

INDUCED MUTATIONS FOR IMPROVEMENT OF GRAIN LEGUME PRODUCTION

REPORT OF A RESEARCH CO-ORDINATION MEETING
ON THE USE OF INDUCED MUTATIONS
FOR IMPROVEMENT OF GRAIN LEGUME PRODUCTION
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF ATOMIC ENERGY
IN FOOD AND AGRICULTURE
HELD IN BANGI, KUALA LUMPUR, MALAYSIA
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FOREWORD

This book contains the proceedings as well as conclusions and recommendations of a research coordination meeting where participants in the FAO/IAEA/SIDA Coordinated Research Programme on Induced Mutations for Disease Resistance in Grain Legumes and in the FAO/IAEA-RCA Coordinated Research Programme on the Use of Induced Mutations for Improvement of Grain Legume Production in South East Asia discussed jointly problems of legume improvement and approaches to solve them with the help of mutation induction.

The meeting was held in Malaysia, hosted by the Universiti Kebangsaan at Bangi, Selangor near Kuala Lumpur. The support in its organization by the Malaysian Ministry of Science, Technology and the Environment and the Universiti Kebangsaan Malaysia is gratefully acknowledged. The assistance by the staff of the Faculty of Science has been essential for the meeting. The cooperation of the Malaysian Agricultural Research and Development Institute (MARDI), the Rubber Research Institute (RRIM) and the Universiti Pertanian Malaysia, in arranging visits to their experimental fields and facilities was much appreciated.

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WELCOMING ADDRESS

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Welcoming Address

by

Y.B. TAN SRI DR. ANUWAR BIN MAHMUD

Vice Chancellor,

Universiti Kebangsaan Malaysia

On behalf of the Universiti Kebangsaan Malaysia it gives me great pleasure to welcome The Honourable Tan Sri Ong Kee Hui, Minister of Science, Technology and Environment Malaysia, our honoured guests and participants.

This University has been given the honour in the past to host workshops and training courses organised jointly by the Ministry of Science, Technology and Environment and the International Atomic Energy Agency (IAEA). It indicates the confidence by the Ministry of IAEA on the ability and suitability of the Faculty of Science to host workshops, training programmes and meeting such as this one.

At the moment, there is in progress a joint research project between the IAEA, local educational and research institutions together with The Universiti Kebangsaan on mutation breeding of soybean. This project is being given the support it needs by the Research Committee of this University because we strongly believe that through this method of breeding it would be possible to produce suitable and high yielding varieties of soybean for the farmers of this country. The success of the project will no doubt assist to a certain extent in our agricultural development programmes aimed at the diversification of crops and also in our desire to produce locally as much as possible of this grain legume which hitherto has to be imported. I am quite sure this meeting and the useful discussions that would emanate from it will further enhance our knowledge in mutation breeding.

In the overall planning of the Faculty of Science of this University the nuclear science building will form an important part of the overall development of the science complex. It will have its own unique structure the plan of which is being given due consideration and in the various stages of preparation. The close proximity of Tun Idmail Atomic Research Centre (PUSPATI) to this campus could stimulate the nuclear science course and research conducted by this University. I have no doubt that both PUSPATI and UKM will be able to cooperate closely not only for the benefit of both agencies but also for joint research activities and the application of results towards higher agricultural production as well as for effective processing and storage of crops.

Ladies and gentlemen, this University is still in the midst of its physical development and it will take a few years more before we could really settle down to beautifying our landscape. This during your stay here you would certainly be exposed to a few inconveniences most of which are beyond our control. Nevertheless, the University will try its best to make your stay here comfortable, beneficial and a memorable one.

I take this opportunity to wish you all the very best and hope your meeting during the next few days will be able to achieve the expected results.

Thank you.

Introductory remarks

A. MICKE

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Vienna, Austria

In 1975, FAO and IAEA convened a meeting of South-East Asian grain legume breeders at Colombo (Sri Lanka), to discuss the need and scope for genetic improvement of grain legumes, to define aims and objectives, to review problems encountered with regard to available germ plasm, and to evaluate feasibility as well as prospects of various approaches and methods.

In this context, particular attention was paid to the potential of mutation induction as a method to solve some of the problems and contribute to improved grain legume cultivars.

There exists a document (No. 203, 1977) where all these problems are nicely written on paper and where conclusions and recommendations can be found as guide lines for further action. Following these recommendations we have tried to stimulate research in this field by concluding research contracts with a number of institutions. In spite of financial constraints we have after all succeeded in establishing so many research contracts that we could consider to hold a Research Coordination Meeting for implementing cooperation and exchange of experiences. We were very grateful to receive an offer from the Malaysian Government to host such a meeting here at Kuala Lumpur.

The meeting has two aspects: One is of a "regional nature". Soon after the mentioned meeting in Sri Lanka a Regional Cooperative Agreement for the peaceful application of nuclear techniques was established among Member States of IAEA in South-East Asia (RCA), and the genetic improvement of grain legumes, recommended by the Member States' experts in Sri Lanka was adopted as subject of common interest to countries in South-East Asia. Funds were set aside within the IAEA Regular Budget for the RCA and about 25% of those were approved to be used to support research projects via IAEA Research Contracts. The amount of financial support that can be given to each contract is rather small, but it has to come out of the Regular Budget which is inelastic and suffers from cost increases that are not fully compensated for by Member States' financial contributions.

A request submitted to UNDP in 1975 unfortunately has not yet been decided upon mainly due to the fact that other organizations and groups have likewise identified the importance of pulses for the diet of the population in South-East Asia and wanted to assist in improving production. So researchers in South-East Asia may have to be satisfied with little support for a longer time, just as Asian people may have to be satisfied with insufficient supply of nutritious pulses.

The other aspects of the meeting is of global nature and concerns specifically the improvement of disease resistance of grain legumes. The Swedish Government has expressed interest in this subject and generously provides financial support through SIDA outside of the Agency's Regular Budget. We felt that it could only be of mutual benefit, if the two groups of research contract holders would meet together to look critically at objectives and methodology.

It is the purpose of this meeting to improve the prospects of success in each individual project. To discuss his project with fellow scientists of wide expertise and often diverging experiences should be a challenging opportunity for each project leader.

Much has been said and written, particularly during the last decade, about the usefulness of induced mutations for plant breeding. Following about 30 years of mostly academic research on genetic effects of radiation and radiomimetic chemicals, since 1960 a greater number of crop plant cultivars were approved and recommended for farmer's use which derived improved characters from induced mutations, thus proving the value of mutagenesis not only for understanding heredity and heritable structures, but also for crop production. Today, we know about

ca. 200 such cultivars released in 30 different countries for agricultural or horticultural commercial use and an equal number of ornamental plant varieties. It is, therefore, not anymore necessary to prove the potential of mutation induction for crop improvement, but rather to realize that potential in the most efficient and economic way.

Mutant varieties of rice and barley reached farmer's fields between 4 and 14 years after the mutagenic treatment, when no further cross breeding was involved. The time required does not need to be longer than 5-6 years if proper selection methods are applied to large enough populations resulting from an effective mutagen treatment. Such a short time would be a definite advantage of mutation breeding compared with other breeding methods. However, often the time till a variety reaches the farmer is unnecessarily extended by governmental authorities who jealously defend their terms of reference. I refer to administrative conflicts between atomic energy and agricultural authorities. These administrative conflicts should be avoided and can be avoided by e.g., early information and cooperation and I would like to urge all of the cooperators in our legume improvement programmes to make every effort to establish such relationships.

Out of the mentioned 200 varieties that we know about as approved and/or recommended by governmental authorities, 26 concern pulses and 4 forage legumes. This is a relatively high proportion considering the fact that much less work has been done with leguminous species than e.g., with barley and rice.

A sharply increasing number of varieties is being released in recent years, in which mutants have been involved as cross breeding partners. This may cause us to remember that mutation induction primarily is not a breeding method but a way to provide additional germ plasm for plant breeding. Till now we observe, however, that mutants are used in cross breeding mainly after they have had a chance to prove their agronomic value as released cultivars. (e.g., of the 31 varieties developed by cross breeding with mutants, 26 derived from crosses with 4 successful parent mutants varieties. This may be explained by a certain scepticism, plant breeders may have against mutants that have not proven their value regarding productivity and adaptation. Mutants may be more often used in cross breeding if they derive from productive cultivars and are made available as an improved line (after crossing) rather than as the raw mutant. I would strongly suggest to all of you who start mutant selection projects, to envisage also a cross breeding programme with the mutants selected.

I feel certain that all participants and observers are fully aware of the purpose of the meeting as being a kind of workshop. We expect detailed and precise presentations and I hope for frank and vivid discussions. Please never mind the criticisms that may have to be expressed here and there as being negative. We all together have an important objective to improve pulse production, its quantity, quality and stability. We want to achieve these objectives in a relatively short time for the benefit of the people, not for our own sake. The donors want their money to be used properly and these donors in our case are not some rich foundations, who give from their surplus but the tax-payers in IAEA Member States.

It remains for me as a pleasure to thank once more the Malaysian Government on behalf of the sponsoring Organizations, FAO, IAEA and SIDA for hosting the meeting and contributing generously to its success. I wish our meeting to be a full success.

PLANT SCIENCE RESEARCH IN MALAYSIA WITH RESPECT TO BREEDING OF CROPS

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Abstract

The status of plant breeding research in Malaysia is reviewed. The lack of breeding efforts in food crops is discussed in the context of the Malaysian experience whereby rubber and oil palm continue to assume a dominant role in the national economy. A discussion of the breeding work and priorities in rice, maize, fruit trees, vegetables, beverages, spices and grain legumes is provided. Conventional plant breeding techniques are widely used but some avenues in mutation breeding are suggested.

Introduction

Malaysia is known as a major producer of natural rubber (*Hevea brasiliensis*) and oil palm (*Elaeis guineensis*). This is brought about by the colonial government which saw the potential of large-scale planting of 'cash crops'. After independence, rubber and oil palm continue to be Malaysia's top foreign exchange earners (Table 1).

The production of crops for human and animal feed to meet local demands has been neglected and Malaysia today still is a net importer of food. In 1975, she had to import approximately 13.0% of her rice requirement, a major portion of her sugar, about 95.0% of her milk and milk products and almost all of her needs in animal feed although she is able to produce 85.0% of her beef, almost all of her fresh vegetables and she is self-sufficient in pork and poultry.

In order to understand clearly the extent to which food and feed production affect breeding programmes or other researches in plant science in Malaysia one has to know the expected production figure of the essential commodities (Table 2). A number of these crops are not indigenous to Malaysia but we have somehow established a record in our ability to introduce non-indigenous species of economic importance and develop their cultivation on a large scale.

The country now has highly trained scientific personnel with interest in food production located at universities and research institutions. A substantial amount of research has been done in these institutions and efforts have been made to have scientists of different institutions working together in clearly defined vital programmes such as the recently started "Joint Malaysian Soybean Project".

Let us now look at some aspects of plant science, particularly genetics and breeding researches in Malaysia. It is intended to divide the crops into two main groups:

- a) Export oriented crops
rubber, oil palm, coconut, cocoa,
pepper, pineapple.
- b) Food (or feed) crops
rice, maize, grain legumes, fruits, vegetables,
spices (e.g., pepper and cloves), beverages (coffee and tea)

Breeding Activities

A. Rubber

Being the world largest producer of natural rubber, Malaysia has been actively engaged in breeding and selection of the rubber tree for the past 50 years except for two brief interruptions caused by the Economic Depression (1933 - 1936) and the Second World War (1940 - 1945). Yield improvement began with the selection of mother trees. This was intensified with the development of 'artificial pollination'. Vegetative propagation by budding provided a major step forward in that elite cultivars can be multiplied as stable homogenous clones. The use of high yielding materials has contributed to the marked rise in production (Mohd Noor, 1976).

