrated on the three following ones: (1) *Early maturity mutants*. At an early stage it was established that the time for early heading and maturity could easily be changed by mutagenesis. 9 different *mat* loci could be indentified among the drastic type of earliness. One of these loci *mat-a* proved to be very striking, it is photo- and thermo-period insensitive. It causes a profound change in photoperiod reaction, making the mutant heading even in short days, and it became important for breeding. (2) *Six-row and Intermedium mutants*. Two-row barley can be mutated in a single step to six-row barley and this affects the development of the lateral spikelets. All 41 Swedish mutants have been localized to one single locus, *hex-v*. But two-row barley can also produce spike development intermediate between the two- and six-row state. 11 different *int* loci could be determined and were studied in more detail. (3) *Mutants affecting surface waxes*. The epicuticular wax coating has a very complex genetic architecture and affects the presence and type of waxes on three different organs (spike, leaf sheath and leaf

blade). In total 1580 mutants have been localized to 79 different *cer* gene loci. Seven different types of mutagenic agents have been applied, and a great range of mutability was found.

IAEA-CN-167-343

THE INDUCED *SD1* MUTANT AND OTHER USEFUL MUTANT GENES IN MODERN RICE VARIETIES

J.N. RUTGER
Woodland, CA, USA
Email: neil.rutger@sbcglobal.net

Induced mutation was accelerated in the USA with the release in California in 1976 of Calrose 76, the nation's first semidwarf table rice variety. Success was due not only to induction of mutants but also to their evaluation and integration into cross-breeding programs. Thus the evaluation of Calrose 76 showed that its *sd1* gene was allelic to *sd1* in the indica Green Revolution varieties DGWG, TN(1) and IR8, and that semidwarfism conferred a yield advantage of 14% over the 6mt/ha yield level of the tall japonicas. Immediate integration of the Calrose 76 source of semidwarfism into cross-breeding has resulted in 25 semidwarf varieties that trace their ancestral source of semidwarfism to Calrose 76: 13 in California, 10 in Australia, and 2 in Egypt. Calrose 76 ancestry also appears in the pedigrees of numerous additional California cultivars derived from crossing the Calrose 76 source with the IR8 source of semidwarfism. In the late 1990s 12 semidwarf mutants were induced in tall tropical japonica varieties at the Dale Bumpers National Rice Research Center in Arkansas. The semidwarfing gene in each of these 12 germplasms was found to be nonallelic to *sd1*. Although selected for productivity, none of the 12 consistently showed yield increases typical of *sd1* sources. The *sd1* source, whether from induced mutation or from the indica source, is truly associated with enhanced productivity. Other induced mutants were found for early flowering, low phytic acid, giant embryo, and marker genes such as gold leaf and extreme dwarfism. The early flowering mutants were recovered in temperate japonicas, in tropical japonicas, and most recently in indicas. The early flowering indica mutants are quite interesting since they provide high yielding or blast disease-resistant indica germplasm which will mature in the USA.

IAEA-CN-167-243

INDUCED MUTATIONS IN PLANT BREEDING AND BIOLOGICAL RESEARCHES IN JAPAN

H. NAKAGAWA
Institute of Radiation Breeding, National Institute of Agrobiological Sciences, Ibaraki, Japan
Email: ngene@affrc.go.jp

More than 200 direct-use mutant varieties generated by using irradiation, chemical mutagenesis and somaclonal variations, have been registered in Japan. About 61% of these were induced by gamma ray irradiation and contribution of Institute of Radiation Breeding with the gamma ray irradiation facilities of Gamma Field, the Gamma Greenhouse and the Gamma Room is great. This high percentage of gamma ray irradiated mutants indicates that mutation breeding via gamma ray irradiation is an effective and highly successful approach for the generation of commercial cultivars. Some mutant cultivars of Japanese pear and apple resistant to diseases induced by gamma ray irradiation and development of a unique bioassay by using toxins of fungi will be discussed. In addition, ca. 200 indirect-use (hybrid) mutant varieties primarily generated in rice and soybean have found values as parental breeding germplasm resources in Japan. The contribution of direct- and indirect-use mutant varieties generated through gamma-ray irradiation is significant. In 2005, two direct-use cultivars and 97 indirect-use cultivars contribute approximately 12.4% of the total area for cultivation in Japan. The semidwarf gene (*sd-1*) generated in rice is perhaps one of the most significant contribution to this. For soybean, similar gamma-ray induced mutants (4 direct-use cultivars and 4 indirect-use cultivars) cover nearly 9.4 % out of the total cultivation area (ca. 142,000 ha) of soybean. These results indicate that agronomically useful mutations, induced by irradiation mutagenesis, have contributed directly and significantly to food production in Japan. On the other hand, molecular genetics based on genome sequencing will be presumably be the most powerful tool for identifying the genes, which control phenotypes, and for selecting mutants of certain phenotypes. This could change the mutation breeding dramatically, especially in rice, and expand its use into the other gramineous crops which show genomic synteny to rice. There are some interesting reports on the characteristics of mutations induced by radiation and on mechanisms controlling mutant characteristics in Japan. These researches, such as low glutelin contents in rice grains caused by the loss-of-function or gene silencing generated by RNAi in the same loci, and advantages of radiation for inducing mutation in the loci, as well as the development of new mutant rice varieties for diet therapy of the patients with kidney disease will be discussed.

MUTATION BREEDING IN OILSEEDS AND GRAIN LEGUMES IN INDIA: ACCOMPLISHMENTS AND SOCIO-ECONOMIC IMPACT

S.F. D'SOUZA

Nuclear Agriculture & Biotechnology Division, Bio-Medical Group, Bhabha Atomic Research Centre, Trombay
Mumbai, India

Email: sfdsouza@barc.gov.in

In India, oilseed and grain legume crops are important food components as they are major contributors for dietary oils and proteins. In order to generate genetic variability in these crops, mutation research using X-rays, gamma rays, fast and thermal neutrons is extensively carried out in several national institutes, state agricultural universities including Bhabha Atomic Research Centre (BARC), Mumbai since half a century. Besides cytogenetic studies, the era of direct mutants as crop varieties began in groundnut, mustard, pigeonpea and mungbean. Induction of modified traits and their incorporation in an ideal genotype was achieved by judicious use of induced mutation and hybridization techniques. So far about 100 mutant varieties in oilseeds and legumes have been released in India. Of these, BARC has developed 33 varieties by incorporating desirable traits like large seed, semi dwarf habit, high harvest index, better partitioning, fresh seed dormancy, yellow seed colour, drought tolerance, powdery mildew resistance, yellow mosaic virus resistance, bacterial pustule resistance. Many of the breeding programmes in national/state systems have been utilizing BARC varieties as parental materials/donors and developed several improved varieties. Several of these varieties have high patronage from the farming community and extensively grown in the country. Groundnut varieties have made considerable impact by giving record yields across the country. Further, mungbean varieties were also surging ahead by virtue of their resistance to yellow mosaic virus, *Rhizoctonia* root-rot and powdery mildew diseases with suitability to rice fallow situations. Blackgram variety TAU-1 has occupied maximum blackgram area in Maharashtra state. These crop varieties also facilitated farmers to develop newer cropping systems. Mutant varieties like Aruna of castor, Pusa 408 (Ajay), Pusa-413 (Atul), Pusa-417 (Girnar) of chickpea, Co-4, Maru Moth-1 of mothbean are among the important varieties of economic significance released by other institutes in India. Thus, induced mutation research remained in the forefront of Indian agricultural research by developing popular varieties with higher productivity potential in oilseeds and legumes.

Poster Presentations

IAEA-CN-167-100P

ACHIEVEMENTS OF GRAIN LEGUME VARIETY IMPROVEMENT USING INDUCED MUTATION OF THE IAEA/RAS/5/040 PROJECT IN THAILAND

S. SRISOMBUN¹, P. SRINIVES², C. YATHAPUTANON¹, S. DANGPRADUB¹, A. MALIPAN¹, W. SRITHONGCHAI¹, B. KUMSUEB¹, V. TEPJUN¹, S. NGAMPONGSAI¹, A. KORNTONG¹, S. IMPITHUKSA¹

¹Department of Agriculture, Bangkok, Thailand

²Faculty of Agriculture, Kasetsart University, Kamphaeng Saen, Nakorn Pathom, Thailand

Email: ksssb@doa.com

The project aims to form a regional cooperation network of mutation germplasm with emphasis on seed-propagated crops among the member states in Asia and Pacific during 2002-2006. It comprised of two components: the establishment and implementation of mutant multi-location trials and the establishment of mutation germplasm network. Thailand participated two crops, soybean and mungbean, of both components. Significant achievements are summarized. Soybean mutant multi-location trials: two introduced mutants were well adapted in the upper and lower north of Thailand. DT84 from Vietnam produced similar yield and matured 18 days earlier than Chiang Mai 60. Bangsakong from Korea gave 11% greater yield with 12 days earlier than Sukhothai 2. Soybean mutants resistant to Soybean Crinkle Leaf: the disease caused by virus and transmitted by whitefly is a major disease in Thailand. Seed of line CM9238-54-1(ST) was irradiated with 200 gray. Mutant lines were selected under natural field infections and tested in laboratory. Six mutant lines resistant to the disease were finally selected. Soybean mutants with high grain protein: seed of vars Chiang Mai 60, SSRSN19-35-4 and EHP275 was irradiated with 200 gray. Pedigree method of selection was used and grain protein of mutants was analysed. The results of a preliminary trial showed that 32 selected mutant lines gave 0.8, 2.0 and 1.0% higher grain protein than the original parents of 41.8, 40.3 and 41.9%, respectively. Soybean mutants with high seed germination and vigor: seed of Chiang Mai 60, a high yielding variety with poor seed germination and vigor, was irradiated with 100 gray. Pedigree selection method was used in late generations. Accelerated Aging Test was also used to test the seed vigor of the mutant lines. In dry season trial, eight mutant lines had seed germination of 65-75% compared with the parent of 30%. In rainy season, 12 mutant lines had seed germination of 75-89% whereas the parent had only 41%. Mungbean mutant multi-location trials: the highest yielding variety across five trials was a Thai mutant, Chai Nat 72. It produced large seed of 70 g/ 1,000 seeds which is a

desirable triat for Thai and international markets but it is susceptible to powdery mildew. An introduction from Philippines, Native Variety, showed resistant to the disease. It can be utilized for further breeding programme. Novel mungbean germplasm derived from induced mutation; Variegated leaf: all F_1 plants from the cross between variegated mutant and normal leaf parent showed normal green leaves without reciprocal while F_2 plants segregated well in a 3:1 ratio. The F_3 lines showing all green plants: segregating: all variegated plants fitted well with the 1:2:1 ratio. The variegated leaf character is controlled by a single recessive gene. Multiple leaflet: a mutant with small pentafoolate was crossed with a large heptafoolate mutant to study the inheritance. It was found that their F_2 plants segregated in the ratio of 9:3:4 with tri ($N_1-N_2^-$), penta ($N_1-n_2n_2$) and heptafoolate ($n_1n_1N_2^-$ and $n_1n_1n_2n_2$). The n_2 may be closely linked to the gene controlling leaf size as well. There are three AFLP markers linked to number of leaflets per leaf and all of them corresponded to the n_1 locus.

IAEA-CN-167-178P

SOYBEAN VARIETIES BRED WITH INDUCED MUTATION AND THEIR APPLICATION IN CHINA

B.G. ZHU

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (CAS), Beijing, China
Email: bgzhu@genetics.ac.cn

Breeding with induced mutation is one of the most effective methods of crop breeding. The physical mutagenesis (PM) and chemical mutagenesis (CM), which are efficient, simple, and cheap, have been utilized for breeding of many crops, and made great progress. The objective of the present article was to summarize soybean (*Glycine max* L.) varieties bred with induced mutation and their application in China. This study began in 1957, and from then on, the largely planted 25 soybean varieties concerned induction mutation were bred, approved, and released in various regions in China. These soybean varieties possess different excellent traits such as high yield, good grain quality, disease/insect resistance, or drought/salt tolerance. A total area of planted these varieties was more than 1×10^7 ha. Among them, 12 varieties, including Youbian30 (from PM, the planted area of 3×10^6 ha, the Third Award of National Technology Invention won in 1988), Baoyou17 (CM, 2.7×10^5 ha, the Second Award of Sci-Tech Progress of Chinese Academy of Sciences won in 1994), Kexin3 (CM), Kexin4 (CM), Kexin5 (CM), Kexin6 (CM), Kexin7 (CM), Kexin8 (CM), Huayou446 (CM), Huayou542 (CM), Huayou4120 (CM), and Huayou5 (CM), were developed by Genetics Institute of Chinese Academy of Sciences; 6 ones, including Heinong26 (PM, 2.33×10^6 ha, the Second Award of National Technology Invention won in 1984), Heinong28 (PM), Heinong31 (PM), Heinong32 (PM), Heinong38 (PM), and Heinong41 (PM), were done by Soybean Institute of Heilongjiang Academy of Agricultural Sciences; one, Tiefeng18 (PM, 4×10^6 ha, the First Award of National Technology Invention won in 1983), was done by Tieling Agricultural Institute of Liaoning Province; And the other ones, Jidou8 (CM), Shidou1 (CM), and Hefeng46 (CM), etc., were done by other breeding institutions.

IAEA-CN-167-260P

DEVELOPMENT OF WHEAT VARIETIES FOR THE MARGINAL AREAS OF KENYA THROUGH MUTATION AND DOUBLE HAPLOID TECHNIQUES

M.G. KINYUA¹, L. KARANJA², P. KIMURTO³, M. SPENCER⁴, Q.Y. SHU⁴

¹Moi University, Eldoret, Kenya

²Kenya Agric. Res. Institue, Njoro, Kenya

³Egerton University, Njoro, Kenya

⁴Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria

E-mail: mgkinyua@africaonline.co.ke

Wheat is an important cereal crop in Kenya and ranks second after maize in its cereal crop priority. Due to increased wheat demand, increased urbanization and migration to non-wheat producing regions this crop has been introduced into the lowlands below 1800m. It is grown in both small scale and large scale. Water stress is a major constraint in wheat production in the non-traditional dry areas of Kenya and tends to cause low yield. Breeding for drought tolerance has proved achievable and over the last 10 years a lot of research work has been done in the dry regions. Various methods have been used to develop drought tolerant wheat varieties, namely conventional breeding, Doubled Haploid technique and mutation breeding. This paper presents achievements from use of mutation and double haploid techniques in development of drought tolerant wheat varieties. Breeding for drought tolerance has improved the status of wheat production in the dryland and increased adaptability. This has formed a very valuable asset in improving the economic status of the people living in the semi-arid regions of Kenya.

IAEA-CN-167-286P

DEVELOPMENT OF MUTANT VARIETIES OF CROP PLANTS AT NIAB AND THE IMPACT ON AGRICULTURAL PRODUCTION IN PAKISTAN

M.A. HAQ

Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan

Email: niabdir@fsd.comsats.net.pk

The Nuclear Institute for Agriculture and Biology is the prime institute of Pakistan Atomic Energy Commission in agricultural sector. It started functioning 40 years ago in 1969. The main objective of the institute is to conduct research in agricultural and biological problems especially in those areas where nuclear techniques have an edge over the conventional methods. The institute has been conducting research and development work related to crop improvement through Mutation Breeding. Mutation breeding involves the use of induced beneficial changes for practical plant breeding purpose both directly as well as indirectly. The main objectives have been to confer specific changes such as improvement of plant architecture, earliness in maturity, resistance against diseases and pests, and improved physiological characters i.e. heat tolerance, cold tolerance, uniform maturity, photoperiod insensitivity etc., in the native well adapted crop varieties/exotic lines to make them more productive. The use of induced mutations for crop improvement has led to the development of 24 improved varieties of different crops at NIAB which clearly indicates the potential of this technique. In addition a wealth of genetic variability has been developed for use in the cross breeding programmes and a few varieties of cotton and chickpea have been developed in Pakistan by using induced mutants as one of the parents. These improved crop varieties in Pakistan have played significant role in increasing agricultural production with positive impact on the economy of the country. The estimated additional income accounted by the selected varieties of NIAB was US \$ 1.744 billion upto 2005.

IAEA-CN-167-326P

OVERVIEW OF MUTATION BREEDING IN SUDAN

A.M. ALI

Agricultural Research Corporation, Wad Medani, Sudan

Email: abdmali@yahoo.com

Mutation breeding has effectively started about twenty years ago with the implementation of the first technical cooperation (TC) project of the IAEA in Sudan. This presentation highlights achievements and prospect of this collaboration. The first TC project (Sud 5/023) was confined to cotton and sugarcane while in the following projects (SUD 5/028 and SUD 5/030) other crops such as sesame, banana, tomato, groundnuts and cereals were included. The mutation program also benefited from the regional projects (RAF5/50 and RAF5/56). Plant breeders involved in mutation breeding increased from less than five in the first project to over 15 in the current one. A banana mutant cultivar (ALBEELY) was released in the year 2003. Albeely excelled the yield of the existing cultivars by 40% and has better crop stand and fruit quality. Albeely is becoming popular and widely preferred by farmers. A drought tolerant ground nut mutant (Barberton-B-30-3) and a number of promising mutants resistant for tomato yellow leaf curl virus (TYLCV) are being evaluated in multi-location trails in preparation for their commercial release. Cotton germplasm has been enriched with a number of useful mutants carrying resistance for bacterial blight and fusarium wilt disease in addition to mutants for weak fiber attachments and high ginning out turn and lint percentage. These mutants are being used in the breeding program and promising lines are under field evaluation for release. The mutation breeding program is strengthened by installing irradiator and establishing tissue culture and molecular laboratories. It is evident that the TC program of the IAEA has contributed significantly to the establishment and sustainability of mutation breeding and related biotechnologies in Sudan. The program is progressively expanding and a number of outstanding cultivars were released or in the pipeline. Intensive work is under way to generate production package for these mutants and set a demonstration plot program to facilitate the dissemination of these varieties and accelerated their adoption by farmers.

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SOCIO-ECONOMIC IMPACTS OF MUTANT RICE VARIETIES IN SOUTHERN VIETNAM

T.K. DO et al.

Institute of Agricultural Sciences for Southern Vietnam, Ho Chi Minh City, Vietnam

Email: iasvn@vnn.vn, dokhacthinh_52@yahoo.com.vn

Rice plays an important role of socio-economic development in Vietnam, especially in Mekong River Delta (MRD) where is more than half of the total and 90% of national export rice. Rice variety improvement is main project in national breeding program. However, no mutant rice variety (MRV) was cultivated in MRD before 1995. Recently, 8 rice mutants occupy 10.3% of total modern varieties in Southern Vietnam. The mutated characters developed so far consist of better resistance to lodging, disease and insect damages, higher tolerance to soil stresses such as acid sulphate soil, drought etc, and also earliness and higher yield potential. Some best mutant varieties: VND95-19, VND95-20, VND99-3, TNDB-100 have been released for large-scale production in MRD. Among them, VND95-20 has become one of the top 5 varieties for export and grown annually more than 300,000ha in Southern Vietnam. In combination with hybridization method, some mutants gave promising recombinants. Selected varieties as VN121, VN24-4, OM2717, OM2718 have been released into production. Successful combination of aromatic character with short duration, high yield, tolerant to new diseases (GSV & RSV) & insects (BPH), consequently reduction of 2-3 spraying times of pesticide / crop, supported for health & environmental protection. For 8 past years under IAEA ' TC project, total cultivated area of MRV was more than 2.54 millions ha in Southern Vietnam. Until 2008, 8 mutant varieties produced the added return of 374 millions USD for past years & continue producing added return for farmers. Eight MRV of VND95-20, VND99-3, TNDB100, VND95-19, OM2717, OM 2718, VN 121 & VN24-4 occupied the added return values as 300.00; 9.0; 37.5; 6.0; 12.0; 8.4; 0.8 & 0.7 millions USD, respectively. Application of MRV is reduced 2-3 spraying times / crop due to their tolerance to diseases & insects. MRV are used in strategy program of "Eradicate hunger and alleviate poverty" of different national projects, particularly for the ethnic minorities in mountainous & remote areas in Southern Vietnam. Due to significant contribution for socio - economic development, achievement of mutant rice varieties have been received many prizes of national & local Government.

IAEA-CN-167-366P

THE ROLE OF MUTATION BREEDING ON PLANT IMPROVEMENT IN MEXICO

E. DE LA CRUZ TORRES

Departamento de Biología, Instituto Nacional de Investigaciones Nucleares, Ocoyoacac, México

Email:ect@nuclear.inin.mx

In Mexico promotion of radioinduced mutation breeding started in 1974. Further research on advanced mutant lines allowed to obtain new varieties. 'Centauro' and 'Bajío Plus', are wheat varieties with increased yield and tolerance to lodging, derived from 'Salamanca' seeds irradiated at 500 Gy, obtained by Colegio de Postgraduados. Regarding to soybean, Instituto Nacional de Investigaciones Forestales y Agropecuarias, obtained two varieties, 'Hector' and 'Esperanza' by irradiation of seeds from variety 'Suaqui 86' at 150 Gy. These new varieties exhibit an increased yield and reduction in dehiscence and lodging, being tolerant to white fly. 'SalCer' is another new soybean variety obtained by CESAEGRO through irradiation of seeds from line ISAEGBM₂ at 200 Gy. Its improved traits are higher yields and increased height to first pod. Currently, in various institutions, several crops are under improvement through mutation breeding, such as Mexican lemon, aiming to increase fruit size and to reduce number of seeds; mango, trying to improve fruit quality and uniform maturity; avocado, looking for reduced height of trees, and agave, trying to select individuals tolerant to stem soft rot. Regarding to ornamental species, several studies are in process to induce variability on genus *Dahlia*, *Euphorbia*, *Tigridia*, *Sprekelia* and *Ficus*. Also a model has been developed to induce variability on endangered species by mutation and biotechnological tools. For example *Mammillaria sanangelensis*, has been rescued and variation has been induced through irradiation and *in vitro* culture. To provide alternative crops for peasants inhabiting marginal areas, a mutation breeding program at ININ allowed to select low saponin mutants of quinoa by irradiation of seeds from cultivar Barandales at 200 and 250 Gy. Also focus has been placed on improvement of ancient crops, such as 'Huauzontle' and 'Chia roja' (*Chenopodium berlandieri* ssp. *nuttalliae*), amaranth and beans. Evaluation of putative mutants of these crops is currently underway.

CONCURRENT SESSION 1 (14:00-18:00)

**Mutational Enhancement of Genetic Diversity and Crop
Domestication**

IAEA Boardroom C04

Tuesday, 12 August 2008

MAKING THE MOST OF AGROBIODIVERSITY TO IMPROVE LIVELIHOODS

E. FRISON

Bioversity International, Rome, Italy

Email: e.frison@cgiar.org

Agricultural biodiversity is generally thought of as simply a collection of traits, to be used in scientific breeding efforts to increase productivity of mainstream crop and livestock species. While those efforts are important, especially in the context of more favoured lands, there is much more that agricultural biodiversity can do. Agricultural biodiversity makes a vital contribution to the stability of food supplies and the resilience of farming systems. This is particularly important in fragile environments where the greatest poverty prevails. The main objective of farmers in these marginal areas is to minimize risks of crop failure and thus famine for the family. In those areas, agricultural biodiversity is often one of the very few assets that poor rural farmers can somehow control. They use this diversity to reduce risk and increase sustainability of their production system. Furthermore, one of the most dramatic consequences of climate change will be greater climatic fluctuations which will further increase the risks of crop failures, especially in marginal areas. It is therefore vital to ensure that sufficient diversity is present in the production system if farming systems are to adapt sufficiently rapidly to keep up with the consequences of climate change. A very important, but often neglected problem is that of malnutrition which affects two billion people. In addition to vitamin and micronutrient deficiencies, non-communicable diseases such as type II diabetes, cardiovascular diseases, cancers and obesity are rapidly increasing among poor people in developing countries as the result of a nutritional transition characterized by a dietary simplification. This is referred to as the “double burden of malnutrition”. By providing for a more diversified diet, agricultural biodiversity can deliver better nutrition and thus better health and is a major underexploited tool to fight malnutrition. For all these reasons, and others, it is important now to consider how to make better use of agricultural biodiversity in farming systems to improve food security, nutrition and environmental sustainability.

IAEA-CN-167-394

GENETICS OF THE INDUCED AND NATURAL PHENOTYPIC VARIATION IN CULTIVATED BARLEY

A. DRUKA

Scottish Crop Research Institute, Scotland, UK

Email: Arnis.Druka@scri.ac.uk

The Bowman series of nearly isogenic lines (NILs) developed by Prof. J. Franckowiak contain almost all of the classically described morphological mutations in barley. The unique feature of the collection is that the original mutated donor plants have all been backcrossed into a single recurrent parent, the cultivar Bowman (generally to $> BC_4F_3$). We applied a 1536-plex SNP genotyping platform developed as part of a transatlantic collaboration (BBSRC / RERAD funded AGOUEB and USDA funded Barley CAP projects) to characterise the introgressed segments containing the mutated alleles in about 1000 of the Bowman NILs. In parallel, to facilitate rapid forward genetics-based gene isolation, we generated high density barley genetic linkage map by integrating recently developed eQTL mapping data in the barley SNP-based genetic linkage map. The SNP map was constructed by genotyping three different bi-parental DH populations with 4600 SNP markers. The integrated barley map was then anchored to the rice genome sequence. We have also developed about 200 F_2 populations representing 40 different genes and alleles from the phenotypic classes such as awn development, grain size, spikelet density and the lateral spikelet development. We will present an update of the strategy and our initial results.

IAEA-CN-167-365

A MUTANT HOMEBOX GENE CREATED SIX-ROWED SPIKE IN BARLEY DOMESTICATION

T. KOMATSUDA, M. POURKHEIRANDISH

National Institute of Agrobiological Sciences (NIAS), Plant Genome Research Unit, Tsukuba, Ibaraki, Japan

Email: takao@affrc.go.jp

Increased seed production has been a common goal during the domestication of cereal crops. Early cultivators of barley (*Hordeum vulgare* ssp. *vulgare*) selected a phenotype with a six-rowed spike that stably produced three times the usual grain number during domestication. We isolated the *SIX-ROWED SPIKE 1* (*Vrs1*) from barley by chromosome walking. We discovered that *Vrs1* encodes a homeodomain leucine zipper I-class protein (HD-ZIP I), a potential transcription factor. RNA *in situ* hybridization revealed that the *Vrs1* is expressed only in lateral spikelet primordia. Sequencing alleles of 54 six-rowed mutant lines revealed a single amino acid substitution in 22 lines, creation of a new stop codon in 12 lines, a nucleotide substitution in the conserved splicing site in 3 lines, a frameshift mutation by a deletion in 5 lines, complete deletion of the gene region in 7 lines, and no DNA changes throughout the coding region with no gene expression detected in the remaining 5 lines. We found three haplotypes among six-rowed barley revealing loss-of-function mutation of the homeobox gene *Vrs1*. We found that two of them independently originated from two different type two-rowed barleys, but origin of the remaining one six-rowed allele remained unclear.

IAEA-CN-167-344

ALLELE-MINING AND NATURAL DIVERSITY IN WHEAT POWDERY MILDEW RESISTANCE GENES

B. KELLER, S. BRUNNER, Z.Q. ZHANG, N. KAUR

Institute of Plant Biology, University of Zurich, Zurich, Switzerland

Email: bkeller@botinst.uzh.ch

Using map-based cloning, we have isolated the *Pm3b* powdery mildew resistance gene from hexaploid bread wheat (*Triticum aestivum* L.). Based on haplotype studies, we have developed molecular tools to isolate all the 10 known *Pm3* genes conferring resistance. We found that the *Pm3* genes form a true allelic series and that they are highly conserved at the molecular level. The molecular work on *Pm3* resistance genes has led to very diagnostic tools for these genes which support the cloning of new functional alleles from this locus by allele-mining. We have used these tools to screen for new *Pm3* alleles in the gene pools of (i) wild and domesticated tetraploid accessions and (ii) hexaploid wheat landraces. The *Pm3* locus is conserved in tetraploid wheat, allowing a comparative evolutionary study of the same resistance locus in a domesticated species and one of its wild ancestors. We have identified 61 *Pm3* allelic sequences from wild and domesticated tetraploid wheat subspecies. These alleles showed low sequence diversity, differing by few polymorphic sequence blocks that were further reshuffled between alleles by gene conversion and recombination. A new functional gene was identified in a wild wheat accession from Syria. This gene, *Pm3k*, conferred intermediate resistance to powdery mildew and consists of a mosaic of gene segments derived from non-functional alleles. From the hexaploid wheat gene pool, a set of 1320 landraces, mostly from Asia, was screened for powdery mildew resistance and the presence of a *Pm3* haplotype. Most of these lines were found to contain a susceptible *Pm3* allele which is closely related to the functional *Pm3* resistance genes. We have also identified resistant lines with new types of *Pm3* allelic sequences, resulting from point mutations, gene conversion and illegitimate recombination events. These new alleles are currently tested for resistance activity in a transient expression assay.

IRRADIATION INDUCED WHEAT-ALIEN TRANSLOCATION LINES AND THEIR APPLICATION IN WHEAT BREEDING

P.D. CHEN, T.D. BIE, Y.P. CAO, S.W. CHEN, X. WANG

State Key Laboratory of Crop Genetics and Germplasm Enhancement, Cytogenetics Institute, Nanjing Agricultural University, Nanjing, Jiangsu, China

Email: pdchen@njau.edu.cn

Wild relatives are rich gene resources for wheat improvement. Transfer of alien useful genes to wheat through development of wheat-alien translocations, especially small alien segment translocations, is important for wheat breeding. Wheat-alien genetic stocks such as amphiploid, addition or substitution lines were irradiated for translocation induction. Mature male or female gametes before flowering on the spikes were irradiated by ^{60}Co - γ -rays at doses ranged from 8 to 22.4Gy. Chromosome C-banding and genomic *in situ* hybridization (GISH) were used to identify chromosome translocation. Backcross of M_1 plants using normal fresh pollen of common wheat was employed to enhance the transmission rate of various structural changes in their progenies. The results showed that the dose of 8~12Gy was suitable for pollen irradiation while 15~20Gy was suitable for female-gamete irradiation. Irradiation treatment just before gamete maturation is advantageous to acquisition of more M_1 hybrids with high frequency of chromosome structural variation. The frequency of plants with at least one translocation chromosome in M_1 could be increased up to 70% through pollen irradiation of *Triticum durum*-*Haynaldia villosa* amphiploid. More than one hundred translocated chromosomes have been identified in the BC_1 and BC_2 . 57 terminal and 80 intercalary translocations with small alien chromosome segments were induced through female-gamete irradiation conducted on *T.aestivum*-*H.villosa* 6VS/6AL translocation line. For the 22.4Gy dosage treatment, the induction frequencies of interstitial translocation, terminal translocation and deletion were 21.02%, 14.01%, and 14.65%, respectively, which were much higher than that previously reported. These genetic stocks will be useful for physical mapping of alien genes such as *Pm21*. Some compensate translocations conveying useful agronomic traits would be useful in wheat breeding. The *T.aestivum*-*H.villosa* 6VS/6AL translocation has been used in wheat breeding and many elite cultivars, such as Nannong 9918, Neimai 9, Shimai 14, etc. have been developed and released.

INDUCED MUTATION IN NARROW-LEAFED LUPIN IMPROVEMENT: AN EXAMPLE OF HERBICIDE TOLERANCE

P. SI¹, B. BUIRCHELL², M. SWEETINGHAM²

¹Centre for Legumes in Mediterranean Agriculture, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, Australia

²Department of Agriculture and Food of Western Australia, WA, Australia

Email: pingsi@cyllene.uwa.edu.au

Spontaneous mutation has been discovered and utilized in domestication of narrow-leafed lupin (*Lupinus angustifolius* L.). As the result of the domestication, lupin has become a dominant grain legume crop in Western Australia. Facing the new challenge of developing herbicide tolerance cultivars, chemical mutagenesis has been used to create new tolerance to herbicide. This paper reports the characterization of two lupin mutants (Tanjil-AZ-33 and Tanjil-AZ-55) that are highly tolerant to metribuzin herbicide. A dose response study over 8 doses revealed that Tanjil-AZ-33 was 6 times more tolerant to metribuzin than the original parental cultivar Tanjil by measure of LD_{50} . This mutant Tanjil-AZ-33 is the most tolerant germplasm in narrow-leafed lupin. Both mutants also maintain the high resistance to the disease anthracnose as cv Tanjil. Seed yield based on small field plots (3.6 m²) under irrigation was 4.2 t/ha for Tanjil-AZ-33 and 1.9 t/ha for Tanjil when the seedlings subject to metribuzin @ 300 g/ha at 6 leaf stage. Seed yields of both Tanjil-AZ-33 and Tanjil-AZ-55 were similar to Tanjil in absence of the herbicide. These facts indicate that the mutation process has created tolerance to metribuzin in Tanjil, but has not altered Tanjil's yield capacity and anthracnose resistance. The mutant Tanjil-AZ-33 has been used as a parent in the lupin breeding program and we expect future lupin cultivars will have increased metribuzin tolerance. Induced mutation proves to be an effective tool in lupin improvement.

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THE GLOBAL PARTNERSHIP INITIATIVE FOR PLANT BREEDING CAPACITY BUILDING (GIPB)

E.P. GUIMARAES, E. KUENEMAN

Plant Production and Protection Division, Food and Agriculture Organization of the United Nations (FAO),
Rome, Italy

E-mail: elcio.guimaraes@fao.org

The demands on crop production are increasing, particularly in the developing countries. Plant breeding and improved agronomy have largely met previous increases in demand. The challenge for the future will be to meet increased demands from a growing population through employing improved agricultural production methods that are sustainable in the long term, with minimal negative consequences for the environment. FAO is carrying out, in collaboration with the CGIAR centres, a global plant breeding and associated biotechnology assessment. The results of this survey singled out lack of capacity building as the most relevant gap to strengthen national capacity to use plant genetic resources for food and agriculture (PGRFA). The Global Partnership Initiative for Plant Breeding Capacity Building (GIPB) was launched to address challenges in training and supporting plant breeders in a concerted and systematic manner, complementing existing efforts whenever possible. The GIPB is proposed as a partnership of public, private and civil society sectors working in consort through a lightweight facilitation mechanism. The goal of the initiative is to strengthen capacities of the developing countries to improve their productivity through sustainable use of PGRFA using better breeding and delivery systems. The main objectives are: 1) **support for policy development** on plant breeding and associated scientific capacity building strategy, to help allocate resources to strengthen and sustain developing countries' capacity to use PGRFA; 2) **provision of education and training** in plant breeding and related scientific capacities relevant to utilization of PGRFA; 3) **facilitate access to technologies** in the form of tools, methodologies, knowhow; 4) **facilitate exchange of PGRFA** that can enhance the genetic and adaptability base of improved cultivars in developing countries; and 5) **sharing of information** focused on plant breeding capacity building to deliver newly available knowledge to national policy makers and breeders in developing country programmes.

IAEA-CN-167-058

RESULTS OF UTILIZATION OF CHERNOBYL RADIO MUTANT IN BREEDING PROGRAMS OF *TRITICUM AESTIVUM* L.

L.A. BURDENYUK-TARASEVYCH

Bila Tserkva Division of Research and Breeding of Institute of Sugar Beet UAAS, Kyiv, Ukraine

Email: azlatska@sops.gov.ua

During 1988-2007 at the research station in Bila Tserkva it was investigated the genetic changes in winter wheat occurred due to the ionizing radiation which appeared as the result of Chernobyl Nuclear Power Plant Accident. 239 accessions of common wheat which during two years in plantations in 1986 and in self-sowing 1987 grew near the Chernobyl Reactor were provided for our station for further investigations and analysis. It was planned to analyze the possibilities of their utilization in breeding by selection among them the mutants with attractive agronomic important traits with further introduction of them in breeding programs. Ever in M_2 the large spectrum of mutations was found out. Because of genetic instability the mutant diversity each year was increased and up to present moment we have in collection up to 2000 mutants. Mainly among mutants in different generations appeared the plants with different kinds of abnormality in structure. We call them chimerical plants and they had no any value for breeding. More over the direct selection from mutants of all studied varieties did not perform the positive results because of high instability of all characteristic in many generations. In the same time the particular lines of mutants, which have advantage by some agronomic characteristics, were applied to the breeding programs. Mutants taken from the different generations (L147/91, BC 47 square head, dwarf 20104/89) were used for development of Lybid, Yasochka and Tsarivna varieties to utilize mutants' traits such as hardiness, drought tolerance, resistance to diseases and lodging, bred quality. The varieties were included in the State Variety Register of Ukraine. Another variety – Lisova Pisnya is included in the list of perspective ones.

SUNFLOWER MUTANTS WITH IMPROVED GROWTH AND METAL ACCUMULATION TRAITS SHOW A POTENTIAL FOR SOIL DECONTAMINATION

E. NEHNEVAJOVA^{1,2}, R. HERZIG², J-P. SCHWITZGUÉBEL³, T. SCHMÜLLING¹

¹Institute of Biology/Applied Genetics, Free University of Berlin, Berlin, Germany

²Phytotech-Foundation (PT-F), Berne, Switzerland

³Swiss Federal Institute of Technology, Laboratory for Environmental Biotechnology (LBE), EPFL Lausanne, Switzerland

Email: nehnevaj@zedat.fu-berlin.de

Over the last two decades, the use of plants has been proposed as an alternative technique to remove toxic metals from contaminated soils. This technique, called phytoextraction, can use either hyperaccumulating species, able to accumulate and tolerate high amounts of metal, but producing low biomass, or high yielding crops compensating moderate metal accumulation by a high biomass. Both types of plants can be considered for metal removal, but soil decontamination still takes quite a long time. Therefore plants used for metal removal need to be improved. This paper summarizes our previous and present work aimed at the improvement of sunflowers for phytoextraction by chemical mutagenesis. Improved yield and metal accumulation in sunflower mutants were already observed in the M₂ mutant generation, where three new sunflower phenotypes were found: mutants with a significantly enhanced biomass production and no changed metal accumulation; mutants with a slightly improved biomass production and an enhanced metal accumulation in shoots; and mutants with reduced metal uptake. The same alterations in growth and metal accumulation were observed in the following generation. The best M₃ sunflower mutants showed a 3-5 times higher Cd, a 4-5 times higher Zn, and a 3-5 times higher Pb extraction, as compared to the control inbred line. The stability of improved traits, yield and metal uptake, was confirmed also in the 4th generation, where mutant lines still provided a significantly enhanced metal extraction. Metal translocation from root to shoot and distribution within the shoot (stem, leaves and flower) of mutant lines and control sunflowers grown on a metal contaminated soil was studied in detail in the 5th generation under greenhouse conditions. Sunflower mutant seedlings show after three months of cultivation on contaminated soils a very good metal translocation capacity; thus the metals were primarily accumulated by sunflower leaves.

CONSTRUCTION AND CHARACTERIZATION OF MUTANT POPULATIONS IN SOYBEAN

S.Y. HAN, M.L. YANG, J.Y. GAI, D.Y. YU

Nanjing Agricultural University, National Center for Soybean Improvement, Nanjing, Jiangsu, China

Email: dyu@njau.edu.cn

Soybean [*Glycine max*(L.) Merr.] is one of the most economically important crops in the world. Collection and creation of germplasm resources have been attractive both for soybean genetics studies and breeding application. In this research, local soybean cultivars, "Nannong 86-4", "Nannong 87c-38" and "Nannong 94-16" were chosen, and treated with NaN₃, ⁶⁰Co γ rays and EMS to construct the mutant populations. Phenotypic characters of M₂ and M₃-generation mutants were investigated for various mutants including morphological characters such as leaf, stem, flower, seed and cotyledon, and results showed 185 mutants from "Nannong 86-4", 62 from "Nannong 87c-38" and 120 from "Nannong 94-16" were created. Meanwhile, protein and oil contents of partial mutants have been studied. The protein content of mutants from "Nannong 86-4" was in the range of 39.5%~52.5% and the oil content range was 15.3%~22.9%. While in the mutants from "Nannong 94-16" the protein and oil content range was 42.1%~53.0% and 14.8%~22.1%, respectively. All these materials are currently under characterization with SSR markers, and will be important both for breeding and for functional genomics studies in soybean. Supported by CRP 12988.

“ANJITHA”- A NEW OKRA VARIETY THROUGH INDUCED MUTATION IN INTER SPECIFIC HYBRIDS OF *ABELMOSCHUS* SPP.

P. MANJU, R. GOPIMONY

Department of Plant Breeding and Genetics, Kerala Agricultural University, College of Agriculture, Vellayani Thiruvananthapuram, Kerala, India
Email: manju_ptn@yahoo.co.in

Studies on inter specific hybrids of okra between *A. esculentus* (cultivated type) and *A. manihot* (wild type) revealed that no useful recombinants were obtained from the conventional combination breeding programme because of the strong linkage between yellow vein mosaic (YVM) resistant genes and wild character of *A. manihot*. The present study envisaged the breaking of undesirable linkage through gamma irradiation (10, 20, 30 and 40 kRad) of F₁ seeds obtained by inter specific hybridization between *A. esculentus* var. Kiran and *A. manihot* and further evaluation and selection of high yielding YVM resistant types from the segregating generations till F₆M₆. The mutagenic effectiveness and efficiency increased with increasing doses of gamma rays. The mutated hybrids and the wild parent showed complete resistance to YVM disease incidence which was confirmed through grafting trials. In the segregating generations, the irradiated treatments were late flowering and had more number of leaves, flowers and fruits per plant. Average fruit weight was maximum in 20 kR while fruit yield was maximum in 40 kR due to larger number of fruits. A few high yielding disease resistant plants resembling the cultivated plants were obtained in 30kR which suggested that 30 kR could be the ideal irradiation dose in okra. Thirteen superior genotypes selected from F₆M₆ generation based on yield and YVM resistance were advanced to three Comparative Yield Trials (CYT). Culture AE18 out yielded the others in CYTs and Farm trials and was released as “Anjitha” by the XXIII State Seed Sub Committee during 2006 for cultivation in Thiruvananthapuram District of Kerala. Anjitha is a high yielding variety having the fruit characters and quality of the cultivated parent *A. esculentus* var. Kiran combined with the YVM resistant character of the wild parent *A. manihot*.

MUTATION BREEDING IN CHILLI (*CAPSICUM ANNUUM* L.) THROUGH INDUCED POLYGENIC VARIABILITY

S. RANGAIAH¹, A. MANJUNATH²

¹Zonal Agricultural Research Station (ZARS), University of Agricultural Sciences Bangalore, Karnataka, India

²Department of Genetics and Plant Breeding, University of Agricultural Sciences Bangalore, Karnataka, India
Email: srangaiah@rediffmail.com

Two genotypes of Chilli viz, Ceylon and Byadagi were subjected to eight doses (50-400Gy) of gamma rays treatments by irradiating the seeds. Shift in mean, coefficient of variation (CV) skewness (sk) kurtosis (ku) and frequency of mutations in each of the treatments was compared with the control for ten characters namely, Days to flowering, days to maturity, plant height, Plant canopy, number of primary branches, number of secondary branches, number of fruits per plant, fruit length, fruit width and fruit yield. In general, in all the treatments in M₂, the CV was higher than in control for all the ten characters in both the genotypes. Also, the treatments deviated for skewness and kurtosis as compared to control for all the characters showing the effect of irradiation. The shift in these variability parameters had no definite correlation with the dose of mutagenic treatment as reported in the earlier studies. The improvement in mean was not distinctly higher in comparison with control. However, the CV had improved considerably for all the characters in all treatments indicating the scope for selection. The maximum observed for yield was 20.34 in 350Gy) as against 10.90 in control. When ten top ranking plants in M₂ were considered (irrespective of the treatments) for yield and important yield components viz, fruit length and number of pods, the maximum improvement over control was 16 per cent for number of pods, 30 percent for fruit length and 1.8 per cent for yield respectively in case of Ceylon, while the corresponding figures for Byadagi were 42, 16 and 76. Thus genotypes superior to control for yield could be realized in M₂ in case of Byadagi though not in Ceylon. Regarding frequency of viable mutations, the order of merit of different treatments differed from one another. In general, in the 300Gy treatment, the frequency of viable mutations was on the higher side.

GENETIC IMPROVEMENT OF CHICKPEA (*CICER ARIETINUM* L.) USING INDUCED MUTATION

J.D. BARSHILE¹, B.J. APPARAOB²

¹Shri Anand College of Science, Pathardi, District Ahmednagar, Maharashtra State, India

²Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar, Taluka Rahata, District Ahmednagar, Maharashtra State, India

Email: jagannath_barshile@rediffmail.com

The main target of breeding programmes has been to develop high yielding chickpea cultivar. In the present investigation an attempt was made to induce genetic variability for improvement of locally popular chickpea cultivar Vijay (Phule G-81-1-1), employing three well known mutagens, sodium azide (SA), ethyl methane sulphonate (EMS) and gamma radiation (GR). The objective was to provide genetic variability in the yield contributing traits that can be exploited in breeding programmes for genetic improvement of chickpea. Seeds of Chickpea cultivar Vijay was treated with three different concentrations / doses of SA (2, 3 and 4 mM), EMS (8, 12 and 16 mM) and gamma radiations (400, 500, and 600Gy). In M₁ generation no dominant mutations were observed, many different mutants were screened and isolated in M₂ generation such as chlorophyll mutations (alnina, chlorina and xantha); leaf mutations (gigas, compact and curly); pod mutations (small, roundish, gigas and narrow elongated); seed mutations (green, dark brown, rough seed coat); flower mutations (white flower and open); morphological mutations (early, sterile, tall and gigas). The true breeding mutant lines in M₃ generation differ considerably in their quantitative traits from the parent cultivar. The early mutant lines were maturing early than parent variety by 10 days. The range in plant height was expanded from 0.02 to 14.91cm. Gigas mutant lines obtained after 400 Gy gamma irradiation was tallest (44.44cm) with 2-3 folds increase in pod and seed size over control. Mutagenic treatments also caused changes in seed size and seed coat. Considerable genotypic variation was observed with regards to the number of seeds and number of pods plant⁻¹. Small leaf mutant showed double the number of seeds and pods plant⁻¹. As a result of mutagenic treatments, genetic variation was induced in mutants with respect to different quantitative characters. Induced mutant lines showed both positive and negative increase in the quantitative traits. As a result of mutagenic treatments, variation observed for crude protein, globulin and albumin content of mutants.

GAMMA RAYS INDUCED MUTATIONS IN SOYBEAN [*GLYCINE MAX* L. Merrill] FOR YIELD CONTRIBUTING TRAITS

A.B. TAMBE, B.J. APPARAO

Padmashri Vikhe Patil College Of Arts Science And Commerce, Pravaranagar, Dist. Ahmednagar, Maharashtra, India

Email: anna_tambe@rediffmail.com

In the present investigation, attempts were made to induce genetic variability in yield contributing traits in soybean [*Glycine max* (L) Merrill], employing gamma radiation. Germplasms of a locally adopted cultivar of soybean, MACS-450 were irradiated with different doses (100, 200, 300, and 400Gy) of gamma rays and were sown in the field during the kharif season of 2006. M₂ progeny was raised from the M₁ seeds. The M₂ progeny was screened for presence of yield contributing traits. The M₂ progeny raised from 300Gy dose of gamma radiation exhibited several induced mutations for yield contributing traits. Important among them was a high yielding mutant. About 10 such high yielding mutant plants were obtained in the M₂ progeny. The High Yielding mutants were all uniformly tall and showed a two fold increase in plant height. They produced double the number of pods per plant and three fold increase in yield per plant as compared to control. No change in pod length and number of seeds per pod were observed between the control and high yielding mutant plants, except for the 100 seed weight, which is almost 1.5 times higher as compared to control. The mutant seems to be very promising in increasing the yield of soybean. These mutants were tall and possessed double the number of pods as compared to their control counterparts.

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ISOLATION AND CHARACTERIZATION OF MUTANTS FROM *INDICA* RICE 93-11 AND GUANGLUAI (*ORYZA SATIVA* L.) INDUCED BY GAMMA IRRADIATION

J.S. BAO¹, J.K. XIE², B.L. HU^{1,2}¹Institute of Nuclear Agricultural Sciences, Zhejiang University, Hua Jiachi Campus, Hangzhou, China²Rice Physiology and Genetic Key Laboratory in Jiangxi Province, Jiangxi Academy of Agricultural Science, Nanchang, China

Email: jsbao@zju.edu.cn

The indica rice (*Oryza sativa* L.) 93-11 and Guangluai were treated by 350 Gy ⁶⁰Co gamma irradiation to induce agronomical and developmental mutants for genetic and functional genomics researches. The M₁ plants were transplanted and harvested by single plant. A total of 5 000 families of M₂ generation of 9311 were grown, the diverse morphology mutations were occurred on 4136 single plants in 1665 families, which were preliminary screened and identified according to their morphological performance during different growing stages, and 2 001 morphological mutants was screened out. Then, the mutants were planted and confirmed again in M₃ generations, a total of 1 996 mutants with defects occurring in leaf, stalk, panicle, fertility and mature time were identified. The calculated mutation frequency of morphological character was 33.3% according to the percentage of mutational families in total families of M₂ generations, which might be derived from the nonrandom harvesting of the M₁ plants. The results also indicated that both single gene mutation and multi-gene mutations were occurred simultaneously in a single mutant. Furthermore, some mutants with good agronomic traits were identified, which can be used directly in the current rice breeding programs. This study was jointly supported by funds from NSFC (30771327), 863 project (2006AA10Z193), Science and Technology Department of Zhejiang Province (2007C32014) and IAEA (12847).

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INDUCED MUTATIONS IN CROP BREEDING PROGRAMMES: ESTIMATION OF INDUCED VARIABILITY IN CHILLIES (*CAPSICUM ANNUUM* L.)

C. LEKHA RANI

Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Trivandrum, Kerala, India

Email: lekha33@yahoo.com

Chillies (*Capsicum annum* L) is a native of Latin America belonging to the family Solanaceae. India is at present the largest producer and consumer of chillies. Breeding for indigenous, adapted and better yielding varieties of chillies is hence gaining importance. So an attempt was made to induce variability through mutagenesis using physical and chemical mutagens. Three chilli varieties were chosen, one each from the low (CA 30), medium (Blue Pendant) and high (CA 12) mutagen sensitivity groups. From each of the three varieties, 200 seeds were subjected to different doses of physical (gamma ray 200 & 300Gy) and chemical mutagens (EMS 0.5 & 1.0%). In order to study the effect of seed collection from different branches of the M₁ on character expression, selfed seeds were collected separately from each of the first three branches and the remaining collected as bulk. The response of chillies to mutagens varied with the genotypes, mutagen and its doses as well as the mode of seed collection from M₁. Significant shift in mean values depending on the type of M₁ branch category clearly demonstrates that there exists the mechanism of diplontic selection in relation to induced mutagenesis in this species. Maximum yield enhancement under 0.5% EMS and 200Gy gamma rays could be achieved by restricting seed collection in M₁ to the first formed branch in varieties CA 30 and Blue Pendant. Under 1.0% EMS highest yield increase was obtained by restricting seed collection to the second branch in CA30 and CA12. Under 300Gy gamma ray treatment, yield enhancement was the highest when seed collection was restricted to the later formed branches in CA 30 and bulk seed collection in CA12.

INDUCED MUTAGENESIS IN MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK]

S.G. AUTI¹, B.J. APPARAO²

¹Department of Botany, H. P. T. Arts and R. Y. K. Science College, Nashik, Maharashtra State, India

²Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar, District Ahmednagar, Maharashtra State, India

Email: autisanjay@yahoo.co.in, bjapparao@yahoo.co.in

A wide range of viable morphological and physiological mutants were observed in M₂ and M₃ progenies of mungbean (*Vigna radiata* (L.) Wilczek) cultivars (Vaibhav and Kopargaon-1) raised from seeds treated with different concentrations of sodium azide, ethyl methane sulfonate and different doses of gamma radiation. The most striking type of mutants obtained in the M₃ progeny include plant habit, leaf structure, flower type, pod type, seed type, early and maturing and high yielding and Lhb mutants. Some of the mutants are new and are being reported for the first time in this crop. The true breeding mutant lines of M₃ generation were compared with their parent cultivar (control) to assess whether the induced genetic variability was statistically significant. These mutants can be better fitted in new cropping patterns and with improved agronomic management and good yielding ability or can be used in the genetic improvement of mungbean crop. Chemical mutagens were more efficient than physical ones in inducing viable and total number of mutations. Along with simple viable mutations, multiple mutagenic effects on two or more characters were also found in all the mutagenic treatments. Differences in the mutation frequency and spectrum will depend on the interaction of three factors such as mutagen, plant genotype, and physiological state of the organism at the moment of treatment. The Kopargaon -1 cultivar was more resistant towards mutagenic treatment than Vaibhav cultivar. All the mutants were analyzed for their protein, albumin and globulin contents by Lowry's method and for protein banding patterns employing SDS Polyacrylamide Gel Electrophoresis. Mungbean mutants with high as well as low protein contents ranging from 29.3% to 14.75% vis a vis 22.2% in control were isolated. Results showed that early flowering mutant and Lhb mutant did differ with each other as well as other mutants and controls in their protein-banding pattern. Obtained results indicated that, the approach of induced mutational breeding was effective and useful for induction of agronomically important mutants in mungbean.

GENETIC ENHANCEMENT OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) FOR HIGH OIL CONTENT THROUGH GAMMA RAY MUTAGENESIS

A. BADIGANNAVAR, S. MONDAL

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: anandgnut@yahoo.co.in

Breeding for increased oil content (OC) is important in groundnut since 80% of the Indian groundnuts are utilized for oil purpose. To induce mutations for higher OC, seeds of TAG 24 were irradiated with 150, 250 and 350 Gy gamma rays. OC in M₃ seeds from 6,000 M₂ plants estimated by Nuclear Magnetic Resonance Spectrometer, ranged from 36.39 to 52.85% as compared to 43.38 to 50.83% in parent. In the M₂, 60 plants had superior OC (50.40 - 52.72%) as well as seed yield (19.3 - 44.7g plant⁻¹), 46 plants had superior OC (48.52 - 50.36%) and 62 plants had superior oil yield (9.5 - 22.1 g plant⁻¹) compared to parent (OC: 46.47%; seed yield: 18.3 g plant⁻¹; oil yield: 8.5 g plant⁻¹). Based on OC and seed yield, 107 plants were advanced. Progeny mean OC in M₄ seeds indicated 14 progenies bred true by recording 1.5 - 4.9% higher OC than parent. Of these, 11 progenies also recorded superior seed yields of 3.0 - 86.0% and oil yields of 6.2 - 92.4%. Further in M₅ generation, four mutants scored significantly higher progeny mean OC, seed yield and oil yield with 2.4 - 5.8%, 46.6 - 67.8% and 54.4 - 71.2% superiority, respectively. True breeding behaviour of high oil mutants was confirmed by progeny evaluation in M₆ generation. All the mutants had significantly superior OC with three mutants having greater seed and oil yields of 27.3 - 35.3% and 29.8 - 48.4% superiority, respectively. Genetic improvement for OC was brought about by gamma ray mutagenesis of TAG 24, wherein seven mutants exhibited consistently superior OC of 4.3 - 6.1% based on pooled mean from M₃ to M₆ generations. Additionally, they also registered an improved seed yield and oil yield of 13.3 - 42.1% and 18.6 - 50.2%, respectively. Thus, induced mutagenesis was successful to bring about genetic improvement for a complex trait like oil content in groundnut.

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DEVELOPMENT AND UTILISATION OF GENETIC VARIABILITY THROUGH INDUCED MUTAGENESIS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

S.J. JAMBHULKAR, A.S. SHITRE

Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, India

Email: sjj@apsara.gov.in

Sunflower is one of the important oilseed crop ranking 4th in India after groundnut, rapeseed-mustard, and soybean. Genetic improvement through induced mutagenesis could pave the way to develop desirable varieties/hybrids for higher seed and oil yields, better nutrition, and environmental stresses. Studies on mutation breeding at Bhabha Atomic Research Centre (BARC), Mumbai, India, were initiated with the objective of isolation of black seed coat mutant from zebra striped seedcoat variety 'Surya' whose yield potential is equivalent to hybrids. Besides 7 black seed coat mutants, large number of morphological mutants was isolated. Prominent among them are fasciation mutation with 125 leaves against 30-35 in parent and extreme dwarf mutant with 11cm plant height against 180cm of parent. Both the mutations are controlled by single recessive gene each. One of dwarf mutant of 'Surya' grows 90cm with sturdy stem. This is being exploited to develop dwarf hybrid/varieties. Besides, variations in number, shape, and size of ray florets were also isolated. In seed coat color black, white, and brown coloured mutations were isolated. Black seed coat mutants were exploited to develop high yielding variety. Sib mating of 7 black seed coat mutants resulted into the development of various high yielding genotypes. Seed yield of one of the black seed coat mutant genotype TAS 82 (1348 Kg/ha) was superior by 17.42%, 12.05% and 53.53% over checks PKVSF9 (1148Kg/ha), Surya (1203 Kg/ha) and Morden (878 Kg/ha) respectively. Oil yield was also found superior over check varieties. Other morphological characters of TAS 82 were similar to parent variety 'Surya'. Besides, TAS 82 was found relatively tolerant to sunflower necrosis disease (SND) and tolerant to low rainfall conditions. Based on these superior characters, TAS 82 was identified for release in the state of Maharashtra and notified by Government of India.

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GENETIC IMPROVEMENT OF SOYBEAN THROUGH INDUCED MUTATIONS

J.G. MANJAYA

Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: jgmanjaya@rediffmail.com

Narrow genetic base of cultivated varieties in soybean is of global concern. Mutations, spontaneous or induced, are an important source for inducing genetic variability. Soybean cultivar VLS -2 was irradiated with 250 Gy gamma rays to induce mutations for morphological characters, high yield, high oil and low trypsin inhibitor content. A large number of mutants with altered morphological characters like plant height, flower colour, sterility, leaf shape, number of pods per plant, seed colour, early or late maturity were identified and characterized. Twenty six selections with high oil content ranging from 20 to 22 percent as compared to the parent VLS-2 (19.7 percent) were identified. Highest oil content was observed in the mutants M-387 (22.7%) and M-126 (20.7%). A modified rapid and reliable micro titration plate technique was developed and used for screening trypsin inhibitor (TI) content in the seeds of 7480 M₂ plants. Three mutants namely M-213 (13.7 TIU/mg seed meal), M-104 (15.4 TIU/mg seed meal) and M-291 (15.9 TIU/mg seed meal) showed lower levels of TI content as compared to the parent VLS-2 (21.8 TIU/mg seed meal). Breeding behaviour and salient features of the mutants were studied through the M₃ - M₅ generations. In the M₅ generation, twenty four mutants with good agronomic traits were evaluated for various quantitative characters. Analysis of variance showed highly significant variation among the mutants for yield per plant. Mutant M-17 showed high yield per plant 13.1gm in comparison to the parent VLS-2 (8.3 gm). This mutant also exhibited more number of branches, more number of pods and high harvest index. Genetic diversity study of the mutants indicated that mutant M-17 had maximum dissimilarity value of 24% from the parent and was most diverse. These identified mutants can be utilised in the breeding programme for developing elite varieties of soybean.

MUTATIONAL ORIGIN OF GENETIC DIVERSITY IN CULTIVATED GROUNDNUT (*ARACHIS HYPOGAEA* L.)

S. MONDAL, A.M. BADIGANNAVAR, S.F. D'SOUZA

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Reserch Centre, Trombay, Mumbai, India

Email: micromandal@rediffmail.com

A disease resistant groundnut genotype TFDRG 5 (derived from VG 9514 X TAG 24) was mutagenized by gamma rays (200, 300 Gy) and NaN_3 (1, 2, 3 mM, pH= 7.0). Of the 73 mutants isolated, molecular variation among the 35 selected mutants, TFDRG 5, VG 9514 and TAG 24 were detected by RAPD, ISSR and SSR markers. DNA amplification produced a total of 141 alleles, of which 105 were polymorphic. Among all the mutated alleles, an allele (C15₁₅₀₀) from RAPD marker was found only in mutant M 52. Similarly an ISSR primer (UBC 840) produced a new mutated allele in mutants, M 118 and M 124. In contrast, six null mutated alleles were detected in M 148 from Ah 282 SSR loci. It was revealed from this study that a large proportion of mutated alleles (59.77%) were of TAG 24 origin. Interestingly, the highest number of mutated alleles (45.0%) was found in M 26 and the lowest in M 40 and M 90 (2.8%). A RAPD marker F16₃₀₀ produced a null allele in mutant M 95 as well as in VG 9514. A sequence characterized amplified region (SCAR) primer from F16₃₀₀ of TFDRG 5 was designed and tested in all the mutants. Interestingly, the SCAR₃₀₀ produced a band in M 95 but not in VG 9514. This confirmed that there is a point mutation in the priming region of F16₃₀₀ in M 95 but not in the VG 9514. Cluster analysis among the mutants and parents revealed that mutant M 26 was the farthest and M 29 and M 90 were nearest to TFDRG 5. Interestingly, diversity between M 26 and TFDRG 5 surpassed the distance between VG 9514 or TAG 24 and TFDRG 5. Thus, genetic diversity induced through mutagenesis in groundnut was confirmed by molecular markers.

CREATION OF MUTANT LIBRARY IN RICE (*ORYZA SATIVA* L.) BY ION BEAM IMPLANTATION AND GAMMA RAYS

X. XU, B.M. LIU, Z.G. ZHANG, Y.J. WU

Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences, Hefei, China

Email: yjwu@ipp.ac.cn

Mutant populations are indispensable genetic resources for functional genomics in all organisms. Rice has become a model plant for molecular studies of monocot crops because of its relatively small genome and the availability of both an efficient genetic transformation method and the DNA sequence of the entire genome. The next major phase of research is to determine the function of each of the approximately 40,000 genes. However, suitable rice mutant populations, induced either by chemicals or irradiation still have been rarely developed to date. In order to find more mutants in the same genetic background, the seeds of two sequenced rice cultivars "9311" and "Nipponbare" were treated with 2.5×10^{16} N⁺/cm², 5×10^{16} N⁺/cm², 7.5×10^{16} N⁺/cm² and 150 Gy, 250 Gy, 350 Gy γ -rays, respectively. Phenotypes of mutants in the M₂ generation were observed in the field and characterized. Some mutants with the variations of leaf, stalk, panicle and other traits were found. In the M₂ generation, 740 and 571 mutants were induced by ion irradiation in "Nipponbare" and "9311", which the mutation frequency were 4.9% and 3.8%, respectively; the other 661 and 781 mutants by γ -rays irradiation, which the mutation frequency were 4.4% and 5.2%, respectively. The results demonstrated "Nipponbare" maybe more sensitive to ion irradiation than "9311". However, "9311" is presumed to be more sensitive to γ -rays irradiation than "Nipponbare". The mutants created will be helpful for gene identification and gene functional analysis.

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INDUCTION OF VIABLE MUTATIONS IN CHICKPEA (*CICER ARIETINUM* L.) EMPLOYING SA, EMS AND GAMMA RADIATION

B.J. Apparao¹, J.D. Barshile²

¹Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar, Ahmednagar District, Maharashtra State, India

²Shri Anand College, Pathardi, Ahmednagar District, Maharashtra State, India

Email: bjapparao@yahoo.co.in

A wide range of viable morphological mutations affecting almost all the parts of the plant and seed characteristics were isolated in M₂ generation of a popularly grown cultivar of chickpea, Phule G-81-1-1 (Vijay), treated with three well known mutagens, sodium azide (SA), ethyl methane sulphonate (EMS) and gamma rays (GR). The objective was to create genetic variability in the yield contributing traits that can be exploited in breeding programmes for genetic improvement of chickpea. Seeds of this cultivar were treated with three doses of SA (2, 3 and 4 mM), EMS (8, 12 and 16 mM) and gamma rays (400, 500 and 600 Gy). The mutagen administered seeds were sown in experimental fields to raise M₁ progeny. Seeds of M₁ plants and control were harvested separately and sown to raise M₂ population. The M₂ progeny were screened for viable mutations. In all twenty five different types of viable morphological mutations were observed. These included 6 types of plant type mutations (gigas, spreading, early, miniature, tall, and compact) and 6 types of leaf mutations (narrow, tiny, small, gigas, compact and curly leaf mutations), three types of flower (white, two tiered and open flowers), five types of pod (gigas, long, small roundish, elongated and small pods) and five types of seed (bold, green, black spotted, rough seed coat and dark brown seed) mutations. Results indicated that all mutagenic treatments were effective in inducing viable mutations in chickpea, during M₂ generation. The spectrum and frequency of viable mutations varied with the type of mutagen. Some mutation types occurred more frequently than others. The frequency and spectrum of viable mutations were relatively high with gamma radiation followed by EMS and SA.

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INDUCTION OF AGRONOMICALLY USEFUL MUTANTS IN SORGHUM THROUGH RADIATIONS AND *IN VITRO* TECHNIQUES

N. KUMARAVADIVEL², M. UMADEVI², P. ANANTHA RAJU², M. JAYARAMACHANDRAN², G. KANDASAMY², S. EAPEN¹

¹Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra, India

²Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India

Email: kumaravadiveln@yahoo.com

Sorghum is an important millet crop known for its multiple usage as food, fodder and fuel. In this crop, irradiation and cell and tissue culture techniques can be very useful for creating new genetic variation affecting major and polygenic traits. In the present study, two grain sorghum varieties (CO 26, COS 28) and two forage sorghum varieties (CO 27, COFS 29) were utilized. LD₅₀ dose for all the four varieties were fixed (400Gy for CO 26, 350Gy for COS 28, 500Gy for CO 27 and 600Gy for COFS 29). The viable mutants for high yield, high biomass, thick stem, tall and dwarf mutant, stay green, bold grain, grain colour (pearly white in CO 26 and COS 28, red seed colour in CO27), glume colour (red glume in CO27 and COFS 29) and forty five non-shattering mutants (COFS 29) were identified. In grain sorghum, fifty families of CO 26 and forty eight families of COS 28 were selected for high grain yield and in forage sorghum, fifteen families of CO 27 and twenty two families of COFS 29 were selected for high biomass in M₄ generation. In grain sorghum for stay green trait, twelve families in CO 26 and seven families of COS 28 were selected in M₄ generation. Agronomically superior mutants are being subjected to further evaluation, selection and stability of performance. *In vitro* culture studies: The explants utilized for the present study were immature inflorescence and young leaf and the medium used was MS the percentage of callus induction was high (82.14%) in CO 27 leaf explant and percentage of regeneration was high in inflorescence callus of CO 26 and COS 28 (80%). Addition of activated charcoal was found to be effective in reducing the phenols. As regards callus induction, 0.4 g/l activated charcoal was found to be optimum. Double the dose of Fe-EDTA was found to be necessary for high frequency of callus induction and regeneration. The best composition of ingredients for callus induction was 0.4 g/l activated charcoal, 2.0 mg/l 2,4-D and 1.0 mg/l kinetin. The best composition for regeneration was 2.0 mg/l BAP, 0.5 mg/l IAA 1.0 mg/l GA₃ and 0.1 mg/l NAA. The first generation of somaclones (SC₁ generation) were studied in the glass house. The SC₂ generation (second generation) of 182 plants (all the four genotypes) were studied in the field along with M₃ generation. Among them, eighty eight families were forwarded to SC₃ generation. A high biomass somaclone (36 tillers and 105 leaves) was identified in COFS 29.

ANALYSIS OF LIBRARIES OF 'MICRO-TOM' TOMATO MUTATIONS INDUCED BY HEAVY-ION BOMBARDMENT.

S. IMANISHI¹, A. NOGUCHI¹, T. HIRAGA¹, M. NAGATA¹, Y. HAYASHI², N. FUKUNISHI², H. RYUTO², T. ABE², I. HONDA¹

¹National Institute of Vegetable and Tea Science (NIVTS), National Agriculture and Food Research Organization (NARO), Mie, Japan

²RIKEN Nishina Center for Accelerator-Based Science, Saitama, Japan

Email: phpabstract_08@yahoo.co.jp

Fruit setting, development, and ripening are complex, genetically programmed processes. Identifying the factors that control these processes is important for understanding the mechanisms of reproductive development. We induced mutations in the tomato cultivar 'Micro-Tom' by irradiation with accelerated heavy ions (HII), recently established as an effective method for inducing mutations in plants, and constructed mutation libraries. 'Micro-Tom' showed lower sensitivities to higher HII doses. The survival rate of 'Micro-Tom' was about 50% with both ions. Screening 6,814 M₁ plants, we found various mutations, including those affecting the sizes of plants, leaves, or fruit, and one conferring a broccoli-like inflorescence. This M₁ mutant resembles tomato *anantha* mutant, in which the development of floral primordia is blocked before organogenesis. Because we could not obtain seeds from this mutant, inheritance and segregation of the trait are unknown, but *anantha* was reported, caused by a recessive mutation. The isolation of monogenic homozygous recessive mutants in the M₁ generation has not commonly been described in other mutational studies using many plant species, although our group isolated such mutants in sweet pepper by M₁ plant selection in HII mutational studies. To date, we have visually phenotyped 1,175 M₂ families (five sibs per line) in the field, and found 262 plants differing from the wild type in one or more characteristics. These results suggest that such mutation libraries could be powerful tools to explain the reproductive development of plants.

INDUCTION OF GENETIC VARIABILITY IN KACHOLAM, *KAEMPFERIA GALANGA* L.

M.T. KANAKAMANY

Agricultural Research Station, Mannuthy, Kerala Agricultural University, India

Email: kanakambalakrishnan@yahoo.co.in

The Indian System of Medicine (ISM) is predominantly a plant based Materia Medica making use of our native plants. It caters to almost the entire rural population of our country. Kacholam *Kaempferia galanga* L. belonging to the family zingiberaceae is a highly valued aromatic herb. It finds its use as a flavouring agent, stimulant, expectorant, carminative and diuretic. The commercial cultivation of this crop is gaining importance owing to its varied uses and the ease with which it can be grown in the tropics. In spite of the medicinal importance of kacholam not much work has been done for the improvement of this crop. Induction of genetic variability in kacholam, *Kaempferia galanga* L. was undertaken in the cultivar Vellanikkara local with eight different doses of gamma rays (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 Gy) and six concentrations of Ethyl Methane Sulphonate EMS (0.25, 0.50, 0.75, 1.0, 1.25 and 1.50 %) and MV₁, MV₂ and MV₃ generations were studied. LD₅₀ of gamma rays was 20.0 Gy and that of Ethyl Methane Sulphonate 1.5 per cent. The highest values for yield and yield contributing characters were obtained for 7.5 Gy gamma rays and 0.75 per cent Ethyl Methane Sulphonate. Gamma rays at 15.0 Gy and Ethyl Methane Sulphonate at one per cent were most effective in inducing variability in rhizome yield and yield attributes. High estimates of heritability (broad sense) coupled with high genetic advance was observed for number of leaves and rhizome number and direct selection for improvement of these traits will be effective. Mutagenic treatments induced alterations in the association between rhizome yield and components. High frequency of positive variants at lower doses and high frequency of negative variants at higher doses were observed. Mutant characters present in MV₂ were not completely expressed in all MV₃ plants.

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INDUCED MUTATIONS IN BREAD WHEAT VARIETY VL404 AND THEIR CHARACTERIZATION

S. AGARWAL, R. G. SAINI, B. KALIA, G. S. MIGLANI

Department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana, India

Email: drshikhas@rediffmail.com

Mutations were induced in a leaf rust resistant Indian wheat cultivar VL404 using 0.4% Ethylmethane sulphonate treatment for 16 hrs. Highest frequency of 1.94 chlorophyll mutations/100 M₁ spikes was obtained. Frequency of different viable mutations varied from 1.20 mutations/100 M₁ spikes to 2.61 mutations/100 M₁ spikes. Of the 161 viable mutants isolated, seventy six mutants showed stable expression in M₃ and M₄ generations. These were characterized for important characteristics. Three mutants having height more than and 58 mutants having height less than VL404 were observed. The spike length of mutants varied from 3.5 to 12 cm, compared to 8.5 cm of VL404. VL404 had 21 spikelets on an average in a spike, whereas 9 mutants had 23 spikelets/spike. Large variation (10 to 69 grains) in the number of grains per ear was observed in the mutants as compared to VL404 (65 grains). Mutants having up to 35 tillers were obtained as compared to 19 tillers of VL404 on an average. Cultivar VL404 has oblong grains whereas four mutants have round grains. Four mutants each having compact and sub-compact ears and twelve mutants having lax ears were recovered. VL404 has speltoid ears. Of the 76 mutants characterized, 18 showed light red to red and 3 mutants showed yellow grain colour as compared to the amber seeds of cultivar VL404. Mutants growing slower than VL404 were also recovered. A mutant bearing measled leaves with similarity to disease lesion mimics was recorded. Three mutants with waxy leaves having cup-shaped leaf tip were obtained. Twenty three of the 76 characterized mutants produced susceptible infection types on adult plants of VL404 indicating break down of resistance of VL404 to leaf rust. Origin of sequence variation in chromosomes 2A and 4B was observed when mutants were tested using 56 SSR markers specific to these chromosomes.

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ESTABLISHMENT OF LARGE MUTANT FAMILIES OF TOMATO FOR GENE KNOCKOUTS AND OTHER IMPORTANT TRAITS

H.F. GALVEZ, J.O. NARCISO, N.L. OPINA, A.O. CANAMA, E.J.U. TONGSON, M.G. COLLE, D.M. HAUTEA

Crop Science Cluster - Institute of Plant Breeding, College of Agriculture, University of the Philippines Los Banos, Laguna, Philippines

Email: haydegalvez@gmail.com

Tomato (*Lycopersicon esculentum* L.) is one of the popular and important vegetable crops grown worldwide. It ranks second after potato in terms of global production area. Tomato is the most important crop in the fresh and processed vegetable market. Current breeding efforts are geared towards the incorporation of disease resistance genes, enhanced quality traits and other important traits required by the tomato crop to sustain productivity under biotic and abiotic limiting conditions. As sources for genetic stocks, breeding materials are resourced from within the *Lycopersicon* and wild relatives. This paper reports the successful establishment of large M₁ and M₂ families of tomato generated using physical (Cobalt 60 gamma ray) and chemical (ethylmethane sulfonate, EMS) mutagens. The mutant germplasm will be used as a rich source of genetic materials to intensify crop improvement and genetic studies in tomato. Based on high-throughput phenotype screening, a total of forty one (41) homogeneous and segregating M₂ families were identified as visible mutants. The most common visible mutations observed in the M₂ screening were the monopodial plant type, different forms of chlorotic mutants, and plants with abnormal leaf morphology. From the large 600Gy and 1.0% EMS mutant families, 12 families were also identified as initial bacterial wilt resistance (BWR) gene knockouts. More gene knockouts, and visible and biochemical mutations will be identified from the remaining 600Gy and 1.0% EMS M₂ families. To confirm mutation, targeted screening will be employed using gene-specific DNA markers like BWR SCAR markers and published EST-derived markers for tomato mutant genes. This is to determine and compare the type/frequency of mutations that have been induced using either gamma ray irradiation or EMS.

BROADENING THE GENETIC BASE AND INTROGRESSION OF MYMV RESISTANCE AND YIELD IMPROVEMENT THROUGH UNEXPLORED GENES FROM WILD RELATIVES IN MUNGBEAN

M. PANDIYAN¹, N. RAMAMOORTHY¹, S.K. GANESH¹, S.JEBARAJ³, P. PAGARAJAN², P. BALASUBRAMANIAN²

¹National Pulses Research Centre (TNAU), Vamban, India

²Centre for Plant Molecular Biology, TNAU, Coimbatore, India

³Tamil Nadu Rice Research Institute, Aduthurai, India

Email: mpandiyand8@yahoo.co.in

Introgression of unexplored genes from the wild relatives could be rewarding for broadening the genetic base of important traits such as yield, yield attributes and resistance to biotic and abiotic stresses in pulses. With an objective to develop superior segregants for yield coupled with yellow mosaic resistance (MYMV), interspecific direct crosses were attempted in *Vigna radiata* var. VRM (Gg) 1 with two accessions of *Vigna umbellata* (yellow and red). Even though crossability barriers were predominant in interspecific crosses, it was possible to recover interspecific hybrids in direct crosses. The F₁ plants of *V. radiata* x *V. umbellata* were found to be intermediate phenotype with light green colour leaves. The reproductive parts tend to resemble towards *V. umbellata* with double peduncle in one leaf axis. There was no pod set observed when selfing was effected in F₁s and also in their corresponding backcrosses with parents. The F₁ plants produced more than four thousand flowers per plant. Spontaneous sterility was observed both in female and male parts of the flower. Detailed cytological studies were carried out for male and female sterility. The reason for male sterility was due to meiotic irregularities viz., unequal separation of tetrad and female sterility was due to degeneration of megaspore during megasporogenesis. To recover fertile plants in F₁ hybrids, irradiation techniques were followed. The parental seeds were irradiated with 100 Gy, 200 Gy, 300Gy, 400Gy and 500Gy doses. The pod set percentage was increased in irradiated parental crosses than normal crosses. In normal crosses, pod set percentage ranged from 2.00 (VBN (Gg) 2 x *Vigna umbellata* red) to 4.40 (VRM (Gg) 1 x *V. umbellata* red). In the crosses resulted from irradiated parents, the pod set percentage ranged from 2.70 (CO 6 x *V. umbellata* yellow) to 4.90 (VRM (Gg) 1 x *Vigna umbellata* yellow) among crosses involving parents treated with 100Gy. The fertile F₁ hybrid plants were obtained from a cross between *Vigna radiata* var. VRM (Gg) 1 and *V. umbellata* red (both parents treated with 200 Gy). The fertile F₁ phenotype was towards female parent. But, traits like orientation of top leaves, tendril ness and number of seeds per pod shifted towards male parent.

INDUCED VARIABILITY FOR POD YIELD, POD TRAITS AND FOLIAR DISEASE RESISTANCE IN GROUNDNUT (*A. HYPOGAEA* L.)

K.G. PARAMESHWARAPPA, M.G. BENTUR
Oilseeds Scheme, MARS, UAS, Karnataka, India
E-mail: ssoparamesh@rediffmail.com

Evaluation of 15 elite M₃ families derived from gamma ray treatment (150Gy) of Dh 86 kernels was carried out to know the extent of induced variability for different productivity traits viz., pod yield, shelling percent, 100 kernel weight and per cent sound mature kernels (SMK %) and late leaf spot resistance. The ANOVA revealed significant genotypic differences among M₃ families for pod yield, shelling percent and 100 kernel weight indicating that irradiation has brought in considerable magnitude of genetic variability in a homozygous parental line Dh-86. The magnitude of genetic variability (GCV) appeared to be considerable for pod yield (35.3) followed by 100 kernel weight (7.02), shelling percent (3.29) and SMK % (1.4) offering further scope for selection. Two superior progenies Dh 86 15 kr-17 and Dh 86 15 kr-12 had higher mean pod yield (2553 and 2108 kg/ha, respectively) compared to untreated Dh 86 parent (1475 kg/ha). The lines also had higher shelling percent (76.8 and 76.2), 100- kernel weight (42.0 g and 38.2 g) besides having high level of resistance to late leaf spot (2 and 3 grade), which were on par with resistant check GPBD-4 (2 grade). Dh-86 is a rabi/summer variety which suffers most due to late spot when grown in rainy season that comes in the way of seed multiplication of a summer variety since seed viability is lost before the next summer season. In this context, Dh-86 15 kr-17 and Dh-86 15 kr-12 having considerable amount of resistance to late leaf spot can be recommended for cultivation both in rainy and post rainy seasons. Therefore, these two lines can be treated as dual season varieties and needs large scale yield test over the locations for confirmation of superiority and stability of resistance.

IAEA-CN-167-287P

HYBRIDIZATION FOLLOWED BY MUTAGENESIS IS A NOVEL METHOD OF ISOLATION OF RARE FREE-THRESHABLE MUTANTS OF DICOCCUM WHEAT (*TRITICUM DICOCCUM* (SCHRANK.) SCHUBL.)

P. KULKARNI, S.A. DESAI, H.C. LOHITHASWA, I.K. KALAPPANAVAR, R.R. HANCHINAL
Dr.Sanjay Rajaram Wheat Laboratory, Main Agricultural Research Station, University of Agricultural Sciences
Karnataka, India
Email: sumadhwa5@gmail.com

Hybridization between elite varieties of *T.dicoccum* x *T.dicoccum*, *T. durum* x *T. durum* and *T.dicoccum* x *T. durum* followed by mutation was undertaken during the rabi season of 1999–2000 at the fields of Dr. Sanjaya Rajaram Wheat Laboratory, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India. The F₁s were obtained from hybridization programme and elite dicoccum genotypes were also treated with 0.5 per cent ethyl methanesulfonate (EMS) in 0.05 M phosphate buffer and also exposed to 150Gy gamma rays treatment. A minimum of 20-30 putative free-threshing plants were selfed to produce the seeds of F₂M₂ generations. The advanced generations of F₃M₃ and F₄M₄ were obtained by selfing in subsequent seasons and similarly advanced generations treated seeds of elite dicoccum genotypes were obtained selfed to advance generation by following pedigree method. More than 20,000 individual ear heads were evaluated and 1261 individual ear heads were selected for putative free-threshable lines derived through hybridization followed by mutagenesis. Based on rachis fragility and glume tenacity, fifty one free-threshable dicoccum lines were selected. It is known that the Q gene confers the free-threshing character. It also pleiotropically influences many other domestication-related traits such as glume shape and tenacity, rachis fragility, spike length, plant height, and spike emergence. The two lines derived from the cross NP-200 x MACS 2912 (line nos. 886 and 915) and one from DDK 1013 x DDK 1001 (line no.206) impersonated the mutation in the q locus as they were nutritionally superior, free threshing types with early flowering, increased seed weight and amber coloured grains. So hybridization followed by mutation can be considered as reliable tool for generating desirable mutants for economically important traits like free threshability, a desired character in dicoccum wheat.

IAEA-CN-167-304P

THE SCREENING AND IDENTIFICATION OF MUTANTS FROM *INDICA* VARIETY 9311 AND *JAPONICA* VARIETY NIPPONBARE OF RICE (*ORYZA SATIVA* L.)

C.H. SHI, J.G. WU, B.T. NGUYEN, W.Q. LI, J. YE
Department of Agronomy, Zhejiang University, Hangzhou, China
Email: chhshi@zju.edu.cn

Among various genetic resources, mutant library with different genetic variation are essential for discovering the gene function. Consequently, there has been growing interest in using chemical and irradiation mutagenesis in model organisms such as rice for functional genomic research. The outstanding two-line hybrid *indica* rice restorer 9311 in China, and the conventional and widely grown *japonica* variety Nipponbare in Asia, which the genomes sequencing were completed, have been chosen to produce the rice mutant library. For radiation mutagenesis, dry seeds were mutagenized by gamma ray (⁶⁰Co) with 10Gy/min at dosage of 300Gy. In chemical mutagenesis process, dry seeds were presoaked in water for 16 hours before soaking for 8 hours with 0.4% EMS (Ethyl methane sulfonate) solution. Germinated seeds of M₂ and other followed generations were sown on the rice seedling bed and about 25-day old seedlings were transplanted to paddy field for screening or validating various mutants. For screening the seedling or roots mutants from population in three-leaf stage, seeds of M₂ or other generations were sown into plastic trays and bred using the hydroponic method. By using hydroponic method, the mutants from 9311 and Nipponbare were screened for morphological and / or root traits at three-leaf stage. The morphological mutants were the seedlings with albino (9311, Nipponbare), greenable-albino (9311, Nipponbare), semi-rolled leaf (9311, Nipponbare), etiolated-leaf (9311, Nipponbare), twins-seedling (9311, Nipponbare), broad leaf (Nipponbare), dwarfism (9311, Nipponbare), white stripe leaf (9311, Nipponbare) and lethality (9311, Nipponbare). The root mutants were long root (9311, Nipponbare), flourishing root (9311, Nipponbare), short root (Nipponbare). Resistant mutants for herbicides (9311, Nipponbare), drought (9311, Nipponbare) and salt (Nipponbare) also have been identified. Among these mutants, the albino was the most frequently observed in all mutagenized populations. To evaluate the rice plant mutants under field conditions, morphological variations at vegetative and reproductive stages were identified in the M₃ and higher generations. Various mutants including plant architecture, growth habit and different physiological characters were screened, which were dwarfism (9311, Nipponbare), tallness (Nipponbare), broad leaf (Nipponbare), narrow leaf (Nipponbare), leaf with white stripe (9311, Nipponbare), spotted leaf (9311, Nipponbare), rolled leaf (9311, Nipponbare), twisted leaf (9311), erected leaf (9311, Nipponbare), reduced tillering (9311, Nipponbare), increased tillering (9311, Nipponbare), earless (9311, Nipponbare), sterility (9311, Nipponbare), puny plant (9311, Nipponbare), early heading (9311, Nipponbare), late heading

(9311, Nipponbare). Furthermore, the visible mutants related to spikelet and panicle could also be found in both populations of radiation and chemical mutagenesis, which involved erected panicle (Nipponbare), larger panicle (9311, Nipponbare), small panicle (9311, Nipponbare), brittle culm (Nipponbare), lower fertility (9311, Nipponbare), dense panicle (9311, Nipponbare), sparse panicle (9311, Nipponbare), degenerated spikelet (9311, Nipponbare), unclosed hull (9311, Nipponbare), larger grain (9311, Nipponbare), small grain (9311, Nipponbare) and loose panicle (9311, Nipponbare). The mutants related to brown rice including round-shape grain (Nipponbare), slender grain (Nipponbare), larger embryo (Nipponbare), large chalkness (9311, Nipponbare) and red color rice (9311, Nipponbare) have been identified from both populations were screened. The above mutants can provide the genetic resources for analyzing the relevant genes, which control the performance of traits and characterizing the relationship between genotype and phenotype. By further study, some of the above mutants can be utilized to identify the functional polymorphism of non-allelic genes, even map and clone the related gene(s).

