



FAO/IAEA International Symposium on
Plant Mutation Breeding
and Biotechnology

27–31 August 2018
Vienna, Austria

ABSTRACTS

Organized by the



Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

www.iaea.org/events

CN-263

BOOK OF ABSTRACTS

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OPENING SESSION
IAEA Boardroom B/M1
M Building

Monday, 27 August 2018

PLENARY SESSION
IAEA Boardroom B/M1
M Building

Monday, 27 August 2018

WORLD FOOD SUPPLY IMPROVEMENT: PROBLEMS AND PROSPECTS**Perry Gustafson***University of Missouri, USA*Email: pgus@missouri.edu

The current world population of well over seven billion is projected to reach nine billion in less than 20 years. The UN projects agricultural output will need to increase by a minimum of 70% in order to maintain current dietary standards, which will still leave approximately one billion malnourished people. Current agricultural production is increasing at a rate insufficient to reach the goal of reducing by one half the number of malnourished people in the world. In spite of declining poverty rates, reducing the number of malnourished people will be very difficult as it is likely that an additional two billion people will be among the poor. Food imports are expected to increase despite any projected increased production. Agriculture can improve the world food supply on the same amount of land currently under production. The prospects of increasing world food production will involve several tools including better agronomy management, better farmer education, better student training, and clearly better varieties of all crops. Technology will be the leader in the tools required to create new cultivars. Mutation technology will be one of the leaders in supplying new genes for improved cultivars. The problems and prospects of the utilization of mutation technology to improve food production are discussed.

SCANDINAVIAN MUTATION RESEARCH DURING THE LAST NINETY YEARS-A HISTORICAL REVIEW**Udda Lundqvist***Nordic Genetic Resource Centre (NordGen), Sweden*Email: udda.lundqvist@nordgen.org

In 1928, the Swedish geneticists Hermann Nilsson-Ehle and Åke Gustafsson started on their idea for the first experiments with induced mutations using a diploid barley crop. They started with X-rays and UV irradiation. Soon after the first chlorophyll mutations were obtained and followed by the first 'vital' mutations 'Erectoides'. Several other valuable mutations were considered as early maturity, high yielding, lodging resistance and characters for barley architecture. The experiments expanded with other different types of irradiation, followed by chemical mutagenesis starting with mustard gas and concluding with the inorganic sodium azide. The research brought a wealth of observations of general biological importance, irradiation problems, difference in the mutation spectrum and directing mutagenesis. This research was non-commercial even if some mutants have become of important agronomic value. Its peaks of activities were during the fifties, sixties and seventies, and barley was the main experimental crop. About 12 000 different morphological and physiological mutants with a very broad biodiversity were brought together and are incorporated in the Nordic Gene Bank. Several important mutant groups have been analysed in more detail genetically, with regard to mutagen specificity and gene cloning. They are: 1. Early maturity mutants (Praematurum), 2. Six-rowed and intermedium mutants, 3. Mutants affecting surface wax coating (Eceriferum), 4. Mutants affecting rachis spike density (Erectoides), they will be presented in more detail. Since the work with induction of mutations began, it was evident that mutations should regularly be included in breeding programs of crop plants. In Sweden direct X-ray induced macro mutants have been released as cultivars, several ones are used in combination breeding and successfully released as cultivars. The importance for breeding will be discussed in more detail.

HISTORY OF MUTATION BREEDING AND MOLECULAR RESEARCH USING INDUCED MUTATIONS IN JAPAN

Hitoshi Nakagawa

Hamamatsu Photonics K.K., Japan

Email: hitoshi.nakagawa@crl.hpk.co.jp

Following the construction of the Gamma Field at the Institute of Radiation Breeding (IRB) in 1960, mutation breeding was accelerated in Japan. The facility is used to induce mutations by applying a higher radiation dose (up to 2Gy/day: ca. 300 000 times that of natural background) and at a higher frequency than occurs in nature. There have been 295 direct-use mutant cultivars representing 70 species generated through irradiation utilizing gamma-rays, X-rays, ion beams and chemicals and somaclonal variation. Approximately 79% of these direct-use cultivars were induced by radiation. There have been 335 indirect-use mutant cultivars, including 298 rice, of which 150 cultivars (50.3%) were derived from the semi-dwarf mutant cv. "Reimei". The economic impact of these mutant cultivars, primarily of rice and soybean, is hugely significant. Some useful mutations are discussed for rice, such as low digestible-protein content, low amylose content, giant embryo and non-shattering. Useful mutations in soybean such as radio-sensitivity, fatty acid composition, lipoxygenase-free, glycinin rich and super-nodulation have been identified. The achievements of biological research include the characterization and determination of deletion size generated by gamma-rays, the effect of deletion size and genomic location and gene function. Genetic studies generated through the use of gamma-ray induced mutations include: phytochrome research, aluminium tolerance and epicuticular wax. Mutation breeding is a very interesting and useful technology for isolating genes and for elucidating gene functions and metabolic pathways in various crops. Records show that mutation induction is a very useful conventional breeding tool for developing superior cultivars. The IRB is well equipped with appropriate facilities and equipment that will contribute to future mutation breeding developments and it will be a contributor in solving various genomic, proteomic and metabolic problems.

IAEA-CN-263-241

IMPACT OF MUTATION BREEDING TO FOOD SECURITY IN VIET NAM

Ham Le Huy, Hoi Pham Xuan

Institute of Agricultural Genetics, Viet Nam

Email: lhham@agi.ac.vn

Viet Nam began mutation breeding relatively later than other countries, but by the end of 2017 already created and released to production 67 new mutant varieties. Among them, 45 varieties of rice, 10 varieties of soybean, cassava, sugar cane, flowers and ornamental plants. The mutant varieties have been contributing significantly to ensuring food security and poverty alleviation in the country since the 1970s, creating hundreds of millions of dollars in profits to small-holder farmers every year. Rice mutant varieties, with productivity of 6-8 ton /ha versus 3.3 ton/ha average at that time in the country, helped to turn Viet Nam from rice importer to rice exporter in 1980-1990s. In 2015 the Joint FAO/ IAEA Division has recognized the contribution of mutation breeders in Viet Nam by granting two awards: Achievement award for breeders from Agriculture Science for Southern of Viet Nam and Centre for Nuclear Techniques and Outstanding Achievement Award for breeders from the Institute of Agricultural Genetics. The main advantage of mutation breeding is that, it does not require expensive equipment, nor staff with high qualification and high investments, but brings fast and stable results leading to great profit. Governments are very supportive to the development of biotechnology in agriculture. But there is a fact that not every country has the capacity to develop biotech crops, due to extremely high requirements in investment and high qualification of staffs involved and very well

managed organization of research system. On the other side, not all traits of agriculture crops are under capacity of biotech to change or improve, like plant structure, growth duration and many others. These traits can be manipulated easily via mutation with very low expenses. Not for all crops biotechnology tools are ready for use for improvement of desired traits, while mutation can be carried out effectively almost with all crops.

IAEA-CN-263-51

ACHIEVEMENTS AND RESEARCH PROGRESS OF MUTATION BREEDING OF WHEAT IN HENAN PROVINCE

Jianwei Zhang¹, Jialin Fan²

¹*Isotope Institute Co., Ltd, Henan Academy of Sciences / Henan Key Laboratory of Nuclear Agricultural Sciences, China*

²*Isotope Institute Co., Ltd, Henan Academy of Sciences, China*

Email: zjw10308@163.com

Henan province is located in central China. It is the core wheat production area with the largest wheat cultivated area, single yield and total production and the highest quantity of export commodity grain, with 5 million hectares, accounting for about 22% of the country's wheat area, the average yield about 6150kg/hm², the production more than 65 million kilograms accounting for 25% of the country. By the end of 2017, Henan province wheat breeding units using mutagenesis technology had bred 21 new mutant varieties of wheat. These varieties have made great contribution to the production of wheat in Henan, of which Taikong 5, Zhengmai 3596, Fumai 2008, Yufeng 11 and Zhengpinmai 8 realized the effective combination of high yield and high quality. The institute of isotopes of HAAS is one of the earlier units of wheat mutation breeding in Henan province. By now, we have bred 6 new wheat mutant varieties including Yutong 843, Fumai 2008, Zhengpinmai 8, etc. In addition, 10 new wheat mutant lines are tested in Henan or national regional trials. At the same time, we have created a large number of dwarf, high-yield, high-quality characteristic wheat germplasm resources. Based on the independent innovation of common wheat cytoplasm background of wheat male sterile line (89AR), we have begun to study the hybrid wheat seed production system. We have now bred some new sterile lines, screening a number of excellent maintainer line, breeding a little of new restorer lines. One excellent restorer line (recovery degree > 99%) was found by measuring the offspring fertility analysis, and it was preliminarily considered that the restorability was controlled by 3 pairs of major genes. This study will provide a new approach for the utilization of hybrid wheat in the world.

IAEA-CN-263-154

HIGH YIELDING NERICA MUTANT RICE FOR UPLAND AREAS AND HOPE FOR BANGLADESHI FARMERS

Mirza Mofazzal Islam, Rigyan Gupta, Shamsun Nahar Begum

Bangladesh Institute of Nuclear Agriculture, Bangladesh

Email: mirza_islam@yahoo.com

Drought is an important stress phenomenon in Bangladesh which greatly hampers in crop production. So, it is imperative to develop drought tolerant rice variety. Low yielding, un-uniform flowering and late maturity Africa rice - New Rice for Africa (NERICA) viz., NERICA-1, NERICA-4 and NERICA-10 rice varieties were irradiated with different doses of gamma rays (250, 300 and 350Gy) in 2010. M₁ plants were grown and M₂ plants were selected based on earliness and higher grain yield. The desired mutants along with other mutants were grown in M₃ during 2011. A total of 37 mutants from NERICA-1, NERICA-4 and NERICA-10 were selected on the basis of plant height, short duration, drought

tolerance and high yielding from M₄ generation. In M₅ generation, six mutants were selected for drought tolerance, earliness, grain quality and higher yield. With respect to days to maturity and grain yield (tha⁻¹) the mutant N1/250/P-2-6-1 of NERICA-1 performed earlier (108 days) and higher grain yield (5.1 tha⁻¹) than the parent and other mutants. The mutant N4/350/P 4(5) of NERICA-4 showed higher grain yield (6.2 tha⁻¹) from the parent and other mutants. On the other hand, NERICA-10 mutant N10/350/P-5-4 performed earlier and higher yield (4.5 tha⁻¹) than its parent. Finally, based on agronomic performances and drought tolerance, the two mutants viz., N4/350/P-4(5) and N10/350/P-5-4 were selected which were evaluated in drought prone and upland areas during 2016 and 2017. These two mutants performed also higher grain yield than the released upland rice varieties and will be released soon for commercial cultivation which will play vital role in food security in Bangladesh.

IAEA-CN-263-41

IMPACT OF MUTANT VARIETIES IN MALAYSIA: CHALLENGES AND FUTURE PERSPECTIVE OF MUTATION BREEDING

Rusli Ibrahim

Elite Scientific Instruments Sdn Bhd, Malaysia

Email: rusli.anm@gmail.com

Malaysia has made a substantial achievement in plant mutation breeding with the use of nuclear techniques and related biotechnologies not only in the development of new mutant varieties but also in the establishment of an excellent nuclear research centre. A total of 53 mutant varieties have been developed including rice (19), banana (1), groundnuts (2), orchids (6), chrysanthemums (7), hibiscus (3), roselles (3), and other ornamental and landscaping plants (12). Most of the new ornamental varieties have been developed by both acute and chronic gamma rays using irradiation of seeds, rooted cuttings, bulbs and tissue cultures. Food crops that have economic impact for sustainable agricultural production are mutant varieties of banana, Novaria and rice, MRQ 74, MR219-9 and MR219-4. Novaria is a selection made from a Grande Naine mutant, GN-60A identified from gamma ray-treated populations of the Biotechnology Laboratory, in Seibersdorf, Austria. It was the first mutant variety officially released by Malaysian Nuclear Agency as a new cultivar in 1995 for its improved characteristics such as early flowering, short stature and high yield. MRQ 74 is a type of high quality fragrance rice which is resistance to blast and was an indirect mutant variety released in 2003. One of its parental lines for cross-breeding was Mahsuri Mutant which was developed through mutation breeding using gamma-rays. In 2014 two new mutant varieties of rice, MR219-9 and MR219-4 have been selected using gamma irradiation which are drought tolerance, high yield and resistant to blast. Despite these achievements, applications of induced mutation have decreased during the last 10 years due to reduced funding. Mutation breeding is still promising techniques for the development of new and novel varieties in combination with advanced molecular genetics that can bring plant mutation breeding into a new era.

IAEA-CN-263-259

SOYBEAN BREEDING THROUGH INDUCED MUTATION IN VIET NAM

Thao Le Duc, Bao Chung Pham Thi

Agricultural Genetics Institute, Viet Nam

Email: leducthao@agi.vaas.vn

In Viet Nam, soybean is one of the traditional crops and plays an important role in crop rotation, soil improvement and in meeting the nutritional need for humans and livestock. With the aim of generating genetic variability in soybean and creating new soybean varieties induced mutation research has been carried out since 80's and resulted in outstanding achievements. Induction of new traits and their

incorporation in an ideal genotype was achieved by judicious use of induced mutation technique. So far, outstanding soybean varieties including DT84, DT90, DT99, DT2008, and several promising lines have been developed in Viet Nam by incorporating desirable traits like high and stable yield (2.0-3.5ton/ha), good grain quality, drought tolerance, disease resistance (rust, powdery mildew, downy mildew), short growth duration (70-100 days), wide adaptability and suitability for crop systems and ecological regions in the whole country. These varieties have contributed to the development of soybean production in Viet Nam, increased 1.8 times of the yield from 0.78 in 1985 to 1.43ton/ha in 2015, and profiting to the production of USD millions. They have been also utilizing as materials for developing several improved soybean varieties. Thus, induced mutation research has played an important role in improving soybean varieties in Viet Nam.

IAEA-CN-263-112

APPLICATION OF MUTATION TECHNIQUES IN THE DEVELOPMENT OF GREEN CROP VARIETIES IN SRI LANKA: WAY FOR WARD

Malathy Parasuraman, Priyantha Weerasinghe

Department of Agriculture, Sri Lanka

Email: mpmalathy@yahoo.com

The Department of Agriculture (DOA) in Sri Lanka has initiated mutation breeding in the 1960's with the introduction of a Cobalt-60 source. The first rice mutant variety, M1273 was released for general cultivation in 1971. M1273, derived from H-4 irradiated with 35 kR gamma rays, was suitable for regions affected with occasional drought. Indirect rice mutant variety, developed by crossing a short mutant line, BW267-3 with a highly adaptable variety, was released as BW372 in 2013. It is moderately resistant/ tolerant to blast, bacterial leaf blight, brown plant hopper, gall midge and iron toxicity thus, increased the productivity on marginal rice cultivation lands to 3-4ton/ha. The popular Groundnut variety "Thissa" is a mutant, previously developed by irradiation with gamma rays at 200Gy. Main improved attributes were high yield, medium maturity (90-100 days) and high oil content (42%). "Thissa" presently covers 80% of groundnut cultivated area in Sri Lanka. A sesame mutant line, derived from the variety MI-3 irradiated at 200Gy with ⁶⁰Co gamma-rays, was released as "Malee" (ANK-S2) in 1993. It is a high yielding variety (1.1-1.8ton/ha) with resistant to Phytophthora blight. A cherry type mutant tomato variety, developed by irradiation of seeds with gamma rays (320Gy), was released as "Lanka Cherry" in 2010. Improved attributes are pear shaped fruits and bacterial wilt resistance. Narrow genetic variability in many crops is a constraint to the development of new varieties adapted to climate change. Hence the DOA is emphasizing integration of induced mutagenesis in conventional breeding programme to develop resistant/tolerant varieties with high yield, quality and health promoting functional properties in field and horticultural crops. The newly installed gamma radiation chamber facilitates to create genetic variability in food crops thus paving way for the development of greener varieties.

MUTATION BREEDING IN RICE FOR SUSTAINABLE CROP PRODUCTION AND FOOD SECURITY IN INDIA

Vikash Kumar¹, Anjali Chauhan¹, A.K. Shinde², R.L. Kunkerkar², Deepak Sharma³, B.K. Das¹

¹*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, BARC, India*

²*Regional Agriculture Research Station, DBSKKV, India*

³*Indira Gandhi Krishi Viswa Vidyalaya, India*

Email: kvikash@barc.gov.in, vikash007barc@gmail.com

Crop improvement program through radiation induced mutation breeding was initiated nine decades ago. India has made significant progress in the field of mutation breeding in different crop species. Rice is a staple food for Indian population with nearly 70% population deriving daily dietary needs from rice. With changing consumer preferences, global climate change, changing pest dynamics and awareness to human health, rice breeding activities, specially in mutation breeding, have been modified with time to cater to these problems. Mutation breeding activities at BARC are focussed with an objective to develop fine and better grain quality rice, salt and drought tolerant rice, bio-fortified rice with high Fe/Zn, medicinal rice with higher yield and better grain quality etc. Recently TKR Kolam (Trombay Karjat Kolam Rice), a very fine grain (short slender: test weight 11.0g), high grain density (250 spikelets/panicle), high yielding (4.5t/ha), high milling% (73%) and tolerance to pest and diseases rice variety have been released through mutation breeding. India has highest diversity for aromatic and scented rice but is becoming extinct due to its poor yield and undesirable agronomic traits. Radiation induced mutation breeding has been instrumental in improving these landraces. A highly scented, dwarf (100cm), high yielding (4.5 t/ha), with better milling recovery (71.6%) and head rice recovery (69.2%), non-lodging and better grain quality mutant of aromatic rice variety 'Dubraj' was released as TCDM-1 (Trombay Chhattisgarh Dubraj Mutant-1). Salinity and drought stress are major abiotic stress affecting more than 15mha land in India and causing significant yield losses. Gamma ray induced mutation breeding have resulted in improved salt tolerant rice selections which will benefit farmers in coastal region on India. Recently advanced high energy ion beams induced mutation breeding activity were initiated for CSR27, IBD-1, Samudhini, Nagina 22 and Dagardeshi rice varieties with an aim to develop climate resilient rice varieties for future. Medicinal values of some traditional rice varieties from Chhattisgarh, India are known to cure different health problems in human and animals. These landraces are being agronomically evaluated and improved through mutation breeding. Mutation breeding will continue to play a significant role in rice improvement program for solving national food security in coming years and decades.

PLENARY SESSION
IAEA Boardroom B/M1
M Building

Tuesday, 28 August 2018

TILLING AS A RENEWABLE SOURCE OF MUTATIONS FOR FUNCTIONAL GENOMICS AND PRAMIRIAMCTICAL BREEDING

Miriam Szurman-Zubrzycka, Iwona Szarejko

Department of Plant Physiology, Faculty of Biology and Environmental Protection, University of Silesia, Poland

Email: miriamszurman@gmail.com

TILLING (Targeting Induced Local Lesions IN Genomes) was first described in 2000 as a reverse genetics tool that combines classical mutagenesis and high-throughput mutation screening. In this presentation the current status of TILLING platforms in different species will be summarized. The lecture will focus on latest achievements of TILLING in functional genomics and in practical plant breeding. The advantages and limitations of this strategy will be compared to other reverse genetic approaches, first of all to CRISPR/Cas 9 based RNA guided genome editing. We will present our *Hor*TILLUS (*Hordeum vulgare*-TILLING-University of Silesia) population, that is TILLING population developed for barley spring variety ‘Sebastian’ after double treatment with two mutagens: MNU and sodium azide. The average mutations density in this population, estimated as 1 mutations per 477 kbp, is relatively high. *Hor*TILLUS platform has proved to be a useful tool for functional genomic studies, as well as for forward selection of mutants with desired phenotypes. We have used this population to study the function of genes related to many aspects of barley growth and development, such as brassinosteroid and strigolactone metabolism, abiotic stress response (drought, aluminum) and DNA repair processes. *Hor*TILLUS population comprises more than 9600 M₂ plants (and progenies) and is being constantly renewed. We would like to share this population within barley community in a cooperative manner.

IAEA-CN-263-89

MUTATION BREEDING FOR POWDERY MILDEW RESISTANCE IN PEA (*PISUM SATIVUM* L.)

José Leitão

Universidade do Algarve, Portugal

Email: jleitao@ualg.pt

The broad-spectrum (mlo) resistance to powdery mildew in barley (*Hordeum vulgare* L.) was demonstrated to be conferred by recessive mutations at the Mlo locus. In the Laboratory of Genomics and Genetic Improvement (LGGI), we induced for the first time, via experimental chemical mutagenesis, two powdery mildew resistant mutants in pea (*Pisum sativum* L.). Via complementation crosses the two recessive mutations were found to affect the same locus, identified as the naturally mutated in the resistant line Mexique locus er1, genetically mapped to the pea linkage group VI. After isolation and sequencing of the barley gene Mlo, other research groups identified the expressed sequence of a homologous gene in pea, which was found to coincide with er1, which, accordingly, was renamed as PsMLO1. The identification by our laboratory of the genomic sequence of this locus allowed the identification of the specific point mutations induced in our mutants, which create stop codons and truncate the cell transmembrane protein coded by this gene. In both cases, the mutations eliminated a restriction enzyme recognition site allowing the easy identification of the mutated loci by CAPS analysis. More recently, we have validated a hyper-variable SSR marker within the PsMLO1 gene as highly useful for marker-assisted selection in multiple pea powdery mildew resistance breeding

programmes. Two advanced lines derived from crosses involving one of our PM resistant mutants will be next year submitted for release as new cultivars. The identification of Mlo homologous genes in other crops and the future prospects of mutation breeding for powdery mildew resistance are discussed.

IAEA-CN-263-214

DEVELOPING NEW COWPEA VARIETIES OBTAINED BY USING GAMMA IRRADIATION INDUCTION

Made Diouf, **Diaga Diouf**, François Abaye Badiane, Sara Diallo
Université Cheikh Anta Diop, Senegal
Email: diaga.diouf@ucad.edu.sn

Cowpea (*Vigna unguiculata*) is one of the most important legume staple food and forage crop in Africa. Because of its low genetic diversity, several breeding programs based on classical techniques have been implemented to develop new resistant varieties to biotic or abiotic constraints. Despite these attempts, the genetic basis of the crop is still narrow and thus, it appears that there is a need to use new approaches such as mutagenesis. The objectives of this study were to contribute to increase cowpea production specifically by identifying and selecting new elite genotypes. For these purposes, the seeds of 40 mutant M₄ mutant lines were selected based on their yield component, sown in pot containing Sangalkam's sand and grown in the shade house and then in the experimental field using single seed descent approach until M₇. Agro morphological characterization based on 11 quantitative and 5 qualitative parameters were evaluated in the population. Our results allowed the identification and the selection of 11 promising varieties among which, early flowering, resistance to nematodes, with large seeds and with long pods. The early flowering mutants were clustering together into the group B of a dendrogram. The mean seed weight of some mutants ranged from 0.23 to 0.35g compared to the control Melakh (0.19g) regardless of the generation. The mutants N°1 and N°14 showed seed size ranging from 10 to 12mm while the control had 9.21mm. The pod length of the mutant 25 ranged from 20 to 25cm but the control had 19cm. Statistical analyses showed correlation among the leaflet parameters and yield components as well. The pod mean length was correlated positively with the seed number per pod. The mean seed weight and mean pod length were highly heritable with respectively 54% and 40.20%.

IAEA-CN-263-199

ISOLATION AND CHARACTERISATION OF YELLOW RUST RESISTANT MUTATION IN WHEAT

Suman Bakshi, Johar Singh, Sanjay J. Jambhulkar
Bhabha Atomic Research Centre, BARC, India
Email: sumansud@barc.gov.in

Stripe rust caused by *Puccinia striiformis* f. sp. tritici is major threat to wheat production leading to yield loss up to 84%. New races of stripe rust are being observed for which no durable source of resistance has been determined in the current high-yielding varieties. Mutation breeding programme was initiated in two popular varieties namely, PBW343 & HD2967 using gamma ray and electron beam irradiation. The gamma ray doses of 250, 300, and 350Gy and electron beam doses of 150, 200, and 250Gy were used for irradiating seeds of PBW343 and HD2967. Six M₂ populations consisting of 75 000 plants of each variety were screened in the field from seedling to adult plant stage by spraying a mixture of urediniospores of Pt pathotypes (77-2, 77-5 and 104-2) and Pst pathotypes (78S84 and 46S119). Disease severity was recorded as the percentage of leaf area covered by rust pathogen following a modified Cobb's scale. A total of 52 putative rust resistant mutant plants in M₂ population

of HD2967 and 63 putative resistant mutants in M₂ of PBW343 were identified. The Number of mutants was higher in the population derived from electron beam irradiation (EBM) as compared to gamma rays. The absence of sporulation and spore production of rust pathogen on mutants indicated resistance. Mutant plants showing seedling resistance also showed hypersensitive response in adult plant. Yield component characters of mutants were comparatively better than in the parents. The mutations responsible for the altered infection phenotypes appear to involve plant resistance genes that interact with the pathogen at later stages of the pathogen development. These rust resistant mutants could be a novel source of stripe rust resistance. The plant to row progenies of these mutants have been raised in M₃ generation and are being characterized.

IAEA-CN-263-297

IDENTIFICATION OF RICE MUTANTS TOLERANT TO COLD STRESS AT THE GERMINATION STAGE BY TILLING

Viviane Kopp Da Luz¹, Vivian Ebeling Viana¹, Daniel Da Rosa Farias¹, Gabriela Magalhães Da Fonseca¹, Ariano Martins De Magalhães Jr.², Luciano Carlos Da Maia¹, Camila Pegoraro¹, **Antonio Costa De Oliveira¹**

¹*Plant Genomics and Breeding Center, Federal University of Pelotas, Brazil*

²*Embrapa Temperate Climate, Brazil*

Email: acostol@cgfufpel.org

The occurrence of low temperatures is a common stress in rice cultivation in temperate regions, making tolerance to low temperatures a desirable trait in rice genotypes grown in Southern Brazil. These low temperatures hinder germination, crop establishment and grain yield. This study aimed to characterize and identify through the TILLING technique, rice families tolerant to low temperature stresses in the germination stage. In germination analysis, seeds of 400 mutant families rice (M₃ generation), and BRS Querência, BR IRGA 409 and Nourim Mochi cultivars were subjected to treatments with different temperatures (13°C and 25°C) and compared as to their relative performance, as measured by the length of coleoptile, root length and shoot length. For the analysis of mutant families by the TILLING technique, 4000 M₃ plants subdivided in 500 pools of eight plants were analyzed. Mutants were obtained by mutagenesis with ethylmethanesulfonate (EMS), for the presence of mutations in *Os03g0103300* (*qLTG3-1*), a gene connected to germination response to low temperature stress in rice. The results indicate that mutation induction was effective in generating genetic variability for tolerance to low temperatures during germination stage. The TILLING technique allowed the identification of a genotype (516 A₃) carrying several SNP mutations in the *Os03g0103300* gene (*qLTG3-1*). SNPs were detected in coding sequences and in the 3'-UTR region, and one mutation was found to be associated to a AP2/ERF domain located downstream the gene and is suggested to be the mutation responsible for the character stress tolerance to low temperatures.

IAEA-CN-263-13

MUTATION BREEDING OF SORGHUM TO SUPPORT CLIMATE SMART AGRICULTURE

Soeranto Human, Wijaya Indriatama

Center For Isotopes and Radiation Application, National Nuclear Energy Agency of Indonesia (CIRA-BATAN), Indonesia

Email: soeranto@batan.go.id

Global climate change effects in agricultural fields often increase plant stress. For mitigating climate change, climate smart agricultural policies should be developed, for example, through the improvement of crop adaptability and productivity in environments impacted by climate change. Attempts to increase

crop genetic variability must be sought in mitigating adverse conditions brought about by climate change. For that purpose, mutation breeding plays an important role since it can increase genetic variability of important crops. By selecting desired mutant genotypes, the plant breeder can advance his germplasm by progressing lines with good adaptability and with high productivity under adverse conditions. For Indonesia, significant adverse impacts of climate change have appeared in some agricultural regions, such as drought problems in eastern regions, more soil acidity problems in western regions and salinity problems along coastal areas. To face the worsening conditions brought about by climate change and variability, a crop was sought that would require less agricultural inputs, being drought tolerant, has a good adaptability and with high economic value. The choice fell on sorghum (*Sorghum bicolor*). In certain areas sorghum is recognized as a source of food, feed and fuel. Mutation breeding of sorghum has been conducted at the Centre for Isotopes and Radiation Application (CIRA), the National Nuclear Energy Agency of Indonesia (BATAN). Sorghum mutation breeding is relevant to the National programme on food diversification and food security. The breeding objectives are to improve sorghum genotypes for improved yield and quality, and tolerant to adverse conditions brought about by climate change, such as prolonged drought. Some sorghum mutant lines and varieties have now been produced and will be presented in the symposium.

IAEA-CN-263-23

PRODUCTION OF HAPLOID EMBRYOS AND PLANTS IN IRANIAN MELON (*CUCUMIS MELO* L.) THROUGH IRRADIATED POLLEN INDUCED PARTHENOGENESIS

Leila Bagheri¹, Mahmoud Lotfi², Mansor Nori¹

¹*Nuclear Agriculture School, Nuclear Science and Technology Research Institute, Iran*

²*Department of Horticulture, College of Aboureihan, University of Tehran, Iran*

Email: lbagheri@nrcam.org

The irradiated pollen technique (IPT) is the most successful haploidization technique within Cucurbitaceae. The influence of gamma ray doses (250, 350, 450 and 550Gy), genotypes and stage of development of embryos obtained by IPT on the induction of haploid embryos were studied in several Iranian melon cultivars as well as their hybrids with alien cultivars. Female flowers were pollinated using pollen that had been irradiated with gamma rays. Different shapes and stages of embryos were excised 21-25 days after pollination and cultured on E20A medium. Direct culture, liquid culture and integrated culture methods were applied for detected the induced embryos. Integrated culture and liquid culture methods showed advantages in increasing the efficiency of haploid plant production in melon breeding programmes. Results revealed that 550Gy of gamma-irradiation was successful in inducing parthenogenesis and fruit development, whereas, lower irradiation doses were not effective in inducing haploid embryos. The percent of embryos per seeds were the highest in 'Samsoori' (1.2%) and 'Saveh' (1.1%) cultivars. Some of the heart-shaped and cotyledon-shaped embryos developed into haploid plants. In total, 52 parthenogenic melon plantlets were recovered from 274 embryos via IPT. Haploid embryos and haploid plants production were strongly influenced by gamma ray dose, embryo stage and genotype. Indirect methods and chromosome counting performed on the root cells of regenerated plants, showed the haploid level ($n = x = 12$).

APPLICATION OF MUTATION BREEDING TO THE IMPROVEMENT OF UNDERSTUDIED CROP TEF

Zerihun Tadele¹, Assefa Kebebew², Solomon Chanyalew²

¹*University of Bern, Switzerland*

²*Ethiopian Institute of Agricultural Research, Ethiopia*

Email: zerihun.tadele@ips.unibe.ch

Tef [*Eragrostis tef* (Zucc.) Trotter] is the most important cereal crop in the Horn of Africa, especially in Ethiopia, where it is annually cultivated on over three million hectares of land, which is equivalent to 30% of the total area allocated to cereals. The large-scale cultivation of tef is due to its resilience to diverse environmental stresses. In addition to being nutritious, tef is considered a healthy food due to the absence of gluten in its grain. Despite its huge importance in the economy of Ethiopia, the productivity of tef is very low. The major yield-limiting factor in tef production is the susceptibility of its stem to lodging (i.e., the displacement of the stem from the upright position). Here, we describe the Tef Improvement Project, which employs mutation breeding to improve the crop. We highlight a new variety, Tesfa, developed in this pipeline and possessing a novel and desirable combination of traits. Tesfa's recent approval for release illustrates the success of the project and marks a milestone as it is the first variety (of many in the pipeline) to be released. We have established an efficient pipeline to bring improved tef lines from the laboratory to the farmers of Ethiopia. Of critical importance to the long-term success of this project is the cooperation among participants in Ethiopia and Switzerland, including donors, policy makers, research institutions, and farmers. Together, European and African scientists have developed a pipeline using breeding and genomic tools to improve the orphan crop tef and bring new cultivars to the farmers in Ethiopia.

IAEA-CN-263-6

IMPROVING SUSTAINABLE COTTON PRODUCTION THROUGH ENHANCED RESILIENCE TO CLIMATE CHANGE USING MUTATION BREEDING

Manzoor Hussain¹, Dana Jawdat², Khin Myat Lwin³, Ljupcho Jankuloski⁴, Massoud Malek⁵, Md Kamrul Islam⁶, Mohammad Reza Rahemi⁷, Tianzhen Zhang⁸

¹*Nuclear Institute for Agriculture and Biology, Pakistan*

²*Atomic Energy Commission of Syria, Syrian Arab Republic*

³*Biotechnology Research Department, Myanmar*

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

⁵*International Atomic Energy Agency, Department of Technical Cooperation, Austria*

⁶*Cotton Development Board, Bangladesh*

⁷*Atomic Energy Organization of Iran (AEOI), Nuclear Science and Technology Research Institute (NSTRI), Iran*

⁸*Zhejiang University, China*

Email: mhmanj@gmail.com

Cotton being a leading commercial fibre crop is grown on 20.5 million hectares in three major cotton producing countries i.e. China, India and Pakistan. Wide difference in yield per hectare exist among these countries and is being aggravated by the changing climate conditions i.e. higher temperature and important seasonal and regional fluctuation in rainfalls. Pakistan is one of the most affected countries. The disastrous effects of extreme period of heat stress in cotton were very prominent in Pakistan during the growing seasons 2013-14 (40-50% fruit abortion) and 2016-17 (33% shortfall) with its alarming threat to the cotton based economy of Pakistan. Poor resilience of main grown

cotton varieties against extreme periods of heat stress are considered as main factors for this drastic downfall in cotton production in Pakistan. Using the approach of induced mutation breeding, NIAB Faisalabad, Pakistan has demonstrated its capabilities in developing cotton mutants that can withstand the changing climatic conditions and help in sustaining the excellent cotton yield. The results of thermo-tolerant cotton mutants (i.e. NIAB-878, NIAB-545, NIAB-1048, NIAB-444, NIAB 1089, NIAB-1064, NIAB-1042 in comparison with FH-142 and FH-Lalazar for their phenological & physiological traits conferring heat tolerance will be presented. NIAB-878 excelled in heat tolerance by maintaining the highest anther dehiscence (82%) and minimum cell injury percentage (39%) along with illustrating of maximum stomatal conductance ($27.7 \text{ mmol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), transpiration rate ($6.89 \text{ } \mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$), net photosynthetic rate ($44.6 \text{ mmol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) and physiological water use efficiency ($6.81 \text{ mmol CO}_2 / \mu\text{mol H}_2\text{O}$) under prevailing high temperature. The author would also like to report the adaptation results of cotton mutants (i.e. NIAB-KIRAN & NIAB-414) shared with MS counterpart (CP) in countries under the Project RAS/5/075.

IAEA-CN-263-225

CREATING VARIABILITY IN COWPEA FOR ADAPTATION AND VALUE ADDITION THROUGH INDUCED MUTATION

Kalaluka Munyinda¹, Kennedy Kanenga²

¹*University of Zambia, Zambia*

²*Zambia Research Institute, Zambia*

Email: kalalukamunyinda@yahoo.com

Cowpea (*Vigna unguiculata* L. Walp) is a major legume crop in Southern Africa. Cowpea provides a highly nutritious and inexpensive source of protein especially for low-income families. Production is characterized by low yields of less than 1.0ton/ha against a potential of more than 4.0ton/ha. The present project aims at developing improved cowpea varieties with high yield potential but with tolerance to abiotic and biotic stresses through induced mutation. Cowpea mutants were evaluated in the greenhouse and in the field. In Region II, LT11-3-3-13 and BB8-1-5-2 out-yielded their respective parental varieties by 52.3% and 40.6% respectively. In Region I, LT3-8-4-6 out-yielded its parental variety by 33.4%. LT11-3-3-12 and BB14-16-2-2 were identified to be tolerant to Al toxicity. LT4-2-4-1 was responsive to local and applied inoculation, while BB10-4-2-3 was responsive to local rhizobia. MS3-14-4, LT11-5-1, LT3-8-4-6, and LT3-8-4-1 showed tolerance to aphids, while LT11-5-2-2, BB 7-9-7-5 and BB-14-16-22 were tolerant to bruchids. Farmers preferred cowpea mutants over currently grown varieties for their desirable traits. BB8-1-5-2 and LT11-3-3-13 will be released early in 2018. Four other mutants will be submitted for pre-release this year. Use of cowpea mutants with desirable traits will increase the food and nutritional security and reduce the cost of cowpea production in the country and create a greater market demand for cowpeas.

GROUNDNUT MUTANTS WITH END-OF-SEASON DROUGHT TOLERANCE FOR THE MARGINAL DRY LANDS OF NORTH KORDOFAN STATE, SUDAN

Elgailani Abdalla, Omar Bakhit, Salih Elsheikh
Agricultural Research Corporation, Sudan
 Email: elgailani_ers@hotmail.com

Groundnut (*Arachis hypogaea* L.) produced in the traditional small-scale rain fed sector of Western Sudan accounts for 80% of the total annual groundnut acreage producing 70% of the total production. Low productivity of groundnut is a characteristic feature in North Kordofan State which is characterized as the most vulnerable state to the impact of climate change. Terminal drought stress resulting from reduction in rainfall amount and distribution at the end of the season is the most deleterious drought period as it coincides with groundnut pod filling and maturation periods. High and stable yields under the subsistence farming conditions in North Kordofan could only be realized by using adapted high yielding drought tolerant genotypes. Mutation induction by gamma rays was utilized to create genetic variability aiming at increasing the chances of obtaining genotypes with the desired drought tolerant traits. Groundnut mutants at M₅ generation advanced by single seed descend method were evaluated for end-of-season drought tolerance. Terminal drought period of 25 days was imposed after 60 days from planting using the rainout shelter. The same mutants were evaluated under optimum growing conditions under sprinkler irrigation system. Mutants which survived 25 days terminal drought stress were further evaluated for agronomic performance under rain fed conditions. Groundnut mutant Barberton-B-30-3 produced 1024kg/ha mean pod yield over 12 seasons compared to 926kg/ha for the check cultivar. The stability and GGE biplot analysis for the last 5 seasons, showed that Baberton-B-30-3 was stable and produced good yield in both high and low rain fall seasons. Hence, Barberton-B-30-3 is a suitable mutant for sustainable profitable yields in the marginal dry lands of North Kordofan State.

ISOLATION OF GAMMA RAY INDUCED URD BEAN (*VIGNA MUNGO* L. HEPPER) MUTANTS WITH INCREASED BATTER VOLUME

Chockalingam Vanniarajan¹, J. Souframanien²
¹Tamil Nadu Agricultural University, India
²Bhabha Atomic Research Centre, BARC, India
 Email: vanniarajan.c@tnau.ac.in

Urd bean is an important legume crop in India useful for the preparation of domestic nutritious breakfast meals called: Idli and Dosa. Idli is a fermented steamed bread-like cake and an important source of protein and energy in the diet of many South Indians. Idli is easily digested and hence preferred by all: from infants to elderly people. The specialty of urd bean in idly preparation is owing to the mucilaginous material present and does not exist in other edible legumes. The mucilaginous material is a complex carbohydrate containing galactose and arabinose. MDU 1 urd bean variety was released from Tamil Nadu Agricultural University, Madurai, India during 2014. It gives higher yield and possesses a high arabinose content of 7.5%. The volume of batter was increased in the variety MDU 1 over other existing varieties. This is beneficial to the farmers, traders and consumers. This variety, MDU 1, has a maturity duration of 70-75 days, has bushy nature, and is susceptible to yellow mosaic virus. To develop a determinate type, MDU 1 urd bean seeds were irradiated with gamma rays using doses ranging from 100Gy to 500Gy with an interval of 100Gy. The determinate types were selected in the M₂ generation and advanced to further generations. After attaining homozygosity in the advanced generations, the mutants were checked for their biochemical characters. The mutants ACM 16-11, ACM 16-15, and

ACM 16-18 were found to have an arabinose content of 8.28%, 8.98% and 8.14% respectively. All the mutants recorded more than 10% increase in batter volume over the variety MDU 1. The albumin (%) and globulin (%) contents were also increased remarkably in the mutants. We suggest subjecting these mutants to multi-location trials and proposed for registration.

IAEA-CN-263-220

A NEW MUTANT CULTIVAR OF BARLEY (*HORDEUM VULGARE*. L.) REGISTERED IN TURKEY

Cuma Akinci¹, Tuba B. Bicer¹, Mehmet Yildirim¹, Onder Albayrak¹, Ferhat Kizilgeçi²

¹Dicle University, Turkey

²Sirnak university, Turkey

Email: akinci@dicle.edu.tr

“Onder”, a two-rowed mutants feed barley cultivar, was developed by the Dicle University Faculty of Agriculture, Department of Field Crops and it has been registered in 2016 by the Ministry of Food, Agriculture and Livestock in Turkey. ⁶⁰Co gamma-ray treatment at the dose of 150Gy was applied to seeds of Sahin 91 two-rowed barley cultivar to induce mutation and “Onder” was derived by mutation breeding from “Sahin 91”. The superiority of “Onder” for some traits in respect to Sahin 91 are 12.8% higher or lower grain yield, 10.1% higher or lower test weight, 6% increased grain protein ratio, and 189% increase over the 2.2 mm sieve grain ratio. A significant lodging tolerance has also been obtained in Onder, which is usually, a major problem in the barley production especially in high input area. Grain weight decreased in “Onder” while plant height increased. “Sahin 91” was normal flowering time, while Onder flowered 13 days earlier than Sahin 91. Seeds and spikes morphology of Onder were also different from Sahin 91. The genotypic differences were determined between both genotype by SSR method. Due to high genotypic and phenotypic differences induced by mutation Onder has proved the power of mutation breeding and it is expected that Onder will take a significant share in Turkey barley farming.

IAEA-CN-263-16

GENOTYPE-BY-ENVIRONMENT INTERACTION OF ELITE VARIETIES OF COWPEA DERIVED THROUGH MUTAGENESIS

Lydia Ndinela Horn¹, Fatma Sarsu², Learnmore Mwadzingeni³ Mark D. Laing³ Shimelis Hussein³

¹Ministry of Agriculture, Water and Forestry, Namibia

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

³University of Kwazulu Natal, South Africa

Email: lnhorn@yahoo.com

Grain yield of cowpea (*Vigna unguiculata* L.) is considerably low in the northern communal areas of Namibia where the crop is predominantly cultivated. This is attributed to: i) the lack of improved and well-adapted cultivars, ii) limited water availability, as well as iii) the effects of genotype by environment (G × E) interaction on the crop productivity. The objectives of this study were to determine G × E interaction and yield stability of elite cowpea genotypes developed through mutagenesis and to identify promising genotypes and to establish the most representative field-test methods and identify the best production environments. The study was conducted in Namibia at three selected sites (Bagani, Mannheim and Omahenene) during two cropping seasons (2014/2015 and 2015/2016) in six different environments. The experiments were laid out using a randomised complete block design with three replications. Thirty-four elite genotypes and three check lines were evaluated. Data were analysed using the Additive Main Effects and Multiplicative Interaction (AMMI) and the Genotype plus

Genotype by Environment (GGE) bi-plot methods. The following four promising mutant genotypes: G9 (ShL3P74), G10 (ShR3P4), G12 (ShR9P5) and G4 (ShL2P4) were identified with better grain yields of 2.83, 2.06, 1.99 and 1.95, ton/ha-1, in that order. The parental lines designated as G14 (Shindimba), G26 (Nakare) and G37 (Bira) provided mean grain yields of 1.87, 1.48 and 1.30 ton/ha-1, respectively. The best environments in discriminating the test genotypes were Bagani during 2014/15 and Omahene during 2014/15. The AMMI model explained 77.49% of the total variation in the present study. The GGE bi-plot showed that 63.57% of the total variation was explained by the first principal component (PC1), while the second principal component (PC2) explained 12% of the variation.

PLENARY SESSION
IAEA Boardroom B/M1
M Building

Wednesday, 29 August 2018

EMERGING BIOTECHNOLOGIES AND THE CONSERVATION AND SUSTAINABLE USE OF PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

Chikelu Mba

Food and Agriculture Organization of the United Nations (FAO), Italy

Email: chikelu.mba@fao.org

The chance discoveries of domesticable spontaneous plant mutants made the transition from hunting and gathering to agriculture possible. The methods for breeding ever higher yielding and more resilient and nutritious crop varieties to sustain a continually growing human population have been evolving rapidly. Cross-breeding; induced mutations (using physical and chemical agents); and biotechnologies-including genetic engineering, marker-aided selection and most recently, genome editing-are all aimed at increasing the efficiency of crop production by creating and utilizing use full heritable variations in plant genetic resources for food and agriculture. First described barely six years ago, the Clustered Regularly Interspaced Short Palindromic Repeats (or CRISPR) has become the genome editing method of choice, thus resulting in the development of crop varieties either formally released or in the pipeline. Its rapid adoption rate, the universal applicability and the low-cost involved mirrors the developments of the closely associated gene drive and digital sequence data and synthetic biology, for instance. The paces of the scientific and technological developments for these methods have far outstripped those of the requisite policy regimes. The ongoing debates include whether the products of genome editing, with or without gene drive, should be considered living modified organisms and, if so subject to the international framework, the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Another debate is whether digital sequence information should be subject to some free access and benefit sharing regime considering that with the powers of synthetic biology, products previously harnessed only from living organisms can now be produced in the laboratory once the DNA sequence is available. To avoid the crippling pitfalls of the past, a call is made for serious intergovernmental dialogues on the safeties, fairness and ethics of the use of these emerging biotechnologies as the stakes are extremely high.

IAEA-CN-263-54

NEW MUTATION TECHNIQUES APPLIED IN CROP IMPROVEMENT IN CHINA

Luxiang Liu¹, Hongchun Xiong², Huijun Guo¹, Linshu Zhao¹, Yongdun Xie¹

¹*Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, CAAS, China*

²*Space Breeding Center, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, CAAS, China*

Email: guohuijun@caas.cn

There are at least 1 billion hungry people worldwide and the Asia and the Pacific region harbour the biggest estimated regional distribution of hunger. Lifting a billion people out of poverty and feeding an extra 2.3 billion by 2050 will require increasing cereal production by 70%, i.e. doubling the output of developing countries. Accelerating the development of agriculture to continually increase productivity should be the final approach to end the poverty. Mutation breeding techniques have played very significant roles in ensuring food security by developing new mutant germplasm and mutant varieties in China, which have generated tremendous social-economic impact. New mutagenesis approaches including space flight and heavy ion beam irradiation used as effective alternative new ways for crop genetic improvement was initiated in 1990s by Chinese scientists and great progresses have been made in the past 20 years. Protocols for crop mutation induction by space flight, energetic heavy ion beams have been established and applied for crop breeding. More than 950 mutant varieties with high-yielding, fine-quality and multi-resistant traits have been developed and officially released in main cereals, oil

and vegetable crops, and have been playing important roles in agricultural production. A number of rare mutant germplasms that may have a breakthrough effect on main economic traits such as yield and quality were also identified and applied for breeding program. New development of space and heavy ion beam breeding will be heavily based on and associated with not only effective use of space research platforms, but also advanced plant omics and molecular biology.

IAEA-CN-263-40

ESTABLISHMENT OF WHEAT-THINOPYRUMELONGATUM-7E CHROMOSOME TRANSLOCATION LINES WITH FUSARIUM HEAD BLIGHT RESISTANCE BY RADIATION

Shiqiang Chen, Xiulan Chen, Zhentian He

Lixiahe Region Agricultural Scientific Research Institute, China

Email: sqchen1116@163.com

Thinopyrum elongatum is one of the important relatives of wheat. It is favoured by many researchers for the disease resistant genes that exist in its E genome. Some studies have shown that the 7E chromosome of *T. elongatum* contains resistance genes related to Fusarium head blight (FHB). Therefore, establishment of wheat-*T. elongatum* 7E chromosome translocation lines with FHB resistance is very important. This study was conducted to establish (wheat) *Triticum* sp./*T. elongatum* 7E chromosome translocation lines from Yangmai16 × DS7E (7A or 7B or 7D) based on ⁶⁰Co radiation induced mutation. Then, we selected the translocation lines by screening for FHB resistance, *T. elongatum* 7E chromosome with specific molecular markers and genomic in situ hybridization (GISH). Finally, we obtained the wheat/*T. elongatum* 7E chromosome translocation lines with FHB resistance and found through further study, that the FHB resistance gene(s) might be located on the long arm of chromosome 7E of diploid *T. elongatum*. The establishment of wheat /*T. elongatum* 7E chromosome translocation lines with FHB resistance will provide important resistant genes and genetic stocks for wheat breeding.

IAEA-CN-263-155

DEVELOPMENT OF CLIMATE CHANGE ADAPTABLE/RESILIENT CROP VARIETIES THROUGH NUCLEAR TECHNIQUES

Mohammad Abul Kalam Azad, Fahmina Yasmine, Hosne Ara Begum, Md. Hassanuzzaman Rani, Md. Kamruzzaman

Bangladesh Institute of Nuclear Agriculture, Bangladesh

Email: makazad.pbdbina@gmail.com

To support rice agriculture, withstand the climate change driven problems like salinity, drought and extreme temperatures and maintain or increase crop production Bangladesh Institute of Nuclear Agriculture (BINA) released a late Boro rice variety, 'Binadhan-14' in 2013 which is tolerant to high temperature (38-40°C), has short duration to maturity (105-125 days), saves 20% irrigation water and gives high yield (average yield is 6.9ton/ha). This variety was developed by irradiating the seeds of Ashfal, a local salt tolerant land race of rice, with 200Gy dose of carbon ion beams. The late transplanting potential of this variety helps avoiding seedling injury due to severe cool temperature. Another variety, 'Binadhan-19' was developed by irradiating the seeds of NERICA-10 rice with 40Gy dose of carbon ion beams. This was released by the National Seed Board of Bangladesh (NSB) in 2017 as a drought tolerant, short duration cycle (95-105 days) and high yielding (average 4.0ton/ha in Aus season and 5.16 ton/ha in Aman season) variety. BINA also developed a salt tolerant wheat variety, 'Binagom 1' by selecting segregating population, collected from NIAB, Pakistan. This variety was released in 2016 and

can tolerate 12dS/m salinity and produces average yield of 2.8ton/ha, which equals the average yield of wheat in Bangladesh. Apart from these, BINA developed four salt tolerant groundnut varieties 'Binachinabadam-5', 'Binachinabadam-6', 'Binachinabadam-7' and 'Binachinabadam-9' by irradiation with gamma rays. All these four varieties can tolerate 8 dS/m salinity during flowering to maturity stages and give 1.8 to 3.4ton/ha pod yields under saline soil conditions. The average yield of groundnut in Bangladesh is 1.6ton/ha. All these varieties are playing significant role in food security and enhancing nutritional status of common people of Bangladesh.

IAEA-CN-263-78

ANTHRACNOSE RESISTANCE INDUCTION IN CHILI BY ELECTRON BEAM IRRADIATION

Vichai Puripunyanich¹, Penjan Sutthanukul², Prartana Kewsuwan¹

¹R&D Division, Thailand Institute of Nuclear Technology, Thailand

²Sukhothai Horticultural Research Center, Thailand

Email: vichaiपुरि@gmail.com

Chili seeds were irradiated with suitable 0.3 kGy dose at energy level 8 MeV electron beam. M₁ seeds were planted in Sukhothai Horticultural Research Centre and found that CA no.1131 line was suitable for growing in this area. Thirty chili anthracnose resistant plants were selected after anthracnose appearance in Sukhothai province caused by fungi (*Colletotrichum gloeosporioides*). Chili fruits from seventeen plants showed resistance after inoculation experiment in the laboratory. Selected chili lines were crossed with Hoarue Huaisai (F1 hybrid). The F₂ progenies were selected for anthracnose resistance plants with healthy and big sized fruits. Two hybrids with anthracnose resistance chili fruits (CA1131 × Hoarue Huaisai) were discovered and used for field anthracnose resistance tests in 2015. Inbred line no. 6-1-4 which had hybrid vigour was discovered in dry season. However, this line did not show strong disease resistance in the rainy season. Meanwhile, the other 63 inbred lines showed anthracnose resistance in the experiment field. Five samples/line of each of the 63 inbred lines were inoculated in the laboratory at Thailand Institute of Nuclear Technology. The fruits of inbred line no. 32-2-8 showed complete anthracnose resistance and seven lines were partial resistant. All the eight lines are being used in the ongoing chili project aimed at developing chili varieties with horizontal resistance to all three-anthracnose causing *Colletotrichum* species prevalent in Thailand.

IAEA-CN-263-209

INDUCED MUTAGENESIS FOR BEAN (*PHASEOLUS VULGARIS* L.) PRODUCTION IMPROVEMENT IN BULGARIA

Nasya Tomlekova¹, Fatma Sarsu², Svetla Sofkova-Bobcheva³, Yordan Muhovski⁴

¹Maritsa Vegetable Crops Research Institute, Bulgaria

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

³School of Agriculture and Environment, New Zealand

⁴Département Sciences du vivant Centre wallon de Recherches Agronomiques, Belgium

Email: nasia.tomlekova@gmail.com

Although historically a surplus food producer, Bulgarian agriculture has faced a downturn in recent decades. Local legume cultivars have lost favour with farmers and the canning industry, due to their low productivity in comparison with the imported ones. Diseases and abiotic stresses are the most important factors limiting the production of edible legumes, costing farmers hundreds of euros in lost

revenue each year. The overall objective of our ongoing bean mutation breeding programme was to enrich the gene pool of *P. vulgaris* L. and to develop genotypes resistant to *Xanthomonas campestris* pv. phaseoli (Smith) Dye and *Pseudomonas syringae* pv. phaseolicola (Gardan) using EMS. The two most common varieties (an heirloom and a snap bean type) in Bulgaria were selected as parents, and their sensitivity to the chemical mutagen EMS was explored. After determination of LD50, 1000 seeds were treated and reproduced in the field in M₁ population. All M₂ mutant plants were grown in field conditions, a number of phenotypic changes were observed on these mutated plants, which were also screened for disease resistance via leaf artificial inoculation. Individual plant selection was performed for the putatively resistant ones. In M₃ generation these lines were screened using artificial inoculation with pathogens (leaves and pods) under field conditions. The expression patterns of genes putatively involved in the resistance reactions towards two races of casual *P. syringae* were determined using qRT-PCR for the specific and reference genes. Selected M₃ M₄ lines with confirmed disease resistance were tested for fresh pod quality. Yield tests were started in M₄ and M₅, according to their yield results. Mutants are being advanced to M₆ or M₇ generation for validation. In conclusion, plants with visible morphological changes and/or increased tolerance to the two targeted bacterial diseases were selected. Mutant lines for beans are in the pipeline, which will boast competitiveness in international markets.

IAEA-CN-263-140

MUTATION INDUCTION IN IMPROVING RESISTANCE TO DOWNY MILDEW IN QUINOA (*CHENOPODIUM QUINOA*)

Luz Gomez-Pando

Universidad Nacional Agraria La Molina, Peru

Email: luzgomez@lamolina.edu.pe

Quinoa is a species native to South America. It is an important crop due to its nutritional characteristics superior to other food crops and its high tolerance to abiotic factors, it is also capable of growing in a wide range of altitudes and annual rainfalls ranging from 200 to 1000 mm even with prolonged periods of drought. This ability to adapt to marginal conditions makes it a crop alternative to face adverse climate changes. However, its development is limited by a number of factors such as its high susceptibility to pests, highlighting among them the disease downy mildew caused by the fungus *Peronospora variabilis*. The genetic control of downy mildew can eliminate or significantly reduce the use of fungicides and maintain the organic quality of the national production of great importance for the national and international market. There is evidence in many species that the induction of mutations can induce mutant lines in valuable commercial varieties; those that in addition to having resistance must maintain their potential for performance and quality. Seeds of quinoa cultivar Amarilla Marangani M₃ generation irradiated with gamma ray were used and near to 420 000 seedlings were established. Considering the severity of the disease, a group of mutant plants were identified with resistance to downy mildew; 735 plants in the dose of 150Gy and 701 in the dose of 250Gy with a frequency of 0.82% and 0.82%; respectively.

BARLEY MUTANT IN HVSNAC1 (STRESS-RESPONSIVE NAC 1) GENERATED THROUGH TILLING STRATEGY SHOWS INCREASED TOLERANCE TO DROUGHT AND OXIDATIVE STRESS

Marzena Kurowska, Agata Daszkowska-Golec, Beata Chmielewska, Dorota Swiergolik, Iwona Szarejko, Janusz Jelonek, Justyna Zbieszczczyk, Magdalena Rynkiewicz, Sabina Lip
University of Silesia, Poland
Email: marzena.kurowska@us.edu.pl

SNAC1 is a member of NAC transcription factor family involved in the response to abiotic stresses, including drought and hydrogen peroxide homeostasis. TILLING (Targeting Induced Local Lesion IN Genomes) strategy is an efficient reverse genetics tool for generating new alleles in genes of interest. In the presented study the TILLING approach was used for functional analysis of HvSNAC1 gene in response to drought and oxidative stress in barley. We used the HorTILLUS platform developed at the University of Silesia in Katowice, Poland, for identification of mutations in the HvSNAC1 gene. One of the mutants, *hvsnac1.c*, carrying a missense change, was back-crossed to the wild type (WT) parent cultivar ‘Sebastian’ and used for physiological and molecular studies. The response of *hvsnac1.c* to drought, salinity and oxidative stress treatment was evaluated at seedling stage. The expression of HvSNAC1 target genes was analysed in mutant and WT under control and stress conditions. The *hvsnac1.c* mutant carries a C90G substitution that led to change of a highly conserved amino-acid residue aspartic acid-30 to glutamic acid. The mutant exhibited a higher relative water content (RWC) than the WT, after severe drought stress as well as after a period of re-watering. The *hvsnac1.c* showed also the lower sensitivity to oxidative stress induced by methyl viologen. The expression pattern of the HvSNAC1 gene and its two potential targets during drought using RT-qPCR technique lead to the conclusion that HvSRO1c (Similar to RDC One) and HvERD1 (Early Responsive to Drought 1) belong to the HvSNAC1 regulon in barley and may be involved in the drought stress response. Using TILLING mutant, we demonstrated an involvement of HvSNAC1 gene in response to drought and oxidative stress in barley. The *hvsnac1.c* mutant can be a valuable material for breeding purposes.

DEVELOPMENT OF FIRST KABULI TYPE CHICKPEA MUTANT VARIETY IN BANGLADESH

Shamsun Nahar Begum, Mirza Mofazzal Islam, Rigyan Gupta
Bangladesh Institute of Nuclear Agriculture, Bangladesh
Email: sbluna98@yahoo.com

Chickpea has a high yield potential, is nutritious importance and has diversified uses compared to other pulse crops. Mutation breeding program was undertaken at BINA with the aim to develop early maturing, large-seeded and high yielding variety(ies) of chickpea. Seeds of a popular chickpea variety, Binasola-2 were treated with different doses of gamma-rays 200, 300 and 400Gy. The treated seeds were grown in dose-wise for raising M₁ generation. M₂ seeds were collected from individual M₁ plants and subsequently grown in plant-progeny-rows in M₂ generation and onwards and made selections from M₂ generations. Five selected M₅ mutants including ‘CPM-kabuli’ mutant along with check varieties were entered in preliminary yield trials. Finally, the mutants were evaluated in advanced, zonal yield, and on-farm and on-station trials in the successive generations along with two check varieties. All the selected mutants were grown at different locations in Bangladesh to observe the yield and other potentiality. Performances of the mutants were evaluated under two management practices i.e., Research management and Farmers’ management practices. The mutant, ‘CPM-kabuli’ was found to be tolerant to root rot, botrytis gray-mold and showed also

higher tolerance to pod borer insect infestation than other mutants and check varieties. Main improved attributes of these mutants are cream seed coat colour which refers to 'kabuli' type, larger seed size and higher grain yield. The mutant 'CPM-kabuli' matures between 115 and 125 days, and is high yielding (1.7 ton/ha). Considering all these results, the bold Kabuli type chickpea mutant (CPM-kabuli) variety has been registered as 'Binasola-9' for commercial cultivation during 2017 for the farmers in dry prone areas in Bangladesh.

IAEA-CN-263-266

EVALUATION OF ADVANCED WHEAT MUTANT LINES FOR FOOD AND FEED QUALITY

Mirjana Jankulovska, Biljana Kuzmanovska, Dane Boshev, Ljupcho Jankuloski, Mile Markoski, Sonja Ivanovska

Faculty of Agricultural Sciences and Food-Skopje, the Former Yugoslav Republic of Macedonia

Email: mirjanajankulovska@yahoo.com

The main goals of the study were to evaluate agronomic performance of wheat mutant lines; to detect the effect of genotype, location and different fertilizer level on analysed traits; to assess seed and feed quality and to select best performing mutant lines for dual purpose growing. 10 wheat mutant lines were sown on two locations in Macedonia, for evaluation of their agronomic performance. On both locations, grain yield, straw mass, harvest index, nitrogen use efficiency, nitrogen and protein content in seed and straw, nitrogen detergent fibre (NDF) and acid detergent fibre (ADF) in the straw were determined. In order to classify the genotypes based on all analysed traits, two-way cluster analysis was applied. According to their overall performance, at both locations and with the three different fertilization treatments, the mutant lines were classified in two main groups. The first cluster was consisted of mutants 5/1-8, 2/2-21, 4/2-56 and 2/1-5, characterized by very high values for seed yield, straw yield and HI, and high to moderate values for all other traits. Only 4/2-56 had very low values for N and protein content in the seed. One mutant line, 6/2-2, did not belong to any of the groups based on very low seed and straw yield and very high values for nitrogen and protein content in the straw and NDF. By these characteristics it differed from all other genotypes. All other mutants belonged to the second group, with low to moderate yield and moderate to high values for the other traits. Mutant lines with the highest seed and straw yield, as well as the best quality of seed and straw under different management systems were identified and after additional evaluation will be submitted for official variety registration.