The objectives of the breeding and selection programme at the Rubber Research Institute of Malaysia (RRIM) are to develop clones that

- are high yielding and responsive to stimulation and low intensity tapping system,
- are vigorous and fast growing with reduced immaturity period,
- are wind tolerant through good branching habit and growth during tapping,
- have disease-free leaves and stems,
- have thick virgin bark as well as good bark renewal,
- are widely adaptable.

Hevea breeding so far consisted of alternating cycles of generative and clonal selection. Selection and budgrafting of the seedling progenies from the generative cycle provide the next generation of clones. The various stages of the procedure are outlined in Fig. 1. Table 3 lists the clones produced between 1928 - 1973.

Recently, other approaches have been tried to bring more rapid results. Among them were germplasm collection in Brazil, the center of origin/diversity of Hevea, in order to widen the genetic base since the modern Hevea brasiliensis cultivars in the Far East are descendants from only 22 seedlings originally introduced to this country (Ho, 1979). Mutation induction, polyploidy and tissue culture research are currently being carried out. Mutation work aims at creating dumpy trees and conferring disease resistance to high yielding clones. Work on polyploidy hopes to increase latex vessel diameter and thereby improving yield. Tissue culture may be able to help in work concerning mutation, polyploidy and heterosis (Mohd Noor, 1976).

Rubber being a perennial crop, its breeding and selection is a time consuming and expensive process. Research is underway to find reliable methods for predicting the performance of clones at the nursery stage. This method would help in economizing and shortening the breeding cycle. Other work is concerned with

- selection and cloning of mother trees from improved seedlings,
- crown budding to produce superior trees,
- biometrical and cytogenetical investigations,
- floral induction and protection,
- breeding for South American Leaf Blight resistance.

After about 50 years of breeding work, the yield has been raised from 600 kg/ha to 2000 kg/ha (Ong, 1976).

B. Oil Palm

Oil palm was introduced into this country during the early part of this century, and since has become a major crop industry. Research on oil palm initially done by the Department of Agriculture, is now taken over by MARDI. Recently the Government set up the Palm Oil Research

Institute of Malaysia (PORIM) to conduct research on all aspects of the crop. Private companies have also been conducting research. The two main economic products are palm oil and palm kernel oil. Both products are derived from the fruit and are considered simultaneously in breeding programmes.

The breeding objectives (Ooi et al., 1976) for oil palm are:

- improvement of oil yield
- improvement of oil characteristics
- reduction of palm height
- development of resistance to diseases.

The objectives will change with changes in technology related to use of the product or in field agronomic practices. A higher level of unsaturated fatty acids in the oil may become an important objective in future programmes.

Most breeding programmes aim at the production of "Tenera" type material which produces 20-50% more oil per bunch than its parents.

Intergeneric crossing has been used in breeding for higher levels of unsaturated fatty acids, namely between Elaeis guineensis and Corozo oleifera or melanococca. The hybrids have intermediate levels of unsaturated fatty acids but the oil per bunch is still low, between 18-20% as compared to good Tenera materials with a value of 23-25%. Reduction in stem height and disease resistance are two other desired properties in these hybrids, besides having a fruit bunch like E. guineensis.

The existing breeding materials derived from a very small number of individuals. As a result existing materials have very low heritable variation (Ooi et al., 1976). Germplasm collections have been made in the natural oil palm grove in Nigeria (Arasu and Rajanaidu, 1975), to broaden the genetic variability of existing material.

Rice

Rice breeding in Malaysia started in the beginning of the 20th century (Van, 1976). There are two types of activities: (a) maintenance of basic materials for breeding and multiplication of breeder seeds for extension to farmers and (b) research projects for varietal improvement (MARDI, 1977). The objectives of the varietal improvement programme are as follows:

- i) yield improvement
- ii) disease/pest resistance
- iii) improvement of grain quality
- iv) early maturing varieties
- v) varieties for semi-deep water conditions
- vi) varieties for acid-sulphate soils
- vii) drought tolerance
- viii) varieties for mechanised farming.

The first rice variety, released in 1915, was Seraup Kecil 36. Varieties such as Mayang Ebos 80, Radin China 4, Nachin 11, Siam 29, Subang Intan 16, Saraup Kechil 48 were later released from various stations. The varieties were developed by pure-line selection. Hybridization was attempted as early as 1927 and a number of varieties were released between 1927-1950. After 1950, emphasis was given to development of varieties for double cropping. Indica-japonica hybridization as was carried out. In 1964, variety Malinja was released, and Mahsuri a year later. Six more rice varieties and two pulut (glutinous rice) varieties with yield ranging from 3920 to 6496 kg/ha were released for double cropping (Table 4).

Maize

Demand for maize grain is high as Malaysia produces less than 20% of her requirement. Local Flint and Metro are popular varieties, but are low yielders. Modified mass-selection (Wong, 1973, 1976), recurrent selection (Lee and Yap, 1974) and half-sib and full-sib breeding (Yap and Chiow, 1974) have been used to improve these varieties.

Two starchy maize varieties were released by MARDI and RRIM namely MARDI Composite I and RRIM Hybrids (Wong, 1976; Chee, 1976). Two sweet corn varieties for fresh consumption were also released. One called Chinta was released from University of Malaya by Graham and Yap (1972). The other variety, named Bakti was released at the beginning of this year by Abdul Halim and Yap (1979). All these varieties are high yielders, ranging from 2500 - 3200 kg/ha.

Vegetables

There has been little work on breeding and improvement of vegetable crops until recently. Mass or pure-line selection methods are used to improve local and imported strains of popular vegetables (Yap, 1977). Hybridization programmes have started and it has been shown that hybrid vigour could be exploited in some of these crops (Jalani and Graham, 1973).

More systematic breeding programmes have been carried in some vegetable crops such as long bean (Vigna sesquipedalis Fruw.), chilli (Capsicum spp), tomato and cabbage (Miller, 1976; Zainal Abidin, 1976; Graham et al, 1977; Soh et al, 1977; Yap et al, 1977). Recently, three wilt-tolerant tomato lines named FP₁, FP₂ and FP₅, yielding 56.9, 43.9 and 48.5 t/ha respectively, have been released for lowland cultivation. (Graham et al, 1977). Tissue culture studies have indicated that this technique could be exploited in the improvement of vegetable crops (Devreux, 1973; Zakri, 1979).

Grain Legumes

Malaysia imports almost all her grain legume (pulses) needs and not much breeding work has been done in the past. Recently, however, systematic breeding programmes have started for the improvement of groundnut (Arachis hypogea L.), soybean (Glycine max (L) Merrill), winged bean (Psophocarpus tetragonolobus L) and mungbean. Although groundnut is widely grown in Malaysia, it is yet to receive full attention from the breeders. Most of the work done consists of varietal evaluation and mass or pureline selection.

Soybean has received a great deal of attention recently and a Joint Malaysia Soybean Breeding Project has been set up by four institutions, namely MARDI, RRIM, UKM and UM, with the aim of developing and releasing within five years varieties which can compete in price and quality with imported soybeans. Soybean could be grown as an intercrop in plantations as well as in rotation with rice and other crops. Studies by Chan (1970), Chee (1975), Yap and Lee (1975), Abu Kassim (1976), West (1976) and Funnah and Mak (1979) have shown a yield range from 345 kg/ha to 3820 kg/ha. Another grain legume which receives attention is winged bean. Germplasm collections as well as breeding and genetics studies have started (Jalani and Wong, 1978).

Fruit Crops

The fruit breeding programmes in Malaysia are still at the initial stage (Yap 1977a) and most of them are confined to selection of desirable types from local fruit orchards or from imported cultivars. Among them are durians (Durio zibethinus Merr.) rambutan (Nephelium laopaceum L.) and mango (Mangifera indica L.), (Arasu, 1976). Hybridization programmes have been established in papaya (Chan, 1970) and pineapple (Lee, 1976).

Beverages & Spices

Not much breeding has been done on beverage and spice crops. The most notable beverages are coffee, tea and cocoa. Among the three crops, cocoa

has become a major export crop. Breeding and evaluation has been carried out, initially by the Department of Agriculture and now by MARDI (Ibrahim and Arasu, 1976). With regard to spices, Malaysia is the world's largest producer of pepper (Piper nigrum L.). On other spices like clove, nutmeg and ginger very little breeding has been done.

Mutation Breeding

Breeding work has been concentrated mainly towards export oriented crops, such as rubber and oil palm, and the staple food in Asia, rice. Other crops such as maize, grain legumes, vegetables, fruit trees, beverages and spices are now being given attention at some institutions. The released varieties or clones of these crops have been the result of classical breeding methods, mutation breeding has only recently been explored. The history of mutation breeding on food crops and rubber is shown in Table 5. The previous lack of interest in mutation breeding is understandable. For instance, the significant achievements made in rice breeding by the International Rice Research Institute (IRRI) have been reached without resorting to induced mutations. However, there are a good number of mutants which have been released as improved varieties in many important crop plants (Sigurbjörnsson and Micke, 1974). Perhaps many plant breeders were unaware of these advances.

The use of mutation breeding should be considered as an adjunct to conventional plant breeding. In some situations the technique may be a way out of a problem. The gene complexes that have been built up during evolution in a particular species are the result of spontaneous mutations and adaptation to a certain environment. Traits retained are those that have a high survival value. These traits need not necessarily be consonant with the characters that modern man is interested in, such as a high amino acid content, grain stickiness, palatable taste, or good cooking quality. Consequently these genes may not be found in the natural gene pool. This is where mutation breeding can contribute in filling up gaps. In addition, induced mutations were useful for pests and disease resistance, shorter growing period, stiff straw in cereals, resistance to lodging and a range of morphological characters. The time taken to release a cultivar can be much shorter than in a programme using conventional breeding methods. Notwithstanding all these, another most important is the additional genetic variability that is generated from induced mutations and can be incorporated in classical plant breeding programmes.

The potential use of induced mutations in plant breeding in Malaysia remains to be exploited.