IAEA-CN-167-362P

ITALIAN MUTANT COLLECTIONS OF *MEDICAGO TRUNCATULA*

O. CALDERINI¹, A. PORCEDDU^{1,5}, F. PANARA¹, L. MOLINARI¹, P. TAVIANI¹, L. LANFALONI², C. SCOTTI³, M. CARELLI³, L. SCARAMELLI³, G. BRUSCHI³, P. RATET⁴, E. PIANO³, S. ARCIONI¹

¹CNR Istituto di Genetica Vegetale Perugia, Perugia Italy

²Dipartimento di Biologia Cellulare e Ambientale, Università degli Studi di Perugia, Perugia, Italy

³CRA-FLC Centro per le Produzioni Foraggere e Lattiero-casearie, Lodi, Italy

⁴Institut des Sciences du Végétal CNRS bat 23 - Avenue de la Terrasse, Gif-sur-Yvette Cedex, France

⁵Present address: Dipartimento di Scienze Agronomiche e Genetica Vegetale Agraria, Università degli Studi di Sassari, Sassari, Italy

Email: ornella.calderini@igv.cnr.it

A functional genomic initiative was undertaken in Italy for *Medicago truncatula* supported by the Italian Ministry of University: Funds for basic Research (MIUR/FIRB). We intended to produce tools in order to unlock the genetic control of traits of agronomic relevance for *Medicago*. Three different mutagenesis strategies were approached: transposon tagging, activation tagging and TILLING. All the three collections proved efficient in isolating mutants of interest for characters such as plant architecture, content of secondary compounds etc. We envisage the integration of our collections into those being produced in both Europe and US with the goal of creating a functional genomic resource of high quality as those available for *Arabidopsis* and rice.

IAEA-CN-167-389P

INDUCED MUTATIONS IN *JATROPHA CURCAS*

V.K. GOUR, V. PRAJAPATI, M. SAHU

Department of Plant Breeding & Genetics, J.N. Agricultural University, Jabalpur, India

Email: vkgour@rediffmail.com

Dry seeds of two *Jatropha curcas* cultivar viz., Acc. No.116 and Acc. No.404 were treated with four different doses of gamma rays 60 Gy, 120 Gy, 180 Gy and 240 Gy at Bhabha Atomic Research center, Mumbai, India. The populations comprising mutants and control in third year exist as plantation. The variation for pollen sterility was observed in both the accessions; most of the plants exhibited pollen size variation, however, few expressed very high degree of sterility. Exceptionally, few plants exhibited high pollen sterility. Higher number of capsules in such plants confirmed sterility limited to male system. The phenomenal changes observed were for sectorial chimera (leaves without lobe, petiole colour in branches and white patches in leaves); and homohistont or solid mutant for stem/foliage colour, leaf margins and petiole with red colour in whole plant and hairy floral parts (calyx, corolla, anthers and ovary). Two accessions have shown differential response to treatments. The accession 116 has shown higher spectrum than 404. The plant height exhibited variation, however, few plant appeared to be vigorous. The extent of variation observed for number of seeds per plant and seed yield per plant in both the accessions at different doses of gamma ray. The 100 seed weight of both accessions revealed variation due to the age and flowering time within season, which showed seed size variation from small to normal size. The variation in seed index is also subjected partly to environmental variation. The phenomenal changes observed were for sectorial chimera and homohistont or solid mutant for stem/foliage colour, leaves margin and petiole with red colour in the full growth and branches. Two accessions have shown differential response to treatments. The accession 116 was shown higher spectrum than 404. The variation observed for all the qualitative and quantitative characters under study is subjected to irradiations. The differential response to treatment is markedly observed in accession 116 as compared to accession 404.

CONCURRENT SESSION 2 (14:00-18:00)

**Plant Mutagenesis – DNA Damage, Repair and Genome
Stability**

UNIDO Boardroom C04

Tuesday, 12 August 2008

RECOMBINATION, EXTRACHROMOSOMAL DNA AND GENOME STABILITY

J.M. WATSON, K. RIHA

Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria

Email: Karel.Riha@gmi.oeaw.ac.at

Eukaryotic chromosomes are faithfully transmitted from one generation to another. However, eukaryotic genomes are much more dynamic than this view permits. Repetitive sequences are among the most rapidly evolving genome constituents and copy number variations are extremely abundant even within species. Rapid evolution of repetitive DNA is particularly apparent during domestication of many crops and is, for example, well documented in cereals such as maize or barley. Importantly, deletions or insertions of such sequences may contribute to variation in protein-coding gene expression. This can occur by altering gene copy number and expression, by disrupting regulatory sequences or by altering regulatory RNA production. One mechanism that may significantly contribute to copy number variation and rapid evolution of crop genomes involves the formation of extrachromosomal circular DNA molecules (eccs). I will present evidence for eccDNA derived from centromeres, 5S rDNA and telomeres in *Arabidopsis*. I will further discuss the role of homologous recombination, telomere metabolism and chromatin modification in eccDNA biogenesis. Finally, I will discuss the impact of eccDNA metabolism on the stability and evolution of plant genomes.

COMPLEX PATTERNS OF T-DNA INTEGRATION: HOMOLOGOUS, NON-HOMOLOGOUS AND “SEMI-HOMOLOGOUS”

C. MELAMED-BESSUDO, M. LIEBERMAN-LAZAROVICH, A.A. LEVY

Department of Plant Sciences, Weizmann Institute of Science, Rehovot, Israel

Email: avi.levy@weizmann.ac.il

The integration of T-DNA vectors, following *Agrobacterium*-mediated transformation, was shown to occur preferentially via non-homologous end-joining (NHEJ) into genomic sites with pre-existing DNA double-stranded breaks. This type of NHEJ is error prone, giving rise to a series of rearrangements at the T-DNA termini (deletions and insertions). By contrast, DNA integration via gene targeting, namely homologous integration between the delivered vector and the genomic target sequence is considered a precise process as we showed in a previous work. Here, we describe the molecular analysis of a series of T-DNA integration events (“semi-homologous”) that involve homologous recombination between the vector and the target on one side, and non-homologous integration at the other end of the vector. This work was done using a new gene targeting assay based on the activation of monomeric RFP in seeds of *Arabidopsis* upon homologous integration between the T-DNA vector and the homologous *Cruciferin* endogenous locus. This assay gives a stronger fluorescence signal than the previously described GFP assay. Using Southern blot and PCR analyses, we found that the target gene was unchanged. However, the vector had invaded the homologous target and synthesized a stretch of DNA of at least 10Kb upstream to the region of homology. On the other side of the integration, we couldn't find sequence homology to the target and moreover the T-DNA right border was integrated at this side. Preliminary sequencing of such complex integration events, using inverse PCR products, together with Southern blot analysis, suggests a model whereby there is a one sided homologous invasion, followed by end elongation of the invading vector and copying of long stretches of DNA. The newly synthesized DNA, rather than being assimilated into the target, becomes dissociated and integrates at an ectopic site, thus generating duplications of large DNA segments.

IAEA-CN-167-352

INFLUENCES OF THE ENVIRONMENT ON PLANT GENOME DYNAMICSB. HOHN¹, I. KOVALCHUK², O. KOVALCHUK², J. LUCHT³, J. MOLINIER⁴, H. PUCHTA⁵, G. RIES⁶¹Friedrich Miescher Institute, Basel, Switzerland²University of Lethbridge, Canada³Internutrition, Zürich, Switzerland⁴University of Strasbourg, France⁵University of Karlsruhe, Germany⁶BioMedinvestigator AG, Basel, Switzerland

Email: barbara.hohn@fmi.ch

A biomonitoring system was established in the model plants *Arabidopsis thaliana* and *Nicotiana tabacum* which allows detection and quantification of point mutation and homologous recombination events in the whole organism. The use of DNA break-inducing agents and UV demonstrated the feasibility of using the model plants transgenic for recombination substrates for detection and analysis of mutants altered in the frequency of somatic homologous recombination events and in reporting environmental influences on chromosome dynamics. The environmental impacts analyzed included gamma radiation, UV, toxic heavy metals and plant pathogens in the form of fungi, viruses and elicitors mimicking pathogen attack. Data collected will be compared to those originating from mutagenesis assays in other organisms.

IAEA-CN-167-395

GENETIC REQUIREMENTS FOR RESISTANCE AND RESPONSE TO PHOTONIC VS. HZE IONIZING RADIATIONA. BRITT¹, P. CONKLIN¹, K. YOSHIYAMA¹, K. CULLIGAN^{1,2}, N. HUEFNER¹¹Dept. of Plant Biology, University of California, Davis, CA, USA²Dept. of Biochemistry and Molecular Biology, University of New Hampshire, Durham, NH, USA

Email: abbritt@ucdavis.edu

Both high and low linear energy transfer (LET) ionizing particles act as effective mutagens in plants. HZE (high atomic weight, high energy) particles are predicted to induce more densely clustered damage than the gamma photon. Here we compare the genetic requirements for resistance to high vs. low LET radiation in a series of *Arabidopsis* mutants. A comparison of effects on the germination and subsequent growth of seedlings led us to conclude that the relative biological effectiveness of the two types of radiation (1 GeV Fe nuclei vs. gamma) are roughly 3:1. Similarly, in wild-type lines, loss of somatic heterozygosity was induced at about a 2:1 ratio (HZE vs. gamma). Checkpoint and repair defects, as expected, enhanced sensitivity to both agents. The "replication fork" checkpoint, governed by ATR, played a more important role in resistance to HZE-induced mutagenesis than in resistance to gamma induced mutagenesis. Effects of the two agents on the transcriptome were also compared, using doses (30 Gy HZE vs. 100 Gy gamma) that have similar (slight, but measurable) effects on seedling germination and growth. The majority of the genes induced by gamma were also induced by HZE, the persistence of this induction was not especially different between the two agents, and the induction was dependent on the DSB induced checkpoint gene ATM. In contrast, there were a large number of genes specifically induced by HZE that were not induced by gamma radiation, and the induction of these genes was not dependent on ATM. We suggest that the first set of genes (induced by gamma, ATM dependent) reflect the induction and persistence of DSBs, while the second set of genes (induced only by HZE, ATM-independent) reflect a response to some as of yet unidentified stressor.

ROLE OF HUMAN DISEASE GENES FOR THE MAINTENANCE OF GENOME STABILITY IN PLANTS

H. PUCHTA, D. KOBBE, K. WANIECK, A. KNOLL, S. SUER, M. FOCKE, F. HARTUNG
University of Karlsruhe, Department of Botany II, Karlsruhe, Germany
Email: holger.puchta@bio.uka.de

In the plant genome a row of homologs of human genes can be found that if mutated are correlated with high incidence of cancer in humans. Here we describe our recent results on homologs of the breast cancer genes BRCA1/BARD1 and RecQ helicase homologs in the model plant *Arabidopsis thaliana*. HsBRCA1 and HsBARD1 are tumor suppressor proteins that are involved in many cellular processes, such as DNA repair. Loss of one or the other protein results in early embryonic lethality and chromosomal instability. The *Arabidopsis* genome harbors one BRCA1 homolog, and we were able to identify a BARD1 homolog as well. AtBRCA1 and AtBARD1 are able to interact with each other as indicated by *in vitro* and *in planta* experiments. Our analyses of T-DNA insertion mutants for both genes revealed that in plants, in contrast to animals, these genes are dispensable for development or meiosis. Nevertheless, we could show that AtBARD1 plays a prominent role in the regulation of homologous DNA repair in somatic cells. RecQ helicases are known as mediators of genome stability. The loss of RecQ function is often accompanied by hyperrecombination due to a lack of crossover suppression. *Arabidopsis thaliana* possesses 7 different RecQ genes. We could show that two of them (AtRECQ4A and 4B) arose as a result of a recent duplication and are still 70% identical on protein level. Disruption of these genes surprisingly leads to antagonistic phenotypes: the AtRECQ4A mutant shows sensitivity to DNA damaging agents, enhanced homologous recombination and lethality in an *Atmus81* background. Moreover, mutation of AtRECQ4A partially suppresses the lethal phenotype of an AtTOP3 α mutant. In contrast, the AtRECQ4B mutant shows a reduced level of HR and none of the other phenotypes described above. Finally, we have started to characterize the different RecQ proteins of *Arabidopsis* by biochemical means and present here the results on AtRECQ2.

IAEA-CN-167-404

DNA REPAIR MECHANISMS OF THE EXTREMELY RADIORESISTANT BACTERIUM *DEINOCOCCUS RADIODURANS*

Y.J. HUA, N.J. YING, G.Z. XU
Key Laboratory of Chinese Ministry of Agriculture for Nuclear-Agricultural Sciences, Institute of Nuclear-Agricultural Sciences, Zhejiang University, Zhejiang, China
Email: yjhua@zju.edu.cn

Deinococcus radiodurans belongs to a family of bacteria characterized by extreme resistance to ionizing radiation, UV, desiccation, and a variety of DNA damaging agents. This dramatic capability is ascribed to its outstanding efficiency in reconstructing a functional genome with high fidelity from hundreds of double strand breaks (DSBs) generated by DNA damaging agents, while few other organisms can tolerate DSBs. Exponentially growing *D. radiodurans* is able to withstand 50-100 times more ionizing radiation than *Escherichia coli* and can survive a 15 kGy acute ionizing radiation dose with no loss of viability. Efficient DNA repair, regulation of cellular responses to radiation damage, and protective role of non-enzymatic Mn (II) ions and carotenoids are attributed to its radiation resistance. *D. radiodurans* recovering from high doses of ionizing radiations demonstrates biphasic kinetics of DSB repair. *D. radiodurans* is able to rejoin many DSBs in RecA-independent manner at early times after irradiation. Subsequently, circular chromosomes are reformed by RecA-dependent recombination repair system. Non-homologous end joining repair system may also play important roles to its radiation resistance. In addition, *D. radiodurans* encodes a number of previously unidentified DNA repair proteins. Approximately 53% of the 3187 open reading frames believed to encode proteins in *D. radiodurans* R1 were assigned a function based on similarity to other gene products found in the protein databases. Two novel regulators, PprI and DrRRA are essential in the radioresistance of *D. radiodurans*. They strongly enhance the activities of catalase and promote the expression of RecA and PprA. One of the striking features of *D. radiodurans* is its intracellular accumulation of Mn (II) and intracellular Mn (II) can be protective by scavenging reactive oxygen species.

IAEA-CN-167-108

MOLECULAR CYTOGENETICS IN AN ASSESSMENT OF DNA DAMAGE AND REPAIR PROCESSES

J. JUCHIMIUK-KWASNIEWSKA, J. MALUSZYNSKA

University of Silesia, Department of Plant Anatomy and Cytology, Katowice, Poland

Email: juchimiu@us.edu.pl

Mutagenesis is one of the simplest and most effective methods for inducing plant variability. The mechanisms, which evoke variability, are chromosomal aberrations, arising from DNA double strand breaks (DSB). The frequency of chromosomal aberrations is correlated with the level of DNA damage and effectivity of cell repair system. Chromosomal aberrations can be detected using simple cytogenetic methods, however to assess the direct DNA damage and the effectivity of repair processes during recovery time after mutagenic treatment, in nucleus molecular methods are required. Comet assay and TUNEL test were successfully adapted and accepted for the detection of DNA fragmentation in mutagenesis. TUNEL test, based on labelling the 3'OH ends of DNA with fluorochrome – conjugated dUTP by terminal deoxynucleotidyl transferase (TdT) allows to distinguish the nuclei with DNA fragmentation. Another method - comet assay, based on the migration of damaged DNA fragments in electric field and forming an image similar to comet, is used for analysis of the level of DNA damage in single nucleus. Fluorescent *in situ* hybridization (FISH), provides new tools for the identification of individual chromosomes/ chromosome arms participating in formation of the aberration. An advantage of FISH is possibility to understand the composition of the micronuclei thus improving an existing micronucleus test. An application of region-specific DNA probes (telomere and centromere) as well as rDNA as probes enables the analysis of the break points in the chromosomes leading to micronuclei. The application of the molecular cytogenetic methods will be presented as the analysis of the level of DNA damage and effectivity of repair processes in *Hordeum vulgare* cells (2n=14) after mutagenic treatment with γ -rays, MH, and MNU in different postincubation times. FISH with rDNA and centromeric/telomeric DNA as probes, to evaluate chromosome aberrations in barley cells caused by these mutagens will show the differences between action of these mutagens.

IAEA-CN-167-048

THE ENHANCED GENOMIC INSTABILITY WAS INDUCED BY ALPHA PARTICLE AND LOW-ENERGY ION IRRADIATION IN SOMATIC CELLS OF *ARABIDOPSIS THALIANA*

B. PO, P. LIU, Y.J. WU

Laboratory of Ion Beam Bio-engineering, Institute of Plasma Physics of Chinese Academy of Sciences, Hefei, China

Email: bianpo@ipp.ac.cn

Although low-energy ion radiation has been proven to have a wide range of biological effects and led to fruitful achievements as a new mutagenic source for genetic modification, there still exist some disputes about its mutagenic mechanisms because of its short-penetrating property. In present research, *Arabidopsis thaliana* transgenic for *GUS* recombination substrate was used to evaluate the genomic instability induced by irradiations of alpha particle (3.3MeV) and Low-energy-Argon ion (30 KeV). A pronounced effects of alpha particle irradiation to *Arabidopsis thaliana* seedlings and Argon ion irradiation to seeds on the somatic homologous recombination frequency (sHRF) were reported. The sHRFs increased 1.88-fold and 2.42-fold, respectively, which indicated that the short-penetrating radiation could effectively induce the plant genomic instability in either dry seeds or seedlings with active metabolism. The local alpha particle irradiation of root was performed. Result exhibited 2.5-fold increase of sHRF in non-irradiated aerial plant, indicating that the enhanced genomic instability in bystander aerial plants was induced by damage signal(s) from the irradiated roots. The Monte-Carlo calculation showed that 3.3 MeV alpha particle only traverse near surface-cells, 17.57 μ m in depth, which suggested that radiation damage signal(s) transmission could also play a key role in the induction of genomic instability in systemic alpha particle irradiation, similarly in low-energy-ion radiation owing to its shorter penetrating power in biological tissue. Treatments of seedlings with DMSO (Dimethyl sulfoxide, ROS scavenger) both in systemic and local alpha particle irradiation significantly suppressed the increase of sHRF. And it showed that the ROS was involved in the induction of radiation responses in non-irradiated portion.

GENOMIC AND GENE-SPECIFIC INDUCTION AND REPAIR OF DNA DAMAGE IN BARLEY

V. MANOVA, M. GEORGIEVA, B. BORISOV, B. STOILOVA, K. GECHEFF, L. STOILOV
Institute of Genetics "Acad. D. Kostoff", Bulgarian Academy of Sciences, Sofia, Bulgaria
Email: vmanova@bas.bg

Repair of DNA damage induced by various mutagenic agents within the barley genomic and ribosomal DNA was a subject of investigation. Reconstructed karyotypes T-1586 and T-35, with normal and increased expression of ribosomal genes, respectively, were utilized to evaluate the relationship between the transcriptional activity and the rate of DNA damage induction and their repair. A tendency towards restoration of rDNA integrity after γ -irradiation was observed, indicative for the efficient recovery of double-strand breaks in barley ribosomal DNA. Ability of barley ribosomal genes to cope with damage produced *in vivo* by the radiomimetic agent bleomycin was further analyzed. Preferential sensitivity of barley linker DNA towards bleomycin treatment *in vivo* was established. Fragments containing intergenic spacers of barley rRNA genes displayed higher sensitivity to bleomycin than the coding sequences. No heterogeneity in the repair of DSB between transcribed and non-transcribed regions of ribosomal genes was detected. Data indicated that DSB repair in barley ribosomal genes, although relatively more efficient than in genomic DNA, did not correlate with NOR activity. Repair kinetics of UV-C induced cyclobutane pyrimidine dimers in barley genomic and ribosomal DNA was also studied. Less amount of CPD in rDNA in comparison to total genomic DNA immediately after irradiation was detected. Results showed that UV-C induced CPD in barley ribosomal genes are as efficiently repaired as in the rest of the genome predominantly by light repair mechanisms.

Poster Presentations

IAEA-CN-167-034P

EFFECTS OF GAMMA-IRRADIATION-INDUCED MUTATION ON UPLAND COTTON POLLEN GRAINS

J.Y. YUE, C.M. TANG
College of Agronomy, Nanjing Agricultural University, Nanjing, China
Email: tang20@jlonline.com, cmtang20@yahoo.cn

Despite the demonstrated value of gamma ray as a tool in plant mutation research, in the genetic plant species upland cotton (*Gossypium hirsutum* L.), such mutations have not been extensively studied. To investigate these questions, the upland cotton cultivar "Sumian 22" pollen grains were irradiated by gamma rays (20Gy). The irradiation effects on pollen grains were tested considering the ultrastructure changes in the exine and interior walls of pollen grains, their germination rate, DNA polymorphism in the pollen grains, the actin filament in pollen tube, fertilization, and boll development after the pistils were pollinated by the pollen grains which were irradiation with γ -rays. As compared with the control, although the cell structures inside the pollen grain were destroyed, its exine and interior walls of the pollen grain were not etched. The amount and the density of pollen grain inclusions decreased and the size of the lacuna and starch granules increased. Pollen grain germination rate decreased by 37%. The number of pollen tubes in the style declined by 38%, but the growth speed of the tubes did not change. All of the pollen tubes reached the end of the style at 13-h after pollination. This result was consistent with that of the control. Also, the weight and the diameter of the ovary decreased and shortened. No evident change of the fecundation time of ovule was observed. The significant difference on DNA polymorphism was found between irradiation pollen grain and control after pollination by Simple sequence repeats (SSR) molecular marker. The actin filament of the apical domain in pollen tube was destabilized, and in the approximately apical domain, the actin cytoskeleton component disappeared. Various mutants were appeared in the M₁ progenies. These results indicate that gamma rays can cause a series of biological changes in irradiated-pollen grains and their progenies of upland cotton.

IAEA-CN-167-046P

POSITION-SPECIFIC EFFECTS IN THE ACTION OF MUTAGENIC AGENTS ON THE CHROMOSOMES OF BARLEY (*HORDEUM VULGARE* L.)

K. GECHEFF, L. STOILOV, V. MANOVA, G. BONCHEV

Institute of Genetics "Acad. D. Kostoff", Bulgarian Academy of Sciences, Sofia, Bulgaria

Email: kgecheff@bas.bg

A comparative analysis of the intrachromosomal distribution of structural mutations induced by maleic hydrazide (MH) and γ -rays in barley was done. The two mutagenic agents have different mechanisms of action; MH as mutagen with delayed effect produces only chromatid rearrangements, and γ -irradiation is a typically mutagenic factor with non-delayed effect, capable to induce both chromosome and chromatid types of aberrations. Two reconstructed karyotypes of barley – T-1586 and T-21, which allow an easy identification of the individual chromosomes of their complements, and differ from each other by the position of the “hot spot” segments, were used as experimental plant material. No significant differences in the sensitivity of the karyotype variants tested were observed after treatment with both MH and γ -rays. As expected, the two agents clearly differ by the types of aberrations induced; only chromatid type for MH and both chromatid and chromosome types in the case of γ -rays. The most interesting finding in this study is that the chromosome constitution may dramatically affect the distribution pattern of aberrations along the individual chromosomes, and this effect was especially strongly pronounced in the action of MH. Two segments, namely 32 and 41, located proximally to the nucleolus organizing regions (NORs) of chromosome 5H and 6H, respectively, were found to be the most sensitive sites (“hot spots”) of barley karyotype. The preferential involvement in chromosomal rearrangements of these segments was mostly expressed for intercalary deletions and duplication-deletions. Interestingly enough, when the two “hot spot” segments are combined in chromosome 5H^{6H} by reciprocal translocation in tandem position (karyotype T-21) approximately 50% of the intercalary deletions (versus 16% in the case of T-1586) and 45% of duplication-deletions (versus 16% in T-1586) were localized in these regions. The same segments showed an increased sensitivity to the action of γ -rays, as well, but the localized breakage was sharply less pronounced. It is remarkable also that the specific constitution of chromosome 5H^{6H} was found to result in the majority of cases in a marked increase of the segment which is involved in intrachromatid exchanges. To throw an additional light on the genetic nature of the “hot spot” segments and the processes concerning the primary induction and repair that may underlie this regional specificity of mutagenic agents, fluorescence in situ hybridization with rDNA probe and analysis of DNA damage by conventional agarose gel electrophoresis under neutral and alkaline conditions were applied.

IAEA-CN-167-134P

ANALYSIS OF A VALUABLE CHROMOSOME REARRANGEMENT INDUCED BY IONIZING RADIATIONS IN A CULTIVATED CHILI PEPPER LINE (*CAPSICUM BACCATUM* VAR. *PENDULUM* – SOLANACEAE)

M.A. SCALDAFERRO¹, M. GRABIELE¹, J.G. SEIJO², H. DEBAT³, D.A. DUCASSE³, A.R. PRINA⁴, E.A. MOSCONE¹¹Multidisciplinary Institute of Plant Biology (IMBIV), Cordoba, Argentina²Botanical Institute from North East (IBONE), Corrientes, Argentina³Institute of Phytopathology and Plant Physiology (IFFIVE-INTA), Cordoba, Argentina⁴Institute of Genetics “E. A. Favret”, Buenos Aires, Argentina

E-mail: moscone@imbiv.unc.edu.ar

Capsicum (chili peppers) is an important genus including five crop species consumed by man as spice and food. Most contributions about induced mutagenesis in *Capsicum* refer to gene mutations while induced changes at chromosome level are scarce. We started a program to achieve chromosome rearrangements by ionizing radiations in *C. baccatum* variety *pendulum* cultivar “cayenne”, which has a karyotype with $2n=2x=24$, 11 metacentrics + 1 subtelocentric pairs, 4 pairs (#1, #3, #10, #12) carrying nucleolar organizing regions (NORs) and associated satellites in short arm. Seeds were treated with different acute doses of X-rays developing M_{1-4} generations. A rearranged chromosome carrying NORs in both arms was found in M_2 seedlings from the only surviving M_1 plant after a 300 Gy treatment. The structural change was analyzed by: 1) Feulgen’s staining to observe chromosome number, size and shape; 2) silver impregnation to detect active NORs; 3) fluorescent chromosome banding to reveal type and position of constitutive heterochromatic regions [triple staining with chromomycin/distamycin/4-6-diamidino-2-phenylindole (CMA/DA/DAPI)]; 4) fluorescent *in situ* hybridization with 18S-25S rDNA repeated sequence as probe. A reciprocal translocation between two NOR-bearing chromosomes in the M_1 plant has occurred, which gave viable progeny carrying the chromosomal interchange without any deviating phenotype after four generations, nor in heterozygous neither in homozygous condition. The lack of chromosome instability suggests a small reciprocal interchange between a member of pair #1 and of #3, both carrying active NORs in shorts arms. The results of this rearrangement were two chromosomes with little change in size, one of them easily recognized by the presence of NORs and associated CMA+/DAPI- heterochromatin in both arms. As the translocation here reported produced a conspicuous

rearranged marker chromosome, the obtained plant line is considered very valuable for studies on chromosome pairing and genetic linkage in the genus, including mapping of crop quality genes. This work was supported by the International Atomic Energy Agency, RBF 12226.

IAEA-CN-167-321P

AN APPROACH TO SCREEN AND IDENTIFY NOVEL MEIOTIC MUTANTS IN AN EMS MUTANT POPULATION

J.A. DA COSTA-NUNES, W. VIEGAS

Secção de Genética, DBEB, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Lisboa, Portugal

Email: jcostanunes@isa.utl.pt

I have produced a novel *Arabidopsis* EMS mutant population with the goal of identifying so far unknown meiotic mutants. The M_2 EMS mutant families were first screened for their reduced fertility. These plants with reduced fertility were subjected to a second screening at the cytological level. Plants with abnormal meiosis, namely abnormal chromosome segregation and chromosome fragmentation were selected for further characterization and SNP mutation mapping. So far 232 sterile and semi-sterile M_2 candidates have been identified in the fertility screen, of which 110 sterile mutants were further analysed at the cellular level. 15 of these have been analysed at the cytological level. Mapping has been carried out.

CONCURRENT SESSION 3 (08:30-12:30)

**Biofortification of Staple Food Crops for Improved
Micronutrient Status**

IAEA Boardroom C04

Wednesday, 13 August 2008

OVERVIEW OF THE HARVESTPLUS BIOFORTIFICATION PROGRAMME

H. BOUIS

Director, HarvestPlus, International Food Policy Research Institute (IFPRI), Washington, D.C., USA

Email: h.bouis@cgiar.org

Biofortified crops offer a *rural-based* intervention that, by design, initially reach these more remote populations, which comprise a majority of the undernourished in many countries, and then extend to urban populations as production surpluses are marketed. In this way, biofortification complements fortification and supplementation programs, which work best in centralized urban areas and then reach into rural areas only in areas with good infrastructure. Initial investments in agricultural research at a central location can generate high recurrent benefits at low cost as adapted biofortified varieties become available in country after country across time at low recurrent costs. HarvestPlus seeks to develop and distribute varieties of food staples (rice, wheat, maize, cassava, sweetpotato, beans, and pearl millet) which are high in iron, zinc, and provitamin A through a global alliance of scientific institutions and implementing agencies in developing and developed countries. Biofortification demands the application of cutting edge interdisciplinary research representing plant breeding, molecular biology, human nutrition, food science, farm extension, communications, and economics. In broad terms, three things must happen for biofortification to be successful. First, the breeding must be successful – high nutrient density must be combined with high yields and high profitability. Second, efficacy must be demonstrated – the micronutrient status of human subjects must be shown to improve when consuming the biofortified varieties as normally consumed. Thus, sufficient nutrients must be retained in processing and cooking and these nutrients must be sufficiently bioavailable. Third, the biofortified crops must be adopted by farmers and consumed by those suffering from micronutrient malnutrition.

IAEA-CN-167-387

APPLICATION OF BIOTECHNOLOGY FOR THE PRODUCTION OF BIOFORTIFIED STAPLE FOOD CROPS: THE GOLDEN RICE CASE

P. BEYER

University of Freiburg, Faculty of Biology, Freiburg, Germany

Email: peter.beyer@biologie.uni-freiburg.de

Golden Rice (GR) is the name given to a genetically modified rice (*Oryza sativa*) that produces β -carotene (provitamin A) in the grain's endosperm. Accumulation of β -carotene confers a yellow color to the grain, which becomes visible after polishing, a procedure that is routinely employed to remove the lipid-rich, oxidation sensitive outer grain layers. Research towards the generation of GR was initiated to help millions of malnourished people in developing countries cope with vitamin A deficiency (VAD), which causes high morbidity and mortality especially among children under five years of age. GR is expected to reach the urban poor and rural target populations through agriculture and local trade. Since the proof of concept in the year 1999, provitamin A content has been considerably increased through intense research and development. Getting GR effectively into the hands of farmers stands nowadays in the foreground of our activities, which represents a novel area of work for public sector research. This effort is handled through a network of partners, mainly based in the Philippines, India, and Vietnam, and encompasses marker-assisted backcrossing into adapted indica rice varieties and the generation of the necessary data to fulfill regulatory requirements. Additional research is underway to further improve the nutritional value of GR by increasing vitamin E, iron and zinc accumulation, and high-quality protein or essential amino acids in the grain.

IAEA-CN-167-400

EFFICACY OF BETA-CAROTENE RICH SWEET POTATO TO IMPROVE VITAMIN A STATUS OF BANGLADESHI WOMEN – PRELIMINARY RESULTS

K.M. JAMIL¹, M. HASKELL², K.H. BROWN²¹International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Mohakhali, Dhaka, Bangladesh²Dept of Nutrition, University of California Davis, Davis, CA, USA

Email: jamil@icddr.org

Vitamin A deficiency is the leading cause of preventable blindness in children, which also increases the risk of disease and death from severe infections. Orange-fleshed sweet potato (OFSP) is a good source of pro-vitamin A and has been successfully tried in African countries as an effective means to ensure adequate intake of pro-vitamin A by mothers and children. We have conducted a randomized controlled trial in an urban poor community of Bangladesh to examine the efficacy of two different preparations of OFSP in women with marginal vitamin A deficiency. The subjects (n=120) were aged between 18-35 years with serum retinol <1.05 µmol/L and hemoglobin >90 g/L and CRP <5 mg/L. They were dewormed with 400 mg albendazole and then 10 mg of deuterated retinol ([²H₄]-retinyl acetate) was given orally to determine total body vitamin A pool size. The subjects were randomly assigned to one of the following four treatment groups to receive 6 d/wk for 60 days: (1) boiled white-fleshed sweet potatoes (WFSP); (2) 600 µg RAE as boiled OFSP; (3) 600 µg RAE as fried OFSP; or (4) 600 µg RAE as a retinyl palmitate capsule, and boiled WFSP. Night vision of the subjects was assessed, as a proxy for vitamin A status, before and after intervention by performing 'dark adaptation test'. Blood samples collected before and after intervention were light-protected and stored on ice until plasma was separated, and then stored at –20C until shipment to University of California Davis under frozen condition. The samples will be analyzed in UC Davis for plasma isotopic ratios using GC-MS following the isolation of retinol from plasma with HPLC, to determine vitamin A pool size before and after supplementation. The preliminary results from partial analysis of the available data will be presented.

IAEA-CN-167-386

EFFECT OF BEAN POLYPHENOLS ON IRON ABSORPTION IN HUMANS

I. EGLI, N. PETRY, C. ZEDER, R. HURRELL

Institute of Food Science and Nutrition, ETH Zurich, Switzerland

Email: ines.egli@ilw.agrl.ethz.ch

Iron deficiency and iron deficiency anaemia are major public health problems in many developing countries. Common beans are a staple food in various Eastern African countries. Beans contain high amounts of iron, but the iron is poorly absorbed due to the presence of the iron absorption inhibitors polyphenols and phytic acid. With the overall aim of increasing the intake of bioavailable iron from beans by plant breeding strategies, this study evaluates the importance bean polyphenols on iron absorption. In common beans the polyphenols are concentrated in the bean hulls. Therefore bean hulls were used as a source of natural bean polyphenols and added in three different amounts to a non-inhibitory test meal (phytic acid free bread rolls). Iron absorption from the test meals was measured in three groups of 16 apparently healthy female volunteers using stable iron isotope techniques. Each volunteer consumed a test meal with and a test meal without bean polyphenols extrinsically labeled with ⁵⁷Fe and ⁵⁸Fe respectively. Iron absorption was determined based on the incorporation of iron stable isotopes into red blood cells 14 days after administration. Isotopic analysis was performed by thermal ionization mass spectrometry. The results of the absorption studies showed a dose dependent negative effect of bean polyphenols on iron absorption in humans. At the lowest polyphenol content tested (20 mg per test meal) no impact on iron absorption was found (p 0.92). A polyphenol content of 50 mg reduced the mean iron absorption significantly from 20.3% to 17.3% (p 0.044). The highest polyphenol content of 200 mg significantly reduced the mean iron absorption from 14.3% to 7.9% (p 0.0001). Further studies are planned to evaluate the relative effect of polyphenols and phytic acid on iron absorption from beans to provide guidance for breeding beans with improved iron bioavailability.

THE CONTRIBUTION OF ZINC BIOFORTIFIED WHEAT TO MEETING ZINC REQUIREMENTS AMONG WOMEN IN MEXICO

J.L. ROSADO, O. GARCIA, K. GONZALEZ

School of Natural Sciences, Universidad Autonoma de Queretaro, Queretaro, México

Email: jlrosado@avantel.net

Zinc deficiency is widespread in different populations in the world. In Mexico about 25 % of children and 30 % of women have deficient plasma concentration of zinc; similar values are found in other countries in Latin America. Zinc deficiency contributes with growth stunting; about 24% of children in Mexico are stunted. Zinc deficiency also contributes with delayed neurocognitive development and an increase in morbidity due to gastrointestinal infections in Mexican children. Several alternatives are available to treat zinc deficiency. Among them supplementation, food fortification and more recently biofortification have been applied in Mexico. The paper discusses the effectiveness of these different strategies to meet zinc requirements and alleviate zinc deficiency. Biofortification refers to the process of breeding food crops with the purpose to increase its content of bioavailable specific nutrients. In a recent study in Mexican women biofortification of wheat with zinc increased zinc intake by 69 and 72 % in 80 and 95% extraction flours respectively, zinc absorption increased about 33 % and 50% respectively. These results are compared with results of food fortification and supplementation. Staple food in Mexico is maize rather than wheat, maize is consumed about 5 times higher than wheat (30 vs 6 million tons respectively); information is given on maize and other cereals consumption by region, age and SES which demonstrates that in order to have a better impact of zinc biofortification, maize will be the crop of choice in Mexico. Studies are needed to evaluate zinc bioavailability of zinc biofortified maize. Other aspects of zinc biofortification of staple crops that demonstrate its feasibility as a strategy to treat zinc deficiency are discussed.

CONCURRENT SESSION 4 (08:30-12:30)

**Induced Mutations for Traits that Affect Abiotic Stress
Tolerance and Adaptation to Climate Change**

UNIDO Boardroom C04

Wednesday, 13 August 2008

UNRAVELLING SIGNALLING CIRCUITS REGULATING TOMATO ROOT DEVELOPMENT USING INDUCED MUTATIONS

R. SHARMA
 School of Life Sciences
 University of Hyderabad
 Hyderabad, India
 Email: rpssl@uohyd.ernet.in

Plant roots perform a multitude of functions such as water, nutrient uptake and anchorage to the soil. The performance of root to carry these functions can be improved by selecting for induced mutants affected in root development and differentiation. We used tomato as a model plant to decipher the mechanisms underlying root differentiation. We screened EMS and γ -radiated tomato M_2 seedlings for mutants defective in root development. In this study, we describe unraveling of signaling circuits regulating root development in tomato. We made detailed physiological, genetic and biochemical characterization of tomato root mutants that are affected in nitric oxide (NO) production and auxin transport. It is now recognized that NO is an important gas molecule that has functions and roles in plants comparable to other gaseous plant hormone ethylene. NO has been reported to participate in many physiological phenomena: such as seed germination, resistance to plant pathogens, stomatal movements, and flowering. Although NO plays a paramount role in plant development, little information is available about mechanisms regulating its biosynthesis in plants. We isolated a tomato mutant displaying extremely short root (*shr*) in seedlings. We observed that in tomato *shr* mutant overproduction of NO caused shortening of root. We used *shr* root elongation as a bioassay to analyze signaling pathway regulating NO formation in plants. We show that a pathway very similar to a mammalian signaling pathway regulates NO levels in plants. The transport of auxin in plants involves a very complex network comprising of proteins, which bring the hormone in and out of the cells. The *polycotyledon* (*poc*) mutant of tomato shows an enhanced polar transport of auxin (PAT). Analysis of the role of auxin transporters in *poc* mutant, especially PIN1, responsible for PAT, at the cellular level would be presented

IAEA-CN-167-391

IDENTIFYING ROOT SYSTEM GENES USING INDUCED MUTANTS IN BARLEY

I. SZAREJKO, A. JANIĄK, B. CHMIEŁOWSKA, M. NAWROT, A. LIGEZA, M. KWASNIEWSKI, A. LABOCHA-PAWŁOWSKA, J. GUZY-WROBELSKA, M. MALUSZYŃSKI
 Department of Genetics, University of Silesia, Katowice, Poland
 Email: iwona.szarejko@us.edu.pl

Root systems play an important role in plant growth and development. They absorb water and nutrients, anchor plant in the soil and affect plant tolerance to various abiotic stresses. Despite their importance, the progress in understanding the molecular processes underlying root development has been achieved only in *Arabidopsis thaliana*. It was accomplished through detailed analysis of root mutants with the use of advanced molecular, genomic and bioinformatic tools. Recently, similar studies performed with rice and maize root mutants have led to the identification of homologous and novel genes controlling root system formation in monocots. The collection of barley mutants with changes in root system development and morphology has been developed in our Department after mutagenic treatments of spring barley varieties with N-methyl N-nitrosourea (MNU) and sodium azide. Among these mutants, the majority was characterized by seminal roots significantly shorter than roots of a parent variety throughout a whole vegetation period. Additionally, several mutants with root hairs impaired at different stages of development have been identified. These mutants have become the material of studies aimed at genetic and molecular dissection of seminal root and root hair formation in barley. The studies included the molecular mapping of genes responsible for mutant phenotype using DNA markers and root transcriptome analysis in the mutant/parent variety system. Using cDNA RDA approach, we have identified the *HvEXPB1* gene encoding root specific beta expansin related to the root hair initiation in barley. We have also initiated the database search for barley sequences homologous to the known Arabidopsis, maize and rice genes. The identified homologous ESTs are now used for isolation of the complete coding sequences and gene function will be validated through identification of mutations related to the altered phenotype. This work was supported by the IAEA Research Contracts 12611 and 12849, and Polish Ministry of Science and Higher Education grant 2 P04C 056 30.

IAEA-CN-167-339

TOWARD UNDERSTANDING THE GENETIC NETWORK CONTROLLING MAIZE ROOT ARCHITECTURE

G. TARAMINO¹, M. KOMATSU¹, F. HOCHHOLDINGER², H. SAKAI¹

¹DuPont Crop Genetics, DuPont Experimental Station, Wilmington, DE, USA

²Center for Plant Molecular Biology (ZMBP), Dep. of General Genetics, University of Tuebingen, Tuebingen, Germany

Email: Graziana.Taramino@cgr.dupont.com

The root system in maize is characterized by different root types, each of which contributes to the development and establishment of the plants in a distinctive manner. During very early development, the embryonic primary and seminal roots are predominant, together with their post-embryonic lateral roots. Shoot-borne roots are formed at each consecutive node of the shoot and are called crown roots when they grow on nodes that reside under the soil line, and brace roots when growing from above ground nodes. In our group we are interested in the genetic mechanism underlying root formation and we take a forward genetics approach to identify genes involved in the molecular pathways that drive maize root development. Several monogenic root mutants have been selected for our map-based cloning activities. Two of them control crown and brace root formation. The *rtl* (*rootless1*) mutant has a variable number of crown roots in the first two nodes, but misses all shoot-borne roots at the higher nodes. In contrast, the *rtcs* (*rootless concerning crown and seminal roots*) mutant was identified because it lacks the embryonically formed seminal roots and post-embryonically formed shoot-borne roots. Other two mutants we are studying control lateral root formation at the seedling level, in the primary root. Among them, the recently characterized *rum1* (*rootless with undetectable meristems 1*) mutant is defective in both the initiation of lateral roots from the primary root, and the initiation of seminal roots. Here, we present data of the strategy we developed for the positional cloning and characterization of two of the maize genes controlling root formation: the *RTCS* gene, which encodes for a LOB domain protein, and the *RUM1* gene, which encodes for a member of the AUX/IAA gene family involved in auxin signalling.

IAEA-CN-167-337

SYSTEMATIC PHENOTYPE ANALYSIS OF *ARABIDOPSIS DS*-TAGGED MUTANTS TO UNRAVEL GENE FUNCTIONS IN ABIOTIC STRESS RESPONSE AS WELL AS GROWTH AND DEVELOPMENT

T. KUROMORI, K. SHINOZAKI

Gene Discovery Research Group, RIKEN Plant Science Center (PSC), Yokohama, Japan

Email: kuromori@psc.riken.jp

By the availability of various mutant resources in *Arabidopsis*, it is now able to investigate mutant lines for almost every gene. *Arabidopsis* is, then, not only a model plant for plant research, but also a model species in which it is possible to carry out "saturation mutagenesis" for all genes, and to totally analyze each gene and mutant of one organism. One of the future goals of "phenome" project is to collect information of knockout-type mutant phenotypes for each *Arabidopsis* gene. We have generated thousands of *Dissociation (Ds)* transposon-tagged lines, which have a single insertion because of an advantage of *Activator/Dissociation (Ac/Ds)* system, and deposited it to the RIKEN BioResource Center. In this resource, we selected 4,000 transposon-tagged lines with a transposon insertion in gene-coding regions, and systematically observed the visible phenotype of each line as a first step of phenome analysis. Totally about 200 clear visible phenotypes were classified into 43 categories of morphological phenotypes. Phenotypic images have been entered into a searchable database. In parallel, we have been selecting homozygous transposon-insertional plants, which would be useful resources to detect other phenotypes than visible phenotypes. We are setting three categories of measurement to search various traits for total phenome analysis, such as physical, chemical or biological-methods. Recently, we started to investigate biological-measured phenotypes, which are stress-responsive or conditional phenotypes, using homozygous mutant resources. We are also collecting any mutant phenotype information from published reports in journal research activity to make a comprehensive phenotype database of *Arabidopsis* genes and mutants.

DEVELOPMENT OF ACID SOIL/ALUMINIUM TOLERANT BARLEY VARIETY THROUGH MARKER-ASSISTED SELECTION AND MUTATION

C.D. LI, R. LANCE

Department of Agriculture & Food, Government of Western Australia, South Perth, WA, Australia

Email: cli@agric.wa.gov.au

Soil acidity with high levels of toxic aluminium is the largest soil constraint limiting sustainable crop production in Australia. Current barley cultivars are generally sensitive to soil acidity. A field study showed that barley yields were decreased at soil pH below 5 and decreased by 40% in the soil with pH 4.7 in topsoil and 4.1 in 10-30 cm layer. Genetic improvement of the tolerance is the best solution to battle acid soil constrains. Over two hundred barley lines were introduced from around the world and tested on acid soils. A glasshouse screening method for acid soil tolerance has been established. The average root length under low pH and high aluminium can be used as the best indicator for acid soil tolerance. The best tolerant lines include Svanhals from CYMMIT, Br2 from Brazil, Dayton and WB229 from Australia. These lines were over yield current variety Stirling by average 30.8%. The best two lines WB223 and WB229 were over yield Stirling by 45 and 42% respectively. Four barley doubled haploid populations were developed for mapping the acid soil tolerance genes from Svanhals, Br2, Dayton and WB229. One major gene for acid soil/aluminium toxic tolerance was mapped on chromosome 4H. A candidate gene HvMATE is believed to control acid soil/aluminium tolerance. The expression level of this gene was associated with acid soil tolerance. Acid soil tolerant gene was transferred into the current malting barley varieties Baudin and Hamelin using MAS. It shortened the breeding cycle by two years. The new breeding lines showed over 30% yield advantage on acid soils. As only one acid soil tolerance gene was identified in the Australian barley germplasm. Two barley varieties Dash and Vlamingh were treated with gamma-rays or exposed to cosmic rays. New mutants were screened for acid soil tolerance with different mechanism.

MAKING THE MOST OF THE HEXAPLOID WHEAT (*TRITICUM AESTIVUM* L.) GENOME: USAGE OF GAMMA RAY MUTANTS FOR HIGH THROUGHPUT POSITIONAL CLONING

F. BASSI^{1,2}, P. LANGRIDGE¹, T. SCHNURBUSCH^{1,3}

¹Australian Centre for Plant Functional Genomics, School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, SA, Australia

²Univerisità di Modena e Reggio Emilia, Padiglione Besta, RE, Italy

³Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Department of Genebank, Gatersleben, Germany

Email: filippo.bassi@acpfg.com.au

The *Triticum aestivum* L. genome went through two polyploidisation events in the course of evolution. The result is a very complex structure that is limiting molecular based studies, making of wheat one of the last species for which a shift toward high throughput approaches has not yet been completed. We discuss here (i) how the wheat genome structure buffers the lethality associated with treatments at high dosage of gamma ray and (ii) how this high dosage generates an easy detectable binary polymorphism, at both phenotypic (1=M₀ vs 0=KO mutant) and genotypic (1=Retention vs 0=Deletion) levels, (iii) how we exploited this 2 point difference to normalise for the genomic background influence when phenotyping early generations and (iv) to develop PCR based markers independent from allelic polymorphism. In particular, we are discussing the usage of a DARK Real Time marker design, free from needs of subgenome specificity, for direct genotyping of radiation mutants. A low resolution corentention map for the positioning of a boron toxicity tolerance gene, Bo1, on chromosome 7BL, is presented and the mapping resolution discussed in comparison with a genetic map. In conclusion, the usage of gamma ray mutagenesis represents a very expedient method to make the most of the very complex hexaploid wheat genome.

IAEA-CN-167-063

MUTATIONAL ANALYSIS TO DISSECT OXIDATIVE AND ABIOTIC STRESS IN *ARABIDOPSIS THALIANA*

T. GEICHEV^{1,2}, N. MEHTEROV¹, M.A. FERWERDA², L. BERNIER², J. HILLE²

¹Department of Plant Physiology and Plant Molecular Biology, University of Plovdiv, Bulgaria

²Department Molecular Biology of Plants, Researchschool GBB, University of Groningen, The Netherlands

Email: tsangech@pu.acad.bg

A genetic approach was used to identify mutants more tolerant to oxidative and abiotic stress. Large collections of *Arabidopsis thaliana* mutant lines generated by chemical and T-DNA mutagenesis were screened for survivors under conditions that trigger oxidative stress-induced programmed cell death (PCD). The fungal AAL-toxin triggers PCD through perturbations of sphingolipid metabolism in AAL-toxin-sensitive plants. While *Arabidopsis* is relatively insensitive to the toxin, the *loh2* mutant is sensitive to AAL-toxin due to knockout of a gene involved in sphingolipid metabolism. EMS mutagenesis of *loh2* resulted in second-site mutants that are more tolerant than *loh2* to the toxin. Nine of these mutants were characterized towards their response to oxidative stress-induced cell death. Either application of the catalase inhibitor aminotriazole, leading to H₂O₂ accumulation was used, or paraquat, leading to superoxide radicals generation. Some mutants were more tolerant to aminotriazole, paraquat, or both herbicides. One of the mutants with tolerance to both aminotriazole and paraquat, called *atr1* (AAL-toxin-resistant 1), was subjected to microarray analyses under conditions that trigger cell death in *loh2* and no visible damage in *atr1*. Majority of the genes showed similar expression pattern in both mutants. Genes encoding for nitrate and ammonium transporters, peroxidases, transcription factors and DNAJ /DNA K were upregulated, while genes related to cell wall extension and cell growth were downregulated in both mutants. Genes from the heat-shock regulon were more clearly induced in *loh2*. In another approach, T-DNA mutagenized wild type seeds were germinated on plant growth media supplemented with aminotriazole and one survivor was recovered. As many types of abiotic stresses are connected with oxidative stress, this T-DNA mutant together with *atr1* and their respective controls were subjected to chilling stress. Both the T-DNA mutant and *atr1* showed reduced chilling damage compared with control, as estimated by chlorophyll fluorescence and cell death measurements.

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DEVELOPMENT OF SALINITY TOLERANT RICE VARIETIES USING BIOTECHNOLOGICAL AND NUCLEAR TECHNIQUES

M.C. GONZÁLEZ¹, N. PÉREZ¹, E. CRISTO¹, M. RODRÍGUEZ², O. BORRAS²

¹National Institute of Agricultural Sciences (INCA), Cuba

²Centre for Genetic Engineering and Biotechnology (CIGB), Cuba

Email: mcaridad@inca.edu.cu

In order to obtain salinity tolerant rice varieties it was developed a Breeding Program using biotechnological and Nuclear Techniques. It was used as donor the Amistad - 82 and Jucarito-104 rice varieties. This work included the increasing genetic variability by means of somaclonal variation and *in vitro* mutagenesis with protons radiations, the establishment of culture medium for callus formation and plant regeneration, the establishment of feasible salt tenors for *in vitro* selection, the identification of morphological markers for the early selection of salinity tolerant lines. The selection in field condition was carried out during five years. It was evaluated the salinity tolerance of the best lines selected in seedling, tillering and flowering stages in two saline concentrations (7dSm/m and 12 dSm/m) were evaluated. To determine the genetic diversity present in rice somaclones and mutants genotypes was used the method of amplified fragment length polymorphism (AFLP). It was possible to select some salinity tolerant lines that overcome the donors J-104 and Amistad-82 in saline conditions. The results of the sequence comparison in BLAST database revealed that several AFLP-fragments from two selected lines showed homology to glutathione S-transferase (GST). In the present work was possible to establish a Methodology for to obtain salinity tolerant rice varieties using Biotechnological and Nuclear Techniques and it was possible to release two salinity tolerant rice varieties that are being uses in rice production. The AFLP markers allowed confirming the adequacy of somaclonal variation as well as the proton irradiation for inducing genetic variability in rice.

RAISING MUTANTS AND TRANSGENIC *ORYZA SATIVA* L. FOR SALINITY STRESS TOLERANCE USING GAMMA RADIATION AND RNAi TOOLS

A. PAREEK¹, RATNAKARAN¹, P. SRIVASTAVA¹, G. KUMAR¹, P.C. SHARMA³, S.L. SINGLA-PAREEK²

¹Stress Physiology and Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

²Plant Molecular Biology, International Centre for Genetic Engineering and Biotechnology, New Delhi, India

³Central Soil Salinity Research Institute (CSSRI), Karnal, India

Email: ashwanip@mail.jnu.ac.in

Plant breeding efforts are being undertaken at warfoot at various National and International Laboratories to yield salinity tolerant genotypes. Random mutations, an approach that causes changes in nucleotide sequences, resulting in expression of genes conferring tolerance against stress has been utilized for generating gene pool for salinity tolerance. In our laboratory, mutations were induced in salt-sensitive rice variety - IR64 using mutagenic gamma radiations. Seeds for M₃ populations were obtained and screened for salt stress sensitive/tolerant lines. Out of 1200 lines 23 putative salinity-tolerant have been identified and further multiplied in order to obtain M₄ lines for screening at microplot levels. Details pertaining to these lines and their characterization will be presented. Employing contemporary tools of genetic engineering - development of novel stress- tolerant plants had so far been attempted by transfer of genes encoding protective protein or enzymes involved in stress responses and adaptation. Since the first step in the stress response is the perception of stress stimulus, we have aimed to clone and characterize an osmosensor or stress receptor gene from rice (*Oryza sativa* L cv IR64). This putative osmosensor (*OsHki1*) is actually a member of two component system inducible by various abiotic stresses like high and low temperature, salinity, drought as well as exogenous application of stress hormone ABA. We have raised transgenic indica rice plants which are altered in expression (either overexpression or underexpression) for this gene to understand the role of this putative osmosensor in salinity stress response. This understanding will be given another leap forward by the analysis of similar transgenic lines which have been created for the *SOS2* gene – believed to play an important role as a component of the the initial signal transduction machinery of plants. The observations and detailed analysis pertaining to these mutants and transgenic plants will be presented.

EVALUATION AND CHARACTERISATION OF MUTANT COWPEA PLANTS FOR ENHANCED ABIOTIC STRESS TOLERANCE

J.A. DE RONDE, M. SPREETH, N. MAYABA, W. JANSE VAN RENSBURG, N. MATOLE

Agricultural Research Council - Vegetable and Ornamental Plant Institute, Pretoria, South Africa

Email: kderonde@arc.agric.za

The objective of the project is to use the radiation induced mutations in cowpea to improve cowpea varieties grown by resource-poor farmers in South Africa. The first aim project was to select cowpea plants with improved levels of drought tolerance without alteration to the colour of the testa or the growth form. It was demonstrated that it was possible to examine mutant lines at seedling stage in wooden boxes. Mature plants were screened in rain out shelters and physiological traits for drought stress were identified among the lines tested. Roots of mature plants were also assessed and variation observed could be correlated with drought tolerance. The data demonstrated that physiological methods can be used to screen mutants. The yield performance of some mutant lines proved to be outstanding under well watered, as well as under drought stress conditions. The second aim was to further characterise the most promising mutant lines using molecular and physiologically techniques. cDNA-Amplified Fragment Length Polymorphism showed differential gene expression at different time points of drought stress. The sequenced transcript derived fragments (TDF) showed high homology to expressed sequence tags of soybean, with a possible function in cell defence/resistance and most importantly, signal transduction. Reverse transcription PCR using a number of primers from published sequences, as well as from the TDF sequences, validated the differential gene expression obtained from the cDNA-AFLP display. The third aim was to evaluate selected mutants on station and at different communities. On station field trials were conducted at the ARC-VOPI's research farm under dryland as well as irrigation conditions for the last two seasons. The long term plan is to introgress the drought tolerance trait from the best mutant line into drought susceptible South African cultivars grown by resource-poor farmers.

ANTIOXIDANT RESPONSE OF AN AMINO ACID ANALOG RESISTANT RICE MUTANTS

D.S. KIM, J.-B. KIM, G.-J. LEE, S.-J. CHUNG, J.Y. SONG, J.-B. JEON, S.-Y. KANG

Advanced Radiation Technology Institute (ARTI), Korea Atomic Energy Research Institute (KAERI), Jeongseup, Republic of Korea

Email: bioplant@kaeri.re.kr

Four M₆ rice mutant lines [MRIIV-9-1 (resistant), MRIIV-11-5 (moderate resistant), MRIIV-31-1 (sensitive) and MRIIV-39-2 (sensitive)] resistant or sensitive to 5-methyltryptophan (5MT), a tryptophan analog, were developed via *in vitro* mutagenesis with gamma-rays. The difference of the ROS production and scavenging activity of the mutants treated with the 5MT were investigated by using an Electron Spin Resonance (ESR) spectrometer. No significant difference in the free radical scavenging activity was observed between the control and the two 5MT sensitive mutant lines, while the resistant MRIIV-9-1 mutant scavenged over 50% of the free radical. We assessed the 5MT stress-mediated responses of the four antioxidant enzymes; catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and aspartate peroxidase (APX). The activity levels of CAT, POD and SOD were significantly increased in the MRIIV-9-1 line compared to control and two sensitive lines. The MRIIV-9-1 line also showed elevated high superoxide (O²⁻) radical scavenging activities in the ultraweak chemiluminescence (CL) assay. This result indicates that the 5MT resistant mutants might activate antioxidant systems which protect the cell from ROS-induced protein and DNA damage, and a lipid peroxidation. In the RT-PCR analysis with antioxidant isoenzyme genes, a difference of the antioxidative response systems (ARS) against the oxidative damage was validated between the control and mutants. Transmission Electron Microscope (TEM) observation for visualizing the H₂O₂ scavenging activity in leaves exposed to 5MT was applied. This work was supported by a grant (Code 20070501034005) from BioGreen 21 Program, RDA (Rural Development Administration), Republic of Korea.

Poster Presentations

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APPLICATION OF SOMACLONAL VARIATION AND *IN VITRO* INDUCED MUTAGENESIS IN CROP IMPROVEMENT

A. ARZANI

Agronomy and Plant Breeding Department, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Email: a_arzani@cc.iut.ac.ir

Manipulating genetic variability is one of the major tasks of plant breeders. Somaclonal variation, gametoclonal variation and *in vitro* induced mutagenesis can be used to create variability from which crop plants can be improved. *In vitro* techniques for the culture of protoplasts, somatic tissues, pollens/microspores, ovules and embryos have been used to create new genetic variation in the breeding lines. The process of *in vitro* selection has been applied to several cell culture systems to generate mutants with useful agronomic traits such as tolerance to biotic and abiotic stresses. Cell and tissue culture techniques have been used to obtain salt tolerant plants employing two *in vitro* culture approaches including selection of mutant cell lines (somaclones) and *in vitro* screening of plant germplasm for salt tolerance. Our study of evaluation of resistance to powdery mildew (*Leveillula taurica*) in wild and cultivated sainfoin (*Onobrychis viciifolia*) indicated a very low variability for reaction to the disease. Induced mutation via ethyl methane sulfonate (EMS) in sainfoin was also not effective in creating variability for resistance to the powdery mildew. In durum wheat, *in vitro* selection for salt tolerance was carried out and the resulting *in vitro* derived salt-tolerant genotypes were compared with those of field-selected under saline field conditions. *In vitro* screening method was comparable to that of field-selected one in recognizing salt tolerance genotypes in durum wheat. Field screening procedures in saline soils are confronted by high spatial and temporal variability problems and the preliminary germplasm assessments can be hence undertaken under either control environments or *in vitro* conditions, and then the selected genotypes can be evaluated under saline field conditions.

WHEAT IMPROVEMENT FOR DROUGHT RESISTANCE AND YIELD STABILITY USING MUTATION TECHNIQUES

A. KOKHMETOVA¹, S. KENZHEBAYEVA², E. BOGDANOVA¹

¹Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan

²Kazakh State University al-Farabi, Almaty, Kazakhstan

Email: gen_kalma@yahoo.com

The main problem of plant breeding is development of varieties with optimal combination of high drought resistance, productivity and yield stability in varying unfavorable conditions. It is especially important for Kazakhstan, the main agricultural areas of which are located in the arid zones characterized by moisture supply deficiency. Mutation techniques have proven to be valuable technique in enhancing crop genetic diversity for selecting new variants with traits of economic importance. Therefore we used M₂ population of spring bread wheat var. Kazakhstanskaya 126 treated by nicotinic acid extracted from tobacco leaves 0,01% and 0,1 %, respectively. Based on germplasm of M₂ there was developed genotype Grekum 476 having changes in leaf shape such as rolling of flag leaf. This trait protects plant from intensive insolation and overheating, prevent losses of water and provide long-term function of leaves and therefore it was used for wheat improvement of commercial cultivars. The objective of this study is the analysis of genotype x environment interaction (GEI) and evaluation of the donors of drought resistance and stability among the winter wheat genotypes. Experimental material has been grown in 2004-2006 at three contrasting ecological zones including irrigated and non-irrigated conditions. To analyze GEI the method Tai (1971) was used. Drought susceptibility index was used for drought resistance assessment (Fisher and Maurer, 1978). The objects of study were wheat genotypes with inserted leaf rolling trait (Grekum 476, Hostianum 88, Albidum 109, Miras), and varieties developed in Kazakhstan and the Ukraine, that differ in the level of productivity and drought resistance. It was found that rolling leaf trait in the main source Grekum 476 is controlled by two dominant RL-genes. The genotypes with RL-genes able to conserve high leaf water potential as the tendency for greater leaf hydration seems to be a consequence of osmotic adjustment connected to drought resistance has a high level of osmotic adjustment. Analysis of GEI allowed differentiating experimental material by the level of stability. The best stability observed in donors of RL-genes – Grekum 476 and Album 109. The biggest level of field drought resistance was observed in varieties Bogarnaya 56, Krasnovodopadskaya 210 and Grekum 476, which were high yielding in stress environments. It is known that ecological reaction of adaptability – the rolling of leaves, is the characteristics for the varieties with RL-genes. This trait allows using water economically by limitation of transpiration, to regulate plant water balance more efficiently. Obviously, the high level of drought resistance of Grekum 476 has been provided by the presence in its genotype of RL-genes. Thus, the use of chemical mutagen allowed widening the spectrum of genetic variability of wheat germplasm. In comparison to the origin cultivar Kazakhstanskaya 126, their mutant derivatives demonstrated higher level of drought resistance and yield stability. These germplasms were ranged by the level of yield stability and drought resistance in wheat. The relationship between field drought resistance and ecological parameters of stability was found.

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DEVELOPMENT OF DROUGHT RESISTANT CULTIVARS THROUGH MUTATION APPROACH IN WHEAT

S. KENZHEBAYEVA¹, A. KOKHMETOVA², E. BOGDANOVA²

¹Department of Plant Biotechnology, Biochemistry and Physiology, Kazakh State University al-Farabi, Almaty, Kazakhstan

²Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan

Email: kenzhebaevas@mail.ru

Better understanding of leaf structure and photosynthetic function is critical to crop improvement in water-limited environments. The aims of this study were to examine photosynthesis characteristics in three spring wheat genotypes differing in degree of rolling leaf under high temperature stress to assess the adaptive potential of photosynthesis. The drought-tolerant wheat genotype with the greatest display of rolling leaf Otan has high adaptive potential of photosynthetic apparatus at action of high temperature (40°C for 2 days) and it is stable to photo inhibition. This ability is conditioned by high (Otan and Alba) photochemical activity of PHS II to the photo inhibitory. The major factors related to PHS II tolerance photo inhibitory is its high photochemical activity and effective stomatal regulation of water transpiration and supply of mesophyll cells by CO₂ are the stable structural organization. The rolling leaf trait results in higher efficiency of a water exchange and decrease in negative influence of high temperature on photosynthesis.

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THE APPLICATION OF THE HAPLOID CELL CULTURE SYSTEM TO OBTAIN THE VARIANTS WITH TOLERANCE TO BIOTIC AND ABIOTIC STRESS IN PLANTS

J.H. HUANG, R.J. LU, Y.F. WANG

Biotech Research Institute, Shanghai Academy of Agricultural Sciences (SAAS), Shanghai, China

Email: swl@saas.sh.cn

The different genotypes of rape haploid cells /tissue tolerated to the oxalic acid were correlated with the tolerance to *Sclerotinia sclerotiorum* in the plant level through the researches. And this phenomenon also occurred in the tolerance to NaCl between the different genotypes of rape haploid cells and the diploid cells, but the diploid cells were generally more tolerant to the haploid ones. In addition, there were similar situations in barely on NaCl tolerance, aluminum tolerance and resistance to scab. So the above results indicated that the haploid cells/tissue tolerant to the stresses could reflect the situations in the plant level in the certain degree. The technology of inducing and screening the variants of the tolerance to rape *Sclerotinia sclerotiorum* by *in vitro* culture of haploid tissue was established. This technical system includes the *in vitro* microspore culture, the regeneration from the haploid cells to plants and the expanding propagation of the haploids populations. A set of oxalic acid tolerance variants was screened through the treatments of pingyangmycin and oxalic acid in stem apexes culture of haploid plants. After the field identification, 3 individuals with the improved tolerance to *Sclerotinia sclerotiorum* was obtained. The technology of inducing and screening the variants with heat tolerance by *in vitro* culture of haploid tissue was established in broccoli. A set of variants with the improved heat tolerance was obtained through the treatment of pingyangmycin and the 45°C heat treatment and 9 variants with higher stability of cell membrane to heat stress than the original varieties was selected. In addition, the technical systems for inducing and screening barley variants tolerant to aluminum and scab stresses according to the above rules were established. And the relevant resistance variants were obtained. Then 1 aluminum tolerance material and 3 scab resistance materials under the field experiments in the plant level were selected.

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MUTAGENESIS AND SELECTION *IN VITRO* FOR SALINITY TOLERANCE AND MOLECULAR CHARACTERIZATION IN SUGARCANE

P. SUPRASANNA, V.Y. PATADE

Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: prasanna@barc.gov.in

Salinity is one the major environmental stresses affecting plant productivity. Combined use of mutagenesis and tissue culture can greatly facilitate the selection and isolation of useful tolerant lines. In the present study, *in vitro* mutagenesis was employed in the selection of salt tolerant lines in popular sugarcane (*Saccharum officinarum* L.) cv. CoC-671. Embryogenic cultures were gamma irradiated (10-50 Gy) and challenged with different levels of NaCl (42.8 - 256.7 mM). Salt stressed calli exhibited lower relative growth rate, decreased cell viability and higher levels of free proline and glycine betaine. The membrane stability (electrolyte leakage) was 3-fold more under salt stress compared to control. The ion levels were drastically affected under salt stress as leached out Na⁺ and K⁺ was much more than that of retained in tissue in both adapted and unadapted callus cultures. The tolerance could also be related to the maintenance of an ample water status and a high to low level of K⁺ to Na⁺ under salinity stress indicating that sugarcane can be a Na⁺ excluder. Plant regeneration was observed in 10 and 20 Gy irradiated calli up to 171.1 mM NaCl selection. A total of 147 plantlets were selected on different salt levels and the tolerant lines are being evaluated at field level. Molecular characterization using RAPD markers revealed genetic polymorphism among selected putative salt tolerant lines and control plants. The proper evaluation of these variants for salinity tolerance may be useful for economic cultivation under the stress regime.

DEVELOPMENT OF VALUABLE TRAITS IN COTTON MUTANTS UNDER THE DROUGHT AND SALINITY CONDITIONS

A. KUSHALIEV, S. ALIKHODJAEVA, O. KUCHKAROV, S. USMANOV, B. KHALMANOV
Department of Mutagens, Uzbek Research Institute of Cotton Breeding and Seed Production, Salar, Kibray,
Tashkent region, Uzbekistan
Email: khalmanov@hotmail.com

Irradiation of cotton seeds using the laser induction give a new mutant forms which have a wide spectrum of valuable traits. There are given the research results on influence of laser irradiation on cotton seeds of the new created lines, which resulted from water deficiency (L-179, L-151, L-198) and salinity (L-1710, L-2074) cases. Induced seeds were planted in conditions of rigid water deficiency (0-1-0) and chloride salinity. Researches results show that the laser irradiation has rendered positive influence on improvement of economic parameters of lines, which necessary for using at all exposures of irradiation in a both conditions of water deficiency and soil salinity, instead of the lines received on the basis of multistage hybridization. Among studied plants it is appeared that, the possibility of plant allocations which have a higher complex of positive traits or excess of parameters on one and two signs under the both researched stress conditions, anyway it depends on the genetic nature of each combination. Under the water deficiency condition at L-179, there were observed the fiber output increasing for 2,1% then standard, fiber strength increasing for 4,25 gs/tex, fiber length increasing for 0,02-0,06 inches and the absolute weight of seeds for 11,0 g. Almost the same picture was observed in both stress conditions at other above mentioned lines. The conducted experiments on practical application of mutagens influences of laser irradiation shows the preferability of its application and opportunity to take of new cotton mutant forms with a wide spectrum of variability of valuable signs.

SELECTION AND PHYSIOLOGICAL RESPONSE OF GLYPHOSATE RESISTANT ZOYSIAGRASS MUTANTS DERIVED FROM A RADIATION BREEDING TECHNIQUE

H.J. LEE¹, G.-J. LEE¹, D.S. KIM¹, S.J. CHUNG¹, J.-B. KIM¹, J.H. KU², S.-Y. KANG¹

¹Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea

²Chungnam National University, Daejeon, Republic of Korea

Email: sykang@kaeri.re.kr

This study was conducted to select of zoysiagrass mutants resistant to glyphosate and to identify their physiological and molecular characteristics. Experiments were conducted to determine the effects of glyphosate on the physiological responses in zoysiagrass and to select mutants resistant to glyphosate. The results indicated that the optimum concentration for a mutant resistant to glyphosate selection is 0.5~1.0%. In order to select mutants resistant to glyphosate, M₂ plants were sprayed with 0.5% glyphosate after propagation. M₂ seeds were collected from the plants that survived after being irradiated with 300Gy gamma ray. Three resistant and susceptible M₂ plants were selected for an analysis of their physiological characters. The electrolyte leakage was increased more in the susceptible plants than the resistant plants treated with 0.5% glyphosate. A difference in the malondialdehyde content was not evident between the resistant and susceptible plants. The chlorophyll and carotenoid contents were decreased in the plants treated with 0.5% glyphosate with a greater reduction in the susceptible plants than in the resistant plants. And, the zoysiagrass 5-enolpyruvyl-shikimate-3-phosphate synthase gene was cloned by RT-PCR and RACE (Rapid Amplification of cDNA Ends) approaches. The derived cDNA sequence revealed a high homology with the genes reported in other species.

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CHARACTERIZATION OF SALT TOLERANT RICE MUTANTS INDUCED BY *IN VITRO* MUTAGENESIS WITH GAMMA-RAYS

J.Y. SONG¹, D.S. KIM¹, G.-J. LEE¹, J.-B. KIM¹, J.B. JEON¹, S.J. YUN², S.-Y. KANG¹

¹Advanced Radiation Technology Institute (ARTI), Korea Atomic Energy Research Institute (KAERI)
Jeongeup, Republic of Korea

²Chonbuk National University, Jeonju, Republic of Korea

E-mail: sykang@kaeri.re.kr

High-proline accumulating rice mutant lines were developed by a selection of Azetidine-2-carboxylic acid (AZCA), a proline analog, resistant embryogenic cell lines induced from *in vitro* mutagenesis with gamma-rays. For a selection of the salt tolerant rice mutants from the selected AZCA resistant lines, we conducted a third selection procedure with 1,500 AZCA resistant lines: 1st, a selection under nutrient solution with 171mM NaCl; 2nd, a selection under *in vitro* conditions; 3rd, a selection in reclaimed saline land. Finally, we selected 7 salt tolerant (ST) lines from the 1,500 AZCA resistant lines. The selected mutants were further characterized with a physiological analysis, e.g. EC, Chlorophyll, MDA and Proline. Proline contents were increased to a maximum of 20%, 100% and 20% in the leaf, seed and callus, respectively, of the selected lines. Compared to the control, the amino acid contents of the mutants were 24 to 29 %, 49 to 143 %, 32 to 60% higher in the leaf, seed and callus, respectively. The ratio of Na⁺/K⁺ for most of the ST-lines was lower than that of the control, ranging from 1.0 to 3.8 for the leaf and 11.5 to 28.5 for the root, while the control had 3.5 and 32.9 in the leaf and root, respectively. To identify the genetic variation of the ST-lines, the expression pattern of P5CS which catalyzes a biosynthesis of proline from glutamate via the proline biosynthetic pathway and NHX1, Na⁺/H⁺ antiporters, which catalyze the exchange of Na⁺ for H⁺ across vacuole membrane, were analyzed using RT-PCR and northern blot. The selected mutants will be useful for the development of a rice cultivar resistant to a salt stress. This work was supported by a grant (Code 20070501034005) from BioGreen 21 Program, RDA (Rural Development Administration), and from the KOSEF (Korean Science and Engineering Foundation) in the MoST (Ministry of Science and Technology), Republic of Korea.

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DROUGHT TOLERANT M₄ SEGREGANTS OF SOYBEAN Cv. JS 335 AND CO (SOY)3

AR. MUTHIAH¹, J.G. MANJAYA², T.S. RAVEENDRAN¹, P.CHEZHIAN¹, V. SUGUNA¹

¹Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India

²Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: armuthaiah@yahoo.com

The cultivation soybean (*Glycine max* L.), crop is constrained by the inadequate moisture availability during the spring and / or summer season, especially at the pod initiation and pod filling stage. Evolution of drought tolerant cultivars through hybridization is tedious because of the very fragile and small sized chasmogamous and cleistogamous flowers of soybean. More over only a very narrow genetic base is available in the germplasm of soybean maintained in India. Hence an attempt was made to irradiate the soybean seeds of the Cv. JS 335 & CO (Soy 3) with gamma rays and Ethyl Methane Sulphonate (EMS) to induce heritable variability for drought tolerance. The seeds of the above mentioned cultivars were irradiated with gamma rays 250 Gy dose, EMS with a concentration of 0.2 and 0.4 per cent, combinations of 100 Gy +0.2 per cent EMS and 100 Gy + 0.4 per cent of EMS. The source used for gamma irradiation was Cobalt 60, available at Indira Gandhi Centre for Atomic Research, Kalpakkam, India. Single plant M₃ and M₄ families segregants were observed with different root densities, leaf thickness with higher grain yield, under imposed drought stress conditions compared to the parental check cultivars. Macro mutants *viz.*, dwarf, mutants with lanceolate leaflets, little leaf, tall and twiny mutants with broader leaves were observed and isolated. A lanceolate leaf mutant from 0.2 per cent EMS treatment having a mean leaf thickness 31.9 µm was observed to be drought tolerant in M₄ generation compared to the mean leaf thickness of 25.8 µm in the control (Co.SOY 3). Similarly a long petiole leaf mutant with thicker leaves of 37.4 µm compared to the leaf thickness of 29.8 µm in the control (JS335) was also observed in the 250 Gy gamma irradiation. In this study it is observed that gamma irradiation is more efficient in inducing leaf thickness compared to that of chemical mutagen.

IAEA-CN-167-163P

DEVELOPMENT OF SALT TOLERANT HIGH YIELDING BARLEY LINES VIA CROSSING BETWEEN A MUTANT INDUCED BY EMS AND A LOCAL CULTIVAR

R.A.K. MOUSTAFA

Nuclear Research Center, Egyptian Atomic Energy Authority, Cairo, Egypt

Email: r_moustafa99@yahoo.com

In winter season of 2002/2003, a high yielding barley mutant line and a local variety were hybridized to obtain in one genotype the salt tolerance of the local variety and the high yield potential of the induced mutant. The obtained hybrid grains were planted in 2003/2004 growing season under normal field irrigation conditions to raise F₁ population, which was grown in 2004/2005 season to advance F₂ generation under saline conditions at El-Fayoum experimental agriculture station belonging to the Nuclear Research Center. Phenotypic correlation coefficients between grain yield and its effective traits were estimated for F₂ plant population. Results revealed that the characters most strongly correlated with grain yield were found to be number of spikes and biological yield/plant. Therefore, these couple traits were used as a selection criterion to screen F₂ plant population in order to detect high yielding variants under salinity conditions. As a result, a considerable number of outstanding individual plants were selected from the large F₂ population and their grains were planted in 2005/2006 winter season to raise F₃ progeny rows. Superior plants from superior rows were selected and carried forward to the next winter season of 2006/2007 as F₄ single plant progenies along with the two parental genotypes and a suitable check. Obtained results indicated that mean values of yield and most of its components for the tested progeny lines were significantly (P= 0.1) surpassed averages of the original parents and the check as well.

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GENOTOXICITY OF CHLORORGANIC PESTICIDES

A.A. NURZHANOVA¹, S. KENZHEBAYEVA²¹Institute of Plants Biology and Biotechnology, Ministry of Education and Science, Almaty, Kazakhstan²Department of Plant Biotechnology, Biochemistry and Physiology, Kazakh State University al-Farabi, Almaty, Kazakhstan

Email: kenzhebaevas@mail.ru

In Kazakhstan there are the warehouses of the obsolete pesticides and their container which should be buried in special burial grounds or neutralized to minimize dangerous genetic and ecological risks. The results of two yr of research were identification of substances stored in 64 former warehouses of pesticides. 64 former warehouses (in the 10 areas of Almaty oblast) are on distance of 250 km from a large city of Almaty (the former capital of Kazakhstan). A total of 352,6 ton of obsolete pesticides and 250 ton of their container were disposed. We determined the residues of DDT metabolites (dichlorodiphenyltrichloroethane) and HCH isomers (hexachlorocyclohexane) in soil around pesticides warehouses where their concentrations exceed MAC (maximum concentration limit) in tens - hundreds times. To analyze a genotoxicity of chlororganic pesticides we used their concentrations that were found in soil from former warehouses. The analysis of structural mutations of chromosomes was carried out by metaphase method in I mitoses meristem cells of barley seeds (*Hordeum vulgare* L.). It was ascertained that HCH isomers and DDT metabolites have genotoxic effect exceeding spontaneous mutation in 5-7 times. High contaminations by pesticides on soil around of warehouses and their ability to induce chromosome aberrations in plant cells indicate that warehouses are a new centre of contamination by POP's (proof organic pollutants).

IAEA-CN-167-175P

GENETIC ENHANCEMENT OF LENTIL (*LENS CULINARIS* MEDIKUS) FOR DROUGHT TOLERANCE THROUGH INDUCED MUTATIONS

J.P. LAL, A.K. TOMAR

Department of Genetics & Plant Breeding, Banaras Hindu University, Varanasi, India

Email: jplalbhu@yahoo.com

An attempt has been made to isolate a number of drought tolerant mutants from four lentil cultivars, two each from small seeded (PL-639 and PL-406) and bold seeded (K-75 and L-4076) groups by treating the seeds with physical (10, 20 and 30 kR of γ -rays) and chemical mutagens (0.04M of ethyl methane sulfonate and 0.05M of sodium azide) separately and in various combinations. The experiment was initiated during the winter season of 1999-2000 and carried over to advanced generations. The selection of environment (water stress or non-stress) for the development of drought resistant varieties still remains controversial, however, the findings from present study suggested that materials ought to be tested in both stress and non-stress conditions so that the favourable alleles under drought can be maintained as well as selection response under favourable condition can be maximized. Yield under drought (Y_d), yield potential (Y_p), drought susceptibility index (S) and geometric mean (GM) were considered as the potential indicators for drought resistance of a family. Correlation coefficients between these parameter were calculated for selecting the parameter(s) which are more effective than others for screening the drought resistant mutant line(s). It was observed that GM was positively and significantly correlated with both Y_d and Y_p , whereas it was negatively but insignificantly correlated with S. There was significant but negative correlation between S and Y_d , while no significant correlation between S and Y_p was observed. From the correlation studies it may be concluded that for the enhancement of yield potential under both the conditions selection should be based on GM rather than on S. Because S is a better measure of drought tolerance than a measure of performance under stress, hence genotypes may be first selected on the basis of high GM and then on the basis of high yield under drought (Y_d). Twenty mutants lines selected on the basis of higher GM than their respective control were further evaluated for their yield performance under rainfed condition and were subjected to drought tolerant tests through M4 to M6 generations. Three chemical tests, viz., nitrate reductase (NR) activity, protein content, and wax content were conducted and data were recorded on grain yield/plant. Nitrate reductase activity and wax content of most mutant lines and was negatively associated with grain yield in that comparison. The lines showing higher nitrate reductase activity, wax content and grain yield appeared to be promising.

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HIGH YIELDING SEMIDWARF *POKKALI* RICE MUTANTS TOLERANT TO ABIOTIC STRESSES OF COASTAL SALINE ECOSYSTEM

K.S. SHYLARAJ, P.L. KORAH, S. SNITHA, B. LOVELY

Rice Research Station, Vyttila, Kerala Agricultural University, Kerala, India

Email: shylaraj_ks@rediffmail.com

The most popular rice varieties in the pokkali tract of Kerala State, India are *Pokkali*, *Cheruvirippu*, VTL-3 and VTL-4. These varieties are tall, lodging genotypes having tolerance to complex abiotic stresses (salinity, acidity & submergence). About 50% yield is lost due to lodging. In the present study, an effort was made to induce semi dwarfism coupled with high yield in these genotypes retaining the complex tolerance. Dry seeds of these varieties were subjected to both physical mutagen (gamma rays 200, 300, 400 and 500Gy) and chemical mutagen treatment (0.5%, 1% and 2% of Ethyl Methane Sulphonate at varying exposure periods of 8h, 16h and 24h). Reduced germination percentage was noticed in all the treatments. Only 25% plants of VTL-3 irradiated with 400 Gy dose of gamma rays produced fertile seeds and 28% in VTL-4. Even in the fertile plants the seed fertility varied greatly (0.5 to 82.4%). The induction of 100% sterility in more than 75% plants of 400 Gy irradiated treatments compared to the maximum of 7% sterility in the untreated control indicated the high mutagenic potency of gamma irradiation in *pokkali* rice. Fifty eight semi dwarf mutants with significant reduction in plant height could be selected from the M_2 generation of 400 Gy irradiated doze of VTL-3. The height of the selected plants varied from 82 cm to 120cm chemical mutagen treatment was not effective in inducing semidwarfism. More than 70% of the selected mutants showed stability for reduced plant height. The segregation data suggested that most of the mutant lines had single recessive mutations with the exception of few lines. The yield of the stable mutants were evaluated in an Initial Evaluation Trial and observed that some of the mutants had the potential to produce about double the yield (10 tons/ha) of its parent VTL-3 (5 tons/ha).

INDUCTION OF SALT TOLERANCE IN HIGH YIELDING RICE VARIETIES THROUGH MUTAGENESIS AND SOMACLONAL VARIATION

T.L. NGUYEN¹, C.B. BUI²

¹Cuulong Delta Rice Research Institute, Co Do, Vietnam

²Institute of Agricultural Science for Southern Vietnam, Ho Chi Minh City, Vietnam

Email: ntlang@hcm.vnn.vn

The objective of the investigation reported here was to produce stable mutants with beneficial traits through somaclone of M_1 plants induced by gamma rays combined with *in vitro* culture. To produce stable mutants from an indica rice (*Oryza sativa* L.) variety, we estimated the mutation efficiency of gamma ray on traditional varieties using somaclone derived from soma of M_1 plants. M_1 seed production and germination were higher in 1,5 NaCl. Sodium chloride (NaCl) tolerant cell lines were selected from irradiated calli that were generated from seed culture. Different level of gamma rays: 0, 20, 30, 50Gy were imposed on the calli, which were subsequently cultured on NaCl callus induction medium with 1.5% NaCl. A total of 68 lines (35.4%) were regenerated by somaclone of M_1 plants. Twenty-one lines (30.9%) were stable mutants, 14 lines (20.6%) were unstable mutants, and the remainder (48.5%) were normal. The frequencies of stable mutants following 1.5% NaCl added to the regeneration medium increased the percentage of calli showing regeneration by 90.79%, 90.12%, 75.00% in Soc Nau, Doc Do (mutant) and Doc Do (original). In a field trial of seven stable mutants for yield potential, five mutants did not show a significant difference in yield as compared with the original variety. Among these five, three glabrous mutants with high yield. These stable mutants could be used as new breeding materials.