IAEA-CN-263-142

INDUCED VARIATION FOR POST-EMERGENCE HERBICIDE TOLERANCE IN LENTIL

Sarvjeet Singh¹, RK Gill¹, Sheetal Raj Sharma¹, Shiv Kumar²

¹*Punjab Agricultural University, India*

²*International Center for Agricultural Research in Dry Areas, Morocco*

Email: sarvjeetm@rediffmail.com

Lentil (*Lens culinaris* L. Medik.) is an important food legume for the cool season, but is a poor competitor to weeds because of a slow growth rate in the early stage of development. If weeds are left uncontrolled they can reduce yield up to 50%. Sensitivity of lentil to post-emergence herbicides warrants development of herbicide tolerant cultivars. In the absence of natural variability, mutation breeding is a powerful tool to create variability for desired traits. Thus, 1000 seeds of the lentil genotype: LL1203 were exposed to gamma radiation at 300Gy with the objective to induce herbicide tolerance. Seeds of all 530 surviving M₁ plants were harvested individually and divided in two parts to raise M₂ generation in two different plots. Each plot was sprayed with imazethapyr (@75g ha⁻¹) and metribuzin (@250g ha⁻¹) herbicides 50 days after sowing using 375 L ha⁻¹ of water. Data on herbicide tolerance for individual M₂ plants were recorded after 14 days of herbicide spray on a 1-5 scale, where 1 = Highly

tolerant (plants free from chlorosis/wilting) and 5 = Highly sensitive (leaves/tender branches completely burnt). Data of putatively herbicide tolerant M₂ plants were also recorded for pod and yield plant⁻¹. None of the M₂ plants showed a high level of tolerance to imazethapyr. However, 14 mutants having higher herbicide tolerance to metribuzin were isolated. Two mutants (LL1203-MM10, LL1203-MM7) were recorded with a score <2.0, while six mutants had <2.50 score as compared to 3.13 score of parent variety. The pods per plant and seed yield per plant of mutants were 383 and 12.4g for LL1203-MM7 and 347 and 12.1g for LL1203-MM10, both were higher than the parent genotype LL1203 (253 and 7.8g). The study indicated that metribuzin tolerant mutants having some other desirable traits can be of use in lentil breeding.

IAEA-CN-263-163

APPLICATION OF MUTATION TECHNIQUES AND GENOTYPE X ENVIRONMENT INTERACTION FOR GRAIN YIELD AND YIELD COMPONENTS OF ION BEAM INDUCED MUTANT RICE LINES TESTED IN MULTIPLE LOCATIONS IN MALAYSIA

Mohd Raffi Yusop¹, Abd Rahim Harun², Asfaliza Ramli³, Ghazali Hussin³, Mohd Razi Ismail¹, Norhani Abdullah¹, Yusuff Oladosu¹

¹*Universiti Putra Malaysia, Malaysia*

²*Malaysian Nuclear Agency, Malaysia*

³*Malaysian Agricultural Research and Development Institute, Malaysia*

Email: mraffii@upm.edu.my

Genotypes evaluation for stability and high yielding in rice is an important factor for sustainable rice production and food security. These evaluations are essential, especially when the objective of the breeding program is to select rice with high yield adaptability and stability. Based on this background, this work was carried out to evaluate and select high yielding rice genotype induced by ion beam irradiation. Rice variety of MR219 seeds were subjected to 0, 10, 20, 40, 60, 80, 100, 120, 160, and 200Gy to determine the optimum doses to produce high mutant frequency and spectrum. After several series of selection and fixation, 15 potential lines with the required adaptive traits were recovered at M₆ generation during the 2009-2012 seasons (M₀-M₆). The selected mutant lines were then tested in five locations in two planting seasons to select high yielding and stable genotype in a randomized complete block design with three replications across the environments. The pooled analysis of variance revealed highly significant differences among genotypes, locations, seasons, and genotypes by environment (G × E interaction) for all the traits. Based on univariate and multivariate stability parameters, rice genotypes were classified into three main categories. First group were genotypes having high stability along with high yield per hectare. These genotypes include ML4 and ML6 and are widely adapted to diverse environmental conditions. The second group is genotype that exhibited high yield per hectare but low stability and, this genotype (ML9) is suitable for specific environments. The last group is genotypes with low yield per hectare and high stability which includes genotypes MR220, Binadhan4, and Binadhan7. The last genotypes group is more suitable for breeding specific traits or perhaps has yield component compensation. Rice mutant ML4 and ML6 were recommended for commercial cultivation in Malaysia.

THE SUCCESS OF IMI TOLERANT RICE VARIETIES IN LATIN AMERICA

Alberto Blas Livore¹, Alejandra Landau², **Alberto Raul Prina**²

¹*EEA Concepcion del Uruguay INTA, Uruguay*

²*Instituto de Genetica E.A. Favret, CICVyA-INTA, Argentina*

Email: landau.alejandra@inta.gob.ar

The Institute of Genetics “Ewald A. Favret” (IGEAF) INTA, has a long tradition in Plant Mutation Research that began in the year 1949. The IGEAF has made important contributions about the effects of different physical and chemical mutagens and mutator genes on barley and described novel uses of barley and wheat mutants in basic research and breeding. Besides, it has done a pioneering work through the interaction with public and private breeding programs to use mutation induction techniques for improving many different crops, like sunflower, sorghum, lemon and orange. The first real success of an induced mutant in Argentina corresponded to the peanut mutant cv Colorado irradiado, which was released by INTA in 1972, reaching some years later a maximum cultivated area of 262 000 ha. The most important impact was more recently achieved by the INTA Rice Breeding Program when looking for mutants having tolerance to imidazolinone (IMI) herbicides to better fight the infestation by weedy red rice, the most harmful weed of this crop worldwide. Through direct selection on M₂ plants derived from sodium azide seed mutagenesis treatments, five different alleles of the acetohydroxyacid synthase (AHAS) gene conferring IMI tolerance were isolated. The allele ala122thr was used to develop several IMI tolerant varieties that in 2016/2017 covered 40% of the rice area in Argentina. Furthermore, they were disseminated in other Latin American countries where rice integrates the daily diet of most families. They covered 70% of the irrigated area in the region, Brazil being the main producer where they were grown in 834 000 ha. Moreover, some varieties carrying this allele have been recently liberated in Italy, Greece, Romania and Portugal. The IMI tolerant rice varieties have contributed to increase productivity and quality, and have been also very useful to recover rice fields abandoned for being severally invaded by red rice.

IAEA-CN-263-84

THE BARLEY CHLOROPLAST MUTATOR (CPM) MUTANT IS AN EXTRAORDINARY SOURCE OF PLASTOME VARIABILITY

Alejandra Landau, Alberto Prina, Franco Lencina, M. Elizabeth Petterson, M. Gabriela Pacheco, Susana Costoya, Vanina Brizuela

Instituto de Genetica, Instituto Nacional de Tecnologia Agropecuaria, Argentina

Email: landau.alejandra@inta.gob.ar

The plastome is usually considered a highly conserved genome. Its current variability is scarce and artificial induction of its variation is not yet sufficiently exploited. Compared to the nuclear genome, it is small and behaves under different genetic rules. Plastome genes have been little used in breeding and their functionalities have been difficult to investigate. Through different molecular methods (TILLING, candidate genes sequencing, amplicons massive sequencing and plastome re-sequencing) applied on barley chloroplast mutator (cpm) seedlings, we detected more than 60 polymorphisms affecting a wide variety of plastid genes and several intergenic regions. The genes affected belonged mostly to the plastid genetic machinery and the photosynthetic apparatus, but there were also genes like matK, whose functions are so far not clearly established. Among mutants, we found the first infA gene mutant in higher plants, two mutants in ycf3 locus and the first psbA gene mutant in barley. The latter is used at present to breed barley cultivars tolerant to PSII toxic herbicides. Most of the molecular changes were substitutions, and small indels located in microsatellites, which apparently originated in mutations. However, particular combinations of polymorphisms observed in rpl23 gene and pseudogene suggest

that, besides an increased rate of mutations, an augmented rate of illegitimate recombination also occurred. Although a few substitutions were so far observed in the mitochondria of cpm plants, we have not yet determined the implications of the cpm in mitochondria stability. The spectrum of plastome polymorphisms highly suggests that the cpm gene is involved in plastid DNA repair, more precisely taking part of the mismatch repair system. All results show the cpm mutant as an extraordinary source of plastome variability for plant research and/or plant breeding. Besides, it looks as an interesting experimental material to investigate the mechanisms responsible for maintaining plastid stability.

IAEA-CN-263-52

A LARGE CAPACITY WHEAT RESOURCE WITH BROAD MUTATION SPECTRUM AND NOVEL ALLELE IDENTIFICATION BY TILLING APPROACH

Huijun Guo¹, Hongchun Xiong², Jiayu Gu¹, Linshu Zhao¹, Shirong Zhao¹, Yongdun Xie³, Luxiang@caas.cn Liu¹

¹*Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, China*

³*Space Breeding Centre, Institute of Crop Sciences, CAAS, China*

³*Institute of Crop Science, China*

Email: guohuijun@caas.cn

Henan province is located in central China. It is the core wheat production area with the largest wheat cultivated area, single yield and total production and the highest quantity of export commodity grain, with 5 million hectares, accounting for about 22% of the country's wheat area, the average yield about 6150 kg/hm², the production more than 65 million kilograms, accounting for 25% of the country. By the end of 2017, Henan province wheat breeding units using mutagenesis technology had bred 21 new mutant varieties of wheat. These varieties have made great contribution to the production of wheat in Henan, of which Taikong 5, Zhengmai 3596, Fumai 2008, Yufeng 11 and Zhengpinmai 8 realized the effective combination of high yield and high quality. The institute of isotopes of HAAS is one of the earlier units of wheat mutation breeding in Henan province. By now, we have bred 6 new wheat mutant varieties including Yutong 843, Fumai 2008, Zhengpinmai 8, etc. In addition, 10 new wheat mutant lines are tested in Henan or national regional trials. At the same time, we have created a large number of dwarf, high-yield, high-quality characteristic wheat germplasm resources. Based on the independent innovation of common wheat cytoplasm background of wheat male sterile line (89AR), we have begun to study the hybrid wheat seed production system. We have now bred some new sterile lines, screening a number of excellent maintainer line, breeding a little of new restorer lines. One excellent restorer line (recovery degree > 99%) was found by measuring the offspring fertility analysis, and it was preliminarily considered that the restorability was controlled by 3 pairs of major genes. This study will provide a new approach for the utilization of hybrid wheat in the world.

**GAMMARAYSAPPLICATIONINTHEDEVELOPMENTOF RICE LINESTOLERANTTO
ARYLOXYPHENOXYPROPIONATE HERBICIDES**

Rubens Marschalek², Adriana Pereira¹, Antonio Mendes De Oliveira Neto³, Augusto Tulman-Neto⁴,
Fernando Adami Tcacenco¹, José Alberto Noldin¹, Alexander De Andrade¹

¹EPAGRI, Brazil

²IFSC, Brazil

³USP, Brazil

⁴UNIVALE, Brasil

Email: alexanderandrade@epagri.sc.gov.br

The aryloxyphenoxypropionate herbicides (APPs) are graminicides with excellent control of many grass weeds species, including weedy rice (*Oryza sativa* L.). These herbicides block the fatty acid biosynthesis by inhibition of the enzyme acetyl-CoA carboxylase (ACCase), and cause the death of the plant. Inducing mutation by applying gamma rays to seeds, two rice lines resistant to APPs herbicides were developed. Plant dose-response assays confirmed the resistance to the APPS herbicides quizalofop-p-ethyl and haloxyfop-p-methyl. The carboxyl-transferase (CT) domain fragments of ACCase from the resistant biotype and susceptible control were sequenced and compared. A point mutation was detected in the amino acid position 2027 (Rice Genome Annotation Project: Os05g22940.1). Results indicated that resistance to APPs is a consequence of an altered ACCase enzyme that confers resistance. The use of APPs herbicide resistant rice lines represents an innovative and promising alternative for weedy-rice control in paddy rice systems.

PLENARY SESSION - PARALLEL SESSION

IAEA Boardroom B/M2

M Building

Wednesday, 29 August 2018

SOMACLONAL VARIATION: CONTAINING THE BAD WHILE EXPLORING THE GOOD

Nicolas Roux¹, Julie Sardos¹, Mathieu Rouard¹, Rachel Chase¹, Sirena Montavo-Katz¹,

Van den Houwe Ines¹Xavier Perrier²

¹BIOVERSITY International, France

²CIRAD, France

Email: n.roux@cgiar.org

Somaclonal variation describes random cellular changes in plants regenerated through tissue culture. It occurs in certain crops that undergo micropropagation, and has been recorded in different explant sources, from leaves and shoots, to meristems and embryos. In banana (*Musa* spp.), a clonal crop conserved *in vitro*, somaclonal variation has been observed after prolonged periods in tissue culture, resulting from an increase in subcultures performed on a given clone. According to scientific literature variants, or off-types, often show characteristics such as abnormal growth and flower or fruit defects, in frequencies ranging from 1% to 32%. This variation poses a problem for gene bank managers, whose mandate is to maintain the genetic integrity of their collections for research and breeding. In the case of the BIOVERSITY International Musa Germplasm Transit Centre (ITC), stress during the *in vitro* process is minimized by various techniques and plants are regenerated after 10 years, making it a long and costly process. Identifying somaclonal variation at an early stage would be an ideal solution; however, this requires suitable molecular markers. Recent studies revealed that techniques such as direct DNA sequencing and single nucleotide polymorphisms (SNPs) are able to detect the underlying factors of somaclonal variation, and are becoming more accessible. On the other hand, somaclonal variation can be beneficial as it allows the natural development of new varieties and supplies genetic stocks used for future genetic studies. Harnessing the diversity of somaclones is easier, faster and cheaper compared to other methods of crop improvement, although it is also less predictable. So far, variants of crops such as apple, strawberry, potato and banana have been successfully adopted in global markets. In this presentation, we will discuss how to minimize the adverse effects of somaclonal variation while maximizing its benefits for greater crop diversity, with a particular focus on banana.

EMS INDUCED *IN VITRO* MUTAGENESIS FOR ABIOTIC AND BIOTIC STRESS TOLERANCE, AND ISOLATION OF MORPHOLOGICALLY AND BIOCHEMICALLY DISTINCT PHENOTYPES IN SUGARCANE

Sunil Dalvi¹, D Theertha Prasad¹, Ghanshyam Dixit¹, P Suprasanna², Pralhad Tawar³

¹Botany Dept. Shivaji University, India

²FB&SP, NABTD, BARC, India

³Tissue Culture Section, Vasantdada Sugar Institute, India

Email: sgdalvi@gmail.com

Chemical mutagenesis was applied in sugarcane to improve desirable characters. In this study, young inflorescence explants of Indian sugarcane cultivar CoC 671 were exposed to Ethyl Methyl Sulphonate (EMS) along with 0.1% polyethylene glycol used in callus induction media. Three hundred shoots were regenerated and transplanted in the field for selecting desired mutants. Eleven mutants were selected and evaluated for smut disease resistance and drought tolerance along with early maturity, high sucrose, higher cane yield and for quality. The PCR based early detection assay for smut resistance with Inter-transcribed region sequence (ITS) indicated smut resistance in the selections. Smut resistance was also confirmed by artificial smut inoculation. The mutants were found superior for juice and sugar quality parameters such as higher Brix %, Sucrose %, Purity % as well as for commercial Sugar cane. Screening of mutants with stress tolerance indicators indicated higher

proline, malondialdehyde and electrolyte leakage in TC 2819 and TC 2813 indicating their improved drought tolerance capacity as compared to CoC 671. Mutant TC 2819 was found significantly superior for sucrose content (20.33%) than parent CoC 671 (17.67%) at early maturity (10th month), while mutant TC 2826 was smut resistant, and was significantly superior for sucrose content (23.97%) than parent CoC 671 (21.39%) at maturity (12th month). The morphological characterization also showed the distinct variation in their stem colour, bud shape, root bands and other characters. The study highlights potential application of EMS-induced genetic variability which can be utilized for direct cultivation or as better parents in sugarcane improvement.

IAEA-CN-263-4

IN VITRO MUTATION BREEDING AND SELECTION FOR RESISTANCE TO FUSARIUM WILT IN BANANA

Behnam Naserian Khiabani, Cyrius Vedadi, Hamideh Afsharmanesh

Nuclear Agricultural Research School-Nuclear Science & Technology Research Institute, Iran

Email: bnaserian@gmail.com

Fusarium wilt, caused by the fungus *Fusarium oxysporum*, is one of the most serious diseases of banana in tropical and subtropical countries. Most research focuses on generating cultivars tolerant/resistant to this disease. Although there are banana cultivars resistant to the disease, transfer of the resistance trait to susceptible cultivars is difficult by traditional cross-breeding. Thus, radiation breeding could prove to be a viable method in banana breeding work. In this research mutation induction in Banana was carried out by shoot tips exposing to gamma ray. Then three times subculture was done. Rooted Banana plantlets in the modified MS medium (1/2 MS salt + 10g l⁻¹ sucrose + 4 g l⁻¹ phytagel) inoculated by *Fusarium oxysporum*. For inoculation, a filter paper disk (5mm diameter) soaked in the suspension containing 10⁶ conidia per ml. Then each plantlet was inoculated by putting one disc on the surface of modified MS medium. Plantlet 7-24 days after inoculation showed disease symptoms such as yellowing, wilting and necrosis. Disease severity rated on a scale of 0 to 6, where score 0 referred to plantlets that were completely clear and without symptoms and scored 6 for those plantlets that were completely wilted. About 6000 mutant plantlets evaluated and scored. All mutant plantlets with a score of 0, 1 and 2 were selected. Selected plantlets transferred to the pot. Our result showed that combination of mutation induction and effective *in vitro* selection methods appears to be the most efficient strategy for improving tolerance to fusarium wilt in banana.

IAEA-CN-263-34

INDUCED MUTATIONS TECHNOLOGICAL ADVANCEMENT FOR DEVELOPMENT OF NEW ORNAMENTAL VARIETIES

Subodh Kumar Datta

Retd. Scientist 'G', C.S.I.R.-N.B.R.I., Assam University, India

Email: subodhskdatta@rediffmail.com

The concept of induced mutation for crop improvement development dates, back to the beginning of the 20th century. Induced mutations have played a major role in the development of superior crop varieties. Here, an overview on past, present and future of induced mutations in ornamental crops will be provided, based on 36 years practical experience and 79 released mutant ornamental varieties. In commercial floriculture there is always demand for new varieties. Mutation has been very successfully utilized in ornamental crops since the effects of mutagens are very visible selection for changed flower colour/shape/size is easy, and almost anything, which is novel, is of value. Many cultivars are heterozygous and mutation frequency is very high. Voluminous literature have been generated on ornamental crops using classical and *in vitro* techniques on different aspects like radio-sensitivity, selection of material, exposure to mutagens, suitable dose of mutagens, combined treatment, recurrent

irradiation, acute and chronic irradiations, ion beam technology, colchicine-mutation, detection of mutation, nature of chimerism, classical and modern methods for management of chimera, *in vitro* mutagenesis, directive mutation, isolation of mutants etc. Step wise advancement/refinement of practical approaches for application of classical and *in vitro* induced mutation techniques will be highlighted for improvement of ornamental crops with special emphasis on interesting mutants with changed flower type, appendage like structure on florets, striped flowers, tubular florets, late/early blooming varieties along with management of chimera. Voluminous knowledge generated on ornamental crops will work as a model system to prepare guide lines for future planning of successful and accurate application of mutation technique in crop improvement programme.

IAEA-CN-263-3

RADIOSENSITIVITY AND PRELIMINARY RESULTS IN MUTATION BREEDING OF 'AMASAYA' APPLE CULTIVAR

Ayşe Nilgün Atay¹, Burak Kunter², Ersin Atay¹, Kadriye Yaprak Kantoğlu², Nejdet Kaplan³

¹*Fruit Research Institute, Egirdir, Turkey*

²*Turkish Atomic Energy Authority, Turkey*

³*General Directorate of Plant Production, Turkey*

Email: nilguntuncer@hotmail.com

Mutation breeding technique is an efficient way to create new genotypes. Gamma-ray induced mutation breeding of apple commenced at the Egirdir Fruit Research Institute in 2011 corresponding to research project TAGEM/BBAD/16/A08/P03/03, collaborated with Turkish Atomic Energy Authority. The aim of the project was to generate variability in 'Amasya' by gamma irradiation and to evaluate performance of the mutants for different morpho-agronomic characteristics. In order to determine Effective Mutation Dose (EMD), seven doses (0, 10, 20, 30, 40, 50, 60Gy) were applied to dormant buds in gamma irradiator, ⁶⁰Co. Irradiated buds were budded onto M9 rootstock by chip budding in a day after irradiation. The EMD dose was calculated 29,01Gy for 'Amasya', in relation to reduced shoot length by 50% compare to control group. In 2012, approximately 2000 dormant 'Amasya' scions were irradiated at EMD. Physiological defects were observed M₁V₁ generation. Genetic uniformity was checked through the growth of the mutant population (M₁V₂-M₁V₄), calculated mutation frequency (%) and preliminary evaluation of the mutants was taken up. A high number of plants were presented that were lower plant height and trunk cross-sectional area compared to non-irradiated control plants.

IAEA-CN-263-152

INDUCTION AND SELECTION OF MANDARIN MUTANTS WITH FRUITS CONTAINING LOW NUMBER OF SEEDS

Rose Mary Pio¹, Augusto Tulmann Neto², Paulo Sérgio Souza³, **Rodrigo Rocha Latado**¹, Vera Lucia N.P. Barros⁴

¹*Centro de Citricultura Sylvio Moreira / IAC, Brazil*

²*CENA/USP, Brazil*

³*IF-Suldeminas, Brazil*

⁴*APTA Sudoeste Paulista, Brazil*

Email: rodrigo@ccsm.br, rlatado@gmail.com

The Brazilian citrus industry has a worldwide presence for production and exportation of sweet orange juice but it has little expression in production of fruits for fresh fruit market. One requirement of this market is the production of seedless fruits. The Fremont IAC 543 mandarin produces fruits with good commercial qualities and presents as an innovative feature, resistance to *Alternaria brown*

spot (ABS), an important disease presents in several countries. However, Fremont IAC 543 mandarin naturally produces fruits with large numbers of seeds (10-12), an undesirable characteristic. The objective of this work was to induce and select mutants of Fremont IAC 543 mandarin using induced mutagenesis (gamma-rays) to obtain seedless fruits or fruits with low number of seeds. The GR30 and GR50 doses for buds were defined as 29.9 and 49.9Gy of gamma-rays. After irradiation and grafting of 2000 buds with each mutagenic dose, nine (9) branches were selected during harvesting period because they produce seedless fruits. After vegetative propagation of these buds, mutation stability was evaluated in another experiment. Mutant clone (# 5) showed plants with good productivity and fruits containing one seed on average, whereas two other mutated clones (# 7 and # 9) had fruits with average of 3 seeds each. All nine mutant clones are in advanced agronomic evaluation experiments, with a greater number of replicates, in order to evaluate productivity, disease resistances and organoleptic quality of the fruits.

IAEA-CN-263-42

⁶⁰CO GAMMA IRRADIATION-INDUCED VARIATIONS IN VEGETATIVELY PROPAGATED *PHILODENDRON ERUBESCENS* 'GOLD'

Dayani Karunananda, Ramya Ranathunga, Wathsala Abeysinghe
Horticultural Crops Research and Development Institute, Sri Lanka
Email: dayani.karunananda@gmail.com

Philodendron erubescens 'Gold', a popular climber with brilliant greenish yellow leaves (D 151 in RHS colour chart), is used in indoor gardening and landscaping. It is commonly propagated through vegetative cuttings, thus incorporation of new traits through conventional breeding is impracticable. As commercial floriculture always demands novel varieties, this study was carried out to induce variations in *P. erubescens* 'Gold' leaves through mutagenesis using gamma ray irradiation. Rooted cuttings (n = 200) of *P. erubescens* 'Gold' were subjected to 70, 100, 150Gy gamma rays and recovered in a propagator. Survived shoots were transferred to pots. Regenerated shoots were multiplied vegetatively and 10 M₁ lines were maintained as M₁V₁-M₁V₁₀ up to 12 generations (V₁₂) to evaluate growth and morphological variations with their genetic stability. Of all 70 and 100Gy treated cuttings, 12 and 1% respectively, survived after 6 months. Most of the irradiated plants had lost the re generation ability except two M₁ plants, which also showed comparatively a reduced growth (1 leaf in 45 days). Only one regenerated M₁ plant showed morphological variations in leaves, which was multiplied and maintained as lines. Several variations including characteristics of leaves (shape, size, colour), stems (internodal length and branching) and plant stature were observed among M₁ lines and in subsequent vegetative generations. Leaves presented 3 different colour patches, however, neither the colour distribution pattern was uniform nor stable. M₁V₄ line shows the highest stability of colour distribution in leaves; the colour composition of leaves ranged as 0-10% dark bluish green, 60-90% strong yellow green and 10-30% brilliant greenish yellow throughout the 12 generations. This study demonstrates that, gamma irradiated *P. erubescens* 'Gold' line M₁₋₄ can be a promising mutant to develop as a new *Philodendron* cultivar.

PLENARY SESSION
IAEA Boardroom B/M1
M Building

Thursday, 30 August 2018

ION BEAM MUTAGENESIS AN INNOVATIVE AND EFFECTIVE METHOD FOR PLANT BREEDING AND GENE DISCOVERY

Tomoko Abe, Yoriko Hayashi, Ryouhei Morita, and Hiroyuki Ichida
Ion Beam Team, RIKEN, Center for Accelerator-Based Science, Japan
 Email: tomoabe@riken.jp

We have developed a unique technology for mutation induction of plants using energetic ion beams at the RI Beam Factory (RIBF) of RIKEN. Ion beams effectively induce mutations at relatively low doses without severely inhibiting growth. The irradiation treatment can be given to various plant materials and mutation can be induced in a short time between seconds and a few minutes. We have collaborated with companies and agricultural experiment stations on ion-beam-induced mutation breeding and generated 30 new cultivars including sterile Verbena ‘Temari Bright Pink’, new color flower of Petunia ‘Safinia Rose Veined’, tearless onion ‘Smile ball’ and short-culm Japanese barnyard millet ‘Nebari-ko No.2’. The linear energy transfer (LET) of ions depends on the species and velocity. For example, C ions provide LET values from 23 to 290 keV/μm in water. Since LET intensively affects the mutation frequency, it is an important parameter to determine the most effective irradiation condition in mutagenesis. According to our investigations, the most effective LET (LET_{max}) for mutation induction is 30 keV/μm in Arabidopsis and 23-50 keV/μm in rice, respectively. Subsequently, we have analyzed on the mutated DNA with morphological mutants. The most mutations were small deletions. Irradiation at LET_{max} is effective for plant breeding because of its very high mutation rate and sufficient energy to disrupt a single gene. We built a new beam line to increase available ion species with higher LET and longer range. We aim at advances in examination of the effects of physical factors (e.g., ion species, LET, and dose) on DNA-mutated regions with detection using whole genome sequencing, and elucidation of the mechanism of mutagenesis with ion beams. In addition, the combination of ion-beam induced mutants and genome sequencing technology may enable discovery of genes and thus lead to new field in biology, ‘mutagenomics’.

SOYBEAN NODULATION: FROM GENE MUTATION, VIA GENE ISOLATION TO FUNCTIONAL CIRCUITS

Peter M. Gresshoff, Brett J. Ferguson
Center for Integrative Legume Research, School of Agriculture and Food Sciences, University of Queensland, Australia
 Email: p.gresshoff@uq.edu.au

Legume plants (18 000 legume species exist) possess a complex root-developmental pathway, leading to nitrogen-fixing nodulation. Rhizobium bacteria induce cortical cell proliferation, then invade concurrently the newly developed tissue to differentiate into nitrogen-fixing bacteroids. This nodule development is of global significance for agriculture and ecology. We have used a variety of analytical approaches to understand one key component of this symbiosis, namely the legume-specific process of ‘Autoregulation of Nodulation’ (AON). The symbiosis (both nodule induction and nitrogen fixation) is inhibited by nitrate. We used induced EMS mutagenesis of soybean to isolate nitrate-tolerant symbiosis (nts) mutants. They lacked AON. The mutant trait was controlled by a single genetic element, shown to be active in the shoot. Thus, communication between root and shoot was assumed. We then developed complex DNA profiling and gene cloning techniques to first clone the mutated gene (2002). It encodes a LRR receptor kinase called GmNARK. *GmNARK* homologues are functional in other legumes such as pea, bean, *Medicago truncatula* (called SUNN) and *Lotus japonicus* (called HAR1). We then found interacting genes and signalling factors. GmNARK is modulated by GmKAPP (Kinase Associated

Protein Phosphatase). GmNARK is also the receptor of inoculation for nitrate-induced, root-derived RIC peptides. These are 11-12 amino acids long, and triarabinosylated at a central proline (P7). These are an excellent example of systemic communication. GmNARK regulates the synthesis of a microRNA (miR2111) in the shoot. This RNA is essential for nodulation, interacts with the *TML* (Too much love) gene. GmNARK, once activated by CLE peptide (either GmRIC1 or GmRIC2), suppresses the synthesis of miR2111, thus inhibiting further nodule induction. We thus understand the overall AON control circuit, its signal peptide and microRNA, and the receptor kinases and transcription factors. Most importantly the data suggest that similar control circuits exist for other processes in plants.

IAEA-CN-263-130

HIGH-RESOLUTION MAPPING OF WHEAT CHROMOSOME ARM 5AS HARBOURING THE FUSARIUM HEAD BLIGHT RESISTANCE QTL Qfhs.ifa-5A

Christian Wagner¹, Maria Buerstmayr¹, Petra Schwarz¹, Andrea Volante², Barbara Steiner¹, Delfina Barabaschi³, Giampiero Valè², Hermann Buerstmayr¹, Klaus Brugger¹, Lisa Blazek¹, Luigi Cattivelli³

¹*BOKU-University of Natural Resources and Life Sciences, Austria*

²*Council for Agricultural Research and Economics, Research Centre for Cereal and Industrial Crops, Vercelli, Italy*

³*Council for Agricultural Research and Economics, Genomics Research Centre, Fiorenzuola d'Arda, Italy*

Email: christian.wagner@boku.ac.at

Fusarium head blight (FHB) is a severe fungal disease of wheat affecting yield and grain quality. One major QTL for FHB resistance in wheat is Qfhs.ifa-5A, which is located on the centromeric region of the wheat chromosome arm 5A. Genetic mapping of Qfhs.ifa-5A resulted in a low resolution in the Qfhs.ifa-5A region: In a near isogenic RIL population, segregating for the Qfhs.ifa-5A resistance allele, four out of 3650 lines recombined within the pericentromeric C-5AS1-0.40 bin, yielding only a single recombination point. To further increase the map resolution a radiation induced deletion mapping approach was performed. Two gamma irradiated wheat deletion panels were created: (i) radiation selfing (RS) panel: seeds of line NIL3 carrying the Qfhs.ifa-5A resistance allele in a susceptible background were irradiated and plants thereof were selfed to obtain deletions in homozygous state and (ii) a radiation hybrid (RH) panel: irradiated pollen of the wheat line Chinese Spring (CS) was used for pollinating the CS-nullisomic5Atetrasomic5B. Overall, 5125 RS and 276 RH plants were pre-screened for deletions on 5AS. Plants having one or more markers deleted were analysed using 102 5AS-specific markers. A consensus map derived from both deletion panels results in a 380-fold map improvement (cR/cM) of the 5AS chromosome compared to the genetic mapping approach, with an average map resolution of 0.77 Mb/cR. This striking improvement in map resolution underlines the superiority of radiation induced deletion mapping over genetic linkage mapping for low recombining regions. Phenotyping the RS deletion lines can help to narrow down the QTL-interval for gene cloning.

WHOLE GENOME SEQUENCING OF RICE MUTANTS INDUCED BY GAMMA RAYS AND ION BEAMS

Feng Li, Akemi Shimizu, Hiroshi Kato, Sayaka Niwa
Radiation Breeding Division, Institute of Crop Science, NARO, Japan
 Email: ami@affrc.go.jp

Gamma rays have been used for several decades in mutation breeding as a physical mutagen. Though gamma ray irradiation has been used to induce many mutants, detailed characteristics of mutated DNA sequence have not been clarified enough. A newly introduced physical mutagen, ion beams, has attracted breeders' attention. We present the results of analysis of DNA mutation of rice mutants induced by gamma rays and ion beams using whole genome sequencing. *Oryza sativa* L. cv. Hitomebore was used for irradiation. Un-hulled dry seeds were irradiated with 250Gy gamma rays, at dose rate 10Gy/h. Hulled dry seeds were irradiated with 220 MeV carbon ions (LET 107KeV/ μ m), at dose rate 30Gy/h. Seven and eight mutants induced by gamma rays and ion beams were selected, by morphological traits, respectively. All DNA in leaves of mutants in M₄ or M₆ generation were extracted and used for DNA library construction and whole-genome sequencing. Paired-end (2 × 150 bp) sequencing was performed in Illumina X Ten to sequence the genome to about 30-fold depth for each sample. Averagely, 81 single base substitution (SBS), 23 deletions and 11 insertions were detected in each rice mutant induced by gamma rays. In contrast, 65 single SBS, 17 deletions and 10 insertions were detected in each mutant induced by ion beams. A t-test showed that the numbers of SBS and deletions induced by gamma rays were significantly higher than those induced by ion beams. The structure variation (SV) analysis averagely detected one SV (large deletion, inversion or duplication) in each mutant induced by ion beams while none in that by gamma rays. Gamma rays trend to induce more point mutations while ion beams induce more SVs, which might be due to notable difference in linear energy transfer (LET) value between these two types of radiations.

IAEA-CN-263-233

EXPLOITING MUTATIONS IN THE ERA OF GENOMICS AND GENOME ENGINEERING

Trude Schwarzacher, Pat Heslop-Harrison
University of Leicester, United Kingdom
 Email: ts32@le.ac.uk

Plant breeding relies on exploiting genetic variation present in crops and their wild relatives. Much natural variation is complex in its genetics and inheritance while induced mutations are generally simpler in their genetics, but usually associated with multiple deleterious characters. Key steps to using mutations include identification of phytotype and the gene (or marker) involved, selection and stable expression of the character. Many crop species are polyploids or hybrids, and the presence of multiple genomes can give advantages and disadvantages. Chromosome engineering can introduce novel traits from mutants and wild relatives into breeding programmes to increase resistance to biotic and abiotic stresses. Our research with use a variety of corps, their wild relatives and hybrids uses high throughput DNA sequencing and repeat finding algorithm to identify tandem repeats and transposable elements. The genomic variation and chromosomal organisation of repeats provide useful markers for identifying alien chromosome segments and diversity linked to mutations and economically important traits. In wheat, we have identified terminal and intercalary translocations of rye, *Aegilops ventri cosa*, *Secale cereale*, *Thinopyrum intermedium* and *T. bessarabicum* involving different recipient wheat chromosomes and transferring resistance to wheat streak mosaic virus and other diseases. Our Brassica and oats programmes exploit the wild relatives, while the banana and Ensete programme use the variation in different accessions of the species and hybrids. High throughput sequencing has

changed genome analysis from mapping and identifying genes, finding new repeats and DNA variation that can be used as markers to GBS (genotyping by sequencing). Our aim is to understand genome restructuring during hybridization and mutation events and to developing super-domestication strategies to exploit biodiversity for sustainable agriculture.

IAEA-CN-263-49

RAPID MAPPING AND CLONING THE CHLORINA MUTANT GENE (VN A1) IN WHEAT BY BULKED SEGREGANT ANALYSIS AND 660K SNP CHIP

Yanzhou Xie, Chengshe Wang
Northwest A&F University, China
Email: yanzhouxie@126.com

Wheat is one of the most widely grown crops in the world. Induced mutations have been used to generate mutant for genetic improvement and functional genomics in wheat. Recently, huge advances in wheat genome sequencing have contributed to the use of single-nucleotide polymorphism(SNP) in fine mapping and map-based cloning of mutant genes. A chlorina mutant in *Triticum aestivum* was obtained by treatment with the chemical mutagen sodium azide. Genetic analysis confirmed that the mutant phenotype was controlled by a recessive gene, which was designated as vn-A1. By applying bulked segregant analysis and Wheat 660K SNP chip, 8 KASP makers were developed. Molecular mapping showed that vn-A1 is located in a 1.1-cM genetic region flanking by KASP markers 660K-7A12 and 660K-7A20 corresponded to a physical interval of 3.48 Mb in the Chinese Spring chromosome 7AL containing 61 predicted genes with high confidence. Further analysis showed that a point mutation occurred in the AAA+ conserved region of Magnesium chelatase I subunit(CHLI) generated an amino acid substitution of Aspartic acid to Asparagine, which led to chlorine phenotype of the mutant. The approach using in this study provides a paradigm for the rapid mapping and cloning of the mutant genes underlying the genetic traits in wheat.

IAEA-CN-263-290

GENOMICS TOOLS TO FACILITATE PLANT MUTATION BREEDING

Bradley J. Till¹, Joanna Jankowicz-Cieslak¹, Owen Huynh¹, Bernhard Hofinger¹, Sneha Datta¹, Andrea Tramontano¹, Luka Jarc¹, Stephan Nielen¹, Brian Forster², Chikelu Mba and Ivan Ingelbrecht¹
¹International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria
²Verdant Bioscience Pte Ltd, United Kingdom
³Food and Agriculture Organization of the United Nations, FAO, Italy
Email: till.brad@gmail.com

Continual genetic improvement of crops is necessary for global food security and climate change adaptation. The use of induced mutations has for more than 80 years been a powerful approach for generating novel genetic variation for breeding. Genomics tools, especially next generation sequencing, have advanced sufficiently in the past decade to consider their routine use in plant mutation breeding. While new technologies hold great promise, the challenge is adapting and applying them in a global context to maximize impact in the field. In terms of induced variation using chemical or physical methods, the density and spectrum of mutant alleles, and mode of propagation of the crop are important considerations for experimental design of a mutation breeding programme. This presentation will highlight collaborative research led by the Plant Breeding and Genetics Laboratory to develop and adapt genomics tools aimed at facilitating mutation breeding projects in the FAO and IAEA Member States. The focus has been on using open-source approaches while keeping assay costs as low as possible. This

includes amplicon and whole-genome sequencing methods for the discovery and use of natural and induced genetic variation. Applications and future prospects for seed and vegetatively propagated crops will be presented.

IAEA-CN-263-0

USING THE IONIZING RADIATION FOR IMPROVING THE DEVELOPMENT AND YIELD OF AGRICULTURAL CROPS

Stanislav Geras'kin, Polina Volkova; Roman Churyukin; Sofiya Bitarishvili
Russian Institute of Radiology and Agroecology, Russian Federation
Email: stgeraskin@gmail.com

Agricultural production is confronted with multifaceted challenges from global climate change. There is now an overwhelming evidence that “business as usual” for crop development will be insufficient to adapt crops to meet the expanding global agricultural demand. Irradiation of seeds can increase field germination, stimulate growth and development of the plants, as well as increase their resistance to unfavourable environmental conditions. In our experiments it was shown that increased size of seedlings after irradiation with stimulating doses was due to the enhancing pace of development rather than an earlier germination. In a study of the activity of the enzymes involved, most of the enzymes showed increased activity in the range of doses that cause stimulation of seedlings development. It was shown that phytohormones were actively involved in the acceleration of the plant development after irradiation of the seeds within the stimulating dose range. Moreover, γ irradiation of barley seeds significantly influenced the development of the plants during the growing season. After irradiation with stimulating doses during the 4-year field study we observed a reduction in the duration of the initial stages of ontogeny; the phase of full ripeness started on 5-7 days earlier than in the controls. The manifestation of the effect of irradiation depends on the conditions in which the plants develop. During the dry 2014 season plants originated from the irradiated seeds showed an increase in the number of productive stems, which led to an increase in yield by 34-38%; during optimal 2015 season an increase in the number of grains per spike caused an increase in yield by 8-29%. Therefore, our field study has shown that at least some hormetic effects can occur in the field with sufficient regularity and predictability for a practical use. A more complete understanding of underlying mechanisms of hormesis is needed to exploit its potential benefits in crop production.

IAEA-CN-263-244

IMPACT OF CROSS-BREEDING ON THE METABOLITE SIGNATURE RESULTING FROM THE OsSULTR3;3 MUTATION IN LOW PHYTIC ACID RICE SEEDS

Chenguang Zhou¹, Karl-Heinz Engel¹, Qingyao Shu², Sophia Goßner¹, Yuanyuan Tan²
¹*Technical University of Munich, Chair of General Food Technology, Germany*
²*Zhejiang University, State Key Laboratory of Rice Biology and Zhejiang Provincial Key Laboratory of Plant Germplasm, China*
Email: chenguang.zhou@wzw.tum.de

Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate), the major storage form of phosphorus in cereals, is considered as an anti-nutrient in food and feed. During the past years, various cereals have been subjected to mutation breeding for generating low phytic acid (lpa) crops. Recently, it was demonstrated that reduction of phytic acid in the rice mutant MH86-lpa obtained by γ -irradiation was due to a disruption of OsSULTR3;3, an ortholog of the sulphate transporter family group 3 genes. The application of a GC/MS-based metabolite profiling approach revealed that the reduction of phytic acid was accompanied by changes in concentrations of metabolites from different classes in the MH86-lpa

mutant. Lpa mutant lines often exhibit lower grain yield and seed viability compared with the wild-type parents. To improve the agronomic performance of the MH86-lpa mutant, cross-breeding with two commercial cultivars was performed. The resulting progenies were genotyped using molecular markers to identify homozygous wild-type and lpa mutants from generations F3 to F8. The objectives of this study were (i) to investigate the impact of cross-breeding on the metabolic phenotype of the homozygous lpa mutant, and (ii) to assess the stability of the mutation-specific metabolite signature in the lpa progenies over several generations and in different field trials. Statistical assessment of the data via multivariate and univariate approaches demonstrated that metabolite profiles of homozygous lpa mutant progenies were dependent on the mutation, the environmental conditions and the phenotypic traits of the commercial crossing partners. However, type and extent of the mutation-specific metabolite signature in the lpa progenies were comparable to the progenitor MH86-lpa mutant and consistent over generations. These findings provide a basis for implementing mutation breeding in the generation of elite lpa cultivars.

IAEA-CN-263-108

DEVELOPMENT AND SCREENING OF GENETIC VARIANTS ON RADIATION MUTATION TECHNIQUES FOR BREEDING AND FUNCTIONAL GENOMICS STUDY IN PEPPER

Yeong Deuk Jo, Han Sol Kang; Jaihyunk Ryu, Jin-Baek Kim, Sang Hoon Kim, Si-Yong Kang
Korea Atomic Energy Research Institute, Republic of Korea
Email: jyd@kaeri.re.kr

Pepper is one of the most important vegetable crops in the Solanaceae family. Although the complete genome sequence has been reported and genetic studies have been performed for many important traits in pepper, a broader range of genetic resources for breeding and functional genomics studies are still required. Therefore, we developed mutant populations using radiation and an optimized a TILLING system for the efficient screening of target mutants. A mutation population which consisted of 4176 M₂ and 414 M₃ individuals was developed from 'Yuwolcho', a pepper (*Capsicum annuum* L.) Korean landrace that has early-flowering characteristics, using gamma-rays and/or a carbon ion beam irradiation. We also developed an M₂ population that consisted of 1666 individuals of 'Habanero', a *Capsicum chinense* cultivar which is famous for its extreme pungency, to provide useful material for practical breeding and the production of functional materials. For efficient and less labour-intensive screening of mutants carrying mutations on the target gene, we optimized a TILLING system based on capillary electrophoresis. Using this system, the screening of the mutants in 4-16 x pools is possible according to the length of the target sequence (4x for 1872 bp and 16x for 672 bp amplicons) without using any acrylamide gel nor labelled primers. We confirmed applicability of this system to a subset of 'Yuwolcho' mutation population which consisted of 2060 M₂ and 384 M₃ lines. By investigating the phenotypes and applying a TILLING technique to the radiation mutation population, we are screening useful genetic variants in pepper.

IAEA-CN-263-102

MUTAGENIC EFFECTS OF ION BEAMS AND DEVELOPMENT OF EFFICIENT MUTAGENESIS TECHNIQUES

Yoshihiro Hase

National Institutes for Quantum and Radiological Science and Technology
Email: hase.yoshihiro@qst.go.jp

We have been working on the use of ion beams for biological research using TIARA facility, established in 1991. The major goal of our research is to elucidate the mutagenic effects of ion beams and the

development of efficient mutagenic techniques. The molecular nature of mutations induced by ion beams is being discovered since the 2000s. It is the current understanding that ion beams tend to induce deletions and structural alterations of chromosomes more frequently than gamma-rays or X-rays, and this tendency is more evident as the LET value of ion beams increases. Generally, mutation breeding is perceived as a random process and no effective methods have been established to control the direction of mutations in practical mutation breeding. We demonstrated that more chrysanthemum flower colour mutants were obtained from cultured petals than from leaves irradiated by ion beams, whereas the regeneration frequency and the stem length of the regenerated plants did not differ between these two tissues. Similarly, in petunia seedlings, sucrose treatment to stimulate pigment biosynthesis resulted in a higher frequency of flower colour mutants after ion beam irradiation compared to mock treated controls, although the frequency of chlorophyll mutants was unaffected. Most recently, we compared the characteristics of mutations in *Arabidopsis* derived from dry seeds and seedlings, both irradiated with carbon ions. Whole-genome re-sequencing of randomly chosen M₂ plants revealed that the frequency of indels was about three times higher in dry-seed irradiation than in seedling irradiation, while the frequency of single base substitution was not significantly different. These results suggested that the physiological status of plant tissue greatly affects the frequency and type of mutations. These findings are highly relevant for developing more efficient mutagenesis techniques and for understanding the molecular mechanism of mutagenesis by ionizing radiation.

IAEA-CN-263-50

A FEATURED STEM DEVELOPMENT PATTERN IS DISPLAYED IN THE WINTER WHEAT MUTANT GENERATED BY HEAVY ION BEAM MUTAGENESIS

Yongdun Xie¹, Hongchun Xiong²; Huijun Guo¹; Linshu Zhao¹; Luxiang Liu¹

¹*Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, China*

²*Space Breeding Center, Institute of Crop Sciences, CAAS, China*

Email: xieyongdun@caas.cn

Induced mutation plays an important role in securing world food demands by developing mutant varieties, introducing new genetic resources, and accelerating vital agricultural gene cloning. Among all the mutation induction methodologies, the newly developed heavy-ion beam mutagenesis is characterized by its capacity to generate new phenotypic features in plants. By using heavy ion beam implantation, we screened out a mutant displaying a novel stem development pattern in winter wheat in the M₂ generation. After satisfying the temperature requirement for vernalization, the development of mutant and wild type plants was monitored, both entered the jointing stage at same time. During the jointing stage, the stems of the mutant elongated much quicker than that of wild type counterparts. As a consequence, the mutants are taller than wild type plants in this stage. However, the final plant height of both mutant and wild type plant was reached at the same time, at anthesis stage. The results from both plant hormone analysis and gene sequence comparison revealed that gibberellins play important roles in regulating the mutant phenotype establishment. Stem internodes length comparison was consistent with the dynamics of gibberellins biosynthesis. The mutation associated with this phenotype remains to be mapped and cloned. This stem development mutant supplies a new gibberellin sensitive semi-dwarf germplasm and a potential gene resource for wheat improvement.

PLENARY SESSION - PARALLEL SESSION

IAEA Boardroom B/M2

M Building

Thursday, 30 August 2018

CREATION AND CHARACTERIZATION OF AN EMS MUTANT POPULATION OF *JATROPHA CURCAS*

Fatemeh Maghuly¹, Bradley J. Till², Joana Jankowicz-Cieslak², Margit Laimer¹, Prateek Gupta³

¹PBU, DBT, BOKU, Austria

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

³Department of Plant Sciences, University of Hyderabad, India

Email: margit.laimer@boku.ac.at

Significant efforts toward the identification, characterization, screening and discovery of mutations in genes of interest have been made successfully in plants and animal by targeting induced local lesions in genomes (TILLING). The combination of induced mutagenesis with high throughput sequencing is a powerful tool for generating allelic series, introducing variation and enables gene function study by reverse genetic approaches in many crop species, especially in plants with a narrow genetic diversity like *Jatropha curcas*, a tropical biofuel crop. *J. curcas* is a semi-wild plant, and an economically important shrub as a useful source of biofuel or in soil reclamation, but it requires genetic improvement, to select the best genotypes for these purposes. A mutant population of *J. curcas* with similar genetic background was generated using three concentrations (0.4, 0.8 and 1.6%) of the chemical mutagen ethyl methanesulphonate (EMS) treated for 3 different durations (0.5, 1.5 and 3 h). 1800 EMS mutation lines (M₁V₆) was used to identify beneficial mutation in 97 genes related to oil composition, toxin production and stress response in *J. curcas*. Three-dimensional pooling strategy were used to generate 112 and 256-fold pooling, which were PCR-amplified. The equal amount of amplicon pools was used for library preparation and sequencing using Illumina MiSeq. Different software programs were used for data analyses. Among different software, CAMBa showed to be the reliable one to identify the mutation changes with lowest false discovery. This study shows that TILLING by sequencing is a high-throughput, fast and cost effective methods to identify allelic variations and gene functions in mutant populations, which control important traits.

IAEA-CN-263-98

NATIONAL REPOSITORY OF RICE EMS MUTANTS OF AN UPLAND RICE CULTIVAR NAGINA 22: PROGRESS UPDATE ON CHARACTERIZATION AND UTILIZATION

Amitha CR Mithra SV¹, Ashok K Singh², Chandra Prakash², Gopalakrishnan S.¹, Jeyaprakash P.³, Meera Kumari Kar⁴, Nagendar K Singh², Neera Yadav², Padmini Swain⁴, Prashant Kale²,

Rameshwar P. Sharma², M.K. Ramkumar², M. Raveendran³, N. Sarla⁵,

Satendra Kumar Mangrauthia⁵, M.S. Sheshashayee⁶, V. Sureshkumar², Trilochan Mohapatra¹

¹ICAR-National Research Centre on Plant Biotechnology, India

²ICAR-Indian Agricultural Research Institute, India

³Tamil Nadu Agricultural University, India

⁴ICAR-National Rice Research Institute, India

⁵ICAR-Indian Institute of Rice Research, India

⁶University of Agricultural Sciences, India

⁷Indian Council of Agriculture Research, India

Email: amithamithra.nrcpb@gmail.com

The Indian initiative for creating a mutant genetic resource in rice has generated 87 000 mutants in the background of a popular drought and heat tolerant upland cultivar, Nagina 22 (N22) through EMS mutagenesis. To enable mapping of the mutants, high quality sequence resources of the wild type (WT), N22, has been created with less than 5% gaps in the total of 113 Mb genic sequences. So far, 541 macro-

mutants from this resource have been identified and are being maintained in the mutant garden. Detailed characterization of these 541 mutants based on 44 descriptors pertaining to distinctness, uniformity and stability (DUS) of rice and their morphological characterization, organic (chlorophyll and epicuticular wax content) and inorganic constituents (phosphorous, silicon and chlorine) are hosted in a database named 'EMSGardeN22'. High throughput capillary based SSR genotyping of these mutants with 57 genome-wide markers revealed that more than 93% of the mutants were identical to the WT with less than one change in SSR repeat motifs. Further, similarity index of the mutants was more than 0.6 for nearly 90% of the mutants with respect to DUS descriptors establishing the validity of the mutants. Recently, a novel herbicide (Imazethapyr) tolerant mutant has been identified and characterized from this resource and is now being used in introgressing the herbicide tolerant trait in 10 major rice varieties of India. One mutant which can grow well in low phosphorous shows monogenic recessive inheritance while two functional stay green mutants show monogenic dominance inheritance were also identified. Mapping populations of these three mutants have been generated, the mutant type plants have been pooled in each population separately and subjected to whole genome sequencing to enable mapping of the mutants. Further, mutants have been identified for tolerance to sheath blight, blast, drought, higher photosynthetic efficiency and root traits from this resource.

IAEA-CN-263-178

RADIATION INDUCED MUTATIONS IN GENETIC ENHANCEMENT AND DEVELOPMENT OF NEW VARIETIES IN BLACK GRAM [*VIGNA MUNGO* (L.) HEPPER]

Souframanien Jegadeesan, Sreenivasulu Kandali Reddy
Bhabha Atomic Research Centre, BARC, India
Email: souframanien@gmail.com

Black gram [*Vigna mungo* (L.) Hepper], popularly known as urd-bean or mash, is a grain legume rich in protein (25-28%), widely cultivated in the Indian subcontinent and to a lesser extent in Thai land, Australia and other Asian and South Pacific countries. Genetic improvement in this crop is hindered due to its cleistogamous nature and narrow genetic base. As genetic variability is a pre-requisite for any crop improvement programme, induced mutation provides an important source for generating variability. Radiation (gamma, X-rays and neutron) induced mutants were identified for various morphological and biochemical traits, creating a pool of genetic variability. These mutants were used in cross breeding programmes to develop high yielding disease resistant varieties in black gram. The effective blend of mutation and recombination breeding at Bhabha Atomic Research Centre has resulted in the release of 5 black gram varieties (TAU-1, TAU-2, TPU-4, TU94-2 and TU-40) by incorporating desirable traits like large seed, wider adaptability, resistance to disease and improved quality. These varieties have been developed from mutants directly or by using them in cross breeding programme. For example, a black gram variety, N0.55 was irradiated with gamma rays (150-750Gy) and fast neutrons (20-60Gy) to obtain large number of mutants. The large seed mutants, UM 196 and UM-201, were used in cross breeding with the elite cultivar T-9 for developing high yielding varieties TAU-1, TAU-2 TPU-4, TU94-2 and TU-40. TAU-1 has become the most popular variety in Maharashtra state, occupying the maximum area under black gram cultivation. Induced mutations will continue to play an increasing role in generating genetic variability for various traits as a major component of environmentally sustainable agriculture.

DEVELOPMENT OF NEW BREAD WHEAT RESISTANT MUTANTS FOR UG99 RUST DISEASE

Abdulwahid Saif

Agricultural Research & Extension Authority (AREA), Yemen

Email: amozaid@yahoo.com

Wheat is an important world crop and continues to play a crucial role in the national food security of Yemen. Rusts are very deleterious diseases on wheat and include stem rust or black rust (*Puccinia graminis* f.sp. *Tritici*) of which a virulent strain developed known as Ug99. M₃ mutant families derived from two bread wheat varieties namely Giza 186 and Saha 93 were planted (13 from Giza 186 and 10 from Saha 93) along with the parents in 2014 in two locations: Tihama, Yemen and Njoro, Kenya (a hot spot for the disease), for screening against Ug99 rust disease resistance. At Tihama results showed that two mutants of Giza 186 and four mutants of Saha 93 were resistant to Ug99 rust disease at both seedling and adult stages while the parents were resistant at seedling stage and susceptible at adult stage. At the Kenya location, the resistance of mutants was slightly different: the two mutants of Giza 186 were confirmed as being resistant to Ug99, but only two mutants of Saha 93, were resistant at both stages while the other two were resistant only at the seedling stage and were susceptible at the adult stage. Continuing evaluation of selected mutants at M₄ stage for yield and agronomic performance resulted in selecting the Giza 186 G-M2 2010-1-28, it has the highest yield among all tested mutants with a 3905 kg/ha followed by the G-M2 2010-41 52 (3729 kg/h) and the S-M2 2010-16-12 (3526 kg/h). All three mutants significantly out yielded the local check Bouth-13 and the two parents, their yields ranged from 3156 to 3312 kg/ha. The new developed material can be used as breeding stock for the wheat breeding programme.

IAEA-CN-263-128

DETERMINATION OF RADIOSENSITIVITY OF *COFFEA ARABICA* VAR. VENECIA SEEDS TO GAMMA RAY IRRADIATION

Noel Arrieta, Reina Cespedes, Miguel Barquero

Costa Rica-Coffee Center Research-Coffee Institute-Technology Institute, Costa Rica

Email: narrieta@icafe.cr

Coffee is one of the most commercially available raw materials, being the tropical product with the highest marketing value in the world. In Costa Rica it is the third most important product for agricultural sector exports and provides the main income for many families in the country. However, coffee is under threat due to the coffee leaf rust disease (CLR). Mutation breeding in coffee a promising approach to develop new varieties resistant to CLR. As the technology is new for coffee, basic tests related to mutation induction need to be done. The plant material used were *Coffea arabica* var. Venecia seeds, with a humidity of 27,3%. The applied irradiation doses were 0, 80, 100, 120, 140, 160 and 180Gy. For each treatment, three replicates of 200g were used. The irradiated seeds were planted on the same day. Eighty days after treatment the number of seedlings was quantified, the hypocotyl height and radicle length were measured, and the opening of cotyledons was determined for each dose. The figure 1 shows the effect of increasing radiation doses on seed germination, whereas seedling growth was not affected. However, a delay in opening of the cotyledons was observed at higher doses. This radiosensitivity test based on seed germination as compared to unirradiated control revealed that the LD50 for the used variety is 127,8Gy. This dose will be used for further bulk experiments aimed. The results show the lethal dose (DL50) in 127,8Gy, for the establishment parameters, being an advance to continue with measurements of genetic and phenotypical parameters to go forward on coffee breeding programs looking for new sources to resistance against CLR.

APPLICATION OF CHEMICALLY INDUCED MUTATIONS USING EMBRYOGENIC CELL SUSPENSIONS AND SEEDS FOR CROP PROTECTION OF COFFEE (*COFFEA ARABICA* L.) VARIETIES IN COSTA RICA

Andres Gatica-Arias¹, Alejandro Bolivar-González¹, Ana Tapia¹, Cesar Vargas-Segura¹, Emmanuel Araya², Karla Sánchez-Aguilar¹, Marta Valdez Melara¹

¹University of Costa Rica, Costa Rica

²CENIBiot, Costa Rica

Email: andres.gatica@ucr.ac.cr

Coffee represents the most important non-alcoholic beverage in the world economy. Nevertheless, coffee is threatened by several biotic and abiotic stresses. Crop improvement via mutagenesis represents a powerful alternative to increase genetic variability and accelerate breeding programs. In this sense, coffee embryogenic suspension cultures (ESC) and seeds were incubated with sodium azide (NaN₃) and ethyl methane sulfonate (EMS). With increasing concentrations of NaN₃ and EMS, the survival of ESC and the germination, seedling height and root length decreased compared to the non-treated controls. In case of ESC the LD50 was determined for NaN₃ (5 mM for 15 minutes) and for EMS (185.2 mM for 120 minutes). The LD50 values for the treatment of seeds with NaN₃ and EMS were between 50-75 mM and 2-3% v/v, respectively. Embryogenic suspension cultures treated with NaN₃ and EMS were cultured on selective media supplemented with NaCl. The results showed that 150 mM NaCl could be used as a selection pressure. Leaves of Caturra and Catuai and the resistant coffee cultivar CR-95 were inoculated with uredospores of *H. vastatrix*. Preliminary results, demonstrated that Caturra is more susceptible than Catuai; whereas CR-95 did not any symptom of the disease. Finally, the induction of genetic variability in coffee seeds in response to the different NaN₃ and EMS treatments was assessed by AFLP analysis. The amplification of six AFLP primer combinations using a pool of plants obtained after mutagenic treatment with sodium azide allowed the identification of four polymorphism. Coffee breeding programs could use mutagenesis combined with screening methods and molecular markers as an additional tool to induce novel traits, such as resistance to coffee leaf rust and produce new and improved coffee cultivars.

VIRULENCE GENES OF A NEW RACE OF RUST (*HEMILEIA VASTATRIX*) AFFECTING LEMPIRA COFFEE AND OTHER RESISTANT/SUSCEPTI VARIETIES IN HONDURAS

Yonis Alberto Morales Reyes

Honduran Coffee Institute, Honduras

Email: ymorales71@gmail.com

The Lempira variety released in Honduras 1998 was qualified as 100% resistant to races of rust I and II by the Coffee Rust Research Centre (CIFC). However, in recent years the disease has been reported in seed foundation plots and producer farms, the most recent epidemic report happened in April 2016, and affected 210 Ha. Considering that this coffee variety constitutes 45% of the cultivated area, there is a need to identify the virulence genes of the new *Hemileia vastatrix* strain, and to determine which coffee varieties may be resistant or susceptible. For this purpose, mass samples of rust inoculated on leaf discs of differential clones 1343/269, 110/5, 147/1, 152/3, 33/1, 419/20, 832/1, 832/2, were used, together with 87/1, 1006/10, 420/10 and 420/2, from the Federal University of Vicosa and inoculated to the main cultivated resistant varieties (Parainema and IHCAFE-90) plus 7 promising genotypes, under controlled temperature conditions and relative humidity. After 20 to 60 days, seven virulence genes were identified (v1, v2, v4, v5, v6, v7, and v9). Of these the v1, v4, v6, v7 and v9 had not been reported in Honduras. It is inferred that this rust population is composed of simple races of 2 genes (v5

combined with v6, v7 or v9) and races with 3,4,5,6 and 7 genes as one of the most complex and aggressive breeds described but lacking the v3 and v8 gene. In addition, it was found that Parainema, H27, T5296-170, Central American, Pacamara yellow and Anacafe-14 are resistant because they possess the SH8 gene absent in Lempira, while IHCAFE-90 and Obatá have 20% susceptibility and Ruiru II is susceptible. This reveals the diversity of rust virulence genes, enables renewal and the increasing reduction of available natural sources of resistance.