Acknowledgement

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Table 1. Agricultural Production in Malaysia 1975

Commodity	Acreage (in thousand ha) 1975	Production (in thousand t) 1975
A. <u>Export oriented crops:</u>		
Rubber - (Pen. Malaysia)		
a) Estate	1400.0	588.0
b) Small holder	2710.0	805.0
Oil Palm - (Pen. Malaysia)	1436.0	1276.0
Coconut (Copra)	895.0	223.0
Cocoa	79.0	17.6
Pepper	23.2	35.3
Pineapple (canned)	37.9	48.0
B. <u>Foods and Feeds</u>		
Rice - (Pen. Malaysia)	944.0	1096.6
Maize	4.0	10.1
Groundnut	20.5	23.4
Tapioca (fresh tubers)	32.1	389.7
Sweet potatoes	5.5	44.0
Fruits (excluding pineapples and citrus)	141.8	773.9
Citrus	8.5	4.5
Vegetables	67.5	523.1
Cloves	0.4	0.2
Coffee	13.0	4.0
Tea ('000 pounds)	6.0	30.7

Calculated from source: Third Malaysia Plan 1976-80
 Ministry of Agriculture & Rural Dev. Malaysia
 Bil. No. 136
 MARDI - 1974

Table 2. Projected Requirement of Principal Foods and Feeds
(in thousand t)

Commodity	Estimated Production in 1975	Estimated Shortage in 1975	Projected Requirement 1985
Rice	1305.0	195.0	1899.0
Wheat	-	263.5	348.4
Maize (or Sorghum)	10.1	189.0	340.0
Tapioca (fresh tubers equivalent)	389.7	24.8	511.2
Sweet potatoes	44.0	-	59.4
Potatoes	-	29.3	43.2
Sugar (refined)	357.3	42.9	540.8
Groundnut	20.0	3.1	28.2
Soya bean (for human)	-	21.9	29.8
Soya bean (for livestock)	-	(65.7)	(89.3)
Vegetables	523.1	4.0	686.2
Onion	-	42.1	63.6
Chillies (dried)	-	8.0	10.8
Fruits (excluding citrus & pineapples)	773.9	40.0	1053.2
Citrus	4.5	12.4	29.7
Cloves	0.21	0.01	0.28
Coconut and copra	198.5	-	217.1
Coffee	4.0	1.2	7.0
Tea ('000 pounds)	30.7	12.9	57.7

Calculated from source: Third Malaysian Plan
Ministry of Agriculture & Rural
Development Bull No. 136
MARDI - 1974

Table 3: Relationships of parental clones used in the RRIM breeding programme (Tan et al, 1975)

Phase	Year	Clones used as parents	Designation of clones selected and tested on large scale trials
I	1928-1931	18 Primary Malayan clones	RRIM 500 series (30) ^b
II	1934-1941	9 Primary Malayan clones (5) ^a 17 RRIM 500 series 6 foreign clones	RRIM 600 series (39)
III	1947-1958	6 Primary Malayan clones (4) 7 RRIM 500 series (6) 18 RRIM 600 series 6 Clones of other selection 9 Foreign clones (2) 3 Dothidella resistant clones	RRIM 700 series (35)
IV	1959-1965	2 Primary Malayan clones (2) 4 RRIM 500 series (4) 8 RRIM 600 series (5) 1 RRIM 700 series 7 Clones of other selection (2) 9 Foreign clones (3) 4 clones from Peruvian <u>H. brasiliensis</u>	RRIM 800 series
V	1966-1973	2 Primary Malayan clones (2) 2 RRIM 500 series (3) 5 RRIM 600 series (5) 5 RRIM 700 series (4) 10 Clones of other selection (3) 19 Foreign clones (4) 2 Clones from <u>H. pauciflora</u> and <u>H. spruceana</u> 1 Clones from Peruvian hybrid	RRIM 900 series

Clones of other selection are secondary or tertiary clones selected in Malaya.

Figures ()^a denote number of clones used in previous phases

Figures in ()^b denote number of clones in the RRIM series.

Table 4: Yield capacity and parentage of 10 rice varieties released from 1964 to 1974

(After Chen et al, 1976; MARDI, 1977)

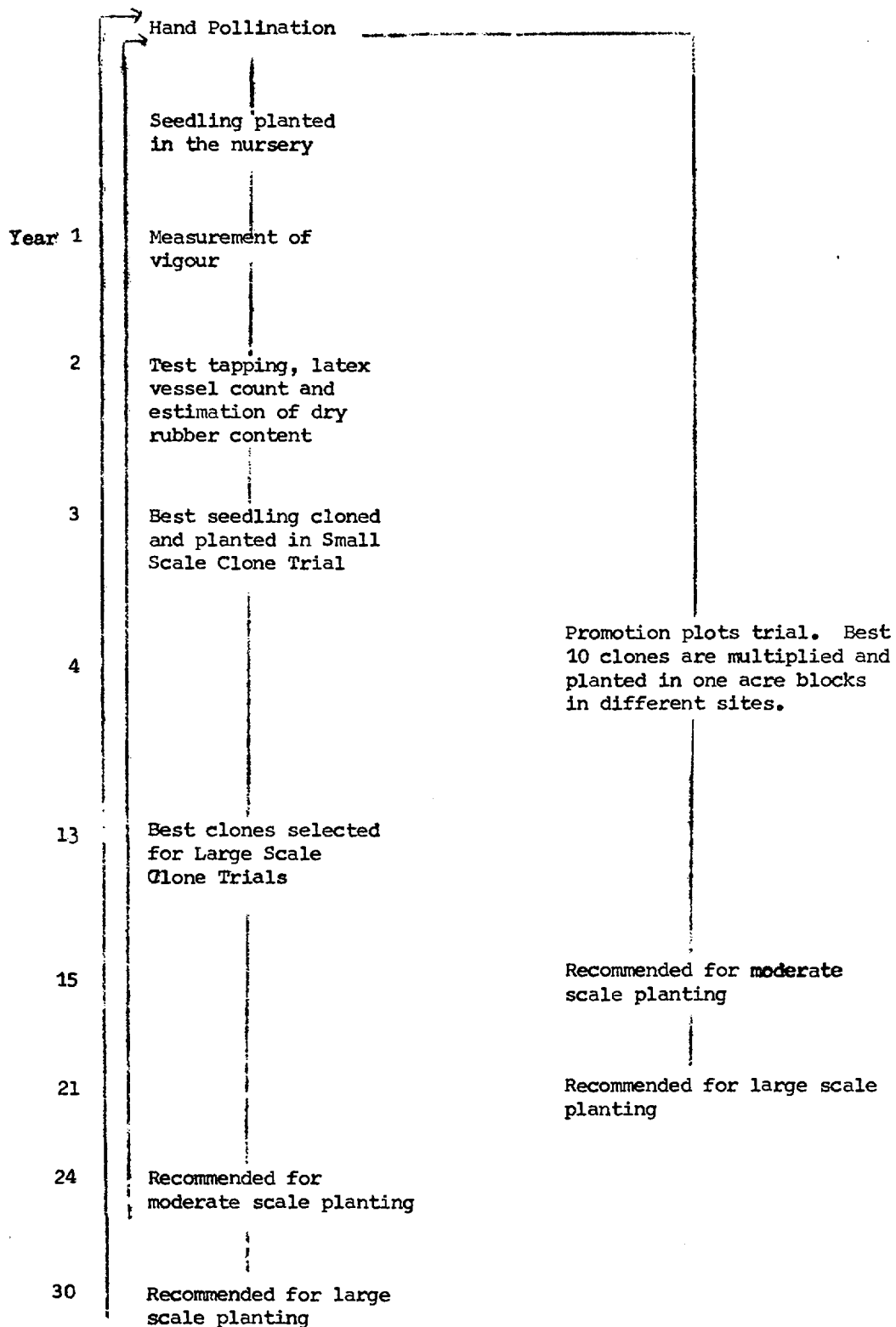
Variety	Year released	Parentage	Yield capacity kg/ha
Malinja	1964	Siam 29 x Pebifun	3920
Mahsuri	1965	Mayang Ebos x Taichu 65	4256
Ria	1966	Peta x Dee-Geo-Woo-Gen	5600
Bahagia	1968	Peta x Tangkai Rotan	5040
Murni	1972	Bahagia x Ria	5600
Masria*	1972	IR8 x Muey Nahng 62M	4480
Jaya	1973	Peta x BPI - 76	4450
Sri Malaysia I	1974	Peta x Tangkai Rotan	5040-6000
Sri Malaysia II	1974	Ria x Pankhari 203	3920-6496
Pulut Malaysia I*	1974	Pulus Sutera x Ria	3920-6160

* Glutinous rice variety.

TABLE 5: MUTATION BREEDING RESEARCHES IN MALAYSIA

Crops	Year	Investigators	Institution
<u>Food Crops</u>			
Legumes	1976	Jalani	UKM
Winged bean	1976	Jalani	UKM
Rice	1978	Zakri, Gaul, Jalani	UKM
		Arasu, Verughese, Saad, Habibbudin	MARDI
Soybean	1978	Jalani, Gaul, Zakri	UKM
		Ng, Mohd. Yusof, Abraham	RRIM
<u>Non-food crops</u>			
Rubber	1973	Ong Seng Huat, Subramaniam	RRIM

FIG. 1. BREEDING AND SELECTION CYCLE IN HEVEA (MOHD NOOR, 1976)



INDUCED MUTATION FOR VIRUS DISEASE RESISTANCE IN SOYBEAN

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Abstract

Soybean necrotic virus became a threat to leading soybean varieties in Korea. Domestic and introduced varieties were evaluated for resistance. The inheritance of resistance is being studied. A mutation experiment was started to make the leading varieties more resistant without losing their valuable characteristics. Attempts are made to develop a reliable method for artificial inoculation of larger populations.

Soybean, Glycine max (L.) Merrill, is an ancient crop in Korea, being used in preparation of a large variety of fresh, fermented and dried food products. Recently soybean production has become more important by developing various nutritionally balanced foods and beverages made of soybeans. On these accounts, soybean acreages have expanded gradually and various diseases have also increased in number and severity. Soybean mosaic virus was regarded as one of the most prevalent diseases of soybean in Korea. In 1975, however, a destructive necrotic disease was found on leading soybean varieties such as Kwangkyo, Kangrim and Dongbuk-tea, especially in fields of central Korea where soybean mosaic virus (common mosaic strain) has been endemic. This necrotic disease was recognized to be incited by a particular strain of soybean mosaic virus. The most striking symptom of the disease was that soybean plants infected while young were severely stunted and proceeded to buds curved downward, necrotic and brittle, and the plants died off later. In a susceptible leading variety Kwangkyo, seedling blight occurred as high as 90% or more and a considerable proportion of the pods contained no viable seeds in an epidemic year. Therefore, we intended to produce resistant mutants from the leading varieties by irradiation without a drastic change of their desirable agronomic characters.

1. Varietal reaction to soybean necrotic virus in the field

The reaction of different soybean varieties to the necrotic virus disease was checked in the field (Fig. 1). Of 230 domestic and introduced soybean varieties which have been kept in our laboratory, 30 seeds each were planted in rows 3m long x 70cm width x 10cm between seeds on May 23. To serve as a source of virus inoculum as well as to compare the disease reaction with tested varieties, the susceptible varieties Kwangkyo and Kangrim were planted between every five testing rows and as border line. Disease was rated as infected individual plants three months after sowing.

Out of 230 varieties tested, 12 varieties were higher than 40% in disease rate and most of them belonged to recommended or leading varieties. There were some varietal differences in the susceptibility to the necrotic virus with symptom variation. The most remarkable symptom of soybean varieties infected at early

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Table 1. Preparation of M₂ seed materials for selection of mutants resistant to soybean necrotic virus in two recommended soybean varieties.