IMPROVING SALT TOLERANCE AND SEED YIELD IN INDIAN MUSTARD (*BRASSICA JUNCEA* L.) THROUGH RADIATION INDUCED MUTAGENESIS

P.C. SHARMA¹, R. PRASHAT¹, G.D. SINGH¹, A. PAREEK²

¹Central Soil Salinity Research Institute, Karnal, Haryana, India

²School of Life Sciences, Jawahar Lal Nehru University, New Delhi, India

Email: pcsharma@cssri.ernet.in

Five genotypes (two high yielding and three with higher salt tolerance) of Indian mustard (*Brassica juncea*) were irradiated with gamma rays (200-1200Gy) for inducing variability for higher seed yield and improved salt tolerance characters. In the mutagenic population of M_4 generation, significant differences were recorded amongst different genotypes with respect to main shoot length, number of primary branches, number of pods on main shoot and seed yield. Mutagenic population showed increase in main shoot length, an important character to be observed for higher yield under salinity, in all the genotypes compared to controls. Further, mean number of primary branches decreased in radiation treatment plants compared to controls (by 8-10% in mean values) in different genotypes. Mean value of number of pods on main shoot increased from 4% (400Gy) to 12% (600Gy) compared to controls. Number of seeds per pod increased by 35% in CS 614-4-1-4 (1000Gy) and 22% in Varuna (1000Gy), compared to controls. CS 245-2 showed maximum 1000 seed weight of 6.54 g in 1000Gy treatments followed by 6.00 g in 400Gy treatment in CS 614-4-1-4. In general, mean seed yield increased with radiation dose but declined with increase in salinity. Amongst different genotypes, maximum increase (35%) in seed yield was observed in Varuna (1200Gy). Further, the evaluation of M_3 mutagenic population for relative salt tolerance potential at seed germination and seedling emergence stage revealed that genotype CS 614-4-1-4 showed 100% germination up to EC 22 dS/m in 800Gy treatments. Radiation treatments also showed positive effects on seedling emergence under salinity stress with 26% increase in CS 614-4-1-4 (200Gy) and 23% increase in CS 245-2 (800Gy). Lower seedling emergence in Rohini under different radiation treatments was associated with higher Na accumulation and higher Na/K compared to controls.

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IN VITRO AND IN VIVO SELECTION OF SOYBEAN MUTANT LINES ON MEDIUM CONTAINING ALUMINIUM

YULIASTI¹, SUDARSONO²¹BATAN Centre for research and Development of Isotopes and Radiation Technology, Jakarta, Indonesia²Bogor Agriculture University, Indonesia

Email: upikyuliasti@yahoo.com, prof_sudar@yahoo.com

The main limiting factors of soybean plants expansion in acid soil are Al toxicity and low pH. The best approach to solve this problem is use of Al tolerance variety. *In vitro* and *in vivo* selection using selective agent and induced callus embryonic of mutant lines are reliable methods to be developed to create new variety. The aim of this research were to evaluate inhibitory effect of Aluminum concentration (0; 7.5; 10; 12.5 and 15 mg.l⁻¹ (ppm) at pH 4.0 on five mutant lines (G1-G5) and three varieties: Tanggamus (Al- toleran), Rajabasa (Al-middle tolerant) and Lumut (Al-sensitive) variety of soybean under *in vitro* and *in vivo* condition. It was decided to concentration 15 ppm Al since it severelly affective in the *in vitro* and *in vivo* response on the explants. In all experiments tolerant mutant lines on Al stress was determined by Aluminum sensitivity index value (S) on observed parameters. Evaluation of response of soybean callus and seedling from some mutant lines and three varieties on the media selection AlCl₃ under *in vitro* culture showed that frequency of tolerance callus of G1 and G2 mutant lines on the medium selection of 15 ppm Al were 3 and 2 times respectively, higher than that of cultivar Tanggamus which has frequency of tolerance 20%. The frequency of tolerances seedling of G5 and G4 on the medium selection of 15 ppm Al, 4 and 2 times respectively higher than that of Tanggamus. Evaluation of response inhibitory effect of Aluminum using sterilized *coco peat* medium on soybean mutant lines was conducted under *in vivo* condition. The soybean mutant lines and three varieties with 7 to 60 days old were watered by nutrition solution containing half-strength modified MS, with 0; 7.5; 10; 12.5 and 15 ppm Al. The results showed that the performance of mutant lines G3 and G4 derived from 15 ppm Al were better and more tolerant than that of Tanggamus based on number of dry pod and dry seed weigh. The results of response inhibitory effect of Aluminum using acid soil medium on soybean mutant lines showed that the performance mutant lines was better than that of Tanggamus. G4 mutant line was more tolerant than Tanggamus based on dry seed weight and number of pod.

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EFFECT OF SALT STRESS AND PHOSPHORUS DEFICIENCY IN MUTANTS OF RHIZOBIUM OBTAINED BY GAMMA IRRADIATION

S. JEBARA, I. CHALLOUGUI, M. JEBARA

Biotechnology Center of Borj Cedria (CBBC), Hammam-Lif, Tunisia

Email: jebarasalwa@yahoo.fr

Two strains of Rhizobium: *Rhizobium Tropici* and *Mesorhizobium Ciceri* nodulating respectively common bean and chickpea were treated by gamma irradiation (⁶⁰Co) source. Radiosensitivity analysis showed that 800 Gy was the biggest dose supported by these two strains. We isolated gamma irradiated resistant strain in order to select mutant of them which can supported salt stress and phosphorus deficiency. Salinity analysis showed that *Mesorhizobium Ciceri* 835 strain, can tolerate up to 18g/l (273 mM NaCl) of salt, whereas, their irradiation mutants tolerate salinity up to 33g/l (564mM. NaCl) *Rhizobium Tropici* CIAT899 can survive at 20g/l (342 mM) either for control strain or mutants. Analysis of phosphorus deficiency showed that either *Rhizobium Tropici* CIAT899, or *Mesorhizobium Ciceri* 835 can survive in medium without phosphore. Our results permit us to screen mutants tolerant to these stresses wide spread in Mediterranean soil. In this study, we choose two mutants strains irradiated by 700Gy and two mutants irradiated by 800Gy in each species, these mutants were characterized by their best growth compared with their reference strains. Our results showed that Gamma irradiation modified antibiotic resistance, such as kanamicyne, tetracycline, vancomicyne, streptomicyne, penicilline, either at 700Gy or at 800Gy, we obtained significant modification of response and persistence of penicilline resistance. Biochemical analysis showed that these strains had a variable superoxide dismutase (SOD, E.C. 1.15.1.1) and catalase (CAT, E.C. 1.11.1.6) activities essentially in *Mesorhizobium Ciceri* 835 mutant strains, these two enzymatic antioxidants was suggested to play an important role in environmental stress tolerance.

SALINITY TOLERANT MUTANT OBTAINED FROM PROTONS RADIATIONS

M.C. GONZÁLEZ¹, N. PÉREZ¹, E. CRISTO¹, M. RAMOS²

¹National Institute of Agricultural Sciences (INCA), Cuba

²Centre for Genetic Engineering and Biotechnology (CIGB), Cuba

Email: mcaridad@inca.edu.cu

A breeding program to obtain salinity tolerant rice varieties using *in vitro* mutagenesis was developed at the National Institute of Agricultural Science. Seeds from Jucarito-104 (J-104) rice variety were irradiated with different doses of protons (10, 20, 30, 40, 60, 90, 110 and 210 Gy) at the Phasotron of DUVNA. The irradiated seeds were cultured *in vitro* for callus induction and plant regeneration. It was carried out the bulk harvest in M₁V₁ population and promising lines were selected from M₂V₁ to M₅V₁ generations. It was evaluated the morphological and molecular difference between the mutant and the donor J-104. It was possible to release the mutant GINES that showing difference with the donor J-104 in cycle, yielding, grain quality, salinity tolerance as well as disease tolerance. The AFLP analysis showed differences between the mutant and the donor J-104. The rice variety GINES is the first mutant release from *in vitro* mutagenesis using protons.

DEVELOPMENT OF DROUGHT TOLERANT TOMATO VARIETIES THROUGH INDUCED MUTATION IN CUBA

M.C. GONZÁLEZ¹, J.P. MUKANDAMA¹, M.M. ALI¹, D. TRUJILLO¹, J.L. FUENTES², S. ALTANEZ², A. FERNÁNDEZ³, B. PETEIRA³

¹National Institute of Agricultural Science (INCA), Havana, Cuba

²Center for Technological Application and Nuclear Development (CEADEN), Havana, Cuba

³National Center of Agricultural Health (CENSA), Havana, Cuba

Email: mcaridad@inca.edu.cu

Having in mind the need to have tomato varieties growing under low water input conditions a tomato breeding program using nuclear techniques was started with the purpose of obtaining adequate yielding-potential cultivars under drought conditions. Seeds from INCA 9-1 variety were irradiated with ⁶⁰Co gamma rays irradiated using of 300 Gy and 500 Gy. Starting from M₂ generation, selection of high yield potential genotypes under low water supply conditions was made during 4 generations. Individual selection was done, taking into account the following criteria: healthy plants, determinate growth habit, yield per plant, fruit number per plant, average fruit weight, equatorial and polar fruit diameters were recorded in individually selected plants. The total soluble solids (Brix), acidity, dry matter and water content were evaluated in fruit of M₅ generation. Different isoenzymatic systems, the protein concentration as well as the Random Amplified Polymorphism DNA (RAPD) were used in order to evaluate the genetic variability between the selected mutants and the donor variety. The most frequent variations observed in each generation were: plant cycle, fruit size, number, shape, colour and yield. It was possible to release two varieties of high yielding under low water input conditions. The tomato mutants Maybel and Mali are being used in tomato production for industrial purposes. These varieties are the firsts tomato varieties obtain from mutation induction in Cuba.

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MOLECULAR CYTOGENETICS OF LYMEGRASS AND WHEAT X LYMEGRASS HYBRIDS

K. ANAMTHAWAT-JÓNSSON

Biology Institute, University of Iceland, Reykjavik, Iceland

Email: kesara@hi.is

The genus *Leymus* (lymegrass) comprises about thirty polyploid perennial grass species in the tribe Triticeae (Poaceae). *Leymus* has its main distribution in the temperate regions of Eurasia and North America. Its natural habitats range from coastal to inland areas, including diverse soil types and climatic conditions. Lymegrass is a pioneer plant in an open or disturbed habitat, due to the ability of its extensive rhizome system to bind soil/sand and the plant's tolerance to extreme environmental stresses such as salinity and drought. The soil binding quality together with its perennial habit, large seeds and tolerance to diverse environmental conditions, makes lymegrass attractive as a potential crop for farming in marginal habitats or in a sustainable, multi-species, and perennial system of future agriculture. Amphiploids have been developed from crosses between wheat and lymegrass (*Triticum* x *Leymus*) with an aim to increase agronomic quality and yield, thus making *Triticoleymus* a viable, perennial grain crop for sub-arctic regions. Numerous *Triticoleymus* genotypes have been generated. Intergenomic translocations and various chromosomal rearrangements have been identified. The objective of this study is to characterize these materials, in order to prepare for more targeted breeding strategies. While wheat chromosomes are well characterized and mapped, lymegrass chromosomes are still relatively unknown. The polyploid genus *Leymus* has only recently been confirmed as being auto- or segmental allopolyploid consisting of the basic N^s genome. In this context, the genus *Leymus* is extended to include N^s species from other Triticeae genera, i.e. *Psathyrostachys*, *Hordelymus* and *Hystrix*. Isolation and characterization of N^s genome specific DNA sequences will be presented, as well as molecular cytogenetic mapping of these sequences on chromosomes of lymegrass species of geographically different origins. Novel repetitive sequences that can be used to identify intragenomic variation within the N^s genome and to differentiate individual lymegrass chromosomes will also be presented.

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DEVELOPMENT AND DISSEMINATION OF BAMBARA GROUNDNUT VARIETIES USING MUTATION INDUCTION AND BIOTECHNOLOGY TECHNIQUES

M. OUEDRAOGO

Crop Husbandry Department, Institute of Environmental and Agricultural Researches, Ouagadougou, Burkina Faso

Email: mahamaouedk@hotmail.com

The project has the aim to breed new varieties of bambara groundnut, for increasing food, income of farmers, reducing protein deficiency in dietary and improving household welfare by using mutation induction and modern biotechnologies. One variety crop which possesses major preferred agronomic, consumers and/or market traits will be enrolled in the procedure of breeding. Breeding objectives in Bambara groundnut improvement are seed yield and drought tolerance. The research methodology includes: Use of physical irradiation (Gamma radiation) for inducing mutation on dry seed; performing radio-sensitivity tests for critical dose of mutagen on dry seed; sowing an initial generation of 800-1000 seeds per retained dose (2 - 3 doses); getting 400-700 M₁ plants at emergence and 35 000-52500 M₂ seeds (2-3 doses). The M₁ plants will be harvest individually and M₂ generation will be grown on head/row or plant/row basis. Finally, at M₃ generation potential mutant will be evaluated and involved in Participatory Plant Breeding with farmers. But, potential mutant variants will be grown in families and progenies in the aim of verifying their mutant characteristics (agronomic, physiologic, phenotypic and molecular levels). Yield and response to drought will be performed in M₃, using individual progenies of M₂ plants. Classical molecular techniques will allow to get genetic markers or design new genetic markers from mutants in M₃ generation. At least one farmers' association and one village per zone will participate in the project. Farmers will also be trained in seed production and processing. Surveys before (baseline data), during (impact) and at the end (impact) of the project on, income, crop yields, household welfare, offer in markets will give main impacts of the projet. The number of farmers which use modern varieties and autonomous farmers associations multiplying improved varieties will be also objectives measures of project success.

INDUCING COLD TOLERANCE IN MALAGASY RICE VARIETIES IR 58614, MALADY AND ROJOFOTSY THROUGH *IN VITRO* MUTAGENESIS

RAKOTOARISOA¹, A. ANDRIANJAKA¹, ZAPATA-ARIAS², R. AFZA², C. MBA²

¹Unité de Biotechnologie et Amélioration des Plantes, Laboratoire de Physiologie Végétale, Département de Biologie et Ecologie Végétales, Faculté des Sciences, Université d'Antananarivo, Antananarivo, Madagascar

²Plant Breeding Unit, FAO/IAEA Agriculture & Biotechnology Laboratory, International Atomic Energy Agency Laboratories, Seibersdorf, Austria

Email: noronathanielle@yahoo.fr

The use of induced mutagenesis to develop cold tolerant mutants from 3 Malagasy rice varieties – Rojofotsy and IR58614 (*indica* subspecies), and Malady (*japonica* subspecies) - was investigated with the aim of developing rice varieties that could be planted during the cold seasons in Madagascar. This would permit the cultivation of the same parcel of land twice annually. The strategy involved the modification of different growth media to achieve the induction of calli from mature embryos of these Malagasy rice varieties and subsequently inducing the regenerants to mutate by exposing the embryogenic calli to gamma irradiation. Putative cold tolerant variants were selected by regenerating plantlets from the irradiated embryogenic at a low temperature of 12°C. The putative mutants were evaluated for agronomic performance under controlled environments and field conditions. Data are presented on the optimal media compositions for both callus induction and plant regeneration. There was interaction between genotype and media composition for callus induction. The induction of embryogenic calli in the Malagasy rice *indica* subspecies was dependent on genotypic responses. The incubation of cultures for two months showed a reduction in the rate of regeneration of plantlets from the embryogenic calli. Also, prolonged incubation of the cultures at 28°C beyond the optimum duration of 1 month significantly impaired the totipotency of the cells. Irradiation of calli seemed to have had a stimulatory effect on plantlets regeneration at 12°C with the low dose of 10Gy being optimal for Malady, and 20Gy for IR 58614. Three high yielding, early maturing and cold tolerant induced mutant lines were identified. These lines showed good germinability under low temperature of 15°C and the 1000-seed weight was significantly enhanced. We discuss the significance for rice agriculture in Madagascar for the integration of these mutants into breeding programmes.

IMPROVEMENT OF BARLEY FOR DROUGHT TOLERANCE BY INDUCED MUTATION

A. FERCHICHI

Institut des Régions Arides, Médenine, Tunisia

Email: ferchichi.ali@ira.rnrt.tn

Barley (cultivar Ardhaoui) is a traditional cereal adapted to the agroclimatic and abiotic stresses of South Tunisia. It's used by local communities as the food plant and source of proteins for animals. It's a heterogeneous mixtures of land races and represent the adapted natural germplasm which needs to be improved in yield, quality and tolerance to stress (especially drought). Irradiation technique is used in this work for the selection of barley lines tolerant to drought. Building on the obtained results, the technique will be used to develop barley lines matching specific environments prevailing in the south of Tunisia. The obtained mutants are characterized by the increased of grain yield and water use efficiency through a greater tolerance to drought. On the level of the physiological behavior, the mutants are more efficient regarding the values of rate of photosynthesis A, the stomatic conductance g_s , the rate of transpiration E, chlorophyll rate and the internal CO₂ concentration C_i . The content of proline shows that this amino acid is strongly concentrated at the irradiated lines. The analysis of nutritional behavior shows that the content of (Ca+Mg) in irradiated lines decreases by increasing the stress. The phosphorus content was also assigned by the water stress. On the contrary, the potassium content increases with the intensity of stress. Under water deficit, the growth parameter, the grain production and the number of spike and tillers are significantly reduced for the control (the reduction reached 42 to 50%). This reduction don't exceed 25% for the irradiated lines. The availability of these improved mutant seeds can contribute to increase food security for the local population.

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SALINITY AND WATER DEFICIENCY TOLERANCE IN RICE: THE ROLE OF RHIZOBACTERIA

R. ARSHAD, S. FAROOQ¹, M. AKRAM, M. AFZAAL, F. NAZ, F. AZAM²

Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan

¹Director (Technical), PAEC, Islamabad, Pakistan²Director, Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, NWFP, Pakistan

Email: arshadrubina@hotmail.com

In attempts to look for water deficiency and salinity tolerance in different rice genotypes, we tested germplasm comprising wild rice species, upland rice genotypes, radiation induced mutants, low land rice cultivars and hybrid derivative of lowland rice varieties and upland wild rice genotypes. One of such genotypes was WAB 56-50 which can be grown at EC level of 8dS m⁻¹. Under these conditions, a soil bacterium was isolated from its rhizosphere and on the basis of differential morphological, biochemical, physiological and salt-tolerance tests; pigmented bacterial isolate was characterized as *Serratia marcescens* (BDCS-N-S1): a Gram-negative bacterium belonging to family *Enterobacteriaceae*, which can be grown on 6% NaCl at 22 to 28°C. Under saline conditions, this strain produces considerable amount of cell associated red pigment called "Prodigiosin" (a linear tripyrrole antibiotic) and chitinolytic enzymes chitinases which function as bio-control agent. These secondary metabolites were further characterized for studying their relationship with rice plants growing under stress. Culture conditions for production of prodigiosin/chitinases were optimized by media manipulations and maximum yield of pigment was observed at 28°C in peptone ethanol mineral broth (PEMB) at pH 8 after incubation of 72 h. The red pigment was extracted from crude bacterial cells and a powder of 2.77 g/liter was obtained against reported international yield of 2.45g/liter. Antimicrobial assay was performed *in vitro* and a strong antifungal activity against rice pathogen *Helminthosporium oryzae* was observed. In this presentation, detailed studies conducted on multiple mode of action of *S. marcescens* (BDCS-N-S1) against fungal pathogen will be presented with special reference to synergism of chitinases and prodigiosin that was exploited *in vivo* for the bio-control of fungal pathogens of rice growing under field conditions. This study is first of its kind which might play significant role in controlling certain fungal diseases that appears on some of the plants growing under water deficient conditions.

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RESPONSE OF NATIVE FLORA TO INDUCEABLE GENOTOXIC DAMAGE FROM INCREASED RADIOACTIVITY AROUND NPP JASLOVSKÉ BOHUNICE, SLOVAKIA

G. MURÍN¹, K. MIČIETA²¹Ústav bunkovej biológie, Bratislava, Slovakia²Katedra botaniky UK, Bratislava, Slovakia

Email: gmurin@fns.uniba.sk

It is not generally known that the first serious failure of nuclear power plant (NPP) technology with loss of human lives occurred in NPP Jaslovské Bohunice (Czechoslovakia) in January 1976. A year later the second accident finally broken reactor A1 with large radioactive contamination. This material was later (in 1980) washed into the nearby drainage by the heavy rain. In cleaning procedure, the contaminated soil particles contaminated the slopes of the drainage. These spots have the shape of "blurs" about 15 cm wide with a scale of contamination from 0,067; 0,15; 2,38; 9,5; 45.5 up to 322 kBq/kg ¹³⁷Cs. The research was done in cooperation with the Institute of Tumorbiology, University of Vienna, within the grant Action Austria – Slovak Republic. Details of radioactivity at the area were obtained thanks to the Research Institute of the Nuclear Energy in Trnava, Slovakia. In our ten years long-term study of contaminated soil around nuclear power plant (NPP) Jaslovské Bohunice 24 species of local flora were used to show impact of these accidents. The 19 km long banks of the Jaslovské Bohunice NPP waste water recipient has been identified as contaminated by ¹³⁷Cs. In total, more than 67,000 m² of river banks have been found as being contaminated at levels exceeding 1 Bq ¹³⁷Cs/g of soil. Used phytotoxic and cytogenetic "in situ" tests were extended by analyses of pollen grains. Although the dose of some samples of radioactive soil was relatively high (322 kBq kg⁻¹) no any significant impact on the biological level of tested wild plant species was observed. Possible explanation (such as adaptation and resistance) is discussed.

DEVELOPMENT OF RICE (*ORYZA SATIVA* L.) WITH SALT TOLERANCE

C.B. BUI¹, T.L. NGUYEN²

¹Institute of Agricultural Science for Southern Vietnam, Ho Chi Minh City, Vietnam

²Cuulong delta rice research Institute, Co Do, Vietnam

Email: buichibuu@hcm.vnn.vn

A series of experiments was conducted to determine the variability and the genetics of tolerance for salinity in Indica rice. OMCS 2000 and OM 1490 and seeds were gamma-irradiated with the following dosages: 0, 10, 20, 30 Gy and the generated M₂ plants were screened for salt tolerance using a salinized hydroponic culture system. Screenings were continued up the M₃ generation until putative salt tolerant mutants were identified. Survival rates of mutants were similar to those of tolerant checks pokkali and IR 29 under artificial salinization and commercial variety AS996 under the 3 level of EC 0,6 and 15 dS/m. Screening techniques developed were depend upon the agronomic traits of the yield component characters. A total of 500 mutant lines M₂ and M₃ indica were tested for tolerance to salinity at the seedling stage. Large variation in salt tolerance among mutants lines was detected. Of the 500 line tested, 25 were found tolerant, 198 moderately, and 277 susceptible. The tolerant lines grown continue to get M₄. Screening at the vegetative and reproductive stages. The parents, 20lines M₄ plants and the tolerant control, Pokkali, Ir 29, AS 996 were grown under saline conditions. Among the 20lines M₄ from OMCS 2000 and OM 1490. Of the 20 lines tested, 7 lines were found tolerant at EC= 15 dS/m 13 susceptibles. Three lines (OM 1490-52 and 55, OMCS 2000-451) of these lines had better agronomic traits and high yield. The success in identifying the salt tolerant mutants could be attributed to the capacity screening. These mutants can now be used by breeders for further genetic studies field –tested for adaptability, and used as sources of salt tolerance in hybridization breeding program

NOVEL IR64 MUTANT LINES WITH CONTRASTING PHENOTYPES UNDER SALT STRESS

B. NAKHODA^{1,2}, H. LEUNG¹, M.S. MENDIORO³, A.M. ISMAIL¹

¹International Rice Research Institute, Los Baños College, Laguna, Philippines

²Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

³Institute of Biological Sciences, University of the Philippines Los Baños, College, Laguna, Philippines

Email: b.nakhoda@yahoo.com

Salinity is one of the major environmental constraints for rice production. Rice is salt-sensitive and its growth and yield can drastically be reduced by salt stress. Rice mutants with altered responses to salt stress can effectively be used to elucidate the biochemical and genetic basis of tolerance and to identify the candidate genes involved, and this can substantially speed the efforts to breed salt tolerant varieties. About 60,000 IR64 mutant lines were generated at IRRI using different mutagenesis techniques e.g. fast neutron, Gamma ray, diepoxybotane, and EMS. Through high-throughput screening of more than 5,000 diepoxybotane IR64 mutant lines in saline hydroponics at seedling stage, mutants with greater tolerance or higher sensitivity than IR64 were identified. Selected lines were evaluated in replicated experiments to confirm their responses under salt stress and advanced to M8 generation. Results of SSR ID test confirmed that all the selected lines were true IR64 mutants. Subsequently, six mutant lines, four of them with better tolerance of, and two with higher sensitivity to salt stress relative to the wild type IR64 were selected. To decipher the key mechanisms contributing to salinity tolerance or sensitivity during the early seedling stage, selected mutants, along with three checks (sensitive IR29, parent IR64, tolerant FL478), were further evaluated. Morphological, physiological, and biochemical parameters were measured in a set of experiments under greenhouse conditions. Results of the detailed phenotyping shaded light on the bases of salinity tolerance and sensitivity of these mutants and the potential pathways involved and this will be further discussed. Tolerant mutants absorbed less Na⁺ and had lower root-to-shoot Na⁺ translocation; while sensitive mutants absorbed more Na⁺ and transferred larger amounts of it to their shoots. Tolerant mutants showed higher ratios of K⁺ to Na⁺ especially in their shoots and were more efficient in upregulating their antioxidant system and in scavenging radical oxygen species generated during stress. These advantages allow tolerant mutants to survive and grow better under saline conditions compared with wild type and their sensitive counterparts.

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FIELD EVALUATION OF IR64 MUTANTS WITH ALTERED RESPONSES TO SALT STRESS UNDER SALINE AND NORMAL CONDITIONS

B. NAKHODA^{1,2}, H. LEUNG¹, M.S. MENDIORO³, A.M. ISMAIL¹

¹International Rice Research Institute, Los Baños College, Laguna, Philippines

²Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

³Institute of Biological Sciences, University of the Philippines Los Baños, College, Laguna, Philippines

Email: b.nakhoda@yahoo.com

More than 5,000 diepoxybotane M₄ IR64 mutant lines were screened for salinity tolerance/sensitivity at seedling stage, during the period of June 2003 to December 2005. Four tolerant and two sensitive mutant lines relative to the wild type IR64 (intermediate tolerance) were identified and advanced to M₈ generation. These selected mutants were then evaluated in a series of field experiments conducted at different locations and in different growing seasons in the Philippines, to assess their performance under natural field conditions. Experiments were conducted either under control conditions to compare the agronomic performance of the selected mutants, or under saline field conditions, to assess the yield responses of the tolerant and sensitive mutants compared to that of IR64. Selected mutants were evaluated along with the wild type IR64 and two other checks, sensitive IR29, and the highly tolerant FL478. Under normal field conditions, all mutants and parent have similar performance. Under saline field conditions, tolerant mutants consistently had better growth, higher survival, and higher grain yield and biomass. Conversely, the sensitive mutants consistently had lower survival rate, poor growth, and lower grain yield and biomass production under saline field conditions. Our results suggest that the tolerant IR64 mutants are potential varieties for areas with high salinity problems during crop establishment as in coastal India and Bangladesh. These mutants have good grain quality as that of the popular variety IR64, and could potentially be released as new varieties in target areas amid further evaluation and acceptance by farmers.

IAEA-CN-167-418P

RICE BREEDING FOR SALINITY TOLERANCE THROUGH IRRADIATED MUTATION IN THAILAND

S. TAPRAB

Bureau of Rice Research and Development, Rice Department, Ministry of Agriculture and Cooperatives,
Bangkok, Thailand

Email: suniyom@ricethailand.go.th

Rice seeds of 3 varieties (KDML105, RD15 and KTH17) had been irradiated by gamma ray and fast neutron with dosages of 200 and 300 grey. Then, M₁ was transplanted in normal soil at Pathumthani Rice Research Center in 2004. M₁ plants derived from 300 grey dosage of irradiation were mostly abnormal and died. A half of selected M₂ population from 200 grey irradiation was transplanted in coastal saline soil at Petchaburi province in wet season of the year 2005. Most plants died and severe injured by salinity and water stress. 200 survived M₃ plants were selected and later considered to be salt tolerant mutants. In 2006, selected M₃ were tested for resistance to leaf blast in seedling stage. There were 5, 4 and 4 salt tolerant mutants of KDML105, RD15 and KTH17 that resistance to leaf blast. Those mutant seedlings were then transferred to be transplanted in concrete pond. Morphological and agronomic characters of these mutants were evaluated. We found 5 glutinous mutants from KTH17 and 14 photoperiod insensitive mutants from RD15. Leaf samples of 14 RD15 mutants were taken to be characterized for salt tolerant gene using molecular markers B1.1-11 and RM140. Double haploid lines from all M₃ mutants were generated. M₄ seeds were harvested and retested for salinity tolerance in coastal saline soil and nutrient solution in 2007.

RICE BREEDING FOR SALINITY TOLERANCE THROUGH IRRADIATED MUTATION IN THAILAND

S. TAPRAB

Bureau of Rice Research and Development, Rice Department, Ministry of Agriculture and Cooperatives, Bangkok, Thailand

Email: suniyom@ricethailand.go.th

Rice seeds of 3 varieties (KDML105, RD15 and KTH17) had been irradiated by gamma ray and fast neutron with dosages of 200 and 300 grey. Then, M_1 was transplanted in normal soil at Pathumthani Rice Research Center in 2004. M_1 plants derived from 300 grey dosage of irradiation were mostly abnormal and died. A half of selected M_2 population from 200 grey irradiation was transplanted in coastal saline soil at Petchaburi province in wet season of the year 2005. Most plants died and severe injured by salinity and water stress. 200 survived M_3 plants were selected and later considered to be salt tolerant mutants. In 2006, selected M_3 were tested for resistance to leaf blast in seedling stage. There were 5, 4 and 4 salt tolerant mutants of KDML105, RD15 and KTH17 that resistance to leaf blast. Those mutant seedlings were then transferred to be transplanted in concrete pond. Morphological and agronomic characters of these mutants were evaluated. We found 5 glutinous mutants from KTH17 and 14 photoperiod insensitive mutants from RD15. Leaf samples of 14 RD15 mutants were taken to be characterized for salt tolerant gene using molecular markers B1.1-11 and RM140. Double haploid lines from all M_3 mutants were generated. M_4 seeds were harvested and retested for salinity tolerance in coastal saline soil and nutrient solution in 2007.

SOMAACLONAL VARIATION OF SUGARCANE FOR SALINITY RESISTANT USING SODIUMCHLORIDE AS SELECTION AGENT

MUH. FARID BDR., R. SJAHRIL

Department of Agronomy, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia

Email: rsjahiril@yahoo.com, rinaldi@unhas.ac.id

Experiments were carried out to study morpho-physiological mechanism of resistance to salinity in sugarcane. Acclimatized plantlets were exposed to selection process to study their growth characteristic which indicated resistance to salinity based on testing under laboratory and screen house condition. Sixteen putative somaclones resistant to salinity were produced *in vitro* from six varieties using mutagenic agent sodium azide (NaN_3). Treatment of NaCl (0 g L^{-1} , 4 g L^{-1} , 8 g L^{-1} , 12 g L^{-1} , 16 g L^{-1}) consisted of three units were repeated three times. Screen house experiment was also carried out using randomized block design consisting of the 16 putative somaclones. Concentration of NaCl treatments were 0 g L^{-1} , 4 g L^{-1} , 8 g L^{-1} of Hoagland's hydroponics media. Laboratory experiment showed that Q81, R579, SM86 and TK26 varieties produced salinity resistance somaclones up to 8 g L^{-1} NaCl. Growth characteristics which can be used as indicator for salinity resistance *in vitro* were callus color and texture, length and volume of root, height and fresh weight of plantlet. There is closed correlation between diluted total protein and morphological character of plantlet regenerated by mutagenic induction under laboratory condition. There may be changes in salinity resistance between somaclones based on their protein and isozyme pattern during mutagenic induction. Screen house experiment showed somaclones from R579 and Q81 varieties can withstand up to 8 g L^{-1} NaCl and may be concluded as resistant clones. Normal growth in screen house experiment indicated by index of root length, root quantity, relative water contents, K-Na absorption selectivity, K-Na translocation selectivity, K, Na and Cl translocation, and photosynthesis index may be used as parameters to determine resistance. Further test of R579 and Q81 mutant clones for field growing experiment using sea water are being planned.

WORKSHOP (11:00-12:30)

**Breeding of Low Phytate Rice for Biofortification and
Reduction of Phosphorus Pollution**

Meeting Room C07IV

Wednesday, 13 August 2008

INDUCTION AND EVALUATION OF LOW PHYTIC ACID MUTANTS IN BASMATI RICE

A.A. CHEEMA, Z.U.-QAMAR, M. RASHID, M. ASHRAF

Mutation Breeding Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisaslabad, Pakistan

Email: aacheema@niab.org.pk

Induced mutations are successfully used to alter a character in an otherwise very well adapted variety. Keeping in view the adverse effects of high phytate contents in various cereal and legume crops, present work was started to develop the low phytate basmati rice through induced mutation. Paddy seeds of Super Basmati (well-adapted variety) were exposed to different doses of gamma rays (150, 200, 250, 300, 350 and 400 Gy) and screened for high levels of inorganic phosphorus (Biologically available form of phosphorus) in the M_2 generation. Subsequently, selected mutants were evaluated up to M_5 generation using the colorimetric assay technique. One progeny in M_5 and two progenies in M_4 generation were found stable for low phytic acid (*Lpa*) mutations and were confirmed through HPLC exhibiting 58.33%, 54.4% and 53.98% inorganic-P respectively as compared to Super Basmati (9.86%). Agronomic traits and physical paddy parameters in the selected progenies were comparable with Super Basmati except one progeny which had short paddy length. The results based on these studies are discussed.

BREEDING OF LOW PHYTIC ACID RICE IN THAILAND

S. TAPRAB

Bureau of Rice Research and Development, Rice Department, Ministry of Agriculture and Cooperatives, Bangkok, Thailand

Email: suniyom@ricethailand.go.th

Mutation breeding of LPA rice in Thailand had been started since 2003. We started on a popular high yielding variety (SPR1) with 200 grey of gamma ray irradiation. Later, we induced mutation in another HYV such as RD23, PSL2, CNT1 and CNT80. In 2004, 619 M3-LPA mutant lines of SPR1 were derived and continuously selected for desirable agronomic traits in advance generation to M6. Three advanced homozygous lines of those M6 such as SPR1'03G₁Cs-PTT-2-3-3-1-1, SPR1'03G₁Cs-PTT-9-6-2-1-1 and SPR1'03G₁Cs-PTT-1-8-2-1-1 are being evaluated for grain yield and quality in various environments. SPR1'03G₁Cs-PTT-2-3-3-1-1 and SPR1'03G₁Cs-PTT-9-6-2-1-1 had been analyzed for IP5, IP6 and Fe contents. The results showed lower quantity of IP5, IP6 and total IP in those two mutants than in original SPR1. Fe content in those two mutants and original SPR1 are almost equal. Allelism tests (*lpa1-1*) are being initiated. Currently, we detected 69 M5-LPA lines from RD23 and 67 M4-LPA lines from PSL2. In addition, M3 seed of CNT1 and CNT80 are being assayed for phytic acid content.

IAEA-CN-167-199

DEVELOPMENT OF RICE (*ORYZA SATIVA* L) WITH LOW PHYTIC ACIDT.L. NGUYEN¹, C.B. BUI²¹Cuulong Delta Rice Research Institute, Vietnam, Co Do, Vietnam²Institute of Agricultural Science for Southern Vietnam, Ho Chi Minh City, Vietnam

Email: ntlang@hcm.vnn.vn

Phytic acid, myo-inositol 1,2,3,4,5,6 – hexakisphosphate (IP 6) is the major storage compound of phosphorous (P) in plants. Rice grain contain anti-nutritional factors, which reduce the bioavailability of iron and zinc. Phytate has for long time been known to lower the absorption of these and other minerals in humans and non ruminant animals. Induced mutations in the most important cereals have been utilized to reduce the phytate content of resting grains. The following is develop the mutants with low phytic acid content have been isolated from four varieties with different genetic backgrounds : OMCS 2000, OM 1490, Rice grains were induced by gamma radiation (200Gy) then screened for high levels of inorganic phosphate in order to identify low phytic acid were analyzed and compared to 101 improve varieties and 600 traditional varieties . LP mutants derived from OM 1490, OMCS 2000 at M₅, M₄ and M₃ advanced generations were uniform in agronomic traits and other improved ones by lines (47, 64, 144, 158, and 274). Three genotypes OM 4498, OM 2517, OM 5731 and 6 local varieties Nang guoc do, Ca Ro, Lua Lun, Nep ao vang, Nep mau luon , Nep Hat to were found to low levels of phytate with level = 0465 µg P. Lua Vang = 0.930 µg P and Lua Hoang = (v) 1.395 µg P. Our findings may help rice breeders develop low phytic acid genotypes with improved nutritional value to overcome anemia symptom and adapt to biofortification strategy demands.

IAEA-CN-167-385

CLONING AND CHARACTERIZATION OF THE RICE LOW PHYTIC ACID 1 GENET.H. TAI¹, S.-I. KIM¹, C.B. ANDAYA², S. GOYAL²¹United States Department of Agriculture, Agricultural Research Service, Crops Pathology and Genetics, Research Unit, Davis, CA, USA²Department of Plant Sciences, University of California, Davis, CA, USA

Email: Thomas.Tai@ars.usda.gov

The rice low phytic acid 1 (*lpa1*) mutant exhibits a 45% reduction in seed phytic acid with a molar-equivalent increase in inorganic phosphorus; however, it does not appear to differ significantly in productivity from its wild-type progenitor. Using a positional cloning strategy, we have identified a single candidate gene at the rice *Lpa1* locus. Sequence analysis of the candidate gene from the original rice *lpa1* mutant and a second recently identified *lpa1* mutant revealed two independent mutations (a single base pair substitution and a single base pair deletion) that confirmed the identification of this candidate as the rice low phytic acid 1 gene, *OsLpa1*. The *OsLpa1* gene has three expressed splice variants. The location and nature of the two mutations suggests that these lesions should only affect the translation of the predicted protein derived from the longest transcript. The proteins encoded by *OsLpa1* do not have homology to any of the inositol phosphate metabolism genes characterized in plants to date, although there is homology to 2-phosphoglycerate kinase, an enzyme found in hyperthermophilic methanogens that catalyzes the formation of 2,3-bisphosphoglycerate from 2-phosphoglycerate. It has previously been shown that 2,3-bisphosphoglycerate is a competitive inhibitor of inositol polyphosphate 5-phosphatases. These phosphatases are known to breakdown inositol polyphosphate intermediates, suggesting a possible indirect role for *OsLpa1* in phytic acid biosynthesis and accumulation. Functional analysis of *OsLpa1* is underway and our progress will be reported.

DEVELOPMENT, CHARACTERIZATION, AND GENE MAPPING OF LOW PHYTATE MUTATIONS IN RICE

X.H. XU,¹ H.J. ZHAO¹, Q.L. LIU², X.L. REN², D.X. WU¹, Q.Y. SHU²

¹IAEA-Zhejiang University Collaborating Centre, Institute Of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou, China

²Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria

Email: xuxiuhong_2000@163.com

Phytic acid (PA, myo-inositol 1,2,3,4,5,6-hexakisphosphate) is the primary storage form of phosphorus (P) in cereal seeds, accounting for about 65-85% of the total P. It is widely regarded as the major anti-nutrient component in cereal and legume grains including rice. By using gamma irradiation, we developed a dozen low phytic acid (LPA) mutant lines in the past 5 years. The LPA traits of those mutant lines were controlled by at least four non-allelic genes; we have mapped four genes to different positions in chromosome 2, 3 and 4, and three genes were identified to be responsible for three non-allelic mutations. Field agronomic trials showed that LPA mutations could affect, mostly at significant levels, the grain weight and yield, seed viability and storability. However, the inferior performance of the LPA lines could be improved through cross breeding and targeted selection.

CONCURRENT SESSION 5 (13:30-17:40)

**Induced Mutations for Enhancing Crop Quality and
Nutrition**

IAEA Boardroom C04

Wednesday, 13 August 2008

INDUCED MUTATION-FACILITATED GENETIC STUDIES OF SEED PHOSPHORUS

V. RABOY

USDA Agricultural Research Service, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID, USA

Email: victor.raboy@ars.usda.gov

Both the chemical composition and total amount of seed phosphorus (P) are important to the end-use quality of cereal and legume seed crops, whether for use in human foods or animal feeds. They are also important to the management of P in agricultural production, and to the long-term sustainability of that production. About 75% ($\pm 10\%$) of seed total P is found as phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisphosphate). Mutations that block the synthesis or accumulation of phytic acid during seed development, often referred to as *low phytic acid (lpa)* mutations, have been isolated in maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and soybean (*Glycine max* L. (Merr.)). Chromosomal mapping has identified as many as six non-allelic *lpa* loci in a single species (barley). Studies of *lpa* mutants has enhanced knowledge of the genes and proteins important to phytic acid P metabolism. While there has been substantial research into the biology of P uptake by plants, there has been little progress in the genetics of seed total P. Genetics that either decreases or increases seed total P might be of value for both enhancing the end-use quality of seed crops and for optimizing the utilization of P during agricultural production. As proof-of-principle, homozygosity for recessive alleles of barley *lpa1* both blocks seed phytic acid accumulation by 50% and reduces seed total P by 15%, while having little impact on yield. The current status of *lpa* genetics and current efforts at isolating "seed-total P" mutants, using both forward and reverse genetics approaches, will be described.

IAEA-CN-167-323

BIOSYNTHESIS AND DEPOSITION OF SEED PHYTATE AND ITS IMPACT ON MINERAL BIOAVAILABILITY

S.K. RASMUSSEN

Department of Agricultural Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

Email: skr@life.ku.dk

In cereal seeds phosphorus is primarily deposited in protein storage vacuoles as phytic acids (PA) together with minerals. Even if the same core set of enzymes should exist throughout the plant kingdom, the organisation of biosynthesis, translocation, site of accumulation and storage vary among species. PA accumulates in the seed beginning shortly after flowering and lasting to seed maturity. During this period of time the growing plant may be challenged with changes in growth conditions such as rain, drought, high temperature and pathogens. It has been shown that the individual inositol-phosphate-kinases, *Ipks*, accept a broad range of substrates and it is also evident that rice and barley *Ipks* have phosphatase and isomerase activity. These multiple activities provide more degrees of freedom for controlling and fine tuning the PA biosynthesis during the seed development. Isolated phytate globoids rice and wheat bran was characterized and $K > Mg > Ca > Fe$ were found as the main minerals. While Fe co-purifies with PA in the globoids, this is less evident for Zn, and although Cu has high affinity to PA, there is no indication that Cu-phytate globoids are the primary storage facility for this element. This difference in seed distribution of Fe and Zn has to be taken into account in breeding strategies for improving mineral content. The dephosphorylation patterns of pure PA and phytate globoids by wheat phytase was established and the kinetics of phytase using either PA or phytate globoids as substrates compared. Results from our recent studies of Fe uptake from phytate globoids using caco-2 cells will be discussed. PA is an important antinutritional factor in the diet of humans because it reduces the bioavailability of iron and zinc. The way food is prepared may actually solve problems with PA by the action of endogenous phytases. Low-PA mutant seeds can potentially reduce the troubles with P management in husbandry and as genotypic variation is also known for mineral content, mutational breeding provides a useful way of creating diversity.

IAEA-CN-167-357

REVEALING OF COMPLEX SYSTEM OF STARCH SYNTHETIC METABOLISM IN HIGHER PLANTS USING RICE MUTANTS

Y. NAKAMURA¹, N. FUJITA¹, Y. UTSUMI¹, A. NISHI², H. SATOH²

¹Akita Prefectural University, Akita, Japan

²Kyushu University, Fukuoka, Japan

Email: nakayn@akita-pu.ac.jp

In this presentation, the concept and perspective of molecular mutation breeding of starch biosynthesis are discussed. Amylopectin, a major component of starch, has a distinct highly-ordered structure referred to as “tamdem-cluster structure”. The starch synthesis system has developed during the process of evolution of plants, and key enzymes involved in the construction of amylopectin tamdem-cluster structure have differentiated into multiple isozymes with specific functions. Detailed analyses of changes in the structure of amylopectin and the physicochemical properties of starch granules in rice endosperm induced by lesion of each isoform of starch branching enzyme, starch synthase, starch debranching enzymes and others have established that the individual mutants exhibit distinct characteristics in terms of the starch structure and properties. These patterns of changes reflected by the specific functions of enzymes in starch biosynthesis enable us to expect to what extent and how the structure and properties of starch can be engineered. In fact, numerous rice mutant lines could be used in the industrial purpose in the future by the production of novel starches in endosperm. Transcriptome analysis established that changes in the transcript levels of starch synthesizing enzyme genes in rice endosperm are largely divided into two patterns. One group of genes are highly expressed in the very early stage of the endosperm development prior to the onset of the rapid starch production whereas the other group genes are greatly expressed in accord with the start of a great amount of starch accumulation in the endosperm. These results strongly suggest that the initiation process of starch biosynthesis and the accumulation process of increases in number of starch molecules and starch granule are regulated by different mechanisms with varied sets of isozymes. Our recent studies showed that plastidial phosphorylase plays a crucial role in the initial stage of starch biosynthesis.

IAEA-CN-167-398

DEVELOPING MUTANT RICE HIGH IN RESISTANT STARCH FIGHTING FOR DIABETES-AFFECTED PEOPLE

D.X. WU, X.L. SHU, C.Z. YANG, L.M. JIA, Q.Y. SHU

IAEA-Zhejiang University Collaborating Center on Mutant Germplasm Enhancement and Its Exploitations in Plants, State Key Laboratory of Rice Biology, Zhejiang University, Hangzhou, China

Email: dxwu@zju.edu.cn

Diabetes is a major socio-economic cost and is a predisposing factor for early onset coronary heart disease and peripheral vascular disease. Type II diabetes reflects a failure of blood glucose regulation, leading to sustained high blood glucose concentrations that have numbers of metabolic effects predisposing to pathology. The risk of Type II diabetes is related to diet, especially the availability of readily digested, highly refined foods and excess energy intake. In the case of diabetes, the ready digestion of carbohydrates (particularly starches) leads to a rapid rise in blood glucose and a greater demand on the pancreas for insulin to normalize concentrations. Starch that is not absorbed in the small intestine but passes into the large bowel (Resistant starch, RS) is a key protector against serious disease (including cancer) in the latter region of the intestines, being fermented by the large bowel microflora yielding short chain fatty acids (SCFA) that promote normal colonic function. Multiple approaches were suggested to obtain high amounts of resistant starch, and most of is to physically modify starch structure through processing technology. Diabetes is also increasing in the Third World and Least Developed Countries (LDCs). The current rice varieties low in resistant starch available to these individuals are not low in GI and the production of high resistant starch rices will be an attractive consumer option to this section of the population. The ability of subsistence farmers to be able sell high-RS rice lines will enable such farmers to continue to survive in those in these countries. The largest producers in the world are China and India with production at 120 and 80 million tonnes per annum respectively. The contents of RS in the hot cooked and processed rice are always below 3% by the traditionally domestic manners. This might lead to a higher glycemic index (GI) and lower butyrate content, subsequently increase the potential risk of metabolic syndrome. To execute the dietary-prevention strategy and the non-insulin-dependent diabetes treatment, developing for rice high in RS is of particular interest and an accepted means of preventing diet-related disease. Serial of mutants high in RS in the hot cooked rice and processed was induced from the leading commercial rice varieties in China i.e. R7954, 9311, II-32B, Zhongzhe B, K17B, Gang46B. Despite obviously low RS content in the raw milled rice, RS content in the cooked and processed rice of mutants were 10-100 times higher than that of the wild type and common rice, the highest RS content is about 15% in mutants. Obvious differences in physicochemical properties, starch granule morphology, pasting properties, thermal properties, and X-ray diffraction pattern were observed among mutants, wild type, and common rice. The high-RS mutants were unique in natural starch structure and were characterized by the higher λ_{max} of absorbance and blue value of iodine-binding starch complex, higher percentage of oval-shaped granules and bigger oval size, lower onset

temperature, peak temperature, final temperature, and enthalpy of gelatinization, lower crystallinity, containing a higher percentage of intermediate chains of amylopectin and displaying a mixture of B- and V-type that was more resistant to starch hydrolysis by alpha-amylase. Starch hydrolyses *in vitro* by porcine pancreatic α -amylase tends to be incomplete with a lower rate and extent in the cooked or processed high-RS rice. In practically consumed as the staple foods by diabetes and animal-feeding test, the GI levels in two hours after eating were significantly lower than that of common rice, and the increased satiety sensation was clearly felt, which is the key important for the diabetes to eat the enough to meet the basic "Always hungry" problem. The reduction in the amount of inhibiting-digestion chemicals and insulin and improvement of health body were quickly observed in the long-term eating testing. Based on the protocols developed, mutants high in RS with 10-50 times increases were also induced from the commercial Chinese and Australia wheat varieties.

IAEA-CN-167-318

MUTANTS PAVE THE WAY TO WHEAT AND BARLEY FOR CELIAC PATIENTS AND DIETARY HEALTH

D. VON WETTSTEIN

Department of Crop and Soil Sciences & School of Molecular Biosciences, Washington State University, Pullman WA, USA
Email: diter@wsu.edu

Wheat has two major nutritional problems for the consumer: (1) The flour or pasta produced from the grain is not acceptable to congenital celiac patients and may induce intolerance of dietary "gluten" in people later in life. (2) The grain is highly deficient in the essential amino acid lysine. Currently there is only one treatment for sufferers of celiac disease: the complete exclusion of wheat, barley and rye grains from their diets. Celiac disease is caused by an autoimmune reaction against undigested proline/glutamine rich peptides (epitopes) that are taken up through the intestinal mucosa and initiate an autoimmune response in human leucocyte antigen DQ2- or DQ8-positive individuals. This leads to chronic erasure of the microvilli of the intestinal epithelium and to permanent intolerance of dietary "gluten". Cereal prolamins are of two types: high molecular weight glutenins (HMWG) with a molecular structure of elastic fibrils that form dityrosine cross-links during dough formation and baking, and gliadins. The gene promoters of the gliadin-type proteins are silenced by DNA methylation in vegetative tissues. This methylation is removed during grain development to permit protein synthesis. Inhibition of the demethylation by mutation specifically inhibits the synthesis of the gliadin-type proteins and only proteins consisting of elastic fibrils are produced. As a proof of principle, a barley cultivar called Lysiba already exists that has such a mutation and provides the rationale creating wheat varieties by mutation of the 5-methylcytosine deglycosylases in the endosperm. Celiac patients are sensitive to a wide variety of different epitopes, which are located in the gliadin-type prolamins. Gliadin-type prolamins are of no importance for baking because wheat HMW glutenin has been shown to be alone sufficient to produce high quality breads.

IAEA-CN-167-164

MAIZE MUTANT *OPAQUE2* AND THE IMPROVEMENT OF PROTEIN QUALITY THROUGH CONVENTIONAL AND MOLECULAR APPROACHES

Y.B. XU¹, R. BABU¹, D.J. SKINNER¹, B.S. VIVEK², J.H. CROUCH¹

¹International Maize and Wheat Improvement Center (CIMMYT), Mexico DF, Mexico

²CIMMYT-Zimbabwe, Harare, Zimbabwe

Email: y.xu@cgiar.org

Maize endosperm protein is deficient in two essential amino acids, lysine and tryptophan. Several spontaneous and induced mutations that affect amino acid composition in maize have been discovered amongst which the *opaque2* gene has been used in association with endosperm and amino acid modifier genes for developing quality protein maize (QPM), which contains almost double the amount of lysine and tryptophan compared to normal maize. These increases have been shown to have dramatic impacts on human and animal nutrition, growth and performance. A range of hard endosperm QPM germplasm has been developed at the International Maize and Wheat Improvement Center (CIMMYT) mostly through conventional breeding approaches to meet the requirements of various maize growing regions across the world. Microsatellite markers located within the *opaque2* gene provide opportunities for accelerating the pace of QPM conversion programs through marker-assisted selection (MAS). Thus, CIMMYT scientists are developing a package of reliable, easy to use markers for endosperm hardness and free amino acid content in the maize endosperm. Recent technological developments in molecular biology at CIMMYT such as single seed-based DNA extraction and low cost, high throughput SNP genotyping strategies promise enhanced efficiency and cost effectiveness of QPM molecular breeding programs. Here we present a summary of QPM research and breeding with respect to the history of conventional improvement methodologies, genetic and molecular basis of *opaque2*, epistasis between *opaque2* and other high lysine mutant genes and recent advances in genomics technologies that could potentially enhance the efficiency of QPM molecular breeding in future.

IAEA-CN-167-250

MUTATION BREEDING FOR OIL QUALITY IMPROVEMENT IN SUNFLOWER

J.M. FERNÁNDEZ-MARTÍNEZ, B. PÉREZ-VICH, L. VELASCO

Institute for Sustainable Agriculture (CSIC), Córdoba, Spain

Email: cs9femaj@uco.es

Although sunflower oil is appreciated as a high quality commodity, new emerging markets and increasing concern about health risks are demanding changes in the oil quality. The optimal quality of oils depends on their intended use either for food or non-food applications. The fatty acid composition and the total content and profile of tocopherols have been the most important traits considered in breeding for oil quality. Applications demanding a high nutritional value (salad oil) require a reduction of saturated fatty acids and enhancement of the vitamin E (alpha-tocopherol) content of the oil. The use of oils in the food industry requiring plastic fats (margarines) demand an increased concentration of saturated fatty acids to avoid hydrogenation. High temperature processes (frying, biolubricants) need oils highly resistant to thermoxidation, with a high concentration of oleic acid and antioxidants (gamma- and delta-tocopherol). In sunflower, the utilization of mutagenesis has been the most successful procedure used to generate genetic variability for these quality components. The mutagenic treatment is usually applied to seeds to obtain M_1 generation and mutants are detected analysing M_2 half seeds, allowing identification of mutants in one year. The most valuable sunflower oil quality mutants produced have been the high oleic acid mutant (>80%), high levels of either palmitic or stearic acid (>25%), low total saturated fatty acids and increased levels of beta- (>65%), gamma- (>95%), and delta-tocopherol (>45%). The novel traits are in all cases governed by a reduced number of genes, which facilitates their management in plant breeding. All this induced variation opens up the possibility of tailoring specialty sunflower oils for specific food and nonfood applications

IAEA-CN-167-301

THE MULTIPLE USE OF BARLEY ENDOSPERM MUTANTS IN PLANT BREEDING FOR QUALITY AND FOR REVEALING FUNCTIONALITY IN NUTRITION AND FOOD TECHNOLOGY

L. MUNCK, B. MØLLER JESPERSEN

Department of Food Science, Life Science Faculty, University of Copenhagen, Frederiksberg, Denmark

Email: lmu@life.ku.dk

The paper is outlining with examples how biochemical seed mutations can be used not only to improve quality in cereals but also to understand functionality in nutrition and in food processing and even to overview gene expression for improved genetic theory. A high lysine barley mutant Risø 1508 (*lys3.a*) cross breeding material developed at Carlsberg 1973-1988 is used to demonstrate how Near Infrared Reflectance (NIR) spectra evaluated by a Principal Component analysis (PCA) score plot can visualize an improved chemical composition due to a changed mutant gene background. By introducing starch and yield improved high lysine *lys3.a* recombinants in pig feeding, protein concentration in the feed and nitrogen load on the animal and also importantly on environment could be reduced by 15-20% without compromising meat production. The decreased nitrogen load on the animal spares the energy that is necessary to catabolize the surplus of non essential aminoacids. The less carbohydrates and lower energy digestibility in the improved *lys3.a* lines is thus likely to be compensated by a more efficient energy metabolism of the protein. Thus the high lysine mutants are contributing to the understanding of animal physiology. In analogy the technological importance of slender cellwalls and low β -glucan was elucidated by a mutant M-737 from Carlsberg speeding up malt modification to spare 1-2 days. The vast majority of mutation scientists are traditionally rather pessimistic regarding the possibility to repair negative pleiotropic effects of mutants by engaging in the hard and time consuming work of cross breeding. An open holistic exploratory strategy is necessary in order to evaluate the great complexity of the pleiotropic effects of a mutant gene that can not be forecasted by prior scientific knowledge. This is now possible by exploiting NIR Spectroscopy as a coarse overview of the phenotype (Phenome) on the level of chemical bonds.

CREATION AND EVALUATION OF VALUABLE TOOLS FOR PEPPER BREEDING THROUGH INDUCED MUTAGENESIS

N. TOMLEKOVA¹, V. TODOROVA¹, V. PETKOVA¹, S. YANCHEVA², V. NIKOLOVA¹, I. PANCHEV³, E. PENCHEV⁴

¹Department of Plant Breeding, Maritza Vegetable Crops Research Institute, Plovdiv, Bulgaria

²Institute of Genetics 'Prof. D. Kostov', BAS, Sofia, Bulgaria

²Agricultural University, Plovdiv, Bulgaria

³Faculty of Biology, Sofia University, Sofia, Bulgaria

⁴Dobroudja Agricultural Institute, General Toshevo, Bulgaria

Email: nasia.tomlekova@gmail.com

Advances of plant molecular biology and screening techniques integrated with the mutation technologies allowed to study and utilize better the potentialities of mutation lines. Application of X-rays, Co⁶⁰ and EMS in pepper breeding programmes contributed to create mutants with applied value: increased β -carotene level in fruit, male sterility, anthocyaninlessness, determinant habit, changes of fruit shape and position. RILs were developed and different mutant genes were combined in the same genotypes. Mutants demonstrated a potential toward to boosted β -carotene levels in different crosses and were selected to be exploited as good donors for development of hybrids. Dramatically increased β -carotene content was assessed in some of the created F₁ hybrids. Obtained results from the cytological, biochemical and physiological studies of carotenoid levels, β -carotene hydroxylase enzyme activity, chlorophylls, observation of the phenotype of plants and fruit suggested breeding to exploit different mutant lines. Molecular study allowed us to establish a marker for high β -carotene useful for MAS. The created and investigated pepper mutant material was assessed as a valuable human food according to the new market requirements.

IMPROVEMENT OF BASMATI RICE THROUGH MUTATION APPROACH

G.J.N.RAO, D.CHAUDHARY, A.PATNAIK

Division of Crop Improvement, Central Rice Research Institute, Cuttack, India

Email: raogjn@yahoo.com

Basmati, an unique group of aromatic long slender grain rices are famous for their distinct aroma, extra linear kernel elongation during cooking and non-sticky and fluffy nature of the cooked rice that confer them a special status in the international market. The cultivation of Basmati rices is confined to a specific geographic demarcation in the foot hills of Himalayas that offer the best climatic conditions for them to mature and flourish. In the export market, Indian Basmati varieties command a premium market price and India earns quite a significant amount of foreign exchange through Basmati exports. The well-known tall, lodging prone Basmati varieties are selections from native land races and only six pure line selections that possess the much appreciated grain quality are recognized as Traditional Basmati in India while six improved types are grouped as Evolved Basmati. With such stringent criteria in place, it is a challenging task to develop non lodging, high yielding Basmati keeping the grain quality intact. Attempts to develop high yielding nonlodging types from Basmati 370, Pusa Basmati 1 and Pakistan Basmati through mutation approach generated several promising mutants and in the multi location trials in the Basmati zone, CRM2007-1, a Basmati 370 mutant, was highly promising. This semi dwarf mutant with Basmati grain quality recorded significant yield superiority over both yield (Pusa Basmati 1) and quality (Taroari Basmati) check varieties. The mutant had also shown great promise in the non Basmati areas and owing to its high yield, excellent grain quality, easy marketability and high economic returns to the farmers, it was released as "Geetanjali" in Orissa. High yielding mutants were also isolated from Pusa Basmati 1 and Pakistan Basmati with good quality traits. The development of promising mutants demonstrates the utility of the mutation approach in Basmati rice development where options of genetic improvement are limited.

INDUCED MUTAGENESIS FOR OIL QUALITY ENHANCEMENT IN PEANUT (*ARACHIS HYPOGAEA* L.)

S.B. KAVERI, H.L. NADAF

National Seed Project, Seed Unit, University of Agricultural Sciences, Dharwad, Karnataka, India

Email: kaveri_pnut@rediffmail.com

Increasing the ratio of oleic to linoleic acid (O/L) in peanut (*Arachis hypogaea* L) significantly improves the nutritional and quality attributes of the crop. The lack of sufficient genetic variation for fatty acid profile, particularly O/L ratio in peanut germplasm and presently grown cultivars has necessitated the creation of variability. Mutation breeding of peanut was therefore initiated with the objective of identifying stable peanut mutants with altered fatty acid composition for improved oxidative stability and nutritional quality. Seeds of peanut cultivars 'GPBD-4' and 'TPG-41' were treated with γ -radiation and/or ethyl methane sulphonate (EMS). Randomly selected mutants were advanced based on single plant selection up to M₄ generation and the harvest of M₄ plants was evaluated for fatty acid composition by gas chromatography. Highly significant variation for palmitic, stearic, oleic, linoleic and arachidic acid was observed. EMS (0.5 %) and 200Gy treatment were found to be effective in increasing the variability for fatty acid content in GPBD-4 and TPG-41 respectively. The variability was skewed towards high levels of oleic (38-66.58%) and low levels of linoleic acid (15-41%). Mutants with improved oil quality selected were significantly superior for O/L ratio and had reduced palmitic acid. Oil with reduced palmitic acid and increased O/L ratio is nutritionally desired. Hence these mutants can be exploited in improvement of oil quality. The mutant GE-87 and T3-105, recorded highest O/L ratio of 4.30 and 3.91 as against control value of 1.75 and 2.60 respectively. A significant negative correlation between oleic acid and linoleic acid, palmitic acid and iodine value and weak inverse relationship with oil content indicates the possibility of selection for improved fatty acid composition. These high oleic acid lines could be utilized further in breeding programs for improvement of peanut oil quality.

INDUCED MUTANTS WITH IMPROVED NUTRACEUTICAL TRAITS IN SESAME (*SESAMUM INDICUM* L.)

K. MADHUSUDAN¹, S. SINGH², H.L. NADAF¹, B.N. MOTAGI¹

¹National Seed Project, University of Agricultural Sciences, Dharwad, Karnataka, India

²Central Food Technological Research Institute, Mysore, Karnataka, India

Email: madhusudan_seeds@rediffmail.com

Induced mutagenesis with gamma radiation and chemical mutagen, ethyl methane sulphonate (0.5%) was employed in sesame (*Sesamum indicum* L.) to enhance the genetic variability for higher seed yield and seed quality components. Isolated mutants were subjected for analysis of nutraceutical components like lignans. ANOVA indicated very highly significant variation between mutants and their interaction. Sesamin content ranged from 1.76 to 10.46 g/kg oil, sesamol ranged from 0.72 to 3.77 and γ -tocopherol ranged from 0.45 to 0.77. Average sesamin content was 5.44 g/kg and the sesamol was 1.92 g/kg, while γ -tocopherol was 0.58 g/kg. Co-efficient of variation for sesamin was 17.99 while it was 15.16 for sesamol and 12.57 for γ -tocopherol. Top ten mutants were selected for the estimation of lignan profiles. Mutant No.1022 recorded significantly higher total lignan content of 14.23 g/kg as against the parent DS-1 (5.79 g/kg). The mutant also had high amount of sesamin (10.46 g/kg). The top four induced mutants (No.1022, 23, 191 and 983) recorded the total lignan content of more than double compared to the parent DS-1 (5.79 g/kg). Chemical mutagens were found to be more effective in inducing more number (25) of mutants for lignan antioxidants. Among the radiation doses treated, 400 Gy was found to be more effective in inducing higher number of mutants (10), followed by 300 Gy which induced nine mutants. The trend was different in 500 Gy, as the dose increased the frequency of useful mutants was quite low, which indicates that, optimum level would be anywhere around 300-400Gy. Promising mutant lines need to be tested over different environments for their adaptability and stability. They can also be utilized in inter-mutant hybridization and recurrent irradiation to further enhancing the lignan profiles. Since, these mutant lines are having higher proportions of lignan they can be better exploited for their anti-diabetic and anti-hypertension benefits.

PROTEIN CONTENT IN HIGH PROTEIN SOYBEAN MUTANTS IN THAILAND

C. YATHAPUTANON, B. KUMSUEB, A. MALIPAN, S. SRISOMBUN, J. BUNYARUT

Nuclear Research in Agriculture Section, Agricultural Chemistry Research Group, APSRDO, Department of Agriculture, Bangkok, Thailand

E-mail: chitima@doa.go.th

Two present studies had been initiated to enhance nutritional quality of seed protein content in soybean varieties using induced mutation techniques. Approximately 5,000 seeds of each variety with uniform size were irradiated with gamma rays at a dose of 200 Gy at Kasetsart University. Kjeldahl method was used to analyze seed protein percentages. Experiment I. Seed of three soybean varieties, Chiang Mai 60, SSRSN35-19-4 and EHP275 were irradiated. M₁ to M₄ generations were grown at Nakhon Ratchasima Field Crops Research Center during 2004-2007. The Pedigree method of selection was used. In M₂, M₃ and M₄ generation, the selected mutant lines had 1.9-2.6, 1.5-2.3 and 0.8-2.2 % higher seed protein content than the three checks, respectively. In preliminary trial, the high protein mutant lines were tested for their protein yield. The mutants had average protein content of 42.5, 42.4 and 42.9 % whereas the check varieties had average protein content of 41.8, 40.3 and 41.9 %, respectively. There were 6, 18 and 8 promising mutant lines selected from Chiang Mai 60, SSRSN35-19-4 and EHP 275. The mutant lines produced both high seed protein content and high yield. They will be tested in replicated trials in the research centers and farmer fields. Experiment II. CM9238-54-1 (ST) was a soybean promising line to be released for farmers. It gave 5–10 % higher grain yield than cv Chiang Mai 60, the most popular variety in the northern and central regions. However, this line was susceptible to Soybean Crinkle Leaf (SCL) Disease. The M₁ plants were grown in dry season 2003 at Sukhothai Technical and Production Resources Service Center (TPRSC). The M₂ and M₃ seed were sown in dry and rainy seasons 2004 and the selected M₄ lines were tested for grain yield in four environments, dry and rainy season 2005, rainy season 2006 and dry season 2007 at Lop Buri TPRSC. From the 2006 to 2007 trials, six selected lines were resistant to SCL under laboratory test giving 74-81% higher grain yield than that of the original parent. In addition, they had 2.1-4.0 and 2.1-7.5 % higher seed protein content than a check variety, Chiang Mai 60, respectively and had 0.5-2.0 and -1.0-3.3 % higher seed protein content than another check variety, SJ4 respectively. The mutants had average protein content of 38.5-43.8 % while the two check varieties had average protein content of 36.3-39.9 %.

RAPID PYRAMIDING OF LOW PHYTIC ACID MUTATION AND FERRITIN GENE FOR IMPROVEMENT OF MINERAL NUTRITIONAL QUALITY OF RICE

S.Q. SHEN¹, M. LI¹, Z.J. GUO², H.X. YE¹, Q.Y. SHU¹, D.X. WU¹, J.S. BAO¹

¹Institute of Nuclear Agricultural Sciences, Zhejiang University, Hua Jiachi Campus, Hangzhou, China

²Department of Plant Pathology, China Agricultural University, Beijing, China

Email: shenshq@zju.edu.cn

Nutritional quality is an important component of the rice grain. Development of low phytic acid (*lpa*) crops, in which the PA phosphorus (Pi) content is significantly reduced in grains, has recently been considered as a potential way to increase bioavailability of Zn²⁺ and Fe³⁺ in the rice grain. Another potential approach to improve nutritional quality is to express *ferritin* gene from legume crops to increase iron content in rice grain. We have isolated a low phytic acid rice mutant (*lpa*-XS110-1) and obtained transgenic rice expressing the *ferritin* gene from pea. Two transgenic lines (Fer34 and Fer65) had iron content about five times that of the parent XS110 (Ye et al 2007). To pyramid the low phytic acid mutation and *ferritin* gene into one line, two crosses were made between Fer34 and *lpa*-XS110-1 and between Fer65/ *lpa*-XS110-1. The F₁ anthers were subjected to anther culture to obtain stable homozygous plants. A total of 43 doubled haploid (DH) lines were obtained from the Fer34/ *lpa*-XS110-1 cross, and 86 DH lines from Fer65/ *lpa*-XS110-1. For individual trait, both low phytic acid and the *Ferritin* gene (indirectly assayed with Gus) were inherited as a single locus. In combination, four recombinant traits were obtained, i.e high inorganic pi (*lpa*)/Gus+, *lpa*/Gus-, low inorganic pi/Gus+, and low inorganic pi/Gus-, the ratio of each recombinant was in accordance with the ratio of 1 : 1 : 1 : 1, indicating that *lpa* and *Fer* gene were not linked, and segregated as a single locus. The results suggest doubled haploid production is a rapid approach to pyramid useful genes from different origin for rice improvement. This study was jointly supported by funds from IAEA (12229), the Science and Technology Department of Zhejiang Province.

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ANALYSIS OF TOMATO PHOTOMORPHOGENIC MUTANTS FOR FRUIT QUALITY

Y. SREELAKSHMI, P. SANTHISREE, S. SHARMA, B. REDDIAH, K. APPENROTH, R. SHARMA
 Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India
 Email: ys1sl@uohyd.ernet.in

Fruits are an important source of minerals, vitamins, fibers and antioxidants for humans and animals. This is due to the presence of various kinds of carotenoids, flavonoids, phenolics, tocopherols, etc. All these compounds are synthesized during the course of fruit ripening. The ripening of fleshy fruits is a complex developmental process influenced by numerous factors including light, hormones, temperature, and genotype. Tomato has long served as a model organism for climacteric fruit ripening. The DNA micro array analysis for expression of genes revealed that nearly 1000 genes might participate in regulation of tomato fruit ripening indicating its complexity. Since plants are photosynthetic organisms, they depend on light for their growth and development. Different photoreceptors like phytochromes, cryptochromes, and phototropins mediate red-far red, and blue light mediated responses during the growth and development of plants. In tomato the process of fruit pigment development is regulated by the light. Recent studies have shown that both red and far-red light can penetrate the epidermis and pericarp of both immature and mature tomato fruits, and photoreceptors such as phytochromes are present in fruit tissues. Similarly, it has been shown that deficiency of cryptochrome affects the pigmentation during fruit ripening whereas the overexpression of *cry2* leads to increase in lycopene content in tomato. But the role played by light is still unclear. In view of the importance of the role played by light during fruit pigmentation, we have undertaken a study on fruit ripening using different photomorphogenic mutants of tomato. We are analyzing the role played by different photoreceptors in the formation of various metabolites in tomato fruits such as carotenoids, flavonoids, phenolics, etc and the results will be presented at the meeting. We believe that such a study would fill the lacunae in the role played by light during fruit ripening of tomato.

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GENETIC ENHANCEMENT OF SPECIALTY RICE THROUGH MUTATION APPROACH - SHORT GRAIN AROMATIC RICE

A.PATNAIK, G.J.N.RAO
 Division of Crop Improvement, Central Rice Research Institute, Cuttack, India
 Email: ashok19512002@yahoo.co.in

India has a rich diversity of widely distributed aromatic rices. They include Basmati, whose cultivation is limited to a well marked out geographic zone while the short grain aromatic rices are grown in localized pockets throughout India and with their unique sensory and cooking traits, cater defined groups of consumers in specific niche markets. Some short grain aromatic rices like Dubraj, Durgabhog, Makarkanda, Badshahbhog are superior to Basmati in traits like high kernel elongation, high volume expansion and high head rice recovery while Bindli, is superior to Basmati in aroma and grain elongation (~200%). The well known Kalajeera is known for the retention of aroma even after long storage. Till date, little attention was paid to short grain aromatic rice improvement as all focus was directed towards improvement of Basmati. The less coverage under the short grain aromatic rices can be attributable to their low productivity, long duration and tall plant stature. As high economic returns are feasible with varieties with shorter duration and shorter height with high yield, mutation approach was attempted to induce erect, semi dwarf, non lodging mutants with high yield potential while keeping the unique grain type and cooking quality traits of the parent cultivar. Twelve popular cultivars from different states i.e. Kalanamak, Dubraj, Tulsiphoool, Randhunipagal, Badshahbhog, Katrani, Improved Raskadam, Kalajeera, Pimpudibasa, Chinikamini, Dhusara and Kalajoha were subjected to gamma (γ) irradiation. From M₂ generation, selection was initiated to isolate mutants with shorter duration and plant height. Mutants with shorter (~20%) stature derived from Kalanamak, Dubraj, Kalajeera and Chinikamini showed high promise in the evaluation trials. Mutants with shortened duration (~10d) were also isolated in all these four genotypes. With the isolation of mutants with desirable traits, expansion in area under the short grain aromatic rices is feasible and the expected rice surplus can augment the farmer's income and also exports from India.

DEVELOPMENT OF HIGH PRODUCTIVE AND LOW NEUROTOXIN LINES IN GRASSPEA THROUGH MUTATION BREEDING

S.KUNDAGRAMI, P.K DAS

Department of Genetics & Plant Breeding, Institute of Agricultural Science, Calcutta University, Kolkata, India
Email: kundagrami@yahoo.co.in

Grasspea (*Lathyrus sativus L*) is a very potential pulse crop in rabi season, which is drought tolerant & improved soil fertility level by fixing atmospheric N₂ in the soil. But the major draw back of this crop is that the seed of it contain the neurotoxin ODAP & continuous consumption of it causes lower limb paralysis in human. Most of the varieties of the *Lathyrus* are low yielder also. So an attempt has been made to induce variability in grasspea through physical mutagen to develop high yielding low neurotoxin genotypes. Two grasspea genotypes Nirmal, a variety from West Bengal and P24, a variety developed at IARI, New Delhi, were treated with three different doses of gamma ray viz 100,200 & 300Gy. In M₁ germination was highly reduced at higher dose of gamma ray. Pollen fertility was affected with high dose of exposure. In M₂ generation at higher dose both the genotypes displayed reduction in pod number & seed yield. However, at 100Gy-dose seed yield tended to increase. In M₂ promising individuals with superior families for yield and pod production were advanced to M₃. In both the genotypes lower level of radiation exposure induced higher order of variability for higher number of pods with increased seed weight. In either of the genotypes maximum seed yield-producing families were from lower dose of gamma radiation. For pods/ plant and seeds/ pod, best families were selected and from those selected families, superior performing individuals were identified for M₃ generation. Grouping of means in M₃ showed that lower dose of gamma rays by and large induced major positive shift for different characters. Majority of the selected individuals were from either 100 or 200Gy populations. These individuals produced very high numbers of pods/plants and high seed yield, almost twice the respective controls. They exhibited in general, higher seed protein content and ODAP level either at par or less than the control. Two individuals in particular derived from 100 and 200Gy treatments in either of the genotypes appeared to be most outstanding because of their high pod production (around 130/plant) very high seed yield performance (Around 30 g/plant) along with high level of protein content (around 29%) and very low level of ODAP content (0.19%).

DEVELOPMENT OF HIGH OLEIC SOYBEAN MUTANT AND ITS STABILITY ACROSS THE ENVIRONMENTS

A. PATIL, S.P. TAWARE, V.S. RAO

Genetics Group, Plant Sciences Division, Agharkar Research Institute, Pune, Maharashtra, India
Email: archie_2180@yahoo.co.in

Modifying seed oil composition has become a major goal in soybean breeding programs. Elevated oleic acid and reduced linoleic and linolenic acid content can improve the oxidative stability, flavor and nutritional value of soybean oil. It is also important to study the effect of the environment on the altered fatty acid content in soybean to determine their stability over different growing conditions. The objectives of this study were to develop a high oleic acid soybean mutant and to determine the stability of fatty acid composition of the same across different environments. A high oleic acid mutant (HOM) containing 40% of oleic acid compared to 27% in parent cultivar "MACS 450" was selected from a mutagen treatment of 200Gy and 0.15% Ethyl Methane Sulphonate (EMS). To study the influence of the environmental factors on fatty acid composition, the HOM along with other four soybean lines MACS 1034, MACS 1055, MACS 1092 and Bragg were grown at 12 locations. Seeds of each genotype from each location were analyzed for fatty acid composition by gas chromatography. Eberhart and Russell's model was used to study the stability of fatty acids. In general, all the fatty acids were influenced by the environmental factors. Elevated oleic acid in HOM was less stable across the environments compared to oleic acid in other four cultivars. The mean oleic acid content in "HOM" was 31.26-45.18% over the 12 locations. Linoleic acid content in "HOM" and "MACS 1034" was also showed significant deviation from unity for regression coefficient showing its unstable nature. This study shows that the elevated oleic and reduced linoleic acids in "HOM" are highly influenced by the environmental factors.

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STUDY OF TOMATO LINES WITH HIGH NUTRITIVE QUALITYN. TOMLEKOVA¹, B. ATANASSOVA²¹Department of Plant Breeding, Maritza Vegetable Crops Research Institute, Plovdiv, Bulgaria²Institute of Genetics 'Prof. D. Kostov', BAS, Sofia, Bulgaria

Email: nasia.tomlekova@gmail.com

Study was performed on tomato lines from the cultivated species carrying mutant genes *hp* and *og*^c, as well as on lines originating from hybridization between *Lycopersicon esculentum* Mill. and *Lycopersicon pimpinellifolium* Mill. or *Lycopersicon chilense* Dunal for evaluate their genetic potential to synthesize high lycopene. The first steps of our study centered on evaluating the methodology of comparison, and the number of screening procedures necessary for determining individual plants or lines possessing potential to synthesize high lycopene content in tomato fruit. A relatively large scale of variation throughout the harvest dates was observed in lycopene content of the lines and hybrids studied but the genotypes investigated ranked almost in the same way despite of the variability in the pigment content. It was found that the genotypes possessing genetic potential to synthesize high lycopene content might be assessed based on one only analysis. Fruit should be collected and analyzed at one harvest date. Studies aiming at fingerprinting and evaluation of DNA variability among tomato lines from diverse origin or possessing genes enhancing lycopene content, as well as on some of their F₁ hybrids, were carried out. The AFLP molecular data indicated very low level of genetic heterogeneity in the studied tomato lines which relative frequency was 0.06. Selective markers with a direct application in the molecular selection of tomato lines and hybrids with economically valuable mutant characters were revealed. The origin of the studied tomato lines make them genetically heterogeneous. Grouping performed on the basis of AFLP patterns corroborated the genotypes origin data in most of the cases.

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IN VITRO MUTATION TECHNIQUES AND *PHYLLANTHUS NIRURI* L. TISSUE CULTURE FOR SECONDARY METABOLITES IMPROVEMENT HAVING AN ANTIPLASMODIAL ACTIVITYN. LUYINDULA¹, L. TONA¹, A.N. DIAMUINI¹, F.O. BULUBULU¹, K. MESIA³, R.S. SANGWAN¹, F. BENOÎT-VICAL¹¹Division des Sciences de la Vie, Commissariat Général à l'Energie Atomique, Kinshasa, RD Congo³Laboratoire de Pharmacologie, Université de Kinshasa, Kinshasa, RD Congo⁴Laboratoire d'Androgenèse et Biotechnologie (A.E.B.), Université de Picardie Jules Verne (UPJV), Faculté des Sciences, Amiens, France⁵Laboratoire de Chimie de Coordination du CNRS, Toulouse, France

Email: sluyindula@yahoo.fr

The research undertaken within the IAEA projects framework ZAI/6/009 and ZAI/5/016 consists in the checking of the antiplasmodial activity allotted to *Phyllanthus niruri* and to the bioguided isolation of the secondary metabolites produced by irradiation and *in vitro* culture. The preliminary *ex-vivo* and *in vitro* tests by the lactate dehydrogenase (LDH) method showed an antiplasmodial activity extract ethanolic of the whole plant higher than that of aqueous decoct. The leaves aqueous extract presented a significant antiplasmodial activity *in vitro* (IC₅₀ = 14±1 µg/ml) by the radioactive microdilution technique (³HP). The preliminary chemical screening tests of the whole plant, leaves and roots revealed the presence of Alkaloids, gallic Tannins, Flavonoïdes, Coumarines, of Terpenoïdes and Steroids. The secondary metabolites present in the extracts ethanolic of the whole plant were separated by the analytical HPLC. The chromatogram obtained gave a majority peak at 26 min. Among the raised signals from various fractions of the majority peak, the spectra MS/MS (of fragmentation) of the ions responsible of the peak in m/Z 353 revealed the bicharged ions with a molecular weight around 660. This major molecule was purified and isolated at 27.97 min. by the coupling LC/MS. A molecule of mass 448 correspondent to that of Quercitrin (Flavonoïd) was among the molecules separated and identified from leaves ethanolic extract by coupling LC/MS. The ethanolic extracts calli of 1 month old revealed a significant antiplasmodial activity (IC₅₀ = 16.3 ± 2.5 µg/ml). The calli aqueous extracts of 3 months old obtained from explants irradiated at the dose of 1Gy showed an activity higher compared to the extracts of the whole plant and calli formed from no irradiated and irradiated explants at 4Gy and 7,5 Gy. In addition, the alkaloids, catechic tanins, saponins and terpenoïdes were identified in the calli from irradiated explants extracts.

MARKER ASSISTED SELECTION FOR FIBER QUALITY IMPROVEMENT IN MUTATION BREEDING PROGRAMME OF COTTON

M. ASIF, M. RAHMAN, Y. ZAFAR

National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

Email: mehboob_pbd@yahoo.com

Thirty four cotton genotypes were screened for fiber quality traits. Four genotypes; FH-883, FH-631S, CIM-707 (tetraploids) and Ravi (diploid) contrasting for quality traits were selected. After surveying 520 RAPDs and 435 SSRs, a genetic linkage map was constructed using 117 F_{2,3} lines derived from a cross FH-631S x FH-883. Twenty loci were mapped into four linkage groups (LG) spanning around 230.2 cM with 5% of the cotton genome coverage. The average genetic distance was 11.5 cM between two adjacent loci. Low level of polymorphism between two parents was might be due to narrow genetic base of cotton. LG1 to LG4 were assigned to chromosome 20, 10, 18, and 15, respectively. QTLs for fiber traits were identified by performing SMA, IM and CIM at LOD > 2 with WinQTLCart. Sixteen putative QTLs were identified for fiber traits including fiber length, fiber fineness, fiber strength, fiber length uniformity, short fiber index, fiber elongation, and fiber color. Nine fiber QTLs were detected on A-subgenome, while seven on D-subgenome, which suggest that fiber traits result from gene interaction of both the subgenomes of cotton. CIM-707 (above 30 mm) and Ravi (17 mm) were irradiated with gamma rays (125, 150, 200, 250 and 300Gy) and treated with EMS (1, 1.5 and 2%), which depressed significantly germination percentage of cotton seed. EMS-treated M₁ population was much better than irradiated M₁ population with respect to germination and plant growth. Moreover, ploidy level also affected the germination percentage. Staple length of selected M₃ lines of Ravi was in the range of 13-17.5 mm, while of CIM-707 M₃ lines were 22-31 mm. It indicates that EMS and gamma irradiation can successfully be used for creating variation among cotton germplasm and hence mutants are valuable tool for fiber quality improvement.

STORAGE PROTEIN MUTATIONS IDENTIFIED IN COMMON WHEAT AND BARLEY ACCESSIONS AND UTILIZATION OF THOSE MUTANTS IN STUDIES OF CROP PROPERTIES

A.V. ZLATSKA, L.V. KOROL. Y.V. GOLOPAPA

Department for Laboratory Analysis of Qualification Expertise of Plant Varieties, Ukrainian Institute for Plant Varieties Examination, Kiev, Ukraine

Email: zlatska@hotmail.com

Three types of mutations in prolamines and glutelins encoding regions which lead to disappearance of whole block of components, the particular component of the block and changing in electrophoretic mobility have been identified as a result of screening of the number of common winter wheat varieties and lines as well as spring and winter barley varieties, which have been treated by both chemical and ionizing radiation mutagens. The importance of such type of plant material for fundamental and applied research is discussed.