IAEA-CN-263-180

MUTAGENESIS OF *IN VITRO* EXPLANTS OF *COFFEA ARABICA* TO INDUCE FUNGAL RESISTANCE

Souleymane Bado¹, Fatemeh Maghuly¹, Margit Laimer¹, Vitor Varzea²

¹PBU, DBT, BOKU, Austria

²CIFC Oeiras, Portugal

Email: margit.laimer@boku.ac.at

Coffee is one of the most valuable commodity tree crops worldwide. However, it suffers from several devastating diseases and pests, e.g. coffee leaf rust and coffee berries borer, whose impact is being amplified by the changing climatic conditions. Development of new adapted varieties remains a laborious effort by conventional breeding due the long juvenile period in tree crop. Plant cell culture represents the ultimate method to produce large amount of true-to type healthy explants and of explants for mutation breeding. In fact, mutation induction combined with *in vitro* cell/ tissue culture techniques has proven to be effective for developing improved cultivars of perennial crops. Prior to mutation breeding, cell and tissue radiosensitivity tests to mutagens need to be performed, so that optimal treatments can be determined for large population development. Thus different *in vitro* explants (plantlets, leaves, callus, embryogenic callus, globular and torpedo stage embryos) of *Coffea arabica* and *Coffea canephora* were exposed to different gamma-ray doses: 0, 10, 15, 20, 40 and 60Gy. Eight weeks after irradiation, a radiosensitivity test was conducted on the different ex plants and the LD50 doses corresponding to 50% of viability or survival of callus, embryogenic callus, globular and torpedo stage embryos and 50% growth reduction of plantlets was also determined. In general, the embryogenic calli showed a relatively higher radio-resistance (LD50 >40Gy) in comparison to entire plantlets. In irradiated plantlets the development of the leaf area was more severely affected by irradiation than the root growth, whereas the shoot growth, number of new nodes and roots formed were less affected.

PLENARY SESSION
IAEA Boardroom B/M1
M Building

Friday, 31 August 2018

MUTATION INDUCTION AND GENOME EDITING IN PLANT BREEDING: COMPARATIVE ADVANTAGES AND PERSPECTIVES

Qingyao Shu

Zhejiang University, China

Email: qyshu@zju.edu.cn

Genome editing has emerged as a powerful tool in plant breeding in recent years, with a trend to replace the 'old' technique of mutation induction. Here, we will review the molecular genetic features of mutations induced by through classical (chemical and physical) mutagenesis and (genome editing based) targeted mutagenesis, the procedures and applicability of these two approaches in different plant species, and based on these findings, discuss their comparative advantages in the context of plant breeding (with the aim of breeding and commercializing new cultivars at lowest costs). While genome editing has its advantages, mutation induction using physical and chemical mutagens still has its own uniqueness (and thus irreplaceable by genome editing) and even has advantages over genome editing for certain traits. Hence, classical mutagenesis can still be a useful tool for plant breeding and genetics, particularly when it is applied properly and together with other molecular techniques.

IAEA-CN-263-224

'GAIN-OF-FUNCTION' MUTANTS FOR GENE DISCOVERY AND FUNCTIONAL GENOMICS FOR MULTIPLE STRESS TOLERANCE IN RICE

Ashwani Pareek¹, Khalid Anwar¹, Nita Lakra¹, Priyanka Das¹, Rajeev Bahuguna¹, Sneha Singla-Pareek²

¹*Jawaharlal Nehru University, India*

²*International Centre for Genetic Engineering and Biotechnology, India*

Email: ashwanipareek@gmail.com

Mutation breeding is a traditional approach to generate crop plants with increased tolerance to abiotic stresses. *Oryza sativa* L. cv IR64 is high yielding rice with incomplete tolerance to critical abiotic stresses, such as extreme temperatures, high salinity, and drought. In the present study, we have generated a population of IR64 mutants by using gamma radiation. Over 4000 independent M₃ lines were screened at the seedling level for improved tolerance to high temperature and salinity stress using hydroponics. The preliminary phenotyping led to the identification of mutant lines which showed a robust phenotype to stress. Apart from this, the selected mutant lines also showed higher tolerance over wild-type plants to both the stress at the flowering stage regarding their growth and yield parameters, including plant height, tiller number, grain weight, harvest index, etc. Further, molecular analysis revealed that several stress-related proteins show higher constitutive levels of HSPs and various anti-oxidative enzymes in the mutant lines as compared to WT and under stress conditions. Proteome analysis using 2-D-DIGE approach, revealed some of the critical stress proteins to be accumulating at higher levels in mutants. These findings suggest that the transcription circuits and ROS scavenging machinery may be working more efficiently in these mutants. Whole genome sequence analysis revealed the 'hot-spots' contributing to the observed phenotypes. Using the tools of functional genomics, a set of the 'candidate genes' identified from the present work have also been validated. Similarly, GC-MS based metabolite profiling of these mutants has also revealed insight into the tolerance pathways contributing to the phenotype. The present study opens new vistas to use mutation breeding as a potential tool for tailoring multiple stress tolerance in rice which can accelerate the breeding programmes focussing towards the improvement of tolerance towards abiotic stresses.

COMPARATIVE STUDY OF MUTATIONS INDUCED BY CARBON ION BEAM AND GAMMA RAY IRRADIATIONS IN *ARABIDOPSIS THALIANA* AT THE GENOME-WIDE SCALE

Libin Zhou, Jiangyan Yang, Lixia Yu, Shanwei Luo, Tao Cui, Wenjian Li, Xia Chen, Yan Du
Institute of Modern Physics, Chinese Academy of Sciences, China
 Email: libinzhou@impcas.ac.cn

Mutation breeding induced by irradiation with high energetic photons and ion beams is one of the important methods used to improve plant varieties, but the mutagenic effects and the molecular mechanisms are often not entirely clear. Traditional research focused on phenotype screening, chromosome aberration test and genetic variation analysis of specific genes. Recently, the whole genome sequencing technique provides a new method for understanding the comprehensive identification of mutations caused by irradiations with different linear energy transfer (LET). In this study, eleven *Arabidopsis thaliana* M₃ lines induced by carbon-ion beams and 10 M₃ lines induced by gamma rays were re-sequenced by using the Illumina HiSeq sequencing platform, and the substitutions and small insertion-deletions (indels) were analysed comparatively. It was found that the ratio of substitutions to indels for M₃ lines induced by carbon-ion beam was 2.58:1, whereas the ratio was 3.69:1 for gamma rays. The ratios of deletions to insertion for carbon ions and gamma rays were 4.9:1 and 2.65:1, respectively. The single base indels were more prevalent than those equal to or greater than 2 bp in both carbon-ion beams and gamma rays induced M₃ lines. Among the detected substitutions, the ratios of transitions to transversions induced by carbon ions irradiation was 0.99 and 1.27 for gamma rays; these values differ greatly from the 2.73 reported for spontaneous substitutions reported. This study provides novel data on molecular characteristics of carbon-ion beam and gamma rays induced mutations at genome-wide scale. It can also provide valuable clues for explaining the potential mechanism of plant mutation breeding by irradiations with different LETs.

IDENTIFICATION OF A NOVEL LOCUS SH2-ON FOR SEED SHATTERING IN RICE (*ORYZA SATIVA* L.) BY COMBINING BULKED SEGREGANT ANALYSIS WITH WHOLE GENOME SEQUENCING

Feng Li¹, Hiroshi Kato¹, Hisataka Numa², Naho Nara³, Naoki Sentoku³, Noriyuki Nishimura¹, Sayaka Niwa¹, Takurou Ishii¹

¹*NARO, Institute of Crop Science, Radiation Breeding Division, Japan*

²*Advanced Analysis Center, NARO, Japan*

³*Institute of Agrobiological Science, NARO, Japan*

Email: rihoumail@affrc.go.jp

Seed non-shattering is an important trait related to the efficiency of seed harvesting. Takanari is a high-yielding Indica Group rice cultivar in Japan displaying an easy shattering phenotype. To reduce its yield loss caused by seed shattering a new cultivar Oonari with moderate shattering was, recently, developed as a mutant induced from Takanari by gamma-ray irradiation. The histological analysis of abscission zone (AZ) revealed no significant difference between Takanari and Oonari grown in paddy field. However, most of the spikelets of Oonari grown in greenhouse had no or reduced AZ formation, and different from that of Takanari. F₃ population derived from a cross between Takanari and Oonari showed single recessive gene's segregation for moderate-shattering. To identify the candidate gene, the bulk DNA of F₃ lines with the same shattering degree as Oonari and their parents, were used for whole genome sequencing. Variation-index (VI) for F₃ bulk was calculated as the ratio between the number of reads with a nucleotide mutation and the total number of reads corresponding to the

mutation. We identified a single base substitution (named TO20) with top VI in the terminal region on long arm of chromosome 2. This novel locus was named sh2-ON. Structure variation analysis showed that a tandem duplication containing a microRNA172 gene occurred near TO20 in Oonari. qRT-PCR analysis indicated that the microRNA172 relative expression in Oonari was about three times as high as that in Takanari. It has been reported that Apetala 2 (AP2) genes, such as SHAT1 in rice and Q gene in wheat which are both involved in controlling of grain threshing, are targets of microRNA172. Therefore, we infer that the duplication of microRNA172 might be the cause of reduced seed shattering in Oonari.

IAEA-CN-263-58

DEVELOPMENT OF A NEW RICE MUTATION BREEDING METHOD FOR THE SELECTION OF HIGHER YIELD PHYSIOLOGICAL MUTANTS

Hiroshi Kato, Akemi Shimizu, Feng Li

Institute of Crop Science, Radiation Breeding Division, NARO, Japan

Email: hkato@affrc.go.jp

The selection of higher yield physiological mutants had not yet been achieved in the 90 years history of crop mutation breeding. This is because physiological traits which cause higher yields than existing high yield varieties were unknown and we did not have any practical methods for selecting unknown physiological traits. We developed a new mutation breeding method of rice in which plant selection by all panicle weight per plant in M₂ and M₃ generations were conducted followed by usual yield trial of rice breeding sectors in M₄ and 10 times replicated yield trials in M₅. By using this method, we could select three higher yield rice mutants. FukuhibikiH6, Yamadawara H3 and Mochidawara H1 showed significantly higher yields compared with their original varieties. Their original varieties, Fukuhibiki, Yamadawara and Mochidawara are the highest yield varieties in some areas or for a specific usage in Japan. The yield advantages of these three mutants were 3 to 5%. In the selection of M₂ and M₃ generations, their yield advantages over surrounding plants were 10 to 40% which were much higher than in M₅ generation. In the M₂ and M₃ fields, they were surrounded by ordinary plants and they must have had advantages against the surrounding ordinary plants which suffered a disadvantage from them and became smaller. However, yield traits differences between them and their original varieties were very small and inconsistent. Higher yield is likely to be caused by physiological traits rather than phenotypical agronomic traits such as panicle and grain size. By gamma ray mutation induction, it is relatively easy to isolate the relevant genes because the number of mutated genes is limited, usually around ten in the whole rice genome. The isolated genes can then be used in genome editing for enhancing gene functions and for breaking the present yield barriers.

IAEA-CN-263-223

THE POWER OF NEXT-GENERATION SEQUENCING IN RAPIDLY IDENTIFYING (EPI) GENETIC CAUSAL MUTATIONS

Joerg Hagmann, Sebastian Schultheiss

Computomics GmbH, Germany

Email: joerg.hagmann@computomics.com

The advent of next-generation sequencing has accelerated forward genetic screens and can simultaneously achieve the typical steps of marker discovery, genetic mapping and identification of causal mutations. Initially applied in model organisms, an increasing number of studies emerge on crops. There is a wide variety of strategies to perform genetic mapping and the choice of an optimal approach depends on many factors, such as the genetic architecture and penetrance of the trait of

interest, the applied mutagenesis and mutational load, the recombination frequency of the species, and the available resources. We will illustrate some of these aspects and their influence on the choice of crossing schemes and on the optimal use of next-generation sequencing. Besides genetic causes of agronomically important traits, a recent landmark study revealed that DNA methylation changes are responsible for a yield-reducing somaclonal variant of oil palm. Current research is focusing on a stable maintenance of epigenetic changes, so that they might become interesting targets for breeding. We will introduce a novel tool that enables the reliable and efficient identification of DNA methylation differences in whole populations of plants. Resulting epi-allele groups can serve as a basis for association studies.

IAEA-CN-263-216

GAMMA RAY INDUCED PEDIGREED MUTANT POPULATION OF TOSSA JUTE (*CORCHORUS OLITORIUS* L.): A KEY RESOURCE FOR FORWARD AND REVERSE GENETICS

Shashi Choudhary¹, J. Souframanien², Neetu Kumari³, Sanjay Jambhulkar²

¹*ICAR-National Bureau of Plant Genetic Resources, Regional Station Ranchi, India*

²*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, BARC, India*

³*Birsa Agricultural University, India*

Email: shashigen@gmail.com

Low natural genetic diversity is a serious limiting factor for academic progress and agronomic improvement of crops like tossa jute (*Corchorus olitorius* L.), an economically important bast fibre crop. Mutation breeding with proven ability to improve both qualitative as well as quantitative yield at tributes can be employed to augment germplasm diversity. In the present study, we used gamma rays to produce a total of 33 000 mutants of *Corchorus olitorius* L. cv. JRO 204. The mutant population was screened for agro-morphological traits (M₂), abiotic stress (drought and water logging) tolerance (M₅) under long day condition and flowering resistance (M₂) under short day condition. A range of morphological variations of agronomic as well as scientific importance were identified at vegetative and reproductive stages. These traits include growth habit, pigmentation, plant architecture, root architecture and flowering behaviour. Conditional mutants such as drought and waterlogging stress tolerance were also identified in a pilot experiment by using a sub-set of the population. Selected mutants (588) have been advanced to M₆ generation by following single seed descent method to constitute pedigreed mutant population. These mutants were subjected to morphological, histological, genomic analysis to understand their genetic regulation using qRT-PCR analysis of key phenyl propanoid pathway genes in lignin deficiency. The study of lignin deficient mutants demonstrated that transcriptional regulation of CCoAMT 1 gene is associated with suppressed phloem fibre intrusive growth and fibre cell bundle formation and hypo-lignifications at early growth stage. In addition, comparative transcriptional analysis of flowering mutants compared to wild type under scored genes regulating flower timing in jute. Overall, the mutant population will be of paramount importance for genetic and functional genomic research in jute.

EARLY ASSESSMENT OF LENTIL AND CHICKPEA MUTANT AND EVALUATION OF LOW COST TILLING ON M₂ CHICKPEA

Meriem Laouar¹, Dahbia Tabti¹, Joanna Jankowicz-Cieslak², Bradley J. Till², Wahiba Tiliouine¹

¹ENSA/INRAA, Algeria

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

Email: till.brad@gmail.com, laouar_m@yahoo.fr

Algeria is the third most importers in the world of lentil (*Lens culinaris* Medik.) and chickpea (*Cicer arietinum* L.). The productions are very low because specially the lack of adapted and productive varieties. Radio-mutagenesis is one of ways to create new variability for selection according to environmental conditions. Mutation was induced using gamma rays in two varieties Idlib-3 (lentil) and Ghab4 (chickpea) by 100 and 258Gy corresponding to the lethal doses (DL50) respectively. For each species, from 10 000 seeds M₁ we generated about 140 families M₁ and about 4000 genotypes M₂ were characterized in the field. Many important agronomic traits were carry on and the generated data were statistically analysed for genetic evaluation among families and correlation between traits. For 87 DNA of chickpea the low-cost TILLING was tested. It was done on 10 candidate genes (resistant to Aschochyta blight and Fusarium wilt, early flowering). To validate the results the sequencing was conducted. Yield, yield component and harbour variability, heritability, and genetic advance were assessed on putative mutant. The range of variability for main traits was high. High correlation showed between number of total pods and seed yield. About 3% of families' mutants (M₂) showed superiority than the control for days to flowering, days to maturity and other traits. High heritability associated with high genetic advance was observed from seed yield and hundred seed weight; this indicates the expected effectiveness of selection for the traits under consideration. The analysis of 10 primer pairs on chickpea showed no bands, neither mutation nor natural polymorphism. It was concluded that the material which was used for mutagenesis was very homogenous. So, no mutations were discovered in this small subset of mutagenesis material that confirmed by the sequencing. Additional M₂ mutants could be analysed by low cost TILLING.

EFFECTS OF LIGHT AND UV-C RADIATION ON THE TRANSCRIPTIONAL ACTIVITY OF COP1 AND HY5 GENE HOMOLOGUES IN BARLEY

Irina Boycheva, Lubomir Stoilov; Ralitsa Georgieva; **Vasilissa Manova**

Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Bulgaria

Email: vmanova@bas.bg

Photomorphogenic regulators COP1 (Constitutive photomorphogenic 1) and HY5 (Hypocotyl5) play a key role in plant development, and are principally important during the transition from dark to light growth. AtCOP1 and AtHY5 are also involved in the transcriptional control of photolyase genes. Here we characterized the expression of barley COP1 and HY5 gene homologues in dark and light grown seedlings in relation to UV-damage response. Etiolated and green 6-day old barley seedlings were irradiated with UV-C and then exposed to light. Leaf samples were collected from control and treated plants immediately (0h) and at different time-points afterwards. Transcriptional activities of barley COP1 and HY5 homologues were assessed by real-time RT-PCR normalizing against 18S rRNA as internal control. In etiolated leaves we found ten-fold less levels of COP1 which, after 1h light exposure reached the amounts detected in the green ones. Barley HY5 mRNA amount was low in the dark-grown seedlings and after 1h lighting increased drastically to levels exceeding those measured in the green leaves. In the irradiated plants both genes were up-regulated but to a lesser extent if compared to the

control ones; however, the increase was still significant in comparison to the 0h time-point. The enhanced transcription of barley COP1 under light was unexpected in view of the well-known role of COP1 as a negative regulator of plant photo-morphogenesis, but it is in line with a previous report for a positive influence on UV-B response. HY5 is recognized as stimulator of light-inducible genes and the data presented here support such a role for barley HY5 homologue as well. According to our knowledge this is the first investigation of the activity of COP1 and HY5 homologues in barley as well as regarding de-etiolation and UV-stress.

POSTERS RELATED TO SESSION 1

**CONTRIBUTIONS AND IMPACT OF MUTANT VARIETIES
IN FOOD SECURITY**

M Building – Common Area

SELECTION OF PROMISING SOYBEAN MUTANTS THROUGH MULTI-LOCATION TRIALS

Reza Mohammad Emon, Md. Abdul Malek

Bangladesh Institute of Nuclear Agriculture, Bangladesh

Email: emonbina@yahoo.com

An experiment was conducted to evaluate the performance of four promising lines along with two check varieties of soybean in respect of plant height, branches per plant, pods per plant, seeds per pod, days to maturity and seed yield, at five different locations during Rabi 2013. Significant variations were observed both in individual location and combined over locations for all traits. Among the mutants, average days to maturity ranged from 105-115 days, 104-108 days and 108-115 days in testing sites of Mymensingh, Rangpur and Magura, respectively. It indicated that, at Mymensingh all the mutant lines as well as check varieties had prolonged days to maturity, which eventually reflected significantly higher grain yield in this location. Plant height ranged from 38.1-65.6cm, 35.1-70.7cm and 31.8-55.1cm in at Mymensingh, Rangpur and Magura, respectively. Higher number of branches/plant in all mutant lines as well as check varieties was found in at Mymensingh. The average number of pods/plant in the mutant lines ranged from 41.6-44.2. The highest number of pods/plant was found in mutant lines SBM-9, SBM-18 and SBM-22 in at Magura, Mymensingh and Rangpur, respectively. At Mymensingh, the mutant line SBM-18 produced the highest seed yield (2969.7 kg ha⁻¹) followed by SBM-15 and SBM-22. Similarly, SBM-18 gave the highest grain yield followed by SBM-22 in at Magura, whereas, the mutant line SBM-22 produced the highest grain yield followed by SBM-9 and SBM-18 in at Rangpur. Among the locations, all mutants as well as check varieties gave the highest seed yield at Mymensingh. Among mutant lines, some of the better mutants will be selected on the basis of yield contributed characters for further trials.

IAEA-CN-263-57

THE BREEDING OF GLUTINOUS HYBRID RICE

Shubiao Zhang

Fujian Agriculture and Forestry University, China

Email: zhangsbiao@aliyun.com

The glutinous rice is the traditional staple food most people in Asia. The glutinous rice is used in many countries for the preparation of the rice cake, and is an important cultural component. Along with living standards' enhancement and market economy's development and the demands for nutritious and healthy foods drive constant increase of the market for glutinous rice demand. The rice breeding programme has adopted the super rice breeding and good progress was made. It is important for us to develop glutinous hybrid rice for improving the yield of glutinous rice. Induced mutants of wx were directly obtained after irradiation on maintainer (B), restorer (R) and photoperiod (temperature) sensitive genic male sterile lines(S) of hybrid rice. CMS(A) lines with wx (referred to as wxA), T(P)GMS lines with wx (referred to as wxS), restorer lines with wx gene (referred to as wxR) as well as hybrid rice with wx gene (referred to as wx-hybrids) were developed. By using the wx-hybrid rice breeding method, most of CMS(T(P)GMS) lines and R lines used in China were transferred to wx-CMS (wxT(P)GMS) lines and wx R lines. Till now, 8 wx-CMS (T(P)GMS) lines and 5 elite wx-hybrid rice were released for commercial production. Compared with the traditional glutinous rice, the yield of the wx-hybrid rice was improved about 1500 kg ha².

GENETIC VARIABILITY INDUCTION IN RICE THROUGH MUTAGENESIS: AN ALTERNATIVE TO MITIGATE CLIMATE CHANGE AND PROMOTE FOOD SECURITY IN COSTA RICA

Andres Gatica-Arias, Griselda Arrieta-Espinoza, Mónica Villalobos-Flores, William Watson-Guido
University of Costa Rica, Costa Rica
Email: andres.gatica@ucr.ac.cr

Rice is a cereal of great nutritional importance in Costa Rica and is mainly cultivated in areas that are prone to accumulate salts due to a combination of bad agricultural practices, climatic conditions, such as low rainfall soil erosion and poor drainage. One alternative to solve this problem is through the genetic improvement of rice, e.g. by induced mutagenesis. Rice seeds and embryogenic calli were irradiated with different doses (0, 20, 40, 60, 80, and 100Gy) from a Cobalt-60 gamma radiation source. As the radiation dose increased, the induction, survival and regeneration of embryogenic calli decreased compared to the non-treated controls. Moreover, the oxidation of embryogenic calli increased as the gamma dose increased. Embryogenic calli irradiated with 40 and 60Gy were cultured on selective media supplemented with NaCl. The results showed that 150 mM NaCl could be used as an initial screen prior to field testing. Screening of mutant plants against drought tolerance and the disease organism *Pyricularia oryzae* (sexual morph *Magnaporthe oryzae*) is planned. Moreover, a molecular characterization of the rice mutants obtained will be carried out to detection mutations at the DNA level. Rice breeding programmes aim to use mutagenesis combined with screening methods and molecular markers to accelerate the development of mutant varieties.

IAEA-CN-263-101

DEVELOPMENT OF NEW VARIETIES USING SPACE INDUCED MUTATION

Xuzhen Cheng, Lixia Wang, Suhua Wang
Institute of Crop Sciences, CAAS, China
Email: chengxuzhen@caas.cn

Space mutation is a new and efficient way to obtain a high frequency of variations for crop breeding. In the past years, we provided a series of food legumes collections for space mutation and continually observed their performances in the field for several generations. The results were as follow: (1) The germination ratio of SP1 obviously decreased shortly after space mutation, compared with the control group, while it became normal in the following generations; (2) A high frequency of variations on agronomic traits was observed within the generations of SP2. The frequency of variation was correlated with the seed sizes of different species, indicating that small seeds were easier to mutate; (3) Variations on the plant height, the number of nodes per stem, the number of branches per stem, the number of pods per plant and the seed size, were observed continuously from SP2 generations, while we didn't find any variation on number of seeds per pod. Some of those variations were steadily inherited after reproduction for several generations, and (4) After reproduction and observation for several generations and selection of lines that had potential advantages, a set of elite lines were finally selected for their stable and good performances on the main agronomic traits. These lines were further developed into new varieties as Zhonglv 8, Zhonglv12 and Zhonglv13, and have been released.

MUTANT VARIETIES DEVELOPED IN THE PHILIPPINES

Fernando Aurigue

Philippine Nuclear Research Institute, the Philippines

Email: fbaurigue@pnri.dost.gov.ph

PARC-1, the Philippine Atomic Rice Cultivar 1, was the first mutant rice variety developed by the Philippine Atomic Energy Commission, now Department of Science and Technology-Philippine Nuclear Research Institute (DOST-PNRI). From 1970 to 1976, four more mutant rice varieties followed but only two passed registration with the Philippine Seed Board, now the National Seed Industry Council (NSIC), Bureau of Plant Industry, Department of Agriculture. Rice improvement programmes then shifted to hybrid development. It was only recently that rice mutation breeding was revived for novel characteristics, like resistance or tolerance to stresses. Mutation induction by gamma irradiation was also tried on other crops, especially those that are asexually-propagated. Radio-sensitivity studies were conducted on planting materials of asexually propagated plants (stem cuttings and tissue culture explants) with effective dose levels of gamma rays from a Cobalt-60 source (Gammacell 220). Mutation detection and selection of desirable mutants based on specific objectives set for the particular crop were conducted in succeeding generations. Line purification and testing of stability of mutated characteristics were done in more advanced generations. The selected mutants were compared with the original material as well as similar existing commercial varieties. For rice and banana, National Cooperative Testing for several cropping seasons is required for variety registration. For cashew, on-site evaluation for three seasons is required, while a new ornamental plant variety must pass evaluation by the Technical Working Group and customer rating in a public exhibition. Mutant varieties were developed and registered by institutions other than DOST-PNRI. Gamma irradiation is a proven means of developing mutant varieties of rice and asexually propagated crops with improved or novel characteristics that are useful to the Filipino people.

DEVELOPMENT OF TROMBAY COWPEA VARIETY 'TC-901' AMENABLE FOR SUMMER CULTIVATION BY INDUCED MUTAGENESIS

Dhanasekar Punniyamorthy, Sreenivasulu Kandali Reddy

Bhabha Atomic Research Centre, BARC, India

Email: dhangene@gmail.com

Legumes/pulses form an integral component of Indian agriculture complementing cereals and ensuring nutritional security of predominantly vegetarian population of the country. To make India self-sufficient in pulses and to meet the buffer stock requirement, it becomes imperative to augment horizontal and vertical growth. Horizontal growth primarily by expanding pulses area could be accomplished by tapping under-utilized niches such as summer and rice fallows. Cowpea [*Vigna unguiculata* (L.) Walp] is one of the predominant arid legume crops potent for summer cultivation. With a view to developing summer cowpea varieties, an induced mutagenesis programme was initiated in 2008 by exposing seeds of an exotic line 'EC394763' (from IITA, Nigeria) to 250Gy of gamma rays. In M₂ generation, a mutant 'TCM 60-1' with more pods and altered seed coat colour was identified and its true breeding nature was ascertained up to M₅ generations. This high yielding mutant was evaluated as 'TC-901' for five consecutive years in the National summer trials of Network Project on Arid Legumes, Indian Council of Agricultural Research. Cowpea entry 'TC-901' (1021 to 1353 kg/ha) out-yielded the national check variety 'RC-101' by 15% and showed field resistance to mosaic and root-rot diseases. Endowed with promising agronomic attributes of semi-determinate growth habit, early maturity (70 days), medium large seeds (12.4 g/100 seeds), more number of pods and seeds per pod and high fodder yield (4954

kg/ha), 'TC-901' has been identified for release for summer season by Varietal Identification Committee in its meeting held on 9th November 2017 at Gwalior, India. 'TC-901' is the first national cowpea variety identified for summer and is expected to enhance the area under summer pulses after its release and notification for cultivation in India.

IAEA-CN-263-190

IRRADIATION OF THE POTATO *SOLANUM TUBEROSUM* CV. DESIRÉE TO INVESTIGATE RESISTANCE TO THE POTATO CYST NEMATODE *GLOBODERA PALLIDA*

Ulrike Gartner, Vivian Blok
The James Hutton Institute, United Kingdom
Email: ulrike.gartner@hutton.ac.uk

Potato cyst nematodes (PCN) pose an increasing threat to the potato industry and continue to be spread into clean land. Management with chemical control is problematic due to restrictions on the use of nematicides. Creating potato cultivars with durable and broad-spectrum resistance to PCN is a priority for potato breeders, especially to *Globodera pallida* for which few cultivars with high levels of resistance are available. Here, we investigate the effects of gamma-irradiation of the tetraploid potato *S. tuberosum* cv. Desirée to generate desirable mutations, such as resistance to the potato cyst nematode *G. pallida* in an established cultivar. Tubers were sent to the FAO/IAEA Plant Breeding and Genetics Laboratory, Seibersdorf, Austria. The first step was to produce *in vitro* materials which were then micropropagated to develop a large population for the gamma irradiation. Irradiated cuttings (with different dose treatments) were then cultured to produce *in vitro* micro-tubers, which were returned to the JHI. The mutated micro-tuber population was planted into root-trainers filled with compost and 20 cysts of a representative UK *G. pallida* population, pathotype Pa2/3. After 7 weeks the number of female nematodes on the root surface were scored and the relative level of resistance determined. Root systems from 2,000 of 2,133 micro-tubers of *S. tuberosum* cv. Desirée were scored; the remainder did not grow or only very poorly and were thus, discarded. 75 plants were re-assessed twice with the same *G. pallida* population with 4 replicates each. As a result, 5 plants with consistently low numbers of *G. pallida* females compared to non-irradiated plants were identified. Tubers were produced from these plants, and further tests to determine their resistance to three *G. pallida* populations that differ in their virulence are in progress. This work developed a new *in vitro* (micro-tuber) system for potato mutagenesis. The phenotypic screening is still in progress, conclusions cannot yet be drawn whether stable resistance in a tetraploid potato cultivar to *G. pallida* has been achieved by induced mutation.

IAEA-CN-263-267

AGRONOMIC PERFORMANCE OF WHEAT MUTANT LINES FOR FOOD AND FEED QUALITY ON FARMER'S FIELDS

Mirjana Jankulovska, Sonja Ivanovska, Biljana Kuzmanovska, Dane Boshev, Ljupcho Jankuloski, Mile Markoski
Faculty of Agricultural Sciences and Food, the Former Yugoslav Republic of Macedonia
Email: mirjanajankulovska@yahoo.com

This research was performed to assess agronomic performance of ten advanced wheat mutant lines on farmers' fields and to determine the best mutant lines for each site, the highest yielding and the most stable genotypes, as well as the most representative locations, by using GGE (Genotype + Genotype × Environment) biplot technique. Mutant lines included in the study were developed by gamma ray treatment (250Gy), the selection was made in M₂ generation for lines with improved quality. The

advanced mutant lines from M_7 generation were planted in 10 different environments in order to assess their stability and adaptability. The experimental sites L4, L5 and L6 had longest vectors from the biplot origin and were the best sites for genetic differentiation of analysed mutant lines. Mutant lines 5/1-8 and 4/2-56 had the best performance on sites L1, L3, L7, L8 and L9, mutant lines 5/1-199 and 7/1-143 on L2 and 2/2-21 and 2/1-51 on L4, L5, L6 and L10. The sites located at the same sectors on the graph, form two separate mega-environments. The mutant line 5/1-8 had the highest average grain yield, followed by 4/2-56, 2/2-21 and 8/2-137/1. The most stable mutant lines in all testing sites were 4/2-56 and 8/2-137/1. Although the mutant line 2/2-21 had high yield, it did not have stable performance in all environments. The mutant line 4/2-56 was the closest to the “ideal” genotype, and it should be considered for cultivation in all environments evaluated within this study. L4 and L5 were the most discriminating as well as most representative environments. These should be considered as the best environments for wheat stability testing and included as experimental sites in future studies. This study provides valuable information about the agronomic performance of advanced wheat mutant lines at different sites of the country which can be used for breeding stable and adaptable wheat varieties for specific regions.

POSTERS RELATED TO SESSION 2

**MUTATION BREEDING FOR ADAPTATION TO CLIMATE CHANGE
IN SEED PROPAGATED CROPS**

M Building – Common Area

IMPROVING FABA BEAN CROP FOR BIOTIC STRESS RESISTANCE THROUGH MUTATION BREEDING USING GAMMA IRRADIATION TECHNIQUE

Sonia Mejri¹, Mouldi Saidi¹, Omrane Belhadj², Yassine Mabrouk¹

¹National Center for Nuclear Sciences and Technologies, Tunisia

²FST, Tunisia

Email: son.mejri@gmail.com

Broomrape (*Orobranche* spp.) a parasitic weed produces serious damage to many legume crops and particularly may become a limiting factor for faba bean (*Vicia faba* L.) production in the Mediterranean basin. Currently, breeding for resistance to this pest remains as one of the most feasible and environmentally friendly methods for managing broomrape infestation. Agriculture has benefited from the use of radiation techniques, which provide plant varieties with unique characteristics. This investigation was carried out to produce new genotypes of faba bean resistant to *orobanche foetida*. At first, from a radiosensitivity test micro-mutations were scored for percentage of germinated seeds, plant growth parameters, morphological modifications, photosynthetic pigments and nutrients contents. According to the seeds percentage germination results, the LD50 was 150Gy. Further, we studied the cellular and enzymatic changes associated with resistance to *O. foetida* in selected fababean mutant that were obtained through radio mutagenesis. Finally, correlation between gamma radio-induced free radicals and antioxidant profiles were studied. At this dose (150Gy), gamma rays greatly induce morphological changes. These modifications in growth traits and morphological changes were accompanied with a marked modulation in the DNA profile. Experiments showed that low induction of seed germination is a major component of resistance in these new lines against *O. foetida*. This is confirmed by *in vitro* experiments with root exudates stimulant effect. In parallel, reduction in infection was accompanied by the continuously enhancement of the peroxidase activity, the polyphenol oxidase activity and the phenylalanine ammonia lyase activity in faba bean roots. These data suggest the contribution of these enzymes in faba bean resistance to *O. foetida* broomrape induced by the use of gamma rays. In conclusion, based on field and *in vitro* M₃ generation results, the studied samples produced by plant breeding program, are promising for the production of new varieties resistant/tolerant to plant parasites.

GENETIC DIVERSITY AND MOLECULAR CHARACTERIZATION OF XA-5 AND XA-21 IN AROMATIC RICE MUTANT LINES DERIVED FROM IRRADIATED GAMMA RAYS

Ishak Ishak, Aryanti Aryanti

Center for The Applications of Isotopes and Radiation, Indonesia

Email: ishakazra@yahoo.com

Molecular characterization of aromatic rice mutant lines used primer 16PFXa, RM122, and pTA248 linked to Xa1, xa5 and Xa21 resistance gene respectively. These primers have been used for Polymerase Chain Reaction (PCR) for identification of DNA fragment and sequences related to *Xanthomonas oryzae pforyzae* (*Xoo*) resistance in rice. Single DNA fragment linked to Xa1 and xa5 in aromatic rice mutant lines and checked susceptible variety of TN1 was found with size 0.375 kb and 0.2 kb for primer respectively, while 0.6 kb of DNA fragments related to Xa21 was found in aromatic rice mutant lines, and 0.7 kb at TN1 and IR64 cultivars. Alteration of nucleotide sequences from each of DNA fragment on rice mutant lines, Sintanur (wildtype), and TN1 cultivars as checked for sensitive varieties to Bacterial Leaf Blight (BLB) were analyzed. DNA fragments derived from PCR products with primer RM122 and pTA248 were taken for DNA sequencing analysis. Results of nucleotides sequences analysis derived from each of DNA fragment of aromatic rice mutant lines (AR.1020 and AR.1030)

and Sintanur cultivar (wildtype) were compared. Results showed that point mutation occurred on aromatic rice mutant lines based on comparison with Sintanur cultivar. These mutation at aromatic mutant lines showed as gamma rays effect hit genomic DNA of AR.1020 and AR.1030 and created nucleotides polymorphism. Based on of Blast analysis software showed that DNA fragment linked to xa5 located at chromosomes 5, while DNA fragment linked to Xa21 at chromosomes 11. Approximately 30 percent of DNA sequences of these DNA fragment was similar with predicted sequences of receptor kinase-2

IAEA-CN-263-9

BAKING QUALITY IMPROVEMENT IN WHEAT FLOUR BY PHYSICAL MUTAGENESIS

Mohammad Reza Rahemi¹, Ahad Yamchi², Hasan Soltanloo², Peter Roepstorff³, Saeid Navabpour²

¹*Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Iran*

²*Department of Plant Breeding and Biotechnology, Gorgan University of Agricultural Sciences and Natural Resources, Iran*

³*Department of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark*

Email: mrahemi@nrcam.org

About 13 million acres of wheat are cultivated in Iran. Wheat is one of the most important cereals for human nutrition and this is mostly related to physical and chemical properties of gluten in wheat seed. Wheat baking quality is under the control of genetic and environmental factors. Genetic factors can be improved through breeding. A significant barrier in plant breeding is the availability of useful genetic variation, and mutation induction can provide this. Seeds of wheat cultivars Roshan and Omid were exposed to a Cobalt-60 gamma irradiation at a dose of 200Gy. Selected mutant lines were subsequently cultivated for seven years to produce genetic purity. Seeds from these mutant lines have been evaluated through rheological and proteomics methods. Some mutant lines showed significant improvement in chemical properties as well as flour strength and stability of the dough. Furthermore, the expression of some proteins such as: vicilin, avenin and gamma-gliadin, known as gluten proteins, were increased in the mutant lines which have improved baking quality traits. Down regulation of some proteins including globulin-1 S allele, globulin-3A, monomeric alpha-amylase inhibitor and glyceraldehyde-3-phosphate dehydrogenase was also observed. These were identified by mass spectrometry as water-soluble proteins which decreases baking quality. Globulin-3A protein is known as a potential allergen and increases the risk of developing type I diabetes in human; it is present in the parental lines, but absent in some of the selected mutant lines. The results of this study showed that nutritional qualitative traits of wheat can be improved by applying physical mutation.

**INDUCED MUTATION THROUGH GAMMA RADIATION IN PEA (*PISUM SATIVUM* L.):
DEVELOPMENTAL CHANGES AND IMPROVED RESISTANCE TO BROOMRAPE
(*OROBANCHE CRENATA*)**

Yassine Mabrouk¹, Kamel Charaabi², Mouldi Saidi², Omrane Belhadj³

¹Centre National des Sciences et Technologies Nucleaires, Tunisia

²LR Biotechnology and Nuclear Technologies, National Center for Nuclear Sciences and Technology (CNSTN), Tunisia

³Université de Tunis, Faculté des Sciences de Tunis, Laboratoire de Biochimie et de Technobiologie, Tunisia

Email: yassine.mabrouk@gmail.com

Legumes are important and widely used in human nutrition, particularly the food industry as due to their high protein and essential amino acids. Despite these nutritional characteristics, a regression of the areas and of the production of these legumes crop is observed in several regions. The Orobanche is among the main factors behind this reduction. The means to fight against this parasitic plant are relatively diversified but remain ineffective or have undesirable collateral effect. Creating variation in the host genetic pathway remains the best way to fight against *Orobanche crenata* infestation. Thus, selection of pea varieties resistant to this parasitic plant appears as an appropriate control strategy. In order to create new desirable genetic variability induced mutation through gamma radiation is one of the best alternatives for the improvement of pea resistance to the parasitic weed *Orobanche crenata*. Seeds (cv. Douce de province) were exposed to increased doses up to 750Gy. Germination rates and plant survival were scored 7 and 15 days after sowing, respectively. The 100Gy dose was determined as the optimum dose limit causing 50% reduction in survival. Multi-shoots and delayed flowering were clearly observed in 150 and 200Gy treatments. Gain in resistance to *O. crenata* in 100Gy mutants was demonstrated in pots and in co-cultures systems. Genetic variation among selected M₂ plants was verified using ISSR indicating high genetic variability induced after gamma irradiation. In conclusion, seed irradiation (LD50) was efficient in pea to create variable initial material in mutation breeding of new lines resistant to *O. crenata*.

**CREATING NEW MUTANTS OF CHICKPEA RESISTANT TO *ASCOCHYTA RABIEI*
USING GAMMA RADIATION**

Yassine Mabrouk¹, Kamel Charaabi², Martina Rickauer³, Mahiout Djamel⁴, Omrane Belhadj⁵

¹Centre National des Sciences et Technologies Nucleaires, Tunisia

²LR Biotechnology and Nuclear Technologies, National Center for Nuclear Sciences and Technology (CNSTN), Tunisia

³Université de Toulouse, Laboratoire d'Ecologie Fonctionnelle et Environnement, France

⁴Laboratoire de Protection des cultures, Faculté des Sciences de la Vie et de la Nature, Université de Mostaganem, Algeria

⁵Laboratoire de Biochimie et de Technobiologie, Faculté des Sciences de Tunis, Université de Tunis, Tunisia

Email: yassine.mabrouk@gmail.com

Chickpea (*Cicer arietinum* L.) is one of the most popular vegetables in many regions of the world. Chickpea is an important pulse crop and a major source of protein in human diet. In Tunisia, because of biotic and abiotic constraints, the cultivated area and production is unstable and is decreasing. The presence of fungi including Ascochyta blight, Fusarium wilt, black collar and root rot, dry root rot, Phytophthora root rot and Pythium damping-off in some chickpea growing areas are considered limiting

factors to the expansion of the crop. Due to ineffective control methods and the lack of resistant varieties, induced mutation is used as an alternative for the improvement of chickpea for resistance to *Ascochyta blight*. This study was conducted to determine the lethal dose of 50% of chickpea seeds (LD50) to determine the optimum mutation induction dose and select chickpea mutant lines resistant to *Ascochyta rabiei*. Irradiation of chickpea seeds with a range gamma ray doses allowed to determine the LD50 (150Gy). Genetic variability among M₂ plants derived by mutagenesis was verified by the ISSR technique. Among 34 studied mutant lines we selected three mutants resistant to *Ascochyta rabiei* in M₂ and M₃ generations.

IAEA-CN-263-12

ADAPTATION OF MUTATION BREEDING FOR ENHANCING COTTON RESILIENCE TO CLIMATE CHANGE IN BANGLADESH

Md. Farid Uddin¹, Dana Jawdat², Khin Myat Lwin³, Ljupcho Jankuloski⁴, Malek Massoud⁵, Manzoor Hussain⁶, **Md. Kamrul Islam**¹, Mohammad Reza Rahemi⁷, Tianzhen Zhang⁸

¹*Cotton Development Board, Ministry of Agriculture, Bangladesh*

²*Atomic Energy Commission of Syria, Syrian Arab Republic*

³*Biotechnology Research Department, Myanmar*

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

⁵*International Atomic Energy Agency, Division for Asia and the Pacific, Austria*

⁶*Nuclear Institute for Agriculture & Biology (NIAB), Pakistan*

⁷*Atomic Energy Organization of Iran, Iran*

⁸*Zhejiang University, China*

Email: islam.mdkamrul@gmail.com

Bangladesh is an agrarian country where, because of climate change the frequency of drought, soil salinity and waterlogging are increasing over the years and adversely affecting the productivity of the field crops including cotton. For sustainable cotton production in Bangladesh, we need to introduce and develop climate resilient cotton mutant varieties. For the introduction of heat tolerant cotton mutant varieties, a field trial was conducted at the Cotton Research Centre, Gazipur in 2016-2017. The performance of two heat tolerant cotton mutant varieties viz. NIAB-KIRAN and NIAB-414 developed at NIAB, Faisalabad, Pakistan was tested against the two local varieties CB-12 and CB-14. Significant differences were found for cotton yield and yield contributing characters among the 4 varieties. The highest node number for initiation of 1st square was recorded from NIAB-KIRON (9.13) and the lowest from CB-14 (7.13). The highest number of bolls per plant (24.67) was recorded from NIAB-KIRON and the lowest (16.33) from CB-12. The highest single boll weight (4.67 g) was obtained from CB-12 the lowest (3.73 g) from NIAB-KIRAN. The highest seed cotton yield, 2.48 t ha⁻¹, was obtained from NIAB-KIRAN and the highest fibre length (33.41mm) was recorded from NIAB-414. As for developing local mutant populations, three local varieties viz. CB-12, CB-13 and CB-14 were irradiated at 25, 30 and 35Gy using Gamma irradiator. at Bangladesh Institute of Nuclear Agriculture (BINA) and the M₁ generations were grown at the Cotton Research Farm, Sreepur, Gazipur during 2016-2017. The M₂ generations are growing in 2017-2018 for the selection of desirable segregant.

MORPHOGENETIC SCREENING OF THE PROMISING RICE GENOTYPES UNDER SALINITY STRESS

Reza Mohammad Emon

Bangladesh Institute of Nuclear Agriculture, Bangladesh

Email: emonbina@yahoo.com

Selection of rice (*Oryza sativa* L.) varieties tolerant to soil salinity is important to reduce the impact of salt stress on rice production. In this study, the morphological characterization was performed on 5 rice genotypes under two salt stress treatments EC 8 and 12 dS/m at seedling, EC 12 dS/m at vegetative and EC 8 dS/m at reproductive stages. Morphological characters and Na⁺/K⁺ uptake ratios were surveyed to evaluate salt stress effects. At vegetative stage, all the genotypes have survived. However, at reproductive stage all the genotypes were affected by salt stress except for the Binadhan10 that survived the whole life cycle. A total of 160 SSR markers were used which revealed 209 alleles among the 5 rice genotypes. Interestingly, 4 SSR markers RM105, RM125, RM178 and RM549 with highest value: 0.67 have scored the highest level of genetic diversity value (0.72). These markers could be used for pyramiding the major and minor salt stress related genes via marker assisted selection in rice. The present investigation on different salt stress responsiveness of 5 promising rice genotypes will be of great value in rice breeding.

IAEA-CN-263-17

IMPROVING SUBMERGENCE TOLERANCE IN THAI RICE USING ELECTRON BEAM INDUCED MUTATIONS

Kanchana Klakhaeng¹, Fatma Sarsu², Udompan Promnart¹

¹*Rice Department, Thailand*

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

Email: kanchana.k@rice.mail.go.th

The devastating 2011 flood in Thailand was the worst in 50 years and caused significant yield losses in rice. Thus, submergence-tolerant rice is highly desirable to enhance food security. RD31 is a nonglutinous, photoperiod-insensitive rice variety with resistance to white-black plant hopper, and due to its several preferable agricultural traits, it is popular with farmers and consumers. RD31 seed was irradiated with 0.44 kGy electron beam. M₁ plants were planted as Hill plots in January 2014 and 500 panicles from the main tiller were collected. M₂ plants were planted as panicle/row for 500 rows (10 000 plants in total). Agricultural traits, such as plant height and maturity date were observed. Seeds from six plants in each row were collected as M₃ seeds. About 3000 M₃ plants were screened for submergence tolerance, and 317 tolerant mutant lines were identified. M₄ plants were screened for blast resistance. The result showed that all mutant lines were highly resistance. Some of them were screened for submergence tolerance and 91 tolerant mutant lines were identified. Furthermore, screening for grain quality, six mutant lines had 23.2-24.4% amylose content (i.e. a moderate amylose content); RD31 has a higher (27.5%) amylose content. Most selected mutant lines also had clear grain or were less chalky. Furthermore, it was found that some mutant lines had early maturity which was 117-124 days while RD31 had 131 days to maturity. M₅ plants were evaluated for important agronomic characters and yield trials were conducted. The experiment is still under progress. Some information is still needed for further conclusion before the release of the new rice variety.

IMPROVEMENT OF IRANIAN RICE VARIETIES FOR SALINITY TOLERANCE THROUGH MUTATION APPROACH

Leila Bagheri¹, Ali Eskandari¹, Alireza Nabipour², Allahyar Fallah², Azam Borzouei¹, Ebrahim Moghiseh¹, Hossein Elyasi²

¹*Nuclear Agriculture School, Nuclear Science and Technology Research Institute, Iran*

²*Rice Research Institution, Iran*

Email: lbagheri@nrcam.org

Rice is staple food for half of the world. Soil salinity is one of the major abiotic stresses leading to the reduction of rice yield. The objective of this research was to induce mutations for salt tolerance, high yield, early maturity and lodging resistance in local rice varieties in Iran (Tarom-mahali, Hasani and Anbarboo). Mutation breeding method by using gamma ray from ⁶⁰Co was conducted during 2010-2015 rice sowing seasons. After determining of the proper dose, seeds of each variety were exposed to 200, 250, 300Gy doses for raising M₁ generations. Twenty-two thousand panicles were harvested on an individual plant basis for evaluation in the next generation. In M₂ generation, seeds of all mutants and control were sown in saline soil (EC = 6-8 ds/m). 430 mutants were confirmed. In M₃ generation, 134 mutants were selected and inspected to confirm their genetic stability as well as to evaluate their agronomic characteristics and salinity reaction in the following generation. The evaluation of M₄ mutants were done in an augment design with 9 blocks at saline field condition (EC = 7.3 ds/m). Parameters of chlorophyll fluorescence were measured during vegetative growth. In flowering stage, plant height, Panicle number in hill, and fifty percent flowering were recorded. In physiological maturity stage, the superior mutant lines than the parent cultivars were harvested. By using data of paddy yield, salinity tolerant indices were calculated. The 30 selected mutant lines were evaluated in the M₅ generation in a complete randomized block design with four replications. Results of yield and components yield trials and morphological and physiological assessments indicated a significant difference among mutant lines and their parents. The 17 promising mutant lines were selected, that in higher generations will be evaluated.

ASSESSMENT OF ALLELIC VARIATION AMONG NEWLY IMPROVED RICE VARIETIES AT SALTOL REGION IN SRI LANKA

R.M.N.H. Senanayake¹, A.A.P.G. Amarasingha¹, D.G.K.P. Wijerathna¹, H.M.V.G. Herath², I.P. Wickramesinghe², U.A.K.S. Udawela¹

¹*Rice Research and Development Institute, Sri Lanka*

²*Department of Agric. Biology. Faculty of Agriculture, University of Peradeniya, Sri Lanka*

Email: nilminihs@yahoo.com

The productivity of rice is greatly affected by soil salinity which is the second most widespread soil problem after drought in rice growing areas in the world. This research was aimed to analyse Sri Lankan rice varieties grown in salinity affected areas, with the SSR markers closer to, previously identified-Saltol QTL-located on chromosome 1 and use those data in rice breeding and gene mapping studies. The research was conducted at Rice Research and Development Institute, Batalagoda, Sri Lanka. 21 improved rice varieties namely Bg379-2, Bg450, At402, Bg403, Bg406, Bg94-1, Bg352, At353, At354, Bg357, Bg358, Bg359, Bg360, At362, Ld365, Bg366, Bg369, Bg300, Bg4-91, At307, At308 and check variety Pokkali were used. Polymorphic bands obtained from SSR markers were analysed by Jaccard's similarity coefficient following the unweight pair group method with arithmetic mean (UPGMA). DNA samples were amplified using 8 SSR markers located between 10.816.4 Mb, on the Saltol QTL region of chromosome 1. The eight markers used were highly polymorphic and 45 alleles were amplified with

band sizes between of 100 and 300 bp. The highest number of alleles was found in RM7075 (8) followed by RM493 (6), RM562 (6), RM10748 (6), RM10582 (5), RM140 (5), RM1287 (5) and RM3412 (4) which gave the lowest number of alleles. Locus RM 562 showed the highest polymorphism information content (PIC) value (0.7831). The mean PIC value was 0.7124 and it ranged from 0.5718 to 0.7831. Out of 8 markers RM562 would be more useful for screening of rice germplasms. Information derived on the polymorphic SSR marker patterns obtained from tolerant and susceptible varieties near Saltol region would be useful in selecting parental lines from the tested varieties for rice breeding and gene mapping programs designed for salt tolerance.

IAEA-CN-263-29

EVALUATION OF DROUGHT TOLERANCE RICE MUTANT LINES AND IDENTIFICATION USING SSR MARKERS

Aryanti Aryanti, Ishak Ishak

Center for Applications of isotopes and Radiation, Nuclear Energy Agency, Indonesia

Email: ishakazra@yahoo.com

Marginal land in Indonesia occupy around 100 million ha, most of these land is dry land. These dry lands can be used for agriculture purposes with Crops tolerance to drought. Developing of rice tolerance to drought through mutation induction is becoming important strategy to stabilize rice production. Seven selected drought tolerance rice mutant lines obtained from mutation induction of Mira-1 variety by the dose of 25 and 50Gy gamma rays at primordial stages have been conducted. SSR markers such as RM 212, RM 302, RM 3825, RM 470 and DRO1 have been used in PCR reaction. The field evaluation of agronomic characters was conducted during dry season at 2016. and sequencing evaluation also done by DRO1 marker. The result shown that mutant lines more tolerant to drought compared their own parent plant, the highest productive tiller found at 26C1 mutant line was 20 compared to Mira-1 variety only 8 productive tillers, the number of grain content per panicle were 78.2%. Based on SSR marker linked to root depth, DRO1 showed that the mutant lines polymorphic, and changing of genetic of mutant lines which were indicating tolerant to drought compared to their wild type. Based on sequencing analysis showed that deletion of 4 bp DNA occurred at 7E1 mutant line and it was similar to Kinandang pathon variety. The objectives of this research were to evaluate the performance of rice mutant lines under drought stress conditions, and the genetic changes of mutant lines using SSR markers.

IAEA-CN-263-35

AGRONOMIC MORPHOLOGICAL STUDY OF SELECTED IRRADIATED COTTON VARIETY DURING M₃ GENERATION

Khin Myat Lwin¹, Aye Aye Thwe¹, San Thandar¹, Than Than Nu²

¹*Ministry of Education, Myanmar*

²*Ministry of Agriculture, Livestock and Irrigation, Myanmar*

Email: khinmyatlwin12@gmail.com

For sustainable cotton production against climate change in Myanmar, well-developed, fresh and uniform cotton seeds were irradiated with 50 to 500Gy of gamma ray. According to the germination test and field condition, we selected the doses from 200 to 350Gy for further generation studies. The desirable mutants having higher yield, early maturity, resistant to CLCuV disease etc. were selected and advanced to M₃ generation. Ten mutant plants of each treatment were evaluated for agromorphological characters compared with the control plants. The mean value of plant height (141.2cm), no. of square/plant (48.7), no. of flower/ plant, (2.5) and total boll number/plant (48.8) of 300Gy were higher

than other treatments and control. Even the shortest plant height was observed in 200Gy, early maturity and larger boll size was also found in 200 and 250Gy. The highest no. of square/plant was observed in 350Gy after 300Gy, however, after heavy rain, defoliation rate is higher than other treatments and control. Yield and fibre quality test will be studied for specific line selection of desirable trait and screening to heat tolerant.

IAEA-CN-263-44

IMPROVEMENT OF RESISTANCE TO KILLER WILT DISEASE THROUGH INDUCED MUTATIONS IN LAND RACES OF PIGEONPEA (*CAJANUS CAJAN* (L.) MILLSP.)

S. Muniswamy, Rachappa Haveri
University of Agricultural Sciences, India
Email: muniswamygpb@gmail.com

Pigeonpea [*Cajanus cajan* (L.) Millsp.] ranks sixth in global grain legume production. India is the largest producer and consumer of pigeonpea with a cultivated area of 5.13 m ha. 'Wilt' is the major disease of this crop, caused by the soil borne *Fusarium udum* Butler. The Northern part of Karnataka state in India is the major producer of pigeonpea. Local landraces viz., 'Bennur' and 'Katti beeja' are predominantly grown in that region to the tune of 40 per cent area because of their high yield and good quality 'dhal' meal. However, these said varieties are highly susceptible of Fusarium wilt disease. Fusarium wilt resistance being a oligogenic trait, mutation induction using gamma (γ) rays at various doses 100, 200, 300 and 400Gy was used with objective of improving wilt resistance in the aforesaid landraces. About 450 dry seeds of each landrace were irradiated and seeds were sown in the wilt screening plot, along with controls (0Gy) following a randomised block design in 3 replications to raise M₁ generation during kharif 2016. About 40 desirable apparently wilt resistant plants with in each treatment were chosen and M₁ generation harvested and planted in a plant to row progenies basis in a wilt prone field to raise M₂ progeny. Seeds from selected M₂ were then planted to raise M₃ progeny. Selection of desirable plants with wilt resistance was carried out in M₂ and M₃ generations. Per cent disease incidence (PDI) = (Number of plants wilted)/ (Total number of plants) \times 100 Results: Per cent seed germination and % wilt resistance in M₁ generation. Percent germination decreased with the increase dose of γ rays and less wilt (%) in higher dose of treatment. Conclusion: In M₃ generation 3 progeny rows of 'Bennur' and 2 progeny rows of 'Kattibeeja' showed resistance to wilt. Non-segregating and high yielding among these progenies will be used for commercial cultivation.

IAEA-CN-263-45

EVALUATION OF THE SERKS FAMILY GENES AND YIELD OF SOME BARLEY INDUCED LINES USING GAMMA RAYS FOR DROUGHT TOLERANCE

Adel Elmaghrabi, Abdulmunam Fellah, Elmundr Abughnia
Biotechnology Research Center, Libya
Email: maghrabiam@hotmail.com

In this study, we assessed the performance of five mutations of barley (*Hordeum vulgare* L) resulting from irradiation of mother variety Ecsad 176 by two doses of gamma rays (150 and 200Gy) under drought stress. Mother variety Ecsad 176 and two controls, California Mariout and Barjuj, were used for comparison. The results showed that three mutants lines showed insignificant increase when compared to the mother and two checks varieties for seed weight/plant, higher number and weight of grains main spike and along main spike. One mutant line, 5/1/150, out-yielded significantly the three controls, which indicates that gamma irradiation produced a new mutant line with increased yield under drought conditions. We also assessed gene expression of somatic embryogenesis receptorlike kinases

(SERKs) in those mutants, controls and other accessions. We found that The HvSERK1/2 transcript was significantly up-regulated and peaked in the leaf of 5/1/150 mutant line. We did not detect significant variation in gene expression of gene HvSERK3 between the mutant lines. In general, SERKs family genes showed the highest expression level in the leaf compared with roots and stems. These results suggest that barley SERKs genes may participate in barley development and plant response toward drought stress. This knowledge could be applied in targeted breeding, with the possibility of increasing the mutation induction of plant drought tolerance.

IAEA-CN-263-53

GENETIC DIVERSITY OF SELECTED CAPSICUM ACCESSIONS DERIVED THROUGH MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION

Kumuduni Senarathne Menike, Dhammika Wickrama Arachchige Rohini

Department of Agriculture, Sri Lanka

Email: bmkumudu@yahoo.com

Capsicum chinense and *C. frutescens* are common cultivated chilli species in some parts of Sri Lanka. Genetic diversity of thirteen *Capsicum* accessions was assessed by morphological and molecular means. Plants were raised in a randomized complete block design with two replicates during yala 2016 and maha 2016/17 at the Field Crops Research and Development Institute, Mahailuppallama, Sri Lanka. Twelve morphological characters were recorded and analysed using analysis of variance (ANOVA) and multivariate methods. ANOVA revealed significant differences among genotypes. In a principal component analysis, the first 3 components explain more than 71% of total variability. Plant height, width, days to 50% flowering, pods per plant and yield were positively correlated with PC1. Pearson correlation coefficient showed a significant positive correlation with some of morphological traits. Dendrogram derived by morphological and molecular analyses given two and three clusters respectively at 0.1 similarity levels and both analysis showed comparable results. A total of 45 alleles were detected in 15 microsatellite markers for the 13 *Capsicum* accessions. Out of these 15 SSR loci 14 loci showed polymorphism. Genetic diversity ranged from 0.00 to 0.75 with an average of 0.51. The PIC value varied from 0.13 to 0.70 with the average of 0.44. To date molecular characterization data of *Capsicum* accessions is limited in Sri Lanka. Therefore, this study will facilitate more detailed characterization of *Capsicum* accessions using morphological and molecular markers.

IAEA-CN-263-59

ESTABLISHMENT OF A HIGH EFFICIENCY ANTHER CULTURE SYSTEM IN WINTER WHEAT

Linshu Zhao, Huijun Guo, Jiayu Gu, Yongdun Xie, Junhui Li, Luxiang Liu, Shirong Zhao, Yuping Ding, Ziwei He

Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, China

Email: zhaolinshu@caas.cn

Anther culture plays important roles in the breeding, construction of doubled haploid (DH) genetic population, mutagenesis and transgenic researches. DH increases efficiency of selection of mutants and accelerate development of varieties. Five characters of anther culture of six winter wheat genotypes were identified through the vernalization, generation acceleration in greenhouse, using anther culture method in spring wheat established by Broughton. We aimed to investigate whether the anther culture method can be applied for the isolated haploid culture of winter wheat (to be reconsidered: is it anther culture of haploid plants, which normally do not bear normal gametes or anther culture to develop haploid plants?). The effects of low temperature pre-treatment for 3-15 days before inoculation were studied. Results showed that the tested parameters showed significant differences among different genotypes. Six winter wheat genotypes exhibited better initiating rate and differentiation ability of green

plantlets. The callus induction rate, green plantlets differentiation rate, and green plantlets productivity rate were 116.5%, 5.8% and 10.3%, respectively, indicating a preliminary establishment of an anther liquid floating separation system for winter wheat genotypes. The young seedling of two genotypes showed reduced callus induction rate and green seedling yield, as well as increased albino seedling after pre-treatment at low temperature. Although the green seedling yield rate in the low temperature groups was lower than that in the control, a certain amount of green seedling was also obtained. Therefore, if the collected samples cannot be used for inoculation immediately, the spikes may be stored at 4°C. In summary, the anther culture method in spring wheat can also be used for winter wheat by the combination of vernalization with generation-adding technique in greenhouse. The optimized procedure expected to contribute to mutation breeding in wheat.