Variety	Radiation dose	No. of seeds irradiated	No. of M ₂ seeds
Kwangkyo	Gamma ray 15 kR	1500	3120
	" 25 kR	"	1640
Kangrim	" 15 kR	"	3070
	" 25 kR	"	2130
T o t a l			9960

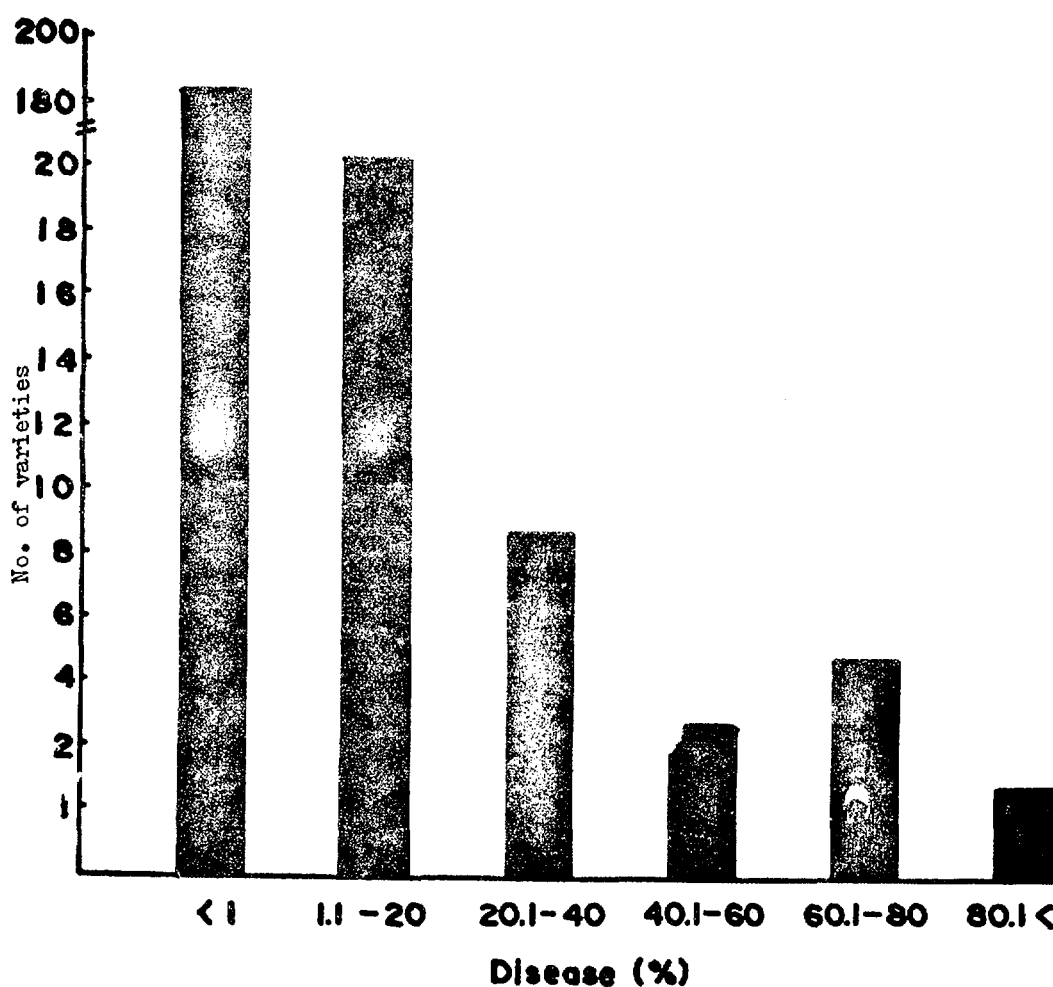


Fig. 1. Varietal reaction to soybean necrotic virus disease observed at early podding stage under field condition.

growth stage was necrosis as a bud blight. However, other soybean plants infected probably at a later stage showed considerable variation in symptoms including systemic brown lesions on leaves and a reddish brown necrosis of veinlets without stunting of plants. Following infection at podding stage, the pods turned black like mature pods without severe symptoms on leaves and stems.

2. Preparation of M_2 seed materials for selection of necrotic virus disease resistance

Soybean necrotic virus disease is a limiting factor in cultivation of the leading variety Kwangkyo, which has made around 80% in soybean production of Korea. Therefore, this variety needs to be improved in virus disease resistance without changes of other good agronomic characters including high yielding potential. For this purpose, mutation induction was thought to be a convenient method. 1500 seeds each of the varieties Kwangkyo and Kangrim were irradiated with gamma rays of 15kR and 25kR (Table 1). The irradiated seeds were planted in the southern area where the necrotic virus disease is not so severe. From the M_1 plants, a total of 9960 M_2 seeds were harvested for screening.

3. Preparation of hybrid seeds and a preliminary study on inheritance of resistance

It must be valuable to know something about gene constitution and mode of inheritance of resistance for further systematic soybean breeding. For this purpose, four "resistant" varieties KEX-2 (a mutant variety), Kumgang-daerip, # 31926 and KAS 390-10 were crossed with the susceptible variety Kwangkyo and another resistant one KAS 150-5 with the susceptible variety Dazzisusnary (Table 2).

In preliminary genic analysis, both F_1 , F_2 and parent plants were inoculated with crude sap of infected leaves by the conventional rubbing method of 2-4 trifoliate leaf stage and aphids were added to enhance natural virus infection. Disease rating was made once when the epidemic had reached the most severe stage close to blooming. Chi-square test was used to compare observed with expected genetic ratio. All of the F_1 plants showed high level of susceptibility approximately identical to that of the susceptible parent Kwangkyo (Table 3). No resistant plant was found, suggesting that susceptibility might be completely dominant over resistance. The F_2 population of the cross between # 31926 and Kwangkyo showed a segregation ratio of 3 susceptible to 1 resistant, indicating a participation of one recessive gene for resistance in the variety. Monogenic segregation was also indicated in the other crosses.

4. Artificial inoculation technique for mass screening of the necrotic virus disease resistance

In order to establish an effective mass screening method for necrotic virus disease resistance, development of artificial inoculation by a spraying technique was intended. The use of the spraying technique for inoculation with plant viruses (TMV, CMV) was attempted by others before.

For soybean necrotic virus inoculation, glass sprayer with air compressor was used. Soybean necrotic virus inoculum was prepared by macerating infected soybean leaves in a homogenizer, and straining the crude sap through two layers of cheesecloths. The extracted sap was diluted in 1:2 (W/V) with 0.01 M phosphorus buffer (pH 7.0) before inoculation. The inoculation was made at the primary leaf stage of the seedlings grown in 23 x 19cm plastic pots and the inoculated seedlings were kept in the room at a temperature of 27°C for 24 hours and then were transferred to the greenhouse. The observation of symptom development was made twice 2 weeks and 4 weeks after inoculation. The effect of varying the gauge pressure of airm compressor and carborundum concentration in the inoculum on symptom development was tested with 5cm of spraying distance. The exposure period under the sprayer of inoculum was around 0.5 seconds in which the leaf was wetted enough to cover the whole leaf surface with inoculum sap. It was noted that relatively little infection was produced when the virus was inoculated by spraying method as compared with mechanical inoculation by rubbing with cloth pads. As shown in Fig. 2, the gauge pressure of the air compressor 4 kg/cm² might be better than lower pressure

for maximum infection within 4 weeks after inoculation. Infected plants were rarely observed when no carborundum was added to the inoculum. The minimum concentration of carborundum for effective inoculation of test seedlings was ca. 1% if the inoculation was conducted with enough air pressure.

Concerning the spraying distance (Fig. 3), (height of sprayer nozzle from leaf surface), 15cm might be appropriate for approximately 42% infection when the gauge pressure of the compressor was set at 4 kg/cm², while at 2 kg/cm² pressure, 5cm in distance was necessary for the same degree of infection. At 1 kg/cm² pressure, little infection was observed, even with the distance of 5cm. The optimum conditions for soybean necrotic virus infection at the primary leaf of soybean seedlings were: 1) 5cm distance between sprayer nozzle and leaf surface, 2) 4 kg/cm² pressure of air compressor, 3) 1% by weight of 600 mesh carborundum as abrasive, and 4) spraying time of about 0.5 seconds to cover the leaf surface completely. However, the infection rate was still quite low so that this technique could not be satisfactory as an inoculation method for mass screening of the virus resistance.

The susceptible varieties Kwangkyo and Kangrim showed high natural infection in the field, and an aphid transmission study for effective inoculation of the virus was also attempted. Seedlings grown in plastic pots were inoculated at the first trifoliolate leaf stage by a number of aphids that were put before in net cage on infected host plants for acquisition feeding of virus. As shown in (Table 4) the infection rate by aphid transmission was about 60%, but was too low compared with natural infection under field conditions, even though it was a little better than the mechanical inoculation method used. Development of epidemic of the necrotic virus in the field seemed to be favoured by other factors.

The result obtained is not satisfactory for the purpose of mass inoculation and still further study should be done to find an effective inoculation technique for mass screening of necrotic virus resistant plants.

Table 2. Hybridization for genetic study of soybean necrotic virus.

Resistant parent (female)	Susceptible parent (male)	No. of seeds harvested (F₁)
KEX-2	Kwangkyo	6
Kumgang-dairip	"	6
KAS 390-10	"	6
# 31926	"	10
KAS 150-5	Dazzisusunary	5
Total		33

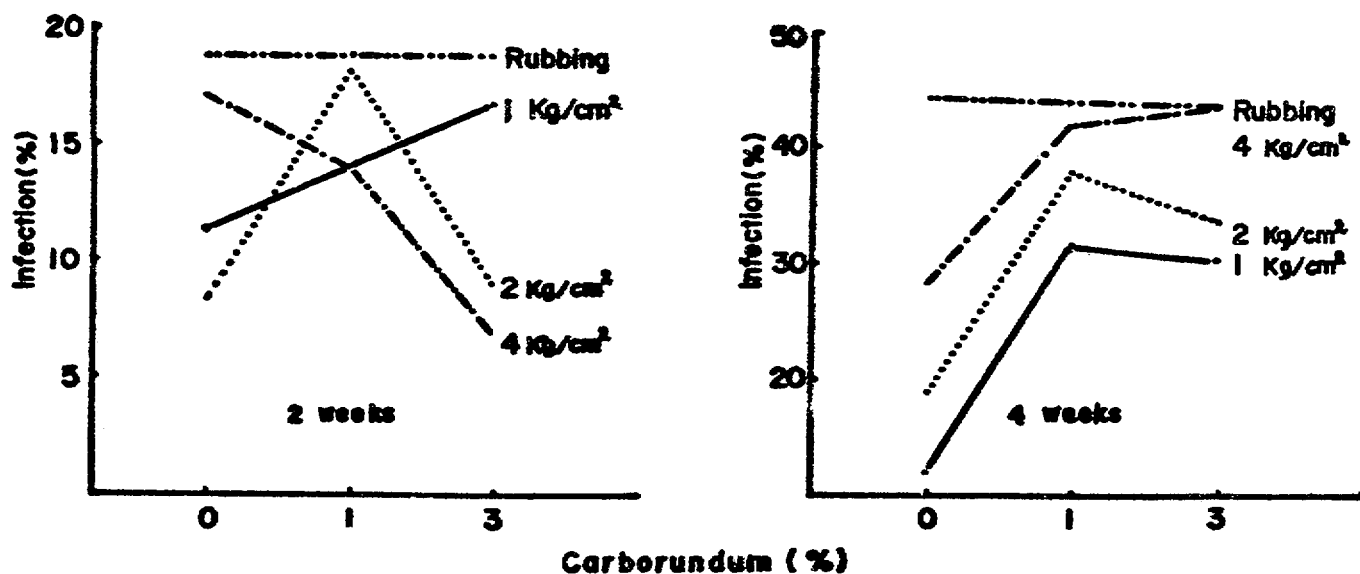


Fig.2. Effect of air pressure and carborundum concentration in the inoculum on symptom development of soybean necrotic virus.