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THE ROLE OF INDUCED MUTATIONS FOR IMPROVING SEED OIL QUALITY IN *BRASSICA CARINATA*

L. VELASCO, J.M. FERNÁNDEZ-MARTÍNEZ
Institute for Sustainable Agriculture (CSIC), Córdoba, Spain
Email: ia2veval@uco.es

Chemical mutagenesis (EMS) was used to broaden the genetic variation for seed quality traits in the high erucic acid Ethiopian mustard (*Brassica carinata* A. Braun) line C101. The identification of putative mutants was conducted by analysing bulk samples of M₃ seeds from 8331 individual M₂ plants for seed oil fatty acid profile by near-infrared reflectance spectroscopy (NIRS). Several mutants with altered fatty acid profiles were identified. Some of them presented different increased levels of oleic acid and/or reduced levels of linolenic acid, other mutants showed reduced levels of erucic acid, and one mutant had increased levels of erucic acid. Mutagenesis was followed by a recombination programme that included the mutants N2-3591, with increased oleic acid content, and N2-4961, with reduced linolenic acid content, together with the low linolenic acid line HF-186 and the zero erucic acid line 25X-1. Crosses between N2-3591 and HF-186 resulted in transgressive segregants with higher oleic acid content than N2-3591, which were subsequently recombined with the zero erucic acid line 25X-1, resulting in the zero erucic acid line AB1 with a very high oleic acid content of 83.9%, compared to 32.9% in 25X-1. Crosses between N2-4961 and HF-186 resulted in transgressive segregants with lower linolenic acid content than both low linolenic acid parents. Subsequent recombination with the zero erucic acid line 25X-1 resulted in the zero erucic acid line AB4, with a very low linolenic acid content of 1.5%, compared to 16.4% in 25X-1. The high oleic acid content of AB1 and the low linolenic acid content of AB4 are similar to the best levels achieved for high oleic acid content and low linolenic acid content, respectively in rapeseed/canola (*Brassica napus* L.), which opens up the possibility of producing high-quality Ethiopian mustard seed oil for a wide range of food and nonfood markets.

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PRODUCTION OF MUTANTS WITH HIGH INORGANIC PHOSPHORUS CONTENT IN SEEDS BY GERMPLASM RESELECTION AND MUTATION TECHNIQUE IN WHEAT

L.S. ZHAO, L.X. LIU, S.R. YANG, H.J. GUO, S.R. ZHAO
Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China
Email: luxiang@263.net.cn

Phytic acid or phytate is commonly regarded as the major anti-nutritional component in cereal and legume grains. Development of low phytic acid or high inorganic phosphorus (HIP) crops has recently been considered as a potential way to increase nutritional quality of crop products. 399 genotypes of winter wheat germplasm including the currently cultivated varieties were colorimetric assayed for inorganic phosphorus content using Chen's reagent. The results showed significant difference in inorganic phosphorus content among different genotypes, and fifteen genotypes (accounting for 3.8%) with high inorganic phosphorus content varying from 0.93µg/mg to 1.39µg/m were selected. Two winter wheat genotypes Zhongyou9507 and Zhongyuan9 were also used to induce HIP mutations by mixed particle field with high energy, ⁶⁰Co γ-rays and ⁷Li ion beams. Dry seeds were irradiated by mixed particle field and ⁶⁰Co γ-rays with doses of 195Gy and 284Gy, and ⁷Li ion beams with the doses of 50Gy, 100Gy and 150Gy, respectively. The results showed that the frequencies of HIP mutations and homozygous HIP mutations induced by 195Gy ⁶⁰Co γ-rays and 284Gy mixed particle field were similar between the two genotypes, while the frequencies of HIP and homozygous HIP mutation in Zhongyou9507 were higher than that in Zhongyuan9 in all the other treatments, revealing the more high radiation sensitivity of Zhongyou9507 than that of Zhongyuan9. Three homozygous HIP mutants from Zhongyou9507 and one from Zhongyuan9 were identified. Characterization of the homozygous HIP mutants in respect of agronomic, biochemical and molecular levels were also conducted.

CONCURRENT SESSION 6 (13:30-17:40)

New Techniques and Systems for Mutation Induction

UNIDO Boardroom C04

Wednesday, 13 August 2008

ACTIVATION OF TRANSPOSABLE ELEMENTS FOR MUTATION INDUCTION

H. HIROCHIKA, C. CHENG, A. MIYAO, E. FUKAI
National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan
Email: hirohiko@affrc.go.jp

Retrotransposons are ubiquitous and major transposable elements in plants. However, most of plant retrotransposons seem inactive under normal growth conditions. Some of the retrotransposons were activated under stress conditions and regulated mainly at the transcriptional level. The transcription of the tobacco retrotransposon *Tto1*, originally isolated as an element activated by tissue culture, was also activated by various stresses. The rice retrotransposon *Tos17* was also activated by tissue culture. In all of the plants regenerated from tissue cultures, five to thirty transposed *Tos17* copies were detected. *Tos17* was shown to transpose preferentially into low-copy-number, gene-rich regions. A collection of 50,000 regenerated rice lines carrying about 250,000 independent insertions was generated. Ongoing reverse genetic studies such as PCR-screening of mutants and cataloguing mutants by sequencing *Tos17*-insertion sites as well as traditional forward genetic studies have demonstrated that tissue culture induced-activation of *Tos17* can be used as an efficient tool for functional analysis of rice genes. Over 35% of the rice genome is composed of transposable elements and several lines of evidences suggested the existence of the silenced quiescent elements. To find such elements, the transcription and transposition of 30 transposable elements with a relatively intact structure were investigated in calli of mutants of the chromomethylase gene *OsMET2a*, an ortholog of the Arabidopsis *CMT3*, induced by the insertion of *Tos17*. Correlated with a decrease of DNA methylation in CNG sites, 10 transposable elements were transcriptionally activated in the mutant. Among these activated elements, two novel transposable elements, a giant *copia*-type retrotransposon *Tos33* and a mutator-type DNA transposon *RiceMu02*, were transpositionally activated, indicating that the rice genome carries silenced quiescent elements. While *Tos17* did not show an accelerated transposition in tissue culture of the *osmet2a* mutant, its transposition was shown in normally propagated mutant plants. This suggests the existence of another layer of regulations.

IAEA-CN-167-041

RESTRICTION ENDONUCLEASES AS A TOOL FOR *IN VIVO* INDUCTION OF CHROMOSOMAL AND DNA DAMAGE IN BARLEY GENOME

L. STOILOV¹, K. GECHEFF²

¹Department of Molecular Genetics, Institute of Genetics "Acad. D. Kostoff", Bulgarian Academy of Sciences Sofia, Bulgaria

²Department of Cytogenetics, Institute of Genetics "Acad. D. Kostoff", Bulgarian Academy of Sciences, Sofia, Bulgaria

Email: molgen@bas.bg

Bacterial restriction endonucleases have been widely utilized to study the significance of DNA double-strand breaks for the formation of chromosomal aberrations based on their ability to produce this particular DNA lesion. Such studies in plants until mid-nineties were very scarce. The stability of maize nuclei towards *in vivo* action of EcoRI was investigated revealing dry embryo cells as less resistant than meristematic ones actively involved in transcription. Restriction endonucleases were also found to induce structural chromosomal damage in barley genome. They exerted S-independent mode of action revealing the transition between the G₁ and S phase the most sensitive stage for aberration induction. Intrachromosomal localization of chromatid aberrations produced by HpaII, MspI and HaeIII displayed similar distribution patterns. The most pronounced aberration hot spots were the Nucleolus Organizing Regions which pointed towards the potential of the restriction endonucleases for damage induction in specific genomic locations. Pattern of the localized chromosomal breakage produced by HaeIII in suitably reconstructed karyotypes showed substantial difference in the aberration hot spot behavior. Position-specific increase in aberration clustering was found indicating that expressivity of aberration hot-spots generated by restriction endonucleases is dependent on their chromosomal environment. Barley karyotypes with normal and increased expression of rRNA genes were further utilized to evaluate the possible relationship between their transcriptional activity and damage induction. Hybridization profiles obtained after treatment with MspI revealed similar induction kinetics. The potential of barley ribosomal genes to accumulate double-strand breaks with different structure was also tested by AluI and band intensity reduction followed the pattern found for MspI. Results indicated that the mode of action of restriction endonucleases applied was not substantially influenced by the activity of the nucleolus organizing regions. The data as a whole are supporting the options for the use of restriction endonucleases for directed induction of damage in plant genome.

IAEA-CN-167-124

ACHIEVEMENTS AND PERSPECTIVES OF CROP SPACE BREEDING IN CHINA

L.X. LIU, H.J. GUO, L.S. ZHAO, J.Y. GU, S.R. ZHAO
Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China
Email: luxiang@263.net.cn

Aerospace provides a special environment with strong cosmic radiation, microgravity, weak geomagnetic field, supervacuum and superclean, etc. A large amount of experimental data showed that space environment conditions might affect plant growth and development as well as induce genetic changes of crop seeds. The frequency of chromosomal aberrations was greatly increased in seeds carried into space and later germinated on the ground. The combined effects of both cosmic radiation and microgravity or other spaceflight factors might be the main causes of genetic changes in spaceflight seeds. Since 1987, China has been conducting experiments of space mutagenesis for crop improvement by using recoverable satellites, *Shenzhou* spacecrafts and high altitude balloons. *Shijian-8*, the first world satellite specially designed for the space breeding program, was launched on 9 September 2006. It carried over 2000 accessions of plant materials from 133 species. So far, 60 new mutant varieties of crops including rice, wheat, cotton, sesame, pepper, tomato and alfalfa developed by space breeding technology have been officially released in China. A serial of useful rare mutant germplasm were also obtained. A new technique and method of mutation breeding by simulating the space environment has been set up. It was concluded that crop space-induced mutation breeding can be as a novel effective way to both breed new varieties and enhance genetic diversity.

IAEA-CN-167-342

ESTABLISHMENT OF ION BEAM BREEDING TECHNOLOGY

A. TANAKA
Japan Atomic Energy Agency (JAEA), Takasaki, Japan
Email: tanaka.atsushi@jaea.go.jp

We have first begun to investigate the characteristics of ion beams for inducing mutation from at molecular level to phenotypic level. Mutation induction rates were investigated using visible known Arabidopsis mutant phenotypes such as *glabra(gl)* and *transparent testa(tt)*, indicating that mutation frequencies induced by carbon ions were 17-fold higher than those by electrons. Molecular analysis showed that half of mutants induced by ion beams possessed large DNA alterations, while the rest had point-like mutations. Both mutations induced by ion beams have common feature that deletion of several bases are predominantly induced. It is plausible that ion beams induce limited number of large and irreparable DNA damage, resulting in effectively producing null mutation that shows new mutant phenotype. On the other hands, novel mutants such as UV-B resistant, serrated petals and sepals, anthocyaninless, etc. have been induced by 220 MeV carbon ions in Arabidopsis. Those genes were also found to encode novel and key proteins for each mechanism. In chrysanthemum and carnation, several kinds of flower-color and flower-form mutants that have never produced by gamma rays or X rays were also induced by carbon ions. It is, therefore, indicated that the characteristics of ion beams for the mutation induction are high mutation frequency, broad mutation spectrum, and producing novel mutants. From these basic researches, recently a lot of practical studies on mutation breeding are being actively carried out and successfully producing useful new varieties.

MUTAGENIC MECHANISM ON ION IMPLANTATION OF PLANTS

H.Y. FENG, Z.L. YU

Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences and Anhui Province, Hefei, China

Email: zlyu@ipp.ac.cn

Ion beam implantation, as a new mutation technique, has been widely used in mutation breeding, and great achievements have been attained in agriculture and fermentation industry. The mechanism underlying ion beam induced mutagenic effects has been the topic of research in recent years. In this paper, we focus on the initial physical process of ion implantation into organisms, discussing that energy deposit, mass deposit and charge transfer of the implanted ions into target organisms are the main contributions to the bio-effects. Recent advances in the study of transferring of damaging signals in plant sample are also included. It has been observed that targeted ion implantation of shoot apical meristem (SAM) of *Arabidopsis* embryos induces damage of root apical meristem (RAM), indicating a long distant bystander effect in intact organism. Further studies showed that generation of reactive oxygen species upon ion implantation and auxin-dependent transcription processes could play important roles in the observed bystander effect.

SITE-DIRECTED MUTAGENESIS IN PLANTS VIA GENE TARGETING

S. TOKI, M. ENDO, H. SAIKA, K.ABE, K. OSAKABE

Plant Genetic Engineering Research Unit, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Ibaraki, Japan

Email:stoki@affrc.go.jp

Many agronomically valuable phenotypes and natural variations seem to be due to point (or only a few) mutations. Thus, site-directed mutagenesis via gene targeting (GT) should be the cleanest, and most direct gene manipulation technique for future molecular breeding in plants. We chose the acetolactate synthase (ALS) gene locus of rice as a target for the introduction of point mutations. ALS catalyzes the initial step common to the biosynthesis of the branched-chain amino acids. Several point mutations in the ALS gene that confer tolerance to several ALS-inhibiting herbicides have been discovered in several plant species. Using a T-DNA-mediated GT strategy, we were able to induce two point mutations in the ALS locus that confer tolerance to the ALS-inhibiting herbicide bispyribac sodium salt (BS). After detailed analysis of GT plants, we confirmed that precise modification of the ALS locus had occurred in several plants. In addition to herbicide tolerance, tolerance against other chemicals is also a potential selectable phenotype. In this context, we are attempting to use GT to introduce point mutations into the rice gene encoding anthranilate synthase alpha subunit 2 (ASA2) -- a key enzyme in tryptophan (Trp) biosynthesis -- to produce Trp-accumulating rice. In this study, gene-modified plants can be selected against the Trp analogue 5-methyl-Trp (5MT). We hope to report the phenotype of ASA2-modified plants. On the other hand, many agronomically valuable phenotypes caused by a small number of point mutations are non-selectable at the stage of transformation using current methods. If the frequency of GT can be improved substantially, co-transformation of a selectable marker gene and a non-selectable GT construct, and subsequent identification of desirable targeting events will cope with this problem. We are currently trying to improve GT efficiency in plant.

IAEA-CN-167-162

ZINC FINGER NUCLEASE-MEDIATED GENE TARGETING IN PLANTS

C.Q. CAI¹, M. AINLEY¹, J. MILLER², P. GREGORY², R. GARRISON¹, L. SCHULENBERG¹, R. BLUE¹, A. WORDEN¹, L. BAKER¹, B. RUBIN-WILSON¹, J.F. PETOLINO¹

¹Dow AgroSciences, LLC, Indianapolis, IN, USA

²Sangamo BioSciences, Richmond, CA, USA

Email: ccai2@dow.com

Zinc finger nucleases were used to facilitate homology driven repair and site-specific transgene integration in transgenic tobacco cell cultures. A target DNA sequence containing a non-functional, partial 3' PAT gene sequence flanked by zinc finger binding sites was stably integrated into BY2 suspension cultures using *Agrobacterium*-mediated transformation. A transgenic event containing a single integrated copy of the target sequence was used for gene targeting through co-transformation with two different *Agrobacterium* strains containing: i) donor DNA sequences comprising the 5' partial DNA fragment necessary to correct the non-functional PAT gene flanked by sequences homologous to the pre-integrated target DNA and ii) DNA that encoded a zinc finger nuclease that specifically recognized binding sites within the pre-integrated target. Two gene targeting strategies differing with respect to the distance between the zinc finger binding site and the homologous sequences were used. Gene targeting was demonstrated for both strategies as evidenced by the reconstitution of a functional PAT gene and was confirmed via molecular and biochemical analyses. Sequencing of recombined DNA confirmed that PAT gene reconstitution resulted from homology-driven repair at the zinc finger nuclease cleavage site. However, imperfect recombination resulting from non-homologous processes was also observed.

IAEA-CN-167-294

TOWARD ZINC FINGER NUCLEASE-MEDIATED SITE-SPECIFIC MUTAGENESIS IN PLANT SPECIES

A. TOVKACH, V. ZEEVI, T. TZFIRA

Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, USA

Email: ttzfira@umich.edu

Double-strand breaks (DSBs) in plant genomes are typically repaired by the plant nonhomologous end-joining machinery, which usually leads to local deletions and mutagenesis at the repair site. Interestingly, artificial induction of DSBs by various restriction enzymes results not only deletions, but also in insertions of foreign DNA molecules into the repair site. This phenomenon could potentially be used for mutating specific sites in the plant genome and targeting foreign DNA molecules into them with zinc finger nucleases (ZFNs). ZFNs are a new type of artificial restriction enzymes that are custom-designed to recognize and cleave specific DNA sequences, producing DSBs. However, technical difficulties in the design, assembly, and analysis of ZFNs have hindered the use of ZFNs for plant gene targeting. We have recently designed a set of constructs and cloning, biochemical, and *in planta* analysis procedures for newly designed ZFNs. Cloning begins with *de novo* assembly of the DNA-binding regions of new ZFNs from overlapping oligos containing modified helices responsible for DNA triplet recognition, and their insertion between a nuclear localization signal and the *FokI* endonuclease domain. Following the transfer of fully assembled ZFNs into *Escherichia coli* expression vectors, bacterial lysates were found to be most suitable for *in vitro* digestion analysis of palindromic target sequences. An *in planta* activity test was also developed to confirm the nucleic activity of ZFNs in plant cells. The assay is based on the reconstruction of GUS expression following bombardment of a reporter and ZFN-expressing plasmids into mesophyll cells, as well as integration of the tested reporter gene in transgenic calli. Our new procedures, plasmids, and assays bring us one step closer to efficient implementation of ZFN based technology for gene targeting in plant species.

LOCUS-SPECIFIC MUTATIONS INDUCED BY HOMING ENDONUCLEASES IN MAIZE

H. GAO, M. YANG, S. C. FALCO, A. LYZNIK

Crop Genetics Research and Development, DuPont/Pioneer Agriculture and Nutrition, Johnston, IA, USA

Email: alex.lyznik@pioneer.com

The double-strand break DNA repair mechanisms are at the core of molecular strategies designed for introducing mutations or integrating foreign DNA at genomic sites. They may differ in different organisms, so a comprehensive evaluation of the double-strand break repair products in particular crop species would be essential for implementing the gene targeting technology for agricultural applications. We designed unbiased (no selection for the DNA repair products) assays that utilize the homing endonuclease I-SceI for introducing DNA double-strand breaks in maize. We found that double-strand breaks induced by I-SceI are predominantly repaired by non-homologous end joining in maize somatic cells. We found an unexpected preference to produce mutations at one side of otherwise symmetrical double-strand breaks in maize. We also found foreign DNA integrated, either by illegitimate or homologous recombination, at about one per one hundred double-strand break sites. Double-strand breaks can be introduced either by genetic transformation with a homing endonuclease expression vector or by genetic crosses to the parental plants that already contain integrated expression vectors. The latter scenario requires activation and subsequent elimination of the homing endonuclease expression cassettes, but it is particularly suited for directed mutagenesis of maize plants.

GENETICALLY UNSTABLE MUTANTS AS NOVEL SOURCES OF GENETIC VARIABILITY: THE CHLOROPLAST MUTATOR GENOTYPE IN BARLEY AS A TOOL FOR EXPLORING THE PLASTID GENOME

A.R. PRINA, A.M. LANDAU, N. COLOMBO, M. JAUREGUALZO, M.C. ARIAS, R.D. RIOS, M.G. PACHECO

Instituto de Genética Ewald A. Favret, CNIA-INTA, Cautelar, Buenos Aires, Argentina

Email: aprina@cnia.inta.gov.ar

The presence of clonally variegated seedlings was used as a criterion to isolate putative genetically unstable mutants (GUMs) from M_2 or further generations coming from X-rays and/or chemical treatments applied on barley seeds. Seedlings analysis in the greenhouse revealed that in some of those isolated families a particular spectrum of mutant phenotypes was repeatedly observed during several generations of auto pollination. By reciprocal crosses it was noticed that some of those GUMs produced maternally-inherited changes and, according to the width of the spectrum induced by them, they were classified in two groups, inducing either a narrow or a wide spectrum of mutant phenotypes. One case of the latter, designated as "chloroplast mutator genotype, has been studied in our Institute since 1985. In several mutants obtained from this GUM, evidences of major plastid-DNA changes were not detected but, interestingly, sequencing of some plastid genes showed that single nucleotide mutations were induced. Three different transitions on the plastid gene *infA* were detected in three independently originated *albo-viridis* mutants. One transition and one base insertion on the *ycf3* locus were observed in a temperature-sensitive *viridis* type. Besides, one transition on the plastid gene *psbA* was observed in families selected for atrazine tolerance. Both, the wide spectrum of mutants and the subtle DNA changes induced by this barley chloroplast mutator genotype suggest that it can be an exceptionally valuable tool to explore the potential functionality of the otherwise highly conserved plastid genome.

INDUCTION OF DNA DAMAGE BY LI-IONS AND GAMMA-RAYS IN BARLEY GENOME

M. GEORGIEVA¹, V. MANOVA¹, L. LIU², L. STOILOV¹

¹Department of Molecular Genetics, Institute of Genetics "Acad. D. Kostoff", Bulgarian Academy of Sciences Sofia, Bulgaria

²Department of Plant Mutation Breeding and Genetics, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China

Email: molgen@bas.bg

Space radiation and heavy ions are capable to produce mutations, chromosome aberrations or inhibit seed growth but the nature of the initial DNA damage remains obscure. Comparative study on the induction of DNA breakage in barley genome after exposure to Li-ion beam irradiation and gamma-rays was performed. Alkaline comet assay was utilized to evaluate the induction kinetics of DNA strand breaks. 55 and 110 Gy of 42.3 MeV Li-ion beams were applied to dry seeds from reconstructed barley karyotypes T-1586 and D-29/46. Cs-137 source was used to deliver the same doses of gamma-rays. Damage estimation was done after short seed germination. Comparative tests for measurement of the overall survival rates were also performed. DNA damage observed after application of 55 Gy indicated for somewhat higher levels of DNA (single) strand breaks induced by Li-ions suggesting for their ability to induce DNA strand scissions. After application of the 110 Gy, however, the respective values were much higher for gamma rays due probably to the accumulation of DNA double-strand breaks. The response of the two barley karyotypes towards irradiation was different, namely mutant line D-29/46 displayed somewhat elevated sensitivity to Li-ions and gamma-rays, more pronounced after application of 110 Gy gamma-rays. Differential sensitivity of the lines corresponded with their survival rates. Lesion-specific enzymes Fpg and T4 Endo V were used for estimation of the type and rate of base DNA damage induced simultaneously with the direct strand breaks after application of the same doses of gamma rays. Data showed higher rate of Fpg sensitive sites, in comparison to the frank strand breaks after irradiation of barley DNA *in vitro*. T4 Endo V sensitive sites were also detected, although at a much lower rate, than the other types of DNA damage.

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MOLECULAR ANALYSIS OF THE IONIZING RADIATION INDUCED GENETIC VARIABILITY IN BARLEY

A. DIMITROVA¹, E. TODOROVSKA², N. CHRISTOV², K. GECHEFF¹, L. STOILOV¹, A. ATANASSOV²

¹Institute of Genetics, BAS, Sofia, Bulgaria

²AgroBioInstitute, Sofia, Bulgaria

Email: anna_d_dimitrova@yahoo.com

Effective use of experimentally induced mutants in breeding programs is highly dependent on the type of the induced mutations. The main goal of the present study is to reveal the molecular nature of ionizing radiation-induced mutational alterations in a stock of originally produced barley structural mutants. Biochemical and molecular tools were applied for obtaining more detailed information about the nature of gamma-rays induced genetic variation in the structural mutant forms (T-16, T-20, T-26, T-48, T-58, T-59, T-63, T-66, T-67, T-68, T-169 and RK 88-4) and their initial parental lines (cv. Freya and T1586). Among the marker systems used (PAGE, RFLP, CAPs, RAPD, REMAP, S-SAP, SSR and AFLP) SSRs and AFLPs have proven to be the most promising markers for evaluation of the ionizing radiation-induced mutational alterations in structural barley mutant forms. AFLP polymorphisms were observed in 2 cases using PstI/MseI and in one case using EcoRI/MseI, out of 20 selective AFLP primer combinations tested. Microsatellite analysis using 26 markers selected from the published barley genetic maps showed new allele variants in mutant lines T-20 and T-68 at the HVM 04 - 7HS locus and in mutant line RK 88-4 at the HVM 03 - 4HL locus. The observed polymorphisms in the above mentioned mutant lines are most probably caused by point mutations either in the respective restriction sites or in the microsatellite repeat or in the vicinity of the adjacent DNA sequences.

IAEA-CN-167-088P

MUTAGENESIS OF GENES FOR STARCH DEBRANCHING ENZYME ISOFORMS IN PEA BY MEANS OF ZINC-FINGER ENDONUCLEASES

H. HUSSAIN

Dept. of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia
Email: hhasnain@frst.unimas.my

Starch debranching enzymes in plants are divided into two groups based on their ability to hydrolyse different substrates. The first group, pullulanases, hydrolyses α -1,6-glucosidic linkages in substrates such as pullulan, amylopectin and glycogen. The second group of debranching enzymes, isoamylases, hydrolyse glycogen and amylopectin and are not active on pullulan. Three isoforms of isoamylase and a pullulanase have been isolated from cDNA library of *Pisum sativum*. These isoamylases have been characterised based on their heterologous expression in *E coli*. Based on the DNA sequence that encodes these debranching enzyme, a specific mutagenesis targeting at these DNA will be attempted. The method that will be employed are based on the techniques developed by Wright *et al.* (2005). This technique involves the homologous recombination of DNA that is mediated by zinc-finger endonucleases. Vectors will be constructed to include a fragment that will modify these genes. Microinjection technique will be used to insert these vectors into pollen which then will be fertilized. Using this technique, it is hoped that null mutant for each enzyme will be created and the exact role of these enzymes for the synthesis and degradation of starch in plants will be elucidat

IAEA-CN-167-104P

MUTATION STUDY ON PLANTS IRRADIATED WITH LOW-ENERGY ION BEAMS

Y.J. WU, P. BIAN, B.M. LIU, Z.L. YU

Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences, Hefei, China

Email: yjwu@ipp.ac.cn

In the mid 1980s, the biological effects of ion implantation were recognized and demonstrated experimentally. In the past two decades, great outcomes were obtained in mechanism and technique of ion-beam irradiation, creating mutants and mutation breeding. The radiobiological effects of ion-beam on rice show that: ion-beam irradiated rice seeds have low damage but high survival rate, high mutation frequency and wide mutation spectrum. Ion beam is an efficient, safe and contamination-free mutagen. On the other hand, new biological effects were found in rice irradiated with ion-beam. Such as, some traits were found to be inheritable and transmitted to latter generations in the first generation irradiated (M₀), e. g. premature mutant with reduction the life period S9042, similar to the phenomenon found induced with laser. Also, there was report suggested that the relation between irradiation dose and the survival of seedling had a saddle-shape curve (dose-abnormal curve). As a new mutation-inducing technique, ion-beam implantation created great success in plant genetic improvement and new mutant creation. According to partially summary, at least 25 new varieties including rice, wheat, maize, soybean and tomato were produced and identified with ion-beam irradiation. Among those new inheritable materials created by ion-beam irradiation, rice dominant semidwarf mutant (Sdd) and maize opposite mutant were precious monocotyledon mutants, which have great theoretical and applied value in genetics, development, plant physiology and rice and maize breeding. The near-isogenic lines induced with ion-beam implantation are very useful in the study about the rice longevity gene (Lox-1). In additional to that, the improvement on physical parameter and miniaturization of ion-beam implantation equipment also build a trustable platform for extension of ion-beam implantation technique.

IAEA-CN-167-146P

INDUCTION AND EVALUATION OF DEB-INDUCED MUTANTS IN BARLEY (*HORDEUM VULGARE* L.)

S. SINGH, G.S. DHINDSA, A. SHARMA

Department of Plant Breeding, Genetics & Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

Email: sarvjeetm@rediffmail.com

Seeds of two malt barley commercial cultivars, VJM201 and DWR28, were treated with 3 concentrations ($T_1=0.10$ mM, $T_2=0.15$ mM and $T_3=0.20$ mM) of a chemical mutagen, 1, 3-butadiene dioxide (DEB) to induce mutations for early heading, short stature, disease resistance and other desirable traits. The germination of both the cultivars was reduced with higher concentration of mutagen. Each M_1 harvested plant was sown in paired rows of 3 m length keeping row-to-row and plant-to-plant spacing of 23 and 10 cm, respectively. The respective parent cultivars were planted after each 20 M_2 progenies for comparison. The number of chlorophyll mutants and morphological mutants was more in DWR28 compared to VJM201. The overall mutation frequency, calculated 1000^{-1} M_2 plants, was higher (4.23) in DWR28 compared to VJM201 (3.09). The segregating progenies were also higher in DWR28 (10.40%) compared to VJM201 (6.36%). According to treatments, the mutation frequency was highest in T_3 followed by T_2 and T_1 in DWR28, whereas it was highest in T_2 followed by T_1 and T_3 in VJM201. Morphological mutants, viz., six-rowed, spreading growth habit, plant height, days to heading, presence or absence waxiness on spike/stem, stem thickness, leaf colour and size, pigmentation on various plant parts, shape of spike, spike density, size of secondary florets, shape and length of peduncle, awn length and tip sterility were recovered in M_2/M_3 generations. Some mutants were recovered with higher degree of resistance to yellow rust, brown rust and leaf blight, whereas some others showed higher degree of susceptibility as compared to the parent cultivars. Besides the morphological mutants, some mutants were recovered with an increase or decrease for protein content and quantitative traits like tiller number, spike length, grains per spike, 1000-grain weight and grain yield. A dwarf mutant, DM2-1 of DWR28, showed multiple mutations as it had six-rowed spikes, initial spreading growth, tip sterility, late heading, high protein content, lower 1000-grain weight, lower grain yield and resistance to yellow and brown rusts. Some other plants having multiple mutant traits were also observed in both parent cultivars.

IAEA-CN-167-228P

GENERATION OF NEW RICE CULTIVARS FROM MATURE POLLENS TREATED WITH GAMMA RADIATION

H.C. WANG, S.M. QIU, J.S. ZHENG, L.R. JIANG, H.Z. HUANG, Y.M. HUANG

School of Life Sciences, Xiamen University, Xiamen, Fujian, China

Email: hym@xmu.edu.cn

Two new high quality and yield early *indica* rice cultivars, named Jiahezaozhan and Jiafuzhan, have been developed, certified, and cultivated by farmers in the provinces of Southern China. These new rice varieties were created by a new mutation breeding technique in which mature rice pollens irradiated with gamma (γ) ray were used to produce crossover progenies. The optimal dose for the irradiation was approximately 46Gy. The accumulative effects of the mutations increased with the initial passages and most of the mutant traits became stable in the fifth generation. These results showed that the mutations generated by γ radiation on rice mature pollen were largely of quantitative trait locus mutagenesis.

APPROACH FOR METABOLIC ENGINEERING OF AMINO ACID PRODUCTION BY T-DNA MEDIATED GENE TARGETING

H. SAIKA, S. TOKI

Plant Genetic Engineering Research Unit, National Institute of Agrobiological Sciences (NIAS), Ibaraki, Japan
Email: saika@affrc.go.jp

Anthranilate synthase (AS) - a key enzyme in tryptophan (Trp) biosynthesis - converts chorismate into anthranilate. AS is a tetramer composed of two alpha subunits and two beta subunits, and alpha subunit is inhibited by high concentrations of Trp or its analogue 5-methyl-Trp (5MT). In rice, the genes *OASA1* and *OASA2* encode the alpha subunits of AS, while *OASB1* and *OASB2* encode the beta subunits. Previous reports suggested that some mutations in the *OASA1* or *OASA2* genes lead to insensitivity to feedback inhibition by Trp or 5MT, and thus to hyper-accumulation of Trp. For example, the S126F/L530D mutation in the *OASA2* enzyme confers insensitivity to high concentrations of Trp and higher accumulation of free Trp in yeast. In this study, we attempted to introduce the S126F/L530D mutation into the endogenous rice *OASA2* gene by T-DNA mediated gene targeting (GT) using homologous recombination. The GT vector for the *OASA2* gene contains 7.0 kb of *OASA2* genomic sequence incorporating the S126F/L530D mutations but lacks the 5'-region sequence encoding the chloroplast-targeting signal. Therefore, only targeted integration of this vector will confer 5MT tolerance. Rice calli (cv. Nipponbare) were infected with *Agrobacterium* harboring the GT vector and selected against 5MT. A total of 25 plants were recovered from 850 calli. Cleaved amplified polymorphic sequence (CAPS) and sequence analysis showed that successful GT of the *OASA2* gene had occurred in only 1 of the 25 regenerated plants. We are currently analysing this plant further.

CREATION OF THE HERBICIDE TOLERANT RICE PLANTS VIA T-DNA MEDIATED GENE TARGETING

M. ENDO, S. TOKI

Plant Genetic Engineering Research Unit, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Ibaraki, Japan
Email: mendo@affrc.go.jp

Precise modification of the plant genome is important both for the study of gene function *in vivo* and for producing publicly acceptable transgenic plants. Thus establishment of an efficient gene targeting (GT) system in plant is a significant goal. Here, we report a successful introduction of point mutations into an endogenous rice gene by T-DNA mediated GT. ALS is the primary target for at least four structurally distinct classes of herbicides. Recently, Shimizu et al. screened an ALS-inhibiting herbicide, bispyribac-sodium (BS) tolerant rice cells. BS tolerance was linked to two point mutations in *ALS* gene: a tryptophan (TGG) to leucine (TTG) change at amino acid 548 (W548L), and serine (AGT) to isoleucine (ATT) change at amino acid 627 (S627I). However, no plants could be recovered from the BS-tolerant rice cells due to prolonged tissue culture. Then we tried to produce BS tolerant rice plants containing these double mutations in *ALS* by T-DNA mediated GT. We obtained 70 GT plants from 1500 rice scutellum-derived calli infected with *Agrobacterium* harboring GT vector. GT rice homozygous for the modified *ALS* locus showed hyper tolerance to BS as compared to BS tolerant plants, which overexpressed W548L/S627I mutating *ALS* produced by a conventional transgenic system. This result indicates that exclusion of the BS sensitive wild-type *ALS* allele is important to confer high levels of BS tolerance. Not only selectable two point mutations, which confer BS tolerance but also non-selectable silent mutations on the targeting vector were incorporated into the GT plants. This result indicates that T-DNA mediated GT system is available for introduction of several point mutations to the target gene. Furthermore, point mutations on the targeting vector were incorporated into the genome with a mosaic manner in 3 plants out of 70 GT plants, suggesting the involvement of DNA mismatch repair system in the course of T-DNA mediated GT in these plants.

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GENE AND PROTEIN TARGETING TECHNOLOGIES CREATE NOVEL OPPORTUNITIES IN PLANT BIOTECHNOLOGY

M. COMPIER¹, P. MAK¹, S. DE PATER², B. VAN DER ZAAL², P. HOOYKAAS², R. OFFRINGA¹

¹Add2X Biosciences BV, Clusius Laboratorium, Leiden, The Netherlands

²Molecular and Developmental Genetics Dept., Institute of Biology, Leiden University, Clusius Laboratorium Leiden, The Netherlands

Email: compier@add2x.com

An important platform technology that is missing in the field of plant science is gene targeting through homologous recombination. Moreover, for many economically important plant varieties, robust and efficient protocols for vegetative propagation and genetic modification are still lacking. The availability of such technologies is essential for the further development of plant biotechnology. Add2X Biosciences BV is a young biotech spin-off from Leiden University that aims to create novel opportunities in plant biotechnology, by developing innovative platform technologies and products for the directed and efficient delivery of DNA and proteins into plant cells. The Add2X portfolio includes: **1. Efficient gene targeting in plants** through suppression of the non-homologous recombination pathway. Proof of concept has been obtained in different yeasts and filamentous fungi. Currently, research is in progress to confirm the applicability of this technology in plant species. **2. Agrobacterium-mediated protein translocation** to produce and transiently 'inject' proteins of interest into plant cells in order to exert their function without permanently altering the host cells. **3. Induction of somatic embryogenesis in hitherto recalcitrant crop species.** A protein family was identified that, when over-expressed, stimulates the spontaneous formation of somatic embryos from vegetative plant cells. The Add2X technologies stand well on their own, but clearly have the potential to be combined into integrated technologies or products. The technologies are developed in close collaboration with Leiden University. In addition, Add2X has established strategic alliances with other academic and industrial partners to explore novel opportunities and to achieve rapid implementation of the technologies in the biotechnology sector.

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SEQUENCE VARIATIONS OF *IN VITRO* PUC18 PLASMID DNA INDUCED BY HIGH ENERGY ⁷LI ION BEAMS IMPLANTATION

H.J. GUO¹, Q.G. XIN¹, L.S. LIU¹, L.S. ZHAO¹, K. ZHAO², L. SUI², S.R. ZHAO¹

¹Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China

²Department of Nuclear Physics, China Institute of Atomic Energy, Beijing, China

Email: luxiang@263.net.cn

High energy heavy ion beams is a new mutagen for crop mutation breeding, but limited data are available on the molecular level induced by this mutagen. The *in vitro* pUC18 plasmid DNA was implanted by ⁷Li ion beams by doses of 0, 20, 40, 60, 80 and 100Gy, respectively, with the energy of 42.3MeV. The results showed that the damage effects induced by ⁷Li ion beams implantation was different from low LET rays, even low doses of ⁷Li ion beam could induce high damage on hydrogen bonds. Percentage of damages on hydrogen bonds of *in vitro* DNA induced by ⁷Li ion beams implantation increased with dosage increase up to 40Gy, then reduced with dosage increase, and higher than those of gamma rays in the same dosage. The relationship of dosage and damage percentage was different from that of gamma rays which was positive-linear correlation. Mutation frequency of ⁷Li ion beam implantation was 1.6 to 4.3 times to that of spontaneous mutation. The relationship of mutation frequency and dosage was similar with that of damage effects on hydrogen bonds, and showed a peak at 40Gy. The above results were identical with biological effects of wheat implanted by ⁷Li ion beams. Ten mutants were used for sequence analysis, which indicated that the types of base changes included base transversion, transition and deletion. Among all base changes detected, the frequency of bases transition (60%) was higher than that of bases transversion (30%) and bases deletion (10%). It seemed that thymine was more sensitive to the implantation than any other bases and base changes were mainly T→C and C→T. Bases between T and C were seemed to be easily induced by ⁷Li ion beams. The high percentage of DNA sequence variations could explain primarily the biological effects caused by ⁷Li ion beams in the M₁ generation of crops.

CONCURRENT SESSION 7 (08:30-13:00)
High Throughput Techniques for Mutation Screening
IAEA Boardroom C04

Thursday, 14 August 2008

GLOBAL TILLING PROJECTS

B.J. TILL, R. AFZA, S. BADO, J. JANKOWICZ, M. MATIJEVIC, C. MBA

Plant Breeding Unit, Joint FAO/IAEA Agriculture and Biotechnology Laboratory, International Atomic Energy Agency Laboratories, Seibersdorf, Austria

Email: b.till@iaea.org

Induced mutation and natural nucleotide variation are powerful tools for probing gene function and improving traits in plants. Traditional mutagenesis has been widely used in forward genetic strategies and has led to the release of over 2000 mutant plant varieties. Mutagens such as ethyl methanesulphonate (EMS) cause stable point mutations and thus produce an allelic series of truncation and missense changes that can provide a range of phenotypes. TILLING (Targeting Induced Local Lesions IN Genomes) uses traditional mutagenesis and nucleotide polymorphism discovery methods for a reverse genetic strategy that is high in throughput, low in cost, and applicable to most organisms. In less than a decade, TILLING has moved from a proof of concept to a well accepted reverse genetic method that has been applied to over 20 different species. Large-scale TILLING services have delivered thousands of induced mutations to the international research community. Advancements in new mutation discovery techniques promise to further increase the efficiency and applicability of the TILLING method. In this presentation I will review progress in TILLING and will describe the work of the Plant Breeding Unit of the Joint FAO/IAEA Program to establish TILLING platforms for banana, cassava, and rice.

IAEA-CN-167-350

TILLING: A NEW TOOL IN THE PLANT BREEDERS TOOLKIT

D. FACCIOTTI, A. SLADE, S. HURST, T. COLBERT, M. MOEH ARCADIA

Arcadia Biosciences Inc., Davis, CA, USA

Email: daniel.facciotti@arcadiabio.com

TILLING (Targeted Induced Mutations IN Genomes) is a powerful reverse genetic tool to identify novel genetic variation in plants and animals at the level of the single nucleotide. This technique has been successfully applied to many different plants with wide-ranging genome sizes including Arabidopsis, rice, soybean, tetraploid and hexaploid wheat. In plant breeding, genetic variation provides the source for differences upon which selection can be made for desirable phenotypes. TILLING can enhance this process by providing a means to identify genetic variation in targeted genes of interest for plant breeding and crop improvement. Mutagenesis can rapidly accelerate development of desirable traits where existing variation is either unknown or non-existent in the preferred varieties for breeding. TILLING can be used to identify single nucleotide polymorphisms (SNPs) that have been induced by mutagenesis. Various mutagens are suitable for use in plants, including the commonly used ethylmethane sulfonate (EMS). Each crop and variety has its unique response to different mutagens that require optimization. Naturally occurring genetic diversity can also be assessed using TILLING in targeted genes of interest. In this method, called EcoTILLING, diversity is identified relative to a reference variety. We will present examples of the optimization of mutagenesis and the application of TILLING for plant breeding in multiple crops, as well as the application of EcoTILLING in tomatoes and castor bean

IAEA-CN-167-376

TILLING WITH TILLMOREV. TALAMÈ¹, R. BOVINA¹, S. SALVI¹, M.S. SANGUINETI¹, P. PIFFANELLI², U. LUNDQUIST³, R. TUBEROSA¹¹Department of Agroenvironmental Sciences and Technology, Bologna, Italy²Genomics Platform, CERSA, Lodi, Italy³Nordic Genetic Resource Center, Alnarp, Sweden

Email: valentine.talame2@unibo.it

TILLING (Targeting Induced Local Lesion IN Genomes) is a reverse-genetic approach that combines a standard strategy of chemical mutagenesis with a sensitive DNA screening-technique to identify single base mutations in a target gene (McCallum et al. 2000). At DiSTA of the University of Bologna, a TILLING resource in barley (cv. Morex), consisting of 4,906 families, has been produced by sodium azide (NaN₃) seed treatment (Nilan et al. 1973) and named TILLMore. TILLMore has been screened for several genes based on the analysis of 8- to 12-fold DNA pools produced from M₂ or M₃ DNA samples, using LiCor and ABI-3730 sequencer. Until now, it was possible to identify an average of ca. seven alleles per gene and an extrapolated rate of one mutation every ca.400 kb; almost all the mutations detected were CG-TA transitions and several (ca. 70%) implied a change in amino acid sequence and therefore possible effects on phenotype. Although the main purpose of our barley-mutagenized population was the implementation of a TILLING platform for reverse-genetics purposes, the same population is also a valuable resource for forward-genetics studies. A high frequency of M₃ families (ca. 33%) showed morphological alterations, which have been scored regularly during the growing seasons in reference to untreated Morex plants. The TILLMore resource is currently available on a cost-recovery basis and/or through collaborations (for details, see www.distagenomics.unibo.it/TILLMore/).

IAEA-CN-167-229

TILLING IN TWO-ROWED SPRING BARLEY: MUTATION FREQUENCIES AND PHENOTYPES

S. GOTTWALD, E. KUGELMANN, J. KUMLEHN, N. STEIN

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Email: gottwald@ipk-gatersleben.de

TILLING (Targeting Induced Local Lesions IN Genomes) searches the genomes of mutagenized organisms for mutations in a chosen gene, and allows functional analysis in the context of the whole organism. Moreover, TILLING extends reverse genetics to mutation breeding, since it can generate large allelic series of economically interesting genes. As part of the GABI-TILL consortium (GABI II) we have established a TILLING platform for the two-rowed, malting barley cultivar "Barke". The population comprises 10,492 M₂ plants, and the respective DNA was arranged in 8-fold two dimensional (2D)-pools for mutation screening. Mutations were detected by following the original TILLING procedure of Cel I based hetero-duplex analysis. By screening ten gene fragments for mutations in 7,348 M₂ lines 81 mutations were identified, yielding an average mutation frequency of approximately 1 mutation per 0.5 Mb. Seventy-nine percent of these mutations were located in coding regions of the analysed gene fragments of which 45% represented missense alleles inducing a change of amino acid in the protein. Five percent of the mutations provided truncation mutations either by elimination of a splice junction or by insertion of a premature stop codon. For instance for the *vrs1* gene (Komatsuda et al. 2007), which is the major factor that controls row-type morphology of the barley spike, thirty-one mutations were identified by screening a 1,270bp fragment. Three of the identified mutants either exhibited a 6-rowed or an intermediate phenotype. One mutant displayed a spikelet morphology that was significantly altered compared to 'Barke' wild-type. The barley TILLING activity is going to be extended into the GABI-Future program. Additionally, tests to improve mutation frequency in barley will be undertaken. Furthermore, attempts for generating an instantly homozygous TILLING-Population by Barley microspore culture mutagenesis are underway in co-operation with the group of Jochen Kumlehn (Co-PI GABI-FUTURE-TILL-Barley), IPK.

APPLICATION OF TILLING TO GAMMA-RAY-IRRADIATED RICE AND USE OF SILENT MUTATIONS FOR TRACING FARM PRODUCTS

T. NISHIO¹, Y. TAKAHASHI¹, M. NISHIMURA²

¹Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

²Institute of Radiation Breeding, NIAS, Hitachi-omiya, Japan

Email: nishio@bios.tohoku.ac.jp

Mutant selection by the reverse genetic approach known as TILLING is a useful tool for gene function analysis and crop improvement. We have reported successful selection of mutants from the progeny of gamma-ray-irradiated rice plants by a modified TILLING technique. Although mutation frequency by gamma-rays is generally lower than that by chemical mutagens, gamma-rays can induce short deletion causing frameshift. Frequency of knockout mutants among the mutants selected by the SNP analysis was higher in gamma-ray irradiation than in chemical mutagen treatments. Even in gamma-ray-irradiation, most of mutations were base substitutions such as transition or transversion. Mutations in introns were also frequent. Therefore, a large proportion of mutants selected by the reverse genetic approach are mutants of silent mutations. Silent mutants are useless in gene function analysis and plant breeding, but we propose a possible use of the silent mutations as a marker for tracing farm products. Difference of cultivars, e.g., that between a high-quality cultivar and a high-yielding cultivar, can be revealed by analysis of DNA markers, but products of the same cultivar produced in different areas cannot be identified by these methods. A silent mutant line of a cultivar, which can be distinguished from the original line of the cultivar by SNP analysis, can be used as a specific line for one area. By using the dot-blot-SNP technique, grains of a Koshihikari line having a silent mutation were distinguished cost-effectively from the grains of the original Koshihikari cultivar in large-scale analysis.

ddSNP: A RAPID METHOD FOR MUTATION DETECTION IN POLYPLOID GENOMES

T. TABONE, M.J. HAYDEN

Molecular Plant Breeding Cooperative Research Centre and the University of Adelaide, Glen Osmond, SA, Australia

Email: tania.tabone@ludwig.edu.au

A method for detecting point mutations as well as insertions and deletions (indels) has been developed for the detection of mutations in polyploid genomes and multi-gene families, and is suited for the simultaneous assay of DNA fragments of variable length. The method, termed dideoxy-terminated single nucleotide polymorphism (ddSNP) detection, has been used to detect sequence variants in candidate genes underlying QTL to assist positional cloning and fine-mapping (forward genetics). In addition, the method has been particularly useful in reverse genetic applications to reveal the presence of chemically induced mutations in TILLING populations. The genetic analysis of TILLING populations ideally involves detecting mutations in coding regions. In polyploids, the intronic regions are poorly conserved across the genomes and contain large indels. The exonic (or genic) regions, under greater selection pressure for conservation, mostly contain homoeologous sequence variants (HSVs). Both indels and HSVs can mask the presence of a TILLING mutation using conventional mutation detection techniques, such as CEL1 cleavage which detects the presence of all heterozygous mutations. The ddSNP method is a low-cost mutation detection technique that addresses these limitations and enables the co-dominant detection of mutations in the presence of homoeologous and paralogous genes. The ddSNP method is based on the principles of manual dideoxy chain-termination sequencing in which an individual dideoxynucleotide is incorporated into the growing chain of deoxynucleotides, resulting in a termination fragment at that position. The ddSNP method saves time and money for high-throughput screening, especially in TILLING populations by avoiding the need to develop and validate primers for genome specificity to amplify a region that may or maybe not contain a mutation. The ddSNP method allows the position of the mutation and nucleotide substitution to be fully characterised in a single assay, with the potential to assign the mutation to a genome

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EMAIL - A HIGHLY SENSITIVE TOOL FOR SPECIFIC MUTATION DETECTION IN PLANT IMPROVEMENT PROGRAMS

L.S. LEE¹, M.J. CROSS^{2,3}, R.J. HENRY^{2,3}¹Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Australia²Centre for Plant Conservation Genetics, Southern Cross University, Lismore, Australia³Grain Foods CRC Ltd., North Ryde, Australia

Email: slade.lee@qut.edu.au

TILLING (Targeting Induced Local Lesions IN Genomes) is a useful tool for discovery of specific point mutations in genes of interest to plant breeders. It employs mismatch cleavage detection using endonucleases, particularly CELI and CELII. During PCR annealing, dsDNA heteroduplexes arise in pooled genomic DNA samples containing one or more Single Nucleotide Polymorphisms (SNP) resulting from, for instance, induced mutation. The cleaved fragments can be distinguished from the larger perfectly-matched homoduplex DNA of the unmutated wild types in the sample of pooled individuals. The ability to efficiently detect individuals with specific mutations within pooled samples provides plant breeders with a powerful screening tool to greatly reduce the numbers of plants requiring phenotypic assessment. Further, it enables geneticists to analyse gene function and associate genotype with phenotype. Such protocols suffer from limited ability to detect mismatch cleavage signal due to non-specific removal, by the nuclease, of 5' end-labelled termini used in the conventional approach. Mutation detection is further limited by high background characteristic of PCR-based end-labelling mismatch scanning techniques. We showed that as nuclease activity increased, internal signal was maintained while 5' signal decayed. Furthermore, internal labelling improved background. The loss of end-signal constitutes a fundamental problem with the conventional approach to mismatch scanning with CEL nucleases. A new mismatch scanning assay called 'Endonucleolytic Mutation Analysis by Internal Labelling' (EMAIL), was developed using capillary electrophoresis, involving internal amplicon labelling by PCR incorporation of fluorescently-labelled deoxynucleotides. Multiple mutations amongst allelic pools have been detected when EMAIL was applied with the mismatch nucleases CELI and CELII. This technique offers greatly increased sensitivity in specific-gene mutant detection in pooled samples, enabling enlarged pool sizes and improving throughput and efficiency. We are investigating the limits of pool sizes to deliver a highly efficient mutation detection and analysis strategy for plant breeders and geneticists.

IAEA-CN-167-358

A TILLING RESOURCE FOR JAPONICA RICE

T.H. TAI¹, P.M. COLOWIT¹, B.J. TILL^{2,4}, J. COOPER², E. GREENE², S. HENIKOFF², R. TRAN³, S. BERARDI³, H. TSAI³, A.A. KHAN³, L. COMAI³¹United States Department of Agriculture, Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA, USA²Fred Hutchison Cancer Research Center, Seattle, WA, USA³Section of Plant Biology and Genome Center, University of California, Davis, CA, USA⁴Current Address: Plant Breeding Unit, FAO/IAEA Agricultural and Biotechnology Laboratory, Seibersdorf, Austria

Email: Thomas.Tai@ars.usda.gov

A TILLING (Targeting Induced Local Lesions In Genomes) resource for rice functional genomics has been developed using the japonica rice cultivar Nipponbare. Two chemical mutagenesis protocols (ethyl methane sulfonate [EMS] and sodium azide plus methyl nitrosourea [Az-MNU]), were employed to generate the mutant populations that constitute this resource. Lines from the EMS and the Az-MNU populations were analyzed to identify induced mutations using the traditional method for mutation discovery which relies on the detection of mismatched heteroduplexes through endonucleolytic cleavage and sizing of resulting fragments by gel electrophoresis (i.e. CEL I nuclease technology). The density of induced mutations in the populations was found to be approximately 1 mutation every 350 kb. This mutation rate was comparable to other plant species for which public TILLING services have been established. We recently completed scale-up of this Nipponbare TILLING resource and experiments are underway to examine the feasibility of employing ultra-high throughput sequencing (Illumina GA sequencing platform) for mutation discovery. A description of this TILLING resource and the results of mutation discovery for various rice genes of interest using CEL I and ultra-high throughput sequencing technologies will be presented.

GENOME-WIDE TILLING: FROM GENE-BASED TO ORGANISM-BASED SCREENS

E. CUPPEN

Hubrecht Institute for Developmental Biology and Stem Cell Research, Royal Academy of Arts and Sciences & University Medical Centre Utrecht, Utrecht, The Netherlands

Email: e.cuppen@niob.knaw.nl

Targeting Induced Local Lesions IN Genomes (TILLING) or target-selected mutagenesis is a versatile and universal method for generating defined genetic mutants and knockouts. The approach is based on chemical mutagenesis to introduce random mutations in genomes, followed by large-scale targeted identification of induced mutations. This method has been shown to work very effectively in a wide variety of plants and animals. For *C.elegans*, we established a clonal library of 6,144 EMS-mutagenized worms. High-throughput screening by dideoxy resequencing resulted in the identification of 1,044 induced mutations in 109 Mbp, which translates into an average spacing between exonic mutations in the library of only 17 bp. We covered 25% of the open reading frames of 32 genes and identified one or more inactivating mutations (nonsense or splice site) in 84% of them. Extrapolation of our results indicates that nonsense mutations for over 90% of all *C. elegans* genes are present in the library. Currently, the effectiveness of the target-selected mutagenesis method is limited by the screening capacity and as a consequence, libraries consisting of thousands of mutant samples are screened on a gene-by-gene basis. To maximally benefit from and to identify all knockouts in mutant resources novel experimental approaches need to be developed. I will discuss our efforts to further optimize and scale the target-selected mutagenesis approach towards genome-wide organism-based identification of gene knockouts in *C.elegans*, zebrafish, and the rat. Implementation of emerging next-generation sequencing technologies and alternative approaches will be presented.

IAEA-CN-167-351

DNA SEQUENCE ANALYSIS OF INDUCED MUTANTS IN SOYBEAN

K.J. VAN, S.-H. LEE

Department of Plant Science, Seoul National University, Seoul, Republic of Korea

Email: sukhalee@snu.ac.kr

Chemical mutagens, such as ethylmethanesulfonate (EMS), caused point mutations were commonly used to induce mutations for both plants and animals due to irreversible mutations with high frequency. Saturated mutagenized populations could be generated with relatively few individuals. High-throughput sequencing is recently available for genome sequencing with GS-20 or GS-FLX from Roche/454 Life Sciences based on the advantages of emulsion PCR and pyrosequencing. Because of frequent appearance and availability of several high throughput genotyping methods, single nucleotide polymorphisms (SNPs) draw the attention to both plant and human communities. Degenerate oligonucleotide primed PCR (DOP-PCR) was used for SNP genotyping in various organisms by massive approach. In this study, we demonstrate the massive DNA sequence analysis using GS-FLX and DOP-PCR with soybean mutants induced by EMS mutagenesis. Three soybean genotypes were used: 'Sinpaldalkong 2', 'SS2-2' and '25-1-1'. Sinpaldalkong 2 is recommended soybean variety in Korea and SS2-2 was generated by EMS mutagenesis from Sinpaldalkong 2. Also, 25-1-1 is the regenerated M₃ plant from EMS-treated immature embryo culture of Sinpaldalkong 2. After genomic DNA from three soybean genotypes were amplified with modified DOP-PCR, nucleotide sequences were analyzed by GS-FLX. With only orthologues shown 100% of identity in BLASTN, average 1,100 contigs and 7,000 singlets were formed in each soybean genotype. A total of 1,187 SNPs were detected with a frequency as 1 SNP per 272 bp, after POLYBAYES was used for surveying sequence polymorphisms.

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HIGH THROUGHPUT MUTATION DISCOVERY USING KEYPOINT™ TECHNOLOGY

D. RIGOLA, J. VAN OEVEREN, N.J. VAN ORSOUW, A. JANSSEN, H. SCHNEIDERS, M.T.J. DE BOTH, M.J.T. VAN EIJK
 Keygene NV, Wageningen, The Netherlands
 Email: Diana.Rigola@Keygene.com

Reverse genetic approaches rely on detection of sequence alterations to identify mutants among a mutant population. Current methods, such as TILLING are based on the detection of single base mismatches in heteroduplexes using endonuclease CEL1. However, there are drawbacks in the use of CEL1, due to its relatively poor cleavage efficiency and exonuclease activity. Furthermore it does not reveal any information about the nature of a sequence alteration and its possible impact on gene function. The KeyPoint™ technology is based on a massive parallel sequencing approach and provides directly the sequence context of the point mutations. KeyPoint was developed using the Roche Genome Sequencer (GS) 20, and it has now been implemented on the GS FLX, which generates around 400,000 sequence reads of around 240 bp per run. Important for KeyPoint, the sequencing error rate of GS FLX is lower compared to the GS 20 due to improved base calling software. To reduce sample preparation cost, the KeyPoint technology incorporates a multi-dimensional pooling strategy of individual DNA samples and sample identification tags to assign sequence reads to individual samples harboring mutations of interest. For this, we modified the original GS 20 sequence library preparation protocol for use with multiple samples, barcoded using KeyGene™ SeqTag technology (van Orsouw et al., 2007). Using a tomato EMS population of 3000 M₂ families, we further optimized the KeyPoint approach by comparing multi-dimensional pooling schemes and determine the optimal pool complexity in relation to the required sequence depth on the GS FLX to discover mutations present in a single sample. Furthermore, mutation discovery algorithms were developed taking into account position-specific variation in background substitution error rates. In conclusion, the power of KeyPoint technology for mutation discovery improved due to the increased throughput and accuracy of GS FLX in combination with optimized pooling schemes and data analysis software.

The KeyPoint™ and KeyGene™ SeqTag technologies are subject to patent applications owned by Keygene N.V. KeyPoint and KeyGene are trademarks of Keygene N.V.

IAEA-CN-167-130

DRIVING FORWARD IN REVERSE

T.L. WANG¹, J. PERRY¹, T. WELHAM¹, J. PIKE³, M. PARNISKE², C. ROGERS¹, G.E.D. OLDROYD¹, P. STEPHENSON¹, L. OSTERGAARD¹, B. MCCULLAGH¹, D. BAKER¹, S. WALSH¹, J. CLARKE¹

¹John Innes Centre, Norwich, UK

²Ludwig-Maximilian-Universitaet, Munich, Germany

³The Sainsbury Laboratory, Norwich, UK

Email: trevor.wang@bbsrc.ac.uk

We describe the use of TILLING in *Lotus japonicus* and the development of deletion (De)-TILLING in *Medicago truncatula*. The evolution of RevGenUK has been driven by the development of reverse genetics technologies in these two model legumes and *Brassica rapa*, which functions as a translational species for brassica crops. TILLING and De-TILLING, are underpinned by populations of plants mutagenised with either EMS (that causes point mutations) or fast neutrons (that cause deletions) respectively. They permit the isolation of either allelic series of mutants or knockouts. Mutation detection will be developed from a number of independent gel-based systems to be carried out on a single platform – capillary electrophoresis. We are currently TILLING in both model legumes, but these developments will be applied to all three species. The resource will develop an open source database-driven system to support laboratory information management, analysis and the cataloguing of mutants in a genome context across all the species.

IDENTIFICATION OF NOVEL STARCH TRAITS IN SORGHUM BICOLOR (*S.BICOLOR*): A REVERSE GENETICS APPROACH

H. HILL, L.S. LEE, R. HENRY

Centre for Plant Conservation Genetics, Southern Cross University, Lismore, NSW, Australia

Email: helen.hill@scu.edu.au

Sorghum (*Sorghum bicolor* L.) is the fifth most important cereal grain crop in the world and reportedly feeds over 500 million people on a daily basis in the developing world providing dietary starch, dietary protein and some vitamins and minerals. In the West, it is predominantly used as an animal feed and is increasingly important for ethanol production. Sorghum has the potential to be increasingly important as drought and global warming impact on cereal production. Mature seeds of *Sorghum bicolor* L (cultivar MR 43) were bombarded with gamma radiation. Over 1000 individuals were grown to M₂; with DNA extracted for PCR and sequencing. The aim of this study is to use a reverse genetics approach to detect DNA base changes in four important starch synthesis genes encoding; soluble starch synthase (SSI and SSIIa), granule bound starch synthase (GBSS) and starch branching enzyme (SBE IIb) in a gamma irradiated mutant sorghum (cv. MR 43) population. Results are presented showing the induced DNA sequence mutations detected in the genes of interest. Whereas mutations, in the form of single nucleotide polymorphisms (SNP) and insertion/deletion (indel) events, predominantly occurred in non-coding introns, some mutations were also found in exons. The latter will induce amino acid variants compared to the parental type plants, and it is possible that the resultant protein changes may be revealed, in subsequent research in this project, to be associated with changes in starch phenotype.

CHARACTERIZATION OF *CHENOPODIUM QUINOA* CHROMOSOMES USING FISH AND REPETITIVE SEQUENCES

B. KOLANO¹, M. SEDERBERG², P.J. MAUGHAN², E. JELLEN², J. MALUSZYNSKA¹

¹Dept. Of Plant Anatomy and Cytology, Silesian University, Katowice, Poland

²Dept. of Plant and Animal Sciences, Brigham Young University, Provo, Utah, USA

Email: bkolano@us.edu.pl

Quinoa is one of the underestimated crops, which recently attracted attention. During last few years many efforts were done to save the natural genetic diversity of quinoa cultivars and landraces as well as to obtain new variability by mutagenesis. Plant characteristics based mainly on morphological and molecular markers. Cytogenetic analysis was not used for these studies. Quinoa is an allotetraploid species with 36 small chromosomes. To follow the chromosomal rearrangement cause by spontaneous or induced mutations it is necessary to find cytogenetics markers for chromosomes and chromosome arms. The physical mapping of repetitive DNAs by fluorescent *in situ* hybridization (FISH) can provide a valuable tool in studies of genome organization and chromosome rearrangements. To characterize quinoa genome several repetitive sequences were used as DNA probes for FISH. Double FISH with rRNA genes as probes allowed to distinguish three pairs of homologue chromosomes. Telomeric repeats hybridisation signals were present only in terminal part of all chromosome arms and no intercalary position was observed. Other tandem repetitive sequence - minisatellite was characteristic for centromeric and pericentromeric region of all quinoa chromosomes although number of repeats differ between loci. It allowed to divide quinoa chromosomes into few groups. Disperse repetitive sequences such as mobile element-like sequences used in this study were detected in all eighteen chromosome pairs. Hybridization signals were characteristic for pericentromeric region of one or both chromosome arms as relatively weak but discrete signals although few chromosomes exhibited signals in intercalary position. Two other repetitive sequences also exhibited disperse organization; however they are not mobile elements. Their FISH signals were spread throughout whole chromosome arms but only one was present on all quinoa chromosomes. The other revealed hybridization signals only on the half of the chromosomes. Presumably it allows identification of the sets of chromosomes belonging to the two ancestral genomes.

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HIGH-THROUGHPUT ANALYSIS FOR PUTATIVE SPONTANEOUS MUTATIONS OF A T-DNA INTEGRATION SITE IN THE GENOME OF *ARABIDOPSIS THALIANA*

M.C. ICHIM¹, A. MILCAMPS², N. PAPAZOVA³, A. DEPICKER⁴, M. DE LOOSE³, G. VAN DEN EEDE²

¹“Stejarul” Research Centre for Biological Sciences/INCDSB, Piatra Neamt, Romania

²European Commission, Joint Research Centre (JRC), Ispra, Italy

³Institute for Agricultural and Fisheries Research (ILVO), Merelbeke, Belgium

⁴Ghent University, Ghent, Belgium

Email: cichim@hotmail.com

The commercial approval of GMOs relies on the assumption that the inserts remain stable in the plant genome. Still, it has been shown that the integration of foreign DNA segments may coincide with minor or major target site mutations and occurrence of filler DNA segments. It is also suspected that instability can arise upon plant exposure to several stresses. A project was initiated with the aim to study putative sequence mutations of a T-DNA insertion site in the genome of *Arabidopsis thaliana* under high light (photo-oxidative) stress, known to induce the formation of genotoxic and mutagenic Reactive Oxygen Species (ROS). A T-DNA insertion line was generated via *Agrobacterium* mediated transformation and the insertion site identified and characterized. For the analysis of the target site in wt plants, a high-throughput method - Single Strand Conformational Polymorphism (SSCP) analysis - was tested and optimized to detect even minor changes that could occur, such as SNPs. The method is based on PCR amplification of the integration site using site-specific dual-fluorescently labeled primers followed by SSCP analysis. If changes in the conformational pattern would have been detected, the fragment had to be cloned and sequenced to determine the exact nature of the mutation. The genomic fragment (264bp) spans a genomic region around the T-DNA insertion point in an *A. thaliana* transgenic line. Plants were grown under regular conditions until the onset of flowering. A first set (control) was then brought to maturity in the same conditions, while a second set was transferred in a phytotron for high light treatment. Seeds from both sets were harvested and the progeny grown in normal conditions. A total of 100 plants/treatments were individually analyzed. The results indicate that in wt plants, both those grown in standard conditions and those exposed to high light stress, the sequence considered does not undergo any major or minor re-arrangements.

IAEA-CN-167-148P

APPLICATION OF THE TILLING STRATEGY FOR ANALYSIS OF MUTATION TYPES AND FREQUENCIES INDUCED BY MNU AND GAMMA RAYS IN *HORDEUM VULGARE* AND *ARABIDOPSIS THALIANA*

A. LABOCHA-PAWLOWSKA, M. KUROWSKA, D. GNIZDA, M. MALUSZYNSKI, I. SZAREJKO

Department of Genetics, University of Silesia

Katowice, Poland

Email: anna.labocha-pawlowska@us.edu.pl

TILLING (Targeting Induced Local Lesions IN Genomes) is a new strategy of reverse genetics (McCallum et al., 2000) that combines the high density of point mutations provided by traditional chemical or physical mutagenesis with rapid mutational screening to discover induced lesions in defined DNA sequences. The main objective of the project was to estimate the type and frequency of DNA changes induced by gamma rays and N-nitroso-N-methyl urea (MNU) in *Hordeum vulgare* and *Arabidopsis thaliana* genomes. The types and frequencies of point mutations were evaluated using M₂ populations of *A. thaliana* and *H. vulgare* obtained after chemical and physical mutagenesis. To ensure the homogeneity of the starting material, the doubled haploid barley line 'H930-36' has been chosen for mutagenic treatments. Applying nontransgenic method of classical mutagenesis, the TILLING populations for *A. thaliana* and *H. vulgare* were created. The library of genomic DNA of M₂ population and the M₃ seed bank were formed. Fragments of *CBP20* (Acc.: AF140219) and *ABH1* (Acc.: AF272891) genes encoding of 5' subunits of the nuclear mRNA cap binding complex were analysed in *A. thaliana*. Recessive mutants of those genes are abscisic acid hypersensitive and have improved drought tolerance. The root-specific gene for β-ekspansin 1 (*HvEXPB1*, Acc.: AY351785) responsible for root hair development and the *HvBRI1* (Acc.: AB088206) gene coding the transmembrane receptor of brassinosteroids (BRs) were tested in barley. The SNPs frequency in *H. vulgare* M₂ population derived from treatment with MNU was at 1.6/1 Mbp level and for population derived from treatment with physical mutagen was estimated to be 0.3/1Mbp. The SNPs frequency in *A. thaliana* derived from treatment with MNU was 1.8/1Mbp. No nucleotide changes were identified in the analyzed gene fragments of physical mutagen-treated plants.

DISCOVERY OF SINGLE-NUCLEOTIDE MUTATIONS IN GENES RELATED TO RICE STARCH SYNTHESIS AND HERBICIDE RESISTANCE BY USING SELF-MADE CEL I EXTRACTS

H.R. CUI, J.Y. LIU, J. SUN, D.X. WU

Institute of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou, China

Email: hrcui@zju.edu.cn

The foundation of CEL I, a specific nuclease isolated from celery, makes the detection of point mutations to be easy and robust and it is essential nowadays in TILLING. However, large amounts of CEL I are consumed in TILLING and its extraction process is time-consuming. Furthermore, the high cost both in isolation and application of commercial CEL I Kit is an albatross for scientists in developing countries. Herein is presented a rapid method for detection of single-nucleotide mutations in rice genes by using self-made CEL I extracts. After tests on mismatch cleavage activity of CEL I extracts at different extraction steps, it was proved that CEL I extracts after clarification and dialysis are sufficiently enriched in mismatch cleavage activity. By optimization of factors related to mismatch cleavage activity, we found that CEL I extract made by ourself showed same function for mismatch cleavage as the commercial CEL I and established a feasible and effective method for detecting point mutation. Understanding and manipulating genetic variation is paramount to elucidating gene function, identifying genes, breeding, and conserving natural diversity. The general applicability of CEL I makes it great potential for detecting and understanding genetic variation in rice. By the method of mutation detection we set up using self-made CEL I, we found single-nucleotide mutations of some rice genes, such as *waxy*, *SSIIa* (starch synthase IIa) and *als* (acetolactate synthase), related to rice starch synthesis or herbicide resistance. The single-based variation (T/G or A/G) were detected both in first intron of *waxy* gene and 8th exon of *SSIIa* gene. For *als* gene, we found the single-nucleotide mutation at the position about 700bp and 400bp in the 1.5kb fragment amplified from different varieties and M₂ plants respectively.

APPLICATIONS OF TILLING TO THE UNDERSTUDIED CROPS FROM AFRICA: THE CASE OF TEF

Z. TADELE, K. ESFELD

Institute of Plant Sciences, University of Bern, Switzerland

Email: tadele@ips.unibe.ch

Induced and natural mutations provide a powerful method for the generation of heritable enhanced traits. The rapid accumulation of plant genomic sequence information has allowed for exploring gene function in a variety of different species. The reverse genetic approaches facilitate the identification of lesions in specific target genes. Such gene-driven approaches promise to speed up the process of creating novel phenotypes, and can enable the generation of phenotypes unattainable by traditional forward methods. TILLING (Targeting Induced Local Lesions IN Genome) is a high-throughput reverse genetic method for the discovery of induced mutations. The method has been applied to many species, including a variety of different crops. Currently, we apply TILLING to alter the stature of tef (*Eragrostis tef*) plant. Although tef adapts to diverse climatic and soil conditions, compared to other cereals, it produces very low yield. Crop lodging is the major constraint to increase the yield. Susceptibility to lodging is due to the height and weakness of the stalk. Once the problem of lodging is tackled by breeding for semi-dwarf cultivars that are responsive to fertilizer application, the yield of tef will significantly increase.

CONCURRENT SESSION 8 (08:30-13:00)

**Mutation Induction and Breeding of Ornamental and
Vegetatively Propagated Plants**

UNIDO Boardroom C04

Thursday, 14 August 2008

THE ROLE OF MUTATION TECHNIQUES AND GENOMICS FOR BANANA AND PLANTAIN (*MUSA* SPP.), MAJOR STAPLE CROPS IN THE TROPICS

N. ROUX¹, F.-C. BAURENS², J. DOLEŽEL³, E. HŘIBOVÁ³, P. HESLOP-HARRISON⁴, C. TOWN⁵, T. SASAKI⁶, T. MATSUMOTO⁶, R. AERT⁷, S. REMY⁷, M. SOUZA⁸, P.J.L. LAGODA⁹

¹Bioversity International, c/o INIBAP-Parc Scientifique, Montpellier, France

²Centre de Coopération Internationale pour la Recherche en Agriculture et le Développement, Montpellier, France

³Institute of Experimental Botany, Laboratory of Molecular Cytogenetics and Cytometry, Olomouc, Czech Republic

⁴University Leicester, Department of Biology, Leicester, UK

⁵The Institute for Genomic Research, Rockville, MD, USA

⁶National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan

⁷Katholieke Universiteit Leuven, Leuven, Belgium

⁸Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) LABEX Europa, Plant Research International (PRI),

Wageningen University & Research Centre (WUR), Wageningen, The Netherlands

⁹Joint FAO/IAEA Division of Nuclear Energy in Food and Agriculture, International Atomic Energy Agency, Plant Breeding and Genetic Section, Vienna, Austria

Email: n.roux@cgiar.org

Bananas and plantains are produced in 126 countries throughout the tropics and sub-tropics, on an area of 12.5 million acres (5Mha), with annual production exceeding 100 million tons. Dessert bananas exported as one of the most widely eaten of the 'five-a-day fruits' in the industrialized countries, with a value of \$US 4.86 billion and underpinning the economy of many developing countries, account for 15% of this total. The remainder provide a staple food and major source of income for as many as 400 million people in developing countries. The banana export industry relies on genetically closely related clones of the Cavendish sub-group (sterile triploids, AAA). Though high-yielding, this sub-group of cultivars is extremely vulnerable to biotic and abiotic stresses and, due to high levels of sterility, is very hard to improve through classical breeding. A high quality genomic sequence of *Musa* has immediate application in assisting localization and identification of genes and alleles related to biotic (pest and disease) stresses, to abiotic (environmental, including drought, flooding, wind and salinity) stresses and to fruit quality (including post-harvest processes and nutrition). The latest information on activities and resources developed by the Global *Musa* Genomics Consortium will be presented. It will be demonstrated that by organizing the Global *Musa* Genomics Consortium (currently comprising 37 member institutions from 24 countries), duplication of effort can be minimized and the results of *Musa* genomics research are rapidly made accessible to taxonomists, breeders and the biotechnology community.

IAEA-CN-167-282

A REPORT ON 36 YEARS PRACTICAL WORK ON CROP IMPROVEMENT THROUGH INDUCED MUTAGENESIS

S.K. DATTA

CSIR, Emeritus Scientist, Madhyamgram Experimental Farm, Bose Institute, Kolkata, India

Email: subodhskdatta@rediffmail.com

Physical and/or chemical mutagens cause random changes in the nuclear DNA or cytoplasmic organelles, resulting in gene, chromosomal or genomic mutations. The author will share his life time experience and achievement on induced mutagenesis. The author initiated induced mutagenesis work in 1971 till July 2007 and used both physical (X-ray and Gamma rays) and chemical (EMS, MMS, Colchicine) mutagens for improvement of vegetables (*Trichosanthes anguina* L., *T. cucurmarina*, *Cucurbita maxima* L., *Cephalandra indica*, *Luffa acutangula* Roxb., *Lagenaria ciceraria*), medicinal (*Trigonella foenum-graecum* L., *Mentha citrate* Ehrh), pulse (Winged Bean (*Psophocarpus tetragonolobus* L. D.C.), oil bearing (*Jatropha curcas* L., *Rosa damascene*, *Cymbopogon flexuosus* (Nees) Wats) and ornamental (Bougainvillea, Chrysanthemum, Dahlia, Gladiolus, Hibiscus, *Lantana depressa* Naud, Rose, Tuberose, Narcissus etc.) crops. All classical and advanced mutagenesis methods have been extensively used for the development of new and novel cultivars of economic importance. Early flowering, late flowering, dwarf, yellow fruit color, crinkled leaf, short thick fruit, increased branching, increased pod and seed number, seed size, seed color (green, brown, chocolate color) high fruit-, seed-, oil- and puniceic acid-yielding mutants have been developed in *T. anguina*, *T. foenum-graecum*, Winged Bean and in *J. curcas* containing 'curcas oil', an efficient substitute fuel for diesel engines. Induction of flower color and chlorophyll variegated mutants in *L. depressa* proved the efficiency of mutation technique for domestication of wild relatives. Author was deeply engaged for the last 30 years for improvement of ornamentals and has been most successful to produce quite a large number of new

promising mutant varieties in different ornamentals. Colchicine has been successfully used to develop new flower color in chrysanthemum and rose and high yielding strains in *T. anguina*. A novel direct *in vitro* regeneration technique has been standardized for management of floral chimeric sector in chrysanthemum. Classical mutation and *in vitro* mutagenesis successfully developed salt resistant strains in ornamentals. Research papers (258), review papers (13), book chapter (29), book (1), edited book (4), bulletins (8), popular articles (78), symposium abstracts (123) etc. have been published which covers different basic aspects for successful application of induced mutagenesis.

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CITRUS IMPROVEMENT USING MUTATION TECHNIQUES

E.S. LOUZADA

Citrus Center, Texas A&M University, Kingsville, Weslaco, TX, USA

Email: elouzada@ag.tamu.edu

Citrus cultivar improvement is hampered by several biological factors inherent to most citrus species. Facultative apomixis, self and cross-incompatibility, long juvenility period, and high heterozygosity are some of the vast arrays of impediments faced by the citrus breeders in conventional hybridization. Since oranges and grapefruits are highly polyembryonic, the production of enough numbers of zygotic offspring for selection of superior genotypes of these species is basically impossible; consequently, most of the commercially important cultivars of these species have originated through natural or induced mutation. Star Ruby, a deep-red-fleshed grapefruit, was developed by irradiation of Hudson grapefruit seeds with thermal neutrons. Unlike Hudson, which contains over 50 seeds per fruit, Star Ruby is nearly seedless. Rio Red, the most planted grapefruit in Texas has also dark-red flesh and originated by thermal neutrons irradiation of the pinkish Ruby Red grapefruit buds in the third vegetative progeny. In the mandarin group, the existence of several monoembryonic cultivars facilitates conventional breeding, but still induced mutation is part of most mandarin breeding programs, and proprietary, new seedless cultivars have been produced in USA, Italy, Israel and elsewhere. Mutation has been also important in lemon breeding, and a seedless lemon, with tolerance to a devastating lemon disease was recently reported, in addition to earlier reports of a thornless lemon mutant produced by gamma irradiation. Gamma irradiation is currently an important component of our breeding program and several potentially improved cultivars of grapefruit, pummelos, and lemons are in the pipeline. Additional details of citrus irradiation programs in USA will be provided.

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MUTATION BREEDING OF CHRYSANTHEMUM BY GAMMA FIELD IRRADIATION AND *IN VITRO* CULTURE

S. NAGATOMI

Institute of Radiation Breeding (IRB), National Institute of Agrobiological Sciences (NIAS), Ibaraki, Japan

Email: nagatomi@affrc.go.jp

The purpose was to clarify the effect of chronic (gamma field) and acute (gamma room) radiations and *in vitro* culture on mutation induction of flower color in chrysanthemum. The combined methods of irradiation and *in vitro* culture yielded a mutation rate 10 times higher than the conventional chronic cutting method, and also produced non chimeric mutants. Somaclonal variation often occurred in plants regenerated from callus, but no significant variation appeared in callus regenerators from non-irradiated plants. Therefore, proper mutagenic treatment on cultured materials is indispensable to effective mutation induction. The chronic culture method clearly yielded the widest color spectrum in chrysanthemum, while the acute culture method resulted in a relatively low mutation rate and a limited flower color spectrum. Flower color mutation could be more readily induced in plants regenerated from petals and buds, than from leaves. In this respect, it is supposed that the gene loci fully expressed on floral organs may be unstable for mutation by mutagenesis or culture, but could perhaps induce mutation in a desired direction. A possible reason why the chronic culture methods showed higher frequencies than the acute, had been discussed. Nine out of ten registered mutant varieties were derived from chronic irradiation, and only one from acute. The combined method of chronic irradiation with floral organ cultures proved to be of particularly great practical use in mutation breeding, not only of flower species but of other species as well.

ENHANCING GENETIC DIVERSITY THROUGH INDUCED MUTAGENESIS IN VEGETATIVELY PROPAGATED PLANTS

R. AFZA, J. JANKOWICZ-CIESLAK, S. BADO, M. MATIJEVIC, B.J. TILL, C. MBA

Plant Breeding Unit, Joint FAO/IAEA Agriculture and Biotechnology Laboratory, International Atomic Energy Agency, Seibersdorf, Austria

Email: c.mba@iaea.org

Conventionally, crop improvement strategies rely not only on the availability of heritable genetic variations within utilisable genetic backgrounds but also on the transferability of the traits they control through hybridizations between the parental stocks. Procedures for producing hybrids of sexually reproducing plants are routine while for vegetatively propagated plants, hybridizations are usually impractical. The improvement of crops that lack botanical seeds necessitate therefore alternative strategies for generating and utilizing genetic variations. Induced mutagenesis generates allelic variants of genes that modulate the expression of traits. Some of the major drawbacks to the widespread use of induced mutations for vegetatively propagated plants include the difficulties of heterozygosity of the genetic backgrounds; the incidence of chimeras; and the confounding effects of linkage drags in putative mutants. In general, the inherent inefficiencies of the economies of time and space associated with induced mutagenesis are further exacerbated in vegetatively propagated crops mostly on account of the need for continual propagation. We highlight the mitigating roles on these drawbacks of the judicious integration of validated biotechnologies and other high throughput forward genetics assays in induced mutagenesis pipelines. Using cassava and banana as models, we demonstrate the use of cellular and tissue biology to achieve homozygosity, minimise or eliminate chimeras, and significantly shorten the duration of the generation of mutants. Additionally, the use of these biotechnologies to attain significantly reduced propagation footprints while evaluating putative mutants without compromising population size is also presented. We also posit that molecular biology approaches, especially reverse genetics and transcriptome assays, contributes significantly to enhancing the efficiency levels of the induced mutagenesis processes. The implications for crop improvement and functional genomics via the concerted application of biotechnologies in the generation, identification, and the tagging of mutation events in the genomes of vegetatively propagated crops are also discussed.

MUTATION BREEDING AND CHARACTERIZATION OF THE GENE TRANSCRIPTS RESPONSIBLE FOR CHANGES IN THE FLOWER COLOR OF CHRYSANTHEMUM MUTATED BY A GAMMA IRRADIATION AND IN-VITRO TISSUE CULTURE

S.J. CHUNG¹, G.-J. LEE¹, I.S. PARK², J.B. KIM¹, D.S. KIM¹, S.-Y. KANG¹

¹Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongseup, Republic of Korea

²Kyungpook National University, Daegu, Republic of Korea

Email: sykang@kaeri.re.kr

Objectives of this study were to breed a new mutant variety and to identify the transcripts of the genes responsible for the flower color of chrysanthemum mutated via an in-vitro culture and gamma-ray irradiation. Stem segments of the cultivar 'Argus' were used to induce a shoot or root formation. Regenerated plantlets were irradiated with various doses (30, 40, 50 Gy) of gamma-ray. The plantlets were transplanted and grown in a greenhouse after 4 subsequent *in vitro* cultures and then some of flower mutants were selected. The flower color of the wild type was pinkish tubular and ray florets, while the mutants had white and purple ray florets and white, purple, and yellow-green tubular florets. To determine the molecular basis of a flower color mutation in the chrysanthemum flowers, we compared the wild type to five flower color mutants using biochemical and molecular approaches. The novel cDNAs fragments of four genes (CHI, F3'H, F3'5'H, ANS) from the chrysanthemum species which have not been reported yet were cloned and sequenced in a series of protocols for the RT-PCR, 3' and 5' RACE, and nucleotide sequence analysis. Expression pattern and level of some genes (CHI, F3'H, F3'5'H, DFR, ANS) were different among not only the colored mutants but also the organ, with no amplification or strong expression for the genes. This is the first report on a gene isolation and a structural comparison of the genes in chrysanthemum, which were characterized by using gamma-ray irradiated mutants, and the results will provide information the genetic mechanisms for a color mutation in chrysanthemum.

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INDUCTION AND IDENTIFICATION OF USEFUL MUTATIONS FOR ROOT QUALITY TRAITS IN CASSAVA

T. SÁNCHEZ², A. ROSERO¹, A.P. TOFIÑO¹, K. DENYER³, A. SMITH³, H. CEBALLOS^{1,2}, N. MORANTE², J.C. PÉREZ²

¹Universidad Nacional de Colombia, Palmira Valle del Cauca, Colombia

²CIAT (International Center for Tropical Agriculture), Cali, Colombia

³John Innes Centre, Norwich, U.K

Email: h.ceballos@cgiar.org

Cassava is an important crop in tropical and subtropical countries. There is little reported variation for root quality traits. Roots spoil 1-2 days after harvest because of a process known as post-harvest physiological deterioration (PPD). These two problems limit the impact of this crop to help farmers out of poverty. Cassava seeds were irradiated with gamma rays or fast neutrons. They were germinated and the M₁ plants transplanted to the field and self-pollinated to produce M₂ seed. Approximately 1500 M₂ plants were harvested, about 800 produced roots, and 38 of them were selected because they showed special characteristics. Best results were obtained with gamma rays (200 Gy from Cobalt 60). Results originally observed in the M₂ genotypes were confirmed in the cloned plants. Two of these mutations will be described here. The first mutation produced a starch whose granules were half the size compared with that of wild type cassava. Interestingly, this phenotype was observed in self-pollinated progenies from two different M₁ plants. In one case, three M₂-genotypes derived from the same M₁ plant expressed this mutation, indicating a genetic origin. Starch had higher than normal amylose content, the gels it produces did not show any viscosity and had low clarity. Most likely this mutation affected one of the isoamylase genes. The small granule size could be useful for bio-ethanol production (reduced need of degrading enzymes) but the higher levels of amylose may neutralize this advantage. High-amylose starches have commercial advantages and are known as "resistant starches". The second mutation involves reduced PPD. Roots were kept for almost three weeks without PPD. Results need further confirmation based on larger number of roots. Most likely the mutation affected one of the self-defense mechanism genes related to PPD. This mutation could have large impact in poverty alleviation.