IAEA-CN-263-62

DEVELOPMENT OF A NOVEL SOYBEAN CULTIVAR SUINONG 44

Chengxi Jiang, Yashu Fu

Suihua Branch Institute, Heilongjiang Academy of Agricultural Sciences, China

Email: chengxijiang@126.com

Suinong 44 was obtained through hybridization breeding of soybean varieties. Kenfeng 16 and Suinong 22 were used as female and male parents, respectively. In April 2016, this variety passed the examination and was approved by Heilongjiang Committee of Crop Variety Examination and Approval because of its outstanding characteristics, such as high-yielding, disease-resistant, strong lodging resistance, high density, as well as good agronomic characters. The protein content of Suinong 44 was 39.59% and the fat content was 20.74%. The growth period of Suinong 44 is 118 days, during which the total sum of temperature to be actively accumulated should be about 2320°C. In 2013 and 2014, the average yield of regional test was 3137.6 kg/ha, and the yield was 10.6% higher than the control variety of Hefeng 51. In 2015, the average yield of production test was 3311.8 kg/ha, and the yield was 83.1% higher than the control variety of Hefeng 51. The cultivar is suitable for growing in the central part of Heilongjiang province. Suinong 44 is suitable for sowing in early May, with seedling density of 240 000-300 000 per hectare, or seedling density of 400 000 per hectare with narrow row close planting. Under normal conditions of cultivation, application of fertilizer diammonium phosphate 135 kg/ha, urea 20 kg/ha and potassium 45 kg/ha is needed for optimal production.

IAEA-CN-263-72

GAMMA-RAY INDUCED MUTATION BREEDING FOR SPEEDY RICE IMPROVEMENTS

Akemi Shimizu, Feng Li, Hiroshi Kato, Sayaka Niwa

Radiation Breeding Division, Institute of Crop Science, NARO, Japan

Email: sayakaniwa@affrc.go.jp

New demands for various traits are arising in plant breeding. Gamma-ray irradiation can correspond to these demands promptly by adding valuable traits to leading varieties more easily. In Japan low beta-carotene and low lignin contents are favorable for rice whole crop silage (WCS). For food and animal feed rice, glabrous glume is desirable for reducing storage cost by decreasing storage space required in country elevators. We irradiated the seeds of WCS high yielding rice variety 'Tachisugata' with gamma-ray. Mutants with yellow-green leaf blades known to have low beta-carotene were selected from about 2500 M₂ lines. The mutants decreased their yields by 6.0 to 9.5%. Around 40% of the beta-carotene decreased mutants were selected and developed within three years. A gold hull mutant known to have a morphological low lignin content was selected from about 7000 M₂ plants. It took two years to develop it. This trait was obvious only just after panicle emergence. The lignin content in M₃ was 22% lower than in 'Tachisugata'. Whole-genome resequencing revealed that a causable mutation was a missense on *GH2*. Although the mutant decreased its yield by 5.6 to 7.0%, lodging resistance was comparable to

'Tachisugata'. Furthermore, another mutant with glabrous glume was selected from about 1900 M₂ plants of high yielding rice variety 'Oonari' within one year. Also, mutants with reduced hair were selected from about 800 M₃ lines and over 10 000 M₂ plants of the leading variety 'Koshihikari' known for good eating quality. Gamma-ray irradiation can promptly add new traits to original leading varieties. It is known that the number of deleterious mutations induced by gamma-ray is few in whole rice genome, which is favorable for the rapid selection of mutants that are almost identical to the original elite varieties.

IAEA-CN-263-77

TESTING M₈ ADVANCED BARLEY MUTANT LINES FOR MATURITY, LODGING RESISTANCE AND YIELD IMPROVEMENT

Nizar Mir Ali¹, Bassam Al-Safadi¹, Dana Jawdat¹, Hussam Al-Faouri¹, Ola Moustafa²

¹Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, Syrian Arab Republic

²General Commission for Scientific Agricultural Research, Syrian Arab Republic

Email: djawdat@aec.org.sy

Mutation breeding has been as a successful strategy for widening the genetic base of major crops. Syria is a centre of origin for barley (*Hordeum vulgare* L.) and it is mainly cultivated for livestock feed. Arid and semi-arid regions in Syria need new barley varieties with vigorous early growth, early maturity, lodging resistance, drought tolerance and increased yield. Seeds of a local cultivar, Furat 9, were treated with 100 and 200Gy doses of gamma radiation. Fourteen selected mutant lines (M₈) with their parent (Furat 9) were tested for the above traits at two locations: South of Syria in Ezra'a (AAR 292 mm), and in the North in Sfeera (AAR 276 mm). Data analysis showed late heading (8-14 days) in genotypes grown in Sfeera as compared to Ezra'a. Nonetheless, Mutant lines M7 and M20 showed accelerated maturity from heading to maturity in less than 8 days compared to their counterparts in Ezra'a, while the parent variety had 16 days difference. Non-significant positive correlations (0.278 and 0.063) were noted between plant height and lodging in Ezra'a and Sfeera, respectively. Both M7 and M20 showed lower lodging resistance than the parent in Sfeera and Ezra'a, respectively. Principal component analysis (PCA) of the agricultural traits studied showed a distinctive separation of the genotypes in the two locations, with M20 and M7 distant from the parent in Ezra'a and Sfeera, respectively. Testing of these advanced mutant lines in multiple locations, enables the segregation of genotypes that meet our objective agricultural traits from others. It also provides the mutant genetic material to study and investigate their physiological and biological processes under different environments towards a better understanding of crop behaviour and adaptability.

IAEA-CN-263-79

HAPLOIDY IN RICE (*ORYZA SATIVA* L.) MUTATION BREEDING FOR STRIGA RESISTANCE

Mehran Enayati Shariatpanahi¹, Samira Tajedini¹, Abdelbagi Mukhtar Ali Ghanim², Baratali Fakheri³, Mahnaz Oroojloo¹, Nafiseh Mahdinejad³

¹Department of Tissue and Cell Culture, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Iran

²Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture Plant Breeding and Genetics Laboratory, Austria

³Dept. of Agronomy and Plant Breeding, Faculty of Agriculture, Zabol University, Iran

Email: mehran.shariatpanahi@abrii.ac.ir

The parasitic weeds *Striga asiatica* and *S. hermonthica* cause a significant reduction in the yield of rice. Identification of resistant hosts by application of mutation breeding and doubled haploidy is one of the

most viable control options. The aim of this research was to develop androgenesis technology for production of mutant doubled haploid population in rice. Different inductive pretreatments including cold shock (4-7°C for 7-21 days), heat shock (30-33°C for 1-10 days), and starvation stress (B medium/ mannitol 0.3 M) were applied on four Iranian rice cultivars. Anthers containing microspores at the mid to late-uninucleate stage were excised from the central part of the panicles. Different induction media including AT3, NLN-13, B5, N6 and MS were applied in onelayer and two-layer culture media for shed/anther cultures. In shed-microspore culture, embryos were formed when anthers excised from pre-treated panicles (4°C for 10-12 days) and cultured in the two-layer culture media (B5 medium with 6% sucrose as the below and B5 medium with 3% sucrose + glutamine as the above). Cultures incubated at 33°C/4d or 30°C/10d resulted in shedding of microspores and then embryos were formed after 20 days. In anther culture, calli were formed in induction L8 medium from pre-treated panicles (4°C for 8-10 days). Calli (2-3 mm) were transferred to the regeneration MS medium supplemented with 2 mg/L Kinetin and 5 mg/L NAA led to the highest rate of shoot regeneration. The haploid induction was proven through flowcytometry and seed setting methods. The protocol developed was successfully led to haploid rice plants. Chromosome doubling methods are being developed. Doubled haploid lines will be produced on irradiated seeds for screening of Striga resistant mutants.

IAEA-CN-263-81

IMPROVEMENT OF POPULAR NEPALESE RICE VARIETIES THROUGH MUTATION BREEDING

Bindeshwar Prasad Sah¹, Binesh Man Sakha¹, Dil Raj Yadav², **Rajib Kumar Yadav**²

¹*Biotechnology Division, Nepal Agricultural Research Council, Nepal*

²*National Rice Research Program/Nepal Agricultural Research Council, Nepal*

Email: ydv.dilraj@gmail.com

Mutation breeding has been widely used to upgrade the well adapted crop varieties by altering one or two major important traits which limit their productivity or enhance their quality. In Nepal, rice mutation breeding works have been started since 2013 aiming to improve the agronomic and disease tolerant traits of four popular rice varieties namely Sabitri, Radha-4, Khumal-4 and Jumli Marshi. In this regard, seeds of these four commercial rice varieties were gamma irradiated at four different doses of 100, 200, 300 and 400Gy at radiation facility of Batan, Indonesia, and the radio-sensitivity test revealed their LD50 to be 271, 300, 294 and 238Gy, respectively. Bulk irradiation at their respective LD50 doses was done at the FAO/IAEA Laboratories, Seibersdorf, Austria. Thereafter, the irradiated M₁ seeds of Sabitri and Radha-4 were sown at the research block of National Rice Research Program, Hardinath, Dhanusha (Terai-plain areas) and that of Khumal-4 and Jumli Marshi at Agronomy Division, Khumaltar (mid-hill). Based on five selection criteria namely, maturity period, % filled grain, number of panicles per hill, panicle length and 1000-grains weight, 10, 11 and 12 promising mutant progeny lines of Radha-4, Sabitri and Khumal-4, respectively were pre-selected from M₃ population and transplanted in June 2017 for generation advancement. Seed of M₄ generation of these varieties are ready for initial evaluation in field in the rice growing season during May 2018. The preliminary results show that the present study would finally lead to rapid enhancement of rice varieties with improved grain yield and increased biotic and abiotic stresses.

ENHANCING EFFICIENCY OF MUTATION BREEDING FOR STRIGA RESISTANCE IN SORGHUM BY HAPLOID TECHNOLOGY

Mehran Enayati Shariatpanahi¹, Samira Tajedini¹, Mukhtar Ali Ghanim², Baratali Fakheri³, Mahnaz Oroojloo¹, Nafiseh Mahdinejad³

¹*Department of Tissue and Cell Culture, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Iran*

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

³*Dept. of Agronomy and Plant Breeding, Faculty of Agriculture, Zabol University, Zabol, Iran*
Email: mehran.shariatpanahi@abrii.ac.ir

Plant breeding programmes are based on creating genetic variation and selection of desired genotypes. Induced mutation and selection has been used extensively to improve yield, quality, disease and pest resistance in a wide range of crops. Root parasitic weeds in the genus *Striga* are the major biological constraints to sorghum production in sub-Saharan Africa and parts of Asia. Progress in haploid/doubled haploid production, such as the rapid generation of large microspore-derived haploid populations via androgenesis, may be exploited in mutation breeding. The present study was conducted to develop and apply haploid protocols in sorghum, which has been a recalcitrant species for this procedure. Starvation stress using B medium, cold (4-7°C for 1-7 weeks) and heat shock (30-33°C for 1-6 days) were used as pre-treatments on cultivars/lines KFS 18, ICSR14001, ICSV112 and S-35 (provided by ICRISAT). Microspores were isolated by different isolation procedures (stirrer and blender) and cultured on various induction media (AT3, NLN-13, B5 and N6) and incubated at 30-33°C for 1-6 days and then transferred to 25°C in the dark. For the first time in sorghum microspore embryogenesis was observed: multi-cellular structures were formed when microspores were isolated from cold-pre-treated panicles, and incubated at 33°C/3d then cultured in AT3 induction medium. The protocol developed is being improved to get embryos and finally doubled haploid plants, and then to exploit the system to produce doubled haploid mutants which can be introduced in a mutation induction programme then screened for *Striga* resistance.

EVALUATION OF TWO ADVANCED COTTON MUTANT LINES IN A DIFFERENT CLIMATIC AREA FROM THEIR GEOGRAPHIC ORIGIN

Dana Jawdat¹, Aghyad Saleh¹, Khin Myat Lwin², Ljupcho Jankuloski³, Manzoor Hussain⁴, Massoud Malek⁵, Md. Kamrul Islam⁴, Mohammad Al-Ali¹, Nowrez Taheir¹, Tianzhen Zhang⁶

¹*Department of Molecular Biology, Atomic Energy Commission of Syria, Syrian Arab Republic*

²*Ministry of Education, Syrian Arab Republic*

³*International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

⁴*Cotton Breeding Programme, NIAB, Pakistan*

⁵*International Atomic Energy Agency, Division of Asia and the Pacific, Austria*

⁶*Zhejiang University, China*

Email: djawdat@aec.org.sy

Crop yields are prone to decrease even with small climatic change due to the predominance of dry lands and their high temperature sensitivity. Understanding the effects of climate change on cotton phenology and yields, may help in assessing and introducing optimal management systems to enhance the quantity and quality of cotton yields. Evaluation of advanced mutant lines in different climatic areas from their geographic origin can provide better understanding of the newly developed line under diverse

environmental conditions. Seeds of two advanced cotton mutant lines, NIAB-KIRAN and NIAB-414, developed in Pakistan using gamma irradiation, were tested in Syrian conditions along with two local cotton varieties (Aleppo-118 and Deir Al-Zour 22) during the growing seasons May-October in 2016 and 2017. The cotton genotypes were evaluated consistently for their two years performance for the attributes like; 1st sympodial node number, 1st sympodial node height, number of bolls up to the 10th sympodia, number of shedding bolls up to the 10th sympodia, plant height, and yield per plant. Data analysis (ANOVA) showed the consistent behavior of NIAB414 in both years, considering all studied traits except for 1st sympodial node number and height. A remarkable steadiness in number of shedding bolls, plant height and yield were noted in this mutant line. Positive correlations ($R = 0.676$) was noted between height and number of bolls, and less but still positive correlation value ($R = 0.246$) between height and number of shedding bolls. Principal component analysis (PCA) of studied agricultural traits displayed the proximity of NIAB414 data in both seasons, whereas, NIAB-KIRAN showed different height and yields. Evaluation of advanced mutant lines in different geographical and climatic areas will fortify analysis and assist in exploring new traits under different environmental conditions.

IAEA-CN-263-91

CLOSE SESAME: MERELY MAGIC OR AN EFFICIENT SCIENTIFIC TECHNIQUE?

M. İlhan Cagirgan

Antalya Mutation Project, Department of Field Crops, Faculty of Agriculture, Akdeniz University, Turkey

Email: cagirgan@akdeniz.edu.tr

Sesame (*Sesamum indicum* L.) is an ancient oilseed crop still very much appreciated and is suitable to different crop management systems in tropics and subtropics. Despite this antiquity, the main problem in its cultivation is the shattering at maturity by splitting of the fruits; the so-called capsules along their sutures and spreading of the seeds to ground. This wild plant characteristic prevents its harvest by combine and requires manual harvest which is expensive and time-consuming. Since the only spontaneous non-shattering mutant found in Venezuela in 1943 not satisfactory in terms of yield, many programs over the globe intended to induce such mutants. The first non-shattering closed-capsule induced mutants were obtained experimentally for the first time by gamma rays in Turkish genetic-backgrounds in the frame of an IAEA Coordinated Research Project. Despite their low fertility and weak agronomic performance at the beginning, modification has been possible in the changed genetic backgrounds and through forced recombination by irradiation. The trait was recessive and allelic to the known spontaneous *id* gene. The aim of this communication is to present all story of selecting closed capsule mutants and its repeatable success both in Turkish and African backgrounds with the conclusion that selecting unique induced closed capsule mutants is not a matter of “lucky chance” but irradiating with effective doses of gamma rays to yield loss of function mutations; growing quite large M_2 populations, preferably in plant progeny rows; and careful screening.

AMINO ACIDS AND CYCOCEL APPLICATION TO ENHANCE CUCUMBER HAPLOID EMBRYOGENESIS WITH GAMMA IRRADIATED POLLEN

Mehran Enayati Shariatpanahi¹, **Hamed Ebrahimzadeh**¹, Ali Eskandari², Behzad Ahmadi³,
Mohsen Niazi¹

¹*Department of Tissue and Cell Culture, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Iran*

²*Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Karaj, Iran*

³*Department of Maize and Forage Crops, Seed and Plant Improvement Institute (SPII), Agricultural Research, Education and Extension Organization (AREEO), Iran*

Email: ebrahimzadeh.h@ut.ac.ir

Polyamines and cycocel (CCC) play important roles in the regulation of embryogenesis. The present study was conducted to investigate the effect of different concentrations of putrescine and spermidine polyamines along with CCC on the number of somatic embryos formed from gamma irradiation of pollen grains in cucumber. The F1 hybrid cv. "Extreme" an Iranian pure line cv. "Dasjerd" were used as the mother plant and the pollen donor, respectively. Mother plants were sprayed with putrescine, spermidine, and CCC each at 0, 50, 500, and 5000 mg/l. Anthers were gamma irradiated at 300Gy using a Cobalt-60 gamma ray source with an activity of 0.2Gy/s. The irradiated pollen was used for pollination of isolated female flowers. Well-developed fruits were harvested at day 28 and then their embryos were rescued. Chromosome counting and flow cytometry were used to confirm haploidy induction. The highest number of haploid embryos were obtained when mother plants sprayed with putrescine at 500 mg/l (5.2 embryos/fruit), spermidine at 50 mg/l (4.8 embryos/fruit), and cycocel at 50 mg/l (5.2 embryos/fruit). The lowest haploid embryos were observed when 5000 mg/l of spermidine (0.4 embryos/fruit) or cycocel (2.0 embryos/fruit) were applied. Spermidine at 50 mg/l also efficiently increased the conversion of derived embryos into fully developed plantlets (up to 100%). Ploidy analysis using flow cytometer indicated that all regenerated plantlets were haploid, i.e. contained the gametic chromosome number ($n = x = 7$). Chromosome counting also confirmed the haploid nature of regenerated plantlets. The induction of haploid embryogenesis from unfertilized ovaries after pollination with gamma irradiated pollen and subsequent conversion of derived embryos into the plantlets could be improved in *C. sativus* L. when appropriate levels of putrescine, spermidine, and cycocel were applied.

GROWTH OF WHEAT MUTANTS UNDER DROUGHT STRESS

Shatha Yousif, Maha Taaen, Nora Abid, Tagreed Abduljabar

Ministry of Science & Technology, Iraq

Email: yousifshatha@yahoo.com

Hydroponic culture was performed to investigate the physiological responses of three mutant lines with their wild-type parent under drought stress. Mutants; Noor 5, Noor6 and Noor7 along with cv. Al Noor (parent) were subjected to 2 levels (0 and 10%) of polyethylene glycol (PEG-6000). Growth at the seedling stage was evaluated. Differential Display Reverse Transcriptase DDRT was applied to study the genetic markers related with drought tolerance using 9 random primers (OPA-1, OPA-5, OPA-11, OPC-4, OPC-8, OPF-12, OPH16, OPO11 and OPR7). The mutants along with cv. Al Noor were also planted under rainfed conditions. Drought tolerance indices (Stress Tolerance Index, Mean Productivity, Geometric Mean Productivity, Stress Tolerance and Stress Susceptibility Index) were calculated. Correlations between stress indices under the hydroponics experiment and grain yield under rainfed

conditions were investigated. Water stress reduced root length and fresh weight in all genotypes, but increased shoot length, fresh and dry weight in mutants Noor 5 and Noor 6. The results revealed that PEG treatment had positive effects in seedling shoot length, shoot fresh weight, root dry weight in the mutant genotypes, but not cv. Al Noor. All primers succeeded to amplify DNA fragments. The result of field experiment revealed that Noor6 surpassed all genotypes in the number of tillers m⁻¹ and grain yield. Grain yield under water stress had positive and significant correlations with major stress indices: shoot and root length, shoot and root fresh weight, shoot dry weight and malic enzyme under hydroponic experiment. Based on these data, it can be concluded that seedling growth parameters could be used as selection criteria for drought tolerance in wheat. DDRT-PCR could differentiate the genotypes and maybe used in developing markers related to drought tolerance.

IAEA-CN-263-96

IMPROVEMENT OF KENAF (*HIBISCUS CANNABINUS* L.) THROUGH GAMMA RAY INDUCED MUTATION

Faiz Ahmad, Abdul Rahim Harun, Affrida Abu Hassan, Ahmad Nazrul Abd Wahid, Mohd Noor Hidayat Adenan, Mohd Zulmadi Sani, Mustapha Akil, Zaiton Ahmad
Malaysian Nuclear Agency, Malaysia
Email: faiz@nm.gov.my

Kenaf (*Hibiscus cannabinus* L.), a tropical crop from the Hibiscus family, is one of the world's most economically important fibre crops particularly in the Asia-Pacific region. In Malaysia, kenaf was identified as one of the main commodity crops and could be the country's next golden crop after oil palm. Currently, kenaf-based products are getting higher demand from local and international market. Thus, the improvement of kenaf crop for high fibre production is highly needed to meet the demand from the industries. Mutation induction is a method to increase genetic divergence associated with selection, recombination, or a combination of these approaches in plant breeding. This method is very promising to plant breeders for developing a new plant variety with desirable traits. In this project, seeds of a kenaf variety (V36) were used as starting materials for mutation induction and were obtained from National Kenaf and Tobacco Board (NKTB). These seeds were irradiated using gamma ray and mutation induced via acute and chronic irradiation methods. The aim of this research project was to develop new mutant varieties with high fibre characteristics. Radiosensitivity tests were done for determination of optimum dose for irradiation to get the higher mutation rate without eliminating desirable traits. Screening and selection for desirable traits will be done for several generations until stable mutants with targeted traits are obtained. At present, several potential mutants with desirable morphological and agronomical traits were observed in M₃-M₄ generations. Thus, gamma ray induced mutation is useful and reliable tools for kenaf breeding program in this modern era.

IAEA-CN-263-97

GENETIC DIVERSITY FOURTEEN SOYBEAN MUTANT LINES USING SSR MARKERS AND YIELD PERFORMANCE UNDER DRY LAND CONDITION

Yuliasti Yuliasti¹, Refflinur Refflinur²

¹Center for Isotopes and Radiation Application, National Nuclear Energy Agency, Indonesia

²Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Indonesia

Email: upikyuliasti@yahoo.com

Development of drought-tolerant varieties is an effective alternative to overcome this abiotic stress. The objectives of this research were to evaluate the genetic diversity and relationship among soybean mutant

lines using SSR markers and to select promising mutant lines with high yield under dry land condition. A total of 14 soybean mutant lines derived from induced mutation (Mutant A, B, C, D, E, F, G, H, Y, K, L, M, N, P) and two control varieties (R=Panderman and S=Muria) were used in present study. Those genetic materials were grown at Gunung Kidul and Bantul (country). Results revealed five mutant lines (E, B, G, P and Y) exhibiting a higher yield (2.63, 2.51, 2.32 t/h, respectively) than the control variety Panderman (2.05 t/h). These mutant lines exhibited significant differences in 100-seed weight (14.3, 14.5, 14.3 gr) number of pot fill (118, 116, 104) and number of seed per plant (215, 218, 205) as compared to national control drought tolerant variety (Dering: 14.1, 103.6 and 201.3). A wide genetic distance between soybean mutant accessions and their wild-type were observed by using 12 simple sequence repeats (SSR) markers. A total of 58 alleles with an average of 4.8 alleles per locus were detected. The polymorphic information content varied from 0.40 to 0.75 with an average of 0.53. The accessions were clustered into two groups according to their genetic background, namely group I (B) and group II (R= the Panderman). The highest genetic diversity value of 0.78 was shown by the Satt294 primers. Six informative markers (GMES3515, GAAT47, Satt009, Satt038, Satt294, and Satt431) provided polymorphic alleles between the parents and their mutants.

IAEA-CN-263-99

INVESTIGATING YIELD AND YIELD COMPONENTS OF 14 CONTROL AND GAMMA-IRRADIATED BARLEY (*HORDEUM VULGARE* L.) CULTIVARS IN THE TEMPERATE ZONE IN IRAN

Ali Eskandari, Azam Borzouie, Saeed Soufizadeh, Sorayya Navid
NSTRI, Nuclear Agriculture Research School, Iran
Email: aeskandari@nrcam.org

In plant breeding, mutations play an important role in the development of genetic variations and new varieties. The use of induced mutations has been found to be very useful not only in practical plant breeding but also in theoretical genetic research. Barley is model species for mutation breeding and an important strategic crop that plays an important role in food security in Iran and the world. A field experiment was carried out by using randomized complete block design with three replications. Treatments included 14 cultivars of barley (13 normal cultivars and one gamma irradiate barley). Various morphological traits, yield and yield components were measured. Results showed that there were significant differences among the 14 barley cultivars in the majority of studied traits. The early maturity, dwarf and six-row cultivars Fajr 30 and Rayhan 03 performed better compared to tall and late-maturity (Valfajr, Makouee) and two rowed (Behrokh and Dasht) cultivars. Although, gamma irradiated barley (Roudasht) had less yield than Fajr 30 and Rayhan 03, it performed better than other cultivars. Results showed that significant and positive correlation existed between grain yield and spike length, awn length, peduncle length, number of seeds per spike, number of spikes, number of fertile tillers per plant, spike, straw and peduncle dry matter, 1000-kernel weight, harvest index and biological yield. Correlations between grain yield and plant height, number of infertile spikelets per spike, number of total tiller (fertile and infertile) per plant, and number of infertile tillers per plant were significant but negative.

IMPLEMENTATION OF MUTATION INDUCTION TO IMPROVE BARLEY PRODUCTION

Habibah Al-Menai, Merlena Babu, Nisha Aneesh, Ouhoud Al-Ragam

Kuwait Institute for Scientific Research, Kuwait

Email: hmanaie@kisir.edu.kw

Production of mutant crop varieties with sustainable high yield under Kuwait's environmental conditions could increase crop production within the constraint of desert ecosystems and reduce its import in Kuwait. A study to address this was implemented under a 'Technical Cooperation (TC) programme' (2016-2017) of the IAEA. It evaluates the potential of mutation breeding to develop new improved barley lines with desirable characteristics and thus enhance barley productivity sustainably under harsh environmental conditions of Kuwait. Three selected barley genotypes were mutated using gamma rays (in cooperation with the FAO/IAEA's Plant Breeding and Genetics Laboratory, Seibersdorf, Austria). Mutated seeds of each barley variety were planted in the greenhouse in Kuwait. M₁-spike progeny were harvested to form the M₂ seed population. The head-rows of M₂ were planted in the field along with parental lines to compare and observe putative mutants. The M₃ family rows were sown in the field and the plants developed were subjected to various phenotyping screening procedures for drought and salinity tolerance. Changes were observed in the germination and growth pattern in the mutant lines tested, these included seedling height, number of tillers, number of secondary spikes, number of spikes, number of spikelets and number of seeds. Presently the M₃ plants are producing the next mutant generation (M₄). The present study forms the preliminary phase of development of a sustainable national strategy for the improvement of barley production in Kuwait. Extensive studies are recommended to release novel barley mutant varieties with improved yield, nutrient quality and drought and salinity tolerance.

EFFECT OF BRACKISH IRRIGATION ON THE GROWTH OF SOME MUTANT BARLEY

Ouhoud Al-Ragam, Abdullah Al-Shatti, Habibah Al-Menai

Kuwait Institute for Scientific Research, Kuwait

Email: oragom@kisir.edu.kw

Increasing agricultural production in an arid land like Kuwait is a major challenge owing to its harsh environmental conditions. A study was implemented by the Desert Agriculture and Ecosystems (DAEP) programme at the Kuwait Institute for Scientific Research (KISR) as part of an IAEA Coordinated Research Project to test the growth and performance of mutant barley lines in Kuwaiti agronomic practices/conditions. The main objective was to evaluate the performance of four mutant lines along with 11 other genotypes of barley grown under irrigation with brackish water. A split plot design with three replicates was used to ascertain the effect of fresh and brackish irrigation on fifteen barley genotypes including four mutant genotypes with the parental lines and six local genotypes were used to select the best varieties with maximum yield adapted to Kuwait environmental condition. Salt stress adversely affected the overall growth and reproductive capacity of the cultivars under study soil, as it decreased almost all the growth and yield attributes under study such as heading and maturity dates, plant height by 37%, number of kernels/spike, 1000-kernel weight by 27.4%, grain yield and straw yield 15%. Number of spikes/m² was significantly affected by the type of irrigation water and was decreased by 41.2% the results showed insignificant interaction between irrigation and cultivars. The type of water irrigation had significant effects on the number of kernels per spike in all barley cultivars under study. Brackish water decreased the number of kernels per spike by 15%, and grain yield was decreased by 19.9%. Mutant lines showed good performance under brackish irrigation in Kuwait.

EVALUATION OF SOME BARELY MUTANT LINES IN KUWAIT**Abdullah Al-Shatti**, Habibah Al-Menai, Ouhoud Al-Ragam*Kuwait Institute for Scientific Research, Kuwait*

Email: alshattiabdullah@gmail.com

Mutant barley genotypes that have evolved under stress conditions using the improved genetic resources have the desired morphological, physiological and agronomic traits. The Kuwait Institute for Scientific Research (KISR) initiated this study in collaboration with the International Atomic Energy Agency (IAEA) to test the growth performance of barley mutant varieties by adopting suitable agronomic practices. The response of the mutant barley variety (Golden Promise) along with five barley genotypes of Kuwait (Kuwait 1, 2, 3, 4 and 5) under Kuwait's environmental conditions was investigated. The experiment was carried out in completely randomized block design (CRBD) with three replications. All the recommended cultural practices were adopted. Plants were harvested in different stages, after two months of plantation and at maturity. Data was collected on parameters including germination percentage, number of days to heading and to maturity, number of spikes/m², kernels/spike, plant height, 1000 kernel weight, and grain yield (ton/ha). Data was statistically analysed using statistical software. The results showed significant differences among cultivars for all the targeted characters. The cultivar Kuwait 2 recorded the highest plant height (111.2 cm). Regarding the number of tillers per plant, Kuwait 6 recorded the highest value (29.79) and ari-e 156 recorded the lowest number of tillers. It was also shown that Kuwait 4 was the earliest genotype in heading while, ari-e 1 was the earliest genotype to get to maturity. The cultivar Kuwait 3 recorded the highest biological yield per plant (91.52) while, Kuwait 6 recorded the highest grain yield (29.54) The heaviest grain was obtained from Kuwait 2 (5.37g). The Maythorpe variety obtained the highest harvest index (41.05%). The findings showed potentiality to grow mutant barley genotypes for good yield production under Kuwait harsh condition.

DETERMINATION OF 50% GROWTH REDUCTION DOSE (GR50) OF GAMMA IRRADIATION FOR LOCAL LANDRACES OF CAULIFLOWER (*BRASSICA OLERACEA* VAR. *BOTRYTIS*) AND CABBAGE (*BRASSICA OLERACEA* VAR. *CAPITATA*) IN MAURITIUS**Ravi Seewoogoolam**, Rita Devi Nowbuth*Food and Agricultural Research and Extension Institute, Mauritius*

Email: rseewoogoolam@yahoo.com

Cauliflower *Brassica oleracea* var. *botrytis* and cabbage *Brassica oleracea* var. *capitata* are two important cruciferous crops in Mauritius. Local landraces of both crops are available which are adapted for growth during the cooler months of the year but are susceptible to black rot disease caused by *Xanthomonas campestris* pv *campestris*. A mutation breeding programme was initiated to improve these two landraces, using gamma rays, for disease tolerance and adaptation to hotter climate. The first step in a mutation induction breeding programme is to estimate the dose of irradiation treatment through determination of the dose which reduce seedling growth by 50% (GR50) for the varieties concerned. The moisture content of cabbage and cauliflower seeds were stabilised to 12-14% and these seeds were irradiated using a ¹³⁷Cesium source. Eight different doses (100, 200, 300, 400, 500, 600, 700, and 800Gy) were used for cabbage and six different doses (500, 700, 900, 1100, 1300 and 1500Gy) were used for cauliflower. The irradiated seeds of cabbage and cauliflower together with the control were sown in seed trays in a complete randomised design with three replicates. For both cabbage and cauliflower plant seedling survival and height decreased with increasing dose of irradiation. The GR50 based on height of seedling for cabbage was found to be 522Gy and for cauliflower to be 756Gy. This

showed that cabbage had lower radio sensitivity to gamma rays compared to cauliflower. The GR50 dose obtained for cabbage and cauliflower will be used to irradiate bulk seeds to produce M₁ generation in the mutation breeding programme.

IAEA-CN-263-111

INDUCED GENETIC VARIABILITY FOR YIELD AND HEAT TOLERANCE IN TOMATO (*SOLANUM LYCOPERSICUM* L.)

Banumaty Saraye¹, Bradley J. Till², Joanna Beata Jankowicz-Cieslak³, Rita Devi Nowbuth¹, Sabinaz Peerboccus¹

¹*Food and Agricultural Research and Extension Institute, Mauritius*

²*Department of Chromosome Biology, University of Vienna, Austria*

³*International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

Email: bballuck@gmail.com

Tomato (*Solanum lycopersicum*) is highly sensitive to heat stress at flowering stage. Flowers drop at high temperature and this may result in 80% yield reduction. Variety MST32/1, commonly grown in Mauritius, is sensitive to heat stress. An improvement program for this variety through gamma irradiation of the seed was undertaken to identify potential high yielding varieties with heat tolerance ability. Irradiation assays were carried out to establish the GR50 dose. 3000 seeds were irradiated at identified GR50 dose (400Gy) to generate the M₁ population. Morphological abnormalities were noted in the M₂ generation for plant growth habit, leaf morphology and fruit shape. These included lines exhibiting determinate to semi-determinate growth habit, standard and potato leaf shape and oblong to round fruit shape. The lines were also screened for heat tolerance in controlled condition at 35°C and 26°C. The sub-sequent mutated populations were screened up to M₆ generations and selection of candidate mutant lines was made based on plant and fruit characteristics. Selected lines were assessed for yield performance in replicated trials at two different agro-climactic conditions. Twenty mutant candidates exhibiting characteristics which may highly correlate with both, higher yield and heat stress tolerance were identified. An average of 7 to 16% higher yield was observed in five mutant lines in the replicated trials. Some also exhibited lower yield but higher tolerance to heat stress during controlled condition screens. Two mutant lines based on instantaneous chlorophyll fluorescence, quantum yield and leaf scorching, were identified as showing heat tolerance ability, which was confirmed in the controlled environmental conditions. Tomato mutants with high yield and heat tolerance characteristics, thus being adapted to climate change will be released to farmers for a constant supply on the market.

A DECADE OF PROGRESS IN COWPEA GENETIC IMPROVEMENT USING MUTATION BREEDING IN ZIMBABWE

Prince M. Matova¹, Collis Mukunguritse², Dumisani kutywayo¹, Edmore Gasura³, Fatma Sarsu⁴, Hussein Shimelis⁵, Onismus Chipfunde⁶

¹*Crop Breeding Institute, Zimbabwe*

²*Soils and Chemistry Research Institute, Zimbabwe*

³*University of Zimbabwe, Zimbabwe*

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

⁵*University of KwaZulu Natal, South Africa*

⁶*Genetic and Biotechnology Research Institute, Zimbabwe*

Email: matova_p@yahoo.com

Cowpea has continued gaining popularity due to its wide stress tolerance and high nutritive value. The crop is highly adapted to areas marginal for crop production. It is a niche crop, improving livelihoods and sustaining crop-livestock farming systems in the wake of climate change. In Zimbabwe different generations of mutant lines have been developed through gamma irradiation and advanced through single plant selections. Desirable characteristics including drought tolerance, pods above canopy and uniform maturity have been developed and fixed. Fourteen advanced pure-line mutants were extensively tested during three seasons in Zimbabwe. The lines were evaluated for grain yield potential, seed size, adaptation and stability across environments under optimum, random stress and farmer managed conditions. Trials were laid out in randomised complete block designs (RCBD) with four replications on-station at 14 sites and, unreplicated under on-farm at 8 sites. Genotype plus genotype-by-environment interaction (GGE) biplots and the, Lin and Binns and Wricke's ecovalence stability coefficients were used to assess the adaptability and stability of the genotypes and depict the most desirable cultivar. The mutant cultivar CM/150/M6-1 was selected and released as it combined stress tolerance, high grain yield potential, adaptability and stability across environments. The cultivar outperformed CBC1 (its parent) and CBC2 (a stable cultivar) on-station ($P < 0.05$) but performed similarly to CBC2 on-farm. Cultivar CM/150/M6-1 has grains that are significantly ($P < 0.001$) larger than those of CBC1 and CBC2 by at least 8%. It is a determinate, short season cultivar that matures uniformly. It has good resistance to major cowpea diseases and insect pests including blights, scab, viruses and aphids. Cultivar CM/150/M6 is a good replacement for CBC1, it is recommended for production in high and low potential production environments.

IAEA-CN-263-114

PROMISING LINES OF LUPINUS MUTABILIS SWEET DERIVED FROM MUTATION INDUCTION WITH IONIZING RADIATIONS

Marco Sinche-Serra¹, José Velásquez², Juan Aguilar-Aguilar¹, Trajano Ramirez¹

¹*Escuela Politecnica Nacional, Ecuador*

²*Instituto Nacional de Investigaciones Agropecuarias, Ecuador*

Email: marco.sinche@epn.edu.ec

The cultivation of *Lupinus mutabilis* Sweet is considered strategic for the alimentary food security of the Andean countries, due to its high content of protein, fat, carbohydrates, minerals and fibre. Ongoing research focuses on developing new lines with disease resistance and improved agronomic traits. Regarding the first goal, the production of *L. mutabilis* is facing a major challenge associated with a destructive disease that affects the stems and pods, called anthracnose. This disease is caused by the fungus *Colletotrichum lupine*, and it is transmitted by infected seeds. To obtain seeds free of this

pathogen, we irradiated them with doses of 1.2, 1.5, 2.0, 4.0, 8.0 and 16.0 kGy in a ELU-6U linear electron accelerator beam. Previously, we had determined that the decimal reduction dose (D10) for *Colletotrichum* spp. was 0.7 kGy. It was observed that the dose of 2.0 kGy allowed a 99.0% elimination of the spore-forming fungus. Then, the effect of the radiation dose on the initial development of the plants was evaluated. The germination and vigour percentages were between 89.37 and 92.08% in the control, and between 83.13 and 89.47% in the plants derived from seeds irradiated with 2.5 kGy. The plant heights, 30 days after sowing, were 24.61 and 12.40 cm, respectively. The Ecuadorian Lupinus breeding programs try to combine higher yields, low alkaloid levels, high seed protein and oil contents, and early flowering and maturity. Mutation breeding has just been incorporated in the program. Currently, plants resulting from seeds irradiated with doses from 25 to 350Gy in a Cobalt-60 source are being evaluated. So far, they show high germination rates and vigour values, and several morphological differences. We expect to have the first harvest in July 2018.

IAEA-CN-263-115

EVALUATION OF *BRACHIARIA RUZIZIENSIS* AND *BRACHIARIA BRIZANTHA* MUTANTS ON FIELD ESTABLISHMENT, DIVERSITY AND PERFORMANCE

Annah Indetie¹, Stephan Nielen²

¹Kenya Agricultural and Livestock Research Organization (KALRO), Kenya

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

Email: indetie2001@yahoo.com

Poor nutrition is one of the major constraints for livestock productivity in Kenya and this is due to recurrent droughts affecting the plants used as fodder. These pasture deficiencies can be mitigated by breeding and selecting highly nutritive pastures that are drought tolerant. *Brachiaria* spp. is a perennial drought tolerant grass. It has been introduced in Latin America, Southeast Asia, and northern Australia where it has revolutionized grassland farming and animal production. The potential of *Brachiaria* spp. in its native land remains unexploited. Seeds of two landraces of *Brachiaria ruziziensis* (KE and BE) and one of *B. brizantha* (LE) were irradiated using gamma rays at different doses (10, 20, 30 and 40Gy) with the objective of identifying agronomically desirable *Brachiaria* mutants. The M₁ plants were observed and from M₂ to M₆ generations selections were performed for agronomic characteristics. At M₅ selected plants were established in randomized complete block design in three replications at different locations. Phenotypic data on germination, nutrition, and yield were collected and analysed. The dry matter yield was significantly enhanced in mutants exposed to 40Gy. Nine M₆ mutant lines were selected. The selected mutants have been isolated and their seeds bulked. The mutant seeds are recommended for adaptability and stability studies in different agro-ecological zones in Kenya. The selected genotypes are valuable genetic resources for genetic enhancement and breeding.

RADIATION BASED INDUCED MUTAGENESIS FOR DEVELOPING NUTRITIONALLY ENRICHED RICE VARIETIES

Parmeshwar Sahu Parmeshwar¹, Ashish Kumar Tiwari Ashish¹, B.K. Das Bikram², Deepak Sharma Deepak³, Samrath Baghel Samrath¹, Satyapal Singh Satyapal¹, Vikash Kumar Vikash²

¹*Indira Gandhi Krishi Vishwavidyalaya, India*

²*Bhabha Atomic Research Centre, BARC India*

³*Indira Gandhi Agriculture University, India*

Email: parmagpb@gmail.com

Rice is the staple food of more than half of the world's population as well as more than 70% of the Indian population, yet it lacks many life-supporting nutrients. People who cannot afford a balanced diet through costly supplementary foods, suffer from multiple micronutrient deficiencies and malnutrition problem. Some 600 rice landraces of Chhattisgarh state, India were agro-morphologically characterized to screen for high yielding rice genotypes. A total of 215 high yielder rice landraces were selected and subjected to grain nutritional and quality profiling. Several landraces were identified as having high nutritive value but they are tall in nature, late maturing and some have undesirable plant stature. Superior rice landraces identified for these traits were subjected to further improvement for reduced height, early maturity and better plant stature by radiation based induced mutagenesis. Gamma radiation was used to irradiate seed of selected landraces with 250Gy and 300Gy at the Bhabha Atomic Research Centre (BARC), Mumbai, India. Field screening and mutation breeding activities have been carried out at the Indira Gandhi Krishi Vishwavidyalaya, Raipur, India. Landraces, 'Luchai, Vishnubhog and Badshabbhog' were identified as superior for overall grain quality traits. Landraces, 'Karhani, Kareni Dhan, Jhilli' had higher amount of both, iron and zinc contents. Landraces, 'Gangachur, Maidubraj, Chhindmauri' had higher quantity of linoleic acid and linolenic acid. Highest oleic acid was observed in 'Kadamphool, Bathrash, Badshabbhog Selection-1'. Out of these, Karhani, Vishnubhog, Jhilli, Luchai, Maidubraj landraces were subjected to further improvement through induced mutagenesis. Some desirable mutants with high nutritive value have been selected at the M₄-M₆ generations. Nutritionally enriched rice mutants with reduced height, early maturity and better plant stature will be very fruitful for poor farmers of Chhattisgarh for increasing their income and for eradicating the malnutrition problem.

INDUCE MUTATION FOR EARLY MATURITY IN PATHUMTHANI 1 (PTT1) RICE VARIETY

Manika Noi-Iam¹, Boonsirichai Kanokporn², Kanchana Klakhaeng³, Vararas Khamvarn²

¹*Chachoengsao Rice Research Center, Thailand*

²*Thailand Institute of Nuclear Technology (Public Organization), Thailand*

³*Rice department, Thailand*

Email: aui_pu@hotmail.com

Phatumtani 1(PTT1) rice variety is famous and well known among Thai consumer for its soft and delicious taste with low amylose content, and high yield. The harvesting day is 120 days, and it is susceptible to rice blast disease and brown planthopper. The electron beam irradiation was used to induce mutation for early maturation and also for resistance to disease and insect pest. Phatumtani 1 was irradiated using electron beam at doses of 300 and 400 gray. It was found that, at M₂ generation the harvesting period was reduced from 120 days to 113-115 days in 174 lines. The number of filled grains per panicle was of about 10-30 seeds. The characteristics regarding the plant height were varying

from semi-dwarf to intermediate height. These 174 rice lines were screening for rice blast disease and brown planthopper resistance. In M₃ generation, 11 lines were identified as quite resistant to the rice blast disease, while most of varieties were susceptible to blast and almost all mutant lines showed susceptibility to BPH. There were 7 lines which were early maturing and could be planted in the salt water intrusion areas during dry season where farmers could harvest before the period of salt water intrusion, or in the flood plain areas by planting before and after flood.

IAEA-CN-263-120

DEVELOPMENT OF DROUGHT TOLERANT MUTANT LINES FROM RICE VARIETY MANAWTHUKHA USING MUTATION BREEDING TECHNIQUE

Myat Myat Minn¹, Khine Khine Zar Linn²

¹*Biotechnology Research Department, Myanmar*

²*Ministry of Education, Myanmar*

Email: minnmya@gmail.com

Water deficit reduces plant growth and development, leading to lower yield. This study was focused on developing drought tolerance rice mutant lines with the same grain quality as the original rice variety and on determining the effects of water stress on promising mutant lines of rice var. Manawthukha. Dry seeds of rice, Manawthukha were irradiated with the dose of 300Gy of gamma rays from ⁶⁰Co source. The treatment of water exclusion was applied to the plants from 90 days after sowing (DAS) and throughout reproductive stage until harvesting time. A selection process was made in the M₂ generation based on agronomic traits. The selected promising M₂ lines were evaluated against non-irradiated control plants in M₃ generation. The desiccation of apical spikelets in stressed panicles was found during the summer cultivation in M₄ generation. Hence, the control plants were not fertile under water deficit condition while other mutant plants were fertile at that time. The two best mutant lines namely MK-D-2 and MK-D-3 were selected in M₆ generation and characterized by using physiological screening techniques such as relative water content (RWC), soil moisture content and yield. In comparison with the well-watered condition, the mutant lines cultivated in drought stress condition produced less grain. The relative water content of mutant lines was between 91% and 93% while those of control plants were ≤ 83%. In M₅ generation, rice grain qualities of drought tolerant potential lines were determined in the laboratory. Finding shows that there were no large differences in amylase content of rice samples from potential lines and control. Among the potential mutant lines, MK-D-3 showed higher husking yield and total mean milling recovery than the control and other mutant lines.

IAEA-CN-263-121

APPLICATION OF GAMMA IRRADIATION FOR THE IMPROVEMENT OF CAULIFLOWER, CABBAGE AND CARROT IN MAURITIUS

Rita Devi Nowbuth, Nema Cahoolessur, Priya Huzar Futtu Beejan, Ravi Seewoogoolam

Food and Agricultural Research and Extension Institute, Mauritius

Email: ritanowbuth@yahoo.com

The main crucifers (cauliflower and cabbage) and carrot are crops of notable economic importance in Mauritius. Black rot disease caused by the bacterial pathogen, *Xanthomonas campestris* pv *campestris*, is causing severe yield losses in these crucifers. The disease has evolved and the new strain causes leaf spot especially in cabbage leading to considerable loss in yield and quality of produce. Introduced varieties have been evaluated to identify tolerant ones with little success. On the other hand, carrot is susceptible to *Alternaria* leaf spot and in the recent years, carrot cultivation has been affected by carrot motley dwarf disease causing stunting of the plants as well as reddening and yellowing of the leaves.

Thus, growers are now heavily dependent on imported hybrid seeds and also on chemical pesticides. Hence, developing mutant lines for these crops using induced mutation techniques could decrease dependence on chemical pesticides and imported varieties, and ensure year-round production. A study using nuclear techniques was initiated in 2016 and irradiation was carried using a Gamma irradiator from a ¹³⁷-Caesium source. Cauliflower was subjected to doses ranging from 500 to 1500Gy, cabbage from 100 to 700Gy and carrot from 25 and 45, 450-750 and 500-1500Gy. The growth reduction dose at 50% was determined as 756Gy for cauliflower, for cabbage it was 522Gy while for carrot the GR30 was calculated to be 1075Gy. Now that the GR50 and GR30 have been identified for all 3 crops, seeds will be irradiated and field testing be undertaken to identify mutant lines adapted in different agro-climatic zones and in different seasons.

IAEA-CN-263-122

INTROGRESSION OF BLAST RESISTANCE GENE INTO RICE CULTIVAR BC15 THROUGH MARKER-ASSISTED SELECTION

Thanh Pham, Hoan Nguyen
Field Crops Research Institute, Viet Nam
Email: thanhpttm@gmail.com

Rice blast, caused by *Pyricularia grisea* is the most serious fungal disease of cultivated rice (*Oryza sativa* L.) causing significant yield loss in the North of Viet Nam. Of the 24 identified blast resistance genes from Blast monogenic lines, 11 of these were evaluated as resistance to blast pathotypes in the North of Viet Nam. In this study, Pita gene was introgressed into cultivar BC15 with good agronomic traits to strengthen its blast resistance levels through marker-assisted backcross breeding. The marker RM7102 linked to Pita gene was used to screen F1 backcross populations as force selection coupled with stringent phenotypic selection. Advance backcross line of BC5F4 generation was produced from the cross of cultivar BC15 with Pita donor line, IRBL12. Phenotypic screening against blast disease indicated that advanced homozygous blast resistant line was strongly resistant against pathotypes 757.6 and 651 in the blast disease endemic areas. The morphological, yield, grain quality, and yield-contributing characteristics were significantly similar to those of BC15. The newly developed blast resistant improved line will contribute to widening cultivation area of the highly adoptable BC15 by farmers.

IAEA-CN-263-132

YIELD VARIATION AND GENETIC DIVERSITY AMONG KEWAL LOCAL RICE MUTANT LINES FROM INDONESIA BASED ON STS MARKERS

Azri Kusuma Dewi¹, Ita Dwimahyani¹, Reflinur Basyirin²
¹*Center for Isotopes and Radiation Application, Indonesia National Nuclear Energy Agency (CIRA-BATAN), Indonesia*
²*Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Indonesia*
Email: azrikusumadewi@gmail.com

Mutation induction is one of the purposeful approaches to increase genetic variability for developing new cultivars. Local rice variety Kewal was exposed to gamma rays with the aim of inducing stable mutants with desirable traits. The objectives of this research were to evaluate yield and yield components of local rice mutant lines, and to analyse the genetic diversity and relationship among mutant lines using STS markers. The study was conducted during the growing season of 2012-2013. The experiment was laid out in a randomized block design with four replications. Six mutant lines and

two parental lines as control were tested for evaluation of yield and yield components under 16 environments. To evaluate genetic variation among selected mutant lines and their discrimination from parental lines in molecular level, a cluster analysis was performed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in the NTSYS software. The results showed that four mutant lines, including OBS-1814/PsJ, OBS-1813/PsJ, OBS-1801/PsJ dan OBS-1802/PsJ produced the highest grain yield compared to the other mutant lines and the parents. Based on 13 STS markers used for clustering analysis, all mutants showed 100% differed from their parent. However, these mutants did not differ from each other at the tested loci. Based on these studies two promising mutant lines could be released as new rice mutant varieties.

IAEA-CN-263-134

GENETIC ANALYSIS AND CORRELATION OF GROWTH AND YIELD COMPONENTS OF RICE MUTANT LINES UNDER THREE DIFFERENT ALTITUDES

Sherly Rahayu¹, Desta Wirnas², Hajrial Aswidinnoor²

¹*National Nuclear Energy Agency Indonesia, Indonesia*

²*Bogor Agricultural University, Indonesia*

Email: sherlyrahayu@yahoo.com

The primary environmental factor which causes weather damage is basically temperature, whether low and high temperature. It causes yield loss through decreasing yield components. This study was aimed to evaluate the phenotypic correlation and broad sense heritability related to low temperature stress as a result of varying altitude. Experimental design was randomized complete block design with three replications. The experiment was conducted in the rainy season at three different altitudes (Banjaran with 700 m above sea level, Ciburuy with 900 m above sea level and Boyongbong with 1200 m above sea level) and examined 20 rice mutant lines and their parents. The result indicated that there was a similar positive correlation of agronomic trait which highly significant for all locations, i.e. tiller number with grain yield, panicle length with flag leaf length, flowering date with grain weight and grain filled percentage with grain filled number as well grain yield. Most of the yield components had broad sense heritability with high category ranged from 0.51 for filled grain numbers to 0.86 for flowering date. The crucial factor to improve grain yield in highland conditions is how to reduce the unfilled grain number which is the main limiting factor affected by low temperature stress during flowering and fertilization. Since the filled grain number trait has high heritability, a low yield could be improved by optimizing the cold tolerance and planting time to escape the low temperature injury during flowering and fertilization.

IAEA-CN-263-139

INCREASING THE GENETIC VARIABILITY FOR THE IMPROVEMENT OF MAIZE FOR ENHANCED TOLERANCE TO DROUGHT AND NITROGEN STRESSES

Freddy Bulubulu Otono, Aimé Diamiuni Ndofunso

Commissariat Général à l'Énergie Atomique, Democratic Republic of the Congo

Email: freddybbl@yahoo.fr

In Democratic Republic of the Congo (DRC), maize (*Zea mays* L.) production encounters constraints such as low soil fertility, low adaptability of genotypes to climate, rains variability, diseases and soils acidity. In order to explore genetic variability maize seed irradiated using a Cobalt-60 gamma source. With doses ranging between 75, 150, 300, 450 and 600Gy. A control maize seeds batch was not irradiated. The optimum dose was calculated precisely from GR50 i. e., the doses leading to a 50% growth reduction. Bulk irradiation of a larger number of seeds was done using the doses of 350 and

348Gy respectively for both accessions. M₁ seeds were planted in the fields at Luvuvamu locality in the Province of central Kongo in DRC. M₂ seeds were planted in the field, the agro-morphological parameters of plants M₃ were evaluated according to maize descriptors while being based on the selected variables. The descriptive analysis showed significant phenotypic variability for 24 agromorphological characters. 12 Katanga M₃ lines presented male flowering between 53 and 57 days whereas the minimum number of days for such flowering are 58 days at M₀ plants (non-irradiated plants). 1 line had 3 ears with a single stalk. For 3 Bukidibukidi M₃ lines male flowering established between 53 and 55 days whereas the minimum was 56 at M₀. 4 ears presented the number of M₄ grains varying between 457 and 610 g. The maximum number of grains obtained on ears was 456 at M₀. In this study, gamma radiation caused morphological changes and also increased variability in quantitative traits of maize lines.

IAEA-CN-263-141

EVALUATION OF BARLEY MUTANT VARIETIES AND ADVANCED LINES FOR FOOD AND FORAGE PRODUCTION FOR THE HIGHLANDS OF PERU

Luz Gomez-Pando

Universidad Nacional Agraria La Molina, Peru

Email: luzgomez@lamolina.edu.pe

In Peru communities located above 4000 meters, live mainly from husbandry and use the land exclusively for this purpose and those located at lower altitudes combine farming with livestock. Barley has been adapted to the highland and grow well above 3000m, and has been cultivated since XVI century in the adverse conditions of the highlands. In the highland region, 70% of barley grain production is used for human consumption directly as pearled grains, flakes and flour. Also, barley straw is used as main feed for farm animals. Prolonged droughts in the highlands have driven incentives to look for crops able to produce high quality food and forage with less water. There is evidence of improved grain yield, biomass production and nutritive quality for barley by mutation induction. 264 barley mutant lines derived of the cultivar UNALM 96 using gamma ray and commercial cultivars were evaluated for food and feed production at highland locations. Parameters such as grain yield, flowering and maturity days, total biomass yield, harvest index, were evaluated to define the agronomic performance. Protein content in grain, test weight and 1000-grain weight were measured to determine food value. Straw protein, ash content, Neutral detergent fiber (NDF), NDF digestibility (NDFD), crude protein (CP) were used to determine feed value. 24 mutant lines were selected for agronomic performance and quality traits for food and feed uses in different experiments, among them UNALM 96 M6h-602 were selected for good agronomic performance and food quality traits. For feed quality UNALM 96 M6h-1 and UNALM 96 M6h-665 because its highest value of relative forage quality (RFQ). The mutant lines selected had better values than the original cultivar UNALM96.

DEVELOPING STRESS TOLERANT TEPARY BEAN THROUGH MUTATION BREEDING

Carmenza Munoz Florez¹, Daniel Deobouck², Idupulapati Rao², Sarsu Fatma³

¹*FIDAR, Colombia*

²*CIAT, Colombia*

³*International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

Email: carmenza.munoz@hotmail.com

Tepary bean (*Phaseolus acutifolius* A. Gray) is better adapted to heat and drought stress than common bean (*P. vulgaris* L.). Mutant lines of two cultivated tepary accessions (G40068 and G40159) were generated by EMS treatment. The M₆ mutated lines: CMT 38, CMT 109, CMT 187 and their original (M₀) accessions were evaluated for heat and drought tolerance under greenhouse conditions. Their performance was evaluated for morpho-physiological attributes, seed yield and yield components. Under high temperature and drought conditions, the CMT 38 mutant (M₆) line and its original tepary (M₀) accession (G40068) showed higher values of pod biomass, pod number and 100-seed biomass. The CMT 109 and CMT 187 mutant lines and their G40159 original accession (M₀) also showed the highest value of seed number. Under combined heat and drought conditions: the SPAD readings, seed biomass, 100-seed biomass and seed number parameters could be incorporated into tepary breeding programs, as selection criteria to screen genotypes for tolerance to heat and drought stress. These parameters explain the observed variance in the principal component analysis. The root biomass and the stem diameter were also identified as useful attributes, based on the univariate analysis. These results show that screening of these mutant lines, based on morphological traits like growth habit, is not negatively affected by the yield variables evaluated. The mutation breeding has the potential to generate phenotypic and genotypic variations in tepary that can be exploited by plant breeders in the development of new cultivars more adapted to heat and drought stress.

IAEA-CN-263-144

DEVELOPING MUTANT RICE GERMPLASM COMBINING GAMMA RAY MUTAGENESIS WITH DOUBLED HAPLOIDY IN NATIONAL AND INTERNATIONAL RICE BREEDING PROGRAMMES

Ayse Sen¹, Necmi Beser²

¹*Department of Biology, Faculty of Science, Istanbul University, Turkey*

²*Department of Genetics and Bioengineering, Faculty of Engineering, Trakya University, Turkey*

Email: senayse@istanbul.edu.tr

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, feeding more than 50% of the world's population. However, an exponential growth in the world's population, particularly in developing countries where rice is the major staple food resource, demands increased rice production. Moreover, recent abrupt climate changes make crop production more difficult. This situation has compelled plant breeders to produce climate change-resilient crops, which can withstand broad-spectrum biotic and abiotic stresses. Breeders urgently need to develop new genetic variation in accelerating crop improvement and achieving sustainable yield increases. Induced mutation through physical or chemical mutagenesis is a useful approach to produce novel variation. This is particularly powerful when combined with enabling biotechnologies such as doubled haploidy. Doubled haploidy is the fastest way of achieving completely homozygous plants (in one generation). After determining optimal irradiation dose treatments through radio-sensitivity analysis, dry seeds from two commercial rice cultivars and one candidate line were exposed to three different gamma ray doses to obtain M₁ mutant populations. During booting stage, panicles were collected for anther and microspore culture to

obtain double haploids *in vitro*. Androgenic calli were obtained using three different culture media. The rate of albino plants obtained from cultured androgenic calli from M₁ plants was higher than in controls (M₀). Gamma irradiations increased the rates of albinism in rice haploid/doubled haploid plants depending on the damage caused by the plant genome.

IAEA-CN-263-146

GENETIC IMPROVEMENT OF COMMON BEAN (*PHASEOLUS VULGARIS*) IN ZAMBIA

Kelvin Kamfwa, Kalaluka Munyinda
University of Zambia, Zambia
Email: kelvinkamfwa@gmail.com

Common bean (*Phaseolus vulgaris*) is an important crop in Zambia. It plays an important role in food and nutritional security of Zambia. Productivity of common bean in Zambia is low, with a national average yield of 500 Kg Ha. Several factors contribute to low productivity including the planting of landraces that have low yield potential, and are susceptible to diseases, pests, low soil fertility, aluminium toxicity and drought. The University of Zambia Bean Breeding and Genetics program is currently developing common bean varieties with multiple resistance to resistance to diseases and pests. Also, varieties with high nitrogen fixation ability and tolerance to aluminium toxicity are being developed. The program is conducting genetic studies using genome-wide association studies and QTL mapping approaches to understand the genetic basis of variation in complex traits such as biological nitrogen fixation, resistance to common bacterial blight, tolerance to drought and aluminium toxicity. The program is using mutation breeding to broaden the genetic base of common bean and create novel genetic variation to support genetic improvement of common bean.