Table 3. Infection types of F_1 , F_2 and parents inoculated with soybean necrotic virus

Exp. no.	Cross	Generation	No. of plants			χ^2 value	P value (3:1)
			Resistant	Susceptible	Total		
I	Kwangkyo (S) ^G	P_1		28	28	1.45	0.10 - 0.25
		P_2	30		30		
	# 31926 (R)	F_1		10	10		
		F_2	52	128	180		
II	Kwangkyo (S)	P_1		30	30	0.06	0.75 - 0.90
		P_2	30		30		
	KEX-2 (R)	F_1		6	6		
		F_2	6	16	22		
III	Kwangkyo (S)	P_1	1	29	30	0.66	0.25 - 0.50
		P_2	30		30		
	Kungang-daerip (R)	F_1		6	6		
		F_2	3	15	18		
IV	Kwangkyo (S)	P_1		30	30	0.04	0.50 - 0.75
		P_2	28		28		
	KAS 390-10 (R)	F_1		8	8		
		F_2	6	20	26		

^G Disease reaction (R) = resistant and (S) = susceptible

Table 4. Soybean necrotic virus infection of three different varieties by aphid transmission.

Variety	Period of acq. feeding	2wks. after inoculation		4wks. after inoculation	
		No. of plants	%	No. of plants	%
Kwangkyo	2 days	4/15	27	9/15	60
	4 days	3/14	21	8/14	57
	Other*	3/14	21	8/14	57
Kangrim	2 days	3/12	25	7/12	58
	4 days	4/14	29	8/14	57
	Other	3/12	25	7/12	58
Riuk-wu No.3	2 days	4/13	31	6/12	50
	4 days	2/12	16	7/13	54
	Other	4/15	27	8/15	53
No inoculation		0/34	0	2/34	6

* Indicates that the test plant was kept with virus infected plant (inoculum source) for two to four weeks.

5. Influence of seed coat mottling associated with soybean mosaic virus (common strain) on seed yield

Therefore evidences that soybean mosaic virus is a primary factor in the expression of soybean seed coat mottling and the mottling seems to affect not only the appearance of seeds but also the soybean yield. In order to test the effect of seed mottling on seed size, germination and transmission of the virus in our experimental field, mottled seeds were harvested from plants which were infected heavily by soybean mosaic virus and healthy seeds were prepared by cultivation of plants in the screen-house to prevent aphid transmission of the virus and eliminating suspected virus infection.

Seedling emergence under field conditions with mottled seed was reduced 25% and 8% in the variety KEX-2 and Bong-Eui, respectively (Table 5). To determine whether seed transmission of soybean mosaic virus is associated with seed mottling, 150 seeds each were planted and the percentage of virus transmission was based on the number of infected seedlings among emerged plants at 3-4 trifoliolate leaf stage. Virus transmission was 80.4% and 64.3% in the variety KEX-2 and Bong-Eui, respectively (Table 6). Infected seedlings from healthy seeds encountered in this experiment may be caused by incomplete elimination of virus infected plants due to masking of symptom or other faults when the healthy seeds were prepared in the screen-house.

In addition, mottled seeds showed a reduced seed size ranging from 8.1% to 29.5% depending upon the varieties and this is likely to cause reduction in seed yield (Table 7). The experimental results indicate that the variety Bong-Eui suffered less than the variety Kwangkyo from soybean mosaic virus infection.

6. Work to be done

- 1) Screening for soybean necrotic virus disease resistance in M_2 generation.
- 2) Preparation of F_2 generation for study on inheritance of resistance to soybean necrotic virus.
- 3) Estimation of yield loss by soybean mosaic virus (common strain).

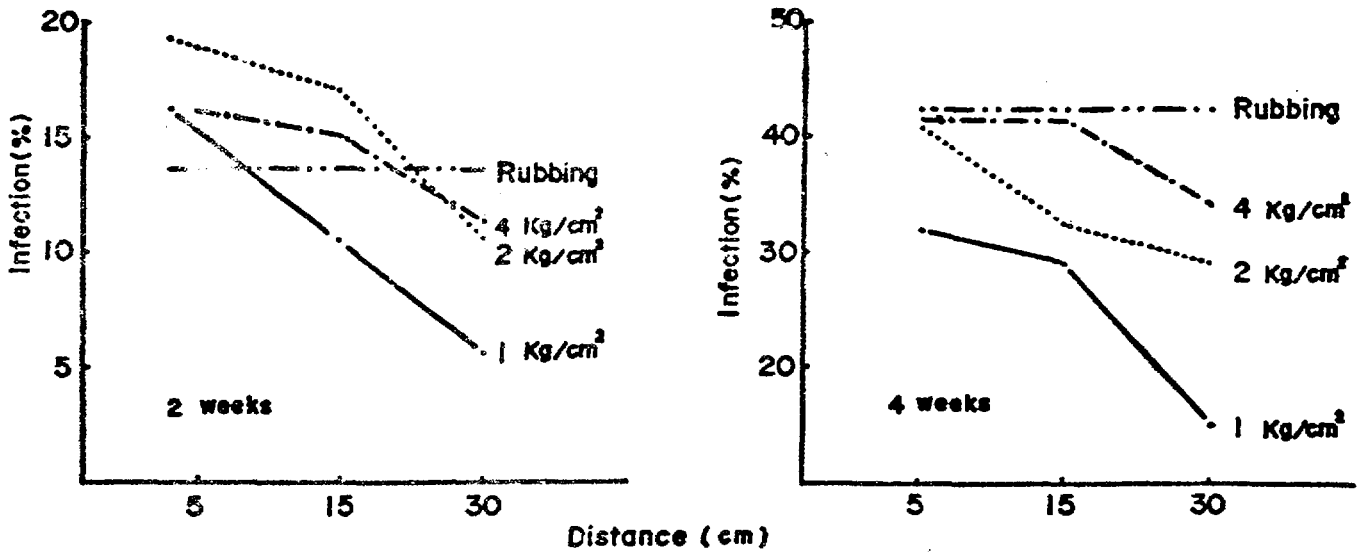


Fig.3. Effect of air pressure and distance by spray nozzle from leaf surface on symptom development of soybean necrotic virus.

Table 5. Percent emergence of mottled seeds associated with soybean mosaic virus in two different soybean varieties.

Rep.	Var.	KEX-2		Bong - Eui	
		Healthy	Mottled	Healthy	Mottled
I		80.0	53.3	76.7	70.0
II		73.3	56.7	83.3	83.3
III		80.0	63.3	83.3	70.0
Average		77.6	57.7	80.1	74.4
Reduction(%)			25.6		7.5

Table 6. Seed transmission percent of soybean mosaic virus through the seed coat mottling in two different soybean varieties.

Variety	Seed	Plants	
		Total No.	% Infected
KEX-2	Healthy*	128	35.5%
	Mottled	107	80.4%
Bong-Eui	Healthy	141	46.1%
	Mottled	133	64.3%

* Healthy seeds were prepared from mosaic symptom-free plants which were grown in screen house.

Table 7. 100 seed weight of mottled seeds associated with soybean mosaic virus in different soybean varieties.

Variety	100 seed wt.(g)		Reduction percent
	Healthy	Mottled	
KEX-2	31.9	22.5	29.5
Bong-Eui	22.5	18.7	16.9
Clark	17.2	12.4	27.9
KAS 100-3	38.9	35.7	8.1
KAS 503-7	28.6	24.2	16.1

INDUCED MUTATIONS FOR RUST RESISTANCE IN SOYBEANS

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Abstract

Soybean rust is a problem in many countries of South-East Asia. In Thailand, losses are between 10 and 40%. An International Working Group on Soybean Rust (IWGSR) has been formed with the Asian Vegetable Research and Development Center (AVRDC) at Shanhua, Taiwan, as coordinating centre. Promising soybean lines and varieties were distributed, but only two moderately resistant lines persist at present. Since 1974, a soybean improvement project exists at the Kasetsart University Bangkok and efforts to develop productive soybean lines with rust resistance by induced mutations are included.

Introduction

Soybean (Glycine max (L.) Merr.) has long been important as hum food in Thailand, but has never been a major crop. According to the Agricultural Statistics of Thailand Crop Year 1976/1977, it ranks 8th after rice, sugar cane, cassava, maize, groundnut, mung bean and kenaf. In 1976, only 113,600 tons of soybean were produced. The production was 186,400 tons below the national target. In the fourth economic development plan, a production target of 431,000 tons of soybean has been set up for 1981. In order to accomplish this target, the planted area would have to be increased to about 450,000 hectares, nearly 4.5 times the soybean acreage in 1976. A large area would have to be planted in the wet season or yields would have to be increased drastically. The soybean yield in Thailand, however, is still low, the national average being less than 1 t/ha. Varieties as well as management are limiting the yield.

Present official varieties are S.J.1, S.J.2 and S.J.4. These varieties are susceptible to soybean rust, caused by Phakopsora pachyrhizi Syd., especially when they are grown in the wet season.

The disease was first recorded in 1966 and it is not known how long before it had been in existence in Thailand. Sangawongse (1973) estimate that losses due to this disease were 10 to 40 per cent in local cultivars. In order to obtain a more accurate assessment of yield loss, experiments were carried out in the wet season of 1976 at Farm Suwan, Pakchong, Nakorn Rajachasima Province. Results show that losses in yield through natural infection by this pathogen were 17.6 per cent (158.8 kg/ha) for S.J.1 and 33.9 per cent (477.4 kg/ha) for S.J.2 (Pupipat, 1977).

The soybean rust is a problem also in other countries, such as Taiwan, Indonesia, the Philippines, India and Australia and many plant breeders and plant pathologists are attacking this problem (Chan, 1977; Sumarno and Sudjadi, 1977; Lantican, 1977; Singh and Thapliyal, 1977; Kochman, 1977; and McLean and Byth, 1977; Yang, 1977b). Scientists actively engaged in soybean rust research organized the "International Working Group on Soybean Rust" (IWGSR) during the Regional Soybean Conference at Chiang Mai in 1976 (Bromfield and Yang, 1977). A year later

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the "Asian-Oceania Soybean Rust Workshop" was convened in Manila and the workshop report "Rust of soybean, the Problem and Research Needs" was published by INTSOY, University of Illinois, Urbana (USA). Recent research findings on soybean rust are disseminated in the Soybean Rust Newsletter published by the IWGSR secretariat at the Asian Vegetable Research and Development Center (AVRDC), Shanhua, Taiwan.

AVRDC serves as a centre for soybean rust research where soybean germ plasm is being collected and screened for rust resistance. In 1975, in cooperation with the USDA Delta Branch Experiment Station, 1080 soybean cultivars were screened and were classified as moderately resistant. These nine cultivars were tested again in two successive screenings in 1976, and PI 230970 (G 8586) and PI 230971 (G 8587) continued to show a moderate resistance with note 323, according to the IWGSR Soybean Rust Rating System (Yang, 1977a; Shanmugasundaram, 1977a). Selected soybean cultivars were sent to 16 scientists in nine countries for further rust testing (Shanmugasundaram, 1977b). Other sources of resistance have been reported from Australia (Kochman, 1977; McLean and Byth, 1977), India (Singh and Thapliyal, 1977), and Taiwan (Chan, 1977). Unfortunately, all were susceptible when screened in a field rust nursery and in the greenhouse at AVRDC (Shanmugasundaram 1977b).