IAEA-CN-167-107

INDUCTION, ISOLATION AND SELECTION OF POTATO MUTANTS RESISTANT TO LATE BLIGHT DISEASE AND TOLERANT TO SALINITY USING *IN VITRO* AND DNA MARKER TECHNIQUES

B. AL-SAFADI, M. ARABI, N. MIRALI, A. AL-DAOUDE, I. NABULSI
Department of Biotechnology and Molecular Biology, AECS, Damascus, Syria
Email: bsafadi@aec.org.sy

A mutation breeding program was conducted to improve potato (*Solanum tuberosum*) resistance to late blight disease caused by *Phytophthora infestans* and tolerance to salinity. Virus free explants of three potato cultivars (Draga, Spunta and Diamant) were irradiated with gamma ray doses 25, 30, and 35 Gy. Growing shoots were cut and recultured every 2 weeks until the 4th generation (MV₄) to obtain chimera free mutant material. Isolates of *Phytophthora infestans* were made from potato leaves showing disease symptoms in different locations of south-west Syria. Around 3000 plantlets were subjected to selection pressure using co-culture technique. Surviving plantlets were propagated and re-incubated with the pathogen for three consecutive generations. Resistant plants were transferred to the greenhouse and inoculated, at the adult stage, with sporangial suspension. Ten plants of Draga were resistant to late blight and one plant from each of the other 2 cultivars. The same technique was used to select potato mutants tolerant to salinity. MV₄ explants were cultured on an MS medium supplemented with NaCl in concentrations from 50 to 200 mM. Tolerant plantlets were grown under greenhouse conditions and were later subjected to a second selection pressure by irrigation with water containing NaCl in concentrations ranging from 50 to 250 mM. Four plants tolerant to salinity were obtained from Spunta and only one from Diamant and none from Draga. In an attempt to determine DNA loci involved in the salinity tolerance, mutants of the three potato cultivars, Draga, Spunta and Diamant found to be salt tolerant in the previous study were used for analyzes. RAPD and ISSR were employed in the study. More than one hundred PCR fragments that were absent in the control (Draga, Spunta or Diamant) and present in the relevant mutations were obtained, gel extracted, cloned in pBluescriptII SK+ and sequenced using plasmid specific primers.

APPLICATION OF INDUCED MUTATION TECHNIQUES IN GHANA: IMPACT, CHALLENGES AND THE FUTURE

K.E. DANSO, O. SAFO-KATANKA¹, Y. ADU-AMPOMAH², V. ODURO³, H.M. AMOATEY³, D.K. ASARE³, E. OFORI AYEH³, G. AMENORPE³, S. AMITEYE³, Y. LOKKO³

Ghana Atomic Energy Commission, Biotechnology and Nuclear Agriculture Research Institute, Department of Plant and Soil Sciences, P.O. Box 80, Legon-Accra, Ghana

¹Department of Crop Science, Kwame Nkrumah University of Science and Technology, Kumasi

²Cocoa Research Institute of Ghana, New Tafo-Akim, Ghana

³Plant Breeding and Genetics, Joint FAO/IAEA Division, IAEA, Vienna, Austria

Email: kaedanso@hotmail.com

Over two decades of application of induced mutation techniques toward crop improvement in Ghana have led to the production of improved mutant varieties in two crops. In cassava (*Manihot esculenta* Crantz), irradiation of stem cuttings using gamma irradiation resulted in the production of "Tek bankye", a mutant variety with high dry matter content (40%) and good poundability from the parental line which was a segregant of a hybrid between the Nigerian landrace Isunikaniyan (ISU) and the breeder's line TMS4(2)1425, both from IITA, Nigeria. Similarly, irradiation of vegetative buds of 'Amelonado' (P30), 'Trinitario' (K5) and 'Upper Amazon' (T85/799) cocoa varieties resulted in the production of Cocoa Swollen Shoot Virus (CSSV) resistant mutant variety. Multi-locational on-farm trials of the mutant line indicate significant increases in yield by farmers with no symptoms of the disease. Despite these achievements, application of induced mutation in Ghana has been challenged by low funding, inadequate statistics on small holder farms, high attrition rate of researchers, and low rate of useful mutant regeneration and lack of indicators for early mutant selection. Recent advances in plant breeding which combine *in vitro* techniques with mutation induction hold better prospects for generating useful mutants. Copy right granted to the IAEA.

IAEA-CN-167-265

MOLECULAR CHARACTERIZATION OF SOMATIC MUTATION IN *MUSA ACUMINATA* 'RED'

A.S. NAIR¹, T. SCHWARZACHER², J.S. HESLOP-HARRISON²

¹Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, India

²Department of Biology, University of Leicester, Leicester, United Kingdom

Email: ashobot2004@yahoo.co.uk

Musa acuminata 'Red' (AAA) is a South Indian dessert banana cultivar ($2n = 3x = 33$) with a characteristic red colour in the pseudostem, petiole and fruit peel. It is a popular edible variety grown extensively in India, Thailand, Sri Lanka, East Africa, West Indies, Myanmar and Continental America. Red banana undergoes the process of somaclonal variation, producing the 'off types' *M. acuminata* 'Green' cultivars. The frequency of the production of this 'green variant' is high during *in vitro* multiplication. In plants, anthocyanin pigments are assembled like all other flavanoids from two different streams of chemical raw materials in the cell. One stream involved the shikimate pathway to produce the amino acid phenyl alanine and the other stream produced 3 molecules of malonyl Co-A, a C3 unit from a C2 unit (acetyl Co A). These streams meet and are coupled together by the enzyme chalcone synthase (CHS), which forms an intermediate chalcone via a polyketide folding mechanism that is commonly found in plants. The chalcone is subsequently isomerized by the enzyme chalcone isomerase (CHI) to the prototype pigment naringenin –the precursor for flavanoids. More than five enzymes are required to synthesize anthocyanin pigments, each working in concert. Any even minor disruption in any of the mechanism of these enzymes by either genetic or environmental factors would halt anthocyanin production. To understand the molecular mechanism for the somaclonal variation in Red banana, the chalcone synthase gene sequences were amplified using PCR products cloned and sequences were compared with those of 'Green variants (AAA)', 'Dwarf Cavendish (AAA)' and diploid 'Pisang lili' (AA). Sequence variations were observed only in amplified product from Red cultivar. Predicted amino acid sequences of the longest ORF indicated changes in seven amino acids such as arginine, glutamine, alanine, aspartic acid, isoleucine, phenylalanine and asparagine to serine, leucine, proline, alanine, valine, tyrosine and serine respectively. In plants a major function of anthocyanins is to provide color to most flowers and fruits but they also protect leaves from ultraviolet radiation. *Musa acuminata* cv. Red with high anthocyanin content might have originated as a natural mutant, selected by farmers and maintained by vegetative propagation through generations. The frequent production of 'off types' during micropropagation indicated the back mutation/reverse mutation.

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GAMMA IRRADIATION INDUCED MUTATION FOR THE IMPROVEMENT OF JOSAPINE PINEAPPLE AGAINST BACTERIAL HEART ROT DISEASE AND IMPROVED FRUIT QUALITY

R. IBRAHIM¹, A. HAMZAH¹, Z. JAN JAM¹, M. BAHAGIA², M. JOYO²

¹Division of Agrotechnology and Biosciences, Malaysian Nuclear Agency, Bangi, Malaysia

²Department of Horticulture, Malaysian Agriculture Research Institute and Development, Serdang, Malaysia

Email: rusli_ibrahim@nuclearmalaysia.gov.my

Bacteria heart rot disease, caused by *Erwinia chrysanthemi*, is one of the most serious diseases of the susceptible cultivars of pineapple in Malaysia, namely, Josapine, Sarawak, Gandul and N36. Using acute irradiation of gamma-rays and *in vitro* cultured meristems, the selection of resistant mutant to bacterial heart rot disease with improved fruit quality has been carried out using radiation-induced mutagenesis of the most popular variety, Josapine. Meristem explants were irradiated with a series of gamma-ray doses of 0, 20, 40, 60, 80, 100, 120, 140 and 160Gy and radiosensitivity was investigated based on shoot formation and survival rate. The lethal dose required for 50% (LD₅₀) and 100% (LD₁₀₀) of meristem tissues for shoot formation was 40Gy and 83Gy respectively. On the other hand, the lethal dose required for 50% (LD₅₀) and 100% (LD₁₀₀) for survival rate was 77Gy and 147Gy respectively. Shoots derived from irradiated meristem explants at 10, 20, 30 and 40Gy were sub-cultured up to M₁V₅ to minimize chimerism. Multiplication of irradiated shoots was carried out in Temporary Immersion Bioreactor System using MS liquid media supplemented with 2.5mg/l benzyl aminopurine (BA). Rooting was promoted on MS media supplemented with 2mg/l indole-3-butyric acid (IBA) and rooted plantlets were hardened in the nursery for 2 months. Preliminary screening of 20,000 irradiated plants in the nursery indicated 60% with smooth leaves, besides vigorous growth. Selected mutant plantlets were field planted in hot spot until fruiting. At lower doses of 10 and 20Gy, there were no potential resistant mutant plants with significant improvement in total sugar content and fruit weight observed. However, at higher doses of 30 and 40Gy, 11 and 5 resistant plants with significant increase in both total sugar content and fruit weight were recorded, respectively. Molecular analysis using AFLP was conducted to identify markers for the selected characters.

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NATURAL GENETIC VARIATION IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ) LANDRACES AS A TOOL FOR GENE DISCOVERY

L.J. CASTELO BRANCO CARVALHO¹, C.R. BATISTA DE SOUZA², J.C. DE MATTOS CASCARDO³, M.A. VALLE AGOSTINI¹, E.A. VIEIRA⁴, J.V. ANDERSON⁵, J. LIPPOLIS⁶

¹Embrapa-Genetic Resources and Biotechnology, Brasília-DF, Brazil

²Universidade Federal do Para, Belém-PA, Brazil

³Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil

⁴Embrapa Cerrados, Brasília-DF, Brazil

⁵USDA-ARS, Plant Science Research Unit, Fargo-ND, USA

⁶USDA-ARS National Animal Disease Center, Ames-IA, USA

Email: carvalho@cenargen.embrapa.br

Cassava landraces are the earliest form of the modern cultivars and represents the first step in cassava domestication. Our forward genetic analysis uses this resource to discover spontaneous mutations in the sucrose/starch and carotenoid synthesis/accumulation and to develop both evolutionary and breeding perspective of gene function related to those traits. Biochemical phenotype variants for the synthesis and accumulation of carotenoid, free sugar and starch were identified. Six subtractive cDNA libraries were prepared to construct a high quality (phred > 20) EST database with 1645 entries. Macroarray analysis was performed to identify differentially expressed gene aiming to identify candidate gene related to sugary phenotype. cDNA sequence for gene coding for specific enzymes in the two pathways were obtained. Gene expression analysis for coding specific enzymes was performed by RNA blot and Real Time PCR analysis. Chromoplast-associated proteins of yellow storage root were fractionated and a peptide sequence data base with 906 entries sequences (MASCOT validated) was constructed. For the sucrose/starch metabolism a sugary class of cassava was identified carrying mutation in the BEI and GBSS mutation. For the pigmented cassava a pink color phenotype showed absence of expression of the gene CasLYB while an intense yellow phenotype showed a down regulation of the gene CasHYb. Heat shock proteins were identified as the major proteins associated with chromoplast. Genetic diversity for the GBSS gene in the natural population identified 22 haplotype and a large nucleotide diversity in four subset of population. Single segregating population derived from F₂, half sib and S₁ population showed segregation for sugary phenotype (93% of the individuals), waxy phenotype (38% of the individuals) and glycogen like starch (2% of the individuals). Here we summarize our current results for the genetic analysis of this variants and recent progress in the direction of mapping of loci and with large-effect genes.

PROSPECTS OF INDUCED MUTATIONS AND BIOTECHNOLOGY IN VEGETATIVELY PROPAGATED CROP IMPROVEMENT

S.M. JAIN

Department of Applied Biology, University of Helsinki, Helsinki, Finland

Email: mohan.jain@helsinki.fi

Nuclear applications in food and agriculture have contributed greatly in enhancing agriculture production of seed and vegetatively propagated crops. As the human population grows continuously and climate changes furthermore, plant breeders are under pressure to adopt new technologies in genetic improvement of vegetative propagated crops for sustainable production. Plant tissue culture has a great potential in plant improvement, provided plants can be readily regenerated in large numbers. It provides the options to reduce costs in generating the useful traits and pre-breeding materials for plant breeders, as well as shortening the screening program. *In vitro* culture techniques together with nuclear technology is effective in generating genetic variability, selection of useful mutants and their multiplication in large numbers, especially in vegetative propagated crops. Shoot tips can be irradiated with an optimal radiation dose, induce direct shoot formation, and shoot multiplication. Excise individual shoots and put them for rooting and rooted plantlets are hardened in the greenhouse for further evaluations. Radiation treatment of somatic embryogenic cell suspension cultures is suitable for mutation induction, mutant selection, and plant regeneration. For example, bayoud disease resistant date palm mutant plants have been regenerated, which are already in the field. In banana, black sigatoka disease resistant mutant lines are in field trials for the final confirmation of the selected mutants before releasing to the farmers. A wide range of mutants of several ornamental plants including chrysanthemum, roses, orchids, gerbera, and curcuma have been isolated by physical mutagen treatment. Some of the selected traits of the mutant ornamental plants are flower colour, flower morphology, plant architecture, compact growth, flower type, and variegated leaves; many flower mutant varieties have been released to growers.

Poster Presentations

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***IN VITRO* MUTAGENESIS FOR PHOTO INSENSITIVITY TO TUBERISATION IN COLEUS ((*SOLENOSTEMON ROTUNDIFOLIUS*) (POIR.) J.K. MORTAN)**

V.V. RADHAKRISHNAN, M. ABRAHAM

Kerala Agricultural University Campus, Trichur, Kerala, India

Email: vvradhakrishnan@yahoo.co.in

Coleus (Solenostemon rotundifolius) (Poir.) J.K.Mortan is a vegetable tuber crop of Kerala, India. The flavanoids present in the tubers of coleus have the property of lowering the cholesterol levels in the blood and used for heart diseases and abdominal/colic disorders. The aromatic flavour makes it a delicacy among vegetables and fetches premium price in the market. It is a highly seasonal (short day) crop and only one crop can be taken in a year during July-November. Due to poor seed setting, genetic variability among the genotypes is very low. *In vitro* mutagenesis was attempted in *Coleus* to induce somaclonal mutants using gamma rays in different doses viz 0.5, 1.0, 1.5 and 2 Gy. The percentage of success in producing rooted hardened plants was 67% for 0.5 GY and 40% for 1 Gy. Calli regenerated from 15 irradiated cultures became green and produced meristemoids after 16h of light period. These meristemoids later developed into shoots with leaves in the same regeneration medium after 3 to 4 weeks with subculture. The adventitious shoots were excised and transferred to MS Basal medium containing IBA 1%. Eleven selected tissue culture mutants and its control 'Paipra local' were raised during the off-season (November 2003-April 2004). The control 'Paipra local' did not produce any tubers during off-season. The tuber yield produced by the mutants ranged from 15 g/plant to 75 g/plant. Number of tubers/plant ranged from 8 to 68. Even though these eleven tissue culture mutants produced tubers, tissue culture mutant 9 (TC-9) produced tubers in a commercially exploitable level (1000kg/ha). This mutant assumes great significance, as there is no photo insensitive variety of coleus available at present. It has the potential for development into a new commercial variety, which can give a stable income to farmers all the year round.

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SCREENING FUSARIUM WILT-RESISTANT PLANTS OF BRAZIL BANANA (*MUSA* SPP., AAA) THROUGH EMS INDUCED MUTATIONS FROM MICRO-CROSS SECTIONS CULTURAL SYSTEM

Y.F. CHEN, W. CHEN, X. HUANG, J.T. ZHAO, Q. GONG, X.J. LI, X.L. HUANG

The Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Zhongshan, (Sun Yat-sen) University, Guangzhou, China

Email: huangxl@mail.sysu.edu.cn

Fusarium wilt is recognized as one of the most destructive diseases of banana worldwide. In this study, we screened Fusarium wilt-resistant plants of Brazil banana (*Musa* spp., AAA.) through EMS induced mutations from micro-cross sections cultural system. Micro-cross sections of pseudo stem of *In vitro* banana plantlets were treated with various concentrations of EMS for different duration, then were cultured in shoots induction medium. The results indicated that the survival index and the shoot forming index of the explants dropped with the increasing of EMS concentration and treatment duration. The optimal treatment for the concentration and duration was 300 mM and 60 min respectively. After the optimal treatment for 21 days, 2.2 regenerated shoots averagely could be produced from the explants of micro-cross sections, and the regenerated shoots were then cultured in shoots multiplying medium for 7 days. The stronger shoots were selected and transferred into roots medium for 4 weeks to obtain healthy regenerated plantlets. Hardened-regenerated plantlets were transplanted in green house for 2 months and 100 regenerated plants with vigorous root systems were selected for screening for Fusarium wilt resistance by using early screening technique. The initial disease symptom, yellowing in lower leaves, of susceptible plantlets could be observed after 2 weeks of inoculation with FOC race 4 and the extensive streaking on the most leaves was appeared after 2 months of inoculation. Only five plantlets survived and grew up healthily, which might be putative Fusarium wilt-resistant plants. Five suckers were selected from these 7-month-old putative resistant plants for screening the tolerance again and two sucker-plants showed to be Fusarium wilt-resistant. Further studies on the Fusarium wilt tolerance of next generation of tissue cultural plants derived from these suckers and their genetic background are conducting. We concluded that the application of microcross sections of banana and plantains for regeneration of plants might be a useful alternative to study on *in vitro* mutagenesis for banana improvement.

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INDUCED MUTATIONS IN COLEUS (*SOLENOSTEMON ROTUNDIFOLIUS*) ((POIR.) J. K. MORTAN) - AN UNDER UTILIZED MEDICINAL TUBER

M. ABRAHAM, V.V. RADFAKRISHNAN

Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Trivandrum, Kerala, India

Email: mareen1961@yahoo.co.in

Coleus (*Solenostemon rotundifolius* (poir) is an under exploited vegetable stem tuber crop extensively grown in Southern peninsular India. Native of Africa and member of family Lamiaceae this produces tubers possessing unique medicinal properties due to flavanoids viz α -thujone and β -farnesene. It is helpful in lowering blood cholesterol level. It is a season bound crop and photoperiod insensitive genotypes are not available in germplasms. They possess little natural variability in the population as seed set is poor. Here an attempt was made to induce variability through physical and chemical mutagenesis. Sixty coleus accessions were collected in an eco-geographical survey in southern states of India. Seed tubers of these accessions were evaluated in the field grown as per Package of practices recommendations (KAU, 1993) in RBD with two replications during 1999-2002 at Agricultural Research Station, Mannuthy (15 m above MSL) and at College of Horticulture, Vellanikkara (22.5 m above MSL) between 10° 32' N latitude and 76° 10' E longitudes. The accessions were grouped in to 10 clusters taking 13 characters using Mahalanobis D² statistics. Ten selected superior genotypes were subjected to physical and chemical mutagenesis. The doses used for gamma ray irradiation was 10, 20, 30, 40 and 50 Gy (LD₅₀ 40 Gy) and that used for EMS was 0.2%, 0.4%, 0.6%, 0.8% and 1.0% (LD₅₀ 0.4 %). EMS at 1% was effective in genetically influencing the economic characters. Fourteen superior ones selected from 140 mutants were advanced to testing for yield and photoperiod insensitivity in 3 seasons along with respective five parents. Eight mutants out of nineteen genotypes produced tubers year long. Some of the mutants showed photoperiod insensitivity to tuberisation, a desirable genetic change. Two mutants M 131 and M 61 were promising and photoperiod insensitive and they may be released as varieties.

RESULTS OF MUTATION BREEDING ACTIVITY ON CHRYSANTHEMUM IN POLAND

M. ZALEWSKA¹, N. MILER¹, B. DRZEWIECKA², J. WINIECKI²

¹Laboratory of Biotechnology, Department of Ornamental Plant and Vegetable Crops, University of Technology and Life Sciences, Bydgoszcz, Poland

²Medical Physics Department, Oncology Centre, Bydgoszcz, Poland

Email: zalewska@utp.edu.pl

Our researches concerning somatic mutagenesis in chrysanthemums induced with ionizing radiation have been carried out since 1977. First significant results were obtained *in vivo* when irradiated leaves detached from flowering chrysanthemums were applied. Induction of mutations in subsequent experiments was conducted *in vitro* with the use of the adventitious buds technique for regeneration. Explants were leaves, callus, internodes and single nodes. Except for callus, all explants were taken from irradiated microcuttings growing *in vitro* during the mutagenic treatment. The dose of X- and gamma- rays involved in our experiments ranged from 15 to 25 Gy. For X-rays therapeutic apparatus THX-250 Medicor was used. Gamma radiation was obtained from Co⁶⁰ cobalt source generated by Theratron 780 C. Regeneration was conducted on MS medium supplemented with 0,6 mg·dm⁻³ BAP and 2,0 mg·dm⁻³ IAA. The most spectacular effects were observed when gamma-rays and *in vitro* regeneration were used. All of mutants were solid, non-mericlinal and non-sectorial chimeras. Our experiments show that over a short period from a single mother cultivar one can obtain numerous attractive mutants, thus creating new cultivar groups.

RESISTANCE OF MUTANTS OF SWEET ORANGE INDUCED BY GAMMA-RAYS TO CITRUS CANCKER (*XANTHOMONAS CITRI* SUBSP. *CITRI*) UNDER ARTIFICIAL INOCULATION

J. BELASQUE JÚNIOR¹, R.R. LATADO², A. TULMANN NETO³

¹Depto. Científico, Fundecitrus, Araraquara, Brasil

²Centro APTA Citros Sylvio Moreira-IAC, Cordeirópolis, Brasil

³Centro de Energia Nuclear na Agricultura, Piracicaba, Brasil

Email: rodrigo@centrodecitricultura.br

The sweet oranges have great economic and social importance for Brazil. However, it is susceptible to citrus canker as the majority of citrus species. This disease is caused by *Xanthomonas citri* subsp. *citri*, bacteria that in case of high incidence can result in great economic damage. More resistant cultivars are the best long-term solution for management of citrus canker and one of the approaches can be the production of mutant plants. In a previous work, several induced mutant clones of sweet orange cv. Pera were selected. They showed lower intensity of symptoms of citrus canker in leaves and fruits in evaluations under natural incidence of the disease, in the field. The objective of this study is to assess the resistance to citrus canker of six mutant clones of cultivar Pera and control plants (three different varieties), in experiments of artificial inoculation. The parameters evaluated were: incubation period, diameter of the lesions and area under the disease progress curve (AUDPC), in evaluations every 15 days, until the 147th day. Only the clones 9-1, 9-2 and 9-3 showed lower incidence of disease, represented by the longest period of incubation of the disease, smaller diameter of lesion and lower AUDPC, in all experiment and using average data of the three experiments. This study is one of the first reports of success in citrus induced mutations aimed to obtaining greater resistance to diseases.

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INDUCED *EX VITRO* MUTAGENESIS IN BANANA VARIETY NENDRAN (*MUSA PARADASIACA L.*)

D.S.RADHA DEVI, N. KRISHNAN NAIR

Department of Plant Breeding & Genetics, College of Agriculture, Vellayani, Kerala, India

Email: drdsradhathampi@yahoo.co.in

Induced *in vitro* mutagenesis in banana variety Nendran (*Musa paradasiaca L.*) through shoot-tip culture was explored. The main objectives envisaged include standardization of shoot-tip culture technique for mutagen treatments and analysis of the extent of created variability for all growth, bunch and fruit characters in vM_1 generation of ex-vitro plants. A medium for shoot tip culture of Nendran variety of banana was standardized for adopting induced mutagenesis. The *ex vitro* plants were also analyzed for various growth, bunch and fruit characters. For *in vitro* mutagenesis, isolated shoot tips were exposed to 5, 7.5, 10, 12.5, and 15Gy gamma rays. On comparative analysis of the modifications of MS medium tried, viz., Bower and Fraser (1982), Swamy et al. (1983) (semi solid media) and Krikorian and Cronauer (1984) (liquid medium), it was seen that the medium described by Krikorian and Cronauer gave a better growth and early tissue differentiation in shoot tip culture of banana. In *ex vitro* analysis, plant height and number of leaves 90 days after planting and at harvest were highest in control population. On increasing the dose of gamma ray exposures plant height and number of leaves decreased. The girth of pseudostem 90 days after planting and at harvest was lowest in control population and it increased as the dose of gamma ray exposure was increased. The days taken to shooting, from shooting to bunch maturity and total duration decreased when the dose of gamma exposure was increased from 5 and 7.5Gy. Increasing the dose of gamma exposure resulted in a decrease in bunch weight, bunch length, number of hands per bunch, number of fingers per hand and bunch, total soluble solids and acidity though it is higher than control in irradiated population. Weight and girth of finger, total sugar and sugar: acid ratio increased with increase in dose of gamma exposures.

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STUDY ON DOSE OF ^{60}Co -R RAY FOR INDUCING MUTANTS FROM WILLOW BRANCHES

L.L. NIE, Y. ZHANG, Z.Q. LIU

Tianjin Research Center of Agricultural Biotechnology, Tianjin, China

Email: xiao433@sina.com

Willow is one of the major native species in Tianjin. It is widely planted along the highway and around houses for greening environment. Unfortunately, the salt tolerance of willow is not so good (only 0.2~0.3%). Therefore, we are trying to enhance the salt tolerance of willow through ^{60}Co -r ray radiation. At first, a proper radiation dose is necessary for obtaining mutants from willow branches. The research means to pick out a proper dose by applying ^{60}Co -r ray radiation to branches of willow in order to induce mutants. Branches of willow were treated with ^{60}Co -r ray in different doses of 10Gy, 30Gy, 50Gy, 70Gy, 100Gy and 200Gy, respectively. Treated branches were cut into about 30cm segments and cultivated in the field with untreated branches from the same tree as a control. Survival rate of treated branch segments, sprouting time of new branches and the shoots length from cultivated stock branches were examined. The result showed that the survival rate and shoots length were negatively associated with the radiation dosage. The sprouting time delayed with increasing of the radiation dose ranged in 10-70 Gy. In 100-200Gy, the branches had no sprout at all. According to the survival rate, the sprouting time and the shoots length, 50Gy can be considered as the proper radiation dose. Furthermore, we are doing experiments to identify the mutants with better salt tolerance.

MUTATION INDUCTION USING ACUTE AND CHRONIC GAMMA IRRADIATION ON SOME VEGETATIVELY PROPAGATED ORNAMENTAL CROPS IN THAILAND

P. JOMPUK^{1,2}, A. WONGPIYASATID¹, S. LAMSEEJAN¹ and K. CHUSREEAEOM²

¹Gamma Irradiation Service and Nuclear Technology Research Center, Kasetsart University, Bangkok, Thailand

²Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Bangkok, Thailand

Email: fsciprk@ku.ac.th

In Thailand, mutation induction has been successfully applied to some vegetatively propagated ornamentals by the researchers from Kasetsart University. Several mutants were created in canna, chrysanthemum, portulaca, adenium etc. Plant materials were irradiated at Gamma Irradiation Service and Nuclear Technology Research Center, Kasetsart University. Treated materials were planted and allowed to produce new shoots. Mutants which differ from the original cultivars were isolated and multiplied for observing phenotypic changes. Irradiation procedures and doses of each material were carried out as follows: (a) canna, rhizomes and young shoots of 11 cultivars were treated with acute (15-30 Gy) and chronic (65-110Gy) gamma-irradiation, (b) chrysanthemum, *in vitro* cultures of Taihei variety were irradiated with chronic(62.8 and 112 Gy) and split doses of acute irradiation (30 Gy = 20+10 and 40 Gy = 20+20), (c) portulaca, stem cuttings of 3 *Portulaca grandiflora* cultivars, were acutely irradiated at doses of 10, 20, 40 and 60 Gy. For, adenium, the project on Technology Transfer of Radiation Induced Mutations on Ornamentals to the Farmers was initiated by the researchers of Kasetsart University and in cooperation with the researchers from the Department of Agricultural Extension. The economically ornamental growers were chosen to be trained on induced mutation technology to improve their own ornamental crops. After training, acute and chronic irradiation were provided to the farmers' materials to be investigated and selected for the mutants by themselves under the guidance of researchers. For example, *Adenium somalense* var. *somalense* seeds were acutely irradiated at doses of 200-300 Gy and growing plants of *Adenium obesum* were irradiated with different total doses at the dose rate of 0.96 Gy/hr. Several mutants were obtained by the farmers. The outstanding mutants from gamma rays treated ornamentals, 37 canna, 6 chrysanthemum, 10 portulaca and 3 adenium were registered as new varieties.

IN VITRO ISOLATION, PURIFICATION, RAPID BULKING AND FIELD ESTABLISHMENT OF A PROMISING RADIO-MUTANT PUSA ANMOL FROM SPRAY CHRYSANTHEMUM CV. AJAY

K.V.PRASAD, S. KUMAR, S. KUMAR, D.V.S.RAJU, K. SWARUP, O. SINGH, M.T.PATIL

Division of Floriculture and Landscaping, Indian Agricultural Research Institute (IARI), Pusa, New Delhi, India

Email: kvprasad66@gmail.com

Promising novel coloured radio mutants in the form of chimeras observed in the irradiated population of chrysanthemum cultivar Ajay (pink coloured spray cultivar) were isolated, purified, proliferated and the micro-plantlets are successfully established in the field as a new mutant variety-Pusa Anmol. Chimeral flower buds with a spectrum of colours appeared when irradiated at 15 and 20 Gy, whereas plant mortality (95%) and negative mutants with abnormalities were higher at 30 Gy. The chimeral portion on florets ranged from 50-100%. The irradiated plants of cv.Ajay produced a wide range of novel chimeras (10-19%) with yellow to yellowish pink florets when irradiated at 15Gy. Complete mutants in the form of yellowish pink flower in a flower bunch and complete branch with brick brown color were noticed at 20Gy. A large number of flowers with altered morphology with spoon or flute or cup or quill shape florets were also noticed. The florets with partial chimeras were rejected and only complete chimeral florets or the florets form complete mutants with novel colour were cultured *in vitro* to isolate the novel mutants. MS medium fortified with 10 mg/l Kinetin + 0.50 mg/l NAA was found to be the best. The florets with limited callus were sub cultured on the same medium where maximum number of florets (82%) regenerated producing as many as 34.40 shoots per explant. A proliferation rate of 5-6 times was achieved using MS medium+3.0 mg/l BAP+0.01 mg/l NAA. Rhizogenesis was achieved (80-90%) when cultured on ½ MS + 0.5 mg/l NAA + 60g sucrose. The yellowish pink coloured mutant is successfully established in the field, evaluated for three consecutive years and is identified for release as Pusa Anmol. The new mutant is also photo/ thermo intensive and flowers twice or thrice in a year along with its parent cultivar.

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INTEGRATING BIOTECHNOLOGICAL ADVANCEMENTS WITH INDUCED MUTAGENESIS: NEW OPPORTUNITIES FOR HORTICULTURE WITH SPECIAL REFERENCE TO *VITIS VINIFERA*

R. PATHIRANA¹, F. CARIMI², A. CARRA², L-H. CHEAH¹¹New Zealand Institute for Crop & Food Research Limited, Palmerston North, New Zealand²Istituto di Genetica Vegetale, Sezione di Palermo, Palermo, Italia

Email: pathiranar@crop.cri.nz

Mutation assisted breeding (MAB) is a highly effective way of enhancing natural genetic resources. Increasing mutation rates is important when natural variation is insufficient, when a single trait in a desirable variety needs improvement or when the species is apomictic or seedless. Development of cell and tissue culture-based regeneration techniques in many horticultural crops and increased knowledge of biochemical pathways provide ample opportunities to exploit MAB at an advanced level. Traditionally buds, bulbs, corms, cuttings or whole plants are treated in vegetatively propagated crops, but this results in chimeras. We have initiated a collaborative project to use embryogenic cell lines of Sicilian and international cultivars of *Vitis vinifera* to produce mutant populations for use in genetic studies as well as for selecting disease-resistant mutants *in vitro*. We have established embryogenic cell lines of Sicilian and New Zealand cultivars and established the LD 20 for ethylmethane sulphonate for several cultivars. The cell lines will be subjected to treatment with mutagenic agents, and mutants with tolerance to Botrytis toxins will be selected *in vitro* and regenerated. With the genome sequencing of *Vitis vinifera* being completed and the resistance to genetically modified crops in both Europe and New Zealand very high, our mutant collection will provide a valuable resource for genetic studies of this species. Acknowledgements: This work is funded in New Zealand by grants from the Sustainable Farming Fund of the Ministry of Agriculture and Forestry and the International Science & Technology Linkages Fund of the Royal Society of New Zealand, and in Italy by the Regione Siciliana, Assessorato Agricoltura e Foreste project "Coltura *in vitro* per la conservazione del germoplasma vegetale siciliano minacciato da erosione genetica".

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MUTATION BREEDING IN PHILIPPINE *SPATHOGLOTTIS* ORCHIDS

F. AURIGUE¹, R. LOBIANO¹, M. GONZALES², T. ROSARIO³¹Philippine Nuclear Research Institute, Diliman, Quezon City, Philippines²Central Luzon State University, Muñoz, Nueva Ecija, Philippines³University of the Philippines, Los Baños College, Laguna, Philippines

Email: fbaurigue@pnri.dost.gov.ph

Attempts to create genetic variability in *Spathoglottis kimballiana* var. *angustifolia* by mutation induction using the chemical mutagen colchicine was not successful. On the other hand, the physical mutagen gamma rays resulted in changes in morphological characteristics on flowering plants that had been subjected to 10 Gy acute radiation at protocorm stage. Most prominent are purple pigmentation on the flower stalk, shorter internodes or distance between flowers, thicker substance of individual flowers and wider or stouter leaves. The most desirable selection was successfully used as a female parent in breeding of *Spathoglottis* 'Lion of Singapore.' The characteristics of the hybrid produced were entirely different from those developed and registered by the Singapore Botanic Gardens. Radiosensitivity studies on this native species as well as *Spathoglottis plicata*, *S. tomentosa* and *S. vanoverberghii* showed that survival of irradiated protocorms decreased with increasing dose from 0 to 50 Gy. The average height of seedlings and length of longest root were significantly affected by gamma radiation. Several qualitative characteristics considered as results of mutations are lack of pigments or albinism, purple pigmentation on leaves, forked leaves, split seedlings or furcation, and multiple branching. However, majority of the putative mutant seedlings did not survive outside the culture vessel or reverted back to normal after growing under ambient conditions. Lethal dose after removal from culture vessel was found to be 20 Gy. For *Spathoglottis plicata*, embryos within the irradiated fruits produced by artificial self-pollination of flowers did not germinate when subjected to 30 Gy and higher dose levels of acute gamma rays. Meanwhile, 10 Gy of gamma radiation enhanced both shoot growth and root elongation as compared to the control and those at 20 Gy.

USE OF IRRADIATION FOR THE INDUCTION OF MUTATION IN OYSTER MUSHROOMS FOR IMPROVEMENT OF STRAINS

P. HUZAR FUTTY BEEJAN, R. NOWBUTH

Crop Department, Agricultural Research and Extension Unit, Quatre Bornes, Mauritius

Email: areucrop@intnet.mu

In order to induce mutants with improved characteristics in terms of good yield and adaptability to a wider range of temperatures, 5 parent strains of *Pleurotus*, a species of edible mushroom, were subjected to two types of irradiation namely gamma-irradiation and UV-irradiation. Mycelial plugs of the actively growing parent strains were subjected to doses of gamma-radiation ranging from 5 to 400 Gy using the ^{134}Cs radioisotope. Irradiated mycelia were assessed in laboratory experiments and were used to prepare mother flasks, spawn and fruiting bags for production experiments. Certain stimulatory effects of gamma-irradiation were noted on mycelial growth rate and yield of fruiting bags at different doses. However, these effects were not consistent. Similarly, for UV-irradiation, multispore suspensions of the 5 parent strains were subjected to varying exposure times from 3 to 20 hours to UV-rays. After plating, vigorously growing mycelia were evaluated in laboratory assessments and production trials. Decrease in viability was noted in several strains after repeated subcultures and storage. Viable UV-irradiated strains exhibited similar stimulatory effect as in gamma-irradiation at certain exposure times. Again, erratic effect at varying exposure times was noted. Based on these results, the irradiated strains showing stimulatory effect on mycelial growth and yield have been selected for further evaluation at different agroclimates in Mauritius and for other breeding works.

AVOCADO BREEDING IN CUBA. STATE OF THE ART BIOTECHNOLOGIES

O. COTO¹, J.L. FUENTES^{2*}, A. ALVAREZ², N.N. RODRÍGUEZ¹, M. MACHADO¹, L. SANTIAGO², Y. VALDÉS², I.M. RAMÍREZ², C. COLLAZO¹, M. VERNHE², M. RAMOS LEAL³, M. GUERRA², S. ALTANEZ², E.F. PRIETO², B. VELÁZQUEZ¹, J.A. RODRÍGUEZ¹, V.R. FUENTES¹, J. CUETO¹, D. BECKER⁴, W. ROHDE⁴, G. BOLAND⁵, A. STECHYSHYN-NAGASAWA⁵, M-A. RENAUD⁵, A. MARTÍNEZ¹

¹Instituto de Investigaciones de Fruticultura Tropical, C. Habana, Cuba

²Centro de Aplicaciones Tecnológicas y Desarrollo Nuclear, C. Habana, Cuba

³Departamento de Microbiología, Facultad de Biología, Universidad de La Habana, C. Habana, Cuba

⁴Max-Planck Institut für Züchtungsforschung (MPIZ), Köln, Germany

⁵University of Guelph, Ontario Agricultural College, Ontario, Canada

*Current address: Escuela de Biología, Facultad de Ciencias, Universidad Industrial de Santander, Bucaramanga, Colombia

Email: orlandocoto@iift.cu

Genetic diversity studies of avocado germplasm: Genetic diversity among avocado varieties cultivated in Cuba was undertaken considering 22 agronomic and morphological traits, 12 AFLP and 16 SSR primer combinations. Using agro-morphological traits, cultivars clustered within racial groups confirming the ecological classification and a catalogue was prepared. AFLP and SSR markers were useful for providing a more accurate estimate of genetic distances between the cultivars. Identification and characterization of soil-borne isolates: A collection of *Phytophthora* spp. and *Phytium* spp. strains isolated from commercial avocado orchards was constructed. Identification and characterization of these isolates was done based on morphological, physiological and molecular markers (ITS and *Lpv* 3 primers pairs) differentiating both genus and confirming the usefulness of using a combined approach for an accurate identification. A first description about *P. palmivora* affecting avocado trees in Cuba was reported based on morphological traits, maximum temperature of growing, amplification pattern using ITS primer combinations and sequencing information of the amplified product obtained with *Lpv* 3 primer pairs. Radiosensitivity curves useful for breeding purposes: Radiosensitivity curves to Gamma rays were determined in 'Duke-7' and 'Hass' cultivars commonly used as rootstocks. LD₅₀ values were calculated to be 28 and 27 Gy for each cultivar, respectively. Selection for salinity conditions using zygotic embryos: Survival curves (LD₅₀) were calculated for 'Duke-7' (56 mM of NaCl), 'Jose Antonio' (66 mM) and 'Catalina' (148 mM). LD₂₀ and LD₁₀ values, useful for breeding purposes, were also determined. Breeding avocado rootstocks using biotechnologies: An *in vitro* propagation method for avocado breeding purposes was optimized using zygotic embryos, combined with the LD₅₀ values for Gamma rays and LD₅₀ values for NaCl to obtain mutant lines from rootstock 'Duke-7' with improved salt tolerance. Putative mutant lines were planted in the field for future segregation analysis. Thus, an avocado germplasm bank was established.

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INDUCTION OF VARIATION IN *COLEUS FORSKOHLII* BRIQ., THROUGH *IN VITRO* MUTATION TECHNIQUE

M. VELMURUGAN¹, K. RAJAMANI¹, P. PARAMAGURU¹, J.R. KANNAN BAPU², R. GNANAM³

¹Horticultural College and Research Institute, TamilNadu Agricultural University, Coimbatore, India

²Center for Plant Breeding and Genetics, TamilNadu Agricultural University, Coimbatore, India

³Center for Plant Molecular Biology, TamilNadu Agricultural University, Coimbatore, India

Email: hortmrvelu@yahoo.com

In vitro mutation in *Coleus forskohlii* was carried with shoot tip and callus explants. The biometrical characters of shoot tip derived *in vitro* mutants of coleus in TS₁M₁ generation exerted a gradual reduction with increase in dose of mutagen for the traits like plant height and number of laterals plant⁻¹. However, the number of leaves plant⁻¹ exerted 2.22 and 2.22 per cent increase over the control at 120 and 180 DAP respectively in 700 μM EMS treatment. Among the mutagenic treatments, the highest forskolin content (0.80 per cent) was observed in 25Gy gamma rays + 750 μM EMS. However, the callus derived *in vitro* mutants in TC₁M₁ generation produced tallest plants (51.00 and 63.85 cm) at 120 and 180 DAP respectively in 5Gy gamma rays + 175 μM EMS treatment. The gradual increase in dose of mutagen expressed a reduction in number of tuber plant⁻¹, length of tuber, fresh and dry tuber yield plant⁻¹. Interestingly among the quality parameters, the treatment 1.50 kR gamma rays + 200 μM EMS produced 188.89 per cent increase over the control for the trait forskolin content.

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EFFECT OF SLOW IRRADIATION OF GAMMA RAYS ON GROWTH, YIELD AND QUALITY OF *COLEUS FORSKOHLII* BRIQ.

M. VELMURUGAN¹, K. RAJAMANI¹, P. PARAMAGURU¹, J.R. KANNAN BAPU², R. GNANAM³

¹Horticultural College and Research Institute, TamilNadu Agricultural University, Coimbatore, India

²Center for Plant Breeding and Genetics, TamilNadu Agricultural University, Coimbatore, India

³Center for Plant Molecular Biology, TamilNadu Agricultural University, Coimbatore, India

Email: hortmrvelu@yahoo.com

Terminal cuttings of coleus cv Garmai is slowly irradiated by using lead filter in the gamma chamber. It has been observed that LD₅₀ of gamma rays was observed at 40Gy dose. Similarly the LD₅₀ for EMS was 1.00 %. Based on this data treatments were formulated in Randomized Block Design and the terminal cuttings were planted in the main field for observation. The results of the study V₁M₁ indicated that the combined effect of mutagens at higher dosage shows reduced growth characters than the untreated control. The treatment with 50Gy gamma rays + 0.5 % EMS exhibited maximum number of tubers (25.50) and maximum length of tubers (17.60 cm) than all other treatments. Maximum fresh and dry weight of tubers (580.50 and 71.20 g) was noticed by the untreated control. The maximum forskolin content (0.66 %) was exerted by the treatment 20Gy gamma rays + 0.5 % EMS. However, most of other treatments exhibited same forskolin content (0.42 %). The secondary shoots were considered as the second vegetative generation. Secondary shoots were obtained by cutting back the primary shoot and planted for the study of V₂M₁ generation. The data on plant height expressed at higher side than that earlier generation. The quality parameters like essential oil (0.09 %) and total alkaloids (1.05 %) was greater at very high doses of mutagen. However, the occurrence of forskolin mutant was stabilized over the generations in 20Gy gamma rays + 1.00 % EMS treatment. From the study it was inferred that sudden exposure of materials causes more lethality with poor field establishment and the chance of occurrence of mutants were comparatively lesser.

IN VITRO MUTAGENESIS IN *DENDROBIUM CV SONIA*

V.L. SHEELA, S. ANITA, S. SARADA, K. RAJMOHAN

Department of Pomology and Floriculture, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Email: sheela_jayagopan@yahoo.com

The heterozygosity and the vast variability in orchids is useful in mutation breeding. *In vitro* propagation methods are available and mutants can be propagated in a short time. It was aimed to evolve mutant varieties of the orchid *Dendrobium cv sonia* with desirable floral traits. Shoot tip explants excised from actively growing adult plants were subjected to initial culture establishment. Vacin and Went medium with BA – 1mg^l⁻¹ and NAA -1.5mg^l⁻¹ was used for bud initiation, then subcultured on half strength MS medium with BA – 1mg^l⁻¹ and NAA -1mg^l⁻¹ to induce protocorm like bodies (PLBs). PLBs developed 3 months after inoculation were subcultured every three weeks for further multiplication. Pilot experiments were conducted exposing the PLBs to different doses of gamma rays (10, 20, 30, 40, 50, 100, 150, and 200Gy from a cobalt-60 source at a dose rate of 10Gy per minute. Cultures were observed for signs of variations or other detrimental effects. Browning of some PLBs were observed at higher doses (40 to 200Gy). Except at 200Gy of gamma rays, most of the PLBs developed small shoots. PLBs were subcultured and transferred to a multiplication medium (half strength MS medium with BA – 2mg^l⁻¹ and coconut water -15 per cent). Multiplication rate of the PLBs was maximum with 20Gy of gamma rays (35) followed by 10Gy (28). The highest number of healthy shoots was at 20Gy (11) followed by 30Gy (10). Most of the PLBs did not survive at 200Gy. Various abnormalities were encountered as a result of the irradiation treatments. Plantlets showing stunted growth as well as high rate of elongation were observed at 20Gy of gamma rays. Some of the plantlets had exhibited fasciation and serration of the leaves. Development of PLBs as well as multiplication of shoots were also observed in all the treatments. Plantlets have started flowering and variations are hopefully anticipated.

THE BREEDING OF *ARTHROSPIRA PLATENSIS* MUTANTS WITH GOOD QUALITY AND HIGH YIELD INDUCED BY SPACE FLIGHT

X.Y. HOU, Y.M. LU, Z.L. ZHANG, W.B. WANG

Shenzhen Nongke Group Corporation, Guangdong, China

Email: houxueying@21cn.com

Arthrospira platensis mutant PNK-2 had been bred from *A. platensis* mutants which had been induced by space flight. PNK-2, with good quality and high yield, suits for outdoor large scale production. Comparing with the initial ST-6: the helix number of PNK-2 was 12~18; the average length of algae body, thread pitch, helix width, diameter of trichome and the rate of large-scale production were 764.31µm, 52.98µm, 18.75µm, 6.02µm and 10g·(m²·d)⁻¹ respectively; the increasing rate was 166.52%, 5.88%, 8.19%, 12.31% and 22.89% respectively. The content of protein, chlorophyll, β-Carotene and phycocyanin in PNK-2 were 69.57%, 1.01%, 0.16% and 14.70% respectively; the raising rate were 8.31%, 8.60%, 6.67% and 6.68% respectively. the γ-linolenic acid content of PNK-2 was 0.63%, reducing 3.08%. The results showed that PNK-2 was a new *A. platensis* strain with good quality and high yield.

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IMPROVEMENT OF TARO (*COLOCASIA ESCULENTA* VAR *ESCULENTA*) THROUGH *IN-VITRO* MUTAGENESIS

S. SEETOHUL, V. MAUNKEE, M. GUNGADURDOSS
Agricultural Research and Extension Unit of FARC, Quatre-Bornes, Mauritius
Email: areucrop@intnet.mu

An *in-vitro* mutation breeding program was implemented to improve taro (*Colocasia esculenta* (L.) schott) for resistance to the fungus *Phytophthora colocasiae*. Apical shoot tips used as explants were cultured on Murashige and Skoog medium (1962) supplemented with varying concentrations of Indole-3-acetic acid, Thidiazuron (1-Phenyl-3-(1,2,3-thiadiazol-5-yl) urea (TDZ) and N6-benzylaminopurine (BA). Optimal culture initiation and multiplication was obtained on MS supplemented with 10 mgL⁻¹ of IAA and 0.9 mgL⁻¹ TDZ/BA at 20 mgL⁻¹ respectively. Explants were exposed to various doses of Gamma radiation and effective mutation dose that causes 30% reduction in growth (LD₃₀) was found to 7.65 grays. 9 accessions of *colocasia species* (dasheen and eddoes type) and 2 from *xanthosoma species* were used for morphological and molecular characterization. 44 morphological characters were assessed and analysed with an unweighted pair group method using an arithmetic average (UPGMA). For RAPD analysis, eight 10 mer random primers were selected as they amplified more than 5 polymorphic bands. UPGMA cluster analysis using Nei and Li's distance coefficient were then performed. Both morphological and molecular analysis revealed low genetic diversity among germplasm accessions. Rapid primers screened will be useful for characterization of mutant line showing resistance to leaf blight and the micropropagation methodology developed will be useful for rapid multiplication of mutants.

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PRELIMINARY RESULTS OF MUTATION BREEDING ON HIGH QUALITY TURKISH SWEET CHERRY CULTIVAR "0900 ZIRAAT"

B. KUNTER¹, M. BAŞ², Y. KANTOĞLU¹, M. BURAK³
¹Turkish Atomic Energy Authority (TAEK), Sarayköy Nuclear Research and Training Center (SANAEM),
Division of Agriculture Ankara, Turkey
²Atatürk Horticultural Central Research Institute, Yalova, Turkey
³Republic of Turkey, Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Research
Email: burak.kunter@taek.gov.tr

In Turkey, sweet cherry production reached at 195,000 tons in the last two years. This value is 13% of the world production. Globally USA was the largest exporter of cherries in 2004, accounting for 21,2 % of world trade, just ahead of Turkey, which accounted for 20,07 %. The major high quality and exporting sweet cherry variety is 0900 Ziraat. It is a mid to late season variety with heart fruit shape, pink and very firm flesh and excellent flavor. Contrary to good traits, 0900 Ziraat is self incompatible, trees tends to grow vigorously with low yield on standard rootstocks. Although has some disadvantages there is huge demand from exterior market for 0900 Ziraat sweet cherry cultivar. In this research, gamma irradiation based mutation breeding technique was applied for improving of 0900 Ziraat. For this aim scions were irradiated 25, 30, 35, 40, 45, 50, 55, and 60Gy doses with Co⁶⁰ as a source of mutagen. After irradiation scions were budded on *P. avium* rootstock in greenhouse, located on Ministry of Agriculture, Yalova Atatürk Horticultural Central Research Institute. At the end of the first year young trees were transferred from greenhouse to orchard. According to 60 days data "efficient mutation dose" was calculated. After the first year, which was including physiological effects, trees were characterized according to pomological traits such as fruit weight (g), peduncle length (cm), fruit width (cm), fruit height (cm), seed weight (g), soluble solid contents (%), yield (g), and cracking rate (%). Among the 371 living mutant trees in M₁V₇ generations, nominee of dwarf, large fruits (>30 mm) and high yield types were observed and data were observed. According to the data 58 mutant variety candidate were selected for advance observations.

CREATION OF A WHITE INFLORESCENCE COLOUR CULTIVAR OF *ALPINIA PURPURATA* THROUGH THE COMBINATION OF INTERGENERIC HYBRIDIZATION AND MUTAGENESIS

L. FEREOL^{1,2,3}, F. LUC-CAYOL¹, L. LUCE⁴, M. GUITTEAUD¹

¹Antilles Biotechnologies, Fort de France, Martinique

²CIRAD, Station de Neuf-Chateau, Belle Eau, Guadeloupe

³INRA, Pointe à Pitre, Guadeloupe

⁴Centre Hospitalier Universitaire, Service Oncologie, Pointe à Pitre, Guadeloupe

Email: leonidas.fereol@cirad.fr

A new cultivar of *Alpinia purpurata* is created that is the result of the combination of intergeneric hybridization and induced mutagenesis. Artificial sexual crosses between the two genus *Alpinia purpurata* and *Etilingera elatior* (Zingiberaceae) have been performed. They generated fruits containing seeds. These seeds were irradiated with gamma rays. Such seeds were planting and gave seedlings which were observed. It was found that a single plant from these irradiated seeds possessed the characteristic of white inflorescence colour. According our knowledge it has never been observed in *Alpinia purpurata* or *Etilingera elatior*. This plant has been propagated through vegetative multiplication. It gave rise to a homogenous clone true-to type called "Madikera white". Getting this white colour is a big improvement relative to the ornamental value of the parents.

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THE RESULTS OF *IN VITRO* MUTAGENESIS BREEDING IN CHRYSANTHEMUM

D.T. BANG, N.P. DOAI, V.T. HANG, L.T. LIEU, N.G. NHUNG, N.G. LY et al.

Institute of Agriculture Genetics (AGI), Hanoi, Vietnam

Email: daothithanhsbang60@yahoo.com

In Vietnam, recent years only, mutation breeding has been applied successfully for ornamental crops and decorative crops. In the past, the number of vegetative propagated crops and flower plant induced through mutagenesis was limited due to the various reasons such as the limitation of multiplication system after mutagenesis or little attention of scientists and researchers and so on. Furthermore, in vegetative crops like chrysanthemum, chimerical appearance after irradiation often cause difficult problems in breeding. Therefore, mutagenesis technique combined with *in-vitro* culture was effectively applied for vegetative crops breeding. Especially, in chrysanthemum thanks to the extremely ability to speed up multiplication a huge number of explants in the short time. Moreover, it can be shorten the breeding cycle. Under sponsor of IAEA/VIE/5014 (period 2003-2007) project, we carried out research on *in vitro* mutagenesis treatment to diverse the flower colour and structure of chrysanthemum. Several beautiful colour mutant lines of chrysanthemum have been developed by our group through mutagenesis induction on callus. There was not segregation at two mutant lines of strong yellow and pink. They are the promising new lines. Those should be evaluated as new varieties in the near future by scientific council, Ministry of Agriculture and Rural Development, Vietnam. Up to now, the promising new lines developing and giving high economical effect in large area in provinces as Hanoi, Hatay, Bacninh, Hungyen, Haiduong.

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GAMMA IRRADIATED VARIANTS OF BANANA CULTIVAR GIANT CAVENDISH (AAA) AND THEIR CHARACTERIZATION USING RAPD MARKERS

T.R. GANAPATHI, MEENAKSHI SIDHA, P. SUPRASANNA, S.F. D'SOUZA

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: prasanna@barc.gov.in

Bananas and plantains are one of the most important fruit crops and a staple food for millions in the tropical and subtropical countries. Owing to the constraints of vegetative propagation, sterility and triploid nature of most of the cultivars, mutation breeding and biotechnological methods are useful tools for developing cultivars resistant to diseases and pests. Herein, multiple shoot cultures of banana Giant Cavendish (*Musa* spp. AAA group) were gamma-irradiated (5, 10 and 30 Gy). Field evaluation of these exhibited variation for several agronomic traits including dwarf stature and early flowering. The selections are now being studied for stability. Molecular analysis using RAPD markers indicated polymorphism and some of the dwarf selections were analyzed using dwarf-specific SCAR marker confirming their dwarf behavior. The results suggest that the gamma irradiation is useful for the isolation of agronomic variants in Cavendish bananas.

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PRELIMINARY STUDY ON RADIATION SENSITIVITY OF *IN VITRO* CULTURES OF *XANTHOSOMA* (MACABO) IN CAMEROON

X. NDZANA, S. ZOK, A.E. SAMA

Jay P. Johnson Biotechnology Laboratory, IRAD Ekona Regional Centre, Buea, Cameroon

Email: xavndzana@yahoo.co.uk

In vitro grown cocoyam genotypes were exposed to ^{60}Co γ -irradiation at varying doses (4 to 20 Gy) in order to determine a suitable lethal dose (LD) for eventual use as orientation for selection of effective mutagenic treatments that can induce useful genetic changes. The LD₅₀ was more appropriate than the LD₃₀ to be used as orientation for dose selection. The three cocoyam cultivars differed in their reaction to the irradiation, with the Red accession being more sensitive than the White. Some phenotypic changes following irradiation included growth reduction and transformation of plantlet leaf shape, indicating thereby the possibility of distinct changes in the plants' genetic makeup. This study indicates that variability within cocoyam species could be increased through induced mutations at a dose rate of ~ 9 Gy.

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PRODUCTION OF DOUBLED HAPLOIDS IN BANANA

L. HAKIM, G. AHMED, H. KABIR, A.N.K. MAMUN, P.K. ROY, M.R. ISLAM, R. KHAN

Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh Atomic Energy

Commission, Dhaka, Bangladesh

Email: lhakim@dhaka.net

The production of doubled haploids in banana was achieved through anther culture of diploid banana *Musa balbisiana* var. Bichikala (BB). Calli were induced by culturing anthers onto MS or N6 based medium. MS medium comprised Morel vitamin and supplemented either with 2.5 mg⁻¹ 2,4-D and 1.0 mg⁻¹ kinetin (MS1) or with 1.0 mg⁻¹ BA, 0.4 IAA mg⁻¹ and 500 mg⁻¹ caesin hydrolysate (MS2) while N6 medium contained basic salts and added with 1.0 mg⁻¹ BA, 0.4 IAA mg⁻¹ and 500 mg⁻¹ caesin hydrolysate. Some of these calli produced embryoids within a span of 8-10 weeks upon transfer to MS medium fortified with 0.5 mg⁻¹ BA and 0.4 mg⁻¹ IAA. These calli/embryoids subsequently developed into shoots when sub-cultured on the same medium but with reduced concentration of IAA (0.1 mg⁻¹). No plants were produced from calli originated from 2,4-D and kinetin supplemented MS medium (MS1). Roots were produced in MS medium devoid of growth regulators. A total of 39 plants were regenerated from anther-derived calli/embryoids. Six of these plants were tested for ploidy level and all of them showed diploid number of chromosome. Regenerated plants were acclimatized and transferred to the field for further study.

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DEVELOPMENT OF A METHODOLOGY FOR THE PROPAGATION OF 'CALCUTTA 4' (AA) AND PLANTAIN GENOTYPES FROM EMBRYOGENIC CELL SUSPENSIONS AND ITS INTERFACE WITH MUTATION BREEDING

J. LOPEZ, N. MONTANO, D. REINALDO, A. RAYAS, M. CABRERA, J.C. VENTURA, V. MEDEROS, A. SANTOS, M. BASAIL R. SANCHEZ, M.I ROMAN
 Instituto de Investigaciones de Viandas Tropicales (INIVIT), Cuba
 Email: jlopezbiotec@yahoo.es

Bananas and plantains represent a major staple food for many millions of people in the tropics and subtropics. In Cuba, this crop constitutes a high priority of the national food program because of its capacity of producing fruit all year round, high demand and diversity of use, however, Black Sigatoka disease, caused by the leaf pathogen *Mycosphaerella fijiensis*, and pests and stress climatic conditions, has resulted in significant yield losses in this culture, which increases the production costs considerably. Somatic embryogenesis combined with mutation induction into *in vitro* culture mutations has not been studied in depth probably due to the low plant regeneration percentage of different *Musa* genotypes. Taking into consideration, the previous information following research objects are being developed: to develop a new methodology for developing somatic embryogenesis in different cultivar AAB, and cv. 'Calcutta 4', to develop a methodology for mutation induction with irradiations gamma rays to embryogenic cell suspensions and study anther culture of diploid cultivars. The genetic stability of plants obtained via embryogenesis through embryogenic cell suspensions showed the possibility to use the new methodology for developing somatic embryogenesis in different cultivar. From the results obtained for each cultivar, it is recommended to irradiate cv. 'Calcutta 4' at 50 Gy and cv. 'CEMSA ¾' at 80 Gy. Irradiations with gamma rays to embryogenic cell suspensions resulted in a shorter stature in regenerated plants. Besides, plants of French plantain type were obtained from cultivar CEMSA ¾. In Calcutta 4 the irradiation effect caused colour changes in plant pseudo-stems, and also deformed leaves were shown, but did not cause changes in the cultivar susceptibility to Black Sigatoka disease. In anther culture of diploid, callus induced from the state of uninucleate development was higher in relation to the other states studied and the regeneration plants are in progress.

IAEA-CN-167-266P

DEVELOPMENT OF CASSAVA GERMPLASM RESOURCES FOR THE IMPROVEMENT OF HIGH VALUE ROOT QUALITY TRAITS THROUGH INDUCED MUTATION AND MARKER AIDED BREEDING IN NIGERIA

E. OKOGBENIN^{1,2}, C. EGESI¹, E. MBANASO¹, F. OGBE¹, M. FREGENE²
¹National Root Crops Research Institute (NRCRI), Umudike, Umuahia, Abia State, Nigeria
²International Center for Tropical Agriculture, Cali, Colombia
 Email: Eokogbenin@yahoo.com

Improvement of cassava for increased beta carotene and protein content, dry matter content and delayed post-harvest physiological deterioration is important for increased income and improved livelihood for poor farmers. Irradiation was used in this study to induce mutation using *in vitro* culture plantlets and botanical seeds. Gamma irradiated *in vitro* culture plantlets of varieties SM 909-25 and Col 2215 identified 12 clones with dry matter content above 40% and five clones with high beta carotenoid content. Cassava mosaic disease (CMD) resistant varieties/land race are presently being used for mutation breeding to reduce the effect of this devastating disease in Africa and irradiated plants are being selfed to generate M₂ populations. Crosses of Latin American varieties having cream or deep yellow coloured-roots and crude protein contents of 3-8% were made to generate populations with combined enhanced beta carotene and high protein contents in cassava roots. A total of 1555 seeds were derived from 17 parents. They were genotyped in marker-assisted selection (MAS) for resistance to cassava mosaic disease (CMD) using one SSR marker, NS158 and a SCAR marker, RME 1. Fourteen percent (138 plants) were selected for CMD resistance and are being evaluated in the breeding scheme. Delayed post harvest physiological deterioration (PPD) have been introgressed from *Manihot Walkerae* and and backcross populations developed. About 150 SSR polymorphic markers were used for mapping in delayed PPD population resulting in the identification of three putative markers for this trait. A BC₂ population for delayed PPD and CMD resistance have been developed. Initial results obtained with irradiated germplasm indicates that induced mutation can rapidly facilitate the development of value-added traits in cassava. The development of new populations for root quality traits using parental lines from Latin America in combination with MAS are also contributing immensely in the breeding for value-added traits in Nigeria.

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MUTATION INDUCTION IN PHILIPPINE BANANAS C.V. 'LAKATAN' THRU GAMMA RAY IRRADIATION

E.K. SALES¹, R.R.C. ESPINO²¹University of Southern Mindanao, Kabacan, Cotabato, Philippines,²University of the Philippines Los Baños, Los Baños, Laguna Philippines

Email: ekalaw@yahoo.com

Banana is the most important crop grown in the Philippines. Among the cultivars grown, 'Lakatan' is the most popular and commands a higher price in the local market. Despite high production, losses due to over ripening, bruising and short shelf life is one of the major constraints in a successful banana industry. The use of chemicals for delayed ripening however, remains an issue of concern due to economic and organic products advocacy. Thus, development and generation of new improved 'Lakatan' cultivar thru gamma ray irradiation was carried out. Mutation was induced in 'Lakatan', a popular Philippine cultivar using gamma ray irradiation. Radio sensitivity was established at 50Gy. Morphological, cytological and molecular analysis done showed significant variations between the irradiated samples and the non-irradiated plants. In terms of morphological parameters, gamma ray irradiation affected leaf traits resulting to increased leaf width, leaf length, and number of leaves. Stem girth on the other hand was significantly reduced. Cytological observations showed that gamma irradiation increased the epidermal width, leaf thickness and size of stomates but reduced the number of stomates. For post harvest attributes, gamma irradiation prolonged the shelf life of banana fruits from 11 days to 14 days. Molecular analysis showed that some markers (RAPD and AFLP) were able to detect unique bands in samples irradiated with 50Gy while the SSR markers did not detect any band difference between the irradiated samples and the control.

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INDUCED MUTATION BREEDING AS A TOOL FOR THE GENETIC IMPROVEMENT OF CASSAVA LANDRACES FOR HIGH STARCH AND DELAYED POST HARVEST DETERIORATION (PPD) IN GHANA

E.Y. PARKES, M. QUAIN, E.A. KWARTENG, S. KANTANKA, B. PREPRAH, E. LOTUS, J.A. AGYEI

Crops Research Institute, Kumasi, Ghana

Email: elizabeth_okai@yahoo.co.uk

Cassava a major staple in Ghana that contributes 22% of Agricultural Gross Domestic Product provides high and reliable cheap source of carbohydrates for over 200 million people has assumed an industrial and cash crop status in Sub-Saharan Africa. However, cassava suffers from pest and diseases, with the starchy roots rapidly deteriorating within 24 to 72 hours after harvest resulting in close to 20% loss in the fresh roots. Cassava crosses for the introgression of useful genes for delayed Post harvest Deterioration where Molecular Markers Assisted selected (MAS) inter-specific hybrids with delayed PPD properties were crossed with preferred land races. Inter-specific hybrids of *walkererea* evaluated showed remarkable disease resistance in all ecological zones tested, PPD score Clone CR 52A-25 additionally showed no signs of post harvest physiological deterioration after the seventh day of harvest and AR 14-10 had unique starch properties for potential industrial use. Induced mutation of tissue culture plantlets of four preferred cassava landraces "Cedi bankye", "Sisipe 166", "Debor" (ancient variety) and "Dabodabo" (zigzag plant architecture with stay green properties) at two levels of doses 15Gy and 12 Gy were at V₅ stage hardened and established in a replicated field trial in three locations. Initial results showed high root yields for doses of 12Gy and remarkable resistance for ACMD (1.5 score) for all mutants at 3MAP, *Debor* at both doses showed the best vigour for all locations. Induced mutation of botanical seeds from landraces and inter-specific hybrids of *walkererea* at 200 and 300Gy showed moderate disease reaction and plant vigor. The mutants would be tested alongside the control for starch and PPD at 9MAP and 12MAP.

ROLE OF CLASSICAL MUTAGENESIS FOR DEVELOPMENT OF NEW ORNAMENTAL VARIETIES

S.K. DATTA

CSIR, Emeritus Scientist, Madhyamgram Experimental Farm, Bose Institute, Kolkata, India

Email: subodhskdatta@rediffmail.com

Mutation techniques by using ionizing radiations and other mutagens have successfully produced and commercialized world wide quite a large number of new promising varieties in different crops including ornamental plants. Considering the importance of induced mutagenesis, extensive work on classical mutagenesis has been carried out by the author and has successfully developed 76 new mutant varieties using gamma radiation in different ornamentals. Research carried out generated voluminous literature on radio-sensitivity, selection of materials, methods of exposure to gamma rays, suitable dose, detection of mutations, isolation of mutants and commercial exploitation of mutants. Different treatment methodology like recurrent irradiation, colchicine treatment, and mutation frequency and spectrum have been precisely determined for successful development of new varieties. Changed flower type, development of appendage like structure on florets, striped flowers and induction of tubular florets are few interesting observations in chrysanthemum. Development of late blooming varieties in chrysanthemum have tremendous commercial impact. Studies have clearly proved that classical mutagenesis can be exploited for the creation of new and novel ornamental varieties of commercial importance.

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MANAGEMENT OF CHIMERA AND *IN VITRO* MUTAGENESIS FOR DEVELOPMENT OF NEW FLOWER COLOR/SHAPE AND CHLOROPHYLL VARIEGATED MUTANTS IN CHRYSANTHEMUM

S.K. DATTA¹, D. CHAKRABARTY²

¹CSIR, Emeritus Scientist, Madhyamgram Experimental Farm, Bose Institute, Kolkata, India

²Floriculture Laboratory, National Botanical Research Institute, Lucknow, India

Email: subodhskdatta@rediffmail.com

Induced mutagenesis has played a major role in the development of many new flower color/shape mutant varieties in ornamentals. The main bottleneck with vegetatively propagated plants is that the mutation appears as a chimera whether developed through bud sport or through induced mutation. The size of the mutant sector varies from a narrow streak on a petal to the entire flower and from a portion of a branch to the entire branch. When a portion of a branch or entire branch is mutated, the mutant tissue can be isolated; on the other hand, a small sector of a mutated branch or flower cannot be isolated using the available conventional propagation techniques. A novel technique has been standardized in our laboratory for the management of chimeric tissues through direct shoot regeneration from chrysanthemum florets. 'Kasturba Gandhi', a large white flowered chrysanthemum, developed few chimeric yellow florets due to spontaneous mutation. Using *in vitro* protocol new yellow florets were established in pure form. *In vitro* mutagenesis experiments were conducted treating ray florets of chrysanthemum cultivars using gamma rays. Induced chimeric yellow, white, light yellow, light mauve and dark mauve floret color sectors and chlorophyll variegation in leaves of cv. 'Maghi' (with mauve floret and green leaves) have been established in pure form. Gamma ray induced sectorial yellow florets of cv. 'Lilith' (white floret) and yellow ray florets in both the cvs. 'Purnima' (with white florets) and 'Colchi Bahar' (with red florets) have been isolated in pure form through *in vitro* management. Induced sectorial flower color/shape mutations in cvs. 'Puja', 'Lalima', 'Flirt', 'Maghi' and 'Sunil' have been isolated in pure form through *in vitro* culture. Gamma radiation procedure and tissue culture techniques have been optimized to regenerate plants from stem internodes, stem node, shoot tip and ray florets. Present technique has opened a new way for isolating new flower color/shape ornamental cultivars through retrieval of single mutated cell.

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SEEDLESS CITRUS DERIVED FROM SELECTED PROMISING MUTANT LINESI. SUTARTO¹, D. AGISIMANTO², A. SUPRIYANTO²¹Centre for Application of Isotopes and Radiation, Technology (BATAN), Jakarta, Indonesia²Research Institute for Citrus and Subtropical, Horticulture Crops, Kota Batu, Indonesia

Email: isutarto@batan.go.id

Conventional breeding on Citrus is laborious, time consuming and expensive because *Citrus* is highly heterozygous, polygenic plants with long juvenile period. Therefore, mutation breeding was carried out; bud woods of two Indonesian local commercial mandarin (*Citrus reticulata* L. Blanco) cv. SoE and Garut and pummelo (*Citrus grandis* L. Osbeck) cv Nambangan were exposed to gamma rays at the doses of 20, 40 and 60 grays, and then irradiated bud woods were then budded onto rootstocks cv. Japanese citron. Three-years-old untreated and irradiated plants grown in pots were checked for fruit characters such as seeds number per fruit, and colour of flesh and skin. Selected promising mutant lines were found in terms of seedlessness in cvs SoE mandarin and Nambangan pummelo, and nearly seedless cultivars were found in cvs Soe, Garut and Nambangan when bud woods were irradiated at the doses 20 and 40 grays. The performance of promising mutant lines obtained are now being observed and propagated in fields to confirm their stability.

IAEA-CN-167-298P

ALTERING AMYLOSE CONTENT IN INDONESIAN CASSAVA THROUGH IRRADIATION: ROLE OF GENOTYPES, DOSAGE AND PLANTING MATERIALSE. SUDARMONOWATI¹, N.S. HARTATI¹, S. SUGIHARTI¹, A. AMZAL²¹Research Centre for Biotechnology-LIPI, Bogor, West Java, Indonesia²National Atomic Energy Agency, Jakarta, Indonesia

Email: s_enny@hotmail.com

Different planting materials i.e. stem cuttings either not yet sprouted and just sprouted, *in vitro* shoots and seeds were irradiated at 0-500Gy, 2-50Gy, and 200Gy respectively, of various Indonesian genotypes (Adira 4, Iding, Gebang and Darul Hidayah) to obtain mutant possessing either high or very low amylose content. Of materials tried, stem cuttings of 300Gy irradiated Iding of M₁V₃ has the highest amylose i.e. 38.98% while M₁V₃ generation of E2 individual of Darul Hidayah irradiated at 100Gy has caused the lowest amylose (12.08%) obtained so far. It was noted that M₁V₂ yield tended to be higher than M₁V₁ regardless genotypes and dosage applied. The highest amylose content of M₁V₁ of Iding was obtained from cuttings irradiated with 100Gy, while that of Darul Hidayah was 200Gy. Whereas the highest amylose of M₁V₂ amylose content of Iding individual A4 (38.94%) was caused by irradiation with 300Gy, and of Darul Hidayah (32.27%) with 300Gy. This value of Iding was higher than the amylose content of all previous values during the selection i.e. 32%. As the other materials have not been propagated till further generation, other materials are also potential for further development. Regardless the planting materials used, morphological abnormality was noted in early growth stage but disappeared at a later stage. Genotypes, types of planting material in combination with dosage have played a role in the success of mutant induction as each genotypes responded differently. In addition to the target trait, other traits such as high yield and high starch content were also noted. Preliminary RAPD and AFLP analyses showed band alterations of irradiated ones as compared to control. RAPD analyses using OPE-15 and OPB-10 random primer have been proven to be suitable for detecting genetic diversity of cassava, seems could differentiate between non irradiated (control) and irradiated ones. OPB-10 has resulted clearer band difference which could differentiate between control and the irradiated plants (20 and 200Gy). AFLP analysis also showed the same indication although more analyses are needed for solid results. Image profiles need to be optimized applying several factors such as quality and quantity of DNA, and selective amplification primer pair. The analyses, however, needs to be confirmed by applying more samples and optimized procedure.

INDUCTION OF MUTANTS WITH USEFUL TRAITS IN A CHINESE CASSAVA GENOTYPE VIA IRRADIATION COMBINED WITH TISSUE CULTURE AND MOLECULAR CHARACTERIZATION OF INDUCED MUTANTS

Z.C. LIU, M.X. FAN, H.R. ZHENG, Y.H. LIU, T. LIN, T.F. LI, L.J. LUO

Laboratory, Shanghai Agrobiological Gene Center, Shanghai Academy of Agricultural Sciences, Shanghai, China

Email: lzc@sagc.org.cn

We concentrated on investigation of the following four aspects: A. Investigate the sensitive dosage of ^{60}Co γ -ray for irradiating stem segments of a Chinese cassava genotype grown in the field, in soil pot or from *in vitro* seedling for the purpose of mutation induction. B. Create and select cassava mutations with improved root yielding ability and lysine content. C. Establishment of a protocol for induction of somatic embryos from auxiliary buds. D. Establishment of molecular marker technique for identification of induced mutations of cassava. Sensitive dosages of irradiation had been determined to be 25-30 Gy for stem segments taken from field or soil pot grown plants, 20-25 Gy for *in vitro* grown stem segments. For either kind of stems, restoring process after irradiation was necessary for about 30-40 days in the soil pot or onto MS medium before the stem could be cut for further culture in MS based medium for trait segregation. In addition, cassava callus from petioles was also irradiated by ^{60}Co γ -ray and 9 Gy irradiation dosage was effective dosage to be used to induce mutation accordingly. Cassava mutations with changed root yielding ability and lysine content through gamma irradiation combined with tissue culture have been obtained from irradiated test tube seedlings after segregations over 4 generations. By the end of last year, 16 mutagenic plant lines were generated. Compared with the non-mutagenic control, some mutagenic lines like MV4, MV1, MV6 harvested more than 2 times of tube root by fresh weight. Lysine content in the root also showed big difference among different lines and the control. And what is interesting is that some mutagenic lines showed higher lysine content than the parent line, especially in the root parenchyma. However, the difference among mutagenic lines in lysine content was not as obvious as in root yield. An efficient and reproducible plant regeneration system via germination of somatic embryos induced from auxiliary buds of a Chinese genotype distributed in San Ya region of Hainan island, southern China, was achieved using MS based media with $12\text{ mg}\cdot\text{L}^{-1}$ picloram, combining with $2\mu\text{M}$ CuSO_4 and 2% sucrose, namely as CBM, CAM, CIM and CMM medium which was a bit different from literatures reported. Further more it was indicated that the sensitive concentration of hygromycin was set up at $4\text{-}8\text{ mg}\cdot\text{L}^{-1}$ for *Agrobacterium*-mediated hygromycin-dependent transformation. Screening of a large number of mutagenic populations is necessary for mutation breeding. And the PCR result revealed the possible application of SRAP marker technique in identification of mutagenic cassava plants. The SRAP marker technique is an applicable approach for identification of induced mutagenic cassava plants.

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ACQUISITION OF *HEMEROCALLIS FULVA* MUTANTS BY γ -RAY TREATMENT

J.O. PARK¹, H.S. CHO¹, M.Y. PARK¹, Y.S. JO¹, G.P. BANG¹, G.J. LEE², S.-Y. KANG²

¹Jeonnam Agricultural Research and Extension Services, Naju, Republic of Korea

²Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeonbuk, Republic of Korea

Email: parkjo63@kornet.net

Hemerocallis fulva which is also known as Orange Daylily, Tawny Daylily, Tiger Lily, or Ditch Lily is a floral plant widely used in flower beds along streets in Korea, of which flower color is simple and plant height is tall. Even though *H. fulva* is widely favored by people, the genetic variation of the species is not diverse enough. To expand the genetic variation and to select favorable variants from wild type, the seeds of the species were irradiated with gamma-ray in 2003. From the first trial, the LD_{50} level was determined as about 150Gy, and 57 mutant-like seedlings were selected in M_1 generation but the phenotype of the M_1 variants did not last to M_2 generation. To increase the genetic variation, the young whole plants were treated with γ -ray in ranges of 50, 100, 150, 200, 250, and 300Gy in the second trial. The survival rate was 80% for the plants irradiated with 50Gy, while all plants in over 50Gy treatments died. Among the survived plants, 12 mutant-like M_1 plants were obtained, among which there were 6 variants with characteristics of 2 white stripes on leaves and twisted leaves, and 11 dwarf type variants with short internodes and plant height less than 20 cm compared with the non-irradiated plants that was 41cm tall. Results indicated that some of the variants were found to have commonly unique features in the leaf and plant height which were different from the original plants. This work was supported by a grant (Code No. 20070301034033) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

IAEA-CN-167-306P

CHARACTERISTICS OF HOSTA PLANTAGINEA MUTANTS DERIVED FROM γ -RAY TREATMENT

J.O. PARK¹, H.S. CHO¹, M.Y. PARK¹, Y.S. JO¹, D.G.LIM¹, G.J. LEE², S.-Y. KANG²

¹Jeonnam Agricultural Research and Extension Services, Naju, Republic of Korea

²Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeonbuk, Republic of Korea

Email: parkjo63@kornet.net

Hosta (Hosta plantaginea) is a flowering plant with excellent fragrance, which has been widely used as a bedding and pot plant worldwide. However, its flower and leaf color is simple and leaf shapes are monotonous, which hinders customers' choice. To increase plant's diversity in morphology and color, *Hosta* seeds were treated with a γ -ray in the dose ranges of 10, 15, 20, 25, and 30Gy in 2003. A preliminary result indicated that LD₅₀ at which 50 % of the treated plants are lethal was around 30Gy for the *Hosta*. A first screening of variants was obtained among the survived plants treated with doses around the LD₅₀ level. In the following M₁V₁ generation, 12 mutants were selected, which had characteristics of short plant height and leaf variations in color and shapes. Based on observation up to a M₁V₄ generation, 8 mutants out of the selected 12 variants were found to maintain their mutated traits. Those 8 mutants include one from each 15 or 20 Gy, four from 25 Gy, and two from 30 Gy treatments. Leaf length and width were 15 and 12 cm, respectively for the non-irradiated plant, while the selected mutants exhibited relatively narrow leaf width and long leaf length. In order to verify their genetic modification, a random amplification of polymorphic DNA (RAPD) approach is being applied by using the selected mutants and original plant materials, which will be reported and updated. This work was supported by a grant (Code No. 20070301034033) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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INDUCED MUTATION EFFECTS ON ECONOMIC TRAITS RADIATED BY γ -⁶⁰CO IN PEARS

W.Q. LE, H.E. ZHANG, J.C. HAN, R.F. CHANG

Changli Institute of Pomology, Hebei Academy of Agricultural and Forestry Sciences, Changli, Hebei, China

Email: lewenquan@tom.com

Induced mutation by radiation was one of effective method to breed new fruit cultivars. Dormant woods of pear tree of cultivars 'Butirra R.M.', 'Bayuehong' and 'Red D'Anjou' were irradiated with gamma rays at doses of 40, 50 and 60 Gy at 1.20Gy/min, and then top-grafted to six-years old pears. The main fruit traits such as fruit weight, fruit shape index, soluble solids content, relative area of over color and fruit core size were evaluated. Compared with the unirradiated control, average fruit weight and fruit shape index were significantly decreased with dose increasing, the fruit weight of Red D'Anjou was reduced by 16.43% and fruit core size by 9-21%. Fruit shape indexes of Bayuehong and Red D'Anjou decreased with dose increasing. The higher the fruit shape index, the larger the fruit in γ -⁶⁰Co dosage 4000- 6000 rad. Relative area of over color was different among cultivars. Relative area of over color of Butirra R.M increased with dose increasing; Bayuehong was higher than the unirradiated, and Red D'Anjou decreased with dose increasing. Soluble solids contents of Butirra R.M and Bayuehong decreased with dose increasing, but Red D'Anjou increased 0.3-1.34%. However, we observed great variation variation of over color such as bright red, flaky, half-red and half russet etc. Two new selections R02-06-23 and R01-8-21 from Red D'Anjou and Butirra R.M were choosed for experiments in different regions.

THE DEVELOPMENT OF NEW GENOTYPES OF THE WHITE YAM BY MUTATION INDUCTION

E.C. NWACHUKWU, E.N.A. MBANASO, K.I. NWOSU
National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria
Email: nwachukwuec@yahoo.com

A method for the development of new genotypes of the white yam, *Dioscorea rotundata*, by mutation induction was investigated by irradiating mini-tubers of the yam with gamma rays. Batches of mini-tubers of a well adopted local cultivar, cvs. "Obiaoturugo" numbering one hundred and fifty each were irradiated with gamma rays at doses, 10, 10, 20, 30, 40, 50, 60, 70, 80 and 90 Gray (Gy) using cobalt ($C0^{60}$) gamma source at the Center for Energy Research and Development, Obafemi Awolowo University, Ile-Ife in the first year. Each irradiated tuber including the control was divided into setts weighing 10-15g and planted in the field to establish the first mutated vegetative generation (MV_1), separating setts from the head (H) and the tail (T) regions. In the second year, the first generation tubers harvested from the MV_1 generation yam plant population were used to establish the MV_2 population. At the MV_1 generation, increasing dosages of gamma ray irradiation progressively inhibited sprouting of setts isolated from treated mini-tubers. These effects were more severe on setts from the tail (T) region than those from the head (H) region. Also plant height, number of leaves, number of nodes and mean tuber yields per stand decreased with increased gamma ray dose. LD_{50} and GR_{50} were observed at 40Gy and 30Gy, respectively. At the MV_2 generation, the observed differences among the treatment means disappeared (were not significant). MV_2 yam lines with modified vegetative characteristic were isolated. Distinct dwarf lines with bunchy and bushy vegetation and bushy with spreading vines that have lost the ability to climb were isolated. One of the genetic improvement objectives apart from high tuber yields include the development of yam lines that may be cropped for high yield without staking (Onwueme, 1978).

IAEA-CN-167-327P

A CLONE OF IRRADIATED BANANA CULTIVAR "WILLIAMS" WITH HIGH YIELD POTENTIAL

M.A. ALI¹, S.B. BAKHIET¹, G.A. HAMID¹, F.A. AHMED¹, E.A. ABDELRAHMAN¹, M.E. ELKASHIF²
¹Agricultural Research Corporation, Wad Medani, Sudan
²Faculty of Agriculture, University of Gezira, Wad Medani, Sudan
Email: mohali5_99@yahoo.com

Efforts to improve banana in the Sudan started effectively with three IAEA mutation breeding projects. The most common banana cultivar is "Dwarf Cavendish", which covers almost 95% of the area under banana production. This cultivar is considered as the most adapted banana cultivar to different climatic conditions, but it is prone to "choke throat" and has low yield potential. Banana cv. "Williams" was irradiated at the IAEA/FAO laboratories, Sebersdorf, Austria. Based on preliminary evaluation of the material, 5 mutants (i.e. W193-3, W188-3, W205-4, W206-1 and W224-4) were selected as single plants and propagated by tissue culture. Multi-location testing was carried out for these mutants with cvs. "Dwarf Cavendish" and "Williams" as standard cultivars in a randomized complete block design with 4 replications and 25 plants per replication. Spacing was 2x2 m (2500 plant per ha) and one sucker was retained. The bunch weight and cumulative yield of clone 193-3 were significantly higher than all banana genotypes. The high yield of clone W193-3 in the plant crop was due to the significantly higher number of hands per bunch and larger fingers. The plant height at shooting and pseudostem girth were significantly higher in clone W193-3. The stability parameters for bunch weight of the different clones showed that clone W193-3 was stable with high yield in all environments. Clone W193-3 was released as a new banana cultivar for farmers under the names "Albeely".