IAEA-CN-263-147

IMPROVING YIELD AND COMMERCIAL POTENTIAL OF WHEAT FOR HEAT TOLERANCE BY MUTATION INDUCTION TECHNIQUES

Sergio Ahumada Flores¹, Cesiah de la Torre Amavizca¹, Eulogio de la Cruz Torres², Fannie Isela Parra Cota³, Luz Rayda Gómez Pando⁴, **Sergio de los Santos Villalobos**⁵

¹*Instituto Tecnológico de Sonora, Mexico*

²*Instituto Nacional de Investigaciones Nucleares, Mexico*

³*Campo Experimental Norman E. Borlaug INIFAP, Mexico*

⁴*Universidad Nacional Agraria La Molina, Peru*

⁵*CONACYT Instituto Tecnológico de Sonora, Mexico*

Email: dlsantosv@gmail.com

Worldwide, wheat is one of the most used cereal in human nutrition. However, its productivity has been affected by the increase of temperature, decreasing about 10% for each increment of 1°C. Therefore, wheat production demands the generation of new varieties with the capacity to tolerate increases of predicted temperature for future years under the climate change scenario. The dose studied in the field experiment were 100, 200 and 300Gy, following the radiosensitivity test from 0 to 600Gy (at intervals of 50Gy). In the field experiment, seeds (M₀) were irradiated using Cobalt-60, the M₁ seeds were sown in the 2016-2017 cycle, the material collected from M₂ was treated with Chlorothalonil following the standard NOM-EM-001-FITO-1994, and was sown in the 2017-2018 cycle for the identification of chlorophyll mutants and the development of germplasm for the M₃ generation. In the M₁ generation, in the field experiment, and in the radiosensitivity test the percentage of germination, survival and height were negatively affected by the doses of gamma rays used, obtaining in the dosimetry test a median lethal dose (LD50) of 433.88Gy. In the M₂ generation, an identification of chlorophyll mutants was

found, which were: albino, chlorine, viridis, maculata, tigrina, Striata, alboviridis, viridoalbina, among others. The dosimetry test helped in the identification of the LD50, which indicated the optimal dose to obtain the highest number of mutations without reducing the population below 50%. In the segregation of the M₂, different chlorophyll mutant's types were found, which may not have an economic value due to their lethal nature, although these can be of great help in identifying the effective dose of a mutagen, which will increase the variability and the number of useful mutants.

IAEA-CN-263-148

IMPACT OF MULCH-BASED CROPPING SYSTEMS USING GREEN MULCH AND RESIDUES ON THE PERFORMANCE OF ADVANCED MUTANTS LINES OF MAIZE (*ZEA MAYS* (L.)) UNDER INFESTED FIELD WITH THE PARASITIC WEED *STRIGA ASIATICA* (L.) KUNTZE IN MADAGASCAR

Harimialimalala Jhonny Rabefiraisana¹, Abdelbagi Mukhtar Ali Ghanim², Alice Andrianjaka¹, Berthe Rasoamampionona¹, Ljupcho Jankuloski², Mbolatiana Alinà Razafindrasoa¹, Nirina Hanitriniaina Ravelonjanahary¹, Noronirina Victorine Rakotoarisoa¹

¹*Plant Physiology Laboratory, Faculty of Sciences, University of Antananarivo, Madagascar*

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

Email: nyjahmalayah@gmail.com

In Madagascar, the cereals yield remains insufficient due to various biotic and abiotic constraints including *Striga asiatica*, a parasitic weed that has contributed to decrease maize yield up to 100%. This work aims at assessing the impact of the practice of two cropping systems on the maize crop infested by *Striga asiatica*. PLATA maize seed of the putative mutant tolerant line from the M₅ generation after gamma irradiation at 300Gy and of the sensitive parent variety were grown in fields naturally infested or artificially inoculated with one pinch of around 3000 of ready-to-germinate *S. asiatica* seeds. The residue of *Stylosanthes* sp., legumes was used as mulch SCVm and the Cowpea legumes was planted with the host plant for intercropping system SCVv. Results have shown that the use of mulch either residues SCVm or green mulch SCVv minimize *S. asiatica* infestation on maize plant. The SCV reduces significantly the number of *Striga* plants emergence of 1.33a for SCVm, 4.33b for SCVv then 15c for the Control. Moreover, M₅ lines have shown significant differences of survival plant rate of 51-80%, versus 13-50% for the parent variety. Yields of the parent and M₅ varieties on SCVm are respectively 3.46 and 4.64ton/ha; 3.3 and 3.61ton/ha for SCVv, while that of the Control block remains low, it varies from 1.39 to 2.29ton/ha. Cover increases the soil humidity and delays the *S. asiatica* development to the host plant then improve the host plant yield. These results demonstrate the benefit of integrated approach of mutation breeding and cultural practice to ensure more durable crop production under heavily *Striga*-infested.

INDUCED MUTATION FOR DEVELOPING MUTANT RICE LINES TOLERANT TO THE PARASITIC WEED *STRIGA ASIATICA* (L.) KUNTZE

Noronirina Victorine Rakotoarisoa¹, Abdelbagi Mukhtar Ali Ghanim², Alice Andrianjaka¹, Berthe Rasoamampionona¹, Harimialimalala Jhonny Rabefiraisana¹, Ljupcho Jankuloski², Xavier Roland Theophile Rakotonjanahary³

¹Plant Physiology Laboratory, Faculty of Sciences, University of Antananarivo, Madagascar

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

³Département de Recherches Rizicoles, FOFIFA, Centre National de Recherche Appliquée au Développement Rural, Madagascar

Email: noronathanielle@yahoo.fr

The parasitic weed *Striga asiatica* causes damage on rainfed rice culture and may reduce production from 75-100% in Madagascar. The lack of resistant variety is one of the main problems for rice production. This work aimed at screening rice mutant lines tolerant to *Striga asiatica*. Two rainfed *Striga* sensitive rice varieties B22 and F154 were used. After radiosensitivity test, 1000 rice seeds per variety were irradiated at 100, 200Gy for F154, and 300Gy for B22, then planted one per hole in the field to develop M₁ then M₂ generation. 200 rice seeds of each 500 M₂ genotypes per dose of irradiation per variety were planted in the field with artificial infestation of *S. asiatica* seeds. 5 rice seeds per pot of all selected genotypes from M₄ to M₆ were planted in the greenhouse under severe artificial infestation. Plant survival rate, infection rate per plant, number of *Striga* plant per pot, were considered for the tolerance evaluation then plant fertility rate, number of fertile panicle per plant, 100 seeds grain weight were assessed for agronomic traits. As a result, of this experiment 10 lines were selected as tolerant to *S. asiatica* from F154 and 12 lines from B22. The survival rate of the two parents was significantly lower (9.74a and 11.83a) than those of mutant lines (52.36b and 74.36b); the *Striga* plants emergence per pot were significantly higher for the parents (13.96c and 14.89c) as compared to the mutants (0.12a to 1.5b); the infection rate for the parents (7.37b and 7.86b) was higher compared to the mutants (2.27a to 2.74a); the fertility rate per plant for the two parents was lower (20.98%b and 22.29%b) but much higher for the mutants (72.19%b and 78.35%b); the average panicle number per plant for the parents was significantly lower (0,5a and 1a) than those of mutants (1,5b to 2,4bc) and the 100 grain weight of the parents are significantly lower (2.35a to 2.56a) than those of the mutants (3.19b to 3.23b). The culture of those mutant lines may increase rice production and contribute to enhancing food security in Madagascar.

GAMMA RAYS, EMS AND SODIUM AZIDE INDUCED GENETIC VARIABILITY FOR QUANTITATIVE TRAITS IN AJARA GHANSAL NON-BASMATI AROMATIC RICE

Nikhil Gaikwad¹, Akash Ramtake¹, Shitalkumar Desai¹, Vinod Dhole²

¹Dept of Botany, Shivaji University, Kolhapur, India

²Nuclear Agriculture and Biotechnology Division NABTD, Bhabha Atomic Research Centre, BARC, India

Email: nbgaikwadsu@gmail.com

Ajara Ghansal, a local non-basmati aromatic rice cultivar (landrace) adapted to the agro-climatic conditions of Ajara Taluk of Kolhapur, Maharashtra, India possesses a good aroma and good cooking qualities, thus is a very good potential for domestic market. However, it has a low yield potential. Five hundred (500) Ajara Ghansal seeds were subjected to mutagenic treatments using gamma rays, Ethyl methanesulphonate (EMS) and Sodium Azide (SA) separately to induce genetic variability. M₁ seeds

were harvested and planted in a plant-to-row basis to develop M₂ generation. Among M₂ populations, several mutations were identified: semi-dwarf plant height, early maturity, and good yield, as well as lodging resistance. Dwarf mutants with shorter culms were identified in plants derived from treatments with 0.8%, 1.0% and 1.2% EMS and 200Gy. Early maturing mutants were detected in EMS treatments (1.0 and 1.2%), SA (0.006%) and 200Gy treatments with 135-137 days of maturity as compared to control (160 days). Mutants with higher number of tillers were also identified in EMS (1.0%), SA (0.006%) and 150Gy, with numbers ranging from 61 to 70 per plant as compared to 19 tillers/plant in control. Mutants with increased panicle length ranging from 24.30 to 28.89cm were observed from treatments using EMS (1.0 and 1.2%), SA (0.006%) and 150 and 200Gy as compared to control (23.03cm). Mutants with higher yield/plant were detected under EMS (1.0%), SA (0.006%) and 150Gy treatments which showed higher yield/plant (138-349g/plant) as compared to control (58g/plant). Lodging resistant mutants were recorded in EMS (1.0 and 1.2%), SA (0.006%) and 150Gy treatments at the time of harvesting; where control plants have shown complete lodging. Among the mutagenic treatments, 1.0% and 1.2% treatments of EMS and 150Gy treatment of gamma rays were found to be the most effective treatments for induction of desirable mutants.

IAEA-CN-263-165

MUTATION BREEDING OF WHEAT FOR FOOD AND FEED PURPOSE

Dolgor Tsognamjil, Myagmarsuren Yadamsuren
Plant Science and Agricultural Research Institute, Mongolia
Email: dolgor_0711@yahoo.com

Wheat is a core agricultural plant, which constitutes about 90% of agricultural production of Mongolia. Wheat is used to necessity daily food requirement of the local population, and wheat straw is used for feed of livestock. There is therefore a need evaluate new varieties and advanced lines with high yield and good quality for food and feed through mutation techniques. Developing dual purpose wheat cultivars is a major activity of plant breeders in Mongolia. The mutant cultivar Darkhan-141 is suitable as a dual-purpose crop as biomass at heading and milk-ripe stage and straw. For food, average grain yield was 1.44 ton/ha, but yield potential is reached at 2.53 ton/ha, it has good bread making quality with 16.5% of protein and 33.3% gluten in seed and 4.0 of bread making score. For feed purposes this variety give good dry matter production at heading and maturity, yield of biomass is reached 8.35ton/ha at heading with 1.52% nitrogen and 16.16 ton/ha at milk maturing with 0.65% nitrogen. Straw yield averaged 5.5 ton/ha with 0.48% nitrogen, 94.6% dry matter, 6.5% crude ash and 36.3% fibre. The GA3-K effects significantly on the plant growth particularly in plant height increased 1.7-13.8cm. In case of biomass treated with PGR, Darkhan-196 had highest biomass yield after three GA-K salt application but Darkhan-209 and Darkhan-141 had higher biomass yield after one application. The yield was increased in the all treatments of Darkhan-210 by 0.1-0.31 ton/ha.

IAEA-CN-263-167

HIGH YIELDING AND EARLY MATURITY BARLEY MUTANT LINE OBTAINED THROUGH *IN VITRO* RADIO-MUTAGENESIS

Dalila Ramla, Mohand-Said Yakhou, Nassima Bilek
National Institute of Agronomic Research of Algeria, Algeria
Email: ramlada@yahoo.fr

In vitro cultures are important tools for plant improvement, they allow increased efficiency of selection and the regeneration of desired mutant plants in the presence of selective agents. They also allow *in vitro* multiplication of selected lines. The combination of tissue culture systems with mutagenesis

increases the effectiveness of creating and exploiting new genetic variabilities. Plaisant is a high yielding and drought sensitive, barley variety that was selected for *in vitro* radio-mutagenesis and subsequent *in vitro* mutation screening for drought-resistant mutants. Callus induced from immature embryos was irradiated with gamma ray (15Gy). After several cycles of maintenance, on a regeneration medium containing a selective drought agent (PEG/ 25%). Four M₁ seedlings were regenerated and placed, after acclimation, in a greenhouse until M₂ seeds were obtained. The four mutant lines (Plaisant1, Plaisant2, Plaisant3 and Plaisant4) were sown in the field for several years to ensure progression in generations. During these progressions, Plaisant1 showed poor field performance, whereas Plaisant3, singularised by showing early maturity (-17 days compared to the parent). Plaisant3 was retained and evaluated, together with Plaisant2, Plaisant4 (in M₈ and M₉ generations) and the parent, for grain yield. During these evaluations, Plaisant2 and Plaisant4 mutants showed lower grain yields than the parent. However, Plaisant3 was distinct in having a grain yield higher than the parent in some years: in year 1 it was less, 21.2 versus 27.9 quintal /ha, i. e 21 200 to 2790Kg to /Ha. The procedure used was effective for regenerating putative drought-resistant mutants, one of these lines showed was high yielding and early maturing and is ready for testing in semi-arid zones.

IAEA-CN-263-168

DEVELOPMENT OF GENETIC VARIABILITY FOR YIELD, EARLINESS AND MORPHOLOGICAL CHARACTERS BY INDUCED MUTATION IN LINSEED (*LINUM USITATISSIMUM* L.)

Joy Manjaya, Umesh Vaidya

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, BARC, India

Email: jgmanjaya@rediffmail.com

Linseed is one of the important rabi oilseed crops of India. Paira/utera (zero tilling) cropping system has been in practice for efficient use of residual moisture in rice fields. About 25% of the linseed area (0.5 million ha) is under utera cropping. Development of early maturing cultivars with high yield will be best suited for utera cropping to overcome the poor yield levels by maximising utilisation of residual moisture and nutrients present in the soil. One thousand seeds of linseed cultivar NL97 were exposed to 250Gy gamma rays from a ⁶⁰Co source and the treated seeds along with the parent were sown as M₁ generation. A total of 725 M₁ plants were harvested individually and the seeds obtained were sown as M₂ generation as plant to row progenies. In the M₂ generation, chlorophyll and viable mutants affecting morphological and physiological characters were identified. The morphological mutants included those affecting plant height, flower colour, sterility, leaf shape, number of pods per plant, seed colour, and days to maturity. Breeding behaviour and salient features of the true breeding mutants were studied up to M₃-M₇ generations. In the M₇ generation, six true breeding, early maturing mutants were evaluated for various quantitative characters. One of the mutants, TL-142, flowered in 39 days and matured in 107 days, in comparison to the parent NL97 (125 days). The early maturing mutant TL-142 (7.6g) showed significantly higher seed yield per plant as against the parent NL-97 (4.5g). In the current study, induced mutagenesis was successful in creating mutants with wide genetic variability, which can be further utilized in future breeding programme.

MUTATION BREEDING IN CHICKPEA (*CICER ARIETINUM* L.) FOR INCREASED GENETIC VARIABILITY, IMPROVED QUALITY AND AGRONOMIC TRAITS

Archana Joshi Saha, Golu Misra, Sreenivasulu Kandali Reddy
Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, BARC, India
 Email: archana.joshisaha@gmail.com

Chickpea is grown worldwide, with its major cultivation area lying in the semi-arid tropics of Asia. Terminal drought is a major abiotic stress for chickpea grown in these areas, causing severe yield losses. Considering the impact of climate change on increasing the severity of terminal drought, this study is focused on inducing genetic variability in chickpea suffering from a narrow genetic base and isolating mutants resilient to terminal drought. A cultivar 'Vijay' was irradiated using 200 to 500Gy gamma rays and electron-beam. Another popular cultivar JAKI9218 was irradiated with 300Gy gamma rays. The M₁ plants were individually harvested and planted as plant-to-row progenies of M₂ generation. The selected mutants were grown as plant-to-row progeny in M₃ and M₄ generation. Agronomically important mutants were identified in M₂, including an early maturing mutant (induced by 400Gy electron-beam) maturing in 80-85 days as compared to parent cv 'Vijay' that matures in 90-100 days. Another mutant showed 2-3-fold higher proline accumulation compared to 'Vijay'. This mutant has elongated phenotype but no adverse effect on plant yield. Proline accumulation might help the plant survive stress conditions. A slow-transpiring mutant of cv JAKI9218 was isolated. The canopy temperature of the mutant (measured using an infrared-thermal camera) confirmed the slow-transpiring phenotype, which can help conserving soil moisture and, hence, a better performance under terminal drought stress. The mutants were found to be true breeding for the traits in advanced generations. Furthermore, both electron-beam and gamma ray-induced mutants accumulating high amounts of iron and/or zinc were also identified. The mutants identified in this study not only constitute an important repository of germplasm useful for basic studies, but also are useful in breeding programs to develop climate resilient varieties.

INDUCED MUTATIONS IN DURUM WHEAT (*TRITICUM DURUM*) FOR IMPROVE PRODUCTIVITY COMPONENTS TRAITS

Aziz Salameh¹, Ismail Hroub², Ljupcho Jankuloski³, Rezaq Basheer-Salimia⁴, Yamen Hamdan⁵

¹*National Agricultural Research centre*

²*Ministry of Health, State of Palestine*

³*International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

⁴*Hebron University, Palestine*

⁵*Khadoorie University, Palestine*

Email: azbarghouthi@hotmail.com

Although durum wheat is the major field crop, old cultivars and landraces are still the predominant planting materials of farmers in Palestine. A sharp decrease in productivity has been noticed over the last few decades. New cultivars are required which would be early maturing, high yielding and drought resistant. Mutation breeding provides a good opportunity to achieve this goal in two-four years breeding programme period. To induce mutation, seeds of durum wheat landrace namely Kahla (M₀) were treated with Gamma ray (200Gy) at the FAO/IAEA's Plant Breeding and Genetics Laboratory, Seibersdorf, Austria. M₁ seeds were planted for seed multiplication only. In the next two growing seasons (2015/2016 and 2016/2017), 27 000 of M₂ plants, and 315 of M₃ mutant wheat lines were grown and subject to selection for earliness, number of tillers/plant, number of grains/spike, number of spikelets

per spike and plant height. The mean value for selected M₃ lines showed higher values for number of tillers/plant (7.27), number of grains/spike (55.17), and number of spikelets per spike (20.6) than the parent, Kahla. There was a range of values in the M₃ families for number of tillers/plant was (1-15), number of grains/spike (21-82), and number of spikelets/spike (14-26). Around 29 lines exhibited earliness in heading compare to Kahla. Currently, 110 mutant lines (M₄) were sown in the field using the spike-row method for a further round of selection. Two to four new Palestinian wheat mutant lines adapted to harsh conditions (early) are expected to be released as varieties.

IAEA-CN-263-177

SPECTRUM FREQUENCY AND SEGREGATING PATTERN OF SOME USEFUL MACRO MUTANTS IN SESAME (*SESAMUM INDICUM* L.) THROUGH INDUCED MUTATION

Sruba Saha¹, Amitava Paul¹, Sanjay Jambhulkar²

¹Department of Genetics and Plant Breeding, Institute of Agriculture, Visva Bharati, India

²Bhabha Atomic Research Centre, BARC, India

Email: sruba.saha@gmail.com

Induced mutagenesis has been effectively employed to improve the productivity and generate variability of morphological and physiological characters. In sesame, the plant architecture is poorly adapted to conventional farming system because of its seed shattering behaviour at maturity, indeterminate growth habit, and long maturity duration. Our aim was: 1) to select early maturing, non-shattering, determinate mutant types, 2) to study the breeding behaviour to make the sesame suitable for multiple cropping system for ensuring higher yield. Two popular variety in West Bengal, Rama and Tillotama were exposed to different doses viz.; 250, 300, 350, 400 and 450Gy of gamma rays at the Bhabha Atomic Research Centre and grown (along with control: unirradiated seeds) during pre-monsoon 2015, 2016 and 2017 as M₁, M₂ and M₃ generation, respectively, at the University experimental farm of Visva-Bharati in West Bengal, India. The spectrum of chlorophyll mutation observed in M₂ generation was narrow because only albina, chlorina and xantha mutations occurred in different treatments. Highest mutation frequency of chlorophyll mutations (1.38%) was induced at 350Gy for both varieties Rama and Tillotama, however, chlorina types were more frequent (0.93%). Different macro mutants were recorded under different doses in M₂ generation viz.; shattering resistant, early maturing types, determinate growth habit, cluster type capsules and combination of these traits. Breeding behaviour of the selected M₂ macro mutants were studied in M₃ generation and found consistent. All the three types of chlorophyll mutations along with macro mutants segregated in the ratio of 15 normal: 1 mutant, hence, suggesting that inheritance of chlorophyll and morphological mutation is governed by the double recessive genes. Induced mutation offers the possibilities of increasing useful variability in sesame, especially improved yield through determinate growth habit, non-shattering plant architecture and early maturity.

IAEA-CN-263-186

ISOLATION OF NOVEL MUTATION FOR DWARFING GENES FOR ENHANCING PRODUCTIVITY IN BRASSICA JUNCEA L

Sanjay Jambhulkar

Bhabha Atomic Research Centre, BARC, India

Email: sanjayjambhulkar41@yahoo.com

Indian mustard (*Brassica juncea*) is the second most important oilseed crops in India. Seed yield potential has stagnated to 1100kg/ha. Lodging and susceptibility to terminal heat are some of the limiting factors. Therefore, development of dwarf and early maturing varieties could help in increasing

crop productivity. Varuna is a high yielding variety used as a national check but it is tall and late maturing. Seeds of parent variety 'Varuna' were treated with 1000Gy dose of Gamma rays. A total of 3752 M₂ population was raised from 150 M₁ progenies. One progeny segregated as tall and dwarf plants. Progeny of dwarf plants was raised in M₃ generation and found true breeding genotypes were selected. Their breeding behaviour was also studied in M₄ generation and the dwarf mutation was confirmed. It was compared with parent and quantitative data was recorded. The plant height was reduced to 93cm and maturity to 70 days compared to 168cm and 130 days of the parent respectively. However, the total number of siliquae was increased to 306 as compared to 263 in the parent. Seeds per silique remained the same but seed yield per plant has increased. The mutant has yellow seed coat colour with increased oil content (41%) and reduced erucic acid content of 25%. Thus, there are more than one mutation in the same plant or it could be pleiotropic effect. It was also used in breeding programme and large number of desirable recombinants with earliness and high seed yield potential has been isolated. Thus, reduced plant height and earliness have not hampered the yield potential and these plants could be suitable to avert the limiting factors. This is the first report on induced mutations for dwarfing genes in Indian mustard.

IAEA-CN-263-188

INDUCED MUTAGENESIS FOR THE IMPROVEMENT OF GREEN SHINY COLOUR MUNGBEAN VARIETY

Shamsun Nahar Begum, Mirza Mofazzal Islam, Rigyan Gupta
Bangladesh Institute of Nuclear Agriculture, Bangladesh
Email: sbluna98@yahoo.com

Mutations were induced in mungbean line MB-149 (susceptible to MYMV) varieties using gamma rays as mutagen. Selection studies were conducted to improve the yield and to generate genetic variability in different quantitative traits viz., earliness, bold seeded, pods per plant and seed yield. The mutant MBM-07 was derived from parent MB-149 found to be promising for several desirable characters like earliness, bold seeded, higher yield, dwarf plant type, shiny green seed coat colour and tolerant to mungbean yellow mosaic virus (MYMV). The distinct features of the selected mutant MBM-07 are medium plant height (35-40cm), early maturing (64-67 days), deep green leaf colour, 23% protein content and average seed yield is 1.8 tha⁻¹. This mutant was released as 'Binamoog-8' for commercial cultivation in Bangladesh. Farmer's in Bangladesh widely adopted and integrated this variety in their cropping system.

IAEA-CN-263-191

COMBINATION OF INDUCED MUTATION AND HYBRIDIZATION METHODS FOR RICE BREEDING

Tan Phuong Tran¹, Quang Cua Ho², Thi Thu Huong Nguyen²
¹*Plant Breeding Center, Viet Nam*
²*Ho Quang Private Enterprise, Viet Nam*
Email: trantanphuong2005@gmail.com

Breeding for aromatic rice varieties having high grain quality, insect and disease resistance ability and increased yield is the objective of this research to satisfy domestic consumers and increase commercial value for exported market. The procedure to obtain the mutants may be summarized as follows: seeds of local Tam Thom TT1 and ST3 were incubated in a water bath at 33°C for 48 h to induce germination to obtain high-frequency gene mutation. They were then irradiated by gamma rays from a ⁶⁰Co facility at 15 krad (150Gy) dose. After 24h, the seeds were sown in a field nursery to obtain the first generation

(M₁). Phenotypic selections began at the M₃ generation. We selected two mutant rice lines in M₅ named Tam Thom T₃ and Mutant ST3. Mutant Tam Thom T₃ line is highly resistant to leaf blast disease, is early maturing and has a semi-dwarf stature. Individuals, including mutants were propagated through self-fertilization, and phenotypes were evaluated and selected at each generation to develop pure-bred lines. Data on plant height (cm), number of effective tillers/plant, panicle length (cm), number of filled grains/panicle, 1000 seed weight (gr), days to maturity and grain yield were recorded in our example. After harvesting, the seeds of each genotype were dehulled for evaluation of the grain quality, viz. grain size (grain length), grain shape (grain length-breadth ratio) and also aroma. But pure-bred lines have small panicles so these lines were used for combination. Thus, a combinatorial approach between hybridization and induced mutation can be used for breeding. Multiline crossing was carried out on mutant rice lines, aromatic rice and others, such as Huong Com 1/mutant ST3//mutant Tam Thom T₃//R15///ST20////ST20. After strict processes of selection through 8 generations by qualitative and quantitative anticipated targets (growth duration, plant architecture, grain shape, high yield, aroma, insect and disease resistance), we selected two aromatic rice varieties named as ST22 and ST24 in 2016.

IAEA-CN-263-193

EFFECT OF GAMMA RAYS, EMS AND SODIUM AZIDE ON QUANTITATIVE CHARACTERS IN KALA JIRGA NON-BASMATI AROMATIC RICE (*ORYZA SATIVA* L.) CULTIVAR FROM KOLHAPUR INDIA

Shitalkumar Desai¹, Akash Ramteke¹, Nikhil Gaikwad¹, Vinod Dhole²

¹*Dept of Botany, Shivaji University, India*

²*Nuclear Agriculture and Biotechnology Division NABTD, Bhabha Atomic Research Centre, BARC, India*

Email: nbgaikwadsu@gmail.com

Kala Jirga, a local non-basmati aromatic rice cultivar (landrace) grown in Kolhapur district, Maharashtra, India possesses a good aroma quality, it has low yield potential and a very good potential for domestic market. 500 seeds bulks of Kala Jirga cultivar were subjected separately to mutagenic treatments with gamma rays (100, 200 and 300Gy), Ethyl methanesulphonate (EMS) 0.8, 1.0 and 1.2% and Sodium Azide (SA) 0.002, 0.004 and 0.006% to induce genetic variability. Seeds from M₁ plants were planted in a plant to row basis as M₂ generation. Various mutants were identified with respect to plant height, maturity and yield. Dwarf mutants were identified among plants irradiated with 0.002% and 0.004% SA and those irradiated with 100 and 200Gy Gamma-rays showed notable reduction in height. Early maturing mutants with 155-157 days to maturity compared with 167 days in control were detected among plants treated with EMS at 0.8 and 1.0%. Plants with higher tiller number were identified in all mutagenic treatments: EMS (1.0 and 1.2%), SA (0.002 and 0.004%) and 100Gy treatments with the number of tillers ranging from 24 to 32 tillers/ plant as compared with 9 tillers/ control plant. Mutants with increased panicle length ranging from 22.08 to 25.96cm from EMS (0.8, 1.0 and 1.2%), SA (0.004%) and 100Gy treatments as compared to control (19.69 cm) were also identified. Mutants with higher yield/plant were detected in treatments with EMS (1.0 and 1.2%), SA (0.002 and 0.004%). Plants derived from 100Gy treatments showed higher yield/plant (29.02-38.84 g/plant) as compared to controls (11g/plant). Among the mutagenic treatments, 0.8% and 1.0% treatments of EMS, 0.002 and 0.004% SA and 100Gy treatments were found to be the most effective treatments for induction of desirable mutants.

EFFECT OF EMS AND SA ON TRYPSIN INHIBITOR CONTENT IN MOTH BEAN (*VIGNA ACONITIFOLIA* (JACQ.) MARECHAL)

Shivram Khadke¹, Vijay Kothekar²

¹Smt. Kasturbai Walchand College, India

²Dept. Of Botany, India

Email: shivramkhadke@gmail.com

Moth bean (*Vigna aconitifolia*, Jacq. Marechal) is one of the most important pulse species of India, however, it contains a great amount of antinutritional factors such as trypsin inhibitors (TI). Seeds of local variety of moth bean (*Vigna aconitifolia* (Jacq.) Marechal) were treated with three different concentrations of each chemical mutagens EMS and SA and the morphological mutants were screened under local conditions for Trypsin inhibitor content. Trypsin inhibitor assays were carried out to find out the trypsin inhibitor activity among seven viable mutants and 78 micromutants of moth bean which were developed by induced mutation breeding. The pertinent studies of seven viable mutants and 78 micromutants of moth bean have indicated considerable variation regarding TI level. The lowest TI (180.00 TIU/min/gm meal) was recorded in 6/IV-mutant, while the 7/IV-mutant revealed the highest TI content (326.16 TIU/min/gm meal). The electrophoretic profiles of trypsin inhibitors on X-rays film revealed 3 to 7 iso-inhibitors in different viable mutants and micromutants of moth bean. The amount of TI in the mutants showing reduced trypsin inhibitor bands were quantitatively estimated. The lowest TI activity (187.98 TIU) was observed in B6 mutant, which was developed from 0.15% EMS treatment. Additionally, to nullify trypsin inhibitor activity in moth bean seeds in order to be able to use it in human diet, heat treatment and germination studies have been tried in some of these mutant lines. In comparison with the control TI profile, some viable mutants showed significant changes. These mutants also showed 25-45% reduction in TI content and seed proteins by quantification of micromutants and viable mutants showed significant variations.

CHARACTERIZATION OF STAY GREEN MUTANTS AND RECOMBINANTS FOR DROUGHT TOLERANCE IN SUNFLOWER

Guruprasad Hiremath, Hajisaheb Nadaf

Department of Genetics and Plant Breeding, India

Email: guruprasad4235@gmail.com

Stay green is an economically important and complex trait for drought tolerance. The present investigation was conducted to identify the drought tolerant stay-green genotypes with desirable root traits which are essential to extract moisture from deeper layers of the soil under drought conditions. The study comprises three non-stay-green checks and seven Alternaria disease resistant staygreen genotypes identified from the mutants: DSR 14 derived from 200Gy gamma irradiation and DSR 57 derived from 0.020 mol/dm³ EMS treatment and recombinant populations. These genotypes have been characterized for root architecture under different soil moisture regimes in factorial RBD design. Analysis of variance revealed significant interaction between the genotypes and different water regimes and among the genotypes. The mutant DSR 14 and recombinant DSR 47 have exhibited higher mean root length of 102.50cm each, higher root to shoot ratio (0.87 and 1.00) and leaf area (151.35cm² and 137.70cm²) under severe stress condition. However, despite of the stay-green nature, EMS mutant DSR 57 and other stay-green recombinants exhibited the poor performance for root traits and seed yield per plant under drought conditions indicating only the cosmetic nature of their stay green trait. The average seed yield per plant for these identified genotypes, DSR 14 (13.74g) and DSR 47 (12.38g) was higher than that of the checks, Morden (5.38g), RHA 6D-1(2.75g) and RHA 95C-1 (3.55g) and over

other stay-green mutant and recombinants under severe low moisture stress condition. Hence, the present investigation revealed that the gamma irradiation treatment yielded a functional stay green mutant which can be further utilized for breeding for drought tolerance and to understand the genetic nature of this functional stay-green trait to further improve the crop.

IAEA-CN-263-200

EVALUATION OF NEW COTTON MUTANT CULTIVARS FOR ADAPTATION TO CLIMATE CHANGE IN IRAN

Mohammad Reza Rahemi¹, Ali Eskandari¹, Ebrahim Moghiseh², Kamran Mozaffari², Ljupcho Jankuloski³, Manzoor Hussain⁴, Masoud Rahimi², Massoud Malek⁵, Mohammad Amiripari², Omran Alishah⁶

¹*Nuclear Agriculture School, Iran*

²*Nuclear Science and Technology Research Institute, NSTRI, Iran*

³*International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

⁴*Principal Scientist/Group Leader Cotton breeding Programme, NIAB, Pakistan*

⁵*International Atomic Energy Agency, Division of Asia and the Pacific, Austria*

⁶*Cotton Research Institute of Iran, Iran*

Email: mrahemi@nrcam.org

In Iran cotton cultivation area is approximately 100 000 hectares and there are some limitations for increasing this area. Several research results reveal that main problems are the fact that the increase in minimum temperatures is more widespread than for the maximum temperature; also, the temperature has risen between 2.5 and 5 degrees centigrade; moreover, the amount of precipitation has also been reduced in Iran. So, cotton production, which is an important source of revenues, has been reduced and our country needs to introduce new cotton cultivars tolerant to abiotic stress. Therefore, two Iranian superior mutant lines (L-M-1425 and L-M-1676) together with two Pakistani mutant cultivars (NIAB KIRAN and NIAB 414) provided through the Regional Agreement Asia project (RAS5075) were entered in a comparative and adaptation study. The mutant genotypes have been cultivated in four repetitions in Karaj. All the activities were in-farm based under normal conditions and without any additional treatment. In the end of the growing season, some traits have been recorded including plant height, number of branch (sympodia and monopodia) boll number. The fibre extracted from selected mutant lines have been sent to the Cotton Research Institute of Iran after harvest to measure traits related to fibre quality including fibre length (upper half mean length (UHML) and uniformity index (UI)), strain, strength, as well as micronaire index. The results show that strain trait has significantly increased in L-M-1425 to the NIAB 414. Also, NIAB KIRAN had significantly lower branch number of monopodial and sympodial. In addition, boll number in NIAB 414 was significantly increased compared to L-M-1676. Finally, the mentioned mutant genotypes have not revealed significant different in the most traits, so that the Iranian mutant lines will be used to next processing of mutation breeding and Pakistani cotton cultivars will be cultivated in other places for introduction in Iran.

HIGH TEMPERATURE EFFECT ON THE MALE GAMETOPHYTE AND THE PHOTOSYNTHETIC ACTIVITY OF TWO *CAPSICUM ANNUUM* L. CULTIVARS

Yanina Arnaoudova, Elena Topalova, Valentina Valentina, Velichka Todorova, Veselina Nikolova
Maritsa Vegetable Crops Research Institute, Bulgaria
 Email: janitwin@yahoo.com

Induced mutagenesis appears as one of the most powerful methods for creating genetic variability to develop starting material for breeding purposes. This also requires extensive research into mutant forms and their hybrid varieties. A prospective trend in selection is the creation of F1 hybrids with high tolerance to abiotic stress. The high temperature (HT) is already a significant factor of the environment when growing different cultural plants and the pollen vitality and pollen fertility occur to be one of the highest temperature stress sensitive indicators. The effect of high temperature stress on two *Capsicum annuum* L. cultivars: Cv. Zlaten Medal 7 and cv. Yasen F1, which was created based on male sterility obtained by irradiating dry seeds with a range of ⁶⁰Co doses, has been investigated. In the bud formation-blossoming period the plants were exposed to high temperature treatment in two regimes 40°C/2h and 45°C/1h. More sensitive to high temperatures was the male gametophyte of mutant cv. Yasen F1 in comparison to cv. Zlaten medal 7 in which decisive role over the vitality occurred to be the treatment duration. According to the chlorophyll fluorescence analyses, the two HT regimes result in a change in the chlorophyll fluorescence parameters. The influence of the higher HT value with a shorter impact duration is more pronounced. The mutant nature of Yasen F1 may be the basis for its higher sensitivity to high temperatures.

MUTATION BREEDING FOR RESISTANCE TO *STRIGA HERMONTHICA* (DEL.) BENTH. IN SORGHUM FOR FOOD SECURITY IN BURKINA FASO

Djibril Yonli, Hamidou Traore, Nofou Ouedraogo, Philippe Nikiema
Institut de l'Environnement et de Recherches Agricoles (INERA), Burkina Faso
 Email: d.yonli313@gmail.com

Sorghum bicolor (Linn.) Moench is the staple crop in terms of cereal production and per capita consumption in Burkina Faso. Sorghum production is highly affected by *Striga hermonthica* which is a major biotic constraint. This study aims at inducing Striga-resistance in popular indigenous sorghum varieties while maintaining the good qualities of the parents. Dry seeds of two sorghum varieties Sarioso14 and ICSV1049 were irradiated with 200, 300, 400 and 500Gy. The irradiated seeds and controls were sown. M₁ panicles were harvested, and planted as M₂ panicle-to-one progeny. M₂ plants were selected and advanced to M₃ and then M₄ generations using pedigree selection method based on plant vigour, tiller number, early maturity and grain and panicle types as selection criteria. M₄ mutants of Sarioso14 (derived from the treatments 96/200Gy, 120/300Gy, 122/400Gy and 164/500Gy and one ICSV1049 mutant derived from the treatment (84/500Gy) were screened for Striga tolerance in rain-fed cropping conditions. Sorghum hills were artificially infested with at least 103 Striga seeds/hill-1 at planting. As results, no Striga plants emerged around the hills of 5 of the M₄ plants of which one Sarioso14 mutant, two Sarioso14 mutants and two ICSV1049 mutants. Field experiments will be repeated 3 times along with bioassays before multi-location evaluation.

INDUCTION OF MUTATION TO IDENTIFY LINES TOLERANT TO SUCKING PESTS IN OKRA (*ABELMOSCHUS ESCULENTUS* L.)

Gangaprasad Sreekantappa, Dushyanthakumar Banur Marulasiddappa

UAHS, Shimoga, Karnataka, India

Email: gangaprasad08@gmail.com

Okra is one of the most popular vegetables of Asian countries. India's okra production ranks first in the world with 3.5 million tonnes (of okra produced from over 0.35 million ha of land). It is quite popular in India because of its easy cultivation, dependable yield and adaptability to varying moisture conditions. Okra is cultivated for its fibrous fruits or pods containing round, white seeds. The productivity of Okra is 11.6 tonnes per ha. There are a quite good number of high yielding varieties and hybrids have been released but all of them are susceptible for many sucking pests viz Aphids, Thrips and white fly. There are no resistant or tolerant varieties in Okra to these sucking pests. In order to develop tolerant lines Arka anamika a popular variety has been subjected to 80 Kr of gamma irradiation. There are some appreciable variations that have been identified based on field tolerance. All these lines are subjected *in vitro* screening. Two lines have been identified with tolerance to sucking pests.

IAEA-CN-263-207

RESPONSES OF CHICKPEA (*CICER ARIETINUM* L.) GENOTYPES INDUCED BY ETHYL METHANESULPHONATE (EMS)

Ferhat Kızılgöçü¹, Behiye Tuba Biçer², Cuma Akıncı², Mehmet Yıldırım², Onder Albayrak²

¹Şırnak University, Turkey

²Dicle University, Turkey

Email: ferhat_kizilgeci@hotmail.com

This study was carried out in the Field Crops Department, Agriculture Faculty of Dicle University, Turkey, in 2017. The aim of the research was to determine the effects of ethyl methanesulphonate (EMS) in M₁ chickpea plants of six cultivars (Gokce, Arda, Azkan, Diyar 95, Çagatay, and Aziziye 94) and six advanced chickpea lines (N5-5, R6, D2-8, D2-5, K2-35, and K3-10). Seeds were pre-soaked in distilled water for six hours. Subsequently they were treated with 16 mM EMS at pH 7 for 12 hours. The treated seeds were thoroughly washed under running tap water and sown in pots in the greenhouse immediately after the treatment along with the controls. The experiment was established a complete randomized block design (CBRD) with three replications and 30 seeds per replicate. Statistically significant differences were observed for several characters in the M₁ chickpea seedlings. EMS has generally caused late heading time and decrease of other characters in the M₁ plants. The results of the study have shown that characters of M₁ plant seedlings changed between 75.0-100.0% for seedling emergence rate, 3.2-29.6cm for seedling height, 11.1-23.1cm for root length, 7.0-17.1g for seedling weight, 1.13-5.30g for shoot fresh weight, 5.60-12.13g for root fresh weight, 0.160-1.090g for shoot dry weight, 1.23-3.00g for root dry weight. Results show that EMS induced sufficient variation to start mutation breeding in chickpea.

BARLEY MUTANT RESOURCES FOR FUNCTIONAL ANALYSIS OF GENES INVOLVED IN RESPONSE TO DROUGHT STRESS

Agata Daszkowska-Golec¹, Anna Skubacz¹, **Iwona Szarejko**¹, Krzysztof Sitko², Marek Marzec¹, Marzena Kurowska¹, Miriam Szurman-Zubrzycka¹, Mirosław Maluszynski¹, Patrycja Gajewska¹

¹*Department of Genetics, Faculty of Biology and Environmental Protection, University of Silesia, Poland*

²*Department of Plant Physiology, Faculty of Biology and Environmental Protection, University of Silesia, Poland*

Email: miriam.szurman@us.edu.pl

TILLING (Targeting Induced Local Lesions IN Genomes) is a reverse genetics strategy that combines the classical mutagenesis and a high-throughput identification of mutations within a gene of interest. We have created a TILLING platform, HorTILLUS (*Hordeum vulgare*-TILLING-University of Silesia) and used it for functional analysis of genes involved in ABA-related response to drought stress in barley. Double-treatment of spring barley cv. ‘Sebastian’ with sodium azide and MNU (N-methyl-N-nitrosourea) was used for generation of HorTILLUS population. Mutation identification in target genes: HvCBP20 (Cap-binding protein20), HvERA1 (Enhanced response to ABA1) and HvABI5 (ABA insensitive5) was performed in M₂ generation. using specific endonuclease and LI-COR Sequencer or Fragment Analyzer. All three target genes are related to ABA-directed response to abiotic stresses. The identified mutants were evaluated for physiological parameters and transcriptome activity under drought stress. The HorTILLUS platform comprises more than 9600 M₂ plants from which DNA was isolated, seeds harvested and deposited in seed bank. The average mutation density in the HorTILLUS was found to be 1 mutation per 477 kb. Here, we report three drought-tolerant barley mutants carrying missense mutations in target genes: hvcbp20.ab, hvera1.b and hvabi5.d. All of them were characterized by a higher relative water content (RWC) after drought stress compared to ‘Sebastian’. Additionally, hvcbp20.ab showed a changed epidermal pattern and lower stomatal conductance, hvera1.b exhibited better photosynthesis efficiency, while hvabi5.d showed faster stomatal closure and better membrane protection. Transcriptome analysis integrated with observed phenotypic traits allowed to draft possible mechanisms in regulation of drought response. The HorTILLUS population proved to be a useful resource, both for functional genomic studies and generation of barley mutants with required phenotypic changes, including tolerance to drought.

EFFECT OF GAMMA IRRADIATION ON SOME CHARACTERISTICS OF DURUM WHEAT (*TRITICUM DURUM* L.)

Mehmet Yildirim¹, Cuma Akinci¹, Ferhat Kizilgeci², Nafiz Sonmez¹, Onder Albayrak¹, Behiye Tuba Bicer¹

¹*Dicle University, Turkey*

²*Sirnak University, Turkey*

Email: myildirim@dicle.edu.tr

This study was carried out during the winter growing season of 2012/2013 and 2015-2016 at the Field Crops Department, Agriculture Faculty of Dicle University, Turkey. The aim of the research was to determine the effects of different doses of gamma ray (0, 100, 200 and 300Gy) in M₁ and M₂ plants of “Guney Yildizi” durum wheat cultivar. Seeds of wheat were irradiated with gamma rays from ⁶⁰Co source at Turkey Atomic Energy Agency, Ankara, Turkey. The research was established for M₁ plants in the greenhouse and in the field and for M₂ plants only in the field. The experiment was laid out in a randomized complete block design with three replications. Increasing gamma ray doses caused

statistically significant differences on most characters of M₁ and M₂ plants. Increasing gamma ray doses also generally increased heading time and decreased other observed characters of M₁ plants. The result of the study showed the following changes in M₁ generation; emergence rate 8.90-88.9%, first leaf length 4.83-12.45 cm, coleoptile length 2.12-3.22 cm, seedling height 4.75-22.95 cm, root length 2.54-17.81 cm, fertile plant rate 3.3-57.0%, heading time 175.0-179.3 date, plant height 54.6-87.8 cm, spike length 5.97-7.37 cm, 1000 kernel weight 47.1-63.5 g, and in M₂ generation; heading time 151.7-153.7 date, spike length 7.33-8.00 cm, chlorophyll content in flowering time 49.96-53.86, grain protein content 15.17-16.08%, grain gluten content 28.92-29.51%.

IAEA-CN-263-221

DOSE OPTIMISATION FOR POLLEN AND EMBRYOS IRRADIATION MUTATION INDUCTION IN OIL PALM

Kenneth Danso¹, Abdelbagi Mukhtar Ali Ghaniam², Daniel Dwarko³, Jankuloski Lupcho², Wonder Nunekpeku¹

¹Ghana Atomic Energy Commission, Ghana

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

³Oil Palm Research Institute, Ghana

Email: kaedanso@hotmail.com

Mutation induction has a great potential to create genetic variability in plant populations for subsequent selection of novel characteristics for human and other needs. In oil palm, the exploitation of this technology is still rudimentary. In this study, oil palm propagules and pollen were irradiated with gamma radiation to determine the optimal irradiation doses for mutation induction in oil palm mutation breeding programme. Lyophilized pollen from dura, pisifera and tenera varieties of oil palm were irradiated at varying doses of gamma radiation ranging from 0-5 kGy. The irradiated pollen grains were then cultured on a sucrose medium containing 5% boric acid and incubated in the dark at 33°C. Eight hundred (800µl) of the medium was plated for light microscopic studies. The irradiation dose conferring 50% lethality (LD50) determined on pollen germinability ranged from 2.4 kGy to 3.2 kGy indicating that oil palm pollen grains are highly radio-resistant, with pisifera being the most sensitive. Pollen obtained from Tenera variety was however, least susceptible to the radiation treatment. The optimal dose for embryos under *in vitro* culture conditions was 18Gy while *in vivo*, optimized dose for whole non-germinated nuts was 15 kGy. Developing plantlets and/or seedlings showed varied morphological variation depending on the dose of irradiation. This study provides the very critical information for mutation induction in oil palm using oil palm embryos, pollen and whole non-germinated nuts.

IAEA-CN-263-230

DEVELOPING WHEAT AND BARLEY LOCAL LANDRACES THAT ARE RESISTANT TO LODGING WITH REDUCED STATURE AND LONGER SPIKE USING NUCLEAR TECHNIQUES IN LEBANON

Faten Raad

MOA/LARI, Lebanon

Email: faten_raad@yahoo.com

A mutation experiment was conducted at the Lebanese Agricultural Research institute (LARI), Lebanon, Tel Amara station in 2014 on two wheat genotypes (Seri 82 and Breiji) and on one barley genotype (Assi), treating with different gamma radiation doses (150, 200, 250 and 300Gy). Several traits were studied over 4 years which started in 2011 on four mutated generations (M₁, M₂, M₃ and M₄

populations) in comparison to untreated controls. The Selected spikes of Barley (Assi) treated M₄ generation (150 and 200Gy) were characterized by shorter stature with an average plant height respectively of 60 and 63cm compared to 67cm for non-treated ones, lengthy spike (6cm for 0Gy compared to 6.5cm for 150Gy and 200Gy) and resistance to lodging (20% for 0Gy to 15% for 150Gy and 200Gy). Selected spikes of Bread wheat (seri 82) (250 and 300Gy) treated M₄ lines showed a shorter stature compared to the mother plants with an average plant height respectively of 44 and 43cm compared to 65cm for non-treated variety. The spike length for parent obtained as 8.6 cm, mutant lines recorded as 9.6cm. Selected spikes of Breiji (Durum wheat) lines (250 and 300Gy) were recorded as 65 and 68cm while parents 75cm. An increase in spike length was observed in mutant lines compared to parents (11cm for parent and respectively 11.3cm and 11.6cm for mutant lines). Those mutant lines showed more lodging tolerance compared to parent plants 25% for the mutant lines and 40% for the parent).

IAEA-CN-263-231

ACHIEVEMENT OF RICE MUTATION BREEDING IN LAO PDR

Siviengkhek Phommalath, Chanthakhone Buolaphanh, Laer Homsengchan, Phatsalakone
Manivong, Sontaya Phethavone
National Agriculture and Forestry Research Institute, Lao PDR
Email: siviengkhek@yahoo.com

Conventional rice breeding started in Lao PDR in 1991, 20 improve rice varieties have been released since 1993. Blast is the major disease and flooding tolerance are major breeding concerns. In 2014, the varieties: TDK8 and TDK11 were irradiated with 150 to 400Gy. They were the most popular varieties and highly productivity but susceptible to blast and flooding. 137 mutant lines were found which segregated for seed colour, plant high and maturity. These were screened for blast resistance and flooding tolerance at RRC in 2016. Five isolates of blast were used for screening and after inoculation responses were measured using a scale of 6 lesion type categories (0 = resistance and 6 = susceptible). Among one hundred thirty-seven (137) mutant lines of TDK8 (150Gy) the lines: 17, 3, 26, 12 and 59 were resistant to isolates H08-040-1, H08-420-1, H08-259-1, H08-243-1 and H08-171-1, respectively. And mutant lines of TDK8 (200Gy): 47, 8, 27, 26 and 84 were resistant to isolates H08-040-1, H08420-1, H08-259-1, H08-243-1 and H08-171-1, respectively; almost of them were score = 0 while TDK8 = 6. The response of mutant line of TDK11 was not significant. Screening for submergence tolerance in 137 mutant lines was done for good recovery after flash flooding at the seedling stage. 16 mutant lines of TDK8 survived with survival from 4 to 34% compare to control TDK8, which did not survive. In TDK1 mutants TDK1-Sub1 showed 15% survival and TDK21_Sub1 showed 18% survival. There were 8 mutant lines which had higher than 20% of survival. While there was only one mutant line of TDK11 that showed survival, this was only 4%. Blast resistant lines and flooding tolerance lines will be screened for eating quality, yield and yield component in next generation

INDUCTION OF NEW GENETIC VARIATION THROUGH MUTAGENESIS IN PIGEON PEA

Inderjit Singh¹, KS Reddy², Manpreet Kaur¹

¹Punjab Agricultural University, Ludhiana, India

²Nuclear Agriculture & Biotechnology Division, BARC, India

Email: inderjitpb@pau.edu

Pigeonpea [*Cajanus cajan* (L.) Millsp] is an important pulse crop useful for both as human food and animal feed. There has been significant decline in pigeonpea production in India in recent years. Naturally existing genetic variability is not sufficient to achieve the desired improvement. Manual emasculation is very tedious and costly because of small size of its flower. Artificial induction of mutations is the best way to generate new variability to develop new varieties in pigeonpea. In Punjab major threat to pigeonpea cultivation is pod borer and other insect pests. Presently recommended varieties AL 201 and PAU 881 are high yielding, early maturing but their very tall stature poses difficulty for insecticide application. To induce genetic variability, seeds of AL 201 and PAU 881 were got irradiated with Gamma rays from BARC, Mumbai with 300Gy and 400Gy. All the M₁ plants were harvested separately. In M₂ generation, 50 desirable mutants of AL 201 with 300Gy and 62 plants with 400Gy dose with desirable traits were identified and classified into different groups. In PAU 881, 46 mutant plants with 300Gy and 41 plants with 400Gy dose were identified. The results obtained in both the doses showed that gamma rays induced variability and the mutants showed dwarfness and earliness over the control. In M₃ generation, thirteen uniform dwarf lines were identified and 36 single plants were selected for further evaluation.

IAEA-CN-263-235

DEVELOPMENT OF LEAFHOPPER AND WILT RESISTANT PISTILLATE LINE IN CASTOR THROUGH MUTATION BREEDING

Lavanya Cherukupalli, P. Duraimurugan, P. Lakshamma, T. Manjunatha, K.T. Ramya, M.

Santhalakshmi Prasad, Senthilvel Senapathy, A. Vishnuvardhan Reddy

Indian Institute of Oilseeds Research, ICAR, India

Email: lavanyador@gmail.com

DPC-9, a popular pistillate line of castor bean (*Ricinus communis*) is highly susceptible to leafhopper which is tightly linked to a monogenic trait absence or zero bloom (no waxy bloom) on different plant parts. Seeds of DPC-9 were treated with 550Gy r-rays at BARC, Mumbai. Generations of M₁ to M₁₀ were raised in winter, at optimal nutrition and irrigation every year and the plants received fundus inoculum. Some plants wilted in M₃, due to high inoculum (2×10^3 CFU/g soil) load. Segregation for bloom varying from triple (all parts waxy) to zero bloom was observed in M₅ and continuous selection pressure for bloom in pistillate background, led to the selection of 9 DPC-9 mutants with green stem, triple or double bloom, spiny capsules and pistillate expression up to sixth order that of spikes. Among the nine mutant DPC-9 selections, IPC-23, a pistillate line with green stem, triple bloom trait, low node number (7-8), short plant height (40-50cm) to the primary spike, early flowering (30-40 days to 50% flowering) and good branching (10-12) was stabilized. Screening for leafhopper resistance using infester row technique, indicated that IPC-23 is highly resistant to leafhopper (grade 0) compared to checks, DPC-9 and DCH-177 (grade 4). It is also resistant to wilt (8.5%), compared to check, JI-35 (97%) in a wilt infested plot. Genetic similarity coefficient between DPC-9 and IPC-23 was 58% in a genotypic data from 130 SNP loci representing all linkage group of castor beans. Pistillate behaviour is similar to DPC-9, with environmentally sensitive ISF and revertants. IPC-23, a physiologically efficient

line with good HI (31.3%) is good combiner for early flowering and maturity. Several hybrids based on IPC-23 yielded 34-134% standard heterosis in preliminary yield trials.

IAEA-CN-263-236

MUTATION INDUCTION IN *DIOSCOREA ESCULENTA* (LOUR) BURK FOR IMPROVED NUTRITIONAL AND FUNCTIONAL QUALITY

Andrew Sarkodie Appiah, Fidelis Ocloo, Joyce Agyei-Amponsah, Kenneth Danso, Kwamina Banson, Mavis OwurekuAsare, Wisdom Agbemavor

Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, Ghana

Email: andysark2000@gmail.com

Gamma irradiation was used to improve agronomic characteristics and postharvest shelf life extension of *Dioscorea esculenta*, a genetically threatened yam species. The lethal dose (LD50) was determined as 100Gy. At this lethal dose, plant height, vine number and sprouting were reduced by 72.5%, 46.7% and 40% respectively, at 60 days after planting (DAP). However, much reduction in plant height and the number of vines was observed at 160 and 200Gy. Reduction in total tuber weight, tuber length and width at all doses was not significantly different from each other and the control. However, the edible weight and the number of edible tubers reduced significantly at 200Gy (41.20% and 30.04% respectively) resulting in marginal increase in tuber size. Furthermore, postharvest shelf life extension of tubers was extended from 2 weeks to 14 weeks after irradiation at 80 to 120Gy. Sprouting was significantly delayed in tubers irradiated at 80, 100 and 120Gy until 8th, 11th and 14th week of storage respectively while rotting was sparingly observed. The effect of irradiation on tuber size, edible weight as well as nutritional qualities at M₁V₂ will be evaluated after harvesting in March 2018. The results presented here show the potential of gamma irradiation in the improvement of agronomic characteristics of *Dioscorea esculenta*.

IAEA-CN-263-237

YIELD AND GRAIN QUALITY IMPROVEMENT OF RICE THROUGH INDUCED MUTATION

Deepthika Kekulandara, Nadeeka Sharmila Thilakarathne

Rice Research & Development Institute, Sri Lanka

Email: deepthikasaman@gmail.com

Even though, there are more than eighty local recommended rice varieties in Sri Lanka, only few are cultivated at present with farmers' preference. At 362 is one of the most popular rice varieties grown island-wide due to its higher yield and adaptability to vast environmental conditions. It is a red pericarp rice variety belonging to the 3.5-month maturity group. However, this trait creates marketing problems in the harvesting period, since consumers do not prefer red pericarp rice in Sri Lanka. Thereby, the objective of this research was to improve at 362 rice variety by developing genotypes with white colour pericarp and longer grain shape, together with other preferred quality characteristics using tissue culture mediated mutation induction on seed calli. Dry rice seeds were de-hulled and surface sterilized. They were cultured in MS medium supplemented with calli induction hormones. Chemical mutagenesis was induced by Ethyl methanesulfonate (EMS). Initiated calli were treated with 0.2% EMS for 3 minutes and plants were regenerated from EMS treated calli. Regenerated green plants were acclimatized and grown in pots. M₁ generation were grown in the field and evaluated for genetic variations for pericarp colour, grain shape, age of maturity, plant height, panicle number and plant architecture. Selected plants were further advanced up to M₆ generation and the line 16TC80, which fixed to the age group 3.5

months and was relatively better for the desired traits was further evaluated for yield, pest and diseases and the overall uniformity. The rice line 16TC80 showed white pericarp colour, earlier maturity, and greater performances in pest and disease resistance and traits for yield and grain quality attributes.

IAEA-CN-263-239

CEREALS MUTATION BREEDING IN TUNISIA

Chahine Karmous¹, Mokhtar Baraket², Sawsen Ayadi³

¹*Ecole supérieure d'Agriculture de Mateur, Tunisia*

²*Institut National de la Recherche en Génie Rural, Eaux et Forêts (INRGREF), Tunisia*

³*Institut National Agronomique de Tunisie, Tunisia*

Email: karmouschahine@yahoo.fr

Cereal breeding programs are mainly released by governmental research institutions targeting disease resistance and yield increase. Most of the programs for durum wheat and barley are closely linked to international research centres such as CIMMYT and ICARDA for providing parental material, although, since the last decade, some of this material is also developed locally. There were few projects involving cereals mutation breeding attempts in Tunisia. Some were undertaken in the frame of IAEA supported projects such as TUN R0/12000 'Identification and pyramiding of mutated genes: novel approaches for improving crop tolerance to salinity and drought' and INT5150, 'Responding to the Transboundary Threat of Wheat Black Stem Rust (Ug99)'. In Tunisia, mutation breeding has been used to develop genetic material for harsh environments (drought and salinity). Many potential mutants tolerant to drought or salinity were isolated. However, the parental cultivars that were used had their resistance genes for major diseases overpassed and the generated mutants could no longer be considered for registration. Black rust Ug99 resistant bread wheat mutant material was generated through the INT5150 project. However, the introgression into durum wheat, the main grown cereal in Tunisia, was not released yet. The former IAEA projects enabled capacity building which accounts as an important component for the ongoing mutation breeding projects. The TUN5029 project is meant to capitalize on successes to develop drought tolerant barley and durum wheat, ready-to-register, mutants. Disease resistant parents will be used as starting material to generate mutants through seed irradiation as well as spikes (X- and gamma-ray) irradiation. Accelerated breeding techniques involving double haploid development will be used for fast development of drought tolerant cultivars.

IAEA-CN-263-248

MUTATION-INDUCED VARIABILITY FOR IMPROVED YIELD IN SPRING WHEAT UNDER HOT IRRIGATED ENVIRONMENTS

Sufian Suliman¹, Abdelbagi Mukhtar Ali Ghanim²

¹*Agricultural Research Corporation ARC, Sudan*

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

Email: sofyaa@gmail.com

Wheat production under dry and hot environments is challenged by heat stresses. Wheat improvement approaches for such stressful environments are constrained by the availability of genetic variability. Gamma-ray is used to induce variability in two Sudanese local wheat varieties (Tagana and Khalifa) to improve grain yield under the hot (30-38°C during the grain filling period) irrigated environments of Sudan. Dry seeds (10-15% moisture content) were exposed to six treatments of irradiation (0, 100, 200, 250, 300, 400Gy). The LD50-60 were 150 and 200Gy for Khalifa and Tagana, respectively, indicating that Khalifa was more sensitive to irradiation than Tagana. Two mutant populations, each with about

20,000 lines, were advanced to M₄ with preliminary visual scoring for agronomic performance. Based on grain yield, 500 M₅ lines from the two populations were selected for tolerance to heat stress at the GRS. The mutant populations were advanced to M₆ with preliminary evaluation of grain yield. Based on M₆ grain yield results, 50 advance lines from the two populations (10 from Tagana and 40 from Khalifa) were selected and tested in addition to the parents (Tagana and Khalifa) and two high yield national checks for grain yield under heat stress condition in 2015/16 and 2016/17. Grain yield was significantly different (P 0.01-0.06) among mutant lines and parents. Three and four lines had significantly higher grain yield than Tagana and Khalifa, respectively. Some of these lines combined early maturity and high grain yield. These top mutant lines will be advanced to multi-location trails to verify their performance before submission for official release as new mutant varieties for hot irrigated environments of Sudan.

IAEA-CN-263-252

THAI RICE BREEDING FOR FLOOD TOLERANCE THROUGH ELECTRON BEAM-INDUCED MUTATION

Udompan Promnart¹, Fatma Sarsu², Kanchana Klakhaeng¹, Peera Doungsoongnern¹, Ruenruedee Kaewchuenchai¹

¹*Rice Department, Thailand*

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

Email: udompan.p@rice.mail.go.th

There are two types of flood in Thailand which has affected to rice. The first is deep water flood which features as a prolonged flooding, and the second is a flash flood which is a sudden and short period flooding. Deep water flood is predictable, on the contrary flash flood is extremely unpredictable and may occur at any stage of rice growth. The flood in 2011 was the worst flood ever experienced in the country and it had largely affected rice field of Thailand. Thus, submergence-tolerant rice is highly desirable and expected to enhance food security. The objective of this study was to improve the variety RD49 for submergence tolerance. RD49 was used as a plant material to be mutated because it has short growth duration (100 days) and is resistant to brown planthopper. Five hundred grams of seed were irradiated with 300Gy electron-beam. The M₁ seed populations were pre-germinated and disseminated, then 500 panicles were collected from the main tiller of each M₁ to obtain M₂ seeds. Five hundred rows or 10,000 plants of M₂ generation were planted as panicle/row. Twenty-six plants were selected from each row as M₃ seeds. Agronomic traits of M₂ plants were observed and the M₃ lines were evaluated for submergence tolerance. Unfortunately, the experiment was affected by salinity due to the rising sea water level. The remaining materials were collected for submergence tolerance screening in the next generation. About 213 lines were identified as submergence tolerant rice lines (M₄). The plants survival was 85-95%, comparable to tolerant check (95%) and significantly higher than susceptible check (0%). Currently, the materials will undergo evaluation in advance experiments.