In Thailand, some soybean cultivars received from AVRDC were tested for their rust reactions and were classified as good to moderately tolerant (Pupipat, 1977) or as susceptible (Nundhapun and Surin, 1977). In field observation of rust reaction in M₂ soybean lines, three lines derived from G 8375 (an AVRDC accession) and one line derived from No. 138 (Taichung) showed a hypersensitive reaction type. Reddish-brown lesions developed on leaves in the upper third of the plant. At the time of observation no spores were found in the lesions (non-sporulating lesions) while the leaves of the other lines were heavily covered with sporulating lesions (Smutkupt, Viparsrinimit, and Pupipat, 1978).

Fuchs (1971) stated that "hypersensitive incompatibility is a result of a very specific process determined by corresponding genes in host and parasite". In soybean, Singh and Thapliyal (1977) classified lines having hypersensitive reaction into a moderately resistant group. This type of reaction might be similar to the infection type RB described by Bromfield (1978). Nevertheless, at present, only the two moderately resistant germ plasms, PI 230970 (G 8586) and PI 230971 (G 8587) identified by AVRDC in 1976 are used in breeding programmes (Shanmugasundaram, Bromfield, and Yang, 1979; Nundhapun and Surin, 1979). It is evident that additional sources of rust-resistant genes are critically needed.

In 1974, the Division of Radiation and Isotopes has initiated a project "Use of Radiation in Soybean Breeding". At the same time, the University realized the need to strengthen soybean research aiming at the improvement of soybean production in the country and developed the Soybean Master Project in which the radiation project has been included. The present research project cosponsored by IAEA under Research Contract No. 2302/SD intends to develop high productive soybean lines with resistance to soybean rust by using induced mutations.

The Project

Location

The field experiments are being carried out at Kasetsart University campus and other locations as follows:

- a) Farm Suwan, Pakchong, Nakoru Rajchasisa Province
(14° 13'N)
- b) Mac Joe Experiment Station, Chiang Mai Province
(18° 30'N), in cooperation with Khun Pricha Surin and Khun Montha Nundhapun, Plant Pathology Division, Department of Agriculture.
- c) Nong Hoi Agricultural Experiment Station of Chiang Mai University, Chiang Mai Province (ca. 1000 m above sea level), in cooperation with Dr. Dumrong Tiyawalee, Department of Field Crops, Faculty of Agriculture, Chiang Mai University.

Experiment I

329 lines derived from irradiation and/or crossing of varieties S.J.2, Sansai, S.B.60 and Wakashima will be tested at 3 locations (Nong Hoi, Mac Joe and Pakchong) for rust resistance. 15g of seeds will be planted in 5 m rows. Every fifth row at Mac Joe and Pakchong will be a check row of S.J.2. At Nong Hoi, every tenth row will be a check row of either S.J.2, S.J.4 or Orba. Artificial inoculation will be carried out only at Mac Joe. For evaluation the IWGSR rating system will be used (Yang 1977a, Shanmugasundaram 1977).

Experiment II

Progenies of 665 M₁ plants (originating from 80 different varieties or lines) will be planted at Farm Suwan as M₂ generation. 60 seeds per progeny will be planted in 3 m rows in July. Artificial inoculation will be carried out in September (60 days after sowing) using rust material collected in the field at Mac Joe Station. Variety S.J. 2 will be used as a control.

Experiment III

The following eleven cultivars are being used for this experiment:

S.J.2
S.J.4
BM 50
BM 52
BM 98
G 8375
G 8377
G 8586
G 8587
Taichung N
Wakashima mut. no. 10

Seeds with ca. 10% moisture will be irradiated in the "gammator" of the Kasetsart University Bangkok with 15 and 30 krad. Irradiated seeds will be planted in July at Farm Suwan in rows 5 m long 50 cm apart with 150 seeds per row in hills spaced 12.5 cm. Ca. 10000 treated seeds will be planted per cultivar and dose, besides 1000 control seeds per cultivar. In addition, ca. 5000 seeds of each cultivar will be irradiated with 15 krad and 20 krad gamma rays and planted in observation plants at the Kasetsart University campus.

At maturity time it is planned to harvest randomly 6 pods from each surviving M₁ plant. M₂ seeds will be bulked and sown at Mac Joe (Chian Mai) during the dry season of 1980 with artificial inoculation. Plants with rust rating 323 or better will be selected for further evaluation. In addition, 2 seeds of each M₂ plant will be harvested and bulked for rust resistance screening during the wet season 1980.

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IMPROVEMENT OF SOYBEAN, PEANUT AND MUNGBEAN BY THE USE OF NUCLEAR TECHNIQUES

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Abstract

Pulses are an important part of the diet in Indonesia. Their production is insufficient. Rust disease is one of the obstacles towards higher yields. Seeds of two soybean varieties, one peanut variety and two mungbean varieties were irradiated with doses causing 15-30% seedling height reduction. M_4 and M_5 generation of irradiated soybean were screened for rust resistance and it appears that irradiated populations contain a few more plants with resistant or moderately resistant reaction. Irradiation of mungbeans with doses of 45-72 krad was rather unsuccessful in terms of obtaining useful mutations. Therefore M_4 seeds were irradiated again with similar doses.

Introduction

Soybean, peanut, and mungbean are the most important grain legumes used as protein supplement to the Indonesian staple food rice, corn cassava or sago. Rice and corn have received most of the attention of plant breeders for many years, legume breeding programmes received very little. One of the major problems in legume production is rust disease. Among 109 soybean varieties and lines only 1 variety and 2 lines were found to be resistant. No variety of peanut and mungbean was resistant (Sudjono 1979). The use of induced mutations will be one of the possibilities to obtain resistance. The main objective of this investigation therefore is to find mutants which are resistant to rust disease.

Experimental materials

The choice of parental varieties is based on their high yielding potential. At the moment the best yielding varieties are ORRA and SHAKTI for soybean, GAJAH for peanut, PR-74 and PR-83 for mungbean. (Table 1). Therefore, we used those varieties as the experimental materials.

Table 1. Important characters of the varieties used for mutation experiments.

Variety	Yielding potential	Maturity (days)	Reaction to rust
<u>Soybean:</u>			
ORBA	High	80	Susceptible
SHAKTI	High	85	Susceptible
<u>Peanut:</u>			
GAJAH	High	100	Susceptible
<u>Mungbean:</u>			
PR-74	High	70	Medium Resistant
PR-83	High	70	Medium Resistant

Irradiation

We choose gamma ray doses which gave a seedling height reduction of about 15 to 30% (Fig. 1.). Dry seeds of the mentioned varieties were irradiated with ^{60}Co gamma rays at the Pasar Jumat Research Centre with the doses given in Table 2.

Table 2. Effect of gamma irradiation on germination.

Variety	dose (krad)	Germination capacity	
		Laboratory	Field
<u>Soybean:</u>			
ORBA	17.5	96%	68%
	25.0	98%	55%
SHAKTI	15.0	98%	61%
	22.5	98%	58%
<u>Peanut:</u>			
GAJAH	25.0	89%	59%
	32.5	87%	52%
<u>Mungbean:</u>			
PR-74	45.0	100%	67%
	72.5	98%	46%
PR-83	47.5	100%	69%
	65.0	100%	58%

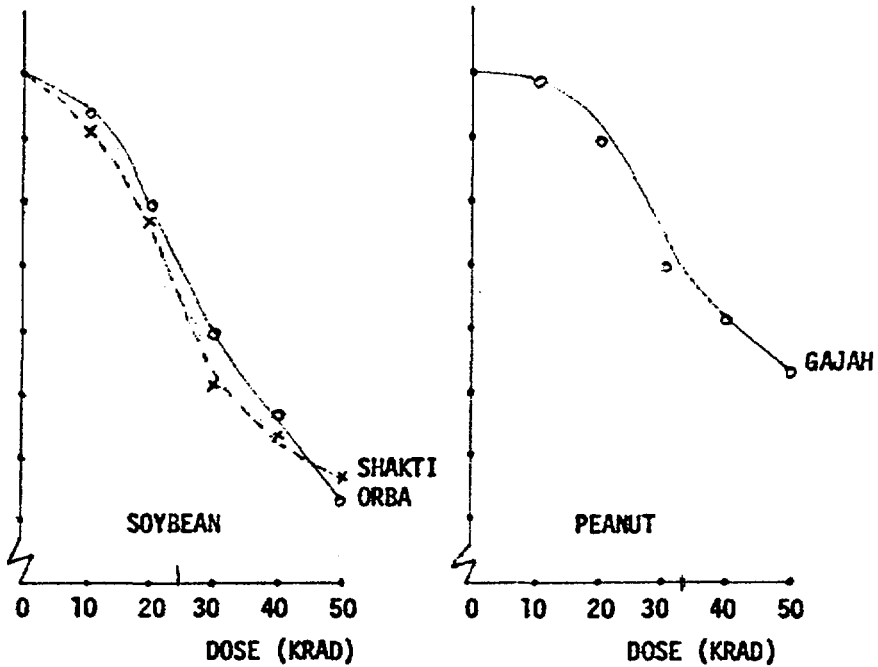
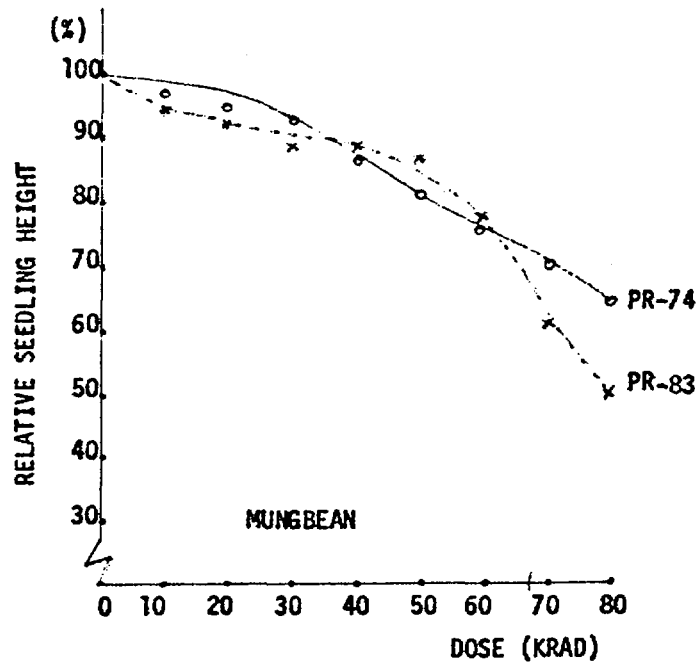


Fig. 1. Effect of gamma radiation seedling height of soybean, peanut, and mungbean.

M₁ Generation

After irradiation seeds were divided into three parts and planted separately at the Pasar Jum'at Research Center, at the Central Research Institute of Agriculture - Sukamandi and at the Bogor Agriculture University.

The M₁ grew well at Sukamandi and Bogor, but due to unfavourable conditions M₁ plantings had to be repeated three times at Pasar Jum'at Research Center.

M₂ Generation

M₂ population was planted at three different locations. We planted each variety-treatment as a bulk. We observed some chlorophyll mutations (Table 3). The type of mutations were xantha and viridis.

Table 3. Frequency of chlorophyll mutations in single seed M₂ bulk.