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INDUCTION OF MUTATION IN ACALYPHA (*ACALYPHA TRICOLOR* L.) THROUGH GAMMA RAYS

M. KUMAR¹, V. KUMAR¹, R.B. SOLANKI²¹Dept. of Horticulture, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut, India²Dept. of Horticulture, A.S. (PG) College, Lakhaoti, Buland Shahar, India

Email: k.mukesh123@yahoo.com

An experiment was conducted to assess the induction of mutation in *Acalypha* through gamma rays at Agriculture Research Farm of A.S (PG) College, Lakhaoti (Bulandshahr), during the year 2001-2002. Ten cuttings of *Acalypha*, each of 10 cm. long rooted cuttings were treated with different doses of gamma rays (0, 10, 20, 30, 40, 50, 60, and 70Gy). It was observed that the treated cuttings decreased significantly the sprouting, maximum longest leaf, number of sprouts, diameter of longest sprout as compared to control. It was also found that the cuttings treated with 3Gy produced the maximum plant height, leaf length and leaf colour with and without variegation. However, maximum breadth of leaf was recorded under 50Gy treatment during the course of investigation.

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ASSESSMENT AND UTILIZATION OF SPONTANEOUS SPORT MUTANT OF GRAPE

Y.Y. WU, Z.G. WANG

Institute of Biotechnology, Zhejiang Wanli University, Ningbo, China

Email: wyy2000@zwu.edu.cn

The spontaneous sport mutant of Fujiminori was discovered in grape garden of Xiaying county at Ningbo city in 1993. The biological, botanical characteristics and fruit quality trait (such as total soluble solid, titratable acid, total water soluble sugar, reducing sugar, free Vc, organic acid and aroma etc.) of the mutant were continuously investigated from 1994 to 1999. The results showed that the sport mutant grew more vigorously, having multiple-bearing capacity in the year cycle. Fruit quality determination demonstrated that total soluble sugar, reducing sugar, soluble solids content and aroma contents of the mutant were higher than those of maternal plant in different degree, while titratable acid content of mutant was decreased. Meanwhile, it was also found that the berries of mutant are firmer and have longer storage life. The RAPD analysis of the genomic DNAs extracted from the young leaves of the spontaneous sport mutant indicated that there were some differential bands in the PCR amplified products using the arbitrary primers, which indicated the genotype diversity happened in the spontaneous mutation of grape. The mutant had been successfully developed the new grape variety named as "Yongyou No. 1" via selection breeding method (see Figure 1). The variety was approved by Ningbo Science and Technology Bureau in 1999 and was rapidly planted at other regions, such as Fenghua County, Yuyao County, Cixi County, Ninghai County, Shaoxing City, Jiaxing City and Hangzhou City, etc. Due to its high quality and productivity, it exhibits the extensive application potential in the future.

TOWARDS THE DEVELOPMENT OF A CHIMERA-FREE *IN VITRO* INDUCED MUTAGENESIS SYSTEM IN CASSAVA (*MANIHOT ESCULENTA*, CRANTZ)

R.H. ESCOBAR-PEREZ, L. MUÑOZ, C. DORADO, J. TOHME

International Centre for Tropical Agriculture, Agrobiodiversity and Biotechnology Project, Cali, Colombia

Email: r.escobar@cgiar.org

Cassava, an herbaceous plant with starchy storage roots, has the potentials for being the cheapest source of starch for varied industries. To achieve this, the starch types must be clearly discriminated into either high preponderance of amylose or the other extreme of amylopectin content (waxy starch). Being a vegetative propagated crop with major crossing barriers, induced mutagenesis holds promise for modifying the starch characteristics of this crop. The efficiency of induced mutagenesis in a vegetative propagated crop such as cassava is severely limited by the occurrence of chimeras. To ameliorate this, the induced mutagenesis strategy must permit the regeneration of plants from one or a few cells that have been induced to mutate. We report the optimisation of protocols for the generation of plantlets from somatic embryos that were exposed to EMS. Different explants (buds and somatic embryos) of a cassava clone with high starch content were exposed to different doses (concentration and duration) of ethylmethane sulfonate (EMS) with the aim of determining the optimal doses for generating induced mutants. A wide range of reactions to EMS, from slightly reduced plantlet regeneration to lethality, was observed leading to the determination of the optimum exposure treatment. The regenerated plantlets were transplanted to pots in the greenhouse for hardening and later transferred to the field. In order to achieve homozygosity of the mutation events, the putative mutants were selfed- crosses. The immature embryos were rescued (cultured on aseptic growth media) in order to speed-up the process of generating the mutant population as well as avoid the possibility of embryo abortion. The resulting plantlets were again subsequently hardened and transferred to the field. Currently, 610 plants, constituting the putative mutant population have been established in the field in Palmira, Colombia. As a pilot assay, this work has demonstrated the feasibility of combining EMS induced mutagenesis with somatic embryogenesis in cassava. The putative mutant population will be evaluated for modifications to starch characteristics and other agronomic traits in cassava. Also, DNA has been extracted from these materials for use in molecular analysis. Perspectives for future activities include a massive production of cassava mutants through this process and integrating a reverse genetics strategy such as Targeting Induced Local Lesions IN Genomes (TILLING) for searching for mutations in the genes controlling the critical pathways for cassava starch biosynthesis. A successful implementation of both the *in-vitro* regeneration of putative mutants and molecular assays for identifying mutations will mark a major enhancement in the efficiency levels for generating homohistants in this crop.

γ -IRRADIATION OF WILD BEET TRANSLOCATION LINES AND MONOSOMIC ADDITION LINES IN SUGAR BEET CARRYING NEMATODE RESISTANCE GENES

S.C. JAEGER, G.G.G. CAPISTRANO, H.J. HARLOFF, C. JUNG

Plant Breeding Institute, Kiel, Germany

Email: s.jaeger@plantbreeding.uni-kiel.de

The beet cyst nematode (BCN, *Heterodera schachtii* Schmidt) is a severe pest in sugar beet (*Beta vulgaris* L.). The only sources of resistance are distantly related wild beet species such as *Beta procumbens*. Sugar beet lines, carrying a translocation from *B. procumbens* chromosome 1, display complete resistance to the BCN. A nematode resistance gene, designated *Hs1^{pro-1}*, had been cloned from the translocation line A906001. This gene gave complete resistance in a complementation study done with sugar beet hairy roots, however only partial resistance was found in whole sugar beet plants transformed with the same construct. There are strong indications for a second resistance gene on this translocation designated *Hs1-1*. Unfortunately, the resistance gene cannot be fine mapped due to complete lack of recombination on the wild beet translocation. In order to narrow down to the target region for *Hs1-1*, a mutant screening among the offspring of γ -irradiated beets was done. 2670 seeds from a translocation line were irradiated with 100 Gy and 578 resistant M1-offspring were analysed with three molecular markers spread around the translocation. Mutants are presently detected by the absence of molecular markers. A second screening at irradiation levels of 200 and 400 Gy was done and the plants are being analysed. Another experiment was started to produce translocations carrying the *B. procumbens* chromosome 7 which houses the *Hs2^{pro-7}* gene for nematode resistance. This resistance has not been broken by virulent pathotypes of *H. schachtii* as demonstrated with monosomic addition lines. To select resistant plants with a translocation derived from chromosome 7, 2826 seeds of monosomic addition lines of chromosome 7 were irradiated with 400 Gy. The M1-families will be tested for their resistance and the size of the chromosome fragment introduced will be determined by molecular marker analysis.

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INDUCTION OF VARIANT IN POTATO CULTIVAR SPUNTA FOR STRESS TOLERANCE VIA TISSUE CULTURE METHOD

Y. AL-SHAYJI, C. SUDHERSAN

Biotechnology Department, Kuwait Institute for Scientific Research, Food Resources and Marine Sciences Division, Safat, Kuwait

Email: yshayji@safat.kisr.edu.kw, schellan@safat.kisr.edu.kw

Potato, one of the world's most important food crop which is highly sensitive to salinity stress. Development of stress tolerant variants through breeding is difficult and time consuming. Attempts were made to develop salinity stress tolerant variant potato via tissue culture techniques. Callus was induced from the shoot tip explants of potato cultivar Spunta under high concentrations of 2,4-D (2,4-dichloro phenoxy acetic acid) and maintained for longer duration (more than 3 months). The callus survived under high concentration of 2,4-D (100 mg/l) were multiplied in the same culture media and screened for salinity tolerance under *in vitro* culture condition. Callus cultures tolerant up to 10,770 ppm TDS (Total dissolved salts) salinity were isolated and plantlets were regenerated from these variants using *in vitro* techniques under low salinity culture media or normal MS media containing 4770 ppm TDS. The regenerated plantlets were transferred to high salinity culture medium containing 10,770 ppm TDS. The plantlets showing good growth under high salinity were multiplied through stem and shoot tip multiplication method. The salt tolerant variant obtained from the potato cultivar Spunta were experimented for micro tuber production under *in vitro* saline culture media and mini tubers under *in vivo* saline conditions. Both *in vitro* and *in vivo* experiments confirmed their tolerance towards salinity. Preliminary field trial with brackish water (11,000 ppm) irrigation also showed normal plant growth and yield.

CONCURRENT SESSION 9 (14:00-17:40)

Induced Mutations in Seed Crop Breeding (1)

IAEA Boardroom C04

Thursday, 14 August 2008

MUTATION TECHNIQUES FOR OILSEED CROP BREEDING IN POLAND

I. BARTKOWIAK-BRODA, S. SPASIBIONEK

Plant Breeding and Acclimatization Institute (PBAI), Department of Genetics and Breeding of Oilseed Crops
Poznan, Poland

Email: ibart@nico.ihar.poznan.pl

At present, in Poland as well as in European Union countries double low quality oilseed rape (without erucic acid and with low glucosinolate content) is the major oilseed crop. The seeds are the source of universal oil which is recognized as perfect oil for edible purposes with very well balanced essential fatty acid composition – linoleic and linolenic, and relatively high oleic acid content which allows to use it in the technology of biofuel and other biodegradable material production. However, the market also demands oil with different fatty acid composition. Induced mutagenesis plays significant role in the development of new fatty acid variability in oilseed crops. The aim of investigations was to find optimal conditions of mutagenesis for the increase of variability of polyunsaturated fatty acids in winter oilseed rape and obtainment of mutated lines with high oleic and reduced linolenic acid content. Different conditions of mutagenesis with the use of ethyl methanesulphonate (EMS) were investigated. The mutation with the use of EMS was performed on double low inbred line PN3756/93. After selection in several subsequent generations $M_2 - M_7$, two mutants M-10453 and M-10464 with significantly increased oleic acid content (over 75%) and one mutant M-681 with high linoleic and low linolenic acid content (respectively 27,5% and 2,7%) were selected. Five mutants obtained in different EMS treatment conditions performed on another double low line PN 5282/98 and selected in $M_3 - M_6$ generations are characterized by increased level of oleic acid (76,2- 81,2%) and reduced linolenic acid content (1,7-3,9%). Significant changes obtained in the content of fatty acids in oil seeds suggest that activity levels of enzymes $\Delta 12$ and $\Delta 15$ desaturases which influence the content of oleic, linoleic and linolenic acids synthesis undergo considerable damages. It is the effect of mutations in genes *fad2* or *fad3* (*fatty acid desaturase*).

IAEA-CN-167-074

DEVELOPING HERBICIDE-TOLERANT CROPS FROM MUTATIONS

S.Y. TAN, S. BOWE

BASF Corporation, Research Triangle Park, NC, USA

Email: siyuan.tan@basf.com

Herbicide-tolerant crops in combination with their corresponding herbicides are able to control many weeds that cannot be or are less effectively controlled with other means. Commercial herbicide-tolerant crops developed from herbicide-tolerant mutants include imidazolinone-tolerant maize, rice, wheat, oilseed rape, sunflower, and lentil; sulfonyleurea-tolerant soybean and sunflower; cyclohexanedione-tolerant maize; and triazine-tolerant oilseed rape. Most of the herbicide-tolerant mutants were developed through chemical mutagenesis followed by herbicide selection. Several herbicide-tolerant mutants were also discovered through direct herbicide selection of spontaneous mutations. All mutations used in commercial herbicide-tolerant crops are derived from a single nucleotide substitution of genes that encode enzymes or proteins targeted by herbicides. Imidazolinone-tolerant maize, rice, wheat, and oilseed rape have a gene variant encoding an altered acetohydroxyacid synthase (AHAS) with the S653N amino acid substitution. Additionally, imidazolinone-tolerant maize and oilseed rape have an AHAS with the W574L amino acid substitution. Imidazolinone-tolerant sunflower has been developed from the A205V AHAS gene mutation. In contrast, sulfonyleurea-tolerant sunflower selected from a farm field has an AHAS enzyme variant with the P197L amino acid substitution. Similarly, sulfonyleurea-tolerant soybean has a P197S AHAS gene mutation. Sulfonyleurea-tolerant sunflower from seed mutagenesis and imidazolinone-tolerant lentil are also derived from AHAS gene mutations. Cyclohexanedione-tolerant maize has an altered acetyl-CoA carboxylase with the I1781L amino acid substitution. Triazine-tolerant oil seed rape possesses a *psbA* gene variant that encodes the D1 protein of photosynthesis with the S264G amino acid substitution. The alleles of all commercial herbicide-tolerant mutations are incompletely-dominant and not pleiotropic except for the triazine-tolerant mutation which is inherited maternally and linked with several agronomic traits.

IAEA-CN-167-037

MARKER-ASSISTED BACKCROSSING TO INCORPORATE TWO LOW PHYTATE ALLELES INTO THE TENNESSEE SOYBEAN CULTIVAR 5601T

D. LANDAU-ELLIS, V.R. PANTALONE

Soybean Breeding and Genetics Program, Plant Science Department, The University of Tennessee, Knoxville, TN, USA

Email: dellis@utk.edu

Development of low phytate soybean is favorable to the environment by reducing phosphorous loads to agricultural lands and surface waters. The trait also provides enhanced nutrition and metabolism for poultry and swine. Our source for the trait was a low phytate germplasm (CX1834-1-2) developed by the USDA and Purdue University through ethyl methanesulfonate (EMS) mutagenesis. The objective of this project was to develop a commercially acceptable, superior quality, high yielding soybean cultivar with low seed phytate. In order to incorporate the low phytate trait into our regionally adapted soybean cultivar '5601T', we have combined a series of backcrosses with marker assisted selection (MAS) at each backcross stage. Simple sequence repeat (SSR) markers have enabled us to *i*) transfer two recessive alleles governing the low phytate trait and *ii*) identify which specific individual backcross plants had DNA of the greatest commonality with the genome of the recurrent parent 5601T. We utilized two low-phytate SSR markers Satt237 (linkage group N) and Satt561 (linkage group L) for dual marker assisted selection for gene transfer of the low phytate trait. Molecular markers dispersed across the genome proved to be effective for facilitating genome recovery of the high yielding 5601T recurrent parent every backcross generation. Chemical analyses confirmed that the low phytate trait was inherited in concert with the molecular markers. Thirty three lines homozygous for both low phytate recessive alleles have been planted in 2008 in a yield trial at the East Tennessee Research and Education Center in Knoxville, TN for field evaluation and seed production.

IAEA-CN-167-251

FUNCTIONAL ANALYSIS OF INDUCED SEMIDWARF MUTATIONS AND APPLICATION TO RICE BREEDING

H. KITANO¹, K. SATO¹, M. IKEDA², M. ASHIKARI¹¹Bioscience and Biotechnology Center, Nagoya University, Nagoya, Japan²Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

Email: hdkitano@nuaf1.agr.nagoya-u.ac.jp

Many dwarf or semi-dwarf mutations have been induced in rice, for both practical breeding and genetic studies. It has demonstrated that plant hormones such as gibberellin (GA) and brassinosteroid (BR) play an important role on the control of plant height or plant shape. The *semidwarf 1* (*sd1*), which is known as the key-gene of rice Green Revolution in 1960's, was identified as a recessive mutation of the gene encoding GA₂₀ oxidase (*GA20ox2*) and its semidwarf phenotype is caused by low GA₁ production due to the loss-of-function of GA₂₀ oxidase. In rice, *sd1* is the only semidwarf gene succeeded in the practical use and at least 7 different *sd1* alleles were used independently in China, USA and Japan. These *sd1* alleles isometrically control the plant growth and reduce the plant height by 80% to 90% of their wild types by diminishing the GA content in the plant body. BR also controls the plant growth and the lack of BR content or its signalling pathway induces dwarfism with some morphological abnormalities. Recessive mutations of *Dwarf 61* (*D61*) encoding BR receptor gene, *OsBR11* (orthologous to *Bril* in *Arabidopsis*) expressed a dwarf phenotype different from that of GA related dwarfs. We have detected successively many mutations including weak to null alleles at the *D61* locus. These mutant alleles expressed a large range of dwarfism from semidwarf with erect leaves to extremely reduced and malformed dwarfs. Some weak alleles of *D61* confer a semidwarf phenotype with erected leaves. Although there is no evidence that BR-related mutations have contributed to the breeding of such cultivars of rice, a barley gene, *uzu*, that has been widely used for dwarfing in East Asia, was proved recently to be a mutation of an ortholog of *D61*. This fact suggests that some alleles of BR-related mutants might be available for rice breeding. Experimental trials examined with weak alleles of *D61* showed that these alleles were keeping the higher yield potential in the greater density of nitrogen fertilization, in which condition their tall parents had depressed the potential throughout over-luxuriant growth. The lodging resistance of rice could be enhanced not only by short culm but also by modifications of the culm structure. A recessive dwarf mutant 1S-18 expressing a modified thick culm structure and its causal gene, *Sbd1* (*Stocky body dwarf 1*), will also be presented.

A DWARF MUTANT RELATED TO BR-DEFICIENCY IN SOYBEAN (*GLYCINE MAX*)

Z.Y. REN^{1,2}, R.H. PIAO^{1,2}, X. LIU^{1,2}, R.X. GUAN¹, W.Y. LI¹, Z.B. HAO², R.Z. CHANG¹, L.J. QIU¹

¹The National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Key Lab of Germplasm & Biotechnology (MOA), Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China

²College of Biotechnology, Northeast Agriculture University, Harbin, Heilongjiang, China

Email: qiu_lijuan@263.net

The use of dwarf genes of the cereal crops resulted in the First Green revolution, which also plays a very important role in soybean cultivation system, but no dwarf gene has been cloned for identification in soybean so far. In this study, a dwarf mutant line obtained by the treatment of the chemical reagent was used in order to illustrate the mechanism of phenotypic variation. A total of 721 SSR loci were used to analyze both mutant line and its wild type and found 10 loci (1.39%) appeared to be difference between two lines. The epicotyl's length of the mutant line could not be recovered from the mutant to the wild type when they were treated with by either light components (red light or blue) or light intensity. Three plant growth-promoting compounds were used to treat both the mutant line and its wild type. No change of plant height was observed when both the mutant line and its wild type were treated with auxin (IAA). Despite the mutant line was responded to the gibberellin (GA3) treatment, but its plant height increased as well as that of its wild type. The GA3 content of both the mutant and wild type was compared and no significant difference was found. The dwarf mutant line was responded to the Brassinosteroids (BRs), which appeared to be similar response to BR-deficient mutants in Arabidopsis, and the plant height of the mutant could be increased by BR treatment compared to the wild type. These results indicated that the mutant might be BR-deficient mutant. By crossing the mutant line with its wild type, the plant height of F1 will be recovered to the wild type, indicating the gene controlling the dwarf might be recessive. Meanwhile, the five homologous genes involving in the BR pathway for Arabidopsis were used to analyze the expression of BR treated mutant and showed that *det-homolog* locus might be the key gene controlled the phenotype of dwarf.

TARGETED MUTATION BREEDING AS A TOOL FOR TOBACCO CROP IMPROVEMENT

E. JULIO¹, F. LAPORTE², S. REIS¹, C. ROTHAN², F. DORLHAC DE BORNE¹

¹Altadis - Institut du Tabac, Domaine de la Tour, Bergerac, France

²UMR Physiologie et Biotechnologie Végétales, IBVI-INRA Bordeaux, Villenave d'Ornon Cedex, France

Email: emilie.julio@altadis.com

Nicotiana tabacum is a model widely used in functional genomics with transgenesis; however, genetically modified organisms are not accepted by consumers in Europe. Targeted mutagenesis as a non transgenic approach was assessed on a demonstration gene, involved in alkaloid metabolism. The *NtabCYP82E4v1* gene is responsible for nicotine demethylation into nornicotine. The secondary alkaloids derivatives (Tobacco Specific Nitrosamines or TSNA) are supposed to be implicated in the increased risks for various pathologies. As a consequence, their reduction in tobacco has become a major goal for tobacco companies. A population of 4.000 EMS-mutagenized M₂ families was created. High throughput Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP) was used to target mutations. Ten mutants were identified by screening 1311 M₂ families. Of 10 alleles isolated by screening 0,532 kb of *NtabCYP82E4* DNA, 1 was silent, 5 were missense and 4 were truncation mutations. Mutations identified in DNA pools were validated by sequencing. Individual plants carrying missense or truncation mutations were studied for their phenotype. Seeds of M₂ families carrying a mutation into the *NtabCYP82E4* gene were sown in greenhouse and plants were individually analyzed by CE-SSCP. Heterozygous and mutant plants could be easily distinguished from wild type plants. Nornicotine synthesis in leaf was induced by a bicarbonate treatment. Nornicotine presence was assessed by a rapid colorimetric test, which highlight nornicotine presence with blue spot. In a family carrying a nonsense mutation, we could observe that no nornicotine was detected in homozygous plants for the mutation. These observations could be confirmed in the field by HPLC analyses of secondary alkaloids. These plants have been used as genitors to introduce this mutation into elite lines. Backcrosses are being performed to recover the elite line background in combination with CE-SSCP analysis to follow the mutation. The amphidiploid nature of tobacco avoids problems related to fertility. Efficiency of this method to create novel genetic variation and to develop cultivars has been demonstrated for the first time in tobacco.

IAEA-CN-167-067

EXPRESSION OF SEQUENCES RESPONSIBLE FOR BRASSINOSTEROID METABOLISM IN BARLEY MUTANTS

D. GRUSZKA, I. SZAREJKO, M. MALUSZYNSKI
 Department of Genetics, University of Silesia, Katowice, Poland
 Email: wintergrun@op.pl

Brassinosteroids (BR) are growth-promoting natural products found at low levels in pollen, seeds and young vegetative tissues throughout the plant kingdom. They are steroid hormones affecting broad range of physiological processes such as: cell expansion, vascular differentiation, etiolation and reproductive development. Defects in BR metabolism may lead to several morphological changes, including dwarfism. The aim of the performed studies was to identify the coding sequences responsible for brassinosteroid synthesis and signaling in barley. Semidwarf mutants that displayed de-etiolation during growth in darkness, a typical feature of BR mutants, were used in this study. These mutants were obtained in the Department of Genetics by MNU (N-nitroso-N-methylurea) treatment. The first step of the analysis was the search in GenBank for rice and Arabidopsis sequences responsible for BR metabolism. These sequences were used to retrieve barley ESTs displaying high level of similarity from TIGR database. These ESTs enabled identification of complete coding sequence of barley homologues and polypeptides encoded by these sequences – *HvDWARF* (GenBank acc. no. DQ832258 and ABH01181, respectively), *HvCYP90D* (GenBank acc. no. EF025184 and ABK30931, respectively) and *HvBAK1* (GenBank acc. no. EF216861 and ABN05373, respectively). *HvDWARF* and *HvCYP90D* encode brassinosteroid C-6 oxidase and brassinosteroid C-3 oxidase, respectively, participating in BR synthesis. *HvBAK1* encodes a transmembrane BR co-receptor, interacting with BRI1 receptor. Function of *HvDWARF* gene in BR metabolism was confirmed by identification of mutations in two allelic semidwarf mutants that carried two different amino-acid substitutions in the conserved regions of the BR-6-oxidase protein. Real-Time Quantitative PCR analyses were conducted to characterize an expression pattern of the identified sequences. *HvDWARF* and *HvCYP90D* are constitutively expressed at very low levels, regardless of organ analyzed, whereas expression of *HvBAK1* is developmentally regulated during seedling growth and proved to be higher in roots than in shoots of barley seedlings.

IAEA-CN-167-208

THE IMPROVEMENT OF TAEK-SAGEL CHICKPEA (*CICER ARIETINUM* L.) MUTANT VARIETY IN TURKEY

Z. SAĞEL, I.TUTLUER, H. PESKIRCIOGLU, Y. KANTOGLU, B. KUNTER
 Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Ankara, Turkey
 Email: zafer@saray.anaem.gov.tr

Chickpea is an important food legume in Turkey. Turkey is one of the most important gene centers in the world for legumes. The most widely known characteristic of chickpea is that it is an important vegetable protein source used in human and animal nutrition. However, the dry grains of chickpea, has 2-3 times more protein than our traditional food of wheat. In addition, because of its high carbohydrate content, it is also energy source. It is very rich some vitamin and minerals basis. In the plant breeding, mutation induction has become an effective way of supplementing existing germplasm and improving cultivars. Many successful examples of mutation induction have proved that mutation breeding is an effective and important approach to food legume improvement. The induced mutation technique in chickpea has proved successful and good results have been attained. Realizing the potential of induced mutations, a mutation breeding programme was initiated at the Nuclear Agriculture Section of the Sarayköy Nuclear Research and Training Center in 1994. The purpose of the study was to obtain high yielding chickpea varieties with large seeds, good cooking quality and high protein content. Seeds of the Ak-71114 and Akçin chickpea varieties were irradiated with 0 (control), 50, 100, 150, 200, 250, 300, 350 ve 400 Gy of gamma rays by using ⁶⁰Co source. One thousand seeds for per treatment were sown in the field for the M₁. At maturity, 3500 single plants were harvested and 20 seeds taken from each M₁ plant and planted in the following season. During plant growth, mutants of the desired traits (earliness, yield per plant, first pot height and Ascochyta blight (*Ascochyta rabie*) resistance) were identified an isolated. 2520 desirable M₂ mutants were selected and grown in progeny rows as the M₃ generation. The protein content was analyzed for the M₃-M₄ seeds. In M₃ generation, preliminary yield trials had been conducted and based on field observations, quality criteria (grain size, grain type, cooking, protein) analyses, 12 mutant lines were selected. The mutants and their controls were evaluated at two locations Sarayköy (SANAEM) and Haymana (TARM) for 2 years (M₇, M₈) for yield trials using randomized complete block design (RCBD) with 3 replications. All the yield, grain size, grain type, first pot height, Ascochyta blight (*Ascochyta rabie*), cooking and protein content data were analysed statistically. After these experiments, 2 mutant promising lines were chosen and given for official registration to the Seed Registration and Certification Center. These 2 promising lines were tested for 2 years (2004, 2005) at five different locations. TAEK-SAGEL mutant chickpea variety had been tested in 2004-2005 for its earliness (95-100 day) with higher yield capacity (180-220 kg/da), higher seed protein (22-25%), higher first pot height (20-25 cm), 100 seeds weight (42-48), cooking time (35 - 40 min), Ascochyta blight (*Ascochyta rabie*) resistance and quality than in the control. After 2 years of registration experiments one of outstanding mutants were officially released as mutant chickpea varieties under the names TAEK-SAGEL in 2006.

DEVELOPMENT OF *B. NAPUS* CANOLA QUALITY VARIETIES SUITABLE FOR INDIAN AGRO-CLIMATIC CONDITIONS BY INDUCED MUTATIONS

S.P. LANDGE¹, Y.Y. BARVE², R.K. GUPTA², S.S. BHADARIA², R.P. THAKRE¹, S.E. PAWAR¹

¹Department of Botany, RTM Nagpur University campus, Nagpur, India

²Dhara Vegetable Oil & Foods Co. Ltd., NDDB Campus, Anand, India

Email: yoganandbarve@gmail.com

The exotic *B. napus* varieties introduced in the country were not commercially successful due to their late maturity and poor seed yield. The present investigation was initiated at RTM Nagpur University by Late Dr. A. S. Khalatkar on induction of mutations in *B. napus* in cv Westar (Canola quality) with the objective of inducing early maturing mutants with high yield potential suitable for commercial cultivation for Indian agro-climatic conditions. In mutation experiments, dry seed and presoaked seeds were used with different doses / concentrations of gamma rays and chemical mutagens EMS and SA. Among several morphological mutants, eleven early maturing mutants were identified. The maturity of these mutants were ranging from 90 -115 days as against 169 days that of Westar in Central India. The maturity of mutants NUDB-38 and NUDB-26-11 was 107 and 105 days respectively. These mutants were further evaluated for their yield and agronomical characters along with non canola quality *B. napus* checks GSL-1 and *B. juncea* varieties Pusa Bold and Varuna under different agroclimatic conditions in the national testing (Indian Council for Agriculture Research) programme in zone II and zone I respectively where *B. napus* is grown. NUDB-38 has shown 33% superior yield performance over check variety GSL-1 (non-canola quality) and the oil yield was comparable with *B. juncea* national check variety Varuna in zone II. Where as NUDB-26-11 was comparable (2% higher) with gobhi sarson check GSL-1 and it has given 24.6% higher yield over *B. juncea* check variety Varuna in zone I. Mutant NUDB-38 has been granted US patent (patent No. US 6706953 B2; dated March 16th, 2004) for its early maturity and high yield potential. Mutant NUDB-26-11 has been identified for release for zone I by All India Coordinated Research Project on Rapeseed Mustard during 2007. These studies have indicated that mutation experiments with specific objectives can result in developing varieties suitable for specific regions with high yield potential.

IDENTIFICATION AND CHARACTERIZATION OF TWO LOW PHYTIC ACID SOYBEAN MUTANTS

F.J. YUAN^{1,2}, H.J. ZHAO¹, S.L. ZHU², X.J. FU², Q.Y. SHU³

¹IAEA-Zhejiang University Collaborating Center, Institute of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou, China

²Institute of Crop Science and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, Hangzhou, China

³Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria

Email: fjyuanhz@126.com

By seed gamma irradiation (150 Gy) of two commercial cultivars, Taiwan 75 and Zhechun no. 3, two soybean low phytic acid (LPA) mutants *Gm-lpa-TW-1* and *Gm-lpa-ZC-2* were obtained. Analysis of seed phosphorus fractions indicated that both two mutants had phytic acid reduction of ~50% comparing with their wild type parents, and the inorganic portion of seed P was increased. Meanwhile, *Gm-lpa-ZC-2* had significantly increased in myo-inositol phosphates containing five and four P ester. Genetic analysis suggested that the LPA characteristics were both controlled by single non-allelic recessive genes in the two mutants. The gene conditioning the LPA mutation in *Gm-lpa-ZC-2* was mapped on LG B2, closely linked with microsatellite loci satt416 and satt168, at genetic distances of ~4.63 and ~9.25 cM, respectively, while the mutation in *Gm-lpa-TW-1* was proven to have happened to the D-myo-inositol 3-phosphate synthase (MIPS1 EC 5.5.1.4) gene 1 (MIPS1), and sequencing results indicated that *Gm-lpa-TW-1* lpa mutation resulted from a 2 bp deletion of the MIPS1 gene. The mutant line *Gm-lpa-TW-1* had a significantly reduced field emergence when seeds were produced in subtropical environments while *Gm-lpa-ZC-2* mutation does not negatively affect plant yield traits and seed field emergence. The novel LPA mutation in *Gm-lpa-ZC-2*, together with linked SSR markers, would be of value for breeding productive LPA soybeans.

CONCURRENT SESSION 10 (14:00-17:40)

Induced Mutations in Seed Crop Breeding (2)

UNIDO Boardroom C04

Thursday, 14 August 2008

EXPLOITING MUTAGENESIS FOR WHEAT IMPROVEMENT

M.A.J. PARRY¹, H.-C. JING¹, P.J. MADGWICK¹, C. BAYON¹, D. HABASH¹, M. BAUDO¹, R. TUBEROSA², J. BORT³, H. BEN HAJ SALAH⁴, G. SLAFER⁵, A. AL-YASSIN⁶, H. OUABBOU⁷, M. LABIHILI⁸, M. NATCHIT⁹, A. PHILLIPS¹

¹Rothamsted Research, Harpenden, Herts, UK

²University of Bologna, Bologna, Italy

³University of Barcelona, Barcelona, Spain

⁴INAT, Cite Mahrajene Tunis, Tunisie

⁵University of Lleida, Lleida, Spain

⁶NCARTT, Amman, Jordan

⁷INRA, Settatt, Morocco

⁸INRA, U.R. de Biotechnologie, Rabat, Morocco

⁹ICARDA, Aleppo, Syrian Arab Republic

Email: martin.parry@bbsrc.ac.uk

The chemical mutagen, ethylmethanesulphonate, is being used to introduce into wheat novel variation that can be exploited for crop improvement. We have created mutagenised populations of diploid (Einkorn), tetraploid (Durum) and hexaploid (bread) wheat. The forward genetic approach enables the identification of high yielding or novel phenotypes that can be exploited in conventional breeding programmes. A powerful reverse genetic strategy, TILLING (Targetting Induced Local Lesions IN Genomes), allows the detection of induced point mutations in the populations of mutagenised individuals and allows gene function to be examined. Genetic redundancy in the tetraploid and hexaploid species allows them to tolerate a high level of mutation (up to one mutation per 25kbp). This mutation frequency makes it relatively easy to identify lesions in each homeologue of a particular gene which can then be combined for crop improvement or functional genomics. Novel variation created can be exploited without the regulatory restrictions imposed on genetically modified organisms. Gene targets have been selected in relation to plant architecture, primary metabolism, disease resistance and stress tolerance and over 50 TILLING mutants have so been identified, including mis-sense, non-sense and splice site mutations.

IAEA-CN-167-373

A *UGPASE1*-BLOCKED MALE STERILITY MUTANT AND ITS POSSIBLE USE IN HYBRID SEED PRODUCTION OF RICE

H.-J. KOH, M.-O. WOO

Dept of Plant Science, Seoul National University, Seoul, Republic of Korea

Email: heejkoh@snu.ac.kr

A rice genic male-sterile mutant was induced by chemical mutagenesis using N-methyl-N-nitrosourea, which had a pleiotropic effect on chalky endosperm. The mutant gene, *ms-h*, was isolated through a map-based gene cloning approach. The suppression of UGPase activity, caused by a splicing error at the 3' splice junction of the 14th intron of the UDP-glucose pyrophosphorylase 1 (UGPase1; EC 2.7.7.9) gene, was the reason for male sterility. This was confirmed by both RNAi- and complementation-transgenic experiments. The starch structure of the mutant had more roundish and smaller starch granules, a higher frequency of long glucose chain amylose, higher ratio of Fr. III to Fr. II chains, and shorter branching of amylopectin than the wildtype parent. A hybrid seed production system was proposed using the pleiotropic effect of *UGPase* gene on male-sterility and chalky endosperm. Relatively low density of chalky seeds may facilitate the early detection of male-sterile seeds in segregating populations prior to sowing by density-gradient method.

IAEA-CN-167-345

BARLEY (*HORDEUM VULGARE*) AND KIWICHA (*AMARANTHUS CAUDATUS*) IMPROVEMENT BY MUTATION INDUCTION IN PERU

L. GÓMEZ-PANDO, A. EGUILUZ, J. JIMENEZ, J. FALCONÍ

Institute of Plant Biology, Cereal Research Program, Universidad Nacional Agraria de La Molina, Lima, Peru

Email: luzgomez@lamolina.edu.pe

In order to increase food availability and household incomes of families in the Andean Region of Peru, mutation induction method was applied to improve barley (*Hordeum vulgare*) and kiwicha (*Amaranthus caudatus*) cultivars. Barley cultivar *Buenavista* was treated with 200 and 300 Gray originating different kinds of mutations. 20 promising mutant lines were selected and have been evaluated at the national trials, from them Mbv-Earlier, from 300 Gray dose; was selected and released in 2006 as a new cultivar denominated *Centenario*. This cultivar has a high yield potential (5 552 kg/ha), resistance to stripe rust (*P. striiformis f sp hordei*) and better food quality than the parental cultivar. Kiwicha traditional cultivar *Seleccion Ancash* treated with 400 Gray permitted to identify a higher yield mutant denominated *Centenario* Cultivar. At farmer location in the coast the yield has a variation of 3500 to 5500 kg/ha and in the highland from 2500 to 3700 kg/ha. The better yield potential, tolerance to *Sclerotinia sp.*, colour and size of its grains have contributed in the preference of *Centenario* over the other commercial cultivars.

IAEA-CN-167-348

UNDERSTANDING THE MOLECULAR MECHANISMS OF RICE BLAST RESISTANCE USING RICE MUTANTS

Y.L. JIA

USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR, USA

Email: yulin.jia@ars.usda.gov

Induced mutation can be useful for studying resistance gene controlled plant immunity. Resulting knowledge should benefit the development of strategies for crop protection. The *Pi-ta* gene in rice has been effectively deployed for preventing rice blast disease-the most devastating disease of rice worldwide. *Pi-ta* was introgressed into diverse cultivars in the US and Japan from landrace indica varieties, Tetep and Taducan, respectively. *Pi-ta* was predicted to be a cytoplasmic receptor that directly binds to the elicitor produced by the pathogen avirulence gene *AVR-Pita* for initiating resistance. Alanine located at position 918 of the *Pi-ta* protein in the region predicted to be involved in ligand binding has been shown to determine the binding specificity. Here I report the identification of a second gene, *Ptr(t)*, required by *Pi-ta* for resistance. Katy, a tropical japonica cultivar from the US, expressing resistance conditioned by *Pi-ta* and *Pi-k^s* to the common races of *M. oryzae*, IB1, IB45, IB49, IB54, IC17, IH1, IE1, and IG1 was treated with fast neutrons. Five susceptible M2 mutants were identified by screening seedlings derived from 10,000 M₁ plants. Among them a stable mutant M2354 was found susceptible to IB1, IB45, IB49, IC17, IH1, IE1, and IG1 conditioned by *Pi-ta* and resistant to IB54 conditioned by *Pi-k^s*. The DNA sequences of the *Pi-ta* gene in M2354 was found unchanged based on PCR-sequencing. Expression of *Pi-ta* in M2354 was also found identical to that of the mother parent examined by qRT-PCR and real time RT-PCR. Thus, mutations in M2354 likely occurred at a new locus specific to *Pi-ta*-mediated resistance. Genetic analysis and genotyping the *Pi-taptr(t)*, *Pi-taPtr(t)*, *pi-taptr(t)* homozygotes revealed that *Pi-ta* and *Ptr(t)* co-segregate and are located within a 9 megabase genomic region on chromosome 12. These findings provide a starting point to isolate *Ptr(t)* and dissect the *Pi-ta* mediated signaling pathways leading to resistance.

AN INNOVATIVE WAY OF DEVELOPING AN IMPROVED VARIETY UTILIZING BOTH GAMMA RAYS INDUCED AND RECOMBINATIONAL VARIABILITY IN BLACKGRAM (*VIGNA MUNGO* L.(HEPPER))

S.T. KAJJIDONI, ROOPALAKSMI, K. SHOBHA IMMADI, R. NAGARAL, I. NAGARAL
University of Agricultural Sciences, Dharwad, India
Email: stkajjidoni@yahoo.co.in

Mutagenesis in association with recombination breeding offers a viable option to improve locally adapted varieties and release the variability hidden in the conserved gene blocks. An attempt was made to compare variability generated through different mating schemes and combination of mating and irradiation in *Vigna mungo* L. (Hepper) to improve productivity of recommended varieties. Four genotypes viz., Manikya and TAU-1 two locally adapted varieties, which lack optimum seed size and number of pods per, plant respectively. And two selected complimentary donor lines viz., No.169, a line for high pod number (50-55) and No. 216 for seed mass (6.5g) were crossed to generate four single crosses, two three way and one double crosses. Four single crosses were further irradiated with 20 Kr gamma rays and advanced to F_2M_2 and F_2 generation for evaluation based on seven agronomic traits. Irradiated progenies of single crosses exhibited higher PCV and GCV values for clusters per plant and pods per plant. Variability generated by irradiation was more compared to recombination variability for clusters per plant and pod length traits. Irradiated single cross (F_2M_2) progenies produced higher frequency of superior progenies for pods per plant, 100 seed weight and seed yield per plant compared to other hybridized progenies involving two or more than two parents. Nature of association between pod length and number of pods per plant under irradiation was improved favorably and even it was changed from non significant negative to positive significant in F_2M_2 progenies of a cross TAU-1x169. Selected superior progenies isolated in F_2 and F_2M_2 (112) and in F_3M_3 and F_3 generation (135) which were advanced separately from F_2 , F_2M_2 to F_3 , F_3M_3 by following three different selection methods and all these selected individual progenies were advanced to F_5 generation and were evaluated in progeny row trial with two replications. Based on their seed yield, which yielded more than mean + two standard deviation values, we found that 29 advance breeding lines were superior. It is interesting to note that when pedigree of 29 advance breeding lines was traced back, we found that 18 lines originated from irradiated single crosses and five lines from single crosses without irradiation and six lines from hybridized populations involving more than two parents revealing the importance of irradiation in creation of desirable variability. We found that based on stability analysis involving 29 advance breeding lines, the stable performance of DBS-14, DBS-16, DBS-24 and DBS-26 genotypes over environments with better mean performance for seed yield. The seed protein content across environments, ranged from 17.9 to 27.2 per cent when grown at Dharwad location. Genotype DBS-15 (27.20%) had highest seed protein content, which was followed by DBS-12 (26%) compared to high yielding check TAU-1 (19.68%). We found that two genotypes DBS-7 and DBS-21 had low seed sugar content across three test environments and these can be considered as low flatulence causing lines upon consumption by human beings. We found that based on results of large scale trials in different agro climatic conditions, genotype DBS-14 was most promising genotype with superior seed yield (22.0%) and seed mass apart from its tolerance to stem fly damage compared to adaptive cultivar TAU-1. Based on the merits, genotype was identified for commercial cultivation in the name of DU-1 in Karnataka provenance and also registered with NBPGR New Delhi for its novel agronomic traits.

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IMPROVEMENT OF SESAME CROP THROUGH INDUCED MUTATIONS IN KOREA

C.W. KANG, K.B. SHIM, C.D. HWANG, S.B. PAE
Upland and Industrial Crops Division, Yeongnam Agricultural Research Institute(YARI), National Institute of Crop Science(NICS), Rural Development Administration, Suwon, Republic of Korea
Email: cwkang@rda.go.kr

Induced mutations of sesame(*Sesamum indicum* L.) in Korea was initiated by developing "Ahnsankkae" occupied 33% of national sesame acreage in 1985 since developed and spreaded in 1984 which had been irradiated with 30Gy X-ray on the seeds of "90days Chamkkae(Early Russian)" in 1978. "Suwon128" the dwarf mutant induced mutation by NaN_3 (sodium azide), and "Suwon129" the determinate mutant crossed between "dt45" and the Korea recommending and "SI90033-14" the determinate with tens of capsule nodes per branch were designed as a model of a mid-parents for crossing to breed "Ideal Plant Type of Sesame" in 1980s. Not only directly induced mutants by irradiation or chemical treatments, but also were considered as a way of induced mutations with indirect way of crossing between direct mutant and cultivar. Totally 15 recommending mutants of directly and indirectly induced mutants with 3 mid-parent of dwarf, determinate and determinate with tens of capsule nodes were bred and occupied 55% of national acreage during two decades since 1984 in Korea. "Yangbackkae" as a high oil content mutant, "Sungboonkkae" as a 2.5 times high sesaminol-triglucoside(STG) one, and "Suhdoonkkae", "Sunbackkae" and "Pyoungankkae" as a phytophthora blight resistant and high yielding and good

quality mutant are much favored from farmers among 15 recommending mutants. Consequently, depending upon those mutants, sesame yielding per unit area in Korea increased two times during two decades from 350 kg/ha in 1970s to 720kg/ha in 2000. Breeding target is being focused to shatter resistance for combiner harvest, with higher content of functional components, disease and disaster resistance, taller and thicker and longer and dense capsule bearing stem, determinate with tens of capsule nodes, tri-carpels hexa-loculi capsule type and male sterile mutants for super high yield, and finally make sesame from world minor crop toward major in the near future.

IAEA-CN-167-212

TOWARDS THE ISOLATION OF A MUTATED GENE CONFERRING RESISTANCE TO POWDERY MILDEW IN *PISUM SATIVUM* L.

J. LEITÃO¹, G. PEREIRA^{1,2}, C. MARQUES¹, R. RIBEIRO¹, M. FARINHÓ¹, M.T. DE SOUSA², M. DÂMASO¹

¹FERN, University of Algarve, Faro, Portugal

²Estação Nacional de Melhoramento de Plantas, Elvas, Portugal

Email: jleitao@ualg.pt

Recently, we have selected two powdery mildew resistant (PMR) mutations in *Pisum sativum* L. after ethylnitrosourea treatment of two commercial cultivars, Frilene and Solara. The two PMR mutations were further determined to be monogenic, recessive and to affect the same locus – *er1*. An F₂ mapping population, Frilene (*er1mut2*) X Solara, was obtained in order to start the way towards the positional cloning of the PMR gene. Although, the use of the Near Isogenic Lines (NILs) strategy did not allow the identification of any molecular marker linked to the resistance locus, the Bulk Segregant Analysis permitted the identification of 4 RAPD and 1 AFLP markers linked to the PMR locus, which were mapped together with multiple markers polymorphic between the paternal lines and specific markers previously identified as linked to the PMR locus by other research groups. Among the 17 DNA-markers that assembled with the PMR gene in the same linkage group, OPO06_1100 was mapped as the closest marker (0.6 cM) to *er1mut2*. In order to increase the efficiency of the identification of markers tightly linked to the PMR locus a new cross was performed between the mutant line Frilene (*er1mut2*) (*Pisum sativum* var. *sativum*) and the more genetically distanced line Gp3151 (*Pisum sativum* var. *arvense*). A very dense map of molecular markers of the genomic region that spans the powdery mildew resistance mutated gene is expected to be obtained soon. The closest linked markers will be used for identification of specific BAC clones in the *Pisum* genomic library, presently under construction.

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INDUCED MUTATIONS AFFECTING ROOT ARCHITECTURE AND MINERAL ACQUISITION IN BARLEY

P.J. WHITE¹, A.G. BENGOUGH¹, I.J. BINGHAM², T.S. GEORGE¹, A.J. KARLEY¹, T.A. VALENTINE¹

¹Scottish Crop Research Institute, Dundee, UK

² Scottish Agricultural College, Edinburgh, UK

Email: philip.white@scri.ac.uk

Root architecture influences the acquisition of mineral elements required by plants. In general, plants with a greater root/shoot biomass quotient and a more extensive root system acquire mineral elements most effectively. In barley (*Hordeum vulgare* L.) induced mutation has produced commercial cultivars with greater root system size, and genotypes with greater root spread, longer roots and roots with denser root hairs. Work is in progress investigating whether these phenotypes improve the acquisition of mineral elements and, thereby plant growth and grain yield.

CURRENT STATUS AND RESEARCH DIRECTION OF INDUCED MUTATION APPLICATION TO SEED CROPS IMPROVEMENT IN VIETNAM

Q.V. MAI, K.T. DO, T.B. DAO, H.A. DO, H.H. LE
 Agricultural Genetics Institute (AGI), Hanoi, Vietnam
 Institute of Agricultural Sciences for Southern Vietnam (IASSV), Ho Chi Minh City, Vietnam
 Email: maiquangvinh@gmail.com

In Vietnam, nuclear techniques and chemical mutagens have been applied in Vietnam since the 1970's in order to improve seed crops as rice, soybean, maize, groundnut, many mutant varieties were recognized as national varieties and some promising regional lines. Main direction and methods using in varietal improvement in Vietnam were exploitation of gene resources, using and genetic methods consisting of hybridization, mutation, gene transformation to create crops having high yield, good quality, tolerance to diseases and unsuitable climate conditions. Up to the year 2007, according to preliminary statistic, in Vietnam 50 mutant varieties were created (as IAEA database, having 43 mutant varieties created, Vietnam is being the ninth of mutant breeding' achievement record in the world), among of those seed crops occupied 47 varieties, rice occupied 32 varieties, soybean was 11, maize was 2 and peanut was 2. At AGI has created 17 rice mutant varieties, 11 mutant varieties of soybean were created and adopted by Ministry of Agriculture and RD as national and regional varieties, among of those occupied more than 50% of Vietnam soybean cultivated area thanks to their grown ability of three crops per year, broad adaptation, good tolerance to hot, cold temperature and good resistance to diseases. These varieties are preponderance in the Northern provinces and every year occupies about 20 - 40% of cultivated area. In present, some best mutant varieties has become one of the top 5 varieties for export and grown recently more than 300,000 ha per year in Southern Vietnam, about double the yield (10 tons/ha) of its parent VTL-3 (5 tons/ha).

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A NOVEL DOMINANT SEMIDWARF MUTANT AND ITS PLANT HEIGHT REVERTANTS INDUCED WITH ION IRRADIATION IN RICE (*ORYZA SATIVA* L.)

B.M. LIU, X. XU, M. ZHAO, Y.J. WU
 Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences
 Hefei, China
 Email: liubm@mail.com

Rice *Dominant Semidwarf* mutant (Sdd) was obtained from its wild type (WT) irradiated by low-energy ion beam. The genetic analysis of Sdd indicated that the phenotype of semi-dwarf was controlled by single dominant gene, termed *Sdd(t)*. We show that Sdd reduced the plant height mainly via inhibiting the first, second and third internodes elongation. In addition, the *Sdd(t)* gene was sensitive to gibberellin (GA) based on the response to extraneous GA₃ and the quantitative determination of endogenous GA₁ and GA₄. To map the *Sdd(t)* gene, we tested molecular markers by bulk segregant analysis. The *Sdd(t)* gene was localized to a 6.4 cM interval on the short arm of chromosome 6, flanked by two sequence-tagged site (STS) markers S9 and S13. To study the function of *Sdd(t)* furthermore, we created six tall revertants of Sdd with ion irradiation. The revertants restored the plant height to WT plants. Agronomic characters investigation indicated the revertants were different from Sdd while similar to WT. And the genetic analysis showed that the revertants were putative different inherited mutation. Furthermore, Sdd, WT and tall revertants were checked for their DNA level differences using SSR (simple sequence repeat) technique. Among 408 SSR primers used, only two primers displayed different SSR bands in two revertants. The result demonstrated the revertants were induced from Sdd by ion beam implantation. This study indicates that ion irradiation may be used as a new effective mutagen to create mutants and revertants for plant functional genomics research.

INDUCED LODGING RESISTANCE IN LITTMILLET (*PANICUM SUMATRENSE*) THROUGH GAMMA IRRADIATION

A. NIRMALAKUMARI¹, N. SENTHIL¹, J. SOUFRAMANIAN², S. ARULSELVI¹, P. DEVAN¹

¹Tamil Nadu Agricultural University, Coimbatore, India

²BARC, Trombay, Mumbai, India

Email: anirmalakumari@yahoo.com

Enlarging the variability and widening the selection for non-lodging and high yielding varieties in littlemillet, induced mutagenesis has been resorted. Recently released littlemillet varieties CO3 and CO(Samai)4 were selected as the parent genotype and they were exposed to 300, 400, 500, 600 and 700 Gy of gamma rays. The treated seeds were sown in the field along with control to raise M₁ and they were forwarded to M₂ and M₃ generation. Lodging susceptibility score were recorded in M₂ and M₃ generation. With reference to lodging susceptibility, the maximum heritability and genetic advance was recorded in the progeny of 600Gy in M₂ generation of CO(Samai)4 and 300Gy in M₃ generation of CO3. The lodging resistance is closely associated with shorter plant height, thick culm and reduced internodal length. Accordingly, selection based on these characters along with lodging resistance in mutagenic dose at 600Gy was found to be effective in CO(Samai)4 variety in M₂ population. Similarly mutagenic dose at 300Gy was found to be effective in M₃ generation of CO3. In M₂ generation of CO3 lodging resistance recorded positive Skewness value (0.26) and the complementary epistasis gene action was confirmed. Where as in M₂ generation of CO(Samai)4 lodging resistance recorded inconsistent negative value (-0.07) for Skewness and indicated that genetic equilibrium of these character was disturbed by the irradiation. The inconsistent trend in frequency distribution suggests that besides selection, other genetic factors might have influenced the induction and expression of micro mutations. Hence, to select lodging resistant genotypes, we have to screen more number of mutated populations to have better combination of lodging resistant alleles.

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INDUCED GENETIC VARIABILITY FOR YIELD AND YIELD COMPONENTS IN PEANUT (*ARACHIS HYPOGAEA* L.)

H.L. NADAF, S.B. KAVERI, K. MADHUSUDAN, B.N. MOTAGI

National Seed Project, Seed Unit

University of Agricultural Sciences, Dharwad

Karnataka, India

Email: sosuasd@yahoo.com

An experiment was conducted during 2005-07 to induce polygenic variability for yield and its components in peanut (*Arachis hypogaea* L.). Two cultivars of peanut, 'GPBD-4' and 'TPG-41' were treated with γ -radiation (200 Gy & 300 Gy) and ethyl methane sulphonate (EMS- 0.5 %). The mutagenized populations showed significantly higher variability in the M₂ generation. Mutant lines showing higher yield/plant than the respective parents and checks were isolated in M₂ and subsequent generation. The evaluation of 14 superior mutants isolated in M₄ over three successive generation yielded few mutants performing better over the parents and checks. In both the genotypes superior mutants were isolated from 200 Gy treatment, indicating effectiveness of the mutagen in obtaining the desired trait. Two of the mutant lines, G2-52 and TG2-30, gave significantly more pod yield (3315 and 2647 kg/ha respectively) than the parents and checks. One of the most interesting features of these mutant lines was the significant increase in hundred seed weight over the parent, contributing to higher yield. The mutant G2-82 recorded highest 100-seed weight of 40.28 g among GPBD-4 mutant population and T2-30 had 67.24 g as against parental value of (62.43 g). Mutants were found to be on par with respective parents for oil content but had improved oil quality with their parental character of disease resistance/susceptible reaction. These promising mutant lines need to be further tested for their adaptability and stability. These can be further utilized in recombination breeding with other mutants and/or cultivars to derive distinct lines with improved agronomic traits.

INDUCED MUTANTS TO STABILISE PRODUCTIVITY UNDER FOLIAR DISEASE EPIDEMICS IN GROUNDNUT

B.N. MOTAGI¹, M.V.C. GOWDA², H.L. NADAF¹, K. MADHUSUDAN²

¹National Seed Project

²Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka, India

E-mail: bnmotagi@rediffmail.com

Though groundnut is very much exposed to mutagenic treatments, the mutation breeding programmes employed exclusively on disease resistance are lacking. Dharwad Early Runner (DER) on mutagenic treatment with Ethyl Methane Sulphonate (EMS, 0.5 %) yielded very high frequency of mutants representing all four botanical types including a homozygous Valencia mutant (VL 1) with resistance to rust. On recurrent mutagenesis VL 1 has yielded high frequency of foliar disease resistant mutants with variations for other characters. Among them, three mutants 28-2, 45 and 100 combined multiple disease resistance and early maturity in Spanish background. Spanish bunch mutant (28-2) was evaluated along with ruling but susceptible Spanish bunch cultivars (TMV 2, JL 24 and TAG 24), a rust resistant Valencia cultivar (ICGV 86590) and a Virginia bunch interspecific germplasm line (ICGV 87165) for disease, productivity and physiological parameters over two rainy seasons. Resistant genotypes ICGV 87165 and mutant 28-2 were superior for pod, oil and haulm yield as they recorded higher values for these productivity parameters. Most of the resistant germplasm are Valencia landraces and Virginia interspecific derivatives with many undesirable attributes. Foliar disease resistant genotypes viz., ICGV 87165 and ICGV 86590 had low partitioning coefficient (42-59%), late maturity (115-125 days), poor pod and seed features. Because of this, they are not popular among the farmers in spite of their higher yield under disease epidemics. On the contrary, mutant 28-2 had desirable combination of foliar disease resistance with early maturity (100-105 days), high partitioning coefficient (63.6%) and desirable pod and kernel features similar to that of ruling but susceptible cultivars. Mutant 28-2 was also resistant to tobacco cut worm (*Spodoptera litura*), thrips and tolerant to *Aspergillus* infection. Hence, the mutant can serve as a superior germplasm in improving the Spanish types for foliar disease resistance and in stabilising groundnut productivity under foliar disease epidemics.

INDUCED MUTATIONS FOR CROP IMPROVEMENT IN YEMEN

A.A. SAIF, M. AL-KBISI, A.A. AL-SAMIRI, Z. NAGI, A.H. KAID, A. ALMAKTARI, R. AL-BASHA

Agricultural Research & Extension Authority, Dhamar, Yemen

Email: a_w_s_mozaid@yahoo.com

Seeds of these crop varieties were irradiated with different doses of gamma rays to induce mutations. In experiment 1 seeds of wheat variety Gemmiza-9 were irradiated with doses 250 and 300 Gy. Five mutants from M₆ generation were selected and evaluated for earliness, yellow rust disease resistance and high yield in the farmers field for two consecutive seasons 2006-2007. Among them, two mutants were selected for the development of mutant varieties that could be released to the farmers. In experiment 2 wheat variety Sonalika seeds were irradiated with 250 and 300Gy, sown at the Research Experimental Station, Dhamar; segregating material were evaluated for rust disease resistance and yield. Two mutants strongly demonstrated resistance against yellow rust disease. In experiment 3 seeds of local wheat variety Arabi-1 were irradiated with 250Gy dose. Few seeds were collected from healthy plants and were grown in M₂ and subsequent generations at the Al-erra Research Experimental Station. A total of 13 mutants were selected and tested in the farmer's field under rainfed condition for two consecutive seasons 2005-2006. Finally two mutants were selected by the farmers that showed resistance to lodging with high yield. In experiment 3 lentil variety D2001 was used for mutation induction. Seeds were irradiated with 150-200 Gy and planted at the Al-erra Experimental Research Station. Plants with useful agronomic traits were evaluated in the M₂-M₆ generations. Six promising mutants from M₆ were selected and evaluated in the farmer's field under rainfed conditions for two seasons on the basis of earliness and yield. Two mutants were selected by farmers which were registered for releasing. In experiment 4 different gamma radiation doses 300, 400 and 500Gy were used to treat seeds of local sesame variety. Irradiated seeds were sown as M₁ population at the Tihama Experimental Station. Through consecutive evaluation from M₂-M₅ generations, which resulted in the selection of 11 mutant lines with different desirable characters such as earliness, resistance to pod fly, high yield, high density of capsules on the stem with short and tall stature. In experiment 5 cotton seeds of a variety ACALSJ2 were irradiated with 400, 500 and 600Gy doses of gamma radiation and were grown at the Tihama Experimental Research Station as M₁ generation. From the segregating M₂-M₆ population, it was developed five mutants showed stability of selected traits such as earliness and high yield. Currently, all the selected mutants are in the process of evaluation in the research farm. Based on their performance, steps will be taken to transfer them in the farmer's field and select the best to register them as mutant varieties.

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NEW RICE VARIETY, DT38 SELECTED SUCCESSFULLY BY GAMMA RAYS IRRADIATION

T.B. DAO, Q.D. LAM, A.T. TO, T.N. NGHIEN, et al.
 Institute of Agriculture Genetics (AGI), Hanoi, Vietnam
 Email: daothithanhsang60@yahoo.com

Mutation breeding has been applied successfully for crop breeding in the world generally and in Vietnam particularly. In many cases, mutagenesis treatment seems to be more efficient than other traditional methods because of this method can create changing only one or two characters but the remains are intact. Under supporting from IAEA/VIE/5/014 and Vietnam Atomic Energy Commission, we carried out scientific research project "Improvement of Khangdan 18 by induced mutagenesis treatment on dry seed with gamma rays". The main objective of the project is through mutagenesis treatment to maintain promising characters at the same time to repair some disadvantage characters of the original rice variety, Khangdan 18. New mutant rice variety DT38 has been released, which is prominent to Khangdan 18 such as: higher grain yield, non lodging, good resistance to some main pests and diseases. DT38 has been producing in some provinces in the north and center of Vietnam. The average yielding of DT38 is higher than that of the origin Khangdan 18 about 10%, even in some locations is 15%. October 2007, DT38 has been officially certified as a new mutant variety by the Ministry of Agriculture and Rural Development, Vietnam. Up to now, DT38 displaying in agricultural variety structure in 8 provinces as Hanam, Hatay, Bacninh, Thabinh, Vinhphuc, Hungyen, Haiduong, Nghean.

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INDUCED MUTATIONS IN CUMIN (*CUMINUM CYMINUM* L.)

Z.S. SOLANKI, B.R. CHOUDHARY, S.R. KUMHAR
 Agricultural Research Station, Rajasthan Agricultural University, Mandor- Jodhpur, India
 E-mail: solankizs@rediffmail.com

Seeds of cumin (*Cuminum cyminum* L.) varieties RZ 19 and RZ 209 were exposed to varying concentration (0, 0.25, 0.50 and 0.75%) of ethyl methane sulphonate (EMS) and colchicine solutions. In M_1/C_1 generation, seed germination was reduced significantly in two concentrations i.e. 0.50 and 0.75% of EMS and in all the three concentrations of colchicine whereas 1000 seed weight increased significantly in two concentrations of colchicine i.e. 0.25 and 0.50% but no effect was observed in EMS treatments as compared to respective check. Variations for plant height (cm), branches per plant, umbellets per umbel, seeds per umbellet, 1000-seed weight (g) and seed yield per plant (g) were recorded in M_2/C_2 in generations. The progenies derived from colchicine treatments had recorded higher means for seed yield per plant, plant height and branches per plant. A few progenies exhibited high coefficient of variation over the control along with high mean hence these may resulted in desirable segregants in their progenies facilitating selection.

IMPROVED EARLINESS AND PRODUCTIVITY IN COTTON BY GAMMA RAYS

M.A. AL-HAMDANY, A.H. KADHEM, H.SH. KETAN, N.N. MOHAMMED ALI
Agricultural Researches Directorate, Ministry of Science and Technology, Baghdad, Iraq
Email: ma_alhmdany@yahoo.com

Cotton seeds of R-5 (Selected from Cultivar Coker 310) were treated with 200 and 250Gy of gamma rays aimed to create host diversity. The first three pods from each survival M₁ plant were chosen for M₂ generation. Selection for earliness was started from M₂ plants. Plants showing more than 70% maturity in their bolls on days 180 from seed sowing were selected. Seeds of the selected plants were sown in line rows as M₃ generation. Five mutant lines namely 21,24,39,41 and 44 were selected from 200Gy treatments, while six mutants namely 64A, 64B, 69A, 69B, 73A, and 73B were selected from 250Gy treatments. Meantime, all undesirable plants or lines were eliminated. The selected mutant lines were evaluated for earliness and yield potential in a comparison with their origin (R-5) and the land race cultivar (Ashor 1). Data of the first picking on day 170 revealed that Boll maturity percentages in 200 Gray mutants ranged from 44.5 to 73.89, while the range was from 35.2 to 57.80. Considering yield potential in the first picking as an extra criterion for earliness, the percentages in all selected mutants ranged from 38.58 to 90.81. However, in contrary to R-5 or Ashoor 1, many mutants showed an excellent tendency for earliness reflected in percent of first fiber yield at day 170th such as mutant 44(86.30%), mutant 39(75.10%), mutant 69A (74.88%), mutant 69B (74.85) and mutant 73A (68.36%). The fiber yield of both R-5 and Ashoor1 at the same time represented 23.78 and 40.65 % of both first and second picking which was at day 210th(November 5th,2007) in Baghdad region. Finally, the level of infestation observed on mutants bolls and their origin (mature or immature) in 5th November ranged from 3.2 to 49.8%.

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SELECTION FOR RESISTANCE TO YELLOW VEIN MOSAIC VIRUS DISEASE OF OKRA BY INDUCED MUTATION

V. PHADVIBULYA¹, K. BOONSIRICHA¹, A. ADTHALUNGRONG², W. SRITHONGCHAI³

¹Thailand Institute of Nuclear Technology (TINT), Bangkok, Thailand

²Horticultural Research Institute, Department of Agriculture (DOA), Bangkok, Thailand

³Crop Protection Research and Development Office, Department of Agriculture (DOA), Bangkok, Thailand

Email:valailak@tint.or.th

Yellow vein mosaic virus disease (YVMD) caused by a begomovirus is the most serious factor affecting okra (*Abelmoschus esculentus*) production for both export and domestic consumption in Thailand. Seeds of Annie and Okra okra varieties were gamma-irradiated at doses of 400 and 600 Gy and planted at Huaysai King's Project in Petchaburi Province. M₃ plants were screened for OYVMD (Okra YVMD) resistance under field conditions at Huaysai King's Project and Phichit Horticultural Research Center (PHRC) in Phichit Province. In addition, M₄ plants were screened for OYVMD resistance under greenhouse conditions at Crop Protection Research and Development Office using whitefly transmission. None of Annie was found resistant but one plant of Okra (B-21) irradiated at 400 Gy was found to be highly resistant. Ten resistant lines obtained through rescreening of B-21 descendants up to M₇ generation were selected for yield trial observations at PHRC and Chiangmai Horticultural Research Station (CHRS). The mutants had good stature and fruit shape but the fruits have spines on the ridges. Selections for OYVMD resistance and spineless fruits were performed at PHRC in three generations and seven of the lines were chosen for yield trial at PHRC. Three of the mutant lines were also screened for OYVMD resistance at Kanchanaburi Horticultural Research Center (KHRC) in Kanchanaburi Province, okra growing area, where OYVMD was seriously widespread. All mutant lines showed resistance against the local OYVMV isolates up to a month before they started showing signs of the disease. Seeds were collected from resistant individuals and planted in farmers's fields for further selection. The farmers were very satisfied with the stature and fruit shape of the mutants when tested against a commercial variety.

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GENETICS OF THE RADIATION-INDUCED YELLOW VEIN MOSAIC DISEASE RESISTANCE MUTATION IN OKRA

K. BOONSIRICHAJ¹, V. PHADVIBULYA¹, A. ADTHALUNGRONG², W. SRITHONGCHAI³,
V. PURIPUNYAVANICH¹

¹Thailand Institute of Nuclear Technology, Bangkok, Thailand

²Horticultural Research Institute, Department of Agriculture, Bangkok, Thailand

³Crop Protection Research and Development Office, Department of Agriculture, Bangkok, Thailand

Email: kboonsir@yahoo.com

The yellow vein mosaic disease (YVMD) is one of the major diseases affecting okra production in Thailand. YVMD-resistant B4610 mutant was generated through gamma irradiation of the Okura variety of okra. In an attempt to develop a DNA marker for YVMD-resistance, a BC₁F₁ and an F₂ mapping population were generated from the cross between B4610 and Pichit 03, a YVMD-susceptible variety. The populations were naturally inoculated with YVMD virus in the field at Pichit Horticultural Research Center, Pichit province, where the disease is widespread. Analysis of F₁ and F₂ progeny revealed the semi-dominant nature of the resistance which appeared to be caused by a single-locus mutation. AFLP and MFLP fingerprintings of the F₂ and the BC₁F₁ population revealed DNA fragments that are potentially linked to the mutation. In addition to the visual assessment of YVMD, a PCR method was developed for the assay of the presence of YVMD virus in leaf tissues. Sequencing of the amplified DNA fragments confirmed the presence of okra YVMD virus in the infected leaf tissues in susceptible plants.

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NEW VARIETIES SELECTING AND MUTAGENESIS MECHANISM OF UPLAND COTTON (*G. HIRSUTUM* L.) BY SPACE MUTATION

M.Z. SONG, S.X. YU, S.L. FAN, X.J. WU

Cotton Research Institute, Chinese Academy of Agriculture Science / Key Laboratory of Cotton Genetic

Improvement, Ministry of Agriculture, Anyang, China

E-mail: songmz@cricaas.com.cn

Cotton space breeding was a newly breeding method. At the first, the seeds were taken into the space by the recoverable satellite, altitude air balloon, and airship, where synthetically physical factors (cosmic rays, microgravity, high vacuum, ebb magnetic field and so on.) of the space were applied to create great genetic variation. The seeds were sent by Shenzhou 4 in 2002, and by the Eighteen science and technology satellite in 2003. There were the long season cotton varieties (Lu9154, Zhong9708, S2498) and the short season cotton varieties (Zhong205806, Zhong206573, SGK Zhong-394, and Zhong108619). Every sample had corresponding ground check. Three generations (SP₁, SP₂ and SP₃) were investigated. The cotton buds were damaged in SP₁, and the induced effect was bigger in the short season cottons. The height had big changed, the leaves and bolls had increased, the function areas, small bolls and opened bolls had positive and negative changes in SP₁, SP₂ and SP₃. The seed cotton yields, the lint yields, the boll weight and the lint percentage had positive and negative changes in SP₁ and SP₂. The induced action to specific strength, elongation and MIC was bigger than to fibre length, uniformity. Molecular polymorphism was existed by SSR in DNA level. There are thirty-five SSR primers pairs amplified marker loci. The polymorphism percentage was 19.4%. There are thirty-seven RAPD primer pairs amplified marker loci. The polymorphism percentage was 25.8%. It was primary approved that the mutations changed from DNA level. Zhongmiansuo50 and Zhongmiansuo24 was released from space mutation offspring.

INDUCTION AND ANALYSIS OF RICE LESION MIMIC MUTANT

Z.H. WANG^{1,2}, Y.L. JIA²

¹Institute of Biotechnology, Zhejiang Wanli University, Ningbo, China

²USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AZ, USA

Email: wang1972@zwu.edu.cn, zhonghuawang@hotmail.com

A lesion mimic mutant of rice (*Oryza sativa* L.), named as *cer1*, was induced from a japonic rice variety Katy with 1.0% ethyl methane-sulfonate (EMS) treatment. Phenotype of mutant was comparatively characterized along with the original parent Katy. The height, the number of tillers, one-thousand-grain weight of mutant was significantly reduced than those of Katy. Genetic analysis indicated that the mutation is controlled by single recessive gene. Lesion mimic phenotype of LmmKaty was rapidly induced by virulent *M. grisea* isolates or by avirulent isolates only at high levels of inoculum. Autofluorescence (a sign of an active defense response) was visible under ultraviolet light 24 h after localized inoculation in the incompatible interaction whereas, autofluorescence was not evident in the compatible interaction. Autofluorescence was also observed in LmmKaty 20 h after pathogen inoculation, thus indicating that rapid cell death is a mechanism of LmmKaty to restrict pathogen invasion. Rapid accumulation of defense related (DR) gene transcripts, phenylalanine ammonia lyase and β -glucanase, was observed beginning at 6 h and was obvious at 16 h and 24 h in an incompatible interaction. Rapid transcript accumulation of PR-1 and chitinase had occurred by 24 h after inoculation in an incompatible interaction. Accumulation of these transcripts was delayed in a compatible interaction. These results indicate that host active defense responses occur 24 h after pathogen inoculation and that LmmKaty exhibits enhanced resistance to *M. grisea*.

CURRENT STATUS OF MUNGBEAN AND THE USE OF MUTATION BREEDING IN THAILAND

S. NGAMPONGSAI¹, A. WATANASIT¹, S. SRISOMBUN², P. SRINIVES³ AND A. MASARI¹

¹Chai Nat Field Crops Research Center, Muang, Chai Nat, Thailand

²OARD1, Chiang Mai University, Muang, Chiang Mai, Thailand

³Department of Agronomy, Faculty of Agriculture, Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand

Email: sumana56@hotmail.com

Seeds of vars Khampang Saen 1 (KPS1) and Chai Nat 36 (CN36) were irradiated with a dose of 500 Gy gamma rays and treated with 1% ethyl methane sulphonate. The objectives of this experiment were to improve seed yield and powdery mildew resistance. A number of mutant lines were selected from M₂ onwards. Three promising mutants, M4-2, M5-1 and M5-5, gave 8-11% and 2-5% higher mean yield than those of KPS1 and CN36, but they showed similar disease infection to their original parents tested during 1997-2006. The objective of the second experiment was to improve mungbean variety tolerant to beanfly, a key pest of mungbean. Seeds of var Khampang Saen 2 (KPS2) were irradiated with 600 Gy gamma rays. A mutant line was selected and subsequently officially released as Chai Nat 72 (CN72) in 2000. It is the first mungbean variety released and developed through mutation techniques in Thailand. CN72 had lower beanfly infestation than a susceptible variety, CN36. The result of an addition trial conducted on a calcareous soil showed that grain yield of mutant CN72 was superior to the KPS2. The third experiment of the Mungbean Mutant Multi-location trials was conducted in two sites during 2003-2005. All mutants retained most traits of the original varieties, including yield. The highest yielding mutant across all five trials was CN72 which was similar with its progenitor (KPS2) and the local check, CN36. These three entries bore large seeds (70 g per 1,000 seeds), which is a desirable trait for Thai and international markets. An exotic entry, Native variety showed least incidence of powdery mildew disease. It would be used as a source of disease resistance in the breeding programme.

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INTERVARIETAL DIFFERENCES IN RESPONSE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) TO DIFFERENT MUTAGENIC TREATMENTS

S. GVOZDENOVIC¹, S. BADO², R. AFZA², S. JOCIC¹, C. MBA²

¹Institute of Field and Vegetable Crops (IFVC), Oil Crops Department, Novi Sad, Serbia

²Plant Breeding Unit, Joint FAO/IAEA Agriculture and Biotechnology Laboratory, International Atomic Energy Agency Laboratories, Seibersdorf, Austria

Email: sandra@ifvns.ns.ac.yu

For much of the past century, mutagenesis has gained popularity in plant genetics research as a means of inducing novel genetic variation. Induced mutations have been applied for the past 40 years to produce mutant cultivars in sunflower by changing plant characteristics that significantly increase plant yield and quality. The present study was focused on generating baseline data to elucidate the role of genotypic differences in the response of sunflower to induced mutagenesis with the aim of expanding the applicability of the use of induced mutant stocks in the genetic improvement of the crop and in its functional genomics. The strategy adopted was to estimate the optimal treatment conditions (doses of mutagens) through relating the extent of damage in seedling progeny to the exposure levels of the initiating propagules to mutagens. Seeds of fifteen elite sunflower genotypes of commonly used as breeding stocks and grown on commercial scales were treated with a range of mutagens: gamma rays (γ rays); fast neutrons and with ethyle-methane-sulphonate (EMS) at different treatment doses. The three mutagenic agents affected seedling height, reducing it with increasing dosage. Based on the mutagen damage on seedling height, the 50% and 30% damage indices (D_{50} and D_{30} , respectively) were estimated for the 15 sunflower genotypes for the three mutagens. The D_{50} (D_{30}) values for the sunflower lines ranged from 120 to 325Gy (5 to 207Gy) for gamma irradiation; 9 to 21Gy (0.1 to 10Gy) for fast neutrons and 0.69 to 1.55% (0.01 to 0.68%) concentration of EMS.

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A NOVEL DOMINANT SEMIDWARF MUTANT AND ITS PLANT HEIGHT REVERTANTS INDUCED WITH ION IRRADIATION IN RICE (*ORYZA SATIVA* L.)

B.M. LIU, X. XU, M. ZHAO, Y.J. WU

Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences
Hefei, China

Email: liubm@mail.com

Rice *Dominant Semidwarf* mutant (Sdd) was obtained from its wild type (WT) irradiated by low-energy ion beam. The genetic analysis of Sdd indicated that the phenotype of semi-dwarf was controlled by single dominant gene, termed *Sdd(t)*. We show that Sdd reduced the plant height mainly via inhibiting the first, second and third internodes elongation. In addition, the *Sdd(t)* gene was sensitive to gibberellin (GA) based on the response to exogenous GA_3 and the quantitative determination of endogenous GA_1 and GA_4 . To map the *Sdd(t)* gene, we tested molecular markers by bulk segregant analysis. The *Sdd(t)* gene was localized to a 6.4 cM interval on the short arm of chromosome 6, flanked by two sequence-tagged site (STS) markers S9 and S13. To study the function of *Sdd(t)* furthermore, we created six tall revertants of Sdd with ion irradiation. The revertants restored the plant height to WT plants. Agronomic characters investigation indicated the revertants were different from Sdd while similar to WT. And the genetic analysis showed that the revertants were putative different inherited mutation. Furthermore, Sdd, WT and tall revertants were checked for their DNA level differences using SSR (simple sequence repeat) technique. Among 408 SSR primers used, only two primers displayed different SSR bands in two revertants. The result demonstrated the revertants were induced from Sdd by ion beam implantation. This study indicates that ion irradiation may be used as a new effective mutagen to create mutants and revertants for plant functional genomics research.

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REVIEWS AND PROSPECTS ON SPACE MUTATION BREEDING

J.Y. LIU, H.X. YAN

Luohe Academy of Agricultural Sciences, Henan, China

Email: jiayou.liu@126.com

Space mutation breeding is a technology that uses beneficial character variations caused by space factors when seed, tissue, organ or individual of crop are carried by satellite, spaceship or other return-type spacecraft to cultivate new crop varieties and create new gene. The utilization of the technology is of great importance for new genes in germplasm gene pool are very poor and genetic resources are exhausted day by day. There are great progresses in creating special mutant gene resources and cultivating new crop variety by space mutation breeding in China. But the bred varieties are not popularized and cultivated in large scale, and satisfied achievements on the utilization of mute gene are not got. There are four reasons as follow. Firstly, the comprehensive quality of materials carried to space is not very well, so bred varieties are lack of competitiveness in yield, quality and resistance. Secondly, researches on basic theory are less than on cultivating new crop varieties in space mutation breeding in China. Thirdly, the establishment and integration of aerospace breeding system should be improved and perfected in the future. The studies on applying basic theory in aerospace mutation breeding technology must be enhanced in future. So the studies on biological effects caused by space factors, mutation mechanism and genetic law of aerospace mutation and the combination with biological technology are most important. In a word, there are special advantages in plant aerospace mutation breeding. It is being given more and more attention because space is special mutation source compared to earth. Space mutation breeding is a new way of cultivating new crop variety effectively and creating new germplasm resources. The promising breeding method with strong vitality shows a fine prospect for human beings entering into the age of space agriculture.

IAEA-CN-167-059P

ENHANCED SENSITIVITY TO MUTAGENS-EMS, MH, SM BY PRE-SOAKING – A TAXOMETRIC STUDY BASED ON M₁ PARAMETERS IN FINGER MILLET

B. SINGH, S.K. SINHA

Plant Breeding and Genetics, OUAT, Bhubaneswar, India

Email: sing_baburam@yahoo.co.in

Pre-soaking seeds before treatment enhances sensitivity to many chemical mutagens; but little work has been done with maleic hydrazide (MH), a chromosome breaking agent and preferential inducer of micromutation and streptomycin (SM), a cytoplasmic mutagen. In the present investigation on pre-soaking (PS) effects in finger millet (*Eleusine coracana*, Gaertn), we included these two mutagens, besides one commonly used mutagen, EMS to determine a common effective PS range using M₁ seedling traits. Since different M₁ parameters, mutagens and their doses showed different peaks of response, we adopted a taxometric approach using all characters together. Combinations of chemicals, doses and six M₁ seedling attributes gave 48 characters for the numerical classification of PS periods (0, 8, 10, 12, 14, 16 and 18 h) as OTUs. Dendrogram from the similarity matrix using UPGMA clustering showed two clusters : (1) Cl. 1 of three OTUs (0, 8 and 10 h PS) and (2) Cl.2 of four OTUs(12-18h PS). We considered 12-18h as effective PS range and 0-10h as ineffective for all kinds of mutagens. The effective range would contain the major peak of sensitivity; the ineffective range might show a small peak. We confirmed these inferences with SM induced albinism as an indicator of plastid mutations. Higher doses shifted the peak within the effective range towards lower PS and low dose towards longer PS. Taxometrics could be usefully adopted in mutagenesis studies.

IAEA-CN-167-062P

APPLICATION OF SPACE-INDUCED MUTATIONS FOR WHEAT BREEDINGZ.S. LEI¹, Z.Q. WU¹, L.X. LIU², X.L. ZHAO¹, M.F. WANG¹¹Wheat Research Centre, Henan Academy of Agricultural Sciences, Zhengzhou, China²Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China

Email: zhenshenglei@yahoo.com.cn

To synchronously improve the yield potential with fine quality in wheat, the breeding method and approach should be innovated and widened. Application of space mutagenesis technique for wheat breeding could be a new way to gain this goal. Since 1991, the experimental studies of wheat seed materials boarding on the retrievable satellites and high-space balloons have been carried out. The results showed that the space-induced mutation was characterized as creating more favourable mutations which were easily stabilized. In one of the boarding experiment carried out in 1996, the main wheat materials used were Yumai 21 and Yumai 49, the extensively popularized cultivars in Henan Province during that period. The SP₁ generation was planted in the fields of 3 locations without screening. In SP₂ generation, 75 from 3291 mutants were identified with grain yields higher than Yumai 21 and Yumai 49, some of which were changed significantly in plant height or leaf types, ear type, disease resistance, quality performance and growth period, etc., compared to their corresponding parent. Two new mutant varieties Taikong 5, which was the first approved variety derived from space-induced breeding technology in China, and Taikong 6 were developed and officially released. The total planting area of these two mutant varieties has exceeded 300,000ha in production up to now. In addition, 8 new mutant lines with large spike and a batch of materials with super-high yielding, good industrial quality or other special characters have been obtained and put into further test at this time. The combination of space-induced technology with conventional breeding, the mutation frequency and mechanism of space mutation induction were also discussed.

IAEA-CN-167-068P

**VERTICILLIUM WILT RESISTANCE EVALUATION IN COTTON
(*G. HIRSUTUM L.*) MUTANTS**B.A. KHALMANOV¹, M.R. BAKHSHI¹, B. AMANTURDIEV¹, M. TASHMATOV²¹Uzbek Research Institute of Cotton breeding and Seed Production, Kibray, Tashkent region, Uzbekistan²Gamma Facility, Institute of Nuclear Physics, Tashkent, Uzbekistan

Email: khalmanov@hotmail.com

Research for resistance evaluation of common important disease – Verticillium wilt (*Verticillium Dahliae Kleb.*) of upland cotton in Uzbekistan have been developed in 2007 cotton crop season in Central Experimental Farm at the Uzbek Research Institute of Cotton breeding and seed production in the frame of IAEA TCP Uzb 5004 -“Development of Mutant Cotton Breeding Lines Tolerant to Diseases, Drought and Salinity”. The research plot is situated near the Tashkent city and *Verticillium* wilt evaluation conducted on the naturally infected fields. There have been used irradiated cotton seed with a different doses of gamma rays to the both 4 upland (*G. hirsutum L.*) and 3 long staple cotton (*G. barbadense L.*) varieties in the Gamma Facility of Nuclear Physics Institute of Uzbekistan. If, strong affection in standard of Djarkurgan shows 4,6 %, so in M₁ plants where used the irradiation dose of 50Gy wilt resistance was less for 1,6% in comparison than standard, but minimum affection in M₁ taken from usage of 150Gy. In some cases the date of evaluation the plants of M₁ were not wilt affected. Even there is no overall picture, in general it could be said that the resistance of M₁ plants are comparatively higher than standard ones. Taking into account of advantages of using gamma irradiation in cotton breeding programs in getting wide spectrum cotton plant mutants, which are comparatively wilt resistance there are quite important to continuing the studies. (IAEA TCP UZB5004 & A-11-004 Projects)

YELLOW RUST RESISTANCE OF WINTER WHEAT'S M₁ IN TASHKENT REGION

B. KHALMANOV¹, M. JURAEV², Z. ZIYADULLAEV², SH. ABDUALIMOV³

¹Uzbek Research Institute of Cotton breeding and Seed Production, Salar, Kibray, Tashkent region, Uzbekistan

²Uzbek Research Institute of Plant Industry, Akkavar, Kibray, Tashkent Region, Uzbekistan

³Uzbek Research Institute of Cotton Growing, Yaderniy, M-Ulugbek, Tashkent city, Uzbekistan

Email: khalmanov@hotmail.com

For the last years, the wheat yellow rust (*Puccinia striiformis f. sp. tritici*) is observed throughout the Central Asia and become one of the popular main diseases. The study of determination a relative resistance of winter wheat's M₁ conducted in the frame of IAEA/TCP RER 5013 -"Evaluation of natural and mutant genetic diversity in cereals by nuclear and molecular techniques" for preparation of high productive, biotic and abiotic stresses tolerant cereal breeding genetic materials using of irradiation mutageneses. There have been given the different doses of gamma rays to the 6 wheat varieties seed. Investigations on the yellow rust resistance evaluation in winter wheat M₁ plants conducted in the natural field conditions. If, we consider evaluation of yellow rust resistance in M₁ of Marjon variety, so maximum disease resistant data taken where 10,0 Kr gamma rays used . Except the Kroshka variety's M₁ (15 Kr), almost the same pictures were observed in the rest varieties. It needs to point that, the higher yellow rust resistance have been observed in M₁ plants than standard in Tashkent region conditions. Investigation on genetics of resistance to yellow rust will be continued in further mutant generations.