A PRE-SCREENING METHODOLOGY FOR THE IDENTIFICATION OF HEAT TOLERANT MUTANTS IN TOMATO (*SOLANUM LYCOPERSICUM* L.)

Banumaty Saraye¹, Bradley Till², Joanna Jankowicz-Cieslak³

¹*Food and Agricultural Research and Extension Institute, Mauritius*

²*Department of Chromosome Biology, University of Vienna, Austria*

³*International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

Email: bballuck@gmail.com

Field survival tests performed in hot-spot environments are a common method to evaluate heat tolerance in plants. However, it is difficult to predict heat conditions in the field. Neither temperature nor other conditions such as wind, irradiance and humidity can be controlled in the field. Thus, a rapid, relatively cheap and reliable pre-field screening methodology is required to screen large populations. Nine tomato mutant lines together with the parent line, MST 32/1 and one reference variety Money Maker were used for the study. Plants were examined at two different growth stages, seedling and mature plant and at different controlled conditions (35°C and 26°C). Plant height, number of flowers per cluster, leaves scorching, chlorophyll content, chlorophyll fluorescence and quantum yield were measured at the flowering stage for mature plant. For seedlings, assessment of leaf scorching was conducted along with analysis of survival rate. Variation in the degree of leaf scorching from moderate to high was observed among the line in the mature plant experiment. Significant difference at $P < 0.05$ was observed among the different lines under study for chlorophyll content, instantaneous chlorophyll fluorescence and quantum yield. Two mutant lines were identified as candidates carrying mutations in genes involved in heat tolerance. In the seedling experiment, leaf scorching was observed in some lines after exposure to 37°C. Most of the lines exhibited leaf scorching greater than 25%, except two lines which had 11 and 12%. No leaf scorching was noted in one mutant line. From the two experiments, it was noted that the same mutant showed tolerance in both experimental settings. This showed that the two experiments can be used as a method for screening large mutant population and pre-selection of candidates for field testing.

MUTATION INDUCTION IN PEARL MILLET (*PENNISETUM GLAUCUM*) AND FINGER MILLET (*ELEUSINE CORACANA*) FOR DRY LANDS IN SUDAN

Omar Hassan¹, Abdelbagi Mukhtar Ali Ghanim², Ivan Ingelbrecht²

¹*Agricultural Research Corporation, Cereals Research Centre, El Obeid Research Station, Sudan*

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

Email: omarbakhit@yahoo.com

The plant breeders before undertaking a large-scale mutagenesis need to determine an appropriate dose of irradiation on target genotypes or germplasm. The objective of this study was to determine an optimum dose of gamma irradiation to induce genetic variation in three Sudanese pearl millet varieties (Okoa, Dar Masa and HSD 1173) and two finger millet varieties (RA and OH). To determine the lethal dose (LD) at 50% (LD50) and 30% (LD30), seeds were exposed to different doses of ⁶⁰Co gamma-ray irradiation (0 control, 75, 150, 300, 450 and 600Gy) in 2016 at Plant Breeding and Genetic Laboratory (PBGL) of the Joint FAO/IAEA Division, Seibersdorf, Austria. The experiment was conducted in glasshouse following the established procedure in the PBGL. The treatments assigned in a randomized complete block design with three replications in plastic trays. Survival rate and seedling height were measured at 15 days after sowing. The results showed that seedlings length and survival rate percentage

decreased dramatically when gamma rays dose increased. In addition, most of the tested varieties showed 100% mortality in 600 doses. For pearl millet, using linear regression model, the LD50 and LD30 values calculated for genotypes Okoa (346Gy, 468Gy), Dar Masa (328Gy, 446Gy) and HSD 1173 (302Gy, 405Gy), respectively. For finger millet, the results for the LD50 and LD30 values for genotypes RA (339Gy, 457Gy) and OH (331Gy, 453Gy), respectively. Based on the findings of this study bulk seeds were treated with three doses; the LD50 plus and minus 20% to produce M₁ seeds for pearl millet mutation breeding for drought tolerance in Sudan.

IAEA-CN-263-262

FIRST SLOVAK AMARANTH VARIETIES GENERATED THROUGH RADIATION MUTAGENESIS

Andrea Hricová¹, Alena Gajdošová¹, Gabriela Libiaková¹, Jozef Fejér²

¹*Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Center, Slovak Academy of Sciences, Slovakia*

²*Department of Ecology, Faculty of Humanities and Natural Sciences, Prešov University, Slovakia*

Email: andrea.hricova@savba.sk

Neglected or minor species like amaranth (*Amaranthus* spp.) can markedly contribute to the promotion of environmental sustainability, agrobiodiversity enhancement, global food production and to the preparation of healthy foods. A nutty-flavoured amaranth grain has an attractive chemical composition and promising nutritional value when compared to other grains, whether cereals or food legumes, with high impact on human health. Amaranth is suitable for diets of people suffering from coeliac disease as only very low amounts of prolamins are present in their grains. Breeding work on grain amaranth has just begun in Europe and shows the necessity of further research for drought resistance, grain maturation, and yield improvement. Our research is oriented towards enhancement of the quality and quantity of amaranth grain using radiation-induced mutagenesis. In the frame of multi-lateral IAEA-coordinated project two amaranth accessions *A. cruentus* L. genotype Fichta and *A. hypochondriacus* x *A. hybridus* L. hybrid genotype K-433 were used for the irradiation treatment. Fifteen generations of mutant genotypes with their non-irradiated (M₀) counterparts were established. Finally, two stable mutants with significantly increased 1000-seed weight were selected. Multi-year phenotypic characterization for important yield parameters were performed in two different fields. The investigated mutants showed a seed size advantage over non-irradiated control seeds, with predictable yield performance. Based on a long-term significantly increased yield parameter and stable nutritional and dietetic value we have passed these mutants on to official DUS trials. Successful DUS trials led to the registration of the tested mutant genotypes as first Slovak amaranth varieties “Pribina” (*A. cruentus*) and “Zobor” (*A. hypochondriacus* x *A. hybridus*). Currently, we are working on the identification of proteins responsible for enhanced traits, that will provide potential biotechnological targets for crop improvement in amaranth and other species.

**INDUCTION OF VARIABILITY FOR YIELD COMPONENTS IN INDIAN MUSTARD
BRASSICA JUNCEA L. CZERN & COSS. UNDER ACIDIC SOIL REGIME OF
JHARKHAND**

Arun Kumar¹, Binay Kumar Agarwal², Rajesh Kumar³, Sanjay J. Jambhulkar⁴, Varsha Rani⁵,
Z.A. Haider³

¹*Birsa Agricultural University, India*

²*Department of Soil Science and Agril. Chemistry, Birsa Agricultural University, India*

³*Department of Genetics & Plant Breeding, Birsa Agricultural University, India*

⁴*Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, BARC, India*

⁵*Department of Crop Physiology, Birsa Agricultural University, India*

Email: arun.bau4@yahoo.co.in

Indian mustard (*Brassica juncea* L.) is the most important oilseed crop of the state of Jharkhand, India, where 78% of the cultivable soil is strongly to extremely acidic. Potential seed yield from such soils cannot be realized. Available varieties and germplasm are not suitable for cultivation and therefore mutation breeding approaches have been followed in attempts to develop novel tolerance to acid soils. Three doses of gamma-rays were applied to seeds: 900Gy, 1000Gy and 1100Gy, and a combination of gamma irradiation plus 0.3% EMS (chemical mutagen) was used for induction of mutation in parent varieties Shivani and Pusa Bold. Large M₂ generations i.e. 139 720 (75 760 Shivani + 63 960 Pusa Bold) were screened under acidic soil (pH 4.8). A wide spectrum of variability for tolerance to soil acidity, earliness, seed yield and yield component traits were observed in the M₂ generation in addition to several morphological variants. These variabilities were confirmed in the M₃ generation under natural acidic soil reaction (pH 4.8) and found to breed true. The variation in selected mutants for secondary branches was up to 38 in Shivani while, up to 24 in Pusa Bold which was many foldshigher than their respective controls. Similarly, siliqua per plant ranged from 6 to 1223 in Shivani and 7 to 562 in Pusa Bold as against the average siliqua ranged between 125 to 160 in their parents. Seed yield per plant also registered wide variation i.e. 0.45 to 45.49g in Shivani, while between 0.62 to 34.84 for Pusa Bold against the average seed yield of 4-5g (Shivani) and 5-7g (Pusa Bold). Large number of morphological mutants with novel traits were also identified. Many of the identified mutants registered yields superior to the parents under acidic soil.

**CHARACTERIZATION OF A NEW GENE CONTROLLING LEAF SENESCENCE USING
PROGENY FROM AN INTERSPECIFIC CROSS IN RICE**

Sang-Nag Ahn, Hyun-Sook Lee, Kyu-Chan Shim, Sun-ha Kim, Yun-A Jeon

Chungnam National University, Republic of Korea

Email: ahnsn@cnu.ac.kr

An analysis of quantitative trait loci (QTL) controlling chlorophyll content was conducted using introgression lines (CR7501 and CR2002) derived from a cross between the *Oryza sativa* japonica cultivar 'Hwaseong' as a recurrent parent and wild species *O. grandiglumis* as a donor parent. These two lines showed higher chlorophyll content than Hwaseong. For QTL analysis, we constructed 58 F₃ and 17 F₄ lines from the cross CR7501/Hwaseong. SSR markers were used for genotyping the lines. One-way ANOVA indicated the presence of a QTL for chlorophyll content (qCC2) on chromosome 2 and qCC2 explained 24.6% of the phenotypic variance. To examine whether qCC2 is also involved in senescence, a series of dark-induced senescence (DIS) experiments were conducted. Detached leaves from Hwaseong and CR2002 were incubated in 3mM MES buffer (pH 5.8) at 27°C under complete dark condition. CR2002 showed higher chlorophyll content with delayed senescence than Hwaseong.

To know whether qCC2 maintains leaf functionality during DIS, ion leakage test and Fv/Fm measurement were performed. The Fv/Fm value displayed significant difference between CR2002 and Hwseong at 6 days after incubation, while ion leakage rate was not significantly different. These results might imply that qCC2 is associated with chlorophyll content and stay-green phenotype. The qCC2 QTL region harbours GW2 locus encoding the RING-type E3 ubiquitin ligase controlling grain size. To know the possible relationship between the GW2 activity and senescence, gw2-knockout mutant (gw2-ko) and the wild type plants were incubated under dark condition. gw2-ko showed delayed senescence in DIS, suggesting that GW2 is possibly related to stay-green phenotype.

IAEA-CN-263-272

INDUCED MUTATION AND BIOTECHNOLOGY IN CÔTE-D'IVOIRE: STATUS AND PROSPECT

Désiré Pokou, Edmond Koffi

CNRA, Centre National de recherche Agronomique, Côte d'Ivoire

Email: desire.pokou@gmail.com

The economy of Côte-d'Ivoire is based on Agriculture. Indeed, agriculture is the main activity in rural area and represent 30% of the GDP and 50% of export earnings. Many crops ranging from cash crop to food crops are cultivated across the country. Due to the climate change and the increasing of effect of pest and diseases, many research programs have been focussing improving varieties and setting reliable screening tests for pathogens. Biotechnology appears as the template to move forward in breeding and pathogen detection. Besides, induced mutation is the tool to generate variation require for specific need. In Côte-d'Ivoire, the mutation breeding started in the 1960 on coffee using chemical mutation to create tetraploid and double haploid in order to make inter-species crosses between *Coffea Arabica* and *Coffea canephora*. This work led to the development of new species named *C. arabusta*. More recently the "Centre National de recherche Agronomique "(CNRA) in collaboration with IAEA has initiated a mutation breeding program on Yam, cassava and plantain using gamma ray. In order to carry out the downstream analysis in biotechnology, the CNRA Central Biotechnology Laboratory conducts research on molecular biology using various markers especially SSR and SNPs for diversity studies and QTLs analysis. The tissue culture facility is working on *in vitro* conservation of genetic resources as well as somatic embryogenesis. In the future, irradiated samples will be subject to TILLING analysis. In addition, functional genomic activities are ongoing for disease resistance on Cocoa tree.

IAEA-CN-263-273

SCREENING PROTOCOLS FOR HEAT TOLERANT MUTANTS IN RICE

Fatma Sarsu¹, Abdelbagi Mukhtar Ali Ghanim¹, Ashwani Pareek², Brian P. Forster¹, Ivan Ingelbrecht¹, Muhammed Ashraf³, Paul Mbogo Kusolwa⁴, Priyanka Das², Rajeev N Bahuguna², Sneha Lata Singla-Pareek⁵

¹International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria

²Jawaharlal Nehru University, India

³Nuclear Institute for Agriculture and Biology (NIAB), Pakistan

⁴Sokoine University of Agriculture, United Republic of Tanzania

⁵International Centre for Genetic Engineering and Biotechnology, India

Email: f.sarsu@iaea.org

Breeding heat tolerant rice is one of several strategies to mitigate the effect of climate change, particularly in major rice growing regions that are vulnerable to increased temperatures. We present

simple, robust pre-field screening protocols that would allow plant breeders to screen for enhanced tolerance to heat stress in rice in a breeding programme using a controlled environment. The protocols are based on the use of a hydroponics system and/or pot experiments in a glasshouse in combination with a controlled growth chamber where the heat stress treatment is applied. The methods include a new protocol for screening heat tolerance of rice at the seedling stage: young seedlings were exposed to heat stress of 45°C/28°C; the seedling test takes 4-5 weeks and involves the visual scoring of symptoms which allows hundreds of seedlings to be evaluated in a short time. The visual screening method was validated through laboratory, glasshouse, and field-based experiments. We also provide a protocol for screening heat tolerant mutant lines at the flowering (reproductive) stage that has been specifically adapted for a mutation breeding program. Here, plants were treated from the first day of anthesis at different temperatures (35.0-39.0°C/28°C) for different durations and spikelet fertility at maturity was determined as a parameter to assess the heat tolerance of the selected genotypes. Selected heat tolerant mutant rice genotypes were tested for physiological and biochemical indicators associated with the pre-field screen protocols. Furthermore, the candidate heat tolerant mutant lines were also tested in the field in hot spot areas in Cuba, Pakistan and the United Republic of Tanzania to evaluate their performance under field conditions in heat stressed growing environments.

IAEA-CN-263-274

THE USE OF GAMMA RADIATION TO GENERATE RESISTANCE TO FUSARIUM VERTICILLIOIDES IN MAIZE INBRED LINES

Lindy Rose¹, Altus Viljoen¹, Brad Flett², Christell Van der Veyver¹, Kobus Slabbert³

¹Stellenbosch University, South Africa

²ARC-GCI, South Africa

³iThemba LABS, South Africa

Email: altus@sun.ac.za

Fusarium verticillioides is associated with maize wherever it is grown. The fungus can reduce yield and grain quality, and this is exacerbated by the production of toxic secondary metabolites, called fumonisins. Plant resistance is the best means to control *F. verticillioides*, but such resistance is not readily available in existing maize cultivars. In this study, gamma irradiation was used to generate genetic variability for resistance to *F. verticillioides* and fumonisin accumulation. The irradiation procedure was optimised on a maize hybrid (CRN3505) and subsequently evaluated on three inbred lines (I-9, I-34 and CB-248). Percentage germination, seedling survival and plant height was measured at 7, 21 and 35 days after irradiation (dai) to determine the effective radiation dosage. The seedling height of CRN3505 was significantly affected 7 and 21 dai at <150Gy. Radio-sensitivity was displayed by lines I-34 and CB-248 at <200Gy at 7 dai, but inbred line I-9 was only significantly affected at 21 and 35 dai. Approximately 7 000 kernels of seven elite maize inbred lines were then irradiated at a dosage of 250Gy and field planted. Plants were self-pollinated and artificially inoculated with *F. verticillioides*. All plants that developed <10% FER (Fusarium ear rot) were retained and replanted the next season. They were analysed for FER severity, fungal biomass and fumonisin production at the M₄ stage. Field testing revealed that four M₄ mutant lines from inbred I-9, five from I-16, six from I-37, three from CB-222 and six from CB-248 were resistant to FER and fumonisin accumulation. These lines can be used to develop *F. verticillioides*-resistant hybrids or serve as donors of resistance alleles for high yielding inbred lines. The comparison of mutated and non-mutated lines could aid in the identification and isolation of resistance genes.

EVALUATION OF HOODED (Kap1), AWNLESS (Lks1) AND ORANGE LEMMA (Rob1) MUTANTS OF BARLEY (*HORDEUM VULGARE* L.) FOR THEIR USE AS FORAGE CROP

Heinrich Grausgruber¹, Christian Emsenhuber¹, Fenja Klevenhusen², Florian Hochhauser¹, Ljupcho Jankuloski³, Qendrim Zebeli²

¹BOKU, Dept. Crop Sciences, University of Natural Resources and Life Sciences, Austria

²University of Veterinary Medicine, Austria

³International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria

Email: heinrich.grausgruber@boku.ac.at

Barley (*Hordeum vulgare* L.) is grown on around 50 million hectares and is the fifth most important crop in the world. Its grain is mostly used as fodder for livestock or as malt for beer and whisky. Within this study, mutant stocks of barley were investigated for their suitability as forage crop. Forage barley can either be grazed at early growth stage or produced into silage or hay at heading, milk or early dough stage. Awns can cause injury in the mouth of animals which can lead to infections and less acceptability of the fodder. The Lks1 mutants have spikes without awns, in the Kap1 mutants the awns are replaced by 'hoods', i.e. reversed sterile florets on the end of the lemma. The rob1 mutant is supposed to produce less lignin and, therefore, a better digestibility is assumed. The barley mutants were investigated in three years field trials in the Pannonian region of Austria for early vigour and growth, resistance to fungal leaf diseases, biomass and grain yield, thousand grain weight and seed plumpness. Moreover, check variety 'Optic' and its EMS induced orange lemma mutant (rob1) were cut at two growth stages (i.e. booting, milk dough), dried to hay and submitted to a fermentation study using the rumen simulation technique (Rusitec). Some hooded and awnless mutant genotypes were identified with good to medium disease resistance, and biomass and grain yield similar to the check varieties 'Optic' and 'Eunova'. The Rusitec experiment showed significant differences between wildtype 'Optic' and its rob1 mutant with respect to chemical composition, fermentation characteristics and diversity of *in vitro* rumen bacterial community. A breeding program was started to combine awnless and/or hooded genotypes with orange lemma genotypes to combine the favourable mutants in one genotype.

RICE BREEDING IN NEW EXTREME ASIAN REGION, MONGOLIA

Ariungerel Mandakh, Ariuntuya Batchuluun, Myagmarsuren Yadamsuren, Odontungalag Tseeren
Institute of Plant and Agricultural Sciences, Mongolia

Email: holylight555@gmail.com

Mongolia is one of biggest regions of East Asia and it's unique and extreme dry and cold climate condition have been severe constraints for rice cultivation. Rice cultivation was first attempted in 1992 among local farmers, but has been unsuccessful. There is a deep interest and attempts are made to cultivate rice in Mongolian among farmers. Therefore, the Institute of Plant and Agricultural began investigations on rice cultivation in 2013. Two major experiments were conducted under the project: 1. identification of growable rice variety, and 2. rice mutation breeding. Under the first experiment, a selection of 209 rice varieties were tested during 2013-2018. Among studied varieties Nanatsuboshi, Hoshinoyume, Kendao 9, Kendao 27, Longjing 27, Longjing 48, Longjing 48, Wir 2040, Hokkaido varieties produced grain 0.55-1.01 ton/ha and were of interest. The maturation period of these varieties was 90-105 day after transplanting and 130-145 days after seedling. Rice mutation breeding using M₃ generations of Hoshinoyume (100Gy gamma-ray) and M₂ generations of Nanatsuboshi (150Gy gamma-ray) started in 2016 and advanced to M₄ generation (40 panicles and 15 rows selected). In the case of mutant generations of Hoshinoyume, 108 panicles and 36 rows were selected for 2018 evaluation.

Several mutant plants of Hoshinoyume and Nanatsuboshi mature 1-6 days earlier than wild types and showed 2 times higher yield than wild types (grain weight in per plant). Rice mutation breeding in Mongolia may allow the expansion of rice cultivation to include Mongolian conditions.

IAEA-CN-263-283

BROADENING THE GENETIC BASE AND NUTRITIONAL ATTRIBUTES OF COWPEA THROUGH MUTATION INDUCTION

Elias Peloewetse¹, Lekgari Lekgari Lekgari², Tidimalo Coetzee¹

¹*University of Botswana, Department of Biological Sciences, Botswana*

²*Ministry of Agriculture, Department of Agricultural Research, Botswana*

Email: peloewee@mopipi.ub.bw

Food and nutrition security at the household level remain significant challenges in Botswana's pursuit of sustainable development goals. The general population of Botswana depends on cereal and pulse crops for their food and nutrition security. Cowpea is one of the main pulse crops and is regarded by many as the poor man's meat due to its richness in protein. However, the presence of phytic acid, an anti-nutrient, limits the food and nutrition security prospects of this crop. This, together with the increasing cases of diabetes within the population of Botswana have provided the much-needed impetus to employ mutation induction to broaden the genetic base of cowpea. This has afforded a platform for selection of mutant lines of cowpea with diverse attributes. DNA analysis has revealed new genetic diversity in gamma-irradiated cowpea. Biochemical analysis of M₃ mutant lines has also revealed polymorphisms in respect of phytic acid content and starch composition of cowpea seeds, with some mutant lines showing significantly high amylose content (approaching 40%). Elucidation of molecular events underlying these phenotype alterations is currently ongoing and results will be shared through the symposium.

IAEA-CN-263-291

PRE-FIELD PHENOTYPING OF LENTIL MUTANTS FOR DROUGHT TOLERANCE USING POLYETHYLENE GLYCOL

Abdelbagi Mukhtar Ali Ghanim, Ljupcho Jankuloski, Ivan Ingelbrecht

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

Email: i.ingelbrecht@iaea.org, l.jankuloski@iaea.org, a.mukhtar-ali-ghanim@iaea.org

Lentil is the most widely preferable pulse crop, especially in Asia. It is predominantly grown under rain-fed condition. Drought is one of the main abiotic stresses that limit the plant growth and productivity in Bangladesh. A robust screening protocol is crucial for successful mutation breeding for drought tolerance. A pre-field screening method was optimized at the PBGL using polyethylene glycol in hydroponic nutrient solution. Three varieties and four mutant lines, from Bangladesh, were used for developing the screening method. Four concentrations (0.0%, 10%, 15% and 20%) of polyethylene glycol (PEG-6000) were used to induce plant-water deficit stress in an aerated hydroponic system. Drought stress was imposed at 14-days old seedlings with frequent changes and replenishment of the nutrient solution every 3-5 days. Observations were recorded on plant growth, chlorophyll content, biomass, stay-greenness and harvest index. Genotypes were classified based on their relative performance in stressed versus normal condition into drought sensitive, intermediate and tolerant. PEG-6000 sufficiently induced drought stress in the aerated hydroponic solution enabling reliable classification of the tested lentil genotypes on the bases of their tolerance during vegetative growth with high correlation to their seed setting and harvest index. Genotype 208 was the most tolerant with

significantly high number of seed sets under the highest level of stress (20% PEG concentration). Field evaluation is needed to validate the outcome of the PEG screening method.

IAEA-CN-263-292

OPTIMIZATION OF DOUBLED HAPLOID PRODUCTION FOR ENHANCING EFFICIENCY OF WHEAT MUTATION BREEDING

Abdelbagi Mukhtar Ali Ghanim, **Ljupcho Jankuloski**, Ivan Ingelbrecht
International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria
Email: l.jankuloski@iaea.org, i.ingelbrecht@iaea.org, a.mukhtar-ali-ghanim@iaea.org

Doubled haploid (DH) technology produces homozygous plants fast and increases efficiency of selection in mutation breeding. The Plant Breeding and genetics Laboratory (PBGL) is carrying out R&D to integrate efficiency enhancing technologies such as DH, rapid generation cycling and marker-assisted selection to speed up the breeding of mutant varieties. Anther culture was optimized to produce haploid plants from four spring wheat varieties and their derived M₁ plants. These M₁ plants were developed by irradiating seeds at 12-15% moisture content with 150, 200 and 250Gy of Gamma and X-ray using in-house irradiation sources. The material was grown in a glass-house following the optimized condition for wheat growth at the PBGL. Established protocol of anther culture was used with minor modifications in pre-treatment and induction media. Spikes were collected at the appropriate early to mid uni-nucleate stage and stored at 4°C for 5-7 days before *in vitro* culture. Different combinations of hormones in solid and liquid induction medium were evaluated for haploid induction. The four wheat varieties and their M₁ generation were comparable in their response to anther culture with varying degrees across different combination of hormones and media. Relative response to the untreated control varied between 55.8 to 112.5% over the three doses (150, 200 and 250Gy). Response of M₁ to anther culture varied with the varieties from comparable between the three treatments such as in Khalifa (90-112.2%) to reduced response with increase in irradiation dose in the other three varieties. The relative response dropped to around 60% with 250Gy however, it is still sufficient to generate reasonable mutant population size for screening purpose. The optimized protocol is expected to enhance efficiency of wheat mutation breeding and accelerate delivery of mutant varieties.

IAEA-CN-263-293

RAPID CYCLING TECHNIQUES TO ACCELERATE PLANT MUTATION BREEDING IN CEREALS

Abdelbagi Mukhtar Ali Ghanim, Ljupcho Jankuloski, **Ivan Ingelbrecht**
International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria
Email: i.ingelbrecht@iaea.org, l.jankuloski@iaea.org, a.mukhtar-ali-ghanim@iaea.org

Although plant mutation breeding is faster than conventional breeding, techniques are available that can speed up the time from mutation induction to mutant variety release. Advances in high-throughput phenotyping and genotyping, and tissue culture provide several opportunities to make efficiency gains and accelerate the delivery of mutant varieties. The PBGL is carrying out R&D to integrate methods in rapid generation cycling, doubled haploidy and marker-assisted selection to speed up the breeding of mutant varieties. Ten wheat, 7 sorghum and 4 barley varieties from Kenya, Sudan and Austria, respectively, were propagated in different pot sizes, day length and watering regimes to shorten the time needed to flowering and seed setting. At milky stage of seed development, immature embryos rescued at 10, 15, and 20 days after pollination. Plants propagated in small pots under continuous light flowered

in less than 35 days. With embryo rescue, an average of 45 days (barley), 48 (wheat) and 60 (sorghum) days were sufficient to complete a generation cycle and produce enough seeds for phenotyping and marker assisted selection in the next generation. These techniques may enable the production of 4-7 generations a year under glass-house with manageable day length options, which is enough to reach sufficient homozygosity to advance a mutant line to evaluation trails.

IAEA-CN-263-294

OPTIMIZATION OF SCREENING FOR SALT TOLERANCE IN SOYBEAN FOR MUTATION BREEDING

Abdelbagi Mukhtar Ali Ghanim, **Ljupcho Jankuloski**, Ivan Ingelbrecht
International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria

Email: l.jankuloski@iaea.org, a.mukhtar-ali-ghanim@iaea.org, i.ingelbrecht@iaea.org

The world faces great challenges to maintain sustainable food production to feed growing population in the era of climate change. Crop production is increasingly affected by abiotic stresses such as drought, salinity and heat stress. Mutation breeding provides attractive options to generate genetic variability for abiotic stresses. The PBGL has been involved in developing efficient and reliable screening methods for development of mutant varieties tolerant to abiotic stresses such as drought, salt and heat stress. Screening packages were developed for different crops under different conditions in the laboratory, glasshouse and open field. Twenty soybean genotypes were used for salinity screening using hydroponic culture. Sodium chloride (NaCl) was used at 0, 5, 10 and 15 dS/m concentrations for salinity induction in hydroponic system with modified Ishida's nutrient solution. Salinity stress was imposed at early vegetative stage (after nine days from transplanting of seedlings in hydroponic) and continued for more than 6 weeks. Measures were taken for plant growth traits like plant height and leaf number, chlorophyll fluorescence etc. Genotypes had shown variation in sensitivity to salinity stress with increasing in the intensity and time of exposure to stress. Combined differential scoring of these parameters enabled classification of the 20 soybean genotypes into five classes; highly sensitive, sensitive, moderately sensitive, tolerant and highly tolerant to salinity. Tolerant genotypes were further validated under soil condition. The extended treatment with different levels of salinity enables the classification of the genotypes based on their tolerance threshold level that can be adapted for screening of mutant populations for salt tolerance. The matrix of salt concentration and time of exposure to stress provides options based on the targeted environment.

IAEA-CN-263-295

IRRADIATION-DOSE OPTIMIZATION FOR MUTATION INDUCTION IN COFFEE

Abdelbagi Mukhtar Ali Ghanim, Ivan Ingelbrecht
International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria

Email: a.mukhtar-ali-ghanim@iaea.org, i.ingelbrecht@iaea.org

Coffee, a perennial tropical crop, can be grown from seeds or cloned plants in the form of cuttings, grafts or tissue cultured plants. Arabica coffee is most commonly grown from seeds while canephora is mostly grown vegetatively from cuttings and other propagules. Improving coffee through conventional breeding is seriously limited by the availability of genetic variation. Mutation breeding provides great potential to induce the novel genetic variation needed for coffee improvement. The process of optimizing dose involved dose-response experiments where the pattern of reduction in germination or growth rate is determined in relation to increasing absorbed doses. From these experiments, the LD/GR50 and LD/GR30 are calculated. In coffee after adjustment trials on seeds and vegetative parts

using our in-house gamma and X-ray irradiators, we came to a range of: 0, 50, 100, 150, 200, 400Gy for *C. arabica* seeds and: 0, 5, 10, 15, 20, 30 and 40Gy for seedlings and cuttings of *C. arabica* and *C. canephora*. The protocol for seed treatment follows the general procedure which starts with sorting clean and viable seeds, moisture equilibration in a desiccator with 60% glycerol, irradiation treatments, planting the treated material in suitable set-up such as moist filter papers in Petri-dish, soil in trays or pots and incubate at appropriate condition under warm condition 28-30°C. Germination or growth rates after 30 days are recorded and plotted relative to the untreated seeds over the series of the doses and from the blotted graph the doses for LD50, GR50 and LD30, GR30 estimated. The estimated dose can be used for bulk treatment. The same follows for vegetative propagules (cuttings, seedling, embryo etc) except that the applied doses here are relatively low in the order of 0 to 40Gy. The protocols will be reviewed for dissemination and wide adoption by the Member States.

IAEA-CN-263-296

MUTATION BREEDING FOR RESISTANCE TO THE PARASITIC WEED STRIGA IN MAJOR CEREAL CROPS FOR SUSTAINABLE FOOD SECURITY

Abdelbagi Mukhtar Ali Ghanim, Adel Ali, Ljupcho Jankuloski, Ivan Ingelbrecht
International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria

Email: i.ingelbrecht@iaea.org, a.mukhtar-ali-ghanim@iaea.org, l.jankuloski@iaea.org

Striga is an obligate hemi-parasitic weed species that attacks roots of major cereal crops such as sorghum, millet, maize and rice. In severe situations of Striga infestation, losses in crop yield may reach up to 90-100%. Forty percent of arable land in sub-Saharan Africa is infested with Striga with annual losses estimated up to 14 billion US\$. For subsistence farmers, growing resistant varieties is one of the most viable control option. A Coordinated Research Project was launched by the IAEA in 2016 for mutation breeding of resistance to Striga in major cereal crops with the objective to develop screening protocols, generate genetic variability and optimize efficiency enhancing technologies to accelerate delivery of resistant varieties. We report in this presentation an overview of the progress made and the prospective. Simple, efficient and reliable screening protocols were optimized for field, glass-house and laboratory screening of *Striga hermonthica* and *S. asiatica* in upland rice and sorghum. These protocols were verified in more than one site in the participating countries and validated on M₂ populations. A total of 82 and 47 putative resistant mutants were, so far, reported in upland rice and sorghum, respectively, by three of the participating countries in Africa (Burkina Faso, Madagascar and Sudan), and the PBGL. Field resistance in M₄ of 20 upland rice and 6 maize lines from Madagascar are in verification and classification at PBGL using pot-phenotyping. Field performance of promising mutants will be evaluated across different environments and crosses will be made between stable mutants and their parents for allelism test, mechanism of resistance and to produce populations for development of molecular markers linked to the resistance. The technologies developed and the generated resistance to Striga together with the markers will enable wide adoption by the Member States for Striga management in vulnerable areas.

DEVELOPMENT OF BARLEY TILLING MUTANTS TOLERANT TO ALUMINIUM STRESS

Miriam Szurman-Zubrzycka¹, Agata Daszkowska-Golec¹, Beata Chmielewska¹, Elena Todorovska², Iwona Szarejko¹, Izabela Bedkowska¹, Janusz Jelonek¹, Malgorzata Nawrot¹
¹*Department of Genetics, Faculty of Biology and Environmental Protection, University of Silesia, Poland*
²*Agrobiointitute (CAB), Bulgaria*
 Email: miriam.szurman@us.edu.pl

Aluminium is the third most abundant element in the Earth's crust. In acidic soils it solubilizes to Al³⁺ ions, that are phytotoxic and cause root growth inhibition. Around 50% of arable lands are acidic. Barley is one of the crops most sensitive to Al it is a good model for studying Aluminium response. The common mechanism of Aluminium tolerance is related to excretion of organic acids. Here we investigate another pathway of Aluminium tolerance through changes in genes related to cell cycle progression and DNA repair to generate Al-tolerant mutants in barley. We used HorTILLUS plat form and TILLING strategy to identify mutants in HvATR (cell cycle checkpoint regulator), HvALT2 (halting cell divisions) and HvSOG1 (Transcription factor (TF) involved in DNA damage response (DDR)). Mutants were screened in hydroponic system in Hoagland solution containing 20µM, 30µM and 40µM of bioavailable Al. After one week, root systems were scanned and analysed in detail. DNA damage caused by Aluminium was investigated on the cytological and molecular levels. We have identified 44 mutations in the three selected target genes. Mutants carrying missense or splice junction mutations were screened for Aluminium response. We found several forms with increased Aluminium tolerance. Their root system was less affected by Al³⁺ ions than the system of the wild types after treatment with different doses of Aluminium. The cell cycle and mitotic index in root meristems were analysed in mutants and wild type after Aluminium treatment. Aluminium is a very important factor limiting crop production on acidic soils. Developing Aluminium tolerant forms is of great importance for plant breeding. Our barley TILLING mutants carrying changes in genes related to cell cycle progression and DNA repair may help to elucidate the alternative pathways of Aluminium response and are a valuable material for breeding barley more tolerant to Al toxicity.

MAIN RESULTS OF PLANT MUTATION INDUCTION FOR ABIOTIC STRESS IN CUBA

Maria Caridad Gonzalez Cepero¹, Rodolfo Guillama Alonso¹, Dayne Horta Fernandez¹, Alba Alvarez Gonzalez², Armando Chavez Ardanza², Novisel Veitia Rodriguez³, Mayra Rodriguez Rodriguez⁴
¹*National Institute of Agricultural Sciences, Cuba*
²*Center for Technological Applications and Nuclear Development, CEADEN, Cuba*
³*Plant Biotechnology Institute, IBP, Cuba*
⁴*Engineering Genetics and Biotechnology Center, CIGB, Cuba*
 Email: mcaridad@inea.edu.cu

The effects of climate change have had an unfavourable impact on food production. Drought and high temperatures affect the production and supply of food to the population in Cuba. The development of new varieties tolerant to drought is an alternative that will be used to minimize the effects of climate change on food production and the induction of mutation has demonstrated its potential in this regard. Faced with this problem in the National Institute of Agricultural Sciences, an improvement program was developed that seeks to obtain new varieties of tomato, common bean and *Hibiscus sabdariffa* highly productive under conditions of drought and high temperature. Tomato seeds, common beans and

Hibiscus sabdariffa were irradiated with different doses of ^{60}Co gamma rays. After four rounds of selection in the field, some genotypes with high yield potential under water supply and high temperature conditions were identified. The best genotypes were evaluated by farmers in different production areas. Three tomato mutants as well as three mutants of *Hibiscus sabdariffa* were released. Some advanced mutant lines of common bean tolerant to high temperature and drought were selected. Biochemistry and molecular evaluation of obtained mutants was performed.

POSTERS RELATED TO SESSION 3

**ENHANCING AGRICULTURAL BIODIVERSITY THROUGH
NEW MUTATION INDUCTION TECHNIQUES**

M Building – Common Area

INDUCED MUTAGENESIS IN *PHASEOLUS VULGARIS* L. USING GAMMA RAY AND DETECTION OF DNA POLYMORPHISM THROUGH RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MOLECULAR MARKERS

Christal Toge¹, Adenubi Adesoye²

¹*Sheda Science and Technology Complex, Nigeria*

²*University of Ibadan, Nigeria*

Email: crystoge@gmail.com

Phaseolus vulgaris L. is a valuable and highly nutritious food legume and highly underutilized in Nigeria as a result of prolong cooking time, low yield and anti-nutritional factors. 7 doses (control, 25Gy, 50Gy, 100Gy, 150Gy, 200Gy, 250Gy) of gamma ray was induced using the gamma facility at the NIRPR, to evaluate its effect on morphological and yield-related traits of *P. vulgaris* varieties (Bokkos and Sardauna) and to identify polymorphism through Random Amplified Polymorphic DNA (RAPD) marker. Improved germination was observed in treated plants (25Gy to 250Gy) in both varieties. There was observed increase in the number of branches, pod length, pod width and number of seeds per pod in the Bokkos variety at 25 to 50Gy. While the Sardauna variety had some observed improvement (25 to 100Gy) in traits namely, plant height, number of branches, number of pods per plant, number of seeds per plant. Flowering and maturity in both varieties were earlier than the control at 200Gy and 250Gy doses. For molecular analysis, of the 12 primers screened, 8 were polymorphic with Primer OPB09 producing the maximum number of bands. Primer OPB01, OPB09, OPB07 and OPT01 showed unique (new) bands in 25Gy, 50Gy, 200Gy and 250Gy of both varieties. In the dendrogram constructed based on the genetic similarity coefficient, the mutants were grouped into four clusters. The treated plants showing their differences in morphological and yield-related traits also showed polymorphism in PCR profile amplified by RAPD marker. Genetic variation was observed among the treated plants and their controls in both varieties. Further research on these mutants can lead to the development of elite line or parent donors for cross breeding to obtain hybrids with improved and better morpho-agronomic characteristics for crop improvement.

EFFECT OF GAMMA RAYS ON SEED GERMINATION AND PLANT GROWTH PARAMETERS OF THREE RICE VARIETIES CULTIVATED IN SRI LANKA

Kamani Wijesena, Amitha P. Bentota, Anoma Nawarathne

Rice Research & Development Institute, Department of Agriculture, Sri Lanka

Email: kamaniwijesena@gmail.com

Development of rice varieties using induce mutation has seldom been applied in Sri Lanka. This study was carried out to determine the effect of gamma irradiation doses on seed germination and different plant growth parameters of three rice varieties. Two hundred grams of seeds of three rice varieties cultivated in Sri Lanka (Bg 94-1, Bg 1165-6, Suwandal) were subjected to gamma rays from ⁶⁰Co source using 3 doses: 200, 300 and 400Gy. Irradiation was undertaken at the IAEA laboratories Seibersdorf, Austria in 2015. The experiment was designed as Split Plot with three replications and conducted at the plant house of Rice Research & Development Institute, Batalagoda. The effects of radiation doses on seed germination and different plant growth parameters were evaluated (radiosensitivity testing). With the increase in gamma doses seed germination, seedling emergence, seedling height, root length and plant survival in the field decreased. Highly significant differences were observed among the varieties for seedling height, root length and percentage of field survival. Gamma irradiation showed significant negative correlation with seed germination (-0.524), seedling emergence (-0.543), seedling height (-0.545), root length (-0.498) and field survival (-0.424). Percentage of field survival was the least

affected character while percentage reduction was 8.56. The most efficient and optimum dose to induce rice mutation of the varieties under investigation is within the range of 200Gy 300Gy. More pronounced adverse effect was observed for all the growth characters at the highest dose in all three varieties. Increasing doses above 400Gy caused severe morphological damages to the rice plants and increasing gamma irradiation doses has negative correlation with the germination and other plant growth parameters. A comprehensive study should be carried out to determine the optimum doses for different rice varieties which could be useful in rice varietal improvement programs in Sri Lanka.

IAEA-CN-263-20

EFFECTIVE SELECTION CRITERIA FOR ASSESSING SALT STRESS TOLERANCE IN BEARD WHEAT LINES INDUCED BY GAMMA RADIATION

Ali Reza Askary Kelestanie¹, Eskandari², **Azam Borzouei**², S. Sanaz Ramezanzpour¹

¹*Department of Biotechnology and Plant Breeding, Gorgan University of Agricultural Sciences and Natural Resources, Iran*

²*Nuclear Science and Technology Research Institute, Iran*

Email: aborzouei@nrcam.org

Mutations induced by radiation with gamma rays has had a major role in improving the most of main agronomic traits in plants. In order to determine some stability indices related to wheat grain yield the fifth generation (M₅) under salinity conditions, 15 selected lines from the fourth mutant generation (M₄) together with two parents' genotypes ('Arg' and 'Bam') and two genotypes produced in Yazd region ('Sivand' and 'Narin') were planted under both conditions of non-stress (2dsm⁻¹) and salinity stress (10dsm⁻¹) in the research field of the National Salinity Research Centre (NSRC) in Yazd province. Analysis of variance for a complete random block design was performed on yield, components and the comparison of means was conducted by LSD test. Tolerant indices of mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), Stress stability index (SSI), Tolerance index (TOL) were calculated to investigate salinity. The results showed that, Line two (Arg-200, 246.67g/m⁻¹) and Line one (Bam-150, 165.55g/m⁻¹) had the highest grain yield. Regarding the positive and significant correlation between grain yield in both conditions and indices of MP, GMP and STI, these indices were selected as the most desirable tolerance indices. According to three-dimensional graph Fernandez were introduced that lines one, two and eight as the most tolerant lines to salinity stress. The experiment results showed, indices of MP, GMP and STI are the most suitable indices for selection of lines tolerant to salinity. Lines one, two and eight had high grain yield under both conditions. So, according to the results lines one, two and eight can be introduced as stable lines and with high yield under salinity stress conditions and the fifth generation and it is recommended to introduce these three lines for cultivation in the sixth generation.

IAEA-CN-263-26

EFFECT OF EMS ON SEED GERMINATION IN CHICKPEA

Mahesh Kharde

Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar, India

Email: maheshkharde@gmail.com

Chickpea (*Cicer arietinum* L.) is an ancient crop cultivated in about 40 countries of the world. India is the largest producer sharing 75% of the world's production. In modern agriculture a remarkable progress has been done in increasing the genetic potential for crop productivity. Ethyl Methane-sulfonate (EMS) is a chemical mutagen which was used to test its effect on seed germination in chickpea. *Cicer arietinum* L. seeds with uniform size were subjected to the chemical mutagen by

soaking seeds in various concentrations; i.e. 0.1M, 0.2M, 0.3M, 0.4M and 0.5M respectively for about 2 hours. Then, the seeds were placed on wet paper towel to germinate and the percent seed germination was determined on the 4th and 7th day. Results revealed that EMS – the chemical mutagen – had inhibitory effect on seed germination. It showed gradual decrease in germination with the increase in the concentration of chemical mutagen. Maximum 90% seed germination was observed in 0.1M concentration whereas a minimum of 70% seed germination was observed in 0.5M concentration. The present investigation concludes that EMS has inhibitory effect on seed germination, which can be employed in mutation breeding programmes.

IAEA-CN-263-27

CONSEQUENCES OF SODIUM AZIDE ON PERCENT SEED GERMINATION AND PEROXIDASE ISOZYME PATTERNS IN COWPEA

Anil Wabale

Padmashri Vikhe Patil College of Arts, Science and Commerce, India

Email: dranilwabale78@gmail.com

Peroxidase is one of the important enzymes present in all tissues and performs catalytic functions. It is suitable as a model enzyme for studying the effect of any mutagen. In the present investigation, an attempt was made to study the effect of sodium azide (SA) on peroxidase composition in cowpea (*Vigna unguiculata* L. Walp.), with an objective of finding its mode of action i.e. whether it has promoting or inhibitory effect on the enzyme synthesis and whether the action is specific or random. The seeds were treated for two hours with various SA concentrations viz. 0.0005M, 0.0010M, 0.0015M, 0.0020M, 0.0025M and 0.0030M. These seeds could germinate on moist filter paper in Petri plates at room temperature (20-25°C) for about three and seven days respectively and later analysed for seed germination. Peroxidase enzymes were extracted from the seedling samples and subjected to electrophoresis. Results indicated that there was a gradual decrease in seed germination percentage with an increase in the concentration of SA. SA retarded the growth of radicle and plumula. PAGE analysis of peroxidase enzyme composition of control cowpea seedlings showed presence of three peroxidase isoforms, but 0.0025M SA treated seedlings showed presence of five peroxidase isoforms. This clearly indicated that, SA induces synthesis of certain peroxidase isozymes. Similarly, certain peroxidase isozymes present in control seedlings were seen missing in the SA treated seedlings. This evidently reveals that SA along with seed germination hampering can also inhibit the synthesis of certain enzymes.

IAEA-CN-263-28

ASCENDANCY OF PHYSICAL MUTAGEN ON CALCIUM CONTENT IN *MEDICAGO SATIVA* L.

Ramdas Borse

Padmashri Vikhe Patil College of Arts, Science and Commerce, India

Email: rdborse@gmail.com

In India, Alfalfa (*Medicago sativa* L.) is an important fodder crop which is rich in calcium, minerals, proteins and other nutrients. It not only increases fat value, but also increases the milk yield. Gamma irradiation has been effectively used to induce useful mutation and enrich the biochemical components in alfalfa. Seeds of *Medicago sativa* L. were irradiated with 5KR, 20KR, 35KR, 50KR and 65KR Cobalt doses respectively. Further, the seeds were sown in the fields under a Randomized Block design. The entire plant material was collected at maturity stage, dried and reduced to powder. The powder was analysed to determine the calcium content using Flame Photometry. Gamma irradiation showed stimulating effect on increasing calcium content of Alfalfa in lower doses of Cobalt-60. It was also

observed that calcium content in aqueous extract was increased as compared to methanolic extract. From the present investigation it can be concluded that gamma radiation can be effectively used in Alfalfa mutation breeding programmes for fodder quality improvement as it is useful in increasing calcium content.

IAEA-CN-263-60

ANALYSIS OF MULTIPLE DIFFERENCES ASSOCIATED WITH CHLOROPLAST IN THE SPACEFLIGHT-INDUCED WHEAT LEAF COLOUR MUTANTS

Jiayu Gu¹, Hongchun Xiong¹, Huijun Guo¹, Junhui Li¹, Kui Shi², Luxiang Liu¹, Yongdun Xie¹, Yuping Ding¹, Linshu Zhao¹

¹*Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, China*

²*Allwegene Technology Inc, China*

Email: gujiayu@caas.cn

Chloroplast development is an integral part of plant survival and growth, and occurs in parallel with chlorophyll biosynthesis. We have obtained three novel wheat leaf colour mutants mtg, mts, and mta from spaceflight-induction. To explore the genetic variations under space environment and uncover the mechanisms, which cause the leaf colour mutations, we performed chloroplast ultrastructural observation, photosynthetic pigments assays, leaf transcriptome sequencing, chloroplast proteomic analyses and chloroplast genome sequencing. Compared to wild type, chloroplast of mts and mta exhibit abnormal morphology and distribution. The photosynthetic pigments content was also significantly decreased. From transcriptome and chloroplast proteome profiling, a total of 4588 differentially expressed genes (DEGs) and 48 differentially chloroplast expressed proteins (DEPs) were identified between mta and wild type, most of which were involved in chloroplast development, chlorophyll biosynthesis, or photosynthesis. The correlation analysis between DEGs and DEPs revealed that the transcript-to-protein abundance was functioned into photosynthesis and chloroplast relevant groups. Chloroplast genome sequencing revealed the impact of spaceflight environmental forces on the variations in the whole plastome sequences. The complete plastomes of three leaf colour mutants mtg, mts, mta and WT were obtained, and the cp genomes were highly conserved in organization. However, the totally length and boundaries of cp genome were slightly distinct. Besides, many variations and gene mutation were found and might affect the leaf colour traits. Integrated omics analyses revealed the main molecular mechanisms regulating leaf colour formation, involving chloroplast development, chlorophyll biosynthesis, and photosynthesis. In addition, plastome variations were identified in the leaf colour mutants. The alterations were induced by space environment, which would be fully utilized of mutagenesis in crop breeding.

IAEA-CN-263-67

IMPROVEMENT OF TRADITIONAL RICE LANDRACES OF CHHATTISGARH (INDIA) THROUGH RADIATION INDUCED MUTAGENESIS

Deepak Sharma Deepak¹, Ashish Tiwari Ashish¹, Bikram Kishore Das Bikram³, Parmeshwar Sahu Parmeshwar¹, Samrath Baghel Samrath¹, Satyapal Singh Satyapal¹, Vikash Kumar Vikash²

¹*Indira Gandhi Agriculture University, India*

²*Bhabha Atomic Research Centre, BARC, India*

Email: deepakhybridrice@gmail.com

Chhattisgarh state of India is dominated by tribal peoples and known as 'Rice Bowl of India' due to its rich heritage in rice biodiversity. More than 23,500 rice germplasm lines are being conserved and

maintained at Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur (Chhattisgarh). A total of 300 aromatic fine grain/short slender grains rice varieties with high nutritive value and good grain quality were selected for improvement by reducing height and maturity duration. These rice varieties are very common and famous among the farmers of Chhattisgarh. Mutation breeding activity in rice started during 2013 at IGKV, Raipur (CG). During this period, a total of 23 rice varieties were irradiated with gamma and electron beam. Physical mutagens (Gamma and electron beam) were used to irradiate the 23 rice varieties with 250Gy and 300Gy at Bhabha Atomic Research Centre (BARC), Mumbai (MH) and Raja Ramanna Centre for Advanced Technology, Indore (MP). Field screening and mutation breeding activities were carried out at IGKV, Raipur. A mutagenized population of 23 rice landraces/farmers' varieties are being maintained at different generations (M_2 to M_8) at field. A total of 12 rice mutants viz., 'Dubraj' (Trombay Chhattisgarh Dubraj Mutant-1, Trombay Chhattisgarh Dubraj Mutant-2, Trombay Chhattisgarh Dubraj Mutant-4,), 'Jawaphool' (Raipur Trombay Rice-31, Raipur Trombay Rice-17, Raipur Trombay Rice-18), 'Safri-17' (Raipur Trombay Rice-602, Raipur Trombay Rice-603, Raipur Trombay Rice-605,), 'Luchai' (Raipur Trombay Rice-501), 'Tilkormel' (Raipur Trombay Rice-204), 'MTU1010' (Raipur Trombay Rice-110) and 'Sonagathi' (Raipur Trombay Rice-702,) were developed with early to mid-late maturity with reduced plant height. These mutants are being evaluated in the state and national yield trials for release in Chhattisgarh State as well as in India.

IAEA-CN-263-100

MUNGBEAN RADIOSENSITIVITY TEST TO GAMMA IRRADIATION FOR MUTATION BREEDING IN MUNGBEAN

Munasingha Jayasundara Mudiyansele Priyanthi Kumararathna¹, Abdelbagi Mukhtar Ali Ghanim²

¹*Department of Agriculture, Sri Lanka*

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

Email: priyanthikumara@yahoo.com

The first stage of plant breeding program by induced mutation technique using gamma rays is to determine the optimum dose for the bulk irradiation treatment as radiosensitivity varies with species, varieties, and genotypes. Therefore, the objective of this research was to determine the gamma ray dose which reduces growth by 50% (GR50) in four mungbean varieties. The trial was conducted at the Joint FAO/IAEA Plant Breeding and Genetics Laboratory, Seibersdorf, Austria. Genetically sound seeds of 4 mungbean varieties: Bino 2, Bino 5, Bino 7 and Bino 8 with a good germination rate and whose moisture has been equilibrated were subjected to gamma rays with the following doses: 0, 75Gy, 150Gy, 300Gy, 450Gy and 600Gy and planted in a RCB design with three replicates. Data were recorded for germination percentage, survival percentage at 15 days after seed sowing, chlorophyll mutation, leaf mutation, variation in plant height (at 21 days and 30 days) and in the number of leaves. Except mungbean variety Bino 7, the other three varieties showed significant reduction of seed germination with increasing doses. Numbers of days to seed germination appeared also to increase when irradiated with higher doses (450Gy and 600Gy) and significant variation was observed among varieties. Variety Bino 2 showed a two-fold shorter duration for seed germination at 600Gy compared to the non-irradiated control. Survival rate of the varieties were drastically reduced with increasing doses after 300Gy. Significant effects on plant height were observed with 600Gy dose in all varieties. Higher chlorophyll and leaf abnormalities were also observed in higher doses. Hundred percent leaf variegation was observed with 600Gy dose irradiation in all varieties. Growth reduction dose was determined by calculating plant height reduction as compared to the non-treated control. The highest GR50 value (523Gy) was observed in variety Bino 8 and the lowest value (484Gy) was observed in Bino 5. LD30 was around 300Gy-310Gy in all varieties.

STUDY TO DETERMINE THE GROWTH REDUCTION DOSE 50 (GR50) FOR GAMMA RAYS INDUCED MUTAGENESIS IN CARROT (*DAUCUS CAROTA* L.)

Priya Huzar Futtu Beejan, Rita Nowbuth

Food and Agricultural Research and Extension Institute, Mauritius

Email: phfuttu@yahoo.com

A study was initiated with the aim to develop new carrot varieties with good nutritive content using nuclear techniques. Mutation using gamma irradiation was selected to induce genetic variability for carrot cultivar improvement. Radio-sensitivity study of carrot to gamma irradiation was investigated to determine the dose which causes 50% growth reduction (GR50) for an indication of the optimal mutagenic irradiation treatment to be applied. After moisture stabilisation, carrot seeds were first subjected to gamma radiation doses of 25, 30, 35, 40, 45 Gray (Gy) and then in a second experiment the doses were increased from 450, 500, 550, 600, 650, 700, 750Gy using a ¹³⁷-Caesium radioisotope. These doses were not effective in inducing growth reduction and mortality. It was concluded that the indicative carrot seeds had to be exposed to gamma irradiation doses higher than 750Gy. Carrot seeds were subjected to dosages of 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400 and 1500Gy of gamma irradiation. The GR50 was determined based on growth reduction in parameters such as root length and plant height. GR50 was determined to be 1075Gy using plant height as the more appropriate parameter. Based on this radio-sensitivity study, the local carrot seeds were subjected to the determined GR50 dose for induction of mutation by gamma irradiation and it was followed by field sowing and evaluation. Mutant line with improved agronomic characteristics will be selected for further evaluation.

EVALUATION OF THE EFFECTS OF PROTON BEAM IRRADIATION ON GERMINATION AND GROWTH IN RICE SEEDS

Hong-Il Choi, Sang Hoon Kim, Si-Yong Kang, Sung-Min Han, Sungil Lee, Yeong Deuk Jo

Korea Atomic Energy Research Institute, Republic of Korea

Email: hichoi@kaeri.re.kr

Ionizing radiations are widely used as physical mutagens in mutation breeding. Proton beams (PBs), which are generally regarded as low linear energy transfer (LET) radiation, are known to have physically intermediate properties between low LET gamma rays (GRs) and high LET ion beams, but have still not been utilized for breeding. This study was conducted to estimate the biological effect of proton beam irradiation compared with gamma irradiation and investigate the optimal dose for mutation induction in rice. Rice seeds were irradiated using a 100 MeV Linear Accelerator (TR103) at the Korea Multi-Purpose Accelerator Complex for PB and a ⁶⁰Co gamma irradiator at the Advanced Radiation Technology Institute for Gamma Ray (GR), with doses of 50, 100, 150, 200, 250, 300, 400, 500, 600, and 700Gy in both cases. The PB irradiated seeds did not germinate from 500Gy or higher doses, whereas the GR irradiated seeds did not germinate from 600Gy or higher doses. The dose showing a half germination, i.e. LD50 against the control were 200Gy and 350Gy for PB and GR respectively. The LD50 and GR50 values estimated by analysing the survival rates (LD50) and growth (GR50) of the four-week-old seedlings were 150Gy and 175Gy for PB, and 260Gy and 300Gy for GR, respectively. These results suggest that the biological damage of PB is more severe than that of the used GR at the same dose. To induce mutations using PB in rice, doses from 100 to 150Gy seem to be proper, and thus we are planning to make an M₁ population with these doses during this year.

THE RELATIONSHIP BETWEEN THE PRESENCE OF BRANCHED EARS, FERTILITY, SEEDING GROWTH AND ENERGY CONVERSION AFTER GAMMA IRRADIATION OF KERNELS IN *TRITICUM AESTIVUM* L.

Eben Von Well¹, Annabel Fossey², Marde Booyse¹

¹*Agricultural Research Council, South Africa*

²*Free State University of Technology, South Africa*

Email: vonwelle@arc.agric.za

The study was conducted to determine whether the efficiency of energy conversion into growth and the presence of branched ears can be used as indicators for the determination of the optimal gamma irradiation dosage for mutation breeding in relation to fertility reduction. To meet this objective, efficiency of energy conversion, fertility and presence of branched ears were studied in two wheat (*Triticum aestivum* L.) breeding lines with 0, 150, 200 and 250Gy gamma-irradiation of kernels. Ten kernels were collected 60 hours after onset of imbibition and then every 12 hours until 132 hours and planted for the determination of the growth parameters. 26 blocks (consisting of 16 plants each) were used for the control together with each irradiation dosage for the determination of the fertility and branched ears. Breeding line 1 displayed better resistance to the retardation effects of the dosages than Breeding line 2 concerning the growth parameters and fertility. In both breeding lines, there were highly significant correlations between the efficiency of energy conversion and fertility with 0.597 and 0.527 respectively for Breeding line 1 and 2. Branched ears were observed at dosages where root growth was $\geq 10\%$ retarded than shoot growth. Branched ears were observed at all the irradiation dosages in Breeding line 2 and only at 250Gy in Breeding line 1. Branched ears were absent at dosages where the efficiency of energy conversion was entangled for specific irradiation dosages with the control. An increase in shoot growth retardation puts an upper limit to the presence of branched ears as observed at 250Gy in Breeding line 2. A combination of the efficiency of energy conversion into growth and presence of branched ears will give the best indication of the optimal gamma irradiation dosage for mutation breeding.

RADIOSENSITIVITY TEST ON GAMMA IRRADIATED *COFFEA ARABICA* VAR. VENECIA IN VITRO ZYGOTIC EMBRYOS

Noel Arrieta, Reina Cespedes, Miguel Barquero

Costa Rica-Coffee Research Center-Coffee Institute-Technology Institute, Costa Rica

Email: narrieta@icafe.cr

In vitro culture is an important tool for coffee breeding programs. Mutation breeding in combination with *in vitro* techniques and other biotechnologies offers opportunities for the generation of new improved coffee varieties in a shorter period as compared to classical plant breeding. The plant material used were fruits of *Coffea arabica* var. Venecia. The fruit pulp was removed, and the seeds washed with soap and water, dried and stored for use. For *in vitro* culture of zygotic embryos, the seeds were immersed in 70% alcohol and disinfected with sodium hypochlorite (2.5% active ingredient). Then, the zygotic embryos were excised, placed vertically in culture medium for subsequent irradiation treatments. Four treatments were performed 0, 10, 20 and 30Gy, each with ten repetitions and 10 embryos per repetition. Cotyledon, hypocotyl and root length and width were determined in the regenerated plantlets, in determined days after irradiation. The results show different effects of the irradiation treatments regarding the measured characteristics. It was observed that the length and width of the cotyledon decrease with increasing applied doses. As to the root length and hypocotyl width there was no specific trend, however, some doses had a stimulating effect to growth in comparison with the

non-irradiated treatment. The hypocotyl length showed a growth reduction different to the non-irradiated treatment although the dose of 20Gy is higher than 10Gy and the root width have no differences in the sizes for all treatments. The results show growth reduction of at least three of the evaluated characteristics in response to the radiation dose.

IAEA-CN-263-133

INDUCED CHLOROPHYLL CONTENT AND MORPHOLOGICAL MUTATIONS IN *PHASEOLUS VULGARIS*

Arjana Ylli, Ilirjana Stamo
Department of Biotechnology, Tirana University, Albania
Email: ariana_ylli@yahoo.co.uk

Beans (*Phaseolus vulgaris*) are major leguminous plants with a high genetic variability for agricultural production. With climatic changes that have occurred in recent years, bean production has been reduced. Due to this reduced production of beans, different studies have been carried that aim to address these losses in productivity. One of the problems is the abortion of flowers of the beans in high temperature and drought conditions. Through induced mutation techniques on bean seeds we tried to extend the time to flowering to eliminate the abortion of flowers. Bean seeds were irradiated with Cs-137 gamma radiation, with three doses, and were also treated with the chemical mutagen dES at three concentrations. The results obtained in the first generation of mutant M₁ showed changes as compared to the control for both treatments. The measurement of chlorophyll a and b in experimental field have been carried out with chlorophyll meter. Regarding the amount of chlorophyll in plant leaves it was observed that the maximum level of chlorophyll (30.6) belong to plants derived from irradiation with the dose 50Gy and the lowest value of chlorophyll amount (7.6) to those derived from treatment with 150Gy. There were changes in the maturation period for two gamma-ray doses (100Gy and 150Gy). On the other hand, related to the chemical mutagens the 0.02M concentration of dES resulted in notable variations, with bigger seeds and larger number of seeds. The M₁ generation was planted to produce the M₂ generation, which was used to evaluate and to select mutant lines with improved properties. The chlorophyll mutations according to Gustafsson, observed in mutant lines during 2016, in greenhouse experiment, belonged to the Viridis, Chlorine, Alboviridis, Xsantha forms.