Variety	Treatment (krad)	M ₂ Germination	No. of M ₂ seedlings (\bar{x})	Chlorophyll mutations per 100 M ₂ seedlings
<u>Soybean:</u>				
ORBA	Control	82.00	447	0.0
	17.5	76.43	863	0.0
	25.0	67.13	782	0.47
SHAKTI	Control	78.00	624	0.0
	15.0	73.17	824	0.0
	22.5	58.23	619	1.11
<u>Peanut:</u>				
GAJAH	Control	73.50	147	0.0
	25.0	58.52	157	0.0
	32.5	58.08	163	0.0
<u>Mungbean:</u>				
PR-74	Control	79.75	638	0.0
	45.0	75.69	899	0.89
	72.5	48.65	506	1.49
PR-83	Control	80.38	643	0.0
	47.5	75.05	816	0.44
	65.0	72.83	894	0.89

x) - Every M₂ seedling represents one M₁ plant.

No serious selection was made in M₂, however some possible mutants with specific morphological changes were harvest separately. Of all the healthy M₂ plants a bulk was prepared separately per variety-treatment. Each bulk was divided into three parts of which two were sent to the other locations. Therefore at every location, we planted three M₃ bulks.

M₃ and later generations

Peanut

Lack of space and facilities with the growing amount of material forced us to discontinue the experiment on peanut.

Soybean

Healthy M_2 plants of soybean were harvested separately and grown as plant to row in M_3 . Healthy M_3 plants of each row were bulked and propagated as a line in M_4 . To stimulate rust infection, we planted a susceptible variety RINGGIT among the M_4 lines. Screening was carried out at Darmaga, Bogor during the dry season of 1978, 70-80 days after planting. The results are shown in Table 4.

Table 4. Reaction of M_4 lines to rust (*Phakopsora pachyrhizi* Sydow)

Variety	dose krad	Total No. of lines tested	No. of lines R/MR
ORBA	0	1412	0
	17.5	1150	4
	25.0	404	15
SHAKTI	0	1249	0
	15.0	1235	3
	22.5	1263	0

Plants which showed R/MR were harvested individually and planted in M_2 in the same manner as in M_4 . The observation was again made 80 days after planting at Darmaga, Bogor, however, during the wet season 1978/79. The environmental conditions in two different seasons may play a very important role. Most of the plants failed to confirm resistance in the M_5 generation (Table 5).

Table 5. The reaction of selected M_5 lines to rust infection.

Original variety	Mutant lines	number of plants			
		S	MS	MR	R
ORBA	1.01	25	0	0	0
	1.02	20	0	0	0
	1.03	13	0	0	0
	1.04	7	2	0	0
	1.05	29	4	0	0
	1.06	22	1	0	0
	1.07	25	1	1	0
	1.10	22	2	0	0
	1.11	20	2	0	0
	1.12	26	3	0	0
	1.13	26	2	1	0
	1.14	20	2	0	0
	1.15	5	0	0	0
	1.17	31	4	1	0
	SHAKTI	2.02	8	2	0

The remnant M_4 plants in each treatment were harvested and bulked. In M_5 they were planted as an independent population, and re-tested to rust disease. The observation was made 60 days after planting. This test was done at the same place and the same time as the test shown in Table 5. It seems that the treated populations had a few more R/MR plants than the control populations (Table 6).

Table 6. The reaction of M_5 populations to rust infections.

Original variety	Dose krad	Replication	no. of plants	No. of R/MR	% R/MR
ORBA	0	I	639	4	0.42
		II	644	3	
		III	639	1	
	17.5	I	644	7	0.88
		II	644	7	
		III	642	3	
	25.0	I	635	5	0.62
		II	646	5	
		III	642	2	
SHAKTI	0	I	610	0	0.0
		II	616	0	
		III	624	0	
	15.0	I	630	1	0.05
		II	620	0	
		III	632	0	
	22.5	I	629	0	0.05
		II	635	0	
		III	629	1	

Selection will continue till M_9 , while the remnant plants in each treatment will be bulked and treated as independent populations. The scheme of future activities is as follows:

M_6	M_7	M_8	M_9
Individual selection	Individual selection	Individual selection	Individual selection
	Population I	Population I	Population I
		Population II	Population II
			Trial
			Collection

Mungbean

We were not able to select useful mutants out of M_2 and M_3 populations.

To increase the frequency of mutations, the M_4 seeds were irradiated again with gamma rays at Pasar Jum'at Research Center. The doses were as follows:

Original variety	First radiation dose (krad)	second dose for M_4 seeds (krad)
PR 74	45.0	55.0
	72.5	77.5
PR 83	47.5	42.5
	65.0	65.0

Reference

Sudjono M.S. 1979

Laporan Hasil Penelitian Ekobiologis tentang Cendawan Karat
(*Phakapsona pachyrhizi* Sydow) dan Ketahanan Varietas Kedelai
(*Glycine max* Merr.). Institut Pertanian Bogor

UTILIZATION OF FAST NEUTRONS AND GAMMA RAYS FOR SOYBEAN IMPROVEMENT*

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Abstract

Soybean is one of the important crops after rice. It is generally cultivated in the lowland and rarely in the highlands.

A mutation breeding programme was started in 1977 with the aim to select types of soybean, having early flowering and maturing time, adaptation to high altitude, good determinate plant type and high yield.

In M₂ generation, 123 plants with desirable plant type were selected and grown as M-3 mutant lines for confirmation. 19 homogeneous mutant lines will be grown in M-4 for yield tests. A few mutants were maturing 3 - 7 days earlier, two others were late flowering with indeterminate growth habit.

Introduction

Soybean is recognized as one of the important crops after rice and occupies a considerable acreage in Indonesia. Because of its value as human food and as a cheap protein source, breeding work in soybean is given high priority in the country's agricultural research programme. SOMAATMADJA (1) reported that in Indonesia 85% of soybean was cultivated in the lowland around an altitude of 100 m, 10% in the land of about 250 m elevation and only 5% was grown in the highland but below 750 m. Soybean consumption in Indonesia is increasing, but the yields are low, 0.7-0.8 t/ha, (2) and therefore a large amount of soybean is imported.

As early as 1950 soybean mutation breeding experiments have started (for reviews see 3, 4 and 5). During recent years, large number of mutants have been released as new cultivars (SIGURBJORNSSON and MICKE, 6) among which 4 were soybeans, i.e., Tainung No. 2, Tainung No. 2, Raiden and Raiko.

To generate genetic variability in crop plants by mutation induction is not anymore difficult, therefore a mutation breeding programme in soybean was started at our Institute in 1977 with the aim to generate variability of potential use and to select for improved plant type, improved yield, adaptation to high altitude cultivation and early maturity.

Materials and Methods

From cultivars available, the variety ORBA was chosen for this experiment mainly because of its high yielding potential of about 2 t/ha. Seeds were obtained from the Central Research Institute for Agriculture, Sukamandi.

Before irradiation, seeds were equilibrated over 60% of glycerol during 10 days in a desiccator to obtain a moisture content of about 13%. Moisture stabilized seeds were then exposed to doses of fast neutrons or gamma rays. Fast neutron irradiation was carried out in the SNIF (Standard Neutron Irradiation Facility)

* Supported by IAEA under Research Contract No. 2231/RB

of the ASTRA swimming pool reactor, Seibersdorf, Austria. Gamma irradiation was carried out using a ^{60}Co source (gamma cell) at the Pasar Jum'at Research Centre, Jakarta, Indonesia. Doses were 20 krad and 25 krad of gamma rays, and 1500 rad and 2200 rad of fast neutrons, 10,000 seeds were used for each gamma dose and 2000 seeds for each fast neutron dose.

The M_2 and M_3 generations were grown at the Horticulture Experimental Station in Lembang at an altitude of 1200 m above sea level. For the M_2 generation, one pod derived from the main stem of good looking (healthy) M_1 plants was randomly harvested. For each dose, 5000 M_2 plants were grown. Visual selection of M_2 plants (including controls) was carried out, followed by single plant progeny lines in M_3 . Selected homogeneous lines will be tested for yield in M_4 generation in replicated randomized trials.

Results and Discussion

The survival rate of M_1 plants in gamma irradiated lots was 90 - 80% and in the fast neutron lots 80 - 70%. Seedlings height tested in the laboratory was somewhat reduced following gamma as well as fast neutron irradiation, but further growth of the treated M_1 plants was not much inhibited. The control plants as well as the treated M_1 plants grew about 30 cm taller at the higher altitude than the control plants grown in the lowland.

Chlorophyll deficient sectorial chimeras were found in the M_1 population, but did not occur among the control plants. In the treated M_1 plants, so-called leaf spots were also found in the first leaves of all plants and these spots became more severe at the higher dosage. KAPLAN (7) claimed that the number of leaf spots increased exponentially with the dose and he concluded that chromosomal aberrations were responsible for the induction of the spots.

In the M_2 generation, 20,000 plants were grown as bulk population. Untreated plants were included as check plants. 123 plants, good in plant type, higher in pod number and phenotypically different from the control plants were selected and raised in M_3 as single plant progeny rows. Control plants were inserted in every tenth row.

19 lines in M_3 looked stable and homogeneous and could be considered as promising mutants of practical value (Table 1). These mutants will be tested for yield in the M_4 generation. Other lines segregated and reselection was carried out. Mutant No. 166-Nf had white flowers and more hairy stem and leaves. Reduced plant height (shorter stature) and an increased pod number could be found in some of the mutants.

Other authors mentioned that earliness could easily be induced by mutation, e.g., ZACHARIAS (4) obtained mutants which were 25 days earlier maturing, ISHIKAWA et al. (3) released 2 soybean varieties, Raide and Raiko, which matured 25 days and 15 days earlier than the parental variety used. However, in the present study the earliest mutants matured only about one week earlier than the control (Table 1).

Acknowledgement

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Table 1. Plant growth and yield characteristics in M_3 of control variety and its 19 mutant lines induced by fast neutrons and gamma rays

Line No.	Dose (krad)	No. of plants	Days to flowering	Days to maturity	Plant height cm	No. of nodes (main stem)	No. of pods per plant	TKW g
Control	0	30	55 (34)*	120 (91)*	90 (61)*	16	111 (152)*	156 (130)*
60 - Gm	20 gamma	19	51	116	79	18	212	195
62 - Gm	20 gamma	25	52	116	77	17	231	184
200- Gm	25 gamma	23	52	117	79	20	219	172
201- Gm	25 gamma	28	48	113	71	15	129	153
202- Gm	25 gamma	24	52	117	72	18	202	178
206- Gm	25 gamma	26	52	116	78	16	123	170
3 - Nf	1.5 N_f	27	51	117	78	16	144	163
8 - Nf	1.5 N_f	24	52	117	70	18	221	150
10 - Nf	1.5 N_f	28	51	116	82	17	187	182
130- Nf	1.5 N_f	20	52	116	84	19	145	168
141- Nf	1.5 N_f	24	52	118	80	18	210	164
143- Nf	1.5 N_f	20	52	117	81	17	144	164
166- Nf	1.5 N_f	30	51	118	80	16	162	123
24 - Nf	2.2 N_f	22	51	116	74	19	216	193
162- Nf	2.2 N_f	17	52	116	70	17	196	169
169- Nf	2.2 N_f	25	52	119	85	18	158	166
175- Nf	2.2 N_f	26	48	114	80	18	168	172
181- Nf	2.2 N_f	30	48	113	63	15	143	164
182- Nf	2.2 N_f	23	48	114	63	15	129	156

* Figures in parentheses show plant characteristics of the control plants grown in the lowland as reported by MARDJUKI and FATIMAH (8).