GENETIC ENHANCEMENT OF PHOSPHORUS USE EFFICIENCY BY INDUCED MUTAGENESIS IN SOYBEAN

R.V.KOTI¹, M. SRIVASTAV², P.U. KRISHNARAJ, N.S HEBSUR, F.H.¹ SAVITA

¹Dept.of Crop Physiology, UAS Dharwad, Karnatak, India

²NA&BTD, BARC, Trombay, Mumbai, India

Email: rvkoti1@rediffmail.com

Induced mutagenesis with gamma radiation (200 and 300Gy) and chemical mutagen, ethyl methane sulphonate (0.5%) was employed in two cultivars (JS-335 and KHSb-2) of soybean to enhance phosphorus use efficiency under phosphorus stress condition. Total dry weight and seed yield in M₂ generation was recorded at harvest. Total P content of the seed was analysed. The phosphorus use efficiency (PUE) was computed as g of dry matter produced per mg of P at harvest and was expressed as g dry wt mg⁻¹ P. Though the mean PUE (g mg⁻¹P) of the M₂ generation plants due to different mutation treatments did not vary much from their respective controls (with or without 'P' application) in both the genotypes, there were considerable numbers of mutants with higher values of PUE than respective parent means. The SD of PUE due to different mutation treatments was higher in JS-335 than in KHSb-2 indicating higher variability in JS-335 than in KHSb-2. The values of PUE of KHSb-2 varied from 0.09 to 0.68 in 200Gy, 0.12 to 0.19 in 300Gy and 0.07 to 0.74 in EMS. Whereas in JS-335, it was 0.07 to 2.97 in 200Gy, 0.10 to 0.38 and 0.09 to 0.59 in 300Gy and EMS treatments respectively. Sixty one mutants of 200Gy, seven of 300Gy and 38 of EMS mutants of KHSb-2 had X+1SD values of PUE. In contrast to this, in JS-335, 36 of 300Gy, 27 of EMS and 12 of 200Gy had higher frequency with X+1SD. Variation in PUE in mutants of both genotypes was attributed to induction of mutation for agronomic traits and as well as in P content. The mutant KE 8-28 recorded highest PUE (0.74) followed by K20 134-1(0.68) in KHSb-2. Whereas in JS-335 mutants J20 39-43 and J20 39-42 recorded highest PUE of 2.97 each. Increase in PUE was higher in JS-335 due to mutation than in KHSb-2.

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ROLE OF MACRO- AND MICROMUTANTS IN COMMON WINTER WHEAT GENETIC IMPROVEMENT

M. NAZARENKO

Department of Experimental Mutagenesis, Institute of Plant Physiology and Genetics, National Academy of Science of Ukraine, Kyiv, Ukraine

Email: nik_nazarenko@ukr.net

62 high-productivity and 41 original mutant lines are created by induced mutagenesis (gamma-rays, nitrosoethylurea (NEU), nitrosomethylurea (NMU)). Over two years intensive field screening of common winter wheat lines revealed a total of exhibiting significantly greater productivity or other value and original traits difference to the parent varieties (Smuglyanka, Odeska 333, Mathilda).

IAEA-CN-167-083P

A NEW MUTANT FOR YELLOW MOSAIC VIRUS RESISTANCE IN MUNGBEAN VARIETY SML-668 BY RECURRENT GAMMA RAYS IRRADIATION

K.S. REDDY

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: ksreddy122@yahoo.co.in

Mungbean variety SML-668 is early, high yielding and large seeded but susceptible for yellow mosaic virus (YMV) disease. To develop YMV resistance in SML-668, mutation breeding programme has been under taken. Seeds of SML-668 were irradiated with 600 Gy gamma rays and planted in the field. Three thousand plants in M_1 generation were harvested separately and planted in M_2 . Ninety lines were showing sterility and only ten lines were showing mutants for chlorophyll, small seed size, short pod length, dwarf plant type and profuse branching but there was no YMV resistant mutant. All the mutants along with normal plants of the segregating lines were harvested separately in M_2 . In M_3 generation 2500 normal lines were planted as single plant progenies and screened for YMV resistance and did not observe any YMV resistant mutant. Hence, the normal M_3 lines were made into two separate bulks and one bulk was irradiated with 500 Gy as a recurrent irradiation and another was sown as it is. In M_3M_1 generation a mutant showing very minor leaf symptoms for YMV and without having any pod symptoms was isolated. The mutant was purified by growing up to M_3M_6 generations. All the mutant plants showed very minor leaf symptoms but not in the pod. The pods and seeds were normal and also gave normal yield as compared to highly resistant check where two recessive genes controlling resistance is reported. The susceptible plants showed leaf and pod symptoms and showed severe yield losses. This mutant will be used in crossing programme to study the genetics of YMV resistance.

IAEA-CN-167-086P

ISOLATION OF EARLY FLOWERING MUTANT IN CULTIVAR C-306 KNOWN FOR ITS GOOD CHAPATI MAKING QUALITY

B. K. DAS, S.G. BHAGWAT

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: bikram_das2001@yahoo.com

About 85% of wheat grains produced in India is consumed in the form of *chapatis* or its variant form. Wheat varieties released in India have acceptable *chapati* making qualities; however, the variety C-306 is quoted for its excellent *chapati* making quality. Good *chapati* making quality requires medium strong dough and it is influenced by protein content and protein quality. The variety C-306 is medium tall and late in flowering and thus not suitable for large scale cultivation. To reduce the duration of the variety, γ -ray induced mutagenesis was used. Mature seeds were irradiated with 200, 300, or 400Gy. About 400 plants in M_1 generation were harvested individually and planted in M_2 generation as plant to row progenies. In M_2 generation mutants were observed which flowered early and showed reduction in height. The mutants were carried forward in M_3 and M_4 generation as plant to row progenies. Although, there were minor segregations in the lines, the early flowering and maturity behavior was consistent. The parent showed anthesis in about 75 days while the mutants showed anthesis from 50 to 63 days. Seven mutant lines were selected for quality analysis. These lines in M_5 generation showed anthesis in 50 days and maturity in 90 days and grain protein content ranging from 11.9 to 14.9% as compared to 13.1% in the parent. SDS-PAGE of total grain protein showed that the mutants had unaltered high-molecular-weight glutenin subunit

pattern. Rheological properties were estimated using Brabender Farinograph. The mutants had comparable water absorption, dough development time, dough stability, degree of softening and quality number. The early mutants are being monitored for yield and quality parameters and are expected to retain good quality and possess improved agronomic characteristics.

IAEA-CN-167-089P

INDUCED GENETIC VARIABILITY FOR QUANTITATIVE TRAITS IN COWPEA (*VIGNA UNGUICULATA* L. WALP)

M.S. UMA, P.M. SALIMATH

Super Elite Sunflower Seed Production Scheme, MRS, University of Agricultural Sciences, Hebbal Campus
Bangalore, Karnataka, India

Email: umauas@yahoo.co.in

Variability in a crop plant can be increased either by hybridization among diverse genotypes or through induced mutation. To harness more variability, mutation has been superimposed on hybridization in several crops. An attempt has been made to study the variability generated by hybridization and mutagenesis in cowpea in the present investigation. The evaluation of about 200 germplasm lines of cowpea, revealed the superiority of C-11 and C-70 in respect of pod length, number of pods per plant and boldness, while KM-1, distinctly determinate in nature, thus acting as source for creation of different plant types which is an important aspect in cowpea breeding. C-152 is a locally adopted variety but lacking in some of the desirable features that are present in C-11, KM-1 and C-70. Therefore it was planned to attempt hybridization with C-152 as base and C-11, KM-1 and C-70 as the different sources of specific features. But as outlined above, since the combination of mutation is expected to release more variability subjected for an appropriate dose of gamma radiation. The resulting M_2 and F_2M_2 populations of different crosses were evaluated for comparing their relative efficiency in generating variability of quantitative characters. The F_2M_2 population of C-152 x C-11 showed highest mean values for plant height, number of pods per plant and yield. Variance and range values were high in F_2M_2 populations for all the characters except number of seeds per pod. The irradiated population of C-152 x KM-1 proved to be superior in producing greater frequency of transgressive segregants for individual traits. Irradiated heterozygous population produced more variability than that of irradiated parent (C-152 M_2) and control.

IAEA-CN-167-091P

THE QUALITY STUDY OF *MEDICAGO SATIVA*. L. VAR LONGMU803 IRRADIATED BY THE MIXED HIGH-ENERGY PARTICLE FIELD AND ^{60}Co - GAMMA RAY RESPECTIVELY

C. SHANG, Y.X. ZHANG, F.L. TANG, R.C. HAO, W.B. HAN

Institute of Pratacultural Sciences, Heilongjiang Academy of Agricultural Sciences, Harbin, China

Email: zyxnky@163.com

Ten respective group dry seeds of LongMu803 alfalfa were irradiated by mixed high-energy particle field (CR) and ^{60}Co γ ray with five doses (109.4Gy, 144.5Gy, 194.7Gy, 284Gy, 559.6Gy). 100 seeds of each group were planted in 2006 Apr, cut on 2006 Sep 27th, when the second batch of bud appeared. All the alfalfa plants were separately reaped in 2006 Sep 27th, airing in shade immediately. The dry plants were triturated by Foss CyclotecTM 1093 Mill, fineness < 1.00mm, then used Wet chemical analysis method measuring the content of crude protein (CP) with the Perten[®] DA7200 Near-infrared Diffuse Reflectance Spectroscopy (NIRS); ether extract (EE) with the Foss[®] SOXTECTM 2045 Extraction System and Crude fiber (CF) with Foss[®] FibertecTM 2010 Fiber Analysis System (Weende Method) The result shows although CR irradiated alfalfa dry seeds have better result than the ^{60}Co γ ray in the plant height and weight, but ^{60}Co γ ray did better in change the alfalfa plant quality. Higher dosage (284~559.8Gy) had better effect on change the CP's content than lower dosage. But high dosage's γ ray will restrain the EE's content.

IAEA-CN-167-092P

IN VITRO MUTAGENESIS FOR *ALTERNARIA* RESISTANCE IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

A.K. BINODH, P. VINDHYAVARMAN, N. MANIVANNAN, R. GNANAM
 Department of Oil Seeds, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University,
 Coimbatore, India
 Email: akbinodh@yahoo.co.in

An attempt was made to find out the possibility of inducing variability for *Alternaria* leaf spot resistance in sunflower variety Morden through *in vitro* mutagenesis. The explant of Morden variety was subjected to callus induction. The callus was treated with appropriate chemical mutagen viz., EMS with concentration of 0.1%, 0.2% and 0.3% and also with *Alternaria* toxin/culture filtrate concentration of 0.5%, 1.0%, 1.5%, 2.0% and 2.5%. The resistant calli would be regenerated. Virulent *Alternaria helianthi* sunflower pathotype toxin was used. In the present study, between the two auxins, NAA was observed as a potent auxin in enhancing the embryogenic callus induction (48.0%). An enhanced callusing (82.1%) was observed in MS + B5 vitamins medium supplemented with NAA (1.5 mg/l) and BAP (1.0 mg/l). A week old embryogenic calli from MS + B5 vitamin + NAA (1.5 mg/l + BAP (1.0 mg/l), when cultured in BAP (1, 1.5 and 2.0 mg/l) containing MS + B5 vitamins medium showed greening response. Embryogenic calli turned greening / greening and browning when BAP was replaced by kinetin (1, 1.5 and 2.00 mg/l) containing MS + B5 vitamins. When *Alternaria* toxin was inoculated browning and blackening response of callus was observed irrespective of media composition and % concentration of EMS. In concluding, the mutated embryogenic calli with greening response when cultured with different concentration of *Alternaria* toxin for the combinations MS + B5 1.0 mg/l, MS + B5 + Kn 1.0 mg/l and MS + B5 1.0 mg/l + Kn 1.0 mg/l with 0.1% concentration of EMS produce no callus regeneration. This might be due to the fact that mutated embryogenic calli did not produce any virulent genetic modification for *Alternaria* resistance.

IAEA-CN-167-102P

EFFECT OF SEED MOISTURE CONTENT ON SATELLITE CARRYING MUTATION

P. FENG¹, J. LI², R.T. LIU¹, Y.W. ZHANG²
¹Grassland College, Gansu Agricultural University, Lanzhou, China
²Institute of Grassland Science, China Agricultural University, Beijing, China
 Email: zywei@cau.edu.cn

There is a new way to get space induced mutant by changing the moisture content of forage seed. The moisture content of alfalfa seed located five levels: 9% (natural water content), 11%, 13%, 15%, 17%. One part of tested seeds were carried by the China's seed-breeding satellite, Shijian-8, from Sep. 9 to 24 in 2006, another part was stored on ground. After the retrieval, performance of key agronomic traits and leaf ultrastructure of plant derived from spaceflight were tested and evaluated. The number of branches, leaf area and height of the nature moisture content were the highest among ground control. The treatment of 9% and 11% was inhibited, the number of branches and leaf area and height of carrying group are lower than the control group ($P < 0.05$); while the 13%, 15% and 17% group were higher than control significantly ($P < 0.05$). And the effect of the 13% and 15% group are significant ($P < 0.05$). Great changes had occurred on ultrastructure on mutations induced by spaceflight factors: bigger chloroplasts of irregular shapes, bigger and more numerous starch grains contained in chloroplasts, less grana and granum-thylakoid stacks of chloroplast. And it aggravated with the moisture content increasing. So we conclude that different moisture content had effect on alfalfa plant growth and development, the 13% to 17% group were lower than the 9% group in the height, number of branches and leaf area; Satellite carrying had different impacts on plant growth and development, especially for seeds of high moisture content. The 13% to 17% carrying groups were higher than corresponding control group in the height, number of branches and leaf area; As the moisture content of carrying seeds increasing, changes of plant ultrastructure aggravated; The moisture content of 13% to 17% of alfalfa seeds was appropriate to satellite carrying.

EVALUATION OF PERFORMANCE OF INDUCED MUTANTS IN MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK]

S. DAS (DEWANJEE), K.K. SARKAR

Department of Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Email: suju_diya2005@rediffmail.com

The importance of mungbean [*Vigna radiata* (L.) Wilczek] has been highly appreciated for its higher protein content with essential amino acids providing balanced diet in combination with cereals. The limited genetic variability available within the existing germplasm does not provide enough scope for genetic improvement in this important crop. Induced mutagenesis has, however, proved its efficiency in generating wider variability with respect to traits of economic importance. To enhance genetic variation for desirable traits, the present study on mutation breeding in mungbean crop was initiated with two varieties K-851 and Sona mung, both treated with four doses of gamma rays (100, 200, 300 and 400 Gy). Fifteen normal looking plants from each of the irradiation treatments of both the varieties were selected to raise the M₂ generation. Fifty seeds from each of these plants along with their parental varieties as controls were sown in M₂ generation for evaluation of variants suspected as mutants and progressed to M₇ generation in succession during the pre-kharif seasons. Families out-yielding the parental variety were only carried forward to advanced generations. For yield and its attributing traits, selection was practised on the basis of the traits showing high positive correlation with primary trait like yield per plant. In Cv. Sona mung, number of branches per plant, clusters per plant, pod per plant, pod per cluster had shown significant positive effect whereas, plant height, number of branches per plant, pod per plant and 100 seed weight showed significant positive effect in Cv. – K-851. Coefficient of variation for most of the traits were successively reduced in advanced generations indicating attainment of uniformity within families in the advanced generations. Four high yielding mutant families accompanied by high harvest index were identified in M₇ generation from 200 and 400 Gy of Sona mung and 200 and 300 Gy of K-851 and such families from 200 Gy showed synchronous maturity for about 80 percent pods. Some of the high yielding families from 300 Gy of K-851 had reduced test weight, thus providing scope for development of high yielding small seeded varieties in mungbean.

ANALYSIS OF HETEROSIS AND COMBINING ABILITY ON THE MAJOR CHARACTERS OF HUANGHUI NO.7, A RICE WIDE SPECTRUM RESTORER LINE BY SPACE MUTATION

Y.Z. LIU, Z.Q. CHEN, J.G. ZHANG, H. WANG, T. GUO, X.D. LIN

College of Agriculture, South China Agricultural University (SCAU), Guangzhou, China

Email: lively@scau.edu.cn

Hanghai No. 7, with strong restoring ability, were obtained by selection and analysis from rice Texianzhan 13, which were carried into space with retrieved satellite for 15 days. Stronger restoring ability were exhibited to three photoperiod-temperature sensitive genic male sterility lines(64S, N9S and N39S, etc.) and eleven cytoplasmic-nucleic male sterile lines of three –line(Hua A, Tianfeng A, II-32A, Bo A, Te A, Qiu A, Mei A, etc.), and the combinations appeared power competition heterosis of F₁ hybrid compared with the control Peizashuangqi, and the superior combination ratio of 1000 grain weight, the per panicle spikelets, filled grains per panicle, seed setting ratio, and panicle length were 100%, 74.3%, 85.7%, 71.4%, and 78.6% respectively. Fifty-four hybrids and their 15 parents (9 male sterile lines and 6 restorer lines) were studied on the combining ability for 8 major agronomic characters. Crosses were made by NCII design. The result showed that Huanghuiqihao appeared a better general combination ability effect value on the grains weight per plant, filled grains per panicle, seed setting rate and 1000-grain weight than the other restorer lines. Furthermore, positive effect of the special combining ability on yield character were observed in 7 combinations of Huanghuiqihao. And three combinations of Huayouhuangqi, Chuanxiangyouhuangqi, Peizahuangqi exhibited higher yields than the other six combinations. Genetic analysis declared the agronomy characters of plant height, panicle length and 1000-grain weight were controlled by the genetic factor mainly and were not easily affected by environment factors. The results indicated that Huanghuiqihao would be a useful material in the improvement of rice breeding.

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GENETIC ANALYSIS OF LOW AMYLOSE CONTENT TRAIT OF MUTANT RICE MLA-1 (INDICA)

T. GUO, Z.Q. CHEN, H. WANG, J.G. ZHANG, Q.M. JI
College of Agriculture, South China Agricultural University (SCAU), Guangzhou, China
Email: guoquot@scau.edu.cn

MLA-1 was a stable rice mutant come from rice cultivar Meixiangzhan by space induced. Compared with its original variety (the amylose content is 17.1%), the amylose content (11.12%) of MLA-1 was decreased obviously and the endosperm character of MLA-1 was translucency. A series of hybrids of MLA-1 with Huahangyihao (high amylose content 27.39%) and Jingxiangnuo (glutinous variety, amylose content 2.40%) were carried out. The amylose content of (MLA-1/Huahangyihao) F_1 showed that the high amylose content was partial dominant to the low amylose content; the amylose contents of (MLA-1 \times Huahangyihao) F_2 seeds population present the separate mode 3:1 of one pair of gene, and all grains with low amylose content exhibited translucency endosperm character; their F_2 plants included low amylose content plants, medium amylose content plants, high amylose content plants, and the separate proportion was 1:2.11:1.24 and fitted in with the separate mode of one pair of gene (1:2:1). The (MLA-1/Jingxiangnuo) F_2 plants included glutinous spikes, mixed spikes and half-appeared spikes, the separate propitiation was fitted in with 1:2:1 too. Based on the results of genetic, it could be presume that the low amylose content trait was controlled by a dominant gene. The mutant gene and *Wx* gene together controlled the low amylose content and the translucency endosperm character. The genomic DNA polymorphism analysis with 180 pairs of SSR (simple sequence repeats) molecular markers which distributed throughout rice genome indicated that genomic polymorphism ratio was 22.8% between MLA-1 and CK. Thanks for support from NSFC (National Natural Science Foundation of China), project number: 30771313.

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THE WIDE SPECTRUM RESTORER OF RICE BREEDING BY SPACE MUTATION

J.G. ZHANG, Z.Q. CHEN, Y.Z. LIU, H. WANG
South China Agricultural University (SCAU), Guangzhou, China
Email: 54601@scau.edu.cn

Dry seeds of pure rice line Te-xian-zhan 13 were carried into space by recoverable satellite, and then the seeds were sent back and planted on ground after flying fifteen days in space. Mutation took place in the seeds mentioned above under space condition. By testing the restoring ability of the mutant progenies, two restorers with wide restoring ability, Hanghui 7 and Hanghui 179, were selected. Though they were derived from the same rice line, their agronomic traits and restoring ability were different. And they showed different combing ability and heterosis too. The results indicated that space mutagenesis can create new restorers.

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INDUCED MUTATION IN PEARL MILLET (*Pennisetum glaucum*)

M.A.M. SALIH, A.H. ABDALLA
University of Khartoum, Faculty of Science, Khartoum, Sudan
Email: salih77@hotmail.com

Pearl millet (*Pennisetum glaucum*) ranks the sixth among cereals in the world following wheat, rice, maize, barely and sorghum. More than 95% of the crop is produced in Asia and Africa, where it is mainly grown for its grain. In America and Europe, it is mainly used as fodder for animals. In this study, 20 different pearl millet genotypes, collected from all over the Sudan, with concentration on the western parts of the country, were used. These genotypes were grown, evaluated and tested for different morphological and agronomical characters. The aim of this experiment was the estimation of variability among pearl millet genotypes. A Radiosensitivity test was carried out to define the suitable level of Gamma radiation for treating pearl millet seeds. Then field performance of the mutants obtained was studied for three successive generations. In the first experiment, the results showed that the 20 pearl millet genotypes exhibited significant difference in most of the parameters studied. Only four were selected to continue with. The radiosensitivity test results reflected that the effective dose ranged between 200-400Gy for the four genotypes. The three mutant generations showed high variations. These variations were reflected in the mean and the range regarding the parameters studied. The second mutant generation (M_2) had the highest number and frequency of mutations, the famous being: chlorophyll deficiency and the male sterility. The third mutant generation also had high variations between the different treatments and within the treatment itself. However, many families

showed a considerable degree of homogeneity. The four pearl millet genotypes reacted differently towards the different levels of Gamma radiation. Mutation induction means proved to be a successful tool for creating variations within a crop variety and inducing desired attributes that can help in far reaching impact on agriculture.

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APPLICATION OF SPACE MUTATION AND IRRADIATION IN THE BREEDING OF RICE VARIETY

X.M. PENG, B.L. PANG

Hunan Institute for Application of Atomic Energy in Agriculture, Hunan, China

Email: pxm889@hotmail.com

The seeds of "96-198" were carried into space by the 18th recoverable science experiment satellite. These seeds were equally divided into 2 portions after returning to earth, one portion did nothing (abbreviated SP), the other portion and 100 original seeds were processed by ^{60}Co - γ irradiation respectively (abbreviated SP+ γ and γ). γ -ray irradiation dose was 200Gy. And 100 original seeds without treatment was taken as checks (CK). The result was shown below. The space mutation combined with irradiation was used in order to breed new rice varieties. This new technology can increase the frequency and type of mutation. a new rice variety with two—lines cross "Peiliang you 72 1", and a series of new materials with good quality were selected.

IAEA-CN-167-132P

EFFECT OF INDUCED MUTATION IN A 7×7 HALF DIALLEL CROSS COMBINATION ON THE GENETIC CONTROL OF QUANTITATIVE TRAITS AND VARIABILITY IN THE ADVANCE GENERATION AT MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR LEVEL IN SESAME (*SESAMUM INDICUM* L.)

T. DASGUPTA¹, T. BEGUM¹, N. MANDAL², D. NATH¹

¹Department of Genetics and Plant Breeding, Institute of Agricultural Science, Calcutta University, Kolkata, India

²Department of Biotechnology, Instrumentation & Environmental Science, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Email: tapashdg@rediffmail.com

Seven sesame genotypes viz. Rama, SI 1666, IC 21706, B 9, B 14, Saheb and BT 893-1 were crossed with each other to make a 7×7 half diallel. Seeds of 21 hybrids were treated with chemical mutagen Ethyl Methane Sulphonate (EMS). The F_1 , F_1M_1 and F_2 , F_2M_2 generations were raised to investigate the gene action and the effect of EMS in the segregating population. Parents Rama, B 9 and B 14 were identified as good general combiner for seed yield and many of component characters both in F_1 and F_1M_1 generations. Ten cross combinations viz. Rama \times B 9, Rama \times B14, Rama \times Saheb, SI 1666 \times B 9, SI 1666 \times B 14, IC 21706 \times B 9, IC 21706 \times BT 893-1, B 9 \times BT 893-1, B14 \times Saheb and B14 \times BT 893-1 appeared to be superior consistently in F_1 and F_1M_1 generations. Rama \times B 9 was the best hybrid combination in both F_1 and F_1M_1 generations. F_2M_2 population performed better than F_2 for seed yield/plant. The combination of hybridization and mutation appeared to have a great impact in generating higher frequency of transgressive segregants in F_2M_2 as compared to only hybridization. Desirable plant types were consistently recorded in mutagen treated hybrids in F_3M_3 and F_4M_4 generations. Out standing F_4M_4 lines also exhibited higher oil content and higher PUFA content. Variation was also observed in protein and DNA banding pattern.

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GAMMA RAYS INDUCED MUTATION IN SOYBEAN (*GLYCINE MAX* (L) MERR.) FOR RESISTANCE TO MOISTURE STRESS, ROOT ROT AND COLLAR ROT

R.S. NANDANWAR¹, AND J.G. MANJAYYA²

¹Regional Research Centre, Dr. Panjabrao Deshmukh Agriculture University, Amravati, India

²Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: rrc_amt25@yahoo.com

The experiment was conducted to develop such a cultivar that yield well in favorable season and suffer with minimum yield losses during water stress. The seed material of two promising cultivars viz. JS-93-05 and JS-71-05 was sent to BARC, Trombay, Mumbai (India) for irradiation with different doses of gamma rays viz. 150 Gy, 250 Gy and 300 Gy, etc. Root rot and collar rot sick plot nursery was developed as per standard procedure. The total 93,689 seeds were obtained from M₁ generation. In M₂ generation more lethality was found in all doses of variety JS-71-05 than JS-93-05. The dose dependent decrease was noticed in most of the characters. The frequency of albino, xantha and chlorina chlorophyll mutants was found to be 0.013, 0.038 and 0.073 per cent, respectively. The common type of morphological mutants found in both varieties are change in flower colour i.e. purple to white (0.0122 & 0.0067), Dwarfness (0.1266 & 0.1760), brown pubescence (0.0142 & 0.0245), high primary root length (water stress tolerant mutant) (0.0326 & 0.1833) and mutants for more number of pods i.e. 270-290 pods/plant (0.2920 & 0.6125) while some of the mutants which are found in JS-93-05 viz. Tall mutant (0.0796), early mutant i.e. maturity in less than 70 days (0.0367), viney type (0.0142), plants with white pubescence (0.0204), change in seed colour (0.0020), and mutants for high secondary root length (water stress tolerant mutant) (0.1960). Two types of mutant found in variety JS-71-05 are mutant for Bold seeded (0.0067) and mutant for elongated leaves (0.0067) with very low frequencies. In general 250Gy dose was found more efficient for getting maximum spectrum and frequency of chlorophyll and morphological mutations. Differential sensitivity of these two varieties to gamma rays was observed. Out of 11,500 seeds which was tested on root rot and collar rot sick nursery plot, only 69 plants survived were harvested separately for further study.

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INDUCED GENETIC VARIABILITY FOR SEED YIELD AND OTHER TRAITS IN CLUSTERBEAN (*CYMOPSIS TETRAGONOLOBA* L.)

S.S.SOLANKI, H.R.BISHNOI

Agriculture Research Station, Rajasthan Agricultural University, Jodhpur, Rajasthan, India

Email:solanki.ss@hotmail.com

The clusterbean (*Cymopsis tetragonoloba*) varieties viz., RGC 936 and Naveen were used for mutation study. Dry seeds of cultivars irradiated with gamma rays (200, 400, 600, and 800Gy), ethyl methyl sulphonaate(0.5%) and their combinations together. Efficacy of mutagens in generation M₂ was studied for expressing macro/micro mutation for seed yield and yield contributing parameters. The mean sum of squares indicated creation of variability for number of branches, number of clusters per plant, total number of pods and seed yield per plant except plant height, length of pods and 100 seeds. The mutation treatments 200Gy+EMS, 400Gy+EMS and 800Gy+EMS significantly increased seed yield per plant in variety RGC 936, whereas 200Gy gamma rays and EMS 0.5% reduced seed yield in this genotype. There was differential behavior of genotypes towards doses and mutagenic agents. The M₂ generation progenies raised from bulk seed of M₁ generation plants are expected to provide higher mean and greater frequency of desirable mutants.

RESPONSES OF EMS-INDUCED DWARF/SEMI-DWARF SOYBEAN MUTANTS TO EXOGENOUS GA₃

W. LI, B.G. ZHU, M.X. XU, L.M. ZHANG, X.W. CHEN, W.P. MA

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (CAS), Beijing, China

Email: bgzhu@genetics.ac.cn

Dwarf and semi-dwarf mutants are the valuable materials for investigating gene function and developing excellent crop variety with high yield and lodging resistance. The objective of the present study is to analyze responses of EMS-induced dwarf/semi-dwarf soybean mutant plants to exogenous GA₃. Seeds of two soybean cultivars, LD4 and JD23, were treated by 0.5% (v/v) EMS. We selected a dwarf (*df*) and a semi-dwarf (*sdf*) mutant from progenies of treated JD23 (WT-d) and LD4(WT-sd), respectively. Plant height (40cm) of *df* mutant is 58cm shorter than that (98cm) of WT-d, and that (76 cm) of *sdf* mutant is 57cm shorter than that (133cm) of WT-sd. The developing plants of *df* and *sdf* mutant and their WTs in different growth stages were treated with exogenous gibberellin (GA₃). The experimental results indicated that the responses of *sdf* and *df* mutant plants to exogenous GA₃(40 mg/L) treatment are significantly different, including that: 1) the plant growth average rate (2.76 cm/d) of *sdf* mutant in different measuring-time intervals was remarkably higher than that (0.92cm/d) of the *df* mutant, whereas in water treatment(control) the plant growth average rates of the *sdf* and *df* mutant were 1.10cm/d and 0.56cm/d, respectively; 2) the average plant height, node-interval length and cell length in vertical section from some node-intervals on the main stem of *sdf* mutant all were much higher than those of *df* mutant; 3) After application of exogenous GA₃, the normal height of *sdf* mutant could be restored, and that of *df* mutant couldn't. Therefore *sdf* was a GA₃-sensitive mutant, and *df* is a GA₃-insensitive mutant. Additionally the endogenous GA₁₊₃ level (4.00 or 5.81 ng/g.FW) in *df* or *sdf* mutant plant was obviously lower than that (9.92 or 8.59 ng/g.FW) in its WT plant, which may be a physiological cause of *df* or *sdf* mutant dwarfism.

GAMMA RAY INDUCED VARIATION IN CUMIN (*CUMINUM CYMINUM* L.)

E.V.D. SASTRY, M.M. SHARMA

Department of Plant Breeding and Genetics, SKN College of Agriculture, Rajasthan Agricultural University, Jobner, India

Email: evdsastry@yahoo.com

Cumin is an important spice crop of Rajasthan, India. Apart from practical problems in emasculation and pollination, the conventional hybridization strategy is hampered due to non-availability of pure lines in cumin. RZ 19 was selected for the present study. Air dried seeds of RZ 19 were subjected to γ rays at 200, 300, 400, 500, and 600Gy. M₁ generation was raised from the treated seed by dibbling 400 seeds per treatment along with untreated check. No specific morphological mutations were observed in this generation. 50 normal looking plants from each radiation dose and control were advanced to M₂. Out of these 50 plants, 25 were self pollinated to produce 'self pollinated' M₂ progenies in each radiation dose. Seeds harvested from each of the 125 self pollinated and open pollinated progenies were sown in single row plots in RBD in the ensuing year along with control to raise M₂ generation. Observations were recorded on important morphological traits. The progenies in each generation were screened for all possible mutations. Only albino (2) and chlorine (5) types were seen which did not survive beyond seedling stage. 3 plants with white flowers were also observed in contrast to pink flowers in RZ 19. Higher estimates of GCV and PCV, heritability in radiation treatments than in control indicated induction of heritable variation. Linear increase in mean values along with the radiation gradient was observed for most of the characters. 300Gy and 400Gy are best doses which induced more mutations per dose, 200Gy dose was ineffective while 600Gy induced more of seed sterility.

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INDUCTION OF NOVEL GENETIC RECOMBINANTS THROUGH CHEMICAL MUTAGENESIS OF MICROSPORES IN INDIAN MUSTARD *B. JUNCEA*

D. PREM, K. GUPTA, G. SARKAR, A. AGNIHOTRI

Plant Biotechnology, TERI (The Energy and Resources Institute), New Delhi, India

Email: abhagni@teri.res.in

The microspore embryogenesis and DH production protocol developed in our lab [*In Vitro Cellular and Development Biology – Plant*, 2005, 41: 266-273] was used for microspores isolation and their chemical mutagenesis to widen the genetic base of three widely cultivated *B. juncea* species, Pusa Bold, Varuna and Bio- 902. The regenerated three to four leaf growth stage plantlets were diploidized, hardened and transplanted to develop doubled haploid plants. The microspores of genotype BIO-902 treated with either ENU/EMS did not produce any embryos while the control produced 85.4 ± 10.9 embryos/ Petri dish. Treatment with 5.0 μM ENU/EMS resulted in maximum embryo induction from the other two genotypes, Pusa Bold and Varuna. Irrespective of the concentration used, EMS mutated microspores produced embryos with higher frequency (239) as compared to those treated with ENU (106). The control embryos exhibited 85 to 90% germination against the mutant microspore derived embryos (16.7 to 31.5%). Overall lower concentrations of EMS (1.0 to 2.5 μM) compared to that of ENU (2.5 to 5.0 μM) promoted higher frequency of positive mutants with promising yield potential. Both EMS and ENU generated considerable variability for agro morphological and biochemical traits; appressed pod phenotype, number of pods, leaf size, total glucosinolate content and FA profile. Desirable phenotypes with reduced glucosinolate ($< 60 \mu\text{M}$) as compared to controls ($> 100 \mu\text{M}$) per g oil free meal were recovered from 2.5 to 5.0 μM EMS mutagenesis. Mutants with $< 2\%$ erucic acid, and $> 45\%$ oleic acid (against 40-45% erucic and 15-20% oleic in controls) were obtained in mutagenized plants from EMS (2.5 μM) and ENU (5.0 μM). Useful variability was identified in mutant plants for their disease response to the most devastating fungal diseases *Albugo candida* (DI 0.6-2.0) and *Alternaria brassicae* (DI 1.3- 2.6) under artificial inoculation.

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GAMMA RAYS INDUCED MUTATIONS IN OAT (*AVENA SATIVA* L.)

A.K. MEHTA, G.S. RATHI, S.K. BILLAIYA

Department of Plant Breeding & Genetics, J.N. Agricultural University, Jabalpur, India

Email: anoop.mehta yahoo.co.in

Livestock play an important role in an agrarian economy of our country, however per day productivity of our animal is poor due to inadequate supply of nutritious forage. Realizing the importance of fodder oat which is one of the source for livestock feeding in northern part of India during the rabi season. The yield trend of oat reach to plateau, to overcome barrier of yield the breeder is left with mutation breeding for improvement. The present study was therefore conducted to augment genetic variability in oat. Dry seeds having 12% moisture content of oat variety 'Kent' and 'JO-1' were treated with 150, 200, 300, and 400Gy of gamma rays. 300 seeds were used in each dose of gamma irradiation. Response to different doses of gamma rays were measured in M_1 generation from (i) seed germination (ii) seedling injury measured in terms of reduction in root shoot length (iii) plant survival (iv) pollen fertility (v) panicle fertility (vi) chlorophyll deficient chimeras. The population size in different treatments in M_2 generation varied from 2670 to 3300 in 'Kent' whereas from 2740 to 3450 plants in JO-1'. The chlorophyll mutations were observed in all the mutagen treatment in both the cultivars in M_2 generation. However, the spectrum of chlorophyll mutants was quite narrow as only three types viz., xantha, straita and albina could be obtained. The spectrum of morphological mutants recorded in M_2 generation were dwarf, semi-dwarf, early maturing, high tillering, broad leaf an extra tall. These mutants showed independent dose relationship as they occurred at random.

INDUCTION OF DOUBLE MUTATIONS IN URDBEAN (*VIGNA MUNGO* L. HEPPER) USING COMBINED TREATMENT OF EMS AND GAMMA RAYS

K. SHARMA¹, V. P. SINGH² R.S. SENGAR¹

¹Direcorate of Research, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut, India

²Department of Genetics and Plant Breeding, Institute of Agricultural Sciences Banaras Hindu University, Varanasi, India

Email: amit78sharma@rediffmail.com

Urdbean (Black gram) is the third important pulse crop of India, and the stupendous breeding efforts have been made for augmentation of yield ceiling and quality in this crop. Almost all the varieties of this crop are generally of rough with black seed. Any breeding effort in the direction of improving the seed colour would be appreciated among the consumers and farmers. Four hundred pure, uniform, healthy and dry (9.5 % moisture) seeds of cultivar Pant Urd-30 were treated with ⁶⁰Co gamma rays (100, 200, 300 and 400Gy doses), EMS (0.2, 0.4, 0.6 and 0.8%) and combination of gamma doses, viz., 100, 200, 300 and 400Gy with 0.2 % EMS. In M₂ generation, four double mutations for seed and pod colour were observed in different frequencies in various treatments of EMS, gamma rays and combination of both. The mutants with black shining seeds and black pods were found maximally followed by the mutant with golden shining seeds and black pods and with yellow and black spotted shining seeds and brown pods. All the mutants were identified and compared with the parent (Pant Urd-30) as well as the standard check (T-9) in M₄ generation in randomized block design with three replications during *Kharif*, 2002. The data were recorded on 10 randomly selected plants from each replication for different yield and yield attributing traits viz., plant height, number of pods per plant, pod length, number of seeds per pod, 100 seed weight and grain yield per plant whereas days to flowering and days to maturity were observed on the plot basis. The Results obtained indicated at SM-3 (Seed Mutant-3) produced maximum grain yield per plant (7.38g) followed by SM-4 (7.08g). The higher grain yield of the mutants could be due to less infection of MYMV and minimum damage by the insects as compared to the check whereas minimum grain yield per plant in SM-6 and SM-8. Maximum 100 seed weight was observed in SM-2 (5.00g) followed by SM-3 (4.96g). The mutant SM-4 required minimum days for maturity whereas maximum was observed in case of SM-5 in comparison to parent and check. Such seed and pod colour mutants accompanied higher grain yield and high protein content may be used as a variety or breeding line directly and indirectly for the improvement of blackgram crop.

THE INFLUENCE OF THE TREATMENT OF WINTER WHEAT WITH IONIZING RADIATION ON THE GROWTH AND DEVELOPMENT OF PLANTS

I. CZYCYŁO-MYSZA¹, F. DUBERT¹, I. MARCIŃSKA¹, A. RESZKA¹, J. JANKOWICZ-CIEŚLAK²

¹Institute of Plant Physiology, Academy of Sciences, Poland

²Plant Breeding Unit FAO/IAEA Agriculture and Biotechnology Laboratory, International Atomic Energy Agency, Seibersdorf, Austria

Email: czyczylo@ifr-pan.krakow.pl

Dry winter wheat seeds var. 'Kobra' were irradiated with the dose of 300 Gy of radiation emitted by a cobalt bomb. The aim of the experiment was to establish whether irradiation could change the vernalization requirements of plants obtained from those seeds. Irradiated and control seeds were germinated after vernalization and under conditions excluding vernalization and then the growth and development of plants obtained in this way were observed. The collected after the first year seeds were vernalized again and sown in order to observe long-term after-effects of radiation. The irradiation of dry seeds slowed down the growth and development of plants independently of the temperature of vegetation inducing or not inducing flowering. Additionally, chosen parameters of crop production were lower for irradiated plants. This means that ionising radiation did not affect the winter genes, especially in the way postulated by the mechanism of dominant genes for spring characteristics. Alternatively, irradiation could affect other genes regulating the level of expression of cold-hardiness genes. The effects of irradiation are more visible in the second year which can point to accelerated ageing processes in irradiated seeds. In the second year the effect of irradiation is still observable, but it is significantly weaker. This can be a result of the active repair processes or of the fact that only some of the effects were inherited. Acknowledgments: We thank Ms. Rownak Afza from IAEA (International Atomic Energy Agency) for providing assistance with seed irradiation.

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INDUCTION OF TEMPERATURE SENSITIVE MALE STERILITY IN RICE USING GAMMA IRRADIATION

T.S. RAVEENDRAN¹, J. SOUFRAMANIEN², S. MANONMANI³¹Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India²Board of Research in Nuclear Sciences, Trombay, Mumbai, India³Department of Rice, CPBG, TNAU, Coimbatore, India

Email: directorcpbg@tnau.ac.in

Temperature Sensitive Genic Male Sterile lines were induced in rice varieties using mutagenesis and used in hybrid rice development. Three agronomically well adapted varieties and one TGMS line were exposed to different gamma ray doses. The highest percent of germination and survival was noticed in 50 Gy in all the varieties and TGMS line. The seeds collected from M₁ plants were raised in M₂ generation as plant to progeny row for screening to identify the best TGMS lines with desirable floral traits. In the M₂ generation 925 plants were raised at Coimbatore. Spikelet sterility was recorded at the time of flowering, through which 160 sterile individual plants were selected and stubble planted at Gudalur to screen TGMS plants. At the time of flowering pollen sterility was observed in plants arising from the stubbles. Fifteen plants showing high degree of fertility (>80%) at Gudalur and behaving as sterile (100% sterile) at Coimbatore was identified for their TGMS nature. These lines will be further exploited for developing two line hybrid combination with higher heterosis.

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A BENTAZON AND SULFONYLUREA SENSITIVE MUTANT AND ITS APPLICATION IN HYBRID RICE

J.W. ZHANG¹, G. PAN², X.Z. WU¹, J.M. TU²¹Institute of Food Crops, Hubei Academy of Agricultural Sciences, Wuhan, China²College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China

Email: zhjwen@public.wh.hb.cn

A rice bentazon-lethal mutant 8077S was obtained by radiation and utilized to develop new hybrid rice systems. Genetic analysis revealed that the bentazon-lethal mutant was controlled by a single recessive gene named as *bel*. The mutant could be killed at the seedling stage by spraying bentazon at the lethal dosage of 300 mg/l or higher, while the dosage was safe for its F₁ hybrids and all other normal rice. This mutant was sensitive to all the sulfonylurea herbicides tested, and the sensitivity was also controlled by *bel*. Interestingly, the another rice bentazon-lethal mutant Norin8m obtained by radiation in Japan was also controlled by a allelic locus of *bel*, which was named as *bsl*. These two mutant genes were cloned by map-based cloning. Both mutant alleles had a single-base deletion, i.e. G deletion in *bel* and C deletion in *bsl*, respectively. The wild-type gene *Bel* encoded a novel cytochrome P450 monooxygenase labelled as CYP81A6. The use of photo-thermo-genic male sterility (P/TGMS) system in two-line hybrid rice breeding was affected greatly by the sterility instability of P/TGMS lines caused by temperature fluctuation beyond their critical temperatures for fertility reversion. To prevent hybrid seeds from contamination, we developed three bentazon-lethal P/TGMS lines using 8077S by backcross, and consequently three new hybrid rice varieties using these P/TGMS lines were registered. If these P/TGMS lines were selfed by temperature fluctuation, seedlings from the selfed seeds could be killed by spraying bentazon at seedling stages, while hybrid seedlings were safe. These new hybrid rice varieties have been widely cultivated in five provinces of China.

DEVELOPMENT OF EARLY MATURING AND SEMI-DWARF IN RICE BY INDUCED MUTATIONS

M.H. FOTOKIAN¹, A.M. NAJI¹, H. AMIRI-OGHAN², A. SADATNORI³, G. AHAMADI⁴

¹Shahed Research centre of Agricultural Sciences and College of Agriculture, Shahed University, Tehran, Iran

²Seed and Plant Improvement Institute, Karaj, Iran

³Aborayhan Campus, Tehran University, Tehran, Iran

⁴Imam Khomeni International University, Qazvin, Iran

Email: fotokian@yahoo.com

Crop improvement using classical induced mutagenesis is now well standardized. A large number of new promising varieties in different crops have successfully been developed world wide using both physical and chemical mutagens. Domsia is one of the best quality and local rice variety in Iran, but it is tall and nearly late in maturity. The dry seeds of this variety were treated with gamma rays at doses 100, 200 and 300 Gy. The irradiated seeds were seeded in nursery, and then transplanted in main land. At the end of first year of experiment, main panicle of some randomly selected plants were harvested and planted as a panicle-to-row at M₂ generation. A semi-dwarf mutant line and an early maturity mutant line were obtained from 300 and 100 Gy respectively, at M₂ generation. These mutant lines were showed no segregation at next generations. The height of semi-dwarf mutant line was 60cm shorter than the control (165cm). The length reduction of internodes 2 through 5 caused the height reduction in semi-dwarf line. Reduction in length of internode closed to panicle was more significant than the other internodes. There was no significant difference in panicle length between semi-dwarf mutant lines and control. The number of tillers in semi-dwarf mutant line was significantly more than the control (#12). The yields of semi-dwarf line were less than control, because of some sterility caused by gamma rays. We are recovering fertility by back crossing of this line with control. Also an early maturity mutant line with 15 days earlier than parent was obtained. The yield of this mutant line was not significantly different from the control. Some traits were studied in mutant lines at M₇ generation, they are includes: plant height, number of culm, uppermost internode length, second internode length, flag leaf length and width, days to heading, days to maturity, panicle length, grain length and width, Spikelet fertility, number of grain in panicle, grain yield, 100-grain weight, amylose (%), gel consistency and gelatination temperature. In this paper I will discuss the details of these traits.

RESEARCHES ABOUT SELECTING RESISTANT MELON TYPES TO *FUSARIUM OXYSPORIUM* F. SP. *MELONIS* RACE 1,2 BY USING TISSUE CULTURE AND MUTATION TECHNIQUES

Y. KANTOGLU¹, E. SEÇER¹, K. ERZURUM², B. KUNTER¹, S. ŞEKERCİ¹, N. KAYABAŞI¹, M. ÖZÇOBAN¹, İ. TUTLUER¹, H. PEŞKİRCİOĞLU¹, Z. SAĞEL¹, S. MADEN², R. YANMAZ³

¹Division of Agriculture, Saraykoy Nuclear Research and Training Center, Turkish Atomic Energy Authority Ankara, Turkey

²Ankara University Agricultural Faculty, Department of Plant Protection, Ankara, Turkey

³Ankara University Agricultural Faculty, Department of Horticulture, Ankara, Turkey

Email: kayaprakta@yahoo.com

Fusarium wilt is a vascular disease of the *Cucurbitaceae* family, especially in muskmelon (*Cucumis melo* L.), caused by the soil fungus *Fusarium oxysporum* f. sp. *melonis* (FOM). This pathogen persists in the soil for extended periods of time, and the only effective control is the use of resistant varieties. *Fusarium oxysporum* f. sp. *melonis* is a very serious disease factor for farmers in Turkey. For this reason researchers are focused on breeding programs to obtain new resistant lines to this factor. During the last three decades, tissue culture techniques have been utilized in crop improvement to generate changes in the genetic material of plants via *in vitro* somaclonal variations (by organogenesis or somatic embryogenesis) and induced mutagenesis. More recently, researchers have been using *in vitro* techniques to investigate the effects of fungal culture filtrates or toxins on susceptible and resistant genotypes of different plant species or cultivars to assess disease resistance. This method is effectively used for cucumber and melon. There are various *in vitro* culture techniques that may be used for cucumber. In this research, we show a method for mass-selection of melon mutants resistant to Fusarium wilt. *In vitro* selection of resistant cells, which are come from irradiated and non-irradiated explants, is done using culture filtrates of different FOM races. According to our results we determined effective irradiation doses and filtrate treatment dose by "Linear Regression Analysis". According to our results 21.75 Gy is effective dose for *in vitro* Yuva cv. Explants to induce mutation and for filtrate treatment 6.73% is the proper dose to select survive calluses and plantlets. This research can lead to the development of new melon cultivars that will be resistant to Fusarium wilt.

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MUTATION BREEDING IN SEED SPICES

K. RAMKRISHNA

Department of Plant Breeding and Genetics, SKN College of Agriculture, Rajasthan Agricultural University,
Jobner, Jaipur, India

Email: krkrishna4@rediff.com

Seed spice is one of the important groups of crops cultivated in India for their large domestic consumption and bright export potential. But while the yield potential of the crops covered under this group is generally low, these crops generally also suffer from lack of usable variation for important yield traits and disease resistance in the germplasm collection and even if present may not be used with ease on account of very small size of their flower, thus restricting the crop improvement programs. We have, therefore, applied mutation breeding using gamma irradiation and chemical mutagens (EMS and Sodium Azide) for creation of variability for improvement of yield of major seed spice crops like Cumin, Coriander, Fennel and Fenugreek. Both M_1 and M_2 generations resulting from treatment of the mutagens were studied in respect of yield and yield attributes and other phenotypic alterations (Chlorophyll and other macro mutations) in certain genotype of these crops. Fenugreek was found relatively most radio and chemo-resistant followed by cumin. Mutagenic efficiency also varied noticeably between crops and mutagens; gamma rays were relatively more potent on cumin as compared to chemical mutagens whereas on fennel it was just reverse. Efficient mutagens more often yielded superior M_2 progenies, i.e. progenies with significantly higher yield than their parent in fenugreek, fennel and cumin. Seed yield per plant of M_2 progenies varied to different extents e.g. the yield was as high as 289% in coriander, 269% in cumin, 122% in fennel and least in fenugreek (83%). In coriander specifically, one of the advance generation mutant showed increase in essential oil content along with seed yield comparable to the parent. The usefulness of induced mutations for improvement of seed yield is discussed.

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DEVELOPMENT OF IDEOTYPES IN URD BEAN: PRESENT AND FUTURE RESEARCH STRATEGIES IN IMPROVING LIVELIHOOD OF FARMERS

N. RAMAMOORTHY, M.PANDIYAN, S.K. GANESH

National Pulses Research Centre, Tamil Nadu Agricultural University, Vamban Colony, India

Email: proframjee@yahoo.co.in

Blackgram culture VBG 69 is a cross derivative from Vamban 1 x UK 17. It matured in 65-70 days and attained 50% flowering after 30-35 days. The average seed yield was 820 kg/ha and 925 kg/ha during rainfed and irrigated condition respectively. The seeds were dull black in colour with 3.85 -4.25.g as 100 – seed weight. The over all average yield for VBG 69 is 820 kg/ha which is 13.10, 22.02 and 9.33 percent increased yield over Vamban 3 (751 kg/ha), ADT 5 (672 kg/ha) and VBN(Bg)4 (750 kg/ha) respectively. Hence, the culture VBG6 9 was released as new blackgram variety VBN (Bg)5 for large scale cultivation in Tamil Nadu during January 2007. Another entry yellow grain type has developed by gamma irradiation which is short duration (55-60 days) high yield with highly resistant to yellow mosaic virus and high nutritive value like protein and phosphorus.

USE OF GAMMA RAYS FOR DEVELOPMENT OF LEAF HOPPER RESISTANT PISTILLATE LINES IN CASTOR (*RICINUS COMMUNIS* L.)

C. LAVANYA¹, GSS MURTHY² AND M. LAKSHMINARAYANA¹

¹Directorate of Oilseeds Research, Hyderabad, India

²Bhabha Atomic Research Centre, Trombay, Mumbai, India

Email: lavanyador@gmail.com

A non revertant stable pistillate line -DPC 9 of castor was developed at Directorate of Oilseeds Research, Hyderabad. It is resistant to a major biotic stress like soil borne *Fusarium* wilt but susceptible to the incidence of leaf hopper - *Empoasca flavescens* (Fabr). Resistance to leaf hopper is linked to presence of waxy coating called bloom on plant parts which is a highly heritable character and controlled by dominant, single to oligogenes. Castor is a sexually polymorphic species and pistillate character in S type is controlled by dominant and epistatic factors. Mutation breeding is resorted to strike a balance between a complex character like non revertant pistillate expression and a simply inherited character like presence of bloom. Seeds of DPC 9 were treated with different doses of gamma rays starting from 400, 450, 500, 550 and 600 Gy rays in 2001. Observations recorded on germination (%), hypocotyl length and root length in 50 seedlings of 15 day age, grown under pot culture conditions indicated that 600Gy gamma rays resulted in 25-30% reduction in these parameters. Three hundred M₁ plants of 550Gy gamma ray treated lot were raised in *Rabi* season, 2002-03 at a spacing of 90 x 45 cm and continued up to 6th to 7th order of spike for selection of stable pistillate expression and selfed. Plant to row progenies were continued in M₂ and selection for pistillate expression continued up to M₅ in *Rabi* season every year. Break down of resistance to *Fusarium* wilt was observed in M₃ and majority of the plant progenies succumbed to wilt under field conditions of the inoculum. In M₅, DPC 9 plants with the presence of bloom on all parts (triple bloom), stem and lower side of the leaf (double bloom) were observed and advanced to M₆. In M₆, DPC 9 pistillate plants with triple bloom and high intensity of bloom on older leaves were identified. The pistillate line is under stabilization for its presence of bloom and stable pistillate character. Selection criteria in the present study included stable pistillate expression, presence of bloom and resistance to *Fusarium* wilt and leaf hoppers under field conditions.

MUTATION BREEDING IN ARID LEGUMES IN INDIA

K. RAMKRISHNA, U. JAIN, S.C. KUMAWAT, O.P. BALAI

Department of Plant Breeding and Genetics, SKN College of Agriculture, Rajasthan Agricultural University, Jobner, Jaipur, India

Email: krkrishna4@rediff.com

Mutation breeding programme using chemical mutagens was taken up with arid legumes popularly cultivated in the state of Rajasthan which is characterized by arid and semi arid nature of the agro climatic conditions. Different genotypes of the three nodulating legumes, namely, mothbean (*Vigna aconitifolia* (Jacq.) Marechal), clusterbean (*Cyamopsis tetragonoloba* (L.) Taub) and mungbean (*V. radiata* Wilczek) were treated with different mutagens, namely, Ethylmethane Sulphoanate (EMS), Methylmethane Sulphonate (MMS), Sodium azide (SA) and Hydroxylamine (HA). While the three crops were more or less similarly sensitive to the four mutagens, clusterbean appeared relatively tolerant to sodium azide than mungbean or mothbean. The efficiency of the mutagen was worked out on different genotypes of the three crops. Efficiency was maximum on mothbean genotypes followed by clusterbean and least on mungbean. Hydroxylamine was least efficient. On genotype CZM1 of mothbean the efficiency of all the mutagens was considerably low. One of the four genotypes of mothbean studied showed high magnitude of reversion from erect habit. The maximum seed yield per plant observed among the M₂ families of different genotypes of the three legume crops varied considerably. Within the species the genotypic differences were easily noticeable. The maximum increment in the magnitude of other characters also varied between the genotypes of a crop and between the species which indicated the importance of the particular yield trait(s) in the expression of seed yield per plant. The M₃ progenies of many superior M₂ progenies have been evaluated for their agronomic performances. Conclusively, it is inferred that chemical mutagenesis may be effectively used for re-isolating an improved derivative of the variety of a legume crop already under cultivation.

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DWARF MALE-STERILE WHEAT: A NEW REVOLUTIONARY BREEDING APPROACH IN WHEAT

L. YANG, B.H. LIU, H.Q. ZHAI, S.H. WANG, H.W. LIU, Y. ZHOU, F.H. MENG, J.P. YANG, G. ZHU, S.L. CHUI, Q.H. ZHANG, Y.L. WEI
 Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China
 Email: luxiang@263.net.cn

Dwarf male-sterile wheat is a new germplasm which linked the Taigu genic male-sterile gene *Ms2* with *Aibian1* dwarfing gene *Rht10* tightly on the same chromosome 4DS with 0.18 crossing-over unit. The progeny of dwarf male-sterile wheat always segregates into 1:1 for male-sterile plants with dwarfing gene *Rht10* and male-fertile plants without dwarfing gene *Rht10*. So the male-sterile plants are shorter than the male-fertile plants. It is very easy to identify male sterility plants based on the plant height. Dwarf male-sterile wheat is favorable tool for wheat breeding in recurrent selection. A simple, effective and practical method and technology in recurrent selection called dwarf male-sterile wheat breeding system have been created. The new dwarf male-sterile wheat technical system consists of construction of basic population, choice of male parent, selection of male-sterile plant and inter-crossing. Three new cultivars, i.e. RS981, RS987, RS518 and RS201 have been developed. Dwarf male-sterile wheat and its breeding technical system is an effective technology platform for wheat breeding in different ecology areas.

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RESEARCH ON MUTANT BARLEY POPULATION UNDER BIOTIC AND ABIOTIC STRESS CONDITION

H. PEŞKIRCIÖGLÜ, I. TUTLUER, Z. SAGEL, G. AYDIN, E. SECER, B. KUNTER, Y. KANTOĞLU
 Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Ankara, Turkey
 Email: hayri.peskirci@gmail.com

Barley is one of the most important cereals with 8.5 million tons production, 3.5 million hectares of sowing area and 2.2 ton/ha yield in Turkey which is also one of the gene centres of barley. Barley is grown in every regions of Turkey where climatic conditions are available for the crop. But barley is the predominant crop in the driest land areas throughout the Anatolian plateau. Winters on that plateau are especially severe. Temperatures of -30°C can occur in the mountainous areas in the east, and snow may lie on the ground 120 days of the year. In the west, winter temperatures average below 1°C. Summers are hot and dry with temperatures above 30°C. Annual precipitation averages about 300 to 400 millimeters and rains mainly in winter. Because of all of these prerequisite conditions, winter barley dominates in Turkey, which indirectly refers to water economy. According to the above mentioned reasons the objectives of this investigation were: a) Improvement of drought resistance, lodging resistance and high yielding barley varieties by mutation breeding in Central Anatolian Region. b) Determination and selection of abiotic stress such as salt resistance and biotic stress such as net blotch (*Dresteria teress*). In our barley mutation breeding programme under Central Anatolian conditions well adapted Tokak 157/37 variety has been used. We applied 50, 150, 250 Gy gamma ray doses. Selection began at M₂ generation. Agronomical characters including earliness, straw length, lodging resistance and disease resistance are monitored in the field and greenhouse. Mutant lines have been tested for salt resistance in the hydroponic culture which contains 180 mMol and 220 mMol NaCl concentrations. Biotic stress characters such as net blotch, (*Drechslera teres*) resistance are tested in the greenhouse. Some parameters have been obtained after harvest. Preliminary yield trial and advanced yield trial are started after M₄ generations. In M₆ generation, we had some desirable lines those are 25-30 days earlier than its parents, so these lines escape from drought period. Some lines that have grown in the hydroponic cultures, contains 180mMol NaCl still surviving. From the result of disease resistance experiments we got some lines which were not been effected by the net blotch disease due to their earliness.

MUTAGENESIS OF TWO CHINESE WHEAT VARIETIES WITH SCAB RESISTANCE

X.E. WANG, H.Y. WANG, J. XIAO, R.H. GAO, P.D. CHEN

State Key Laboratory of Crop Genetics and Germplasm Enhancement, Cytogenetics Institute, Nanjing Agricultural University, Nanjing, Jiangsu, China

Email: xiuew@njau.edu.cn

Fusarium head blight (FHB), or wheat scab, is a destructive disease of wheat. Epidemic of wheat scab not only affects the grain yield but also grain quality, and threaten the food security worldwide. Sumai#3 and Wangshuibai are two wheat varieties developed in China. They are considered to be the best genetic resources in wheat breeding for scab resistance. However, development of new varieties using these two varieties as parents was hardly successful because of their poor agronomic traits, such as high plant height. QTL mapping of scab resistance of the two varieties were also inconsistent because different researchers used different susceptible varieties for mapping population construction. In the present research, physical (Fast neutron) and chemical (EMS) mutagenesis was used to induce mutants of Sumai#3 and Wangshuibai for two purposes. First, induce mutants with improved agronomic traits and scab resistance and they can be used as parents in breeding program; Second, induce scab susceptible mutants for mapping and cloning of scab resistance related genes. Among the M₃ population, some mutants with good agronomic traits including dwarf, more tillers, big spikes etc. have been identified. These mutants have been used as parents in breeding program. Several heavy scab susceptible mutants were also identified. A mapping population between Wangshuibai and its susceptible mutant has been constructed. This mutant was also used for microarray analysis.

CREATION OF MUTANT SOYBEAN INITIAL MATERIALS TOLERANT TO BIOTIC STRESS

L. COREȚCHI, E. VEVERIȚA, A. BUDAC, L. COREȚCH, A. CORNESCU, I. ROMANOVA

Institute of Genetics and Physiology of Plant of the Academy of Sciences of the Moldova Republic, Radiation Protection and Radiation Hygiene Centre, Chisinau, Republic of Moldova

Email: lcoretschi@mail.ru

Soybeans are susceptible to many species of fungi and bacteria in the seed and seedling stage. Root rots and seedling blights of soybeans are generally worse when soybeans are planted under cool, wet conditions. The species of fungi responsible for disease in a soybean field depends on several factors: species complex present, temperature and moisture conditions and the genetics of the soybean variety. Our study about the variability of the *Fusarium* species composition demonstrates that the different soybean genetics form (variety, hybrid populations, mutants form) have been affected by: *F. oxysporum* (27,17 % strains), *F. oxysporum var. orthoceras* (42,09 %), *F. solani* (9,57 %), *F. solani var. coeruleum* (4,59 %), *F. javanicum* (2,30 %), *F. javanicum var. redolens* (1,40 %), *F. javanicum var. radicola* (3,95 %), *F. merismoides* (2,30 %), *F. moniliforme* (2,04 %), *F. gibbosum var. bullatum* (1,79 %), another species –2.81 %. Under the consideration of creation of mutant soybeans initial materials tolerant to *Fusarium*, we have studied in the field condition the reaction to this infections of 6 soybean genotypes: Kizelniska, KOO3, Ki237xKOO3, Glia, Mida and Alina, the wet seeds of which were treated with γ radiation on RXM- γ -20 installation with ⁶⁰Co radiation source. Seeds were treated with 10 Gy, 30 Gy and 50 Gy doses. The dose debit consisted 0, 67 Gy/s. The modification of the response reaction of the soybean genotypes under the γ radiation treatment to *Fusarium* diseases attack has been revealed. In general under the influence of all used doses of γ radiation treatment all genotypes manifested higher resistance to *Fusarium* root rots by 9, 52 % and to seedling blights by 5, 75 %. Simultaneously, the specific reaction in the function of genotype has been elucidated. Varieties Glia and Mida manifested higher susceptibility to γ radiation treatment. So, under γ radiation treatment (30 Gy and 50 Gy) the intensity of rots root and seedling blights development of Glia variety decreased by 25,5 % and 18,5 %, respectively and by 22,5% and 16,5 %, respectively, at Mida variety.

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STATUS OF COWPEA MUTATION BREEDING IN ZIMBABWE

P.M. MATOVA

Crop Breeding Institute (CBI), Agricultural Research and Extension Services (AREX), Zimbabwe

Email: matova_p@yahoo.com

Opportunities for developing elite cowpea varieties through mutation breeding were explored in Zimbabwe. Seeds of a commercial cowpea variety CBC1, were irradiated with different doses (150, 200, and 250Gy) of gamma rays. CBC1 is a small seeded, early maturing, high yielding and bushy variety. The irradiated seeds were advanced to M₂ and M₃ in 2003/04 and 2004/05 seasons. Selections were done in 2005/06, the selected lines were put in trials in 2006/07 in which different parameters were measured. The objective was to evaluate the effect of gamma irradiation on agronomic performance of cowpeas. There was no significant difference on days to emergence between the doses 0, 150, 200, and 250Gy ($p < 0.05$), however the data showed that gamma irradiation had negative effects on percentage germination rates. The control (CBC1) had the highest germination rate whereas dose 150Gy had the least. Gamma irradiation had a negative effect on the number of days to flowering. Irradiation was observed to cause an increase in the number of days to flowering with a corresponding increase in the number of days to maturity compared to the control, which took the least number of days to both flowering and maturity. Dose 200Gy was seen to increase podding, whereas dose 150Gy, and 250Gy reduced podding in cowpeas. A corresponding increase in seed yield with dose 200Gy was seen whereas a corresponding decrease in yield was observed with dose 150Gy and 250Gy compared to the control. However, despite the increase in podding and seed yield with dose 200 Gy, gamma irradiation generally reduces seed weight/size and plant height. Gamma irradiation was concluded to have both negative and positive effects on cowpeas but opportunities for developing desirable mutants are high. Various yield parameters in various genotypes can be improved through gamma irradiation. Dose 200 Gy proved to be a useful dose in improving cowpea yield, experimental yields of 2000–3000 kg/ha have been realized.

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THE USE OF INDUCED MUTATION IN THE DEVELOPMENT OF NEW CULTIVARS IN MOROCCO: ACHIEVEMENT AND PROSPECTS

C. AL FAÏZ¹, M. EL AZHARI², M. TAGHOUTI¹, N. EL MRABET², A. AMRI³

¹Institut National de la Recherche Agronomique, CRRA-Rabat, Morocco

²Institut National de la Recherche Agronomique, CRRA-Tanger, Morocco

³ICARDA, Aleppo, Syria

Email: alfayz@hotmail.com

Under the AFRA project RAF5050, induced mutation programme was initiated on wheat, barley and lentils. Results showed that from mutation induced on three ancient varieties (BD 1658, BD 2777 and Karim) we select seven varieties, characterized by reduced growing cycle and short height. These genotypes are under multiplication for quality test. On barley however, results are less promising, since the obtained mutant material are less productive due to their lateness, susceptibility to diseases and lodging. On lentils, we obtained three high productive mutants by irradiating the local variety Bakria. We also select one mutant with 20 days later than the mother variety and two mutants resistant to Fusarium. Furthermore, nitrogen fixation studies showed a high ability of two mutants to fix the atmospheric nitrogen, which was evaluated at 10 to 20%. The selected mutants are growing to improve their genetic stability to start multilocation yield trials. Currently and under the extended phase of AFRA project RAF5056, induced mutation concern the safflower, capper and sugar beet. The objective is more oriented on the increasing of the genetic variability of these species.

PRODUCTION OF DOUBLED HAPLOIDS IN TUNISIAN DURUM WHEAT (*TRITICUM DURUM* DESF.) CULTIVARS THROUGH UNPOLLINATED OVARY CULTURE

O. SLAMA AYED¹, M. BARAKET¹, Y. TRIFA¹, H. SLIM AMARA¹, J. DE BUYSER², E. PICARD²

¹Laboratoire de génétique et Amélioration des plantes, Institut National Agronomique de Tunisie, Tunis, Mahrajène, Tunisia

²Laboratoire de Morphogenèse Végétale Haploïde, Université Paris, Orsay cedex, France

Email: olfa.slama@planet.tn, olfayed@yahoo.fr

The use of doubled haploid improves the efficiency of cultivars development and homozygous genotypes can be obtained in one generation. The major problem with this approach is the low efficiency of green plant regeneration. We describe here an efficient method for regenerating green plants from *in vitro* unpollinated ovary culture of durum wheat that was considered as recalcitrant species to androgenesis because of the high level of albina regeneration. Three Tunisian genotypes (Khlar, Hmira, Azizi) were cultured in this study. Spikes were pretreated at 4°C for 14 days, at 4°C in mannitol solution (0.3M) for 7 days and at 4°C in PEG₄₀₀₀ 1% solution for 5 and 10 days. Induction was performed using two media. The cold pretreatment for 14 days was more efficient than the cold treatment in a mannitol solution or in PEG solution. The addition of 2,4-D, vitamins and glutamine, and the use of maltose as sugar source in induction medium was the most effective condition for the regeneration plants. All the haploid plants regenerated by gynogenesis are green.

INDUCED MUTATIONS FOR DEVELOPMENT OF *B. JUNCEA* CANOLA QUALITY VARIETIES SUITABLE FOR INDIAN AGRO-CLIMATIC CONDITIONS

Y.Y. BARVE¹, R.K. GUPTA¹, S.S. BHADAURIA¹, R.P. THAKRE², S. E. PAWAR²

¹Dhara Vegetable Oil & Foods Co. Ltd., NDDDB Campus, Anand, India

²Department of Botany, RTM Nagpur Uni. Campus, Nagpur, India

Email: yoganandbarve@gmail.com

'Heera', a canola quality *B. juncea* selection, was developed at Department of Botany, RTM Nagpur University, Nagpur, India, by Late Dr. A. S. Khalatkar during 1992-93. The mutation breeding / hybridization programme was initiated during 1994-95 for developing canola quality early maturing varieties with high yield potential. Dry and presoaked Heera seeds were treated with 0.01, 0.02 and 0.03% EMS with three hours and six hours mutagenic treatments. Five mutants with early maturity (93-95 days) as against 140 days that of parent were evaluated in trials. EH-1 was found superior in yield potential; however, the yield was lower than the control. NU-6 was selected in the segregating generation of EH1 X Pusa Bold with high erucic acid and medium glucosinolate with medium seed size. In another experiment, the mutagenic treatment of 0.04% sodium azide was given to 12 hour water soaked seeds of advanced selection derived from cross EH1 x Pusa Bold. The large seeded mutant PB-7 with zero erucic acid, medium glucosinolate was identified. Extensive hybridization programme using mutants EH-1 and PB7 and mutant derivative NU-6 was initiated. Several selections with low glucosinolate, high erucic acid and canola quality were identified from the cross EH-1 x NU-6. Selection NUDH-YJ-6 with low glucosinolate, high erucic acid, 3.6g test weight and high oil content (46%) was at par in seed yield but 12% higher in oil yield in the multi-location trials of four years during 2003-04 to 2006-07 at ten locations in zone III and zone IV of India. The advance selection derived from EH1 X NU6 was crossed with PB7. Several '00' selections were developed and studied for their agronomic characters. Two selections along with checks were evaluated for two years during 2005-06 and 2006-07. Both these selections were resistant to white rust disease and given seed and oil yield comparable to national check Varuna. Another selection NUDYJ- 5 with canola characters having maturity like Indian mustard varieties with small seed size has been registered with the National Bureau of Plant Genetic Resources (INGR NO-03034), ICAR, New Delhi.

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DEVELOPMENT OF IMPROVED VARIETIES OF RAPESEED AND MUSTARD THROUGH *IN VIVO* MUTAGENESIS AND HYBRIDIZATION IN PAKISTAN

A.S. SYED, K. RAHMAN

Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan

Email: sanwarshah@nifa.org.pk

Pakistan is facing edible oil shortage for the past many years despite modest progress made in the development of agriculture sector. More than a billion US \$ are being spent annually to meet the domestic requirement of edible oil of the country. This huge import bill can be reduced considerably by increasing the domestic oilseed production. Rapeseed (*Brassica napus* L. and *Brassica campestris* L.) and mustard (*Brassica juncea* L.) are the second important oilseed crops of Pakistan and their share in total area and production of all oilseed crops grown in the country is over 31% and 28% respectively. However, these crops have been pushed to marginal lands due to their low productivity that resulted in narrow genetic base. Mutation breeding research in conjunction with classical breeding techniques was, therefore, initiated at NIFA in 1989 to induce useful genetic variability in characters of economic importance in Oilseed Brassicas. The research efforts of NIFA Scientists resulted in the development of three varieties namely Abasin-95 in 1996, NIFA-Raya in 2003 and Durr-e-NIFA in 2005. These varieties were approved by Seed Council of North West Frontier Province for commercial cultivation in the irrigated and rainfed areas of the province. Abasin-95 and NIFA-Raya are the first ever-mutant varieties respectively of rapeseed and mustard in Pakistan. Durr-e-NIFA was developed from hybridized population of a cross between Australian canola variety 'Dunkeld' and NIFA mutant variety 'Abasin-95'. All the three varieties possess high yield potential, medium to high oil content, early maturity and broader adaptability to rainfed and irrigated environments in comparison with the local check varieties and respective parents. These varieties are being cultivated by growers on appreciable areas. The paper reports the developmental history and performance of these varieties.

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EARLINESS IN MATURITY AND SEMI DWARF-NESS IN BARLEY INDUCED THROUGH MUTATION

I.N. MAMUYA, O.F. NGULU, S.A.J. SECHONGE, R.B. SHEKIBULA

Selian Agricultural Research Institute, Arusha, Tanzania

Email: ibmamuya@hotmail.com

In Tanzania barley is used for malting, and to accelerate the release of new varieties, breeding strategies have been on improvement of existing cultivars. Under the project "Enhanced Crop Productivity through Radiation URT/5/023" three barley varieties; Bima, Kusini and 8519 were treated with chemical mutagens. The cultivars were treated for three hours with 1.0mM NaN₃ followed by three hours in 0.5mM MNH. From M₂ generation individual plants showing earliness and semi dwarf-ness were selected. Evaluation of selected lines has been going on, agronomic the lines look similar to the mother cultivars except for the induced traits and now they are at M₆ generation. The release of new promising cultivars (direct or after hybridization) in the near future is possible. Earliness in maturity helps as an escape mechanism to drought and semi dwarf-ness is important to avoid lodging in more humid and fertile areas.

M 127- A PROMISING TOMATO VARIETY DEVELOPED THROUGH INDUCED MUTATION TECHNIQUE

R. PEIRIS, T.K.WICKRAMASINGHE, S.P.INDRASENA

Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka

Email: peirisranjani@yahoo.com

Bacterial wilt (BW) is the most serious constraints for tomato cultivation in Sri Lanka. At present, the producers and consumers are more interested in yield and quality of produce. The objective of this study was to develop genotypes having BW resistance, high yield potential (>20 t/ha) with desirable fruit qualities. Application of induced mutations was practiced on Manik variety. It is a well adapted variety with BW resistance, large fruit size with low fruit weight (76 g) due to large empty locular cavities. Several beneficial mutants better than the parent variety were identified in M₂ generation and confirmed in M₄ generation. The most promising 05 mutants were evaluated for BW resistance, fruit quality and yield. During dry and wet seasons, the yield evaluation studies were conducted in research and farmer fields. The mutant M 127 gave significantly higher yields (32.2 t/ha) than the check variety T 245 (21.7 t/ha) during the both seasons. Bacterial wilt screening in the field and laboratory demonstrated that M 127 was moderately resistant. The National Coordinated Varietal Trials confirmed that it was a promising mutant under different Agro-ecological zones in both dry and wet seasons. On farm trials indicated that farmer acceptability was higher for the mutant than the check variety. The mutant M 127 possesses high fruit weight (158.6 g), red, slightly flattened firm fruits. It is highly acceptable for table purpose. In near future the mutant M 127 will be officially released to farmers and at present, it is utilized as a donor parent in the development of new HF3 hybrid under the heterosis breeding programme.

IAEA-CN-167-296P

INDUCTION OF DORMANCY IN SPANISH GROUNDNUT SEEDS (*ARACHIS HYPOGAEA* L) USING COBALT-60 GAMMA IRRADIATION.

N. BENSLIMANI¹, L. KHELIFI²

¹Nuclear Research Center of Algiers, Algiers, Algeria

²National Institute of Agriculture, El-Harrach, Algiers, Algeria

Email: benslimaninabila@yahoo.fr

Irradiation has been used in several countries to create genetic variability in groundnut (*Arachis hypogaea* L). Several mutated lines were isolated. Our research aims to induce genetic variability to make a selection to improve a local population of groundnut especially with regard to the dormancy characteristic of the seeds. In this context, dried seeds (14% moisture content) of four Spanish type groundnut local populations were treated with the dose range of 50 to 450 Gy in order to study their radiosensitivity in first instance at the laboratory level. The optimal irradiation doses were determined for two groundnut populations Berrihane (P1) and Tonga oust (P3). The measurement of field agronomic characters allowed us to choose a single population that was investigated during two generations. The obtained results have shown a significant effect of irradiation through statistical analyses. Concerning the seeds dormancy tested on every M₂ plant, the obtained results demonstrated the existence of such a feature. However, one has to wait for the next generations in order to evaluate the evolution of the dormancy characteristics with respect to time.

IAEA-CN-167-302P

GENERATION OF PROMISING LINES OF BEAN (*PHASEOLUS VULGARIS* L.) INDUCED BY MUTATIONS TO INCREASE COMPETITIVENESS OF COSTA RICA

P. RAMÍREZ^{1,2}, M. VALDÉZ², F. MORA^{1,4}, R. ARAYA³, N. BARBOZA¹, A. GATICA², E. HERNÁNDEZ¹, J. MUÑOZ²

¹ Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica

² Escuela de Biología, Universidad de Costa Rica

³ Estación Experimental Agrícola Fabio Baudrit Moreno, Universidad de Costa Rica

⁴ Servicio Fitosanitario del Estado, Ministerio de Agricultura y Ganadería, Costa Rica

Email: andresgat@gmail.com

Biotechnology techniques combined to molecular and nuclear energy (Biotec/EN) can be used for inducing mutations in plants and therefore to develop new lines of crops with new characteristic such disease resistance, better quality or enhanced production. In Costa Rica, there is a National Project on Technical Cooperation of the International Atomic Energy Agency (IAEA) for the improvement of beans. This crop is the main source of protein and folic acid in the diet of Costa Ricans. The fungal disease known as Web blight (*Thanatephorus cucumeris*), is the main constraint in the production of the crop and there are not any naturally resistant lines. For this reason the main objective of this project is to select resistance lines and contribute to the increased competitiveness of the national production. In addition, the project will provide an effective technology transfer to the agricultural sector in Costa Rica and enhance the quality of life for farmers and the strengthening of alimentary security in the country. Seeds of "Bribri" and "Brunca" bean varieties were supplied by the Experimental Station Fabio Baudrit Moreno of the University of Costa Rica. A method has been improved for *in vitro* culture in the regeneration of the seedlings. The embryonic shoot apex irradiated with 0, 10, 20 and 30 Gy were cultivated in culture medium MS without growth regulators. The plants were evaluated at 15, 30, 60 and 90 days after transplanted. The variables evaluated were: survival rate, percentage of abnormalities, number of leaves, plant size, number of flowers per plant, number of seeds per plant, seed size, fresh weight of seed per plant and flowering days. Lines resistant to *Tanathephorus cucumeris* through disconnected leaves were evaluated and selected. The currently obtained results are preliminary, because the experiments have not been finished yet. We hoped that this research project will help promote interest in the coordinated use of biotechnology and nuclear energy for peace.

IAEA-CN-167-308P

BIOLOGICAL STUDY OF *MEDICAGO SATIVA*. L. CARRIED BY CHINESE RETURNABLE SATELLITE SHIJIAN 8

W.B. HAN, Y.X. ZHANG, F.L. TANG, R.C. HAO, L.J. LIU

Institute of Pratacultural Sciences, Heilongjiang Academy of Agricultural Sciences, Harbin, China

Email: alclever@163.com

Mutation induction technique has played an important role in alfalfa improvement. In this paper, eight alfalfa (*Medicago sativa*. L.) varieties were carried by Chinese returnable satellite Shijian 8, each variety was 5000 dry seeds. Same number seeds no carried on the earth as control. The satellite was sent by Long March 2C launch vehicle, which was launched in November 3 2003, and returned successfully in November 22 2003 in Sichuan province. The result shown seeds of Caoyuan1, Longmu801, Pleven6, BeZa87 and WL323HQ carried by satellite have higher germination rate than control seeds on the earth, germination rate of Pleven6 has increased 8.93% than control, is the most among eight varieties. But seeds of Zhaodong, Longmu803 and WL232 carried by satellite have lower germination rate than control seeds on the earth, germination rate of WL232 has decreased 19.76%, is the lowest among eight varieties. Zhaodong has shown most heavy susceptibility, but WL323HQ has shown lest susceptibility.of germination rate among 8 alfalfa varieties. There were micronucleus in seed root tip cell carried by satellite, different gene type has different micronucleus rate, Pleven6 has lowest micronucleus rate, but BeZa87 has highest among eight varieties. The seedling shown different aberrations, including cotyledon dry rot; cotyledon kraurosis; dry rot of cotyledon tip; single cotyledon; nick of cotyledon fringe; three cotyledons; four cotyledons etc. al. Seedling aberration rate of WL232 has reached 12.04%, is the highest among eight varieties.

INDUCTION AND EVALUATION OF UMBRELLA TYPE PANICLE MUTANT IN *JAPONICA* RICE

G.L. BAO¹, T. ZHENG², R.T. LUO¹, X.X. ZUO², J.M. WANG¹, F.M. YU¹

¹Institute of Crop Research and Atomic Energy Utilization, Zhejiang Academy of Agricultural Sciences Hangzhou, China

²Yuhang District Station for Extension of Seeds and Techniques, Hangzhou, China

Email: bao8815@yahoo.com.cn

The dried seeds of *japonica* rice pure line U5 with compact type panicle, were irradiated by 200Gy ⁶⁰Co γ -rays. Based on the phenotype of its descendant, a mutant with umbrella type panicle, named as ET2, was developed. Compared with its parent U5, several different phenotypic characteristics were found in ET2: 1) an obvious difference in panicle type showing that the angle of its primary rachis-branches was enlarged upto 30~40°; 2) unripened grains, chalky grain rate and the chalkiness degree was decreased by 41.8%, 39.8% and 59.1% respectively; and 3) yield was increased by 4.15%. The analysis on the traits of grains at different positions within a panicle was indicated that improvement of unripened grains and chalkiness in the bottom grains were better than the ones in the middle, and much better than the ones in the top of the panicle. Induction of ET2 might be an effective way for improving the quality of rice grain in *japonica* varieties with compact panicle.

IAEA-CN-167-317P

***IN VITRO* SELECTION OF BEANS (*PHASEOLUS VULGARIS*) FROM COSTA RICA FOR RESISTANCE TO FUNGAL PATHOGEN *THANATEPHORUS CUCUMERIS* (*RHIZOCTONIA SOLANI*)**

F. MORA UMANA¹, A. LEBEDA², B. NAVRATILOVA²

¹Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, San Jose, Costa Rica

²Department of Botany, Faculty of Science, Palacky University, Olomouc-Holice, Czech Republic

Email: fmorauma@hotmail.com

The research has been realized in the Laboratory of Plant Pathology and Laboratory of Plant Tissue Cultures of the Department of Botany in the Palacky University, Olomouc during the period 3 September – 21 december 2007. *In vitro* cultivation of beans seeds. The culture medium AC agar (Sigma) was used for *Thanatephorus cucumeris*. The next three isolates from Costa Rica were tested: 007-3242, 007-3241, 007-3077. For each pathogen isolate three different doses were evaluated: 1%, 5% and 10%. The medium was added before it was autoclaved. Two bean varieties (Brunca /black color/ and Bribri /red color/) were used during this procedure. In order to cultivate the bean seeds the Murashige/Skoog (MS) (Duchefa) culture medium was used. The bean seeds were sterilized with Chloramine B (2.5%) for 30 min, washing them three times with distilled sterile water and then leaving the seeds into distilled sterile water for 24 hours. The seed coat was eliminated, and then the embryos were extirpated, and placed in Petri dishes for two days, after two days of incubation the roots were cut and transferred to Erlenmeyer flasks. They were cultivated in the growth chamber at a temperature of $24 \pm 2^{\circ}\text{C}$ and a photoperiod of 16 hours /8 day/night. The evaluations were made during the 30, 40 and 50 days of incubation. The spray method was used in order to inoculate *Thanatephorus cucumeris* from the *in vitro* bean seedlings. After three weeks of incubation the seedlings were inoculated (by using a glass sprayer) with *T. cucumeris* (treatments of 1%, 5%, 10%, 2 ml per/ Erlenmeyer flask) in aseptic conditions. The evaluation was made 12 days after the inoculation. The results obtained so far are preliminary, the collected data needs to be analyzed statistically, so it can be published this year.

IAEA-CN-167-319P

STUDY ON THE SPACE MUTAGENIC EFFECTS OF LEYMUS CHINENSIS

D.F. PAN, Y.X. ZHANG, Z.B. SHEN, J.L. WANG

Institute of pratacltural sciences, Heilongjiang Academy of Agriculture Sciences, Haerbin, China

Email: nxpanduofeng@yahoo.com.cn

5000 dry seeds of *Leymus chinensis* were carried by No 21 recoverable reseach satellite (Express by SP) and the same number on the earth as control (Express by CK).Investigation of cytogenetic characteristics showed that space mutation could increase the number of micronucleus and micronucleus rate,germination rate of *Leymus chinensis* which had been treated by space mutation was 13.33%,higher than that of control (Germination rate of CK was only 3.33%). Biological characteristics such as plant height, leaf length,leaf breadth,spike length, above ground biomass, number of tillers and growth velocity were also investigated,the results showed that all of those indexes except number of tillers increased due to space mutation.Germination rate,number of cell micronucleus and chromosome variation rate of *Leymus chinensis* increased after space mutation which were beneficial to variation of *Leymus chinensis*. Also space mutation redounded to increase productivity and seed yield of *Leymus chinensis* in a way.