IAEA-CN-263-136

DEVELOPMENT, AGRONOMIC AND MORPHOLOGICAL CHARACTERIZATION OF A LARGE EMS MUTANT POPULATION IN COTTON *GOSSYPIUM HERBACEUM* CV. VAGAD

Umesh Kumar Umesh, Hemant Kumar Yadav Hemant
CSIR-National Botanical Research Institute (NBRI), Rana Pratap Marg, India
Email: maurya.umesh1990@gmail.com

TILLING (Targeting Induced Local Lesions IN Genomes) is a mutagen-based, non-transgenic, effective, reverse genetic technology, which is utilized for functional genomics studies. *Gossypium herbaceum* (2n = 2x = 26) withstand drought, thus it maximum cultivation occurs in the rain-fed regions of Asia. The cotton genome(s) and transcriptome(s) sequencing studies of *Gossypium* spp. provide information for candidate genes that determine different traits. Mutagenesis generated several new alleles of the interesting gene(s). They need functional validation before being used in breeding. In current EMS mutagenized TILLING population of *G. herbaceum* (cv. VAGAD), 70 mM EMS was used and we developed 5473 M₃ plants. The morphological data for 11 agronomical traits were recorded for 4453 matured M₃ plants. The range of dispersion, mean performance and coefficient of variation

(CV), was more variable in the mutants as compared to control plants. The phenotyping of M₃ population showed 31.63% plants having some variation as compared to the control untreated plants. The visual inspection of plant morphology showed that 2.29% of plants have visible changes in leaf morphology, leaf colour, sterility, and plant habits. This mutant population provides the opportunity for functional genomics studies of cotton that might potentially be useful in breeding.

IAEA-CN-263-161

CARBON ION BEAM IRRADIATION TECHNIQUE SHORTENS BREEDING CYCLE AND INDUCES NOVEL MUTANTS IN RICE

Mohammad Abul Kalam Azad, Ara Begum, Md. Hassanuzzaman Rani, Mirza Mofazzal Islam
Bangladesh Institute of Nuclear Agriculture (BINA), Bangladesh
Email: makazad.pdbina@gmail.com

Rice cultivars Ashfal, NERICA-10 and Kasalath were submitted twice to 26.7 MeV/n carbon ions beam irradiation to induce stable mutants even in M₁ generation. In the cultivar Ashfal, M₁ fixed mutant was obtained with 200Gy dose but in the other two cultivars NERICA-10 and Kasalath the dose range was 40-80Gy. The irradiation was accomplished from Japan Atomic Energy Agency, Takasaki, Gunma, Japan through the FNCA forum as assistance to Bangladesh Atomic Energy Commission in 2009 and 2013, respectively. From the M₁ fixed mutant of Ashfal, the rice variety, 'Binadhan-14' was released in 2013 by the National Seed Board of Bangladesh (NSB) that took four years and two months from irradiated seed sowing to release of the variety. On the other hand, from the M₁ fixed mutant of NERICA-10, the rice variety, 'Binadhan-19' was released in 2017 that took four years and one month from irradiated seed sowing to release. The shortening of breeding cycle in these two varieties was attributed to the fixed M₁ plants together with growing of two generations in a year. The fixed mutant of Kasalath has not yet been released due to the presence of some undesirable characters. 'Binadhan-14' is tolerant to high temperature (38-40°C), has a short duration cycle (105-125 days), saves 20% irrigation water and gives high yield (average yield is 6.9 ton/ha). The late transplanting potential of this variety helps escaping seedling injury as well due to severe cool temperature. 'Binadhan-19' is a drought tolerant, short duration (95-105 days) and high yielding (average 4.0ton/ha in Aus and 5.16 ton/ha in Aman) variety suitable for both Aus (April to July) and Aman (July to December) seasons. Proper and sincere application of this technique will enhance rice breeding worldwide.

IAEA-CN-263-172

COMPARATIVE EFFICACY OF ELECTRON BEAM OVER GAMMA RAYS TO INDUCE NOVEL MUTATIONS IN MUNGBEAN (*VIGNA RADIATA* L. WILCZEK)

Vinod Dhole, Sreenivasulu Kandali Reddy
Bhabha Atomic Research Centre, BARC, India
Email: vjdhole@yahoo.co.in

Induced mutation is very useful in mungbean as natural genetic variation is limited. The present study was conducted to induce variability through induced mutation, compare efficiency and effectiveness of gamma rays with electron beam based on frequency and spectrum of mutant and efficiency to detect novel mutations in the M₂ generation. Seeds of mungbean variety TM 96-2 were irradiated with doses of 200, 300, 400 and 500Gy gamma rays and electron beam. Observations in the M₂ generation showed that electron beam treatments induced a greater frequency of chlorophyll mutations than gamma rays. The total mutation frequency in all doses of electron beam (2.0%) was found to be higher than gamma rays (1.3%). The highest mutation spectrum was observed in the 200Gy dose of electron beam, followed by 200Gy gamma rays. Mutagenic efficiency of electron beam was higher in 200 and 400Gy treatments

as compared to gamma rays, while it was less than gamma rays in 300 and 500Gy treatments. Mutagenic effectiveness was found to be highest in 200Gy dose of electron beam. Four novel mutants: four primary leaves, lanceolate leaf, yellow pod and seed coat colour were identified in 200Gy treatment of electron beam. Desirable mutants like early and synchronous maturity, large seed size and drought tolerant were identified. The drought tolerant mutant showed significantly greater root length (40cm), leaf thickness (0.704 to 0.803mm) and lower leaf canopy temperature (32.4°C) than control (20.5cm root length, 0.573mm leaf thickness and 40.9°C leaf canopy temperature). True breeding nature (inheritance) of these mutants was confirmed in M₃ generation.

IAEA-CN-263-173

GAMMA RAY INDUCED MUTAGENESIS FOR DEVELOPING NEW MUTANT GENOTYPES WITH HIGHER YIELD POTENTIAL IN RED KERNEL RICE VARIETY 'SURAK'

Vikash Kumar¹, Anjali Chauhan¹, Avinash K. Shinde², Bikram K. Das¹, Deepak Sharma³, Ramesh L. Kunkerkar²

¹*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, BARC, India*

²*Regional Agriculture Research Station, DBSKKV, India*

³*Indira Gandhi Krishi Viswa Vidyalyaya, India*

Email: kvikash@barc.gov.in

Improving rice yield is a major challenge in India, but recently breeding efforts are also focused on developing grain rice quality. Red rice, which looks red in colour due to its high anthocyanin content has a high fibre, high iron, manganese and zinc content and low glycemic index (GI). Modern day rice varieties have higher yield but poor micronutrients status. One of the red kernel rice varieties 'Surak', common in Maharashtra, India has completely red endosperm, probably due to high anthocyanin and high iron. Agronomic performance of this variety is very poor because of its tall stature, easy lodging, short-bold grain, spreading panicle and grain shattering resulting in yield losses. Gamma ray irradiation of Surak with 250Gy dose was done at BARC, Trombay. In M₂ generation, 12 dwarf mutants (90-102cm) including 8 red-kernel and 4 white-kernel mutants were identified. Among the red-kernel mutants, five were higher in yield, ranging 22-35g/plant as compared to Surak 18 g/plant. White-kernel mutants were significantly higher in yield ranging 20-40g/plant. Significant increase in branches/panicle (9-15), grains/plant (756-1792) and grains/panicle (39.7-136) was found as compared to Surak with 8 branches/panicle, 871 grains/plant and 48 grains/panicle. However, tiller number/plant and panicle length were less affected in both type of mutants. All white grain mutants were either MS or LS type while all red kernel mutants were SS or MS type, which is desirable for its acceptability among consumers. Stable lines will be tested for their functional attributes like high micronutrients, anthocyanin, lutein and different flavonoids and their effect on cell lines in future. These mutants may serve as high yielding functional substitutes to the tribal and marginal farmers of India.

DEVELOPMENT OF HIGH YIELDING AND LARGE SEED MUTANTS OF TROMBAY GROUNDNUT (*ARACHIS HYPOGAEA* L.). USING ELECTRON BEAM IRRADIATION

Suwendu Mondal¹, Anand Badigannavar², Poonam Bhad¹

¹Bhabha Atomic Research Centre, BARC, India

²NABTD, BARC, Mumbai, India

Email: suwendu@barc.gov.in

Electron beam accelerators have been used as radiation sources for a variety of industrial and biological applications. Such a facility has been used for mutation breeding experiments involving improvement of agronomic traits in groundnut (*Arachis hypogaea* L.). A 10 MeV linear accelerator facility was used for seed irradiation and standardized for low dose application (0.1 to 1 kGy) in pulse mode using un-scanned scattered beam. A linear dose response was found for the growth reduction of seedlings of irradiated M₁ plants in five groundnut genotypes (TAG 24, TG 26, TG 51, TG 68 & TG 69). Significant genotypic differences were observed (probit and ANOVA analysis) for seedling height reduction for different electron beam dose treatments. Among these genotypes, TAG 24, TG 26 and TG 68 showed the same GR50 value (240Gy) followed by TG 69 (175Gy) and TG 51 (111Gy) to electron beam. After the dose standardization for each genotype, TG 26 and TG 68 were irradiated with 150Gy, 200Gy and 250Gy of electron beam for improving their yield contributing traits. From the mutagenized population of TG 26, two high yielding mutants (one with large seed) were isolated. Another four high yielding mutants, a disease mimic leaf mutant and a small leaflet mutant were isolated from TG 68 electron beam irradiated population. These high yielding mutants were field evaluated and exhibited significant higher yield compared to their parents and check varieties.

CHARACTERIZATION OF REMARKABLE MUTANTS AND ECOTYPES OF BRACHIARIA (*UROCHLOA* SPP.) AND NEW COLLECTIONS OF FORAGE GRASSES FROM KENYA

Regina C. Oliveira¹, Stephan Nielen²

¹Universidade de Brasilia, Brazil

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

Email: regina711@hotmail.com

Native Kenya brachiaria grasses (= *Urochloa* spp.) samples and their radiation-induced mutants are cultivated in the experimental field at the Beef Research Centre, Nakuru, Kenya for genetic improvement to increase productivity of smallholder dairy farms. The aim of the present work was to collect new samples of germplasm, characterize ecotypes and mutants. Non-irradiated controls and mutant lines of *Urochloa brizantha*, ecotype Lanet, *U. ruziziensis*, ecotype Busia, and *U. ruziziensis*, ecotype Kisii were analysed. Fertile samples of all analysed plants and new collected germplasm from the Nyanza and Rift Valley regions were deposited at the Nairobi National Museum Herbarium. Lanet is the most difficult ecotype to pull up, it has a uniform morphology, hard and glabrous plants, however, hairy mutants have been developed. Busia control has greenish leaves with 19-33 × 0.5-0.8cm, rough-haired on the adaxial surface and uncommon axillary inflorescence. Busia mutants have yellowish leaves, ca. 8 × 1.7cm, hairy, soft trichomes, longer internodes, common axillary inflorescence and a great variety of stamen colours. The Kisii ecotype has the greatest morphological variation, and the mutant plants are almost impossible to pull up. Indications for cross-contamination between these mutants were found. This could be due to the presence of many granivorous birds, which cause seed dispersal of *Urochloa* species. Mutants form great swards and have remarkable features: vivid green

and soft leaf blades, long, stems rooting at lower nodes and late flowering. Busia and Kisii mutants were selected because Lanet is highly lignified and shows a tendency to spread out of control. Little is known on the reproductive capacity of these mutants, so the consequent effects on the wild diversity and dispersal outside the trial should be further investigated. Moreover, five new accessions of the *Urochloa* spp., and two other species were collected.

IAEA-CN-263-201

ENRICHING GENETIC DIVERSITY AND CONSERVING PLANT GENETIC RESOURCES USING NUCLEAR TECHNIQUES AND RELATED TECHNOLOGIES IN THE STATE OF QATAR

Elsayed Elazazi, Aisha Al-Kuwari, Maryam Al-Qahtani, Nafeesath Shahsil
Ministry of Municipality and Environment, Qatar
Email: emazazi@mme.gov.qa

Over the past 50 years, induced mutation has played a major role in the development of high genetic variability and helped develop many new superior crop varieties: both in vegetative and seed propagated plants. Heat and salinity are the major abiotic stresses limiting crop production in Qatar. Food availability is an essential situation for food security. Qatar has developed an ambitious strategy to increase crop productivity. Moreover, resource rich Qatar can play a vital role in the global market for developing novel food production-related technologies, by developing agricultural research. Mutation is an important resource to generate and identify genetic variation of useful traits for improvement crops and native plant. We used one of the general methods for mutation induction using X-rays and gamma irradiation on seeds of some wild plant and wheat varieties treated in the Joint FAO/IAEA Laboratories and germination experiments were conducted in Qatar. We achieve high variation between different treatment and radiation. We found albino in barley after treatment with Gamma ray at 75Gy, and chlorina (light green), and only wheat and wild plant completed their life cycle.

IAEA-CN-263-210

CREATION OF VARIABILITY IN BAMBARA GROUNDNUT USING MUTATION BREEDING

Busiso Mavankeni, Shylet Tsekenedza
Crop Breeding Institute, Zimbabwe
Email: bmavankeni@gmail.com

Bambara groundnut (*Vigna subterranea* L. Verdc.) is a grain legume crop with a great potential of contributing to food and nutrition security in Zimbabwe. It has high protein content (17-23%) with high levels of methionine and lysine. The major constraints faced by farmers in Zimbabwe in the production of Bambara groundnut include the long maturity duration exhibited by the crop, unavailability of improved disease resistant, early maturing high yielding and drought tolerant varieties. Mutation breeding has been identified as one of the strategies to improve the crop. Bambara groundnut has a narrow genetic base and mutation breeding was identified and used to create variability. Seeds were irradiated using gamma rays at the following doses 150, 200, 250, 300 and 350Gy. Two varieties namely Mana and Kazuma were irradiated. Selections were made from M₂ generation to M₄. The following traits were observed from the second generation; early flowering, high yield per plant, spreading growth habit, changes in leaf shape and size, seed coat colour, disease and drought tolerance. Many mutants with desired traits were selected. Three populations' namely the early maturing group, disease resistant (Fusarium wilt) and drought tolerant populations were developed. The mutation technique of irradiating seed with gamma rays has proved to be a tool that can be used in the creation of variability and desired

traits in Bambara groundnut. Mutation techniques can therefore contribute significantly to Bambara groundnut improvement thereby contributing to nutrition and food security in Zimbabwe.

IAEA-CN-263-243

INFLUENCE OF CROSS-BREEDING ON THE PHYTIC ACID CONTENTS OF LOW PHYTIC ACID RICE AND SOYBEAN MUTANTS

Sophia Goßner¹, Chenguang Zhou¹, Fengjie Yuan², Karl-Heinz Engel¹, Qingyao Shu³, Yuanyuan Tan⁴

¹Technical University Munich, Chair of General Food Technology, Germany

²Institute of Crop Science and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, China

³Zhejiang University, State Key Laboratory of Rice Biology, Institute of Crop Sciences, China

⁴Zhejiang University, State Key Laboratory of Rice Biology and Zhejiang Provincial Key Laboratory of Plant Germplasm, Institute of Crop Sciences, China

Email: sophia.gossner@wzw.tum.de

Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate), the major storage form of phosphorus in crops, is considered as an anti-nutrient for monogastric animals and humans, and phytic acid-derived P in animal waste plays an important role in water pollution. Therefore, various efforts have been made to obtain low phytic acid (lpa) crops, e.g. by mutation breeding. Lpa mutant lines often exhibit lower grain yield and seed viability compared to their wild-types, and there have been attempts to minimize these negative effects by cross and selection breeding. However, the impact of these crossing steps on the contents of phytic acid in the resulting progenies have not been investigated. In this study, three lpa mutants of rice (*Oryza sativa* L.) called: Os-lpa-XQZ-1, Os-lpa-XS110-2 and Oslpa-MH86-1; and three lpa mutants of soybean (*Glycine max* L. Merr.) including: Gm-lpa-ZC-2, Gmlpa-TW-1 and Gm-lpa-TW-1-M developed through γ -irradiation mutation induction were used for cross-breeding experiments with different commercial wild-types. The progenies were genotyped using molecular markers to identify homozygous WT and lpa plants from generations F3 to F8. For rice, the contents of phytic acid in the homozygous lpa mutant progenies grown at different field trials were dependent on the phytic acid contents of the crossing parents, the environmental conditions and the type of mutation. For soybean, all homozygous lpa mutants carrying the GmIPK1 mutation showed a significant signature of lower inositol phosphates. For each cross the generated homozygous lpa mutant progenies displayed consistently decreased levels of phytic acid contents compared with the corresponding homozygous wild-type progenies and the original parental wildtypes. The elaborated data provide an important analytical basis for further implementations of mutation breeding in the generation of lpa crops.

IAEA-CN-263-246

APPLICATION OF ION-BEAM IRRADIATION ON CASSAVA SEEDS

Anh Vu Nguyen, Ham Le Huy

Agricultural Genetics Institute, Viet Nam

Email: nguyen.anh.vu.agi@gmail.com

Cassava (*Manihot esculenta* Crantz) is an important food crop, providing staple starch as well as material for a range of different industries including biofuel production. Viet Nam has identified cassava as the most suitable crop supplying starch for bioethanol production. KM94 is currently the most popular variety occupying more than 70% of the cassava growing area. However, KM94 has a number of undesirable traits including high-branching, easy lodge and susceptible to many diseases. In order to overcome these drawbacks of KM94, we studied heavy-ion beam irradiation of seeds from KM94 at

different doses of 100Gy, 200Gy, 300Gy and 400Gy. The irradiated embryos were isolated and cultured in embryo rescue media. Explants developed from irradiated embryos are designated M₁ plants. From each well-developed M₁ plant, 15-20 new plants were asexually reproduced and maintained *in vitro*. Our result indicated that heavy-ion beam irradiation dose of 100Gy already created a wide-range of observable morphological changes from the beginning of cassava embryo development. Irradiation doses of 200Gy and higher severely affected embryo germination and its ability to regenerate a whole plant. As far as we know, this is the first study in Viet Nam using heavyion beam irradiation in combination with plant tissue culture for the improvement of cassava KM94 as well as crop plants in general.

IAEA-CN-263-251

MUTATION INDUCTION FOR SORGHUM AND RICE USING GAMMA AND X-RAY RADIATIONS

Al-Ghaliya Humaid Khamis AL-Mamari¹, Abdelbagi Mukhtar Ali Ghanim²

¹Ministry of Agriculture and Fisheries, Sultanate of Oman

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

Email: g.humaid@gmail.com

It is well established that different mutagens have different effects on plants, resulting in different types and levels of damage, and consequently produce a range of induced mutations at different frequencies. We present a comparison between the effect of gamma and X-ray irradiations on one variety of sorghum and upland rice. Pure seeds of both sorghum and upland rice were exposed to gamma and X-rays radiations at six different doses: 0 (control), 75, 150, 300, 450 and 600Gy. Three replicates of 20 seeds per dose were planted in standard soil mixture in plastic trays and maintained in a glass-house at Plant Breeding and Genetics Laboratory (PBGL). Different parameters such as germination %, survival % and plant height (cm) were scored on M₁ plants after two weeks and one month of planting. The germination and survival percentage, after two weeks, for both sorghum and rice were high following the gamma radiation compared to X-ray radiation. The sorghum showed higher germination and survival percentage than rice at different doses of both gamma and X-ray radiation. The reduction of 50% in growth rate (GR50) for sorghum and rice using gamma radiation were 300 and 250Gy, while the GR50 for both crops using X-ray radiation were 250 and 150Gy, respectively. The rice germinated from seeds exposed to higher doses (600Gy), but did not survive more than two weeks. The results of the experiments indicated that higher dosage of gamma and X-ray radiation reduced germination percentage, number of survival plants and plant height and rice was more sensitive to irradiation than sorghum. Three doses, GR50 + 20%, are proposed for bulk treatments of sorghum (240, 300 & 360Gy) and rice (200, 250 & 300Gy) using gamma-ray compared to 200, 250 & 300 for sorghum and 120, 150 & 180Gy for rice using X-ray.

EVALUATION OF GAMMA RAY INDUCED MUTANT POPULATIONS OF TWO EGYPTIAN BREAD WHEAT VARIETIES (*TRITICUM AESTIVUM VULGARE* L.) FOR PROLINE GENE EXPRESSION IN TWO CULTIVARS UNDER SALT STRESS

Amina Aly¹, Rabab Maraai¹, Sayed Mohamed², Youssef Trifa³

¹National Centre for Radiation Research and Technology, Atomic Energy Authority, Egypt

²Agriculture Research Institute, Egypt

³National Agronomic Institute of Tunisia, Carthage University, Tunisia

Email: aly_amina@yahoo.co.uk

Wheat (*Triticum aestivum* vulgare L) is the major human consumable commodity in most areas of the world including Egypt. The productivity of wheat is adversely affected by salt stress, which is associated with decreased germination percentage, reduced growth, altered reproductive behaviour, altered enzymatic activities and disrupted photosynthesis. The irradiation of seeds may cause beneficial genetic variability that enables plant breeders to select new genotypes with improved characteristics. In the present study, grains of two wheat Egyptian cultivars: Sids-1 and Sakha-93 that had been exposed to gamma irradiation at dose levels of: 100, 200 and 300Gy. These M₁ seeds. Were sown and the M₁ plants were under sodium chloride concentrations of 60 and 120 mM NaCl to get M₂ grains. The content, activity and expression level of delta 1-pyrroline-5-carboxylate synthetase were carried out to evaluate the changes which may occur at biochemical and molecular levels for proline. Both cultivars Sids-1 and Sakha-93 showed an increase in proline level contents accumulation in their leaves under the different treatments. There was also an upregulation in the expression the genes involved in proline biosynthesis under stress. The relative expression was more pronounced in the salt stress concentration of 60 and 120 mM NaCl following the treatment 300Gy. Comparable observations were also made in Sakha-93 cultivar. In conclusion, the results showed that the relative expression of proline gene increased more under sodium chloride treatments than in the combination treatments between sodium chloride and gamma rays. Gamma ray can be used as a useful tool for gene expression in plants and salt tolerance could be attributed to the constitutive induced changes, leading to more efficient genes in plants.

IMPROVEMENT OF SPRING WHEAT GRAIN MICRONUTRIENTS CONCENTRATIONS THROUGH MUTATION BREEDING

Gulina Doktyrbay¹, Saule Kenzhebayeva¹, Alfya Abekova², Dauren Tashenev¹, Fatma Sarsu³, Nargul Omirbekova¹

¹Al-Farabi Kazakh National University, Kazakhstan

²Kazakh Institute of Agricultural and Breeding, Kazakhstan

³International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria

Email: kenzhebs@gmail.com

Bread wheat is a main crop with global importance for food security and one of the major cereal source of human nutrients. Cultivated wheat grains have low micronutrients content. Improvement of agronomic traits including grain quality require their genetic variation, which should be separable from non-genetic impacts. The main focus in wheat breeding was the replacement of traditional by modern high yielding varieties which, also led to the reduction of genetic diversity accompanied by reduced end-use quality characteristics and nutrition quality. Therefore, there is need for cereals fortification with micronutrients thereby contributing to reducing malnutrition in a cost-effective and efficient way. To broaden the genetic variation of spring wheat in Kazakhstan, mutant populations of three nationally released varieties, Zhenis, Almaken and Eritrospermum-35, were developed by irradiation with 100Gy

and 200Gy doses and advanced to M₅ lines. The grain protein content (GPC) in mutant lines was significantly higher (3.7 to 16.9%) as compared to the respective parents. The highest increase of GPC was revealed for the Zhenis lines. Depending on the genetic background of the parent varieties, the Fe concentrations (GFeC) were significantly increased at varying degrees between 1.3 to 3.4 times. The highest GFeC were identified for both the Zhenis and Almaken in M₅ mutant lines. The enhancement of grain Zn concentrations (GZnC) in mutant lines was less distinct compared to that of GFeC, with means of 1.36-2.9 times higher. These ranges of GZnC were not depended on variety background. Several mutant lines showed increase of both GFeC and GZnC. A dose-dependent pattern for grain micronutrients parameters was not determined. Wheat mutant resources with high GPC, GIC and GZnC have the potential to be used in a diet aimed at reducing human nutrition deficiencies.

IAEA-CN-263-264

GAMMA RAY INDUCED BLACK GRAM (*VIGNA MUNGO* L. HEPPER) MUTANT DIFFERING IN FLORAL CHARACTERS

Vanniarajan Chockalingam¹, Priyadharshni Sambath Kumar¹, Selva Kumar Thangaraju¹, J. Souframanien²

¹Tamilnadu Agricultural University, India

²Bhabha Atomic Research Centre, BARC, India

Email: priyadharshnis13697@gmail.com

An investigation was carried out in Blackgram (*Vigna mungo* L. Hepper) genotype MDU 1. The MDU 1 Blackgram variety is indeterminate and duration 75 days and has high arabinose content 7.5% and has high batter volume. It was highly susceptible to Yellow Mosaic Virus (YMV) and cultivated only during September-October season. Thereby, to develop a short duration, determinate and YMV resistant genotype, the Blackgram variety MDU 1 was subjected to Gamma ray treatment from 100Gy to 500Gy. Four high yielding mutant lines (ACM 11, ACM 15, ACM 17, ACM 25) were isolated and advanced to yield trails. To distinguish from the MDU 1 Blackgram variety, the floral traits were studied in detail in these four mutants. Yellow standard petal with purple tinge on the margin was observed in ACM 17 and found to be absent in MDU 1. The stigmatic hairs are dense in MDU 1 and sparse in ACM 17. The YMV incidence was less than 10% when compared to MDU 1 which recorded 75% of infection. From the investigation, we found that ACM 17 mutant was high yielding, determinate, YMV resistant and also differs in floral characters from MDU 1 Blackgram variety. This mutant ACM 17 must be subjected to further detailed study.

IAEA-CN-263-278

AN APPROACH TO OBTAIN THE OPTIMAL DOSE OF SEED IRRADIATION FOR MUTAGENESIS IN *COFFEA ARABICA*

Kimberly Ureña, Noel Arrieta Espinoza

Instituto del cafe de Costa Rica, Costa Rica

Email: narrieta@icafe.cr

Coffee is one of the most important crops in Latin America and has a significant socio-economic impact for thousands of families. The coffee crop is under threat due to exponential increases in biotic and abiotic factors. The use of mutation induction techniques has been successfully implemented in certain crops to obtain desirable agronomic characteristics. In coffee, mutation breeding has been initiated by the determination of the optimal dose of irradiation of seed. This is necessary to obtain high efficiency and efficacy in experiments of mutagenesis induction. This can be determined by identifying the 50% reduction in seed germination, known as LD50 or by reducing the average seedling growth by 50%,

with respect to the control treatment, known as RD50. Seeds of *Coffea arabica* were irradiated with gamma rays at doses of 80, 100, 120, 140, 160 and 180Gy, and 0 (as a control). The seeds were placed in a suitable substrate for germination (peat moss), 80 days later, the germinated seeds were transplanted to individual containers and the percentage of cotyledons involved was determined 30 days after sowing. An unrestricted randomized experimental design was used with six treatments and a control without irradiation, with 120 replicates per treatment. We performed a linear correlation analysis ($P \leq 0.01$), for the determination of the RD50. According to the preliminary results: the RD50 is in the range of 100-120Gy. In previous experiments it was found that the lethal dose (LD50) is in the same range. This dose range will now be used to create an M_1 population in developing advanced mutant populations and lines for screening for traits of interest.

POSTERS RELATED TO SESSION 4

**MUTATION BREEDING FOR ORNAMENTAL
AND VEGETATIVELY PROPAGATED CROPS**

M Building – Common Area

CAN CRYOTREATMENT BE A TOOL USEFUL IN MUTATION BREEDING?**Dariusz Kulus***UTP University of Science and Technology, Faculty of Agriculture and Biotechnology, Department of Ornamental Plants and Vegetable Crops, Laboratory of Biotechnology, Poland*

Email: dkulus@gmail.com

The impact of cryo-treatment (storage in liquid nitrogen) on the stability of plant material is still ambiguous. It would be interesting to learn, whether liquid nitrogen could be a potentially valuable tool in mutation breeding. Therefore, the aim of the study was to evaluate the stability of three chrysanthemum cultivars cryopreserved via encapsulation-dehydration technique. Shoot tips were precultured on MS medium supplemented with 10 μ M ABA for 7 days, encapsulated in sodium alginate, and then, osmotically dehydrated in sucrose gradient for four days, followed by 3-hour air desiccation. The pre-treated shoot tips were stored in liquid nitrogen (-196°C). After thawing, the recovered micro-shoots were acclimatised and cultivated in glasshouse conditions. The plants were subjected to molecular, cytogenetic, biochemical and phenotypical analysis. By applying RAPD and ISSR markers, 18 polymorphic bands within 5-6% of cryopreservation-obtained chrysanthemums were detected. Usually no changes in the flower pigment content were detected. Only one plant produced a completely new phenotype, i.e. with mosaic leaves (forma variegata) and a double-coloured inflorescence. Interestingly, the colours of the flower got reversed in the second vegetative generation, and no alternation of this parameter was observed in the third generation, as compared with the control. The variegated leaves, however, remained stable the whole time. The recovered after cryopreservation and control plants had the same diameter and fresh weight of inflorescences, as well as, ray florets length. On the other hand, the analysis revealed that the bottom and central leaves of all cryopreservation-derived plants were shorter and thinner, and had a reduced amount of chlorophyll, while their internodes were shorter as compared to the untreated control. Furthermore, inflorescences of one cultivar opened slower, but faded faster.

BANANA MUTAGENESIS *IN VITRO*: SENSITIVITY OF SHOOTS TO GAMMA RADIATION**Ramon Scherer¹, Alexander De Andrade¹, Augusto Tulmann Neto², Giuliano Rigo³**¹*Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri), Brazil*²*Centro de Energia Nuclear na Agricultura (CENA/USP), Brazil*³*Universidade Federal de Santa Catarina (UFSC), Brazil*

Email: ramonscherer@epagri.sc.gov.br

Epagri is carrying out a project aimed at the induction of mutants for resistance to banana diseases by irradiation of *in vitro* shoots of the cultivar SCS451 Catarina. The first step was the determination of sensitivity to gamma rays using doses of 0; 12.5; 25; 37.5 and 50Gy. As it is known if in a shoot tip after treatment with any mutagen the mutation may be induced in M₁V₁, however, there is the occurrence of chimerism and further subcultures (M₁V₂, M₁V₃, M₁V₄, etc.) are needed to select and isolate the stable mutants (periclinal chimeras or solid mutants). The shoots used were cultured using the appropriate multiplication and growth media for banana. The experimental unit was composed of 3 flasks in M₁V₁ and 5 flasks in the others (M₁V₂ to M₁V₄) and 5 shoots were always inoculated in each flask. The mean height and shoot regeneration rate (number of shoots formed at the number of inoculated shoots) were evaluated at the end of each subculture (30-40 days interval). The treatments that received the two highest doses (37,5 and 50Gy) presented the lowest regeneration rate (1,91 and 1,48, respectively) and the shortest shoot height (2,09 and 1,72 cm, respectively) at the end of M₁V₁.

However, with the advance of the subcultures, the results of these treatments were like the others, so that in M₁V₄ there were no significant differences. As in mutation breeding whenever possible it is recommended to use more than one dose, based on the obtained results it was concluded that these two doses (37,5 and 50Gy) can be used for the continuation of the research. For the continuation of the work shoots were irradiated with each of these doses and the material is being led up to the M₁V₄ generation for the selection.

IAEA-CN-263-36

POLLEN GRAIN CHARACTERS-A USEFUL PARAMETER FOR TESTING RADIO-SENSITIVITY AND CHARACTERIZATION OF MUTANTS

Kalyani Datta, Subodh Kumar Datta

Retd. Scientist & Head, Palynology Laboratory, C.S.I.R.-National Botanical Research Institute, Lucknow, India

Email: subodhskdatta@rediffmail.com

Determination of radio-sensitivity is pre-requisite for large scale irradiation in mutation induction for breeding experiments and a wide range of parameters (growth inhibition, chromosomal aberrations, mutation etc.) have already been standardized. Appreciable amounts of literature have been accumulated on pollen grains after mutagen treatment. Pollen grains and their morphological features are genetically stable characters and the effects of mutagens on these features have been studied critically on different ornamental plants (canna, chrysanthemum, mesembryanthemum, Narcissus tazetta, Lantana depressa etc.) and other crops (*Trichosanthes anguins*, *T. cucumarina*, *Cucurbita maxima*, *Cephalandra indica*, etc.). Changes in pollen grain sterility, size and morphology (ornamentations) were significant after mutagen treatment. Increase in pollen grain sterility after mutagen treatment was observed in all the experimental materials. The responses to irradiation with regard to pollen sterility/fertility appeared to be plant specific and may be the outcome of the mode of chance mutation in different plants. Apertural character and exine ornamentation pattern are the two most stable morphological features of pollen to be considered for diagnostic value of a taxa. Palynological characters are often found helpful for assessing taxonomic treatments, not only for delimitation of higher taxonomical grouping like genus or species, but also for providing additional parameters for differentiation of microtaxa as low as cultivar level. Differential conspicuous changes in exine and apertural characters were recorded both after gamma irradiation and in mutants. Significant changes in pollen apertural and exine surface sculptures due to mutation at several independent loci controlling these characters and their differential sensitivity to mutagens have been clearly established. Such study will provide additional information for understanding the genetic control over pollen aperture and exine surface ornamentation which are of potential as markers in plant biosystematics.

GENETIC CHARACTERIZATION OF A NOVEL POLLEN-PART SELF-COMPATIBLE MUTANT OF JAPANESE PEAR IN PROGENY OF A CROSS USING POLLEN FROM A CHRONICALLY GAMMAIRRADIATED TREE

Nobuko Mase¹, Toshihiro Saito²

¹Radiation Breeding Division, Institute of Crop Science, NARO, Japan

²Institute of Fruit Tree and Tea Science, NARO, Japan

Email: nobuko@affrc.go.jp

Most of the important fruit crops in the subtribe Pyrinae of the Rosaceae, such as apple and pear, exhibit S-RNase-based self-incompatibility. In 2013, we selected a pollen-part self-compatible mutant of Japanese pear (*Pyrus pyrifolia*), designated 415-1, from the progeny of a cross using pollen from a 'Kosui' tree chronically exposed to low-dose-rate gamma-irradiation in the gamma field at the Radiation Breeding Division, Institute of Crop Science, NARO. We developed a new self-compatible mutant selection (designated 391-1) of Japanese pear, which was identified from among male-derived progeny of a gamma-irradiated 'Nijisseiki' tree. CAPS analysis of the S-RNase genes indicated that the S haplotype of 391-1 was S2S4. Crosses between 391-1 and self-incompatible cultivars with S2 and/or S4 haplotypes revealed that the styles of 391-1 accepted S4 pollen but rejected S2 pollen. Furthermore, the pollen of 391-1 was not rejected by self-incompatible cultivars containing the same S haplotypes. Thus, this selection was determined to have mutations in both pollen and stylar functions of self-incompatibility. Segregation analyses of S haplotypes of the progeny of 'Niiitaka' × 391-1 revealed a duplication of the S2 haplotype; thus, the accurate S haplotype of 391-1 is S2S2S4. Furthermore, segregation analyses of SSRs in the same linkage group as the S haplotype revealed a segmental duplication encompassing an S2 haplotype. Similarly, 415-1 (S4S5S5) has a segmental duplication encompassing an S5 haplotype. These results indicate that pollen-part self-compatibility in *Pyrus* does not require mutation of a pollen S factor, but can be caused by duplication of an S haplotype. Further, they demonstrate the effectiveness of using pollen from irradiated plants to obtain self-compatible mutants of fruit crops in the Pyrinae.

CHIMERISM IN MUTANT WOODY CROPS

Hiroaki Yamanouchi

Radiation Breeding Division, Institute of Crop Science, The National Agriculture and Food Research Organization, NARO, Japan

Email: yamanouc@affrc.go.jp

Because somatic mutations begin in a single mutated cell surrounded by wild-type cells, the mutants are inevitably in a chimeric state for some time after they first occur. The periclinal chimera state (e.g., only one layer in a shoot apical meristem consists of mutant cells) is usually held to be stable. Here, we used mutant cultivars of Japanese pear (*Pyrus pyrifolia*) and mulberry (*Morus* spp.) to study periclinal chimera patterns and mutant stability in woody crops. The mutant cultivars included gamma-ray-induced, spontaneous, and ploidy mutants. To separate the chimeras, we obtained regenerated plants from adventitious shoots generated by *in vitro* culture. The immature leaves in winter buds were isolated aseptically and cultured on solid medium containing 1-50 μM thidiazuron. After several sub-cultures, the regenerated shoots were acclimatized by grafting or cutting. The regenerated plants and their original cultivars were analysed by morphological observation, crossing experiments, flow cytometry, PCR, and other methods. Flow cytometry analysis of polyploid mutants (cytochimeras) and the regenerated plants derived from them indicated that the regenerated shoots were predominantly derived from L1 cells. For example, the majority of regenerated plants from the 4-2-2 cytochimera mulberry

strains, which had tetraploid L1 layers, were tetraploid. Among the regenerated plants derived from mutant mulberry and pear cultivars and strains, wildtype (original-type) plants occurred at a high frequency, depending on the particular cultivar or strain. These results indicate that wild-type cells were maintained though long-term cultivation or repeated mutagenesis in some cultivars and strains. Periclinal chimerism was stable for a long time, indicating that additional procedures are needed to isolate pure (non-chimeric) mutant plants if all cells need to be mutant. These findings have implications for the utilization of somatic mutants of woody crops. This research was partially supported by SATREPS, JST/ JICA (Japan).

IAEA-CN-263-75

IN VITRO MUTATION BREEDING OF CARNATION BY GAMMA RADIATION

Hamid Reza Sabaghi¹, Gholamreza Sharifi Sirchi¹, Pejman Azadi²

¹*Horticultural Science Department, Agriculture and Natural Resources College, University of Hormozgan, Bandar Abbas, Iran*

²*Agricultural Biotechnology Research Institute of Iran (ABRII), Iran*

Email: h.sabaghi@ut.ac.ir

The production of new cultivars in ornamental plants is of great economical significance to this industry. Gamma irradiation causes mutagenesis that significantly increases genetic diversity. The objective of this research was production of new carnation cultivars using *in vitro* mutation breeding. Six carnation cultivars (White Liberty, Liberty, Tabor, Tabasco, Eskimo, Mariposa and Grand Slam) were considered in this study. The best treatment for highest percentage of callus induction were obtained from leaf explant cultured on MS medium supplemented with 0.5mg/l 2,4-D and 0.2mg/l BA. The 3 weeks old calli induced were Gamma irradiated with different doses (0,15, 25, 35, 45, 55Gy). After radiation, calli were transferred to MS medium supplemented with 3mg/l BA, 0.6mg/l NAA, 4mg/l AgNO₃ and 40mg/l adenine hemisulfate. Regenerated plants cultured onto MS medium with 1mg/l IBA for root induction. The rooted plantlets were transferred to greenhouse for adaptation. Different traits including plant height, inflorescence length, leaf length and width, inter node space, number of flower in florescence, number of petals, the number of true leaves below the flower, flower diameter and number of days to flowering will be considered.

IAEA-CN-263-88

DEVELOPMENT OF POLYPLOIDS OF SOME CITRUS SPECIES BY IN VITRO COLCHICINE TREATMENTS AND EARLY SELECTION BY FLOW CYTOMETRY

Murat Seker, Engin GUR, Mehmet Ali Gundogdu, Neslihan Ekinci, Nilufer Kaleci

Çanakkale Onsekiz Mart University, Turkey

Email: mseker@comu.edu.tr

Developments in citrus biotechnology, especially in callus cultures have opened new opportunities to develop new rootstocks and cultivars. Polyploidy is one of the main breeding goals in order to obtain new genotypes with improved characteristics to develop citrus germplasm. Flow cytometry is a highly developed early and rapid selection technique that aims to analyze mutant cells and its components like total genome sizes in mutant population. In this research, embryogenic callus lines of six Citrus species including orange (*Citrus sinensis*), lemon (*Citrus limon*), mandarin (*Citrus reticulata*), kinkoje (*Citrus obovoidea*), hyokan (*Citrus ampullaceae*) and sanbokan (*Citrus sulcata*) were investigated. Colchicine as a chemical mutagen applied with different concentration (0.0%, 0.01%, 0.05% and 0.1%) on embryogenic callus lines cultured on Murashige and Tucker (MT) medium supplemented with 500mg/l malt extract for 8 weeks. Callus lines were transferred to MT medium without colchicine and

subcultured during 12 weeks after treatment. Somatic embryos arising from the callus in petri dishes were transferred for germination to tubes containing MT medium with 5mg/l GA3. Obtained plantlets were evaluated for genome sizes for ploidy determination by flow cytometry analysis. According to the obtained results colchicine treatments were resulted different polyploidy ratios in different Citrus species. The highest polyploidy ratio (27%) was obtained in oranges from 0.1% colchicine treatment followed by lemon (22%) from the same concentration of colchicine.

IAEA-CN-263-104

HIGH-THROUGHPUT GENOTYPING OF VCGs *FUSARIUM OXYSPORUM* PATHOTYPES FOR POTENTIAL SELECTION OF BANANA RESISTANT MUTANTS

Mohammed Jawhar¹, Altos Viljoen², Mohammed Arabi¹, Nizar MirAli¹, Bradley J. Till³

¹Atomic Energy Commission of Syria, Syrian Arab Republic

²University of Stellenbosch, South Africa

³International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria

Email: mjawhar@aec.org.sy

Fusarium wilt of banana (Panama disease), is a lethal fungal disease caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). Development of stable forms of resistance to this disease depends upon identification of resistances effective against the prevalent Foc populations in banana growing areas. Yet, many methods are not suitable for developing countries with varying levels of laboratory infrastructure. Here, we optimized low cost method for genotyping vegetative compatibility groups (VCGs) Foc pathotypes for selecting banana mutants to ensure durability of the resistance. An enzymatic mismatch cleavage assay for the haploid *F. oxysporum* was developed. It was optimized in different Foc VCGs to detect allelic variants of target pathogenicity genes (SNF1 and FOW2). Enzymatic mismatch cleavage using PCR primers specific for SNF1 and FOW2 genes has detected different allelic mutations among the VCGs. Differences of band patterns among the digested products of different isolates were clearly observed by agarose gel electrophoresis. Novel nucleotide variation was detected among Foc "Tropical Race 4" VCG 300, 194, and 1036 strains. This method is potentially of great value in the characterization of the banana fusarium wilt VCGs pathotypes. For example, in laboratories without sequencing facilities and where VCG testers of Foc cannot be used because of national quarantine regulations, this low-cost method can be used to rapidly and accurately detect Foc "Tropical Race 4" VCGs 300, 194, and 1036 strains. This may be especially important in new geographical regions where this pathogen is introduced, and immediate management practices are needed. In principle, the strategy proposed here might be suggested to be widely applied to a variety of necrotrophic and biotrophic plant pathogens to be used for selection disease resistant mutants.

IAEA-CN-263-119

IMPROVEMENT OF STEVIA REBAUDIANA BERTONI THROUGH GAMMA IRRADIATION AND MICROPROPAGATION

Norazlina Noordin, Mohd Fajri Osman, Nashimatul Adadiah Yahya, Norellia Bahari, Norhafiz

Talib, Nurhayati Irwan, Saw Peng Chong

Malaysian Nuclear Agency, Malaysia

Email: azlina@nm.gov.my

Stevia rebaudiana Bertoni is a perennial herb that belongs to the family of Asteraceae. It is a natural sweetener plant known as sweet leaf, estimated to be 300 times sweeter than cane sugar. It is one of the 154 members of genus stevia which produce sweet steviol glycosides. The potential of stevia has been

regarded worldwide and fast becoming the best alternative sweetener due to the health conscious of excess sugar intake that linked to obesity, diabetes, heart disease, high blood pressure, tooth decay along with various other diseases. Since its first introduction to Malaysia 10 years ago, there are few setbacks in stevia cultivation and plantation. Low, insufficient and inconsistency supply of high quality planting materials for large scale commercial plantations is a major setback for stevia industry in Malaysia. Since 2012, Nuclear Malaysia has started a project on mutation breeding of stevia using acute and chronic gamma irradiation. From this project, several outputs have been successfully achieved such as potential stevia mutant lines, advanced tissue culture and mass propagation procedures. One stevia mutant line that exhibits desired agronomic traits and is adaptable to Malaysian climate has been successfully transferred through a collaboration with local stakeholder in Sabah, East Malaysia for pre-commercialization of stevia seedlings using advanced tissue culture technology. Recently, we are working closely with the community and farmers in Tuaran, Sabah on the propagation and cultivation of stevia as a continuous effort to enable sustainable stevia cultivation to become a source of new income generation for the farmers in near future. This paper will discuss on stevia improvement that includes mutagenesis using gamma irradiation, micropopagation, pre-commercialization and cultivation.

IAEA-CN-263-138

CHILEAN FIRST STEPS IN PLANT MUTATION BREEDING

Ethel Velasquez, Adriana Nario, Oscar Duran, Paulina Aguirre

Chilean Nuclear Energy Commission, Chile

Email: ethel.velasquez@cchen.cl

Due to its large geographic coverage and diverse climatic regions, Chile has a large variety of plant species. As a consequence of natural processes and human activities, climate change, wildfires and a decrease of cultivable lands, many crop variants run the risk of extinction. Furthermore, Chile needs to improve the quality and productivity of its crops. In this context, induced mutagenesis represents a valuable tool to the development of improved vegetable crops. From 2005, the Chilean Nuclear Energy Commission (CCHEN) in collaboration with other national research institution teams, through private and public funds proposed projects aligned with local strategies to obtain desired plant varieties by applying gamma radiation induced mutagenesis. As an example, radiosensitivity studies and radiation induced mutagenesis experiments were conducted on citrus twigs for developing specific characteristic such as seedless fruits. In 2006 new approaches using this powerful technique promoted the production of new commercial orchid varieties adapted to cold climatic conditions. In 2008, various biotechnology techniques were added to obtain the first Chilean ornamental and fruit variety. Since 2012, CCHEN has been involved in IAEA activities related with sustainable agricultural development and plant mutation breeding topics, participating in regional projects as RLA5063 and RLA5068 focused on the generation of local relevant crops varieties tolerant and resistant to abiotic stress. These projects have strengthened CCHEN's capabilities and are part of the institution and national strategic plan.

PHENOLOGICAL AND POMOLOGICAL DIFFERENCES IN GAMMA IRRADIATED '0900 ZIRAAT' SWEET CHERRY MUTANTS

Murat Seker, Engin Gur, Mehmet Ali Gundogdu, Neslihan Ekinci, Nilufer Kaleci
Çanakkale Onsekiz Mart University, Turkey
 Email: mseker@comu.edu.tr

'0900 Ziraat' is the leading cultivar with high quality fruit characteristics among sweet cherry cultivars in Turkey. Pollination and compatibility problems result in poor fruit set in different ecological conditions of the country. Improved fruit quality such as size, appearance, firmness, flavour, extending harvest season, self-compatibility, compact growth habit are main breeding goals in '0900 Ziraat' breeding. Induced mutations may play significant roles in increasing new '0900 Ziraat' types. Gamma irradiation is a physical mutagen widely used for mutation breeding. The doses applied in this study were 0Gy (control), 10Gy, 20Gy, 30Gy, 40Gy and 50Gy; the irradiated scions consisted of 5 buds in each. The buds were grafted immediately after mutation treatment on mahaleb (*Prunus mahaleb*) rootstocks. Mutations were stabilized in three vegetative generations. In this study, several observations were carried out to determine plant growth and fruit characteristics in mutant plants. This study showed considerable potential for enhancement of '0900 Ziraat' sweet cherry genetic variation.

IN VITRO MUTAGENESIS IN CONGOLESE CASSAVA ACCESSION, BOMA AND REVERSE GENETICS STRATEGIES (TILLING) STUDIES

Aime Diamuini Ndefunsu, Freddy Bulubulu Otono
Commissariat General à L Energie Atomique, CRENK, Democratic Republic of the Congo
 Email: aimediamuini@yahoo.fr

Cassava is contributing to food security in the Democratic Republic of the Congo. Major constraints for cassava production for are the biotic and abiotic stresses. The feasibility of overcoming these problems by only classical breeding is hampered by several genetic factors. Thereby, plantlets of cassava line Boma obtained from meristems culture and/or cassava 2 node-explants were cultured on liquid MS medium free of plant growth regulators and complemented with 20g sucrose for the determination of the optimal dose. Ten (10) explants each having 2 nodes were irradiated at 5, 10, 15, 20, 25 and 30Gy. After 4 to 5 weeks of growth at 26°C, plantlet height and weight, number of nodes and leaves were measured and expressed as a percentage of the control. The optimal dose was calculated using the linear regression equation. For bulk irradiation and Chimeras dissociation 1000 explants with 2 nodes each were placed in 10 ml liquid MS media and irradiated at the determined optimal doses: 3, 6, and 9Gy. Irradiated materials were sub-cultured and chimeras were dissolved after 14 days. For TILLING (Targeting Induced Local Lesions IN Genomes) of cassava genes involved in coding for starch synthesis: SSII, SSIII, susy, susy2, sps and X77012 were used. 3000 putative cassava mutant clones were obtained. The TILLING cassava method showed some very low frequencies of mutations.

RADIATION INDUCED MUTAGENESIS FOR GENERATING USEFUL GENETIC VARIATION IN SUGARCANE

Suprasanna Penna

Bhabha Atomic Research Centre, BARC, India

Email: prasanna@barc.gov.in

Induced mutation technology is a powerful means to introduce novel genetic variability. Sugarcane is an important commercial crop cultivated primarily for sugar and bioenergy. It is vegetatively propagated, has a complex genome, high polyploidy, long life cycle, poor fertility and a narrow genetic base, hence mutagenesis can be a good approach for the induction of genetic variation. A blend of radiation mutagenesis with *in vitro* culture technique can augment the induction of novel genetic variability through selection of new genotypes. At BARC, strategies of radiation mutagenesis, selection at the cellular level, followed by *ex vitro* evaluation and field testing have been successful in the isolation of sugarcane mutants for improved traits. Gamma irradiation has been used on embryogenic calli of commercial sugarcane cultivars, agronomically desirable mutations for morphological, quality and yield contributing traits have been detected and isolated. The mutant clones showed better performance under saline field condition, indicating that the agronomically superior mutants can be useful in sugarcane improvement. Molecular characterization of the mutants using *de novo* transcriptomics has provided interesting information on genome-wide effects. The physiological basis of salt tolerance was investigated in one of the high yielding salt tolerant mutants and the results showed that sodium exclusion, ROS homeostasis and consequent maintenance of photosynthesis and WUE were the key components of the mutant's salt tolerance trait. The study highlighted the application of radiation induced mutagenesis for the creation of novel genetic variability in sugarcane.

THE INDUCTION OF POLYPLOIDS IN 'IRIS SARI SCHOTT EX BAKER' VIA *IN VITRO* TECHNIQUES

Selay Dogan

Republic of Turkey Ministry of Food, Agriculture and Livestock, Turkey

Email: selay.eldogan@tarim.gov.tr

Iris sari SCHOTT ex BAKER is an endemic plant which has a natural distribution area in Turkey. The plant, which has attractive flowers, is also known as "Ana kurtkulağı, Bahar çiçeği". Tetraploids were induced successfully from *in vitro* plantlets of *I. sari* by treating micro-bulbs with colchicine. The colchicine doses tested were: 0.1 and 0.5% during 2, 4 and 6 hours for each dose. Ploidy levels could be easily determined by flow cytometry. From a total of 45 surviving plantlets, 3 tetraploids were detected. The most efficient condition for inducing tetraploids seemed to be the treatment with 0.1% colchicine for 4 hours. Comparison of the survival rate of the controls and tetraploid plants *in vitro*, showed significant differences. Additionally, the induced tetraploids in *I. sari* also presented larger stomata and decrease in stomata density, compared to control plantlets.

MUTATION BREEDING OF ORNAMENTAL CROPS

Viktor Kozhevnikov¹, **Aleksander Pavlov**², Anton Kozhevnikov¹, Rena Mikailova²

¹*Stavropol Botanic Garden, Russian Federation*

²*Russian Institute of Radiology and Agroecology, Russian Federation*

Email: 49434@mail.ru

The Stavropol botanical garden located in the city of Stavropol (45°02'10.4" N 41°54'28.3" E) at 640m above the sea level, has an aster collection of some 68 genotypes. In 2003 investigations were initiated to study the effects of ⁶⁰Co (gamma) irradiation of aster seeds for mutation induction of commercially interesting traits. The work was new as there were no data on aster seed irradiation at the time. Initial experiments involved ten aster varieties, which were irradiated with gamma rays with a total absorbed dose of 90-100Gy (using a gamma emitter of 1.3Gy/min), but seed germination of varieties Utrennyaya Zarya and Solnechnaya was zero and seedlings of all other varieties died 25-30 days after germination. In 2012, reduced dose treatments were given (60-90Gy) and germination was successful in all but one variety. However, although germination was increased by the 50th day after germination all the plants had died. To extend M₁ plants life, dose rates were reduced further to 20-90Gy (at 10Gy intervals). The results show that the various aster varieties had different radiosensitivity. Seeds of all the varieties resulted in viable and fertile plants, from which M₂ seeds were obtained. During the following years, a wide range of mutant traits was observed. Plant selection was performed annually. By 2018, in M₅ generation ~200 lines with features different from the original parental varieties were selected. According to the requirement for the new varieties of plants to be resistant to fusariose, the varieties that were obtained have a resistance to the complex disease. These plants have the potential to produce highly decorative features and additional resistance to diseases and pests by further breeding.

BROADENING THE GENETIC VARIATION OF VEGETATIVELY PROPAGATED CROPS USING NUCLEAR TECHNIQUES IN NICARAGUA

Guillermo del Carmen Reyes-Castro¹, Donald Juárez-Gámez², Heeidy Corea-Narváez¹,

Rosario García-Loáisiga¹, Stephan Nielen³

¹*Universidad Nacional Agraria, Nicaragua*

²*Instituto Nicaragüense de Tecnología Agropecuaria, Nicaragua*

³*International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

Email: greyes@ci.una.edu.ni

An IAEA-funded four years national project will be carried out with the objective of expanding the genetic variability of cocoyam, taro and plantain through the induced mutations on *in vitro* plants of two cultivars per crops, searching for diseases tolerant and climate change adapted genetic variants. These crops are sources of protein and carbohydrates for consumers and income for farmers in tropical countries. Nevertheless, their narrow genetic base and way of vegetative reproduction put in risk the production and make difficult to meet the challenges of climate change (emergence of new pests and diseases) and the new market demands. In Nicaragua the areas, yields and production of cocoyam has been reduced due to the attack of root rot disease (RRD) caused by *Pythium myriotylum* in rainforest region and the reduction and unpredictable distribution of rain in the Pacific region. Taro production depends on only one non-resistance cultivar to Taro leaf blight (*Phytophthora colocasiae*), which recently reduced totally the production in Puerto Rico and Dominican Republic. Plantain production relies on few cultivars susceptible to diseases and pests. The gamma-ray irradiation dose per crop will be determined. 500 *in vitro* plants per each two cultivars per crop will be entered in a mutation induction

programmes. The *in vitro* chimeric tissues will be disaggregated through four subcultures. Induced variability will be assessed at *in vitro*, greenhouse and in field conditions. Molecular techniques will facilitate the mutant selection. New cocoyam, taro and plantain mutants adapted to the main effects of climate change (drought) and current diseases will be generated.

IAEA-CN-263-228

EXPLORING INDUCTION OF DOUBLED HAPLOIDS IN CASSAVA THROUGH WIDE CROSSING WITH CASTOR BEAN

Julius Baguma¹, Robert Kawuki¹, Settuba B. Mukasa²

¹*National Crops Resources Research Institute (NaCRRRI), Uganda*

²*Makerere University, Uganda*

Email: bagumakj@gmail.com

The increasing demand for cassava for food and non-food uses necessitates that its breeding be made faster to respond to these demands. The characteristic long breeding cycle and heterozygous nature of cassava pose major obstacles to the rapid genetic improvement of the crop through breeding. This study was aimed at inducing doubled haploids (DHs) in cassava using wide crosses with castor bean (*Ricinus communis*) pollen. A total of 3349 cassava flowers from twelve elite cassava varieties were pollinated with castor bean pollen. Early embryo rescue and ovule culture were done. 803 fruits were harvested, out of which 800 were dissected to obtain 1312 young ovules and from the remaining three fruits, seven unique embryos were excised, all of which were cultured *in vitro*. 82 (6.25%) of the cultured ovules formed callus of interest, originating from the embryo sac region and four of the rescued embryos (57.1%) regenerated into plantlets. A microscope was used to assess and screen the morphology of calli, flow cytometry was used to determine ploidy level and single nucleotide polymorphism (SNP) genotyping was used to determine the level of homozygosity in regenerated plantlets and calli. Ploidy analyses of 24 samples of the regenerated plants and calli revealed 95.8% diploids (23 samples) and 4.2% aneuploids (1 sample). SNP genotyping revealed increased level of homozygosity of up to 84.2%. The knowledge generated in this study is an important contribution towards on-going efforts in developing protocols for generation of DH cassava.

IAEA-CN-263-245

REDUCING POST-HARVEST PHYSIOLOGICAL DETERIORATION IN CASSAVA ROOTS: THE NIGERIAN EXPERIENCE

Damian Ndubuisi Njoku

National Root Crops Research Institute Umudike, Nigeria

Email: njokudn@yahoo.com

Cassava roots are notable for their short shelf-life due to post-harvest physiological deterioration (PPD). PPD is initiated by mechanical damage, which occurs during harvesting, making the roots unpalatable and unmarketable within 72 hours. Extending the shelf life of cassava to several weeks would reduce financial losses by \$2.9 billion in Nigeria over a 20-year period. Our main approaches at NRCRI aimed at reducing Cassava PPD include conventional breeding, mutagenesis, molecular breeding and genetic engineering. Over 150 land races and 120 exotic genotypes have been screened for delayed PPD. Limited variability was observed amongst land races in this trait. Thirty-three genotypes with delayed PPD at 7 days after harvesting (DAH) and 22 with delayed PPD at 14 DAH have been identified from backcross populations of *Manihot walkerae*. Gamma radiation was used to induce genetic variation for delayed PPD in these local germplasms. Mutagenized populations were developed using *in vitro* plants and OP seeds of farmer preferred varieties and land races. Few genotypes from these populations had

low PPD (7DAH). Genetic mapping for PPD using genomic DNA isolated from young leaves of parental genotypes indicated that genetic factors, most likely major QTLs, were likely involved in the expression of delayed PPD in cassava. Another strategy adopted was to use the synergistic effect of over expressing nuclear encoded gene, alternative oxidase (AOX) and increased accumulation of beta carotene content in cassava roots to delay onset of PPD. Constructs carrying appropriate genes were successfully transferred into cassava and plants expressing the inserted genes have been obtained and are being evaluated appropriately in different agro-ecologies of Nigeria. Furthermore, markers for PPD are being developed, and image analysis method of assessing roots for PPD is being introduced to help straighten and fast-track the selection of genotypes with delayed PPD.

IAEA-CN-263-249

IMPROVEMENT OF LOCAL DESSERT-TYPE BANANA FOR DISEASE TOLERANCE AND AGRONOMIC TRAITS THROUGH NUCLEAR TECHNIQUE APPLICATION

Babita Dussoruth, Vivian Vally

Food and Agricultural Research and Extension Institute FAREI, Mauritius

Email: babita3jdussoruth@gmail.com

The demand exists for a local dessert-type banana ('Gingeli'), appreciated for its soft, dry-textured and sweet-acidulated taste. The Gingeli banana is a tall and slender variety highly sensitive to Fusarium wilt (FoC), weevil borer, and wind, thus limiting its commercial exploitation and causing its near-extinction. The 'Gingeli' banana, however, holds untapped commercial prospects both for local and export markets. Thus, a mutation breeding project was initiated in 2007 with the support of IAEA, to induce desirable traits such as reduction in height and tolerance to FoC. *In vitro* cultures were irradiated at 18, 20 and 25Gy and the generated putative mutants were field planted. In absence of a Fusarium 'hot spot', the mutated population was planted and allowed to grow for two to three years, to be naturally affected by weevil and FoC. Eight improved lines with 11-16kg bunches and 80 to 125 fingers as compared to an average of 6-9kg bunches with 65 to 80 fingers borne on tall but stout trees were selected. These improved lines and other lines were brought back to *in vitro* conditions and 2 months old rooted plantlets were subjected to Fusarium assays, using a modified double-tray system. The roots were inoculated with spores of FoC race 1 and planted in the double tray containing sterile soil-manure mix. Suckers produced on lines that died were selected and planted in the field to confirm their response. The major constraint faced with the modified double tray system was that the disease pressure was too high making the selection process difficult. The soil/manure mix later became too compact which could have affected the adequate screening. Screening under field conditions remains a more reliable, yet slow, method. All the improved lines were mass multiplied for distribution to growers.

IAEA-CN-263-255

IRRADIATION AS MEANS FOR INCREASING GENETIC DIVERSITY IN BANANA: THE NEED TO CARRY OUT RADIOSENSITIVITY TESTS ON *IN VITRO* CULTURES AT APPROPRIATE STAGE OF GROWTH

Babita Dussoruth¹, Joanna Jankowicz-Cieslak²

¹*Food and Agricultural Research and Extension Institute FAREI, Mauritius*

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

Email: babita3jdussoruth@gmail.com

Banana in Mauritius is affected by several biotic and abiotic stresses and genetic improvement through conventional breeding is limited by the low-female fertility, fruit parthenocarpy and timeconsuming

methods. Worldwide, mutation induction through use of radiation and chemicals has been widely used to induce variation at a higher frequency in banana. Banana mutation breeding in Mauritius was initiated in 2007 through support of an IAEA through a technical research project, using irradiation of banana shoot-tips ('Gingeli' banana) with gamma rays. At that time, with exception of Cavendish types, the genomic group and ploidy level of most varieties were poorly documented as they were known by local names only. Historical records referred the 'Gingeli' banana to Figue Sucrée or Sucrier (AA). The first radiosensitivity test, with doses from 0 to 100Gy, was carried out in a batch of shoot-tips derived from cultures that were sub-cultured over ten times. The same was repeated in another batch that was sub-cultured around 8 to 10 times. A GR30 of less than 9 and 15Gy were obtained respectively. As this was within reported recommended range for diploids following gamma irradiation, it was thus assumed that the 'Gingeli' banana was probably a diploid. However, subsequent flow cytometric analysis of accessions in Mauritius revealed that most local bananas were triploid. Molecular studies also revealed that 'Gingeli' belonged to the Silk (AAB) group. A GR30 of 1825 was then obtained on fresh *in vitro* cultures of 'Gingeli'. The range of 15 to 18Gy was also obtained with triploids, Petite Naine (AAA), 'Ollier' banana (AAA, Cavendish-type) and Mamoul (ABB, P. awak group) based on survival and proliferation rates.

IAEA-CN-263-263

DEVELOPING DISEASE RESISTANT HIGH YIELDING FARMER-PREFERRED CASSAVA VARIETIES IN UGANDA THROUGH INDUCED MUTATION BREEDING

Emmanuel Ogwok, Titus Titus Alicai
National Crops Resources Research Institute, Uganda
Email: emmyogwok@gmail.com

Cassava is a tropical food security crop that is grown by many farming families in Uganda. The crop is resistant to drought conditions, does well in low fertility soils, and low input production systems. The roots are a very handy source of energy for humans and livestock, and a focus for many industrial applications. The dual epidemic of cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) are the most pressing constraint to cassava production. CBSD specially, is the most damaging as it causes necrotic root rots, which limit its utilization for food and feed. Conventional breeding efforts generated durable CMD resistance, but has not been successful for CBSD control. All CMD-resistant farmer-preferred cassava varieties are highly susceptible to CBSD. Biotechnology strategies have also been tested for CBSD resistance in cassava and transgenic plants generated were highly resistant to CBSD. However, genetic engineered crops face many challenges including perceptions and attitudes especially on issues concerning human nutrition, health, and wildlife security. Uganda also lacks biotechnology related law, which hinders commercialisation of genetically modified crops. Fortunately, mutation breeding does not have such ethical issues regarding human health and sustainability, and is invaluable in developing unique germplasm within a short timeframe when there is limited genetic variation. Besides, gamma irradiation has been used to generate mutant cassava varieties with improved yield, starch and dry matter content. This project seeks to artificially induce mutagenesis in selected cassava varieties using gamma irradiation for the purposes of improving yield and disease resistance, and for broadening the genetic base of cassava to circumvent genetic erosion.

THE PRESENCE OF STEM SPLITTING AND FASCIATION IN CHRYSANTHEMUM AS RESPONSE TO GAMMA IRRADIATION AND PHOTOPERIOD

Sasanti Widiarsih, Ita Dwimahyani
 CIRA-BATAN Indonesia
 Email: sasanti.widiarsih@gmail.com

Chrysanthemum is the second economically most important floricultural crop. Photoperiod controls growth and flowering for many floriculture crops. A small change in photoperiod can trigger the switch between vegetative and flowering phase. For commercial production in tropical countries, additional light hours were commonly applied to prolong vegetative phase. This practice prevents the inflorescence emergence until the population homogenously reaches optimum height. Mutation breeding in chrysanthemum has been developed for decades. Many experiments have been carried out to learn the effect of irradiation to both *in vitro* and *ex vitro* plants. However, most experiments were conducted only in natural light condition. The combined effect of irradiation and photoperiod is still poorly understood. The purpose of this experiment is to investigate morphological changes on plant growth as response to different levels of gamma irradiation in both natural and additional light treatments. The experiment was conducted in 2006 at PT. Saung Mirwan, a floriculture company in Ciawi, Indonesia. The materials used were shoot cuttings of chrysanthemum cv. Pink Fiji, subjected to gamma irradiation with doses of 0, 10, 15, 20, and 25Gy, subsequently. The materials were grown in a green house and divided into two separate plots: one with 4 hours additional light and the other with natural light. Plant growth rate and morphological changes were observed. Plant growth was slower with higher irradiation doses given. Additional light delayed flower initiation by 2 weeks, induced faster plant growth and more primary branches compared to natural light treatment. Morphological changes in form of stem splitting and fasciated stems were found only in irradiated plants grown under additional light, and occurred mostly at 15-20Gy. Under both light treatments, the optimal irradiation dose to increase genetic diversity in chrysanthemum cv. Pink Fiji stem cuttings was 15-20Gy.

IAEA-CN-263-280

IN VITRO TREATMENT OF 'GROS MICHEL' BANANA APICES WITH ETHYL-METANESULFONATE (EMS): A POSSIBILITY FOR OBTAINING BANANA RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* RACE 1

Jorge A. Sandoval
 Research Department, Corbana, Costa Rica
 Email: jsandoval@corbana.co.cr

Banana's research community is nowadays finding new options to control *Fusarium oxysporum* f. sp. *cubense* (Foc race 1 and Foc TR4). Although there is a wide number of scientific reports about different techniques for inducing genetic variation for crop improvement, recent information on banana mutation breeding is limited. However, mutation techniques can be considered for finding solutions to control the disease. In order to induce possible genetic variations for resistance or tolerance against *Fusarium* wilt (by Foc race 1), experiments using the chemical Ethyl methanesulphonate (EMS) for mutation induction in Gros Michel (*Musa* AAA) banana shoot tips were performed at the biotechnology laboratory at Corbana. Four EMS doses were tested: 0.025, 0.050, 0.075 and 1.0 molar (M). Two controls were included: sterile water and the buffer solution. Total number of treatments were six. A group of 100 apices were separately submerged-in-agitation in each treatment for nine hours. The more effective dose was determined between 0.05M and 0.025M of EMS. A total of eight *in vitro* subcultures was performed to the apices. Resulting plantlets showed 90% of surviving, 5% of albinism and a propagation rate of 3.2 plantlets by each shoot tip. A total of 250 plants from each of the more effective

EMS treatments were exposed to selection pressure in soil inoculated with a solution of Foc race 1 (1×10^6 spore \times water ml). During the experimental period more than 95% of the plants from both EMS treatments showed Fusarium wilt symptoms. The other 5% showed a temporary and partial better performance against the disease. These results confirm that mutation techniques in banana have the potential to be an integrative part in a disease control strategy.

POSTERS RELATED TO SESSION 5

**NEW CHALLENGES AND TECHNOLOGIES IN
PLANT GENOMICS AND BREEDING**

M Building – Common Area

RADIATION EXPOSURE OF BARLEY SEEDS CAN IMPROVE PLANTS' DEVELOPMENT

Roman Churyukin, Polina Volkova, Stanislav Geras'kin
Russian Institute of Radiology and Agroecology, Russian Federation
 Email: r.churyukin@mail.ru

Identification of mechanisms of plant adaptive response to weak external radiation exposure is a complex and interesting problem that might need consideration in plant biology. Such responses include the effect of radiation hormesis which is the stimulating effect of low doses of ionizing radiation. The reactions of barley seeds in terms of the root and sprout lengths, the germination rate and the root mass variation were studied after γ -irradiation with doses in the range of 2-50Gy. The dose range in which plants' growth stimulation appear to occur was identified as being between 16 and 20Gy. It was shown that increased size of seedlings after irradiation with stimulating doses was due to the enhancing pace of development rather than an earlier germination. The activity of most of the enzymes involved in plant germination and early growth notably increased within the range of doses that cause stimulation of seedlings development. The dose rate, the quality of seeds, their moisture and the time interval between irradiation and initiation of germination has a major influence on the manifestation of the effects of radiation. The experimental data were significantly better explained by mathematical models that consider the hormetic effect. It was shown that irradiation of seeds significantly influenced the development of plants throughout the vegetative period. The duration of the initial stages of ontogenesis was shortened, and the phase of full ripeness came on 5-7 days earlier than in the control. Various agronomic traits such as, the length of the stems, the weight of 1000 grains, the number of grains per ear, the number of productive stems, the weight of straw and ears showed notable increase. Our study showed that the dependence of traits, including economically important traits on the dose of γ -irradiation of seeds may be significantly better described by models that ponder the effects of hormesis. It was shown that realization of the effect of hormesis specifically depends on the conditions in which the plants developed.

IAEA-CN-263-39

FINE MAPPING AND CANDIDATE GENE ANALYSIS OF THE QUANTITATIVE TRAIT LOCUS GW8. 1 ASSOCIATED WITH GRAIN WEIGHT IN RICE

Sang-Nag Ahn¹, Hyun-Sook Lee¹, Kyu-Chan Shim¹, Yeo-Tae Yun², Yun-Joo Kang¹
¹*Chungnam National University, Republic of Korea*
²*Chungcheongnamdo Agricultural Research and Extension Services, Republic of Korea*
 Email: ahnsn@cnu.ac.kr

A quantitative trait locus (QTL) gw8.1 was identified in the population derived from a cross between the elite japonica cultivars; 'Hwaseong' and *Oryza rufipogon* (IRGC 105491). Near isogenic lines (NILs) harboring the *O. rufipogon* segment on chromosome 8 showed increased grain length and weight compared to those of the recurrent parent, Hwaseong. This QTL was mapped to a 175.3-kb region containing 28 genes, of which four were considered as candidates based on the presence of mutations in their coding regions and as per the RNA expression pattern during the inflorescence stage. Leaves and panicles obtained from plants harvested 5 days after heading showed differences in gene expression between Hwaseong and gw8.1-NILs. Most genes were upregulated in *O. rufipogon* and gw8.1-NIL than in Hwaseong. Scanning electron microscopy analysis of the lemma inner epidermal cells indicated that cell length was higher in gw8.1NIL than in Hwaseong, indicating that gw8.1 might regulate cell elongation. Among the candidate genes, LOC_Os08g34380 encoding a putative receptor-like kinase

and LOC_Os08g34550 encoding putative RING-H2 finger protein were considered as possible candidates based on their functional similarity.

IAEA-CN-263-46

ANALYSING THE SPECTRUM OF SPACEFLIGHT-INDUCED MUTAGENESIS VIA HIGH THROUGHPUT SEQUENCING

Tao Guo¹, Hui Wang¹, Wenlong Luo¹, Zhiqiang Chen²

¹South China Agriculture University, China

²Institute of Agricultural Sciences of Lixiahe Region in Jiangsu, China

Email: guo.tao@vip.163.com

Rice is an important staple food crop, and it is also the first crop that has been subjected to spaceflight-induced mutagenesis. In the present study, whole-genome sequencing of rice mutants selected following mutagenesis induced by space flight was conducted. The main results were as follows: (1). Three mutants named H153, H398 and H399 were selected following spaceflight-induced mutagenesis, the genome mutation number observed was more than 10 000 via high throughput sequencing. Single nucleotide variation (SNV) and small insertion or deletion (indel) shorter than 5 bp made up the most majority of all mutations. (2). Validation of mutations in the mutant H153, H398 and H399 were performed by using single molecule sequencing (SMRT). The validation revealed a very low false positive rate in the mutations detected, indicating that the mutation discovery by using illumina sequencing was highly accurate. Based on the discovered mutation data, the mutation rate in the H153, H398 and H399 was 7.1×10^{-4} , 6.8×10^{-5} and 6.6×10^{-5} , respectively. (3). Many structural variants (SVs) with size larger than 50 bp were detected in the three mutant H153, H398 and H399. Moreover, SNVs were densely distributed around the SVs and therefore formed many mutation clusters as revealed by visualized analysis. (4). Effects of all the mutations detected were analyzed and predicted, and the percentage of mutations that put highly impact on the gene was 1.98%. For these genes predicted to be highly impacted, nonsignificant GO term was found, indicating that the genomic mutations may put an unbiased impact on biological processes, cellular functions and pathways.

IAEA-CN-263-47

CHARACTERIZATION OF NOVEL SORGHUM MUTANTS FROM AN EMS-MUTAGENIZED POPULATION

Li Zhu¹, Guiying Li², Xiaoduo Lu¹, Yubin Li¹

¹Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, China

²Institute of Crop Science, Chinese Academy of Agricultural Sciences, China

Email: zhuli01@caas.cn

Sorghum (*Sorghum bicolor*) is ranked as the fifth most important grain crop and serves as a major versatile C4 model crop for research in gramineae. High-quality genome sequence is available for sorghum functional genomics, but the availability of genomic and germplasm resources remains limited for functional validation of genes. Chemical mutagenesis of sorghum germplasm, followed by screening for mutants altered in important agronomic traits, represents a rapid and effective means of addressing this limitation. Ethyl methanesulfonate (EMS) was found to be the most potent mutagen in inducing mutation. Here, we reported that a sorghum mutant population consisting of 3000 lines was generated from the cultivar Jiutian1 by treatment with EMS. Numerous phenotypes with altered morphological and agronomic traits were observed from M₂ and M₃ lines in the field. A dwarf mutant (named SbME6-1) with a pale leaf phenotype and stunted growth and reduced chloroplast contents relative to wild-type (WT) was isolated from this EMS-mutagenized M₃ population. To identify the genetic determinant of this trait, four DNA pools (two each from normal and dwarf mutants) were isolated and performed by

the Gene Mapping by Sequencing (MBS). Comparison of allele frequency of the single nucleotide polymorphisms (SNPs) between the pools with contrasting phenotype showed that a locus in chromosome 9 between 51M and 57M had an allele frequency of 1. There were 15 mutation genes in the locus, out of which in 5 mutation genes showed unknown function. This finding is expected to contribute to future research on the identification of the causal factor of dwarfing. Our results show an EMS mutant resource as an efficient platform for gene discovery in sorghum, thereby accelerating sorghum breeding.

IAEA-CN-263-48

GENOMIC VARIATION CHARACTERISTICS OF GAMMA RADIATION INDUCED MUTATIONS IN M₁ POPULATION OF THE RICE JAPONICA VARIETY GAOGENGNUO

Li Zhang, Xuanming Peng, **Zhen Yang**

Hunan Institute of Nuclear Agricultural Science and Space Breeding, China

Email: yz473@163.com

Radiation-induced mutation can generate useful genetic variation in crops and has been widely used for rice germplasm innovation. To determine the pattern of DNA polymorphism in radiation induced mutations in M₁ population, we performed whole genome sequencing of rice cultivar Gaogengnuo lines treated with 300Gy and 400Gy ⁶⁰Co gamma radiations. The Gaogengnuo resequencing data of 4×fold coverage was 96.24% compared with the *Oryza sativa* L. Japonica Nipponbare reference genome, in Gaogengnuo genome, a total of 1 002 114 polymorphic sites were detected, including 878 381 single nucleotide polymorphisms (SNPs), 123 733 insertion/deletion polymorphisms (indels), and 12 805 structural variations. Compared with Nipponbare, the two M₁ populations that received the different doses of gamma radiation, 300Gy and 400Gy, contained 429 809 and 684 221 polymorphic sites, and 9075 and 8852 structural variations, respectively. Through integrative analysis of resequencing data, we identified polymorphisms between Gaogengnuo and 300Gy and Gaogengnuo and 400Gy. We identified 67 885 and 69 899 indels and 609 209 and 632 745 SNPs between 300Gy and Gaogengnuo and between 400Gy and Gaogengnuo, respectively. We identified 2682 homozygous SNPs and 2988 homozygous indels present in Gaogengnuo, 300Gy and 400Gy. These genetically stable variants will be useful for rice breeding and functional research. Gene ontology analysis revealed that genes associated with metabolic process, binding function, and membrane may be susceptible to gamma radiation.

IAEA-CN-263-55

RNA-SEQ-BASED TRANSCRIPTOME ANALYSIS OF MOLECULAR VARIATIONS IN COMMON WHEAT MUTANTS DERIVED FROM GAMMA-RAYS AND EMS

Hongchun Xiong, Huijun Guo, Linshu Zhao, Luxiang Liu, Yongdun Xie

Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, China

Email: xionghongchun@caas.cn

Induced mutation has been widely used for crop improvement due to acceleration and enhancement of genetic variations. However, the molecular basis induced by physical or chemical mutagens is still largely unknown in common wheat. And mechanisms behind the phenotypic variations induced by mutagens are interesting to be clarified. Transcriptomic sequencing was performed for investigation molecular basis of induced mutation, the dwarf or salinity-resisting mechanisms in wheat mutant lines. The results showed that mutations in the EMS-induced dm20 dwarf mutant resulted in higher SNP numbers, transitions and DEGs between the wild type (WT) and dm20 compared to that in γ -rays-induced dm19 dwarf mutant, suggesting that the variation in the transcriptome of dm20 was higher than

that in dm19. Most of genes encoding heat shock proteins (HSPs) showed higher expression in both dm19 and dm20 compared with WT. Furthermore, transcript levels of three genes involving auxin metabolism were significantly down-regulated in dm20. In addition, we have also screened one salinity-resisting mutant and analysis of mutated genes suggested that two mutated genes were enriched in GO term “sodium ion transport”. Moreover, “oxidation-reduction process” was the only significantly enriched GO term by the up-regulated genes between salinity-treated mutant and WT. This study supplied important information of transcriptomic sequence variation in the mutant lines and also revealed the potential mechanisms of phenotypic variations. In the dwarf mutants, the HSPs may play an important role in dwarfism and auxin may contribute to the reduction of plant height in dm20. Genes associated with sodium ion transport may directly contribute to salinity tolerance and the homeostasis of oxidation-reduction process play important roles in salt resistance in the salinity-resisting mutant.

IAEA-CN-263-56

WHOLE TRANSCRIPTOME MAPPING REVEALS COMPLETE TRANSCRIPTION AND SIGNALS OF EUKARYOTIC PROCESSING IN PLANT MITOCHONDRIA

Lucretia Ramnath

South African Sugarcane Research Institute, South Africa

Email: lucretia.ramnath@sugar.org.za

The plant chloroplast has become a vector to deliver transgenes. Recently, however, the mitochondrion has become a prospective target for plant genetic engineering. Transformation of mitochondria can result in synchronized expression of transgenes between all three plant genomes via interorganellar communication pathways within the cell, resulting in effective engineering of the whole plant cell factory. In addition, the mitochondrial genome (mitogenome) can shuffle genes between the chloroplast and the nuclear genome, making it a unique vector for transgenes, and of great potential to plant breeders. However, there is evidence that plastomes can be transferred across plant species boundaries by contact. As a result, understanding the plant mitochondrion is of paramount importance in identifying the risks of their use as transgenic vectors. Mitogenomes are known to have evolved from bacterial genomes and include operons and spliced genes. However, in this study, whole transcriptome mapping reveals indicators of eukaryotic processing never reported before in plant mitochondria, which will aid in our understanding of the mitogenome. The transcriptomic data of various plant and animal species available on NCBI were mapped to mitochondrial reference genomes. Mappings of reads to mitochondrial genomes revealed, for the first time, that selected monocot, dicot, non-seed plant, fungal and animal mitochondrial genomes were expressed in their entirety. Moreover, when poly-A selected transcript sets were mapped they were found to only cover part of the mitogenome. Thus, mitochondrial transcripts may undergo eukaryotic-like processing that need to be further investigated. Overall, the plant mitochondrial genome shares many features with the size-relaxed nuclear genome.

IAEA-CN-263-63

THE PROTEOMICS RESEARCH IN MAIZE MUTANT SEEDS BY ⁶⁰CO GAMMA-RAY RADIATION

Xinmei Guo, Xinxin Sun, Xiyun Song

Qingdao Agricultural University, China

Email: xmguo2009@126.com

In this study, dry seeds of Chang7-2 and P138 were radiated by ⁶⁰Co γ -ray radiation with 100, 150, 200 and 250Gy. In order to research the changes of the growth and development in maize, seeds germination and major agronomic traits were measured. The results showed different dosage obviously inhibited the

growth of the maize of M₁, especially root length. While there was no apparent effect on M₂, M₃ germination, but significant influence on the 100-kernel weight of M₃ and M₄. The reaction of 2 maize inbred lines to ⁶⁰Co γ-ray radiation was different. Chang7-2 was more sensitive. Four mutants from M₄ Chang7-2 were elected which had higher 100-kernel weight, named mutant1,2,3 and mutant 4. Albumin, globulin and glutelin from mutants were analyzed by SDS-PAGE. Results showed that mutant2 had obvious different bands on albumin compared with control, while mutant2 and mutant4 had obvious different bands on glutelin. Through the analysis of Imagemaster 2D, mutants had different variation points in albumin, globulin, glutenin and total protein. 6,6,7 and 8 albumen were respectively chosen in mutants, in which 11 albumen increased, 13 albumen decreased, 3 albumen induced expression. 4,4,11 and 4 globulins in mutants were chosen, in which 9 globulins increased, 9 globulins decreased, 5 globulins induced expression. 5,2,8 and 4 glutenin in mutants we chosen, in which 6 lutenin increased, 12 glutenin decreased, 2 glutenin induced. 9,3,9 and 4 total proteins in mutants were chosen, in which 11 proteins increased, 13 proteins decreased, 1 protein induced. These proteins were identified by MALDI-TOF-MS and their functions were analyzed. 8 proteins were identified successfully. These proteins were classified into 3 categories: stress-related proteins; sugar metabolic pathways related proteins; other proteins.

IAEA-CN-263-76

HIGH-RESOLUTION MELTING ANALYSIS FOR THE IDENTIFICATION OF SNP MUTATIONS IN THE bZIP DOMAIN OF HvWRK38 GENE AMONG BARLEY GENOTYPES

Dana Jawdat¹, Bernhard Hofinger², Nizar Mir Ali¹, Ola Moustafa³

¹Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, Syrian Arab Republic

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

³General Commission for Scientific Agricultural Research, Syrian Arab Republic

Email: djawdat@aec.org.sy

Drought tolerance is a complex trait that is governed by many genes and pathways, where transcription factors (TFs) play an important role as regulator molecules. HvWRKY38 gene is a transcription factor of the WRKY family in barley, with two conserved domains: the leucine zipper and WRKY. It is known for transcriptional reprogramming in response to abiotic stress conditions in plants. High Resolution Melting (HRM) procedure has been applied on DNA samples of barley genotypes that include: the wild type *Hordeum spontaneum* L., M₈ mutant lines (HvM 7, HvM 58, HvM 64, HvM 66, and HvM 69) and their *H. vulgare* L. parent variety (Furat 9), together with local and introduced varieties (IC9, Furat 3, Tadmour and Banteng). The Type-it®HRMTM PCR kit was used for amplification and HRM analysis was carried out using the Rotor-Gene Q system. The following forward and reverse primers were used to amplify the bZIP motif of HvWRKY38 (NCBI: AAS48544.1): Hv_bZIP_F (5'AACTTCATGCCGCTCAAGAA -3') and Hv_bZIP_R (5'TGACCATGTCTGGTGAAGTGG -3'). HRM analysis showed a distinctive curve pattern of the mutant line HvM7. This variant pattern of normalized melting curve and its corresponding difference curve for the 230 bp amplicon, was verified by Sanger sequencing to contain two SNPs, a nonsynonymous transition at 189T>C, and a synonymous transversion at 213 C>A corresponding to the bZIP motif within the ORF of HvWRKY38 sequence. The identification of functional variation among members of TFs gene families, and further links with physiological and morphological data, will enable a robust assessment and selection process of candidate parents.

MARKER-ASSISTED BACKCROSSING FOR RECOVERY OF RECURRENT PARENT GENOME FROM A CROSS BETWEEN MR264 AND PONGSU SERIBU 2 RICE VARIETIES

Noraishah Hasan, Abdul Rahim Harun, Norida Mazlan, Nusaibah Syed Ali, Rafii Mohd Yusoff, Shamsiah Abdullah

University of Technology MARA, Faculty of Applied Sciences, Malaysia

Email: aishahnh@ns.uitm.edu.my

Background selection is the most crucial step in marker-assisted backcrossing breeding (MABC). The primary goal in this selection is to develop backcross progenies carrying the gene of interest with maximum recovery of the recurrent genome, a process which can take many generations. MABC is the most significant technique to recover the recurrent genome within 2 to 3 generation and minimize the drawbacks of conventional breeding. In this study, MABC technique was adopted for crossing between MR264, a mutant rice susceptible to blast and Pongsu Seribu 2, a traditional Malaysian variety possessing resistant genes (donor) to produce a new blast resistant rice variety. In the present study introgression of the blast resistance genes Pi-kh and Pi7(t) was identified through presence of the tightly linked markers RM5961 and RM206. Out of 375 SSR markers, 72 polymorphic SSR markers between parental lines were used to monitor the recovery of recurrent parent genome in backcross population. Backcross populations, BC1F1 and BC2F1 revealed 76.1 to 87.9% and 86.5 to 95.2% of recurrent parent genome recovery in background analysis, respectively. The average percentage of recipient genome recovery in selected BC2F2 lines was 94.4%, which indicated a close similarity at phenotypic resemblance to the recurrent parent MR264. Seven homozygous plants carrying blast resistance genes having maximum genetic backgrounds of MR264 were selected as improved blast resistant lines for development of a blast resistant variety. Dendrogram analysis revealed that the selected BC2F2 lines were clustered together with MR264, which indicates a strong relationship with MR264. This study concluded the on effectiveness of marker-assisted backcrossing for rapid recovery and maximization of the restoration of recurrent parent genome in backcross populations in a mutation breeding programme.

DNA DAMAGE RESPONSE DURING EARLY GERMINATION IN RELATION TO SEED AGING AND RADIO-SENSITIVITY IN BARLEY

Vasilissa Manova¹, Christopher West², Lubomir Stoilov¹, Wanda Waterworth²

¹*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Bulgaria*

²*University of Leeds. Faculty of Biological Sciences, United Kingdom*

Email: vmanova@bas.bg

Accumulation of DNA damage is a hallmark of aged seeds; therefore, the successful repair during imbibition is an important prerequisite for seed germination and further seedling development. In this work we investigated the effects of aging and ionizing radiation on the germination potential of barley seeds and their ability to activate cellular DNA damage repair response systems. Aged and unaged seeds were subjected to 100Gy X-ray irradiation for 5 hours after imbibition and the accumulation of DNA lesions and the activation of DNA repair mechanisms were observed and recorded; 2, 6 and 24 hours afterwards. The levels of oxidative DNA damage were measured at the whole genome level by alkaline gel-electrophoresis and in specific nuclear and extra-nuclear genes by Long-range PCR. Transcriptional profiles of barley gene homologues involved in various DNA repair pathways were assessed by semi-quantitative and quantitative RT-PCR. Actin, tubulin and 18S rRNA genes were tested as reference controls for the time periods analysed. Accelerated aging increased the radio-sensitivity of imbibed barley seeds in terms of delayed germination and reduced growth. The results obtained with naturally

aged seeds showed the presence of formamidopyrimidine-DNA glycosylase (FPG)-sensitive sites in barley nuclear, chloroplast and mitochondrial genomes early after imbibition in both irradiated and control seeds and lesions were still detectable at the 24h time-point. In response to ionizing radiation RAD51 and PARP2 were found to be specifically modulated during the early imbibition periods. The obtained results reveal molecular mechanisms which might underlie the tolerance of aged and unaged barley seeds to ionizing radiation. Knowledge on the processes responsible for the fate of DNA damage during seed germination has the potential to influence the efficiency of mutation induction protocols utilized for breeding purposes based on the irradiation of dry seeds.

IAEA-CN-263-124

BARLEY ACT7 HOMOLOGUE IS UP-REGULATED IN RESPONSE TO UV-C RADIATION

Vasilissa Manova, Irina Boycheva, Lubomir Stoilov, Ralitsa Georgieva
Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Bulgaria
Email: vmanova@bas.bg

Plant genome reacts to ultraviolet radiation by altering the transcriptional pattern of some genes leading to the activation of complex cellular defence mechanisms. Whilst, intensive research and achievements have been obtained in this area, new networks and players of plant's response to UV stress continue to emerge. In this study we found that barley actin gene was strongly up-regulated in the leaves barley seedlings exposed to UV-C. Plants were subjected to acute UV-C irradiation and left for recovery under dark condition or under high-intensity light. RNA was isolated from the irradiated and non-irradiated leaves collected at different time-points up to 24 hours post-irradiation. The expression pattern of actin gene was characterized by both semi-quantitative and quantitative RTPCR; 18S rRNA served as reference control. Several-fold induction of barley actin gene was found in the green leaves 3h after treatment and increased further at 6h time-point. In the etiolated leaves the up-regulation was delayed and registered at 6h and 24h post-irradiation. Significant increase in the mRNA levels was also detected in the irradiated etiolated seedlings kept in dark for 24h. No changes were found in the respective non-irradiated controls. High-resolution melting analysis followed by sequencing revealed that the amplified fragment corresponded to a unique mRNA designated as actin 7 in the IPK-Gatersleben Barley Database and the NCBI blast analysis matched the predicted protein predominantly to actin 7 in the closely related species. In plants ACT7 isotype is known as the actin responsive to external stimuli such as hormone treatment and pathogen attack. Our study showing transcriptional activation of ACT7 homologue in the UV-C irradiated barley seedlings is the first one linking a member of the actin family with the UV defense response in plants.

EVALUATION OF WHEAT AND BARLEY GERMPLASM STRESS RESPONSE BY NUCLEAR, OMICS AND PHYSIOLOGICAL APPROACHES

Elena Todorovska¹, Atanas Atanassov²; Georgi Boncheva³, Gergana Mihailova³, Goritza Rakleova¹, Irina Boycheva³, Katya Georgieva³, **Lubomir Stoilov**³, Lyudmila Simova-Stoilova³, Rossitza Rodeva³, Stefan Tsonev¹, Svetlana Misheva³, Tanya Karceva³, Valya Vassileva³, Vasilissa Manova³, Zlatina Uhr³, Zornitsa Stoyanova³

¹*Agrobiointitute, Bulgarian Agricultural Academy, Bulgaria*

²*Joint Genomic Center, Sofia State University, Bulgaria*

³*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Bulgaria*

Email: molgen@bas.bg

Breeding priority worldwide is the development of cereals adapted to stress factors like extreme temperatures, salinity and drought, limiting the growth, productivity and distribution of agricultural crops. Knowledge of the mechanisms governing stress response is a prerequisite for development of advanced breeding practices and new varieties. National IAEA project BUL5014 for technical cooperation is aimed to highlight the adaptive potential and enhance the involvement of wheat and barley in breeding programmes directed towards more sustainable, environmentally friendly and cost-effective agriculture. Complex stress response to radiation, temperature and fungal diseases of representative wheat and barley genotypes from the national seed gene bank was evaluated using molecular, biochemical and physiological approaches. Gene expression profiling by Real-time PCR, SNP-based genotyping, gene cloning and characterization, PCR-mediated screening of DNA repair activities, HRM and expression analyses of stress-related proteins was performed. Genotype characterization was complemented by estimation of physiological and biophysical parameters like photosynthetic activity, electrolyte leakage and malondialdehyde content, nutrient use efficiency and protease activity. Research was focused on improvement of wheat for disease resistance, radiation induced barley stress response, nutrient use efficiency in wheat and characterization of abiotic stress response of wheat and barley through DNA fingerprinting and functional analyses of stress-related genes as well as physiological and biochemical studies under stress conditions. Advanced knowledge on the protective capacity and adaptability of wheat and barley genotypes, based on the molecular mechanisms underlying their response to abiotic stress and radiation was obtained. Transcriptional profiling of radiation-responsive genes revealed some features of the genetic control of DNA repair in barley. Complex molecular, biochemical and physiological screening of wheat and barley stress tolerance supports further development of effective breeding strategies towards alleviation of the harmful impact of biotic and abiotic stress factors on the productive capacity of cereals.

ASSOCIATION MAPPING STUDY OF AGRONOMIC TRAITS IN BACKCROSSED MUTANT WHEAT GERMPLASMS UNDER DROUGHT STRESS AND NON-STRESSED CONDITIONS

Ayşe Sen¹, Irfan Ozturk²

¹*Department of Biology, Faculty of Science, Istanbul University, Turkey*

²*Trakya Directorate of the Institute of Agricultural Research, Turkey*

Email: senayse@istanbul.edu.tr

Increasing aridity of semi-arid regions and limited water resources have led to a crucial necessity for improving crop resistance to drought. Induced mutations play a significant role in adaptation to stress conditions, mutants are valuable germplasm resources in crop improvement programs. Molecular association mapping technique through linkage disequilibrium is one of the other approaches

successfully used in breeding programs. As reported in publications, it can be effectively used to tag genomic regions involved in drought stress tolerance. In view of the above, during the presented work, we have focused on investigating population structure, linkage disequilibrium, and association mapping between pairs of SSR marker loci in F₂ segregated population derived from reciprocal backcrossed mutant wheat germplasms under drought stress and non-stressed conditions. To associate markers with drought tolerance at vegetative stage, we examined over the 170 polymorphic SSR markers that were nearly evenly distributed on the 42 wheat chromosomes. A total of 100 segregated individuals grown under normal water supply and stressed conditions were observed for morphological and physiological parameters variations. Phenotypic and genotypic data were associated using TASSEL 2.1 to find associated markers with agronomic traits. The distribution of phenotypic variance was measured as varying between 7% and 17%. Three SSR markers were associated with more than one morpho-physiological trait under drought treatments. Important genomic regions were identified on chromosomes 5A and 7A. The results have clearly shown that genetic mapping is one of the feasible options to identify drought-related marker(s) in wheat mutant germplasms.

IAEA-CN-263-145

ASSOCIATION MAPPING FOR MORPHOLOGICAL AND GRAIN QUALITY TRAITS IN RICE WHITE PONNI MUTANTS

Andrew Peter¹, Arumugam Pillai¹, S. Ramchander¹, J. Souframanien²

¹Tamil Nadu Agricultural University, India

²Bhabha Atomic Research Centre, BARC, India

Email: mapillai1@hotmail.com

Improved White Ponni (IWP), a tall and medium duration rice variety is famous for its superior grain qualities. However, the variety is susceptible to lodging and takes long duration for maturity. In this study, twenty M₆ generation of IWP mutants primarily developed for semi-dwarfism and early maturity were assessed for the level of morphological and molecular variability. Possible marker-trait associations were studied using 53 SSR markers. The twenty IWP mutant lines and WP-Control plants were assessed for morphological variability and grain quality assessments. Fifty-three SSR markers were utilised for assessment of molecular variations and tree construction. Marker-trait associations were studied using STRUCTURE 2.3 and TASSEL 2.0.1. Significant reduction in plant height (up to 63.4cm) and days to fifty per cent flowering (up to 13 days) was observed among the mutants. Significant increase in single plant yield than WP-Control was observed in 13 mutants. WP_15-5 recorded highest single plant yield of 59.44 g. Fifty-three SSR markers revealed variations which were consistent with morphological observations. The phylogenetic tree showed two prominent branches, which separated WP-Control and the semi-dwarf and early maturing mutants. The population structure analysis using STRUCTURE extracted four clusters (optimum K = 4). The IWP mutants studied here showed potential divergence both at morphological and genetic levels. WP_15-5, WP_16-1, WP_16-2, WP_22-2, WP_23-3 and WP_6-3 are promising mutants with high yield, semi-dwarfism and earliness with grain quality characters equivalent to WP-Control.

MODIFICATION OF UNMETHYLATED CYTOSINE INTO THYMINE IN SD1 CAUSES SEMI DWARFING IN WHITE PONNI RICE MUTANT

Arumugam Pillai¹, K. K. Kumar¹, Yasin Jeshima Khan²

¹TNAU, India

²NBPGR, India

Email: mapillai1@hotmail.com

Mutants of improved white ponni rice (IWP) along with non-irradiated control lines were evaluated over 6 generations. Test lines and controls were genotyped using SNPs and a few haplotypes were identified. Bioinformatic analyses revealed the presence of multiple mutations in each locus and the presence of splice variants and multiple ORFs were analysed. DNA methylation at C5 of cytosine is a mechanism of gene expression regulation. This indicates the presence of chromatin modification and transcriptional gene silencing. From SNP data sets of the tested samples and control white ponni, the conversion of unmethylated Cytosine to Thymine were found in regions of SD1 gene located in chromosome 1. White ponni control sample was having two Cytosines (CC) which were completely converted into Thymines (TT) in Nipponbare. Whereas, in BRNS-WP-15-5 mutant conversion of C to A at second C is found. BRNSWP-22-2 is exactly like white ponni control in three base positions vulnerable for mutations. The in-depth haplotype analyses of these SNPs revealed the presence of four different forms of SD1 with its three alternate forms viz. SD1 (protein kinase), SD1 isoform x2, SD1-8, SD1-8 isoform X1. With these four variants six more loci viz., Os01g0784700, ARK1, OsJ_19099, OsJ_11431, OsJ_03701, Os07g0550500 were found to exist in multiple ORFs. Conversion of unmethylated C into T changes the functional SD1 ORF into silenced locus. In chromosome 1 SD1 loci position 33252230 bases and 33253569 bases are vulnerable for CC to TC and TT mutation. This information could be very significant in terms of either generating desired phenotype by modifying the loci or by transfer by conventional breeding tools. The base change specific markers if developed could help in screening larger germplasm sources for identification of desirable genotype.

ASSOCIATION MAPPING OF ASCORBIC ACID CONTENTS, ANTIOXIDANT ACTIVITY AND SEED WEIGHT IN PEANUT (*ARACHIS HYPOGAEA* L.) MUTANTS

Poonam Bhad, Anand Badigannavar, Suwendu Mondal

NABTD, Bhabha Atomic Research Centre, BARC, India

Email: poonam@barc.gov.in

Cultivated peanut (*Arachis hypogaea* L.) is valued for its edible oil and for direct consumption. It is rich source of nutrients and antioxidants. These traits are polygenic in nature and association mapping is an ideal approach for efficiently exploring the genetic basis for these traits. In present study, 98 peanut genotypes including diverse mutants were used to identify molecular markers associated with ascorbic acid, seed weight and antioxidant activity. For this, 58 polymorphic markers consisting of SSRs and transposable element (TE) markers were employed. The ascorbic acid content and the antioxidant activity [measured as 2, 2Diphenyl-1-picryl hydrazyl (DPPH) reduction assay and ferric reducing antioxidant power (FRAP) assay] were measured spectrophotometrically in all the genotypes in two replicates for two seasons. Seed weight was determined as hundred-kernel weight. The 58 markers amplified a total of 115 loci. The average polymorphic information content (PIC) of the markers was 0.48, ranging from 0.12 to 0.91. Subsequently, these markers were scored in tetraploid fashion to access population structure. The most significant LnP (D) was observed at K=3 in these genotypes. Using mixed linear model by simultaneously accounting for structure and kinship matrix, significant association ($p < 8.3 \times 10^{-3}$) of four markers (TE 129, TE 426, TE 457, TE 121) with ascorbic acid, two

(TE 129 and GM 2032) with DPPH reducing activity, three (TE 129, TE 459 and GM 2032) with FRAP and one (GM 1742) with seed weight was identified. All these markers explained 7.5-18.0% of phenotypic variation (PVE) for each trait. The marker TE 129 was simultaneously associated with ascorbic acid and DPPH and GM 2032 with FRAP and DPPH content. The favourable alleles of these markers can be potentially utilized for marker assisted selection in peanut breeding programs.

IAEA-CN-263-183

ESTABLISHMENT OF CRISPR/CAS9-MEDIATED GENE EDITING APPROACHES IN JATROPHA CURCAS

Fatemeh Maghuly¹, Margit Laimer², Markus Freudhofmaier¹, Wayne Parrott²

¹PBU, DBT, BOKU, Austria

²University of Georgia, USA

Email: margit.laimer@boku.ac.at

Jatropha curcas L. is an undomesticated perennial plant belonging to the Euphorbiaceae family. It is native to America, but is distributed widely in the tropical and subtropical areas. The high production of oil bearing seeds makes it a potential candidate for a sustainable biofuel crop. To achieve a competitive biofuel producing cultivar it is necessary to eliminate existing deficits by improving the genetic background of the plant. The current work aims at establishing the molecular tools to modify the biosynthetic pathways related to commercially important traits by CRISPR/Cas9 knockout constructs. Genes related to fatty acid, protein and toxin biosynthesis, were selected. The gene structures were analysed, the number of isoforms identified and guide RNAs for potential target sites were designed. We successfully introduced the Cas9 gene, the gRNA expression cassettes and the nptII selectable marker to *J. curcas*. In each gene of interest frameshift inducing indel mutations could be detected at the expected positions. *J. curcas* leaf discs were transformed via *Agrobacterium tumefaciens*. Plants will be regenerated from mutant lines and the successful gene knockout validated by phenomic and genomic analyses. This work provides the fundamental knowledge to construct gene knockout constructs for *J. curcas* based on the CRISPR/Cas9 system. The designed gRNAs make it possible to fine-tune the expression of selected genes. Furthermore, the established tool set can lead to a better understanding and improvement of commercially important genes of *J. curcas*.

IAEA-CN-263-218

A NEW VARIETY ZHONGLYU NO.8 FROM SPACE MUTATION INDUCTION

Suhua Wang, Xuzhen Cheng

Institute of Crop Sciences, Chinese Academy of Agricultural Sciences CAAS, China

Email: chengxuzhen@caas.cn

Zhonglyu No8 is a new variety derived from CN36 by using space mutation. CN 36 is a cultivar introduced from Thailand in 1996, and this cultivar is early maturing, has large seeds, but with narrow adaptability. After space mutation, the new variety appears to be early maturing too, with growth period about 70-80 days in Beijing. The plant erects well and somewhat lodging-resistant, with a height of about 60cm. The branches on main stem vary from 2-4, and the number of pod vary from 20-30, sometimes could reach to 50 pods per plant. Most of pods mature within a focus period, making mechanized harvesting available. Mature pods are black with pod length about 10cm and 10-11 seeds per pod. The seeds are bright green with 7.2g for 100-seed-weight. The yield of the new variety is usually about 1650-1725kg per hectare. It can be mono cultivated or intercropped with other crops, especially after the harvest of wheat in north part of China. From the observation in the field, Zhonglyu

No.8 is moderately resistant to leaf spot, powdery mildew and rust, the new variety has higher tolerance to drought, lodging and barren soil than other cultivars.

IAEA-CN-263-219

A NEW VARIETY ZHONGLYU NO.12 FROM SPACE MUTATION

Lixia Wang, Xuzhen Cheng

Institute of Crop Sciences, Chinese Academy of Agricultural Sciences CAAS, China

Email: chengxuzhen@caas.cn

Zhonglyu No12, a new variety derived from Zhonglyu No 2 by using space mutation. Zhonglyu No 2 is an old cultivar originally derived from VC2917A by system selection. Zhonglyu No 2 is high in plant height and easily to be lodging. After space mutation, the new variety appears to be early mature too, with growth period about 70 days in Beijing. The plants erect well and somewhat lodging-resistant, with the height about 50cm. The branches on main stem vary from 2-4, and the number of pod vary from 20-30, sometimes could reach to 40 pods per plant. Most of pods mature within a focus period, making mechanized harvesting available. Mature pods are black with pod length about 8.5cm and about 10 seeds per pod. The seeds are bright green with 6.5g for 100-seedweight. The yield of the new variety is usually about 1500-2250kg per hectare. It can be mono cultivated or intercropped with other crops, especially after the harvest of wheat in north part of China. From the observation in the field, Zhonglyu No.8 is moderately resistant to leaf spot, powdery mildew, and rust, the new variety has higher tolerance to drought, lodging and barren soil than other cultivars too.

IAEA-CN-263-229

IMPROVING RICE LINE Ld 99-12-38 FOR BACTERIAL LEAF BLIGHT RESISTANCE THROUGH MARKER ASSISTED SELECTION (MAS)

Deepika Weerasinghe¹, Akila Amarathunga¹, Darsha Withanawasam¹, Dimanthi Jayatilake², Kapikasiri Udawela³, Mihirini Gunasinghe², Pradeepa De Silva²

¹*Regional Rice Research and Development Center, Sri Lanka*

²*University of Peradeniya, Faculty of Agriculture, Sri Lanka*

³*Rice Research Institute, Sri Lanka*

Email: deepika.weerasinghe@yahoo.com

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is an emerging issue in rice cultivation. There are no chemicals used effectively as a control method in the world. Use of resistant varieties is the most appropriate mode of control. Dominant genes Xa21 and Xa4 are known to convey durable resistance against BLB. The advanced rice line, Ld 99-12-38 is a farmer demanding, high-yielding rice line, and is susceptible to BLB. In the current study, an attempt was made to improve its resistance to BLB through marker-assisted breeding (MAB). Through MAB of Ld 99-12-38/IRBB 60 the resistance alleles coming from the IRBB 60 were introgressed into the Ld 99-12-38 and progeny lines were advanced up to BC3F6. Two linked-markers, pTA248 (Xa21) and PM1+MP2 (Xa4) were assayed on the thirty-five BC3F2 progeny to evaluate resistance allele diversity of Xa21 and Xa4. Selected four progenies in BC3F6 were evaluated in yield trial. Out of the tested 35 progeny lines, eight were confirmed phenotypically as resistant for both Xa21 and Xa4, they carried the resistance alleles, twenty-one carried only the resistant allele at Xa4, however, they were phenotypically categorized as resistant/moderately resistant. A paired t-test revealed that there is a significant difference in the resistance level exerted by progeny carrying resistance alleles of two rather than with one ($p = 0.0064$). Selected four lines with Xa 21 and Xa 4 performed well with significant yield advance compared to

Ld 99-12-38. Therefore, it can be concluded that resistant alleles of Xa21 and Xa4 have been successfully introgressed into rice lines derived from a cross between Ld 99-12-38/IRBB 60 with desirable morphological characters of Ld 99-12-38 to withstand BLB.

IAEA-CN-263-242

INITIAL SURVEY OF MUTANTS ASSOCIATED WITH BACTERIAL LEAF BLIGHT RESISTANT GENES IN RICE BASED ON LINKED MARKERS

Hong Nguyen Thi, Tuyen Vo Thi Minh
Institute of Agricultural Genetics, Viet Nam
Email: lhham@agi.ac.vn

In Viet Nam, bacterial leaf blight is one of the most destructive diseases in rice. A set of 138 mutant rice lines obtained from the original variety by ion beam irradiation was surveyed for mutations associated with Xa4, Xa7 and Xa21 genes based on linked markers MP1-2, P3 and pTA248. When checking PCR products of materials with these markers, the original variety presented the positive control bands at detail size: 170 bp (by MP1-2); 300 bp (P3) and 1000 bp (pTA48). In the set of 138 mutant rice lines, results of PCR by marker MP1-2 showed that there were 95/138 lines (68.8%) at positive control band (170 bp, the same with the original type) and 43/138 lines (31.2%) at negative control band (150 bp, different from the original type). By marker P3 linked with Xa7 gene, results showed 80/138 lines (42%) at positive control band (300 bp) and 58/138 lines (42%) at negative control band (250 bp). By marker pTA248 marker linked with Xa21 gene, there were 60/138 lines (43.5%) at positive control band (1000 bp) and 78/138 lines (42%) at negative control band (800 bp). Ratios of mutants associated with bacterial leaf blight resistant genes in the initial survey based on linked markers are: 31,2% mutants associated with Xa4 based on MP1-2; 42,0% mutants associated with Xa7 based on P3) and 56,5% mutants associated with Xa21 based on pTA248. It is indicated that there were different effects of ion beam on different gene regions. In other side, this result is also helpful to select the mutant rice line remaining the resistant genes for breeding program.

IAEA-CN-263-265

MOLECULAR AND GENETIC ASPECTS OF THE RESISTANCE OF LEGUMINOUS PLANTS TO BIOTIC STRESS FACTORS

Liuba Coretchi¹, Dinu Cliciuc², Ecaterina Bondarenco²
¹*National Centre of Public Health, Academy of Sciencis of Moldova*
²*Institute of Genetics, Plant Physiology and Protection*
Email: coretchiliuba@gmail.com

Biotic stresses are a serious threat to global food security. The origin of new pathogens/insect races due to climatic and/or genetic factors is a major challenge for plant breeders in breeding biotic stress resistant crops. Molecular genetics approaches like mutation, marker assisted selection (MAS), genomics, recombinant DNA technology, targeted induced local lesions in genome (TILLING), and virus induced gene silencing (VIGS) were adapted by breeders to develop effective resistance in crop plants in a shorter time. Significant progress can already be seen in the development of genomic resources for model species of Medicago, Lotus, major legumes (soybean, common bean, peanut), and minor legume crops (cowpea, chickpea, pigeon pea). The objectives of this research were to evaluate soybean and chickpea mutant lines, obtained by gamma-irradiation at the Institute of Genetics, Plant Physiology and Protection, through simple sequence repeat (SSR) molecular-genetic and immunological analysis. SSR analysis has been provided with the use of 22 SSR primers. Development of dendrograms based on the immunological and molecular genetics analyses of each particular line and determination of genetic

features of studied genotypes have been done. The production indices of mutants were assessed in vivo. The gene effects involved in controlling soybean genotypes interactions with fungal/bacterial pathogens, and polymorphism of SSR markers for soybean and chickpea have been established. A high degree of similarity has been found for soybean, chickpea plant response to *Fusarium oxysporum* Schlecht. emnd Snyd., and SSR markers polymorphism from different linkage groups, which indicates a polygenic control of the reaction and the opportunity to use molecular markers to identify resistant genotypes. SSR molecular markers which show high polymorphism of soybean and chickpea genotypes have been identified. The results show a future perspective for utilization of SSR analysis in the national soybean and chickpea breeding programme.

IAEA-CN-263-286

FRAGMENTATION OF POOLED PCR PRODUCTS FOR DEEP AMPLICON SEQUENCING

Andrea Tramontano¹, Luka Jarc¹, **Joanna Jankowicz-Cieslak**¹, Bernhard J. Hofinger¹, Katarzyna Gajek², Miriam Szurman-Zubrzycka², Iwona Szarejko², Ivan Ingelbrecht¹, Bradley J. Till¹
¹*Plant Breeding and Genetics Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*
²*Department of Genetics, Faculty of Biology and Environmental Protection, University of Silesia, Poland*
Email: i.ingelbrecht@iaea.org, till.brad@gmail.com

Improvements to massively parallel sequencing have allowed the routine recovery of natural and induced variations in genomic DNA sequence. Thus, such approaches can be considered for routine application in plant mutation breeding projects. The need for high sequence coverage to accurately recover single nucleotide variants and small insertions and deletions limits the applicability of whole genome approaches. This is especially true for the many important crops with a large genome size or for applications requiring the screening of thousands of individuals, such as the reverse-genetic technique known as TILLING. Using PCR to target and sequence chosen genomic regions provides an attractive alternative as the vast reduction in interrogated bases means that sample size can be dramatically increased while maintaining suitable coverage for recovery of small mutations. Direct sequencing of PCR products is limited, however, due to limitations in read lengths of many next generation sequencers. Here we show the use of ultrasonication for the simultaneous fragmentation of 32 PCR products produced from large pools of barley mutant lines. Analysis of these fragmented PCR products using Illumina 2x300PE sequencing showed consistently high coverage and quality across the amplicons suitable for single nucleotide variant (SNV) calling.

IAEA-CN-263-287

INDUCED MUTAGENESIS FOR GENERATING BANANAS RESISTANT TO FUSARIUM WILT TR4

Joanna Jankowicz-Ciesla¹, **Florian Goessnitzer**¹, Chih-Ping Chao², Shih-Hung Huang², Sneha Datta¹, Ivan Ingelbrecht¹, Bradley J. Till¹
¹*Plant Breeding and Genetics Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*
²*Taiwan Banana Research Institute, Taiwan*
Email: i.ingelbrecht@iaea.org, till.brad@gmail.com

Cavendish bananas are sterile, parthenocarpic and an obligate vegetatively propagated crop. This makes them particularly susceptible to diseases including Fusarium wilt caused by *Fusarium oxysporum* f. sp.

cubense (Foc) tropical race four (Foc TR4). Foc TR4 has been identified in nine countries and its recent spread is threatening global banana production. Previous experiments showed that when using the chemical mutagen EMS, mutations can be induced in the banana genome at a high density, and chimeric sectors rapidly dissolved. Further, induced point mutations were shown to be stably inherited in sibling lines through multiple generations. We combined this approach with a previously developed protocol for gamma treatment of meristematic tissue to increase the genetic diversity in Cavendish bananas. A large mutant population of the cultivar Grande Naine was produced. Following treatment, plantlets were allowed to recover and transferred to the greenhouse. Plants were screened for their response when challenged with fungal pathogen. To date, 19 banana plants showing no symptoms of pathogen attack have been recovered. Four percent of material treated with EMS showed no disease symptoms, whereby in gamma treated material, 18% was observed for 30Gy treated material and 17% for 40Gy. Plantlets were next re-established as *in vitro* cultures for multiplication and multilocational distribution. A subset of treated material was also used to develop a low-coverage whole genome copy number variation analysis for fast and efficient recovery of large induced mutations. Material showing no disease symptoms will be further analysed with the use of the established whole genome sequencing technique.

IAEA-CN-263-288

MAPPING THE LANDSCAPE OF GAMMA- AND X-RAY INDUCED MUTATIONS IN RICE

Joanna Jankowicz-Cieslak¹, Bernhard J. Hofinger¹, Luka Jarc¹, Sini Junntila², Attila Gyenesei², Ivan Ingelbrecht¹, Bradley J. Till¹

¹*Plant Breeding and Genetics Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

²*Bioinformatics & Scientific Computing, BioComp, Austria*

Email: till.brad@gmail.com

Crop monocultures and the disappearance of small-farms is having a negative impact on the genetic diversity used in global agriculture. Enhancing agrobiodiversity plays a key role in adapting to a changing climate and feeding future generations. Expanding genetic diversity through induced mutagenesis is highly successful with thousands of improved varieties released worldwide. Advanced molecular techniques such as massively parallel sequencing opens new opportunities to accelerate crop mutation breeding. While X-ray irradiation was the first mutagen employed to induce novel genetic variation in the 1920s, treatment with gamma-rays had become the predominant physical mutagen for crop mutation breeding by the middle of the 20th century. To explore why physical mutagens have been so effective in breeding we investigated the landscape of mutations induced by gamma and X-rays in a rice landrace from Madagascar, Marotia. Eleven mutant lines advanced via self-fertilization for 7 generations were sequenced using a HiSeq Illumina platform. We have identified a broad spectrum of mutations including SNPs, small indels, and larger structural variants. Overall, thousands of putatively induced SNP's and indels were identified across all mutant lines. Over 80% of these mutations were located within intergenic regions while in case of SNPs and indels, only 0.1% and 2% are predicted to impair the function of genes, respectively. Interestingly over 90% of the large deletions (>150 bp) mapped to transposons within the reference genome. Approximately 40% of randomly selected putative SNPs, indels and large structural variants could be validated as radiation-induced mutations using standard PCR and Sanger sequencing. Overall, our study shows that (1) X-ray and gamma rays induce similar spectra and frequency of mutations, predominately SNPs and small indels; and (2) points towards an important role for transposons in radiation-induced mutagenesis.

WHOLE GENOME SEQUENCING OF ADVANCED MUTANT LINES OF HEAT TOLERANT TOMATO INDUCED BY GAMMA IRRADIATION

Joanna Jankowicz-Cieslak¹, Binita Saraye¹, Sini Junttila², Attila Gyenesei², Ivan Ingelbrecht¹,
Bradley J. Till¹

¹*Plant Breeding and Genetics Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

²*Bioinformatics & Scientific Computing, BioComp, Austria*

Email: j.jankowicz@iaea.org

Tomato (*Solanum lycopersicum* L.) is an important economic crop worldwide. In Mauritius, three types of tomatoes are grown: cooking, salad and cherry tomatoes. The salad and cherry tomatoes are grown under hydroponic systems whereas the most widely consumed tomato, the cooking type, is cultivated in open fields and thus is subject to adverse climatic conditions. Tomato with increased tolerance to abiotic stresses, especially heat, is essential for the small island economy. To address this, a mutant population of cooking tomato was developed using gamma irradiation. Two advanced mutant lines were identified exhibiting increased tolerance to heat stress. These mutant lines also showed an early flowering phenotype, with different fruit shape and plant architecture compared to the parental line. Whole genome (re)sequencing was applied at the M₇ stage to identify sequence variations between the two mutant lines and their parent. Our data shows differences in the frequency and spectrum of sequence variants in the two mutant lines. In L25P10, 175,116 unique SNPs were detected, whereas in L5P12, 49,690 SNPs could be detected. Likewise, differences were observed in the case of small indel with 42,435 and 12,370 detected in L25P10 and L5P12 respectively. Variants can produce effects of different types and affecting different regions of the genome. Less than 1% of SNPs and indels identified in both mutants are predicted with high impact of the effect. Similar differences were noted in the number of Structural Variants identified in both mutants with L25P10 showing twice more Deletions, Inversions and Duplications. We are currently conducting in silico analysis to identify candidate causative mutations for the observed traits.

DEVELOPMENT OF A FUNCTIONAL MARKER FOR MARKER-ASSISTED SELECTION OF 'ORANGE LEMMA' MUTANTS TO IMPROVE FEED QUALITY IN BARLEY

Ivan Ingelbrecht¹, Bernhard Hofinger¹, Eda Akgun¹, Mirta Matijevic¹, Adel Ali¹, Joanna Jankowicz-Cieslak¹, Luka Jarc¹, Ljupcho Jankuloski¹, Abdelbagi Mukhtar Ali Ghanim¹, Heinrich Grausgruber²

¹*International Atomic Energy Agency, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria*

²*BOKU, Dept. Crop Sciences, University of Natural Resources and Life Sciences, Austria*

Email: l.jankuloski@iaea.org, i.ingelbrecht@iaea.org, a.mukhtar-ali-ghanim@iaea.org

Barley is a major cereal crop for feed consumed by a wide range of animals including cows, pigs and poultry as green forage, hay, silage or seed. Multiple mutations exist in barley that can be exploited to develop specific forage barley varieties. Barley 'orange lemma' (rob1) mutants show similar phenotypic characteristics compared to mutants in maize or even trees such as poplar that have reduced lignin content. A range of barley 'orange lemma' cultivars exist either as natural variants or induced using chemical and physical mutagenesis techniques. Most genes in the lignin biosynthesis pathway have been well characterized opening perspectives to develop a functional marker for orange lemma using a candidate gene approach. Here we describe the molecular and genetic characterization of orange lemma in different spring barley accessions. Candidate causative mutations underlying the orange lemma phenotype were identified. An allele-specific assay was developed for rob1 in the Bowman genetic

background. Genetic and phenotypic analysis of the F1 derived from the rob1 mutant and its parental line showed that orange lemma is a monofactorial recessive trait. Using a segregating F2 population, trait and marker linkage could be demonstrated. Experiments have been initiated to validate the marker assay in additional barley genetic backgrounds to enable marker-assisted backcrossing of rob1 for feed barley improvement.

IAEA-CN-263-299

CREATING DESIRED TRAITS FOR AFRICAN SORGHUM, SEMI-DWARF AND EARLY MATURING, AND MOLECULAR CHARACTERISATION OF THEIR GENETIC ARCHITECTURE

Norman Warthmann, Abdelbagi Mukhtar Ali Ghanim, Adel Ali, Mirta Matijevic, Joanna Jankowicz-Cieslak, Ivan Ingelbrecht

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

Email: a.mukhtar-ali-ghanim@iaea.org, i.ingelbrecht@iaea.org, n.warthmann@iaea.org

Wad Ahmed is a Sorghum variety popular among farmers in Sudan, except that it matures slightly late and is (too) tall making it prone to yield losses caused by terminal drought and lodging. Mutagenesis and breeding work was undertaken to obtain early-maturing and semi-dwarf mutants and we are developing molecular markers to facilitate use of these new traits in breeding programs. Seed bags of Wad Ahmed were subjected to different doses of gamma radiation (200, 300, 400Gy). M₂ seeds were planted 'head-to-row', i.e., one row per M₁-head. From 500 rows, six promising mutants (D1-D6) were picked and progressed to the M₆ stage by single seed descent. For marker development we created segregating populations to genetically map the causal variant(s): M₆-plants for each mutant were backcrossed to Wad Ahmed (M6BC1). The resulting F1 generations (M6BC1F1) were allowed to self-pollinate and thus F2 populations (M6BC1F2) were developed. Phenotyping of two of the F2 populations for flowering time and final plant height shows that the traits are segregating and accessible to genetic mapping. For genome wide identification of candidate loci we produced 2nd-generation DNA sequencing data (Illumina) for all six mutagenized lines (D1-D6, M6) and wild-type Wad Ahmed Sorghum plants. The sequencing reads were aligned to the available Sorghum reference genome and searched for distinct variants between the mutants and wild-type Wad Ahmed. Across the six mutants we called several thousand candidate variants (SNPs and small IN/DELS). They are scattered across the genome. We also identified regions of statistically significant differences in read coverage, indicative of large deletions. Genetic mapping with the segregating F2 populations will determine which of the candidate mutations are causal for the observed phenotypes. We will then convert those into PCR-based molecular markers to facilitate Marker Assisted Selection (MAS).

Note: Both first authors contributed equally to the work presented.