IAEA-CN-167-322P

MUTATION BREEDING FOR RICE IMPROVEMENT IN TANZANIAA. LUZI-KIHUPI¹, J.A. ZAKAYO², H. TUSEKELEGE², M. MKUYA³, N.J.M. KIBANDA³, K.J. KHATIB⁴, A. MAERERE¹¹Sokoine University of Agriculture, Department of Crop Science and Production, Morogoro, Tanzania²Dakawa Agro-Scientific Research Centre, Morogoro, Tanzania³Agricultural Research Institute, KATRIN, Ifakara, Tanzania⁴Kizimbani Agric. Research Institute, Zanzibar, Tanzania

Email: aluzikih@yahoo.com

The mutation breeding based at Sokoine University of Agriculture (SUA), Morogoro, Tanzania aims at reducing plant height and maturation period of the popular indigenous cultivars while maintaining some of the good qualities of the parents. Dry seeds of the indigenous popular cultivars were irradiated with 170, 210, 240 and 250 Gy gamma rays from ⁶⁰Co at IAEA Seibersdorf Laboratories in Vienna in 1987, 1994 and 2001. The irradiated seeds and controls were sown at SUA. M₁ panicles were harvested, and planted as M₂ panicle-to-row progenies. M₂ plants were selected and advanced to M₃ and subsequent generations using pedigree selection method using plant height, early maturity and grain type as selection criteria. In another procedure, Single Seed Descent (SSD) method was used whereby, one seed was randomly selected from each M₂ plant to raise the M₃ generation. Apart from this, some improved mutants have been used in the cross-breeding programme. The selected variants with improved plant type have been evaluated in multi-locational trials and on farmers' fields. Mutants which were selected using single seed descent were found to be very early in maturity and were resistant to rice yellow mottle virus (RYMV). After several years of multilocation and on-farm trials, SSD 35 was released in 2005 as a new variety under the name of Mwangaza. On the other hand, the improved mutants originating from cultivar 'Salama' also combined high yield potential and resistance to RYMV. Semi-dwarf Supa mutant, M-100 was backcrossed to 'Supa' variety and one high yielding line selected from this cross has been recommended for cultivation in Zanzibar. Other lines originating from crosses between mutants and other varieties have been found to be resistant to rice yellow mottle virus and also combine high yield potential and acceptable grain quality.

USE OF INDUCED MUTATIONS TO ADOPT AROMATIC RICE TO LOW COUNTRY CONDITIONS OF SRI LANKA

R. PATHIRANA^{1,2}, T.D.VITIYALA², N.S. GUNARATNE²

¹New Zealand Institute for Crop & Food Research Limited, Palmerston North, New Zealand

²Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka

Two aromatic rice accessions, Au 27789 and IR Basmati were used in mutation breeding by subjecting 12000 seeds of each variety to γ -ray doses of 200 or 300 Gy from a ⁶⁰Co source. Based on agronomic characteristics, 635 M₂ plants were selected and grown as M₃ progenies. Sixty plants were selected from non-irradiated parental varieties using the same criteria, and tested along with mutant plant progenies. Both doses of γ -rays were effective in creating genetic variability for agronomic characteristics, with high heritability values when M₂ parent to M₃ progeny regression based heritabilities were compared with selection in non-irradiated control varieties. Three mutant lines with compact plant type, erect and larger flag leaf, compact panicles and acceptable quality recording the highest yield were tested in five locations over four seasons using two recommended cultivars as controls. The mutant line 22/3 with a medium level of aroma recorded more than 2.5 t/ha, higher than the average yield of rice (1.5 -2 t/ha) in low-country wet zone. It has a compact panicle and narrow leaf angle allowing denser planting, which may help further increase the yield. The mutant lines maintained superior kernel length, linear elongation ratio and expansion index, all of which are important characteristics of aromatic long grain rice. High-quality aromatic Basmati rice is almost triple the price of rice produced from standard varieties, making their cultivation more profitable. Such grades fetch approximately two times the price of average-grade rice in international markets. Investigation and implementation of agronomic practices that enable the optimisation of the yield and quality of new mutant lines will help to increase profitability of rice cultivation in the marginal areas. Their further improvement may be possible through hybridisation among mutants.

IAEA-CN-167-338P

MUTAGENESIS IN GUAR [*CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.]

R.N. ARORA, S.K. PAHUJA

Forage Section, Department of Plant Breeding, CCS Haryana Agricultural University, Hisar, India

Email: arora15@rediffmail.com

Guar or clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] (2n=14) is a multipurpose legume crop. In India, it is mainly grown for feed, green fodder, vegetable, green manuring and grain purposes. It is also cultivated as a cash crop in Australia, Brazil, South Africa, Pakistan and U.S.A. The guar endosperm which contains galactomanan gum has several diversified industrial uses. Mutagenesis is a powerful tool for creating variation in a crop like guar where exploitable and favourable genetic variability is very meager. The first successful attempt to induce mutations in guar through physical mutagen like gamma radiation was made in India. Various types of manifestations such as reciprocal translocations, trisomics, reduction in seed germination, seedling survival, pollen fertility, seed yield, number of seeds per pod, and pod length have been reported in the treated material. However, some authors have observed increase in peduncle length, plant height, and number of clusters, number of pods per cluster, number of pods per plant, seed yield, protein content, gum content and early maturity in the mutated material. Various types of chromosomal abnormalities such as chromosome stickiness, fragments, univalents, translocations, anaphase bridges, leggards, partial fertility, self sterility etc. have been observed in the progenies obtained from treated seeds. The doses beyond 1000Gy have been quoted to be lethal. The application of chemical mutagens like EMS, hydroxyl amine, hydrazine hydrate, kitazin, saturn, sodium nitrate, NMU, and sodium azide have manifested in chlorophyll mutations, profuse vegetative growth, single stem, regular pod bearing, changed leaf texture or shape and pod size, late flowering, changes in seed colour, determinate and spreading growth habit etc. Heterophylly in guar has also been reported. The effect of hybridization and mutagenesis on the inheritance of various morphological traits in guar has also been studied. Further research and understanding on mutation research is needed to generate more desirable genetic variability for traits of economic importance to develop better ideotype in guar.

IAEA-CN-167-346P

CHARACTERIZATION OF PRE BREEDING GENETIC STOCKS OF URDBEAN (*VIGNA MUNGO* L. HEPPER) INDUCED THROUGH MUTAGENESIS

V. KUMAR¹, A.K. SHARMA, V.P. SINGH², M. KUMAR¹

¹Directorate of Research, Sardar Vallabh Bhai Patel University of Ag. & Tech., Meerut, India

²Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India

Email: vipinch1@yahoo.co.in

Pre-breeding genetic stocks using different doses of EMS, gamma rays and combination of both (EMS and Gamma rays) in two urdbean cultivar viz., PU-19 (Pant Urd-19) and PU-30 (Pant Urd-30) were induced. Out of a total 14 macro mutation selected from the different treatments of the mutagens in PU-19, narrow leaf mutant exhibited significantly higher yield/ plant as compared to the parent and some other mutants viz., Non hairy, Tall, and tendriller showed at par grain yield. All the seed and pod colour double mutations selected from the PU-30 showed significantly higher yield as compared. Such breeding stocks can be used for the further genetic enhancement of this crop.

IAEA-CN-167-360P

DESIGNING POLYMORPHIC ISSR PRIMERS IN ORDER TO STUDY GENE SEQUENCES X AND Y TYPES GLUTENIN SUBUNITS IN 1D LOCUS CONTROLLING FAVORABLE BAKING QUALITY IN ELITE MUTANT LINES OF BREAD WHEAT

M.T. HALLAJIAN , B. NASERIAN KHIABANI

Agriculture, Medicine and Industry Research School, Nuclear and Technology Sciences Research Institute Karaj, Iran

Email: hallaji_2003@yahoo.com

Baking quality is one of important traits in qualitative improvement of bread wheat. Gluten prolamins determine wheat flour quality for different technological process such as bread making. Between gluten proteins, High Molecular Glutenin (HMW) group and specially, d allele in 1D locus with x-type and y-type subunits are very valuable in baking quality. In this study, amino acid sequences of x-type subunits (2.1, 2.2, 2.2*, 5) and y-type subunits (10, 12) related to 1D locus were searched, found and compared together using Genedoc software. After amino acid sequences alignment of y-type subunits and x-type subunits, it was characterized that deletion, insertion (duplication) and point mutations in these subunits involved in biological function of proteins. most important insertion and deletion mutations were 185 amino acids sequence insertion of 2.2* subunit and 102 amino acids sequence insertion of x2.2 subunit in position 486 of amino acid sequence and six amino acid sequence deletion IGQGQQ in position 203 of y10 subunit. From important point mutations can be pointed to conversion of serine to cysteine in position 118 of x5 subunit and substitution of glutamine to histidine in position 626 of x5 subunit. Finally, polymorph ISSR primers in repetitive domains were designed on similarities and differences in subunits of x and y types. These primers show good banding polymorphisms in elite mutant lines, standard commercial cultivars and F₂ populations from crosses.

SELECTION STUDIES ON MUTANT DURUM WHEAT (*TRITICUM DURUM* DESF.) POPULATIONS IN TURKEY

T. KUSAKSIZ¹, S. DERE²

¹University of Celal Bayar, Alasehir Vocational High School, Manisa Turkey

²University of Ordu, Faculty of Agriculture, Department of Agronomy, Ordu, Turkey

Email: sahindere@hotmail.com

Three durum wheat populations, namely Gediz, Salihli, Ege, the effects of gamma irradiation were studied. Various doses of gamma irradiation i.e. (0-150-300 Gy) were applied to seed. The research was carried out at two locations (Bornova and Alasehir) in the 2002-2003 and 2003-2004 growing season. A total of 100 single plants were selected at each mutant and control populations in 2002-2003 growing season. Twenty five percent selection pressures were applied and 25 mutant lines from each population was selected. The progeny rows of the selected mutant plants were grown at two locations in 2003-2004 growing season. A second stage selection was applied in each progeny populations and selected mutants were advanced to the micro yield testing. Genetic gains at each stage of selection done for single plant yield and expected progeny means for plot yield was estimated. In some mutant populations, heritability and phenotypic standard deviation values are higher than control population. Hence, expected genetic gain obtained has become out. Expected genetic gain in Bornova at 150 Gy for Salihli genotype notably has over uppermost value (260.09 g). The value was followed by Ege variety at 150y in Alasehir (258.66 g). The progeny means of the selected mutant populations were higher compared with control populations. The expected gains were also found to be high for the mutant selections. This research has been financially supported by the Celal Bayar University Scientific Research Projects Unit, Projects No. ALS 2002-082.

IAEA-CN-167-377P

EVALUATION OF EFFECT OF DIFFERENT GAMMA RAY DOSES ON ANTHR CULTURE RESPONSE IN TWO IRANIAN WHEAT LANDRACES

M. GHAFARI¹, B. NASERIAN¹, A. SHAHNEJAT BOOSHEHRI², S. VEDADI¹

¹Agriculture, Medicine and Industry Research school of Atomic Energy Organization of Iran, Karaj, Iran

²Dept of plant breeding and biotechnology, faculty of agriculture, University of Tehran, Tehran, Iran

Email: maziar_ghaffari@yahoo.com

Wheat according to its importance as a source of food is the most important strategic agricultural product and has the highest level of under planting lands around the world. Double haploid induction methods are able to raise wheat breeding efficiency through decreasing length of breeding programming and increasing selection efficacy. In this experiment for determining the effect of gamma radiation of seeds on anther culture response of wheat, two recalcitrant Iranian wheat landraces, *Graecum* and *Nigricum* with no response to anther culture were chosen. Callus induction and plant regeneration traits from anther culture of these landraces and twenty of their random selected mutant genotypes which were produced through seed radiation with 100, 150, 200, 250Gy of gamma ray doses were evaluated. A factorial design based on completely randomized design with five replication was used to compare callus induction and plant regeneration percents of genotypes. Gamma ray doses levels and genotypes were two factors of this experiment which had a significant effect on both of callus induction and plant regeneration traits. An evaluation of the means revealed that the highest level of callus induction is noticed in 200Gy (12.5%) and that of plant induction is noticed in 150Gy (67.28%). the highest mean of callus induction (68.6%) was obtained in genotype L9.200.2 as a mutant of *Nigricum* landrace and The highest mean of plant induction (93.12%) was obtained in genotype L8.150.1 as a mutant of *Graecum* landrace.

IAEA-CN-167-382P

THE THREE PISTILS MUTATION ENABLES TO PRODUCE THREE KERNELS IN A FLORET IN BREAD WHEAT (*TRITICUM AESTIVUM* L.)

P. MARTINEK¹, Z.-S. PENG², M. POUCH¹, N. WATANABE³

¹Agrotest Fyto, Ltd., Kroměříž, Czech Republic

²Department of Biology, China West Normal University, Nanchong, Sichuan, China

³College of Agriculture, Ibaraki University, Inashiki, Ibaraki, Japan

Email: martinek.petr@vukrom.cz

The spontaneous mutation of bread wheat (*Triticum aestivum* L.) producing three pistils (TP) in a floret is presented. The TP mutant was found in a spring Chinese landrace and can form up to three kernels in a floret and thus to increase grain number per spike. Restricted space in florets causes kernel flattening. Doublets or triplets of kernels form clusters where the basal parts arise from the receptacle and ventral groove of kernels is outside oriented. In some cases, these groups of kernels are visible after threshing. The TP mutant is determined by the dominant *Pis1* gene located on long arm of chromosome 2D. The original Chinese landrace was grown in field tests at Kromeriz (Czech Republic) in 2007. Its yield was only 44 % in comparison with mean yield of check registered cultivars of spring wheat Vánek, Granny and SW Kadrij (6.46 t.ha⁻¹). TP wheat exhibited low resistance to fungal pathogens, low 1000-kernel weight (TKW) (27.9 g), low volume weight (75.4 kg.hf⁻¹), lower germination vigour, high protein content (17.7 %) and was 10 and 7 days earlier at heading and maturity, respectively. The TP mutant was crossed to significant cultivars of winter wheat aiming to transfer the gene *Pis1* to the genetic background of currently grown cultivars. TKW of the harvested F₁ plants was around the average of parents. The TP can be used as a potential gene resource for increasing reproductive spike capacity (a kernel number per spike) and spike sink capacity. The significance of the *Pis1* gene cannot be exactly evaluated unless the comparison of near-isogenic lines distinguishing in the TP trait and identical in genetic background is carried out.

IAEA-CN-167-383P

CREATING MUTATIONS IN PLANT RESISTANCE GENES TO PARASITIC WEED

Y. MABROUK¹, S. MEJRI^{1,2}, O. BELHADJ²

¹Unité des Radiopharmaceutiques, Centre National des Sciences et Technologies Nucléaires, Sidi Thabet, Tunisia

²Laboratoire de Biochimie et Technobiologie, Faculté des Sciences de Tunis, Campus Universitaire Elmanar, Elmanar, Tunisia

Email: yassine.mabrouk@fst.rnu.tn

The parasitic weed *Orobanche crenata* Forsk. (crenate broomrape, Scrophulariaceae) represents a major limiting factor for production of food legumes. Current management methods are not ideal. Indeed herbicide selectivity remains insufficient to control the parasite without decreasing crop yield significantly. Creating such mutant legumes, by mutagenesis has become an important tool to have resistance to broomrape. Gamma radiation from cobalt 60 source was selected as the mutagen to create such mutant plants. Seeds were exposed to the radioactive source to determine the proper dose of radioactivity for mutagenesis. A kill curve has been generated using doses exposures ranging from 0 to 200 Gy. The kill curve data was used to expose legumes seeds to enough radiation to create a survival rate of 50%. The surviving plants will be exposed to the parasitic plant *Orobanche crenata* and scored for resistance to infection. All phenotypic plant mutations will be logged for future reference.

PLENARY SESSION 2 (08:30-13:00)

**Induced Mutation in Genomics Era: New Opportunities
and Challenges**

IAEA Boardroom C04

UNIDO Boardroom C04 (via CCTV)

Friday, 15 August 2008

MUTATIONAL AND FUNCTIONAL GENOMIC ANALYSIS OF SYSTEMIC AND LOCAL REGULATION OF LEGUME NODULATION

P.M. GRESSHOFF

Australian Research Council Centre of Excellence for Integrative Legume Research, The University of Queensland, St. Lucia, Brisbane, Australia
Email: p.gresshoff@uq.edu.au

Nitrogen fixation is critical for sustainability of food, feed and biofuel production. Legumes are very efficient in this process, known to reduce the fertiliser requirements of crops and farming systems. This issue has increasingly become critical in a world attempting to capture solar energy in biomass and biofuels, without a clear recognition of the significance of nitrogen inputs. Our research program has utilised a genetic-molecular genomics-biochemical approach to dissect the process of nodulation, and its control by internal mechanisms. Using induced mutagenesis of soybean, subsequent developmental and molecular genetic characterisation, we isolated several genes critical for nodule initiation and control of nodule numbers (Searle et al, *Science* 2003). Nodule formation in legumes is minimally controlled at two levels: a) the locally-acting perception of the mitogenic signal from the *Rhizobium* bacterium and b) the systemically-acting Autoregulation of Nodulation (AON), which involves closely related CLAVATA1-like LRR receptor kinases (GmNARK/LjHAR1/MtSUNN) expressed in phloem of most legume tissues; yet its biological activity in nodulation is almost exclusively within the leaf. To dissect AON, the soybean NARK promoter was analysed to reveal tissue expression domains and a putative phloem specifying promoter domain. Purified kinase domain of GmNARK was able to autophosphorylate and transphosphorylate itself as well as the newly discovered GmKAPP (kinase associated protein phosphatase). A bioassay for the AON shoot-derived inhibitor was developed to indicate that the inhibiting principle was extractable, *Bradyrhizobium*-induced, NARK-dependent, RNAase A and Proteinase K resistant, and of small size. Downstream molecular events from GmNARK revealed coordinate expression of genes leading to the synthesis of jasmonic acid. Mutations in *LjHAR1* also lead to inhibited main root growth, which was reversed by ethylene insensitivity (ETR1 controlled) in shoots, suggesting a dual role in nodulation and root growth. Increased nodulation, nitrogen gain and ability to nodulate effectively at sub-optimal *Bradyrhizobium* titres were achieved after overexpression of the *GmNFR1a* gene. Cloning of *GmNFR1* and *GmNFR5*, complementation of mutants, and nodulation efficiency analysis suggest that the AON circuit acts by perception of the NF signalling cascade and subsequently targeting its efficiency.

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INDUCED MUTANTS FOR RICE FUNCTIONAL GENOMICS

J.M. WAN

Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China
Email: wanjm@caas.net.cn

Induced mutations have been playing important roles in both crop germplasm enhancement and new variety development. With the completion of the rice genome sequence, the study on functional genomics in rice has become a major task. Construction of rice mutant library is an essential approach for rice functional genomics study. This paper briefly reviewed several common techniques for generation of rice mutant library and its application in rice functional research, taking examples of developing rice chloroplast development related mutant library to provide the basic materials for functional genes cloning. A rice Chlorophyll (Chl) deficient mutant, *yellow-green leaf1* (*yg11*), was isolated, which showed yellow-green leaves in young plants with decreased Chl synthesis, increased level of tetrapyrrole intermediates, and delayed chloroplast development. Genetic analysis demonstrated that the phenotype of *yg11* was caused by a recessive mutation in a nuclear gene. The *yg11* locus was mapped to chromosome 5. A missense mutation was found in a highly conserved residue of YGL1 in the *yg11* mutant, resulting in reduction of the enzymatic activity. Another green-revertible albino leaf (*gal*) mutant involved in chloroplast development was screened from a M₂ population induced by 300Gy ⁶⁰Co gamma rays irradiation to the seeds of rice male sterile line PA64S with the collaboration of Zhejiang University. The mutant seedling leaves exhibit albino firstly but turn to normal green after the sixth leaf extended thoroughly. Systematical research including photosynthetic pigment, chloroplast microscopic observation and gene cloning was carried out on the *gal* mutant.

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THE USE OF MUTANTS FOR DISSECTING AND UNDERSTANDING PLANT SMALL RNAs AND THEIR FUNCTIONS

B.C. MEYERS

Delaware Biotechnology Institute & Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA

Email: meyers@dbi.udel.edu

Small RNAs (21-24 nt) such as miRNAs and siRNAs are a powerful regulatory force in most eukaryotes because they can function to shut off genes at multiple levels. Deep sequencing of the small RNA component of the transcriptome is an important step toward elucidating the impact of small RNAs on individual genes and the genome as a whole. We have developed and applied small RNA profiling methods based on novel parallel sequencing technologies. Using these approaches in wildtype and mutant Arabidopsis lines, we have identified numerous new miRNAs and siRNA-generating loci from Arabidopsis (<http://mpss.udel.edu/>). For example, by analyzing an *rdr2* loss-of-function mutant, we characterized the complement of miRNAs expressed in Arabidopsis to considerable depth. We have been analyzing the small RNA component of grasses and other plant species. In maize, we've examined the small RNA complement of wildtype and a mutant of the *mop1* (*mediator of paramutation1*) gene. More recently, we have also developed a high-throughput and global approach to simultaneously identify miRNA-target RNA pairs; this represents an advance over predictive methods, as our approach simultaneously provides experimental data for both the miRNA and its targets. Applied in Arabidopsis, this approach has shown that most miRNA targets show a single abundant signature at the miRNA cleavage site, particularly in libraries from a mutant deficient in the 5' to 3' exonuclease AtXRN4. Taken together, these data are providing insights into the small RNA populations present in complex plant genomes.

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METABOLITE PROFILING OF INDUCED MUTANTS OF RICE AND SOYBEAN

K.-H. ENGEL

Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Freising-Weihenstephan, Germany

Email: k.h.engel@wzw.tum.de

The study objects of the investigation were two low phytic acid (*lpa*) rice (*Os-lpa*-XS110-1, *Os-lpa*-XS110-2) and soybean (*Gm-lpa*-TW-75-1, *Gm-lpa*-ZC-2) mutants generated by γ -irradiation. The aim was to compare these mutants to the corresponding wild-types by means of capillary gas chromatography metabolite profiling and to explore the usefulness of this approach to assist in the elucidation of the types of mutation resulting in the reduced contents of phytic acid. Metabolite profiling aspires to provide a comprehensive picture of the metabolites present in biological systems. It aims at extracting, detecting, identifying, and quantifying a broad spectrum of compounds in a single sample to provide a deeper insight into complex biological systems. The extraction and fractionation method used in the study allowed a comprehensive coverage of a broad spectrum of low molecular weight metabolites ranging from lipophilic (fatty acids methyl esters, hydrocarbons, free fatty acids, sterols, tocopherols) to hydrophilic (sugars, sugar alcohols, organic acids, amino acids) compounds. For rice, considerable amounts of the peaks detected were statistically significantly different between wild-types and *lpa* mutants within one field trial. However, only a few of these differences could be consistently observed in all analyzed field trials indicating a strong influence of the biological variability. Metabolites shown to be consistently statistically significantly different between wild-type and *lpa* rice mutants were found to be closely related to the biogenetic pathways leading to phytic acid. This allowed a prediction of the mutation targets for the *lpa* rice mutants in the biosynthetic pathway of phytic acid. Similar effects, e.g. clustering of wild-types and *lpa* mutants on the basis of metabolite profiling data, were observed for soybean.

A MICROARRAY APPROACH TO IDENTIFY GENES KNOWN ONLY BY THEIR MUTANT PHENOTYPE

M. HANSSON

Department of Biochemistry, Lund University, Lund, Sweden

Email: mats.hansson@biochemistry.lu.se

The Scandinavian barley (*Hordeum vulgare* L.) mutant collection contains 357 mutants representing 105 loci deficient in chloroplast development and chlorophyll biosynthesis. A few of the mutants are spontaneous, but the majority has been induced by various irradiation and chemical treatments. Less than ten of the loci (corresponding to 30 mutations) have been connected to a gene at the DNA level. In order to identify other genes deficient in the collection we have developed a cDNA microarray approach. Three barley mutant strains, *xantha-h.57*, *xantha-f.27* and *xantha-g.28*, with known mutations in the genes encoding the three subunits of the chlorophyll biosynthetic enzyme magnesium chelatase, were used for the development of the microarray method. The mutation *xantha-h.57* prevents transcription of *Xantha-h* mRNA. Microarrays were prepared by robotic spotting of PCR products from 968 clones at 1760 positions. Most material was from cloned ESTs (expressed sequence tags). The barley *Xantha-h* gene was printed at six positions in duplicate. cDNA from the three mutant strains were differentially labeled with fluorescent nucleotides. Labeled cDNA from one mutant was mixed in equal amounts with labeled cDNA of another mutant and competitively hybridized to the microarrayed clones. The combination of labeled cDNA from *xantha-h.57* with that of *xantha-f.27* or *xantha-g.28* specifically highlighted the positions representing the *Xantha-h* gene on the microarrays. We regard these experiments as a demonstration that microarrays provide a very promising method for screening large DNA libraries in order to clone genes known only through their mutant phenotype. This opens up a new way of using the microarray technology for cloning genes from eukaryotes with complex genomes for which genome sequencing is unlikely to proceed. Our results also put the many available plant mutant collections in focus as treasuries for gene hunters.

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GENOMICS MEETS INDUCED MUTATIONS IN CITRUS: IDENTIFICATION OF DELETED GENES THROUGH COMPARATIVE GENOMIC HYBRIDIZATION

G. RIOS, M.A. NARANJO, D. IGLESIAS, O. RUIZ, M. TALON

Centro de Genómica, Instituto Valenciano de Investigaciones Agrarias (IVIA) Moncada, Valencia, Spain

Email: talon_man@gva.es

We report on the use of genomic approaches to identify pivotal genes in induced citrus mutants. Citrus is the most economically important fruit crop in the world while Spain is the first fresh citrus producer. The survival of the Citrus industry is critically dependent on genetically superior cultivars but improvements in fruit quality traits through traditional techniques are extremely difficult due to the unusual combination of biological characteristics of citrus. Genomic science, however, holds promise of improvements in breeding. In this work, we reported the successful identification of genes included in hemizygous deletions induced by fast neutron irradiation on *Citrus clementina*. Microarray-based CGH was used to identify underrepresented genes in a citrus mutant that shows color break delay. Subsequent confirmation of gene doses through quantitative PCR and comparison of best hits of putative deleted citrus genes against annotated genomes from other eudicots, specially poplar, enabled the prediction that these genes were clustered into a 700 kb fragment. The availability of *Citrus* BAC end sequences helped to draw a partial physical map of the deletion. Furthermore, gene content and order in the deleted segment was established by PCR location of gene hits on the physical map. Finally, a lower chlorophyll a/b ratio was found in green tissues from the mutant, an observation that can be related to the hemizygous deletion of a ClpC-like gene, coding a putative subunit of a multifunctional protease complex located into the chloroplast. Analysis of gene content and order inside this *Citrus* deletion led to the conclusion that microsynteny and local gene colinearity with *Populus trichocarpa* were higher than with the phylogenetically closer *Arabidopsis thaliana* genome. In conclusion, a combined strategy including genomics tools and induced citrus mutations has been proved to be a successful approach to identify genes with major roles in citrus fruit development.

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MUTAGENESIS AS A FUNCTIONAL GENOMICS PLATFORM FOR PHARMACEUTICAL ALKALOID BIOSYNTHETIC GENE DISCOVERY IN OPIUM POPPY

P.J. FACCHINI, I. DESGAGNE-PENIX, J.M. HAGEL, E.-J. LEE
 Department of Biological Sciences, University of Calgary, Calgary, Canada
 Email: pfacchin@ucalgary.ca

Opium poppy (*Papaver somniferum*) accumulates the analgesic alkaloids morphine, codeine and thebaine, and remains one of the world's most important medicinal plants. The development of varieties that accumulate valuable compounds, such as thebaine and codeine, but not morphine precludes the illicit synthesis of heroin (*O,O*-diacetylmorphine) and has created opportunities to establish alternative cash crops. Novel cDNAs encoding more than a dozen biosynthetic enzymes have been isolated, and substantial EST databases and DNA microarray chips have been established. The full potential of functional genomics as a tool for gene discovery in opium poppy remains limited by the relative inefficiency of genetic transformation protocols, which also restricts the application of metabolic engineering for both experimental and commercial purposes. We are establishing an effective functional genomics initiative based on induced mutagenesis and TILLING (Targeting Induced Local Lesions IN Genomes) and with the aim of identifying biosynthetic genes that can be used to engineer opium poppy to produce copious levels of high-value pharmaceutical alkaloids. Mutagenesis involves the treatment of seeds by fast-neutron bombardment (FNB) or with ethyl methane sulfonate (EMS). Mutagenized opium poppy plants are cultivated in a secure underground growth facility in partnership with a Canadian biotechnology company. In preliminary experiments with EMS-treated seeds, the screening of 1,250 independent M₂ plants led to the isolation of four mutants that displayed two distinctly altered alkaloid profiles. Two lines accumulated the central pathway intermediate (*S*-reticuline and only low levels of morphine, codeine and thebaine. Two other lines showed the unusual accumulation of the antimicrobial alkaloid sanguinarine, which is the product of a branch pathway distinct from that leading to morphine, in the latex. The present status of -omics resources and functional genomics platforms available to study alkaloid biosynthesis in opium poppy will be discussed with a focus on the application of approaches involving induced mutagenesis.

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FROM DISCOVERY OF HIGH LYSINE BARLEY ENDOSPERM MUTANTS IN THE 1960-70 TIES TO NEW HOLISTIC SPECTRAL MODELS OF THE PHENOME AND OF PLEIOTROPY IN 2008

L. MUNCK, B. MØLLER JESPERSEN
 Department of Food Science, Life Science Faculty, University of Copenhagen, Frederiksberg, Denmark
 Email: lmu@life.ku.dk

As documented by eight IAEA/FAO symposia 1968-82 on nutritionally improved seeds, a wide range of high lysine endosperm mutants were isolated in maize, sorghum and barley. These mutants observed by new spectroscopic screening methods can now be exploited to advance basic biological research and theory. Since 1982 effective methods to overview the physiochemical composition of seeds by Near Infrared Spectroscopy evaluated by chemometric data analysis have developed. Spectroscopic analyses by calibration have now substituted for the wet analyses in industry. In genetics there has traditionally been a differentiation between major genes for qualitative and minor "polygenes" for quantitative traits. This view has been coupled to an incomplete understanding of pleiotropy. It is shown that seed spectra from isogenic barley endosperm mutants represent a coarse-grained physiochemical overview of the phenome that can be classified by chemometrics. Pleiotropy expressed by a gene is quantified as a whole pattern by the gene specific mutant spectrum subtracted by the spectrum of the parent variety. Selection for an improved plumpness (starch) in a breeding material with the *lys3.a* mutant visualises in spectra the effect of enriching "minor polygenes" for an increased content of starch in a mutant gene background. Morphological, spectroscopic and chemical analyses suggest that mutant genes have both qualitative and quantitative expressions. They produce qualitative pleiotropic phenomenological patterns that can be observed as more or less severe changes in macro and microstructures of the plant and seed phenotype. Behind are quantitative chemical changes that by spectroscopy and chemometrics can be transferred to qualitative patterns. In fact one major gene for a qualitative trait can act as several apparent minor polygenes for quantitative variables. This explains the reduced need for the previously expected several hundred thousands of genes and gene modifiers down to the about 30.000 genes that now are sequenced in barley.

THE EFFECT OF PLANTS WITH NOVEL TRAITS (PNT) REGULATION ON MUTATION BREEDING IN CANADA.

G. ROWLAND

Crop Development Centre, Plant Sciences Department, University of Saskatchewan, Saskatoon, Canada
Email: gordon.rowland@usask.ca

In 1988 the Parliament of Canada passed the Canadian Environmental Protection Act (CEPA) into law. Within this Act is a definition for biotechnology which reads “the application of science and engineering in the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms”. The definition was placed in CEPA to deal with concerns regarding Genetically Modified Organisms (GMOs) and would allow Environment Canada to regulate all GMOs. In response to CEPA the Canadian Food Inspection Agency (CFIA), which is responsible for registration of plant varieties in Canada, developed the concept of a Plant with Novel Traits (PNT) defined as “a plant variety possessing a characteristic that is intentionally selected or created through a specific genetic change and is either not previously associated with a distinct and stable population of the cultivated plant species in Canada or expressed outside the normal range of a similar existing characteristic in the plant species”. Not only does this definition capture GMOs it also includes induced mutations, natural mutations and exotic germplasm that have not previously been grown in Canada. It is, as CFIA has argued, a system that is product not process based. However, apart from questions regarding the novelty of traits in new plant varieties, breeders are asked by CFIA to identify the process used to develop the trait or traits in question. Field trials involving breeding lines with a PNT may be subject to confined testing. This conference celebrates 70 years of unconfined development and testing of induced plant mutations. This regulation is time consuming, expensive and an innovation barrier for Canadian plant breeding. It can only be hoped that other nations, and particularly those that have successfully used induced mutations, will not emulate Canada’s approach.

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TURNING PLANT MUTATION BREEDING INTO A NEW ERA: MOLECULAR MUTATION BREEDING

Q.Y. SHU

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria
Email: q.shu@iaea.org

The advance in molecular genetics and DNA technologies has brought plant breeding including mutation breeding into a molecular era. With the ever increasing molecular genetics and genomics knowledge and rapidly emerging molecular techniques, breeders can now more wisely and efficiently than ever before using mutation techniques in breeding new varieties. Plant molecular mutation breeding is here defined as mutation breeding in which molecular or genomic information and tools are used in the development of breeding strategies, in the screening, selection and verifying of induced mutants, and in the utilization of mutated genes in the breeding process. It is built upon the science of DNA damage, repair and mutagenesis, plant molecular genetics and genomics of important agronomic traits as well as induced mutations. Mutagenic treatment, super-mutable genetic lines, molecular markers and high throughput DNA technologies for mutation screening such as TILLING (Targeting Induced Limited Lesions IN Genomes) are the key techniques and resources in molecular mutation breeding. Molecular mutation breeding will significantly increase both the efficiency and efficacy of mutation techniques in crop breeding. A perspective molecular mutation breeding scheme is proposed for discussion.

DEVELOPMENT OF EMS MUTANT POPULATIONS FOR WHEAT FUNCTIONAL GENOMICS

T.X. ZHAO, R.G. ZHOU, J.Z. JIA, X.Y. KONG

Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Email: xykong@mail.caas.net.cn

Ethyl methan sulfonate (EMS) is a chemical mutagen, which causes primarily point mutations. Recent years, EMS mutants have demonstrated a wide range of applications in functional genomics research. Here we reported two EMS populations generated from wheat cultivar Yanzhan 4110. Yanzhan 4110 represents an elite wheat variety that is planted in south of Huanghuai winter wheat region in China. In order to determine the optimum treatment condition for mutagenesis of wheat, the seeds of hexaploid wheat cultivar Yanzhan 4110 were treated with different concentrations of EMS at pre-experiment. The results indicated that the treatment of seeds with 0.7% EMS at room temperature for 16 hours was the best choice, and the germination rate was about 70%. Based on the pretest results, 9000 and 6000 seeds of Yanzhan 4110 were treated with 0.7% EMS and 1.2 % EMS respectively, and the mutagenized seeds were grown into M₁ plant in the field. The germination rates of M₁ were 72% and 65% for 0.7% EMS and 1.2 % EMS respectively. M₂ seeds were collected from individual plants to generate the family structure. About 6500 and 3900 families of M₂ were harvested from the above treatments. The germination rates of M₂ were 85.5% and 80.9% for 0.7% EMS and 1.2 % EMS respectively. During the growth period after sowing the M₂ seeds, some mutants with the phenotype variations of seedling, leaf, stem, panicle traits or physiological properties were observed in field. M₂ mutants will be validated through M₃ generation. All the mutants identified from Yanzhan 4110 EMS populations will play an important role in wheat functional genomics research.

PHYSICAL MAPPING AND CLONING OF GENOME-SPECIFIC REPETITIVE DNA SEQUENCES IN *O. RUFIPOGON*

N. XIAO, Y.H. HONG, R.X. XIA, J.M. CHEN

College of Bioscience and Biotechnology, Yangzhou University, China

Email: jmchen@yzu.edu.cn

The repetitive sequences play an important role in genetic evolution of *Oryza*. Over 20 candidate positive BAC clones containing specific repetitive sequences have been identified by dot blot hybridization using DNA of *O. rufipogon* (AA genome) and *O. officinalis* (CC genome) as genomic probes. Four true positive BAC clones were subcloned and select positive subclones were sequenced. BLASTn analysis showed that eleven out of sequenced 20 subclones belonged to *RIRE3*-like, three *RIRE2*-like, one *RIRE8*-like, and one *Retrosat1-2*-like retrotransposon repetitive sequences. Southern blot were performed using A6, A8 and A15 (belong to *RIRE8*, *RIRE3* and *RIRE2*-like retrotransposon, respectively) as probes. The results showed that they were genome-specific repetitive sequence with high copies in AA genome of rice. The above subclones were selected to hybridize with metaphase chromosome of *O. rufipogon* by FISH. The hybridization signals could be found at sub-terminal sites, the middle parts and centromere region on one to three pair of different chromosomes. Based on the conservative sequences of LTR from A8, A15 subclones and sequence of classical reverse transcriptase, three pairs primers were designed for developing new molecular markers by transposon display (TD) technology. Different amplification bands were detected among different genomes including AA, BB, BBCC, CC. The result also showed that obvious different bands were identified between *indica* and *japonica*. These TD markers would be very useful markers to distinguish *indica* and *japonica*. Our results indicated that the species or subspecies-specific markers would be developed from genome-specific repetitive sequence, which can be used as molecular markers for evolution study, identification of species in *Oryza* and varieties in rice.

GENETIC FINGERPRINTING OF MUTANT ROSE CULTIVARS

S. KUMAR, K.V. PRASAD, K.P. SINGH, A.P. SINGH

Division of Floriculture and Landscaping, Indian Agricultural Research Institute, Pusa, New Delhi, India

Email: kvprasad66@gmail.com

Six rose mutants evolved at the Indian Agricultural Research Institute, New Delhi from four parent cultivars were characterized based on RAPD markers. Contrary to the earlier findings our effort has conclusively proven that the RAPD markers are indeed robust tools to discern the mutants from their parents. Among 40 primers screened, 7 primers produced inconsistent banding pattern. The number of polymorphic bands varied between 4 (OPA 14) and 10 (OPA1) with an average of 6.5 bands per primer. The percentage polymorphism ranged from 62.5 (OPM 9) to 100 percent (OPA 1). Most of the primers produced monomorphic bands between parent and mutant rose cultivars. When primer OPA 2 was used a specific band of 2.5 kb was noticed in mutant cv. Pusa Urmil and cv. Pusa Abhishek but was absent in parent cv. Jantar Mantar. A polymorphic band of 750 bp was noticed in the parent Kiss of Fire and helped in differentiating the parent from its mutant when amplified with OPK 3. Primer OPS 16 produced discriminatory band of 800 bp in mutant cv. Pink Sport of Montezuma while it was absent in its parent cv. Montezuma. Another specific band of 650 bp was present in parent cv. Montezuma and absent in its mutant cv. Pink Sport of Montezuma signifying the uniqueness of the mutant. Primer OPM 5 brought out distinct polymorphism among the parent Jantar Mantar and its three mutants with absence of a specific band of 1.5 kb in the parent. The four parents and 6 mutants were divided into four distinct groups in the Dendrogram constructed by UPGMA method. The most genetically similar cultivar among the 10 cultivars analyzed are Montezuma and its pink sport of Montezuma whereas Abhisarika a mutant of cv. Kiss of Fire was distinctly different and formed a separate cluster.

MOLECULAR GENOTYPING OF GA₃ INSENSITIVE REDUCED HEIGHT MUTANT OF EMMER WHEAT (*TRITICUM DICOCUM*)

S. SUD¹, K.A. NAYEEM², S.G. BHAGWAT¹

¹Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

²College of Agriculture, Pune, India

Email: sood004@yahoo.co.in, sbhagwat@barc.gov.in

Emmer wheat (*Triticum dicocum* Schubler) is cultivated in parts of peninsular India. Grains of emmer wheat contain higher amounts of protein and dietary fibre and hence are being recommended for inclusion in diet. Traditional varieties of emmer are tall, susceptible to lodging and are low yielding. An induced semi dwarf mutant was obtained in tall emmer wheat variety NP200. The seeds of variety NP200 were subjected to 100, 200, 300 or 400Gy of γ -rays. In the M₂ population of 200Gy treatment, a reduced height mutant with vigorous growth and high tillering was observed. The reduced height mutant its parent and other emmer varieties were tested for their response to GA₃ treatment in seedling test. The mutant was found to be insensitive to externally applied GA₃. The mutant, its parent, and also tall and semi-dwarf varieties of emmer were subjected to *Rht* genotyping. Allele specific primers for dwarfing gene (*RhtB1b*) and their wild type allele (*RhtB1a*) were used. The validity of primers in emmer varieties was confirmed. All semi-dwarf emmer varieties showed a band of 237bp with primer pair BF-MR1. The mutant (HW1095) showed absence of amplification for both *RhtB1a* and *RhtB1b* alleles with respective primer pairs indicating that the mutant carried a different mutation than the existing allele (*RhtB1b*). The mutant allele was amplified with another primer pair resulting in a product of about 400bp. In a comparative yield trial the mutant gave higher yield than the other emmer wheats.

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DNA FINGERPRINTING OF SAFFLOWER IRRADIATION INDUCED MUTANTS BY RAPD MARKERS

A.I. RAGAB, M.R. AL-BAKERY, M. KASSEM

Plant Res. Department, Nucl.Res.Center, Atomic Energy Authority, Inshas, Egypt

Email: ragabeaea@yahoo.com

RAPD markers were utilized to identify the genetic differences and the genetic relationship between 8 safflower genotypes i.e. seven induced mutants namely Mut 1 H, Mut 2 H2, Mut3, Mut4, Mut 5, Mut6, Mut 7 and the parental variety Giza1. Ten arbitrary primers were used; different primers generated polymorphic RAPD profiles. The number of amplified DNA amplicons across the ten primers ranged from seven amplicons for the primer OBC-18 to 17 amplicons for the primers OPA-03 and OPA-04. However the number of polymorphic amplicons ranged from 1 for the primer OPB-3 to 14 amplicons for the primers OPA-03 and OPA-17. The percentage of polymorphism ranged from 9.09 % for the primer OPB-03 to 100% for the primer OPC-17. The highest genetic similarity (94%) was found between Mut 4 and Mut 7 and the lowest (79.0%) was found between Mut 1 and Giza 1. Seventeen positive and four negative unique RAPD markers were identified across the 8 safflower genotypes. The parent Giza 1 was characterized by one positive unique marker amplified by OPA-03 primer at the molecular weight of 2000 bp as well as, two negative unique markers generated by the OPB-6 and OPB-5 primers at the molecular weights of 1150 and 800 bp., respectively. The mutant 1 showed highest number of positive unique markers (8) generated by OPA-3 primer at the molecular weights of 1400, 800, 700 and 600 bp, OPB-04 at the molecular weight 2000 bp., OPB-06 primers at the molecular weight of 900 bp., OPB-05 primer at the molecular weight of 500 bp., and OPA-04 primer at the molecular weight of 600 bp. Mut 2 was identified by two positive unique markers generated by the OPB-05 and OPA-03 primers at the molecular weights of 1500 and 500 bp respectively, However the Mut 3 was characterized by one positive unique marker amplified by OPC-17 primer at the molecular weight 550 bp., there is no unique number was found to characterize the mutant 4. The Mut 5 identified by one positive unique marker generated by OPA-04 Primer at the molecular weight 950 bp. Mutant 6 was identified by one positive unique marker generated by OPB-06 primer at molecular weight 350 pb as well as two negative markers generated by OPB-05 and OPC-04 primers, at the molecular weights 1400 and 1300 bp., respectively, however Mut 7 was identified through three positive unique markers generated by OPC-17 primer at the molecular weights 2000 and 1500 bp and OPA-03 primer at the molecular weight of 1800 bp.

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GENETIC ANALYSIS AND GENE MAPPING OF A DWARF MUTANT RICE CHA-1

H. WANG, Z.Q. CHEN, T. GUO, Y.Z. LIU, H.Z. LI

College of Agriculture, South China Agricultural University (SCAU), Guangzhou, China

Email: wanghui@scau.edu.cn

A special dwarf mutant rice CHA-1, studied in this paper, was a stable and inherited rice material selected from the induced generation derived from rice variety Tehuazhan which had been flown on a high space balloon. In order to investigate the inheritance of the dwarf genes in CHA-1, the crosses between CHA-1 and high variety Huiyangzhenzhuo were carried out. Genetic analysis of the height in their F_2 generation showed that the new mutant gene *h* (named as *h* temporarily) from CHA-1 was a major recessive gene which linked with *sd-1* in a certain extent. The two genes had complementary interaction and controlled the dwarf trait of CHA-1 together. Therefore, the genotype of CHA-1 was *hhsd-1sd-1*. To map the locus of the dwarf gene *h*, bulked-segregate analysis and recessive-class analysis in F_2 generation from the cross between CHA-1 and variety 02428 were used to screen SSR molecular markers linked with the dwarf gene. The result showed that the dwarf gene *h* was located on chromosome 1, with a genetic distance of 4.715 cM from SSR marker RM302 and a genetic distance of 5.915 cM from the semi-dwarf gene *sd-1*.

GENETIC ANALYSIS AND GENE MAPPING OF A MUTANT DWARF GENE *IGA-1* IN RICE

Z.Q. CHEN, T. GUO, H. WANG, J.G. ZHANG, D.H. RAO

College of Agriculture, South China Agricultural University (SCAU), Guangzhou, China

Email: chenlin@scau.edu.cn

The rice material, Hangai-1, which studied in this paper, was a stable dwarf mutant by space mutation of rice cultivar Texianzhan 13 (*indica*). Genetic analysis showed that its dwarf trait was controlled by two recessive semi-dwarf genes, *sd1* and a new semi-dwarf gene, named as *iga-1*. The new semi-dwarf gene *iga-1* was located between microsatellite markers RM6645 and RM3837 on chromosome 5, the genetic distances between them were 0.07cM and 1.21 cM, respectively. The *iga-1* gene is possibly a multiple allele to the *d-1* gene. The semi-dwarf mutant with the new semi-dwarf gene *iga-1* was found insensitive to gibberellin 3(GA₃).

SELECTION OF WHEAT MUTANT GENOTYPES CARRYING HMW GLUTENIN ALLELES RELATED TO BAKING QUALITY BY USING PCR (STS METHOD)

M.J. ZAMANI¹, M.R. BIHAMTA², B. NASERIAN KHIABANI³, M.T. HALLAJIAN³

¹Islamic Azad University, Branch Roodehen, Agriculture Department, Tehran, Iran

²Tehran University, Biotechnology Department, Tehran, Iran

³Agriculture, Medicine and Industry Research School, Nuclear Science and Technology Research Institute, Karaj, Iran

Email: mj_zamani1980@yahoo.com

For considering the bread quality of some mutant genotypes (Roshan, Omid, Tabasi, Azar and Azadi) with their parents and some other cultivars like (Chamran, Enia, Bezostaya, Tajan, Pishtaz and Chinese spring) this study accomplished in the Agriculture, Medicine and Industry Research School, Nuclear Science and Technology Research Institute of Iran in 2005-2006 through Polymerase Chain Reaction by using Sequence Tagged Site (STS) method. Twelve pairs of primers were used in this study. Seven pairs of them were extracted from the references and the others were designed from the D genome subunits sequences of wheat. Some studies like drought resistance, salt resistance, etc, have been accomplished for these mutant genotypes that some of them showed good results in that studies but there were not any studies about baking quality on these genotypes. The alleles Dx2+Dy12 (with negative effect on bread quality) and Dx2*, Dx5+Dy10 (with positive effect on bread quality) have the main effect on wheat bread quality. Special primers of these subunits were used to amplify these alleles. Except for the cultivars that had Dx5+Dx10, six mutant genotypes T-66-58-60, Ro-5, Ro-4, Ro-3, Ro-1 and O-64-1-10 that their parents did not have these alleles, showed Dx5+Dx10. Also for being assured of the results of molecular experiment, SDS-PAGE method was accomplished which there were not any contradictory results. Significant differences were on protein percentage for mutant genotypes that have polymorphism, showed in Ro-1, Ro-3 and Ro-5 with Roshan (their parent) at 1% probability level.

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GENOME-WIDE TRANSCRIPTOME PROFILING ACCORDING TO SEED DEVELOPMENTAL STAGES IN A HIGH AMINO ACID ACCUMULATING RICE MUTANT

D.S. KIM, J.-B. KIM, G.-J. LEE, S.-J. CHUNG, J.Y. SONG, J.-B. JEON, S.-Y. KANG

Advanced Radiation Technology Institute (ARTI), Korea Atomic Energy Research Institute (KAERI), Jeongseup 580-185, Republic of Korea

E-mail: bioplant@kaeri.re.kr

High amino acid accumulating rice mutant line (MRIIV-33-1) resistant to 5-methyltryptophan (5MT), a tryptophan (Trp) analog, were developed via *in vitro* mutagenesis with gamma-rays. In order to obtain detailed information about the genes expressed at rice seed developmental stages, we investigated the gene expression profiles in the wild-type (WT) and MRIIV-33-1 (MR), at 5 grouped seed developmental stages [Stage I (SI), 2-3 days after pollination (DAP); Stage II (SII), 4-5 DAP; Stage III (SIII), 7-10 DAP; Stage IV (SIV), 12-15 DAP; Stage V (SV), 20 DAP] by microarray analysis. The amino acid contents of the WT seed were decreased with increasing seed developmental stages. But, in the MR, the contents were maintained after S III by a mature seed. In the mature seed, the amino acid content of the mutant line was 2-fold higher than that of the WT. Approximately 7,366 reliable genes with a p-value less than 5% by the ANOVA test were selected from a replicated array. In the GeneTree analysis, the reliable genes showed a significant difference of the expression patterns between the WT and MR. They were divided into 20 groups according to the expression pattern by a Self-Organizing Maps (SOM) clustering analysis. In addition, 99 amino acid biosynthesis related genes were selected and analyzed according to their expression patterns. Transcriptomic analysis by microarray validated by RT-PCR further demonstrated that a large number of genes showed altered expression in the MR line. Some Trp-related genes, putative anthranilate synthase a (AK069031), anthranilate N-hydroxycinnamoyl/ benzoyltransferase (AK070381, AK070440), phosphoribosyl anthranilate transferase (AK064915), indol-3-glycerolphosphate synthase (AK059358), tryptophan synthase a (AK066734) showed a slightly enhanced expression in the MR line, suggesting the absence of an inducible regulatory mechanism that deals with Trp accumulation. This work was supported by a grant (Code 20070501034005) from BioGreen 21 Program, RDA (Rural Development Administration), and from the KOSEF (Korean Science and Engineering Foundation) in the MoST (Ministry of Science and Technology), Republic of Korea.

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WHOLE GENOME SCANNING FOR MUTATIONS INDUCED BY CHEMICAL AND PHYSICAL MUTAGENS IN BARLEY

M. KUROWSKA, A. LABOCHA-PAWLOWSKA, I. SZAREJKO, M. MALUSZYNSKI

University of Silesia, Department of Genetics, Katowice, Poland

Email: mkurowsk@us.edu.pl

The presented research focuses on estimation of the types and frequencies of DNA changes induced by gamma rays and N-nitroso-N-methyl urea (MNU) in barley (*Hordeum vulgare* L.) genome. The analysis was performed in the M₂ generation obtained after mutagenic treatment of doubled haploid (DH) line 'H930-36' with different doses of gamma rays (180, 210 Gy) and MNU (0.5, 1.0, 1.5 mM/3h). The main approach used in the study was the scanning of the whole genome for amplified fragment length polymorphism (AFLP). In the presented study, the combination of enzymes *EcoRI/MseI* and seven different primer combinations were used. The AFLP fragment sizes ranged from approximately 50 to 500 bp. The analysis was conducted on 1700 M₂ plants derived from both populations. In all applied doses of mutagens plants with changes in AFLP profile were observed. In total, 6,821 kb were scanned for AFLP polymorphism in the MNU treated population. Assuming that each polymorphic band (67 total) results from a single nucleotide change, this indicates 1 mutation per 102 kb. In gamma rays treated M₂ population 5,600 kb barley genome sequence was scanned and only 18 polymorphic bands were detected, what corresponds to 1 mutation per 313 kb. The longest polymorphic AFLP bands were extracted from the polyacrylamide gels, cloned and sequenced. We found lack of homogeneity in all explored products. The NCBI database was used to find annotation for analyzed sequences. So far, the majority of investigated DNA fragment appear to be LTR retrotransposons. The repetitive sequences constitute the main part of barley genome. In order to determine the mutation type which caused an appearance of the additional band, the isolation of flanking regions was performed using thermal asymmetric interlaced (TAIL)-PCR method.

DEVELOPMENT OF RETROTRANSPOSON BASED MOLECULAR MARKERS FOR FINGERPRINTING ANALYSIS OF HEXAPLOID WHEAT AND *TRITICALE SPHAEROCOCCUM* MUTANT FORMS

G. BONCHEV¹, S. PEARCE², S. GEORGIEV³

¹Department of Genetics, Institute of Genetics "Acad. D.Kostoff", Bulgarian Academy of Sciences, Sofia, Bulgaria

²Plant Stress Unit, University of Sussex, Brighton, UK

³Department of Genetics, Faculty of Biology, Sofia University "St. Kl. Ochriski", Sofia, Bulgaria

Email: g_bonchev@mail.bg

Retrotransposons have been an excellent source of useful polymorphic markers for genome characterization because they are one of the most mutable components of plant genomes. Several biotic and abiotic stress factors have been known to influence and regulate retrotransposon activity. Retrotransposon based techniques SSAP, IRAP and REMAP have been applied to assess the behavior of BARE-1/ Wis 2 retrotransposons within a population of *T. aestivum* and *Triticale sphaerococcum* mutant forms obtained by chemical mutagenesis with EMS (ethylmethane sulphonate). The polymorphism level was high (up to 17% for SSAP and 21% for IRAP and REMAP) despite the genetic closeness and common ancestral origin of mutant forms. Several polymorphic markers have been identified which could be successfully used to distinguish different mutant groups. Sequencing analysis of polymorphic markers demonstrated the retrotransposon preference for integration into transposon sequences ("nested transposition") and into or near gene rich regions like glutenin and gamma-gliadin loci in wheat genome. A dendrogramme based on IRAP was constructed revealing genetic distance between mutant forms which is consistent with the previous results obtained using SSR markers. All methods exploited in this study turned out to be reliable, perspective and as powerful as SSR and other genetic molecular markers for mutant diversity and fingerprinting analysis even in closely related variety's individuals. The polymorphic pattern within the mutant and control forms enables us to speculate about the increased retrotransposon activity and rearrangements spanning retrotransposons which have occurred in sphaerococcum mutant forms during their formation and the role of EMS as an inducing agent in this process.

ISOLATION AND CHARACTERIZATION OF RETROTRANSPOSONS IN WILD AND CULTIVATED PEANUT SPECIES

S. NIELEN¹, F. CAMPOS FONSECA¹, P. GUIMARÃES¹, S.C. LEAL-BERTIOLI¹, D. BERTIOLI²

¹Embrapa Recursos Genéticos and Biotecnologia, Brasília, Distrito Federal, Brazil

²Universidade Católica de Brasília, Brasília, Distrito Federal, Brazil

Email: stephan@cenargen.embrapa.br

Retrotransposons are considered as a possible source for mutations due to their potential of spreading in the genome using a "copy and paste"-like mechanism. We report about the isolation and characterization of a new Ty3-gypsy retrotransposon from allotetraploid peanut (*Arachis hypogaea*, 2n=4x=40) and its diploid ancestors *A. duranensis* (AA-genome, 2n=20) and *A. ipaënsis* (BB-genome, 2n=20). We have identified two repetitive sequences, one showing high similarity at amino acid level to the reverse transcriptase of Athila-type retrotransposons, the other being AT-rich with no similarities to genebank sequences. Results from genome walking experiments gave first evidence that both sequences represented parts of the same Ty3-gypsy retrotransposon, the 5'-LTR (long terminal repeat)- and the *pol* (polyprotein)-region respectively. Fluorescent *in situ* hybridization (FISH) experiments showed that the element is dispersedly distributed on the chromosomes, absent from centromeres and telomeric regions, and more prominent in chromosomes of the A-genome. The element appeared to be moderately repetitive with copy numbers of about 430 (*A. ipaënsis*), 1350 (*A. duranensis*), and 3000 (*Arachis hypogaea*) per haploid genome. Phylogenetic analysis of the deduced amino acid sequences of 80 isolated reverse transcriptase clones from the three species shed light on its evolution within the peanut species. The isolated sequences contained multiple stop-codons and so far, no evidence has been found that the element is still active. An outlook is given regarding finding new tools for the advancement of *Arachis* breeding programmes aimed at the transfer of resistance to biotic and abiotic stresses to peanut.

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FINE MAPPING OF THE MUTATED GENE OF A GENIC-MALE-STERILE MUTANT IN RICE

S. SHIOKAI¹, Y. HORI¹, M. NISHIMURA², T. NISHIO¹¹Graduate School of Agricultural Science, Tohoku University, Sendai, Japan²Institute of Radiation Breeding, NIAS, Hitachi-omiya, Japan

Email: shiokai@bios.tohoku.ac.jp

Male sterility is a useful trait for F₁ hybrid production. Identification of the mutated gene may enable practical use of GMS in F₁ hybrid breeding, and may also contribute to elucidation of the mechanism of anther and pollen development. From 28 sterile mutant lines obtained by gamma-ray-irradiation in rice, we selected one mutant line of 'Koshihikari', C204, having stable male-sterility. C204 produced pollen grains, but pollen fertility investigated by the staining with I₂/KI solution was 0%. The male sterility of C204 was found to be controlled by a single recessive nuclear gene. Since F₁ and some F₂ plants between 'Koshihikari' and Indica cultivars show hybrid sterility, we crossed C204 with a different Japonica cultivar, 'Akihikari', for rough mapping of the mutated gene. Linkage analysis using 23 dot-blot-SNP marker, one SSR marker, six SCAR markers, and two CAPS markers revealed that the GMS gene of C204 is located between a SCAR marker named T1423 and an SSR marker named SSR87 on chromosome 9. For fine mapping of the GMS gene of C204, this mutant was crossed with a chromosome substitution line, CSSL228, which has a segment of the chromosome 9 from 'Kasalath' with 'Koshihikari' background. From 551 F₂ plants, 38 recombinants between T1423 and SSR87 were selected. Investigation of pollen fertility and genotyping of five DNA markers showing polymorphism between 'Koshihikari' and 'Kasalath' revealed the GMS gene to be between SCAR511 and SSR520. The physical distance between SCAR511 and SSR520 is about 350 kb.

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CHARACTERIZATION OF RESISTANCE GENE ANALOGS IN *MUSA ACUMINATA* CULTIVARS CONTRASTING IN RESISTANCE TO BIOTIC STRESSES

F.L. EMEDIATO, F.A.C. NUNES, C. DE CAMARGO TEIXEIRA, M.A.N. PASSOS, D.J. BERTIOLI, G.J. PAPPAS JR, R.N.G. MILLER

Post-graduate program in Genomic Sciences and Biotechnology, Universidade Católica de Brasília, Brasília, DF, Brazil

Email: rmiller@pos.ucb.br

The majority of commercial banana cultivars (*Musa* sp.) have evolved via asexual vegetative propagation, with diversity dependent upon somatic mutation. Restricted variation has resulted in a crop with little resistance to pests and disease, and conventional breeding efforts are limited due to limited viable seed production. Numerous disease resistance genes (R-genes / R-proteins) have been characterized in plants, recognizing and conferring resistance to bacteria, virus, fungi and nematodes. The identification and cloning of R-genes in *Musa* would contribute to germplasm improvement. To date, five main R-gene classes have been identified, based upon protein domains, with the most abundant coding for nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins. Primers designed from conserved protein motifs have enabled amplification of NBS homologues across diverse plant species. In the case of *Musa*, our group has identified over 50 distinct NBS-LRR type resistance gene analogs (RGAs) in the resistant wild diploid *M. acuminata* Calcutta 4. The objective of this work was to characterize RGAs in *M. acuminata* cultivars contrasting in resistance to Black leaf Streak Disease. PCR amplification was conducted using DNA from *M. acuminata* cultivars Calcutta 4 (resistant) and Pisang Berlin (susceptible). Degenerate primers targeted sequences homologous to the NBS-LRR R-gene family. Following sequencing and processing of cloned PCR products, 63 out of a total of 136 high quality sequences showed homology to R-genes or RGAs. Phylogenetic analysis was conducted on deduced amino-acid sequences. Degenerate primers were also developed targeting an R-gene family of cytoplasmic serine-threonine (Ser/Thr) receptor-like kinases (RLKs) with extracellular LRRs, for application across cultivars. Studies are also planned for selection and full length sequencing of clones from *M. acuminata* and *M. balbisiana* BAC libraries containing novel RGAs characterized in this study, as an approach for complete R-gene sequence characterization, applicable both in transformation and breeding programs for banana genetic improvement.

DEVELOPMENT OF MICROSATELLITE MARKERS IN *MYRICA RUBRA*– A SUBTROPICAL FRUIT TREE SPECIAL IN CHINA

X.B. XIE^a, Y.Y. QIU¹, L.P. KE², X.J. QI¹, X.L. ZHENG¹, G.T. WU², J.Q. CHEN², S. AHN³

¹Institute of Horticulture, Zhejiang Academy of Agriculture Sciences (ZAAS), China

²Institute of Virus and Biotechnology, ZAAS, China

³Department of Agronomy, Chungnam National University, Republic of Korea

Email: qixj@sohu.com, xiexiaobo@yahoo.com

Myrica rubra Sieb & Zucc, namely Red Bayberry, which also named Yangmei in Chinese, is a subtropical fruit tree bearing delicious berry-like fruit native to China. Its most important growing region is vested to the Zhejiang province where the planting area has recently expended to 74,667 ha and listed at the second position of the provincial fruit production with both of area and yield. Nowadays Red Bayberry has become into one of important resources of the local rural families' revenue increasing because of its good economic benefits. But its breeding progress has been lagged greatly for various reasons that its short shelf-life of fruit has become the most distinct bottleneck of the fruit-tree further developing. The purpose of the study is hammered at the development of polymorphic microsatellite markers by using ISSR-suppression-PCR method. We adapted three blunt cutting restrict enzymes: *Ssp* I, *Alu* I and *EcoR* V to digest the genome of *M. rubra* var. Heijing, and obtained three corresponding libraries. Then they were ligated to a specific blunt adaptor consisting of a 48-mer: 5'GTAATACGACTCACTATAGGGCACGCGTGGTTCGACGGCCCGGGCTGGT 3' and an 8-mer: 5'ACCAGCCC-NH₂ 3', and were used as DNA template for PCR reaction. The long adaptor-primer AP1 (5'CCATCCTAATACGACTCAC TATAGGGC3) and one ISSR primer GSG(GT)₆ were used as the primer pair for enriching simple sequence repeats (SSR). The smear PCR products were produced in results. And then recombinant plasmid were constructed for above smear PCR products with pMD18-T vector. By the procedure we get 205 positive clones comprising insertions between 300bp to 700bp, 187 sequences hold SSR information in one end after successfully sequenced and three of them hunted SSR at intra-position, but which were all mono-polymorphic by checking with 10 *myrica rubra* varieties or ecotypes and two *M. nana* genotypes. Temporary we have developed three polymorphic SSR markers tested with above 12 *myrica* materials from five of the 187 sequences. Now more *M. rubra* genome specific SSR markers are under developing.

UNLOCKING NATURALLY OCCURRING VARIATION FOR STARCH QUALITY BY GENE-TAGGED MARKERS IN RICE

J.S. BAO

Institute of Nuclear Agricultural Sciences, Zhejiang University, Hua Jiachi Campus, Hangzhou, China

Email: jsbao@zju.edu.cn

Quantitative trait loci (QTLs) mapping and association mapping are currently used to dissect the natural occurring variations for traits of agronomical importance. We found that the major QTLs for starch quality co-locate at the starch-synthesizing gene loci, e.g. *Wx* locus controls the genetic basis of amylose content, pasting viscosity, gel texture and retrogradation properties, while *starch synthase IIa* (*SSIIa*) locus controls the gelatinization temperature (GT) and amylopectin structure. Some of other genes involved in starch biosynthesis and other minor QTLs were also detected. Gene tagged markers such as simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) that were inside or close to those starch-synthesizing genes were designed. Among 499 nonwaxy rice accessions, polymorphisms of SSR in the *Waxy* gene, soluble starch synthase I gene (*SSI*) and starch branching enzyme I gene (*SBE1*), SNPs in *Waxy* and starch branching enzyme III gene (*SBE3*) and *SSIIa*, and a sequence tagged site (STS) in *SBE1* were surveyed. Ten SSR alleles were found at the *Wx* locus and four SSR alleles were found at the *SBE1* and *SSI*, respectively. Two continuous SNPs (GC/TT) alone can differentiate rice with high or intermediate GT (possessing GC SNPs) from those with low GT (possessing the TT SNPs). Association test was conducted using all starch gene markers, results indicated that *Wx* SSR and SNPs were strongly associated with amylose content, pasting viscosities, gel hardness, and retrogradation properties, whereas the *SSIIa* GC/TT SNPs were strongly associated with the pasting temperature and retrogradation properties, which confirmed the findings from QTL mapping. These markers are useful in molecular breeding for improvement of rice eating and cooking qualities. This study was jointly supported by funds from NSFC (30771327), 863 project (2006AA10Z193), Science and Technology Department of Zhejiang Province (2007C32014) and IAEA (12847).

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MOLECULAR GENETIC TOOLS TO MODULATE POST-HARVEST PHYSIOLOGY IN CASSAVA

J. BEECHING

University of Bath, UK

Email: J.R.Beeching@bath.ac.uk

Within 24 – 72 hours of harvest the starchy storage roots of cassava deteriorate rapidly depending on variety and environmental conditions. This post-harvest physiological deterioration (PPD) necessitates their prompt consumption or processing. In traditional village society, cassava roots are left in the ground until required; but, with increased urbanisation and the entry of cassava into the cash economy, distances have increased and PPD has become a major constraint to the development of this important crop, which impacts on farmers, processors and consumers alike. Improvement of cassava with respect to its PPD response via breeding is fraught with difficulties due to the high heterozygosity of the crop, a strong association between PPD and high dry matter content, and a high genetic X environment interaction. Molecular genetic tools may offer alternative approaches via insights into the PPD response itself, the provision of molecular markers for use in marker assisted selection and via the direct manipulation of the cassava genome. cDNA microarrays identify 73 genes whose expression changes significantly during the time-course of PPD; these clones are available to the cassava community for mapping or other research. These data support the hypothesis that reactive oxygen species mediated programmed cell death is at the heart of the PPD response. Currently we are further testing this hypothesis through the genetic modification of cassava using genes with the ability to alter the reactive oxygen defence status of the roots or to enhance the root's anti-programmed cell death response. These modifications have the potential to extend the shelf-life of the cassava roots and ultimately to benefit resource-poor farmers.

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ENHANCING DROUGHT AND SALINITY TOLERANCE IN WHEAT CROP GROWN IN THE MEDITERRANEAN REGION

K. MASMOUDI, F. BRINI, M. HANIN, H. KHOUDI

Centre of Biotechnology of Sfax (CBS), Plant Molecular Genetics Unit, Sfax, Tunisia

Email: khaled.masmoudi@cbs.rnrt.tn

Drought and salinity are major constraints on crop production and food security, and adversely affects entire countries over several years and result in serious social, economic, and environmental cost. Water is in extremely short supply in up to 10 eastern and southern Mediterranean countries. Although Mediterranean climates, characterized by hot, dry summers and cold, wet winters (when 85% of annual rainfall occurs) are notoriously variable, severe drought is clearly a major problem. This situation is compounded by the predicted change in climate with increased temperatures and decreased precipitation as a result of global warming. Wheat production in the Mediterranean region is limited mainly by the availability of water resources. Investigating the mechanisms by which wheat physiologically adapts to plant water deficits points to a salinity tolerance strategy showed that varieties of wheat which are able to maintain photosynthesis and growth at low soil Ψ_w often display a relatively greater capacity for leaf osmotic adjustment. Recent work at the molecular level has led to the identification and cloning of cDNAs encoding proteins which are involved in the cellular-level physiological system which facilitates this adaptive response. Transgenic *Arabidopsis* plants over expressing wheat candidate genes encoding ion transport proteins (TNHX1, TVP1), dehydrins (DHN-5) or water channel proteins (AQP1) are much more resistant to high concentrations of NaCl and to water deprivation than the wild-type strains (Brini et al., 2007a; b; c). Over expression of the isolated genes from wheat in *Arabidopsis thaliana* plants is worthwhile to elucidate the contribution of these proteins in the tolerance mechanism to salt and drought. The rationale for improving abiotic stress tolerance in the crop cultivars grown in the Mediterranean countries by over expressing genes that were shown to confer improved salt and drought tolerance in other plants offers a first-rate possibility to transfer these traits into local crop varieties. Testing candidate genes in TILLING available wheat population will allow the identification of new alleles conferring abiotic stress tolerance.

CYTO-PALYNOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF ORIGINAL AND INDUCED MUTANTS OF GARDEN CHRYSANTHEMUM

S.K. DATTA¹, K. DATTA, J. CHATTERJEE²

Palynology Laboratory, National Botanical Research Institute, Lucknow, India

¹CSIR, Emeritus Scientist, Madhyamgram Experimental Farm, Bose Institute, Kolkata, India

²Bose Institute, Kolkata, India

Email : subodhskdatta@rediffmail.com.

A large number of new somatic flower color/type mutants have been evolved by induced mutations in different ornamentals. Few reports are available on the systematic work being done on comparative analysis of the original and the mutant cultivars. Attempt has been made for the comparative analysis of original cultivars and their respective induced mutants on cyto-palynological, biochemical and molecular characters for better and clear understanding of the exact mechanism involved in the origin and evolution of flower color mutations. Proper characterization and identification of new mutant cultivars is extremely important to protect plant breeder's rights for commercial exploitation. Chrysanthemum original varieties and their gamma ray induced mutants were selected as the materials for the present analysis. Critical cytological analysis with special reference to chromosome number, chromosomal aberrations, ICV, INV and DNA content showed no differences. The karyotypes were reasonably symmetrical. No mutant specific chromosomal aberrations could be detected. Thin layer chromatographic and spectrophotometric analysis of floret pigments indicated that somatic flower color changes in chrysanthemum are due to both qualitative and/or quantitative changes in pigments as a result of mutation induced by gamma rays in pigment biosynthesis pathway. Significant increase in pollen grain sterility was found in all the mutants. The pollen grains of all the cultivars and their mutants are basically 3(-4) zonocolporate with tectate spinose exine having perforations. No appreciable variation in pollen apertural character was noticed in any of the mutants. Significant changes in pollen exine surface pattern were found in 4 mutant varieties. The changes are inconsistent and do not correspond to the intensity of radiation. The similarity among the cultivars and mutants varied from 0.17 to 0.90 using RAPD analyses. Cultivars with different flower colors could be clearly distinguished. But all the mutants could not be distinguished by bands specific for changed flower color due to the resolution capacity of tested primers. It is however possible that some of the specific bands present for some of the mutants may code for flower color, but this can only be verified by using SCAR markers and cloning cDNA. Results of present cyto-palynological, biochemical and molecular characterization provide important information for the identification of induced mutant varieties.

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FINE MAPPING OF A MUTANT PITH STEM IN RICE

X.D. ZHU¹, H.Q. CHEN¹, W. Huang², H.X. LIN²

¹State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, China

²National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Email: ricezxd@126.com

The overground inter-node of rice was normally hollow. A rare rice mutant SKZ-ps with pith stem was induced by γ -ray from *indica* variety Shuang-Ke-Zao (*Oryza sativa* L.). Besides pith stem, epiphenomenon of the mutant represented the thick and wide leaf with dark-green, decreased in plant height, tiller number and seed-setting, increased in grain size, stem-wall thickness and lodge resistance. The genetic analysis of populations F₂ and BC₁F₁ (SKZ-ps crossed with normal parents) indicated that the pith stem was recessive mutation and controlled by a single gene. The mutation gene *ps(t)* based on F₂ populations (SKZ-ps / Nipponbare) was located initially on the long arm of Chromosome 10 between the molecular marks RM258 and RM496. Further the *ps(t)* was finely mapped between SSR5 and SSR21.

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DETECTING MUTATIONS IN THE DIHYDROCHALCON 2'-GLYCOSYLTRANSFERASE AND KUNITZ PROTEASE INHIBITOR GENE AMONGST A SEGREGATING APPLE POPULATION AS A SNPS-BASED GENOME MAPPING STRATEGY

T. GARCÍA-LIBREROS¹, C. GOSCH², A. PEIL³, H. FLACHOWSKY³, V. HANKE³, R. MILCEVICOVA¹, I. MATUSIKOVA¹, B. TROGNITZ¹, K. STICH², H. HALBWIRTH², M. BERENYI¹, K. BURG¹, E. WILHELM¹, S. FLUCH¹

¹Austrian Research Centers, Department of Bioresources, Seibersdorf, Austria

²Technical University, Vienna, Austria

³Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst. Julius Kühn Institut (JKI), Dresden, Germany

Email: tgl040802@gmail.com

Fire blight, caused by the bacteria *Erwinia amylovora*, is a major production constraint for fruit trees of the family Rosaceae such as apples for which resistance to the disease is thought to be polygenic. We have in this study sought to develop a molecular genetic linkage core map; to identify molecular markers linked to the genes involved in fire blight resistance in apples; and to map the genes, dihydrochalcon 2'-glycosyltransferase and Kunitz protease inhibitor, which are thought to be involved in resistance to this disease. A population of 140 individuals being the progeny of the cross between *Malus robusta* 5 (wild, disease resistant) and Idared (cultivated, susceptible) that segregated for the incidence of disease symptoms were screened using molecular markers and the segregation data used to create male and female linkage core-maps. For the Idared parent, the core-map constructed from the segregation of 130 AFLPs, 60 SSRs and 1 SCAR was made up of 20 linkage groups, spanned 1082 cM and covered 94 % of the genome. The *Malus robusta* 5 core-map with 19 linkage groups was anchored by 150 AFLPs, 60 SSRs and 1 SCAR, spanned 1033 cM and covered 75 % of the genome. Additionally, we report the localization of a QTL on linkage group 3 of the resistant parent that explained 84.1% of the phenotypic variation. Using the SNP technology we developed, the dihydrochalcon 2'-glycosyltransferase and Kunitz protease inhibitor genes implicated in resistance to this disease were mapped to linkage group 11 and linkage group 3 respectively of the *Malus robusta* 5 core-map. The implications of the results are discussed.

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QUANTITATIVE ¹H NMR METABOLITE PROFILING AS A FUNCTIONAL GENOMICS PLATFORM TO INVESTIGATE ALKALOID BIOSYNTHESIS IN NATURAL MUTANTS OF OPIUM POPPY

J.M. HAGEL, A.M. WELJIE, H.J. VOGEL, P.J. FACCHINI

Department of Biological Sciences, University of Calgary, Calgary, Canada

Email: hagelj@ucalgary.ca

Opium poppy (*Papaver somniferum*) produces a diverse array of bioactive benzyloquinoline alkaloids and has emerged as a versatile model system to study plant alkaloid metabolism. The plant is widely cultivated as the only commercial source of the narcotic analgesics morphine and codeine. Variations in plant secondary metabolism as a result of genetic diversity are often associated with perturbations in other metabolic pathways. As part of a functional genomics platform, we have used ¹H NMR metabolite profiling for the analysis of primary and secondary metabolism in opium poppy. Aqueous and chloroform extracts of six different opium poppy cultivars were subjected to chemometric analysis. Principle component analysis of the ¹H NMR spectra for latex extracts clearly distinguished two natural mutants, including a low-alkaloid variety, and a high-thebaine, low-morphine cultivar. Distinction was also made between pharmaceutical-grade opium poppy cultivars and a condiment variety. Such phenotypic differences were not observed in root extracts. Loadings plots confirmed that morphinan alkaloids contributed predominantly to the variance in latex extracts. Quantification of 34 root and 21 latex metabolites, performed using Chenomx NMR Suite v. 4.6, showed major differences in the accumulation of specific alkaloids in the latex of the low-alkaloid and high-thebaine, low-morphine mutants. However, few significant differences were found in the levels of other metabolites, indicating that the variation was highly specific for alkaloid metabolism. Exceptions included the accumulation of the alkaloid precursor tyramine in the low-alkaloid cultivar, in addition to altered levels of sucrose, select amino acids and malate. Real-time PCR analysis of 42 genes involved in primary and secondary metabolism showed differential gene expression mainly associated with alkaloid biosynthesis. Reduced alkaloid levels in the condiment variety were associated the reduced abundance of transcripts encoding several alkaloid biosynthetic enzymes.

A COMPARISON OF RESEARCH ON PLANT-INDUCED MUTAGENESIS AND TRANSGENESIS IN BELARUS

I.D. VOLOTOVSKI

Institute of Biophysics and Cell Engineering, National Academy of Sciences of Belarus, Minsk, Belarus

Email: volot@biobel.bas-net.by

During many decades of last centuries the main approach to develop the plants with improved properties has been their mutagenesis induced by either chemical compounds or ionizing radiation. Unfortunately, this required the subsequent prolonged selection of obtained species to choose one with corresponding quality of interest. For example, the experiments using this technique were performed on white beet in Belarus. This fodder crop was intensively cultivated in this country in the sixties-seventies to increase the sugar production in former Soviet Union. The researches have been conducted at the Institute of Genetics and Cytology of Belarus Academy of Sciences with help of neutron irradiation source of experimental atomic reactor at the Institute of Nuclear Research of Belarus Academy of Sciences. The description of these experiments is presented. Beginning from late eighties the plant transgenesis began to be used at the laboratories under model conditions at first using the tobacco plants and then other plants species including those of agriculture importance. If to compare the advantages and disadvantages of both approaches it becomes to be evident the preferentiality of the second one. This has definite direction, predictable outcome and higher opportunity to get the positive results. At the moment one can enumerate a number of transgenic species obtained during last years. As an example, it is tempting to present the experiments to get the transgenic potato plants expressing antimicrobial peptide. The data concerning the structure of genes of this peptide and construct containing this gene to be introduced into potato plant were presented. All stages of transgenesis procedure are described in details. The prospect of these experiments is discussed.

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ANALYSIS OF EMS MUTAGENIZED SOYBEAN BY COMBINATION OF DOP-PCR AND GS-FLX

K.J. VAN, M.Y. KIM, T.-H. JUN, S.-H. LEE

Department of Plant Science, Seoul National University, Seoul, Republic of Korea

Email: kvan@snu.ac.kr

Ethylmethanesulfonate (EMS) causing point mutations were commonly used to induce mutations for various organisms because this led to irreversible mutations with high level of frequency. With relatively few individuals, saturated mutagenized populations could be generated by chemical mutagens. Since high-throughput sequencing instruments, such as GS-20 or GS-FLX from Roche/454 Life Sciences, are now available, characterization of nucleic acids and massive mutant analysis are more feasible. Due to the requirement of sequence information and high cost for designing primers, degenerate oligonucleotide primed PCR (DOP-PCR) instead of direct sequencing was used for single nucleotide polymorphisms (SNPs) survey. In this study, we screen point mutation in soybean mutants generated by EMS mutagenesis using combination of DOP-PCR methodology and GS-FLX. Four different modified DOP-PCR primers were used for amplifying genomic DNA of three soybean genotypes, Sinpaldalkong 2, SS2-2 and 25-1-1. And then, nucleotide sequences of these amplified PCR products were analyzed by GS-FLX. Different number and length of contigs and singlets were constructed depending on soybean genotypes and nucleotide identity, after sequences were trimmed and aligned. With 100% in identity, average 1,100 contigs and 7,000 singlets were formed in each soybean genotype. In order to survey sequence polymorphisms, POLYBAYES was used with base quality consideration. A total of putative 1,187 SNPs were detected and these polymorphisms should be reconfirmed by direct sequencing after homology search against GenBank databases.

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HETERODUPLEX MAPPING IN THE MEDICINAL PLANT *ARTEMISIA ANNUA*

T. CZECHOWSKI, D. VYAS, C. WHITEHEAD, B. KOWALIK, T. WINZER, I. GRAHAM, D. BOWLES
 Centre for Novel Agricultural Products, Department of Biology, University of York
 York, UK
 Email: tw518@york.ac.uk

A Chinese herb, *Artemisia annua* (huang hua hao), is currently the sole source of the leading anti-malarial drug, artemisinin (qinghaosu). In the face of increasing resistance to anti-malarial drugs such as chloroquine, 69 countries have adopted the WHO recommendation to use artemisinin combination therapies (ACTs) instead. However, there are considerable price barriers to widespread use as artemisinin yields from *Artemisia* plants are low, making artemisinin expensive to produce. Also, the rapid adoption of ACTs has created shortages, keeping the prices at high and sometimes volatile levels despite increased agricultural production. The aim of our project is to produce new, non-GM, varieties of *Artemisia* with increased artemisinin yields. These new varieties should help to secure a stable supply of artemisinin and reduce its cost of production, making treatment with ACTs cheaper and more accessible to malaria victims in developing countries. Artemisinin synthesis and storage occurs in specialised groups of cells, known as glandular trichomes, which are found on the leaves, stems and flowers of the plant. Artemisinin yields are typically less than 1% of leaf dry weight, whilst other species produce similar compounds in similar trichomes at 13% dry weight, so there is considerable scope for improvement. A seed treatment with chemical mutagens, widely used in plant breeding has been applied to an existing *Artemisia* cultivar (*Artemis*) in order to boost its genetic diversity. Around 10,000 M₂ plants from this treatment are currently being screened using heteroduplex mapping technique on a set of target genes with the potential to impact on artemisinin yields. Mutations which might impact on the function of the selected gene targets have been identified. Selected mutants will be fed into a fast track breeding program to bring the mutations to homozygosity in the most appropriate genetic background. This route should result in at least a doubling of artemisinin content. Gene targets for other important traits alongside artemisinin content will also be screened for advantageous mutations to further improve the agronomic performance.

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IMPROVEMENT OF MUTANT WHEAT FOR BAKING QUALITY USING MARKER ASSISTED SELECTION

B. NASERIAN KHIABANI, M.J. ZAMANI, M.T.HALLAJIAN, C.VEDADI
 Agriculture, Medicine and Industry Research School, Karaj, Iran
 Email: bnkhiabani@yahoo.com

The experiment was conducted in the Molecular marker lab. Of nuclear agriculture Dep. In Agriculture Medicine and Industry, Research School. Cultivars carrying the alleles HMWx2 and HMWx5 were classified according to results of SDS-PAGE in poly acryl amid gel. According SDS-PAGE results we made crosses between Bezostaya, Enia, Tajan, Chamran, Pishtaz as pollinators (with high bread making quality) and Tabasi mutant lines (low bread making quality, but have desirable traits), as recipient parents. F₁ plants were planted in greenhouse, subsequently F₂ Plants that obtained from F₁ hybrids were planted in greenhouse. Selection for high baking quality were performed using STS Marker, related to Glu1D (subunits 5+10). This marker showed a sharp band (450bp) in all genotypes that have 5+10 subunits. The identification of *Glu-D1* HMWx5 carrier genotypes is more straightforward at the gene rather than at the gene product level. Furthermore, in all blind experiments including a wide array of wheat genotypes the PCR system correctly detected the presence of the *Glu-D1* HMWx5+y10 pair. F₂ individuals that having this allele selected and in April 2007 were planted in field condition. Selection of F₃ individuals considered according to some agronomical traits such as earliness, vigor and yield component such as seed no per spike, weight of seed per spike, etc.

DEVELOPMENT AND MOLECULAR CHARACTERIZATION OF A GAMMA-IRRADIATED HEXAPLOID WHEAT POPULATION

V. SAYS-LESAGE, K. JARLETON, M.-C. DEBOTE, C. FEUILLET
INRA, Génétique Diversité et Ecophysiologie des Céréales, Clermont-Ferrand, France
Email: says@valmont.clermont.inra.fr

The bread wheat genome is large (17 Gb), highly repetitive (>80%) and composed of three homoeologous A, B, D genomes with 7 chromosomes pairs each ($2n=42$). With the development of efficient map based cloning programs and future genome physical mapping and sequencing project, it is essential that functional tools are in place to validate gene function in wheat. This can be performed through reverse genetics where populations are established after mutagenesis and screened with candidate genes to identify knock-out mutants for each homoeologous gene copy. To establish a reverse genetic tool for functional genomics in a cultivated elite wheat cultivar, we have mutagenized seeds from the hexaploid wheat (*Triticum aestivum* L.) French cultivar Renan.