SOYBEAN MUTATION BREEDING PROGRAMME IN MALAYSIA

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Abstract

The problem of breeding soybean in Malaysia is discussed with special reference to the use of induced mutation. Soybean is envisaged for planting as an intercrop in the rubber and oil palm plantations as well as a rotational crop with rice and/or other food annuals. An outline of the UKM breeding programme is described. EMS and gamma rays are used for induction of mutations. Three varieties, Acadian, Jupiter and Palmetto are selected for experimentation. Using two different dose levels 80,000 seeds per variety per season are treated with EMS and gamma rays respectively. Doses are chosen for obtaining 70% and 90% killing. The results of pilot experiments conducted in the greenhouse are presented.

I. Introduction

Growing soybean (*Glycine max* (L) Merrill) in Malaysia is hampered by the lack of adapted high yielding varieties with desirable grain quality. Cheam and Tan (1975) estimated that the local consumption of soybean per capita per year averaged 5.3 kg for the years 1971 to 1973. Based on the assumption of an annual population growth rate of 2.7 percent the demand projected for 1980 would be 71,000 t. However, almost all soybean and its products are imported and in 1975 the import amounted to 45 million Malaysian Ringgit.

Soybean is a potential crop for extensive cultivation in Malaysia judging from the literature about the capacity of soybean in the humid tropics (FAO, 1977; Hinson and Hartwig, 1977; IAEA, 1977; Sinha, 1977).

It is envisaged that the soybean plant can profitably be grown in Malaysia in two main ways:

- 1) as an intercrop in immature plantations of rubber and oil palm. This is particularly significant to smallholders who can obtain quick cash return.
- 2) as a rotational crop with rice and/or other annual crops.

Part of our soybean mutation programme will be conducted jointly with the Rubber Research Institute (RRIM)

II. Plan of work for 1978/79

1. PILOT EXPERIMENTS IN UKM LABORATORIES AT FI CAMPUS, PETALING JAYA

Seedling growth experiments will be conducted to work out mutagenesis parameters which are necessary for effective main experiments. Seedling height, seedling growth and leaf spotting will be measured. Three varieties, Palmetto, Jupiter and Acadian will be used. Each experiment will take between two six weeks.

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Gamma irradiation and EMS treatment will be used and dose effect curves will be established. The influence of presoaking and of postwashing and of redrying after postwashing will be investigated.

2. STUDIES WITH UNTREATED SOYBEANS AT BANGI

50 relatively well adapted varieties, including Palmetto, Jupiter and Acadian, will be grown in observation plots. Notes will be taken on germination, pests and diseases, days to 50% flowering, plant height at flowering, days to maturity, plant height at maturity, branches per plant, nodes per plant, internode length, pods per node, pods per plant, seeds per pod, 100-seed weight grain yield and some other characters.

Yield trials will be conducted with 10 varieties, including Palmetto, Jupiter and Acadian, plot size will be 6 m x 2 m and planting distance of 50 cm x 5 cm with two seeds per point; a randomized block design with 3 replicates will be used. Seeds Palmetto, Jupiter and Acadian will multiply.

The experiments will be duplicated. One set will be sprayed against diseases and insects, the other one not.

3. M₁ GENERATION AT BANGI WITH SPRAYING AGAINST PESTS AND DISEASES

Using gamma rays and EMS, 80,000 seeds per variety of each of the three varieties, Palmetto, Jupiter and Acadian, will be treated with each mutagen. Two doses are applied, one aims at 70% killing, the other at 90%. The seeds are sown in rows. For the distance of the seeds in the row, the killing rate will be considered. Branching will be reduced by a dense stand. Germination survival and M₁ sterility will be measured.

4. M₂ GENERATION GROWN AT BANGI

One third of the harvested M₂ seeds will be grown with sufficient space in and between the rows, in order to be able to detect mutants easily. Notes will be taken on germination, chlorophyll mutations, extreme dwarfs and other macro-mutants. Selection will be carried out for high yield, earliness, non-shattering, hard seed coat, seed size, indeterminate growth habit. Mutants interesting for other reasons, e.g., (taxonomic value, interspecific macro-mutants, 'monsters') will be harvested as well.

The M₂ population will be grown in duplicate, one set with, one without spraying against pests and diseases. Selection for disease and pest resistance will be carried out primarily in the unsprayed part.

5. M₂ GENERATIONS GROWN AT A 'HOT SPOT', WITHOUT SPRAYING AGAINST PESTS AND DISEASES

The location will be selected close to Kuala Lumpur; either at the RRM Experimental Station, Sungai Buloh or at a private estate. That location will be developed into a 'hot spot' through growing, for at least two seasons, a mixture of highly susceptible varieties. This experimental field will be between immature rubber tree rows. The last third of the harvested M₂ seeds will be grown for mass screening of resistant mutants at this 'hot spot'. The selection criterion will be a healthy looking plant.

III. Plan of work for 1979/83

Pilot experiments (1), studies with untreated soybeans (2), handling of the M₁ generations (3) and of the M₂ generations (4,5), will be similar as in 1978/79. However, new parent material for mutagenic treatment will be introduced, according to the results obtained in the studies of the Joint Malaysian Soybean Project.

In the M₃ generation, all mutants selected will be grown in observation plots and notes will be taken as to their characters.

In the M₄ generation, mutants of agronomic value will be put in micro yield trials with two replicates at three locations: One location will be in the open field with spraying against pests and diseases. Another location will be between immature rubber tree rows with spraying against pests and diseases. A further location between immature rubber trees without spraying against pests and diseases. A mutant collection will be built up.

In M₅ generation the best M₄ families will be used for a multi-location trial with more locations and larger plot sizes.

In the M₆ generation, the number of families tested will again be reduced. They will be tested, as to their performance and acceptability to the farmers and the market, at more locations in the evaluation system of the Joint Project. In the M₇ generation, the testing is repeated, however, in a different season and again with a further reduced number of families.

In the M₈ generation, testing in as many locations as possible in Malaysia is repeated, including large-scale trials in farmers' fields. In the M₉ generation the testing repeated, however, in a different season. In the M₁₀ generation, following the sixth selection step, the first variety can be released for commercial growing.

From the M₃ generation onwards (a) purification of the mutant families and (b) seed multiplication of interesting lines will be done by normal plant breeding techniques. Maintenance breeding will start from M₆ generation onwards.

It is expected that the major part of the mutants selected are of no direct use because the grain yield is too low. However, some of them are expected to have outstanding specific agronomic characters which are of value for use as parents in cross-breeding programmes. These mutants will be handed over early to partners in the Joint Project for use in their hybridization programmes together with mutants which have a potential value for direct use.

IV. Results of pilot experiments

We have conducted pilot experiments to work out mutagenesis parameters for main experiments, using gamma-irradiation from a Cobalt-60 source radiation doses ranged from 0 - 70 krad with a 5 krad interval between each dose. Each treatment group was split into 5 replicates with 20 seeds per replicate. The seeds are sown in flats, 60 x 90 cm, with garden soil. The seedling height is measured from the surface of the soil to the cotyledon or the tip of the first leaf respectively. The results show a progressive decrease in cotyledon height (Fig. 1). A similar pattern is observed for the first leaf height.

Pilot experiments using various concentrations of EMS treatment was applied for a three hour period at 26°C but the duration of post-washing (under running tap water at 26°C ± 0.5) varied from 0 - 24 hours. Generally we observed an inverse relationship between concentration of EMS and the height of cotyledon and the first leaf. The duration of postwashing seems to be critical (Fig. 2 and 3). After three hours postwashing the seedlings seem to recover from the effects of EMS. One important point is the sensitivity of soybean to soaking in water. This can be seen by the reaction of the control (0.0%), as both cotyledon and first leaf height were reduced after three hours of soaking in water, but then they recovered after another three hours soaking. This result has been reproducible.

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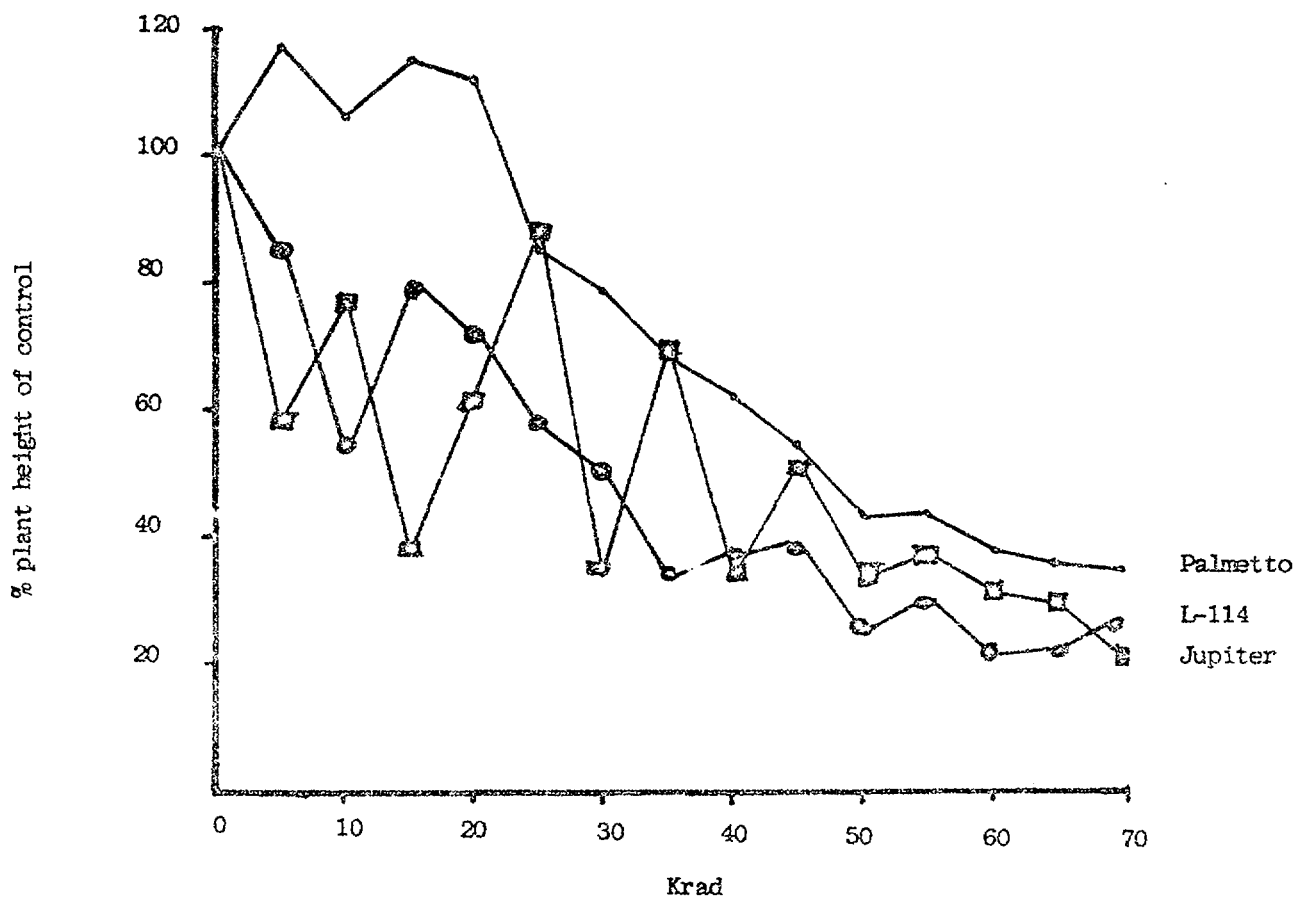


Fig. 1. Relationship between Gamma-irradiation doses and mean height of cotyledon in 3 soybean varieties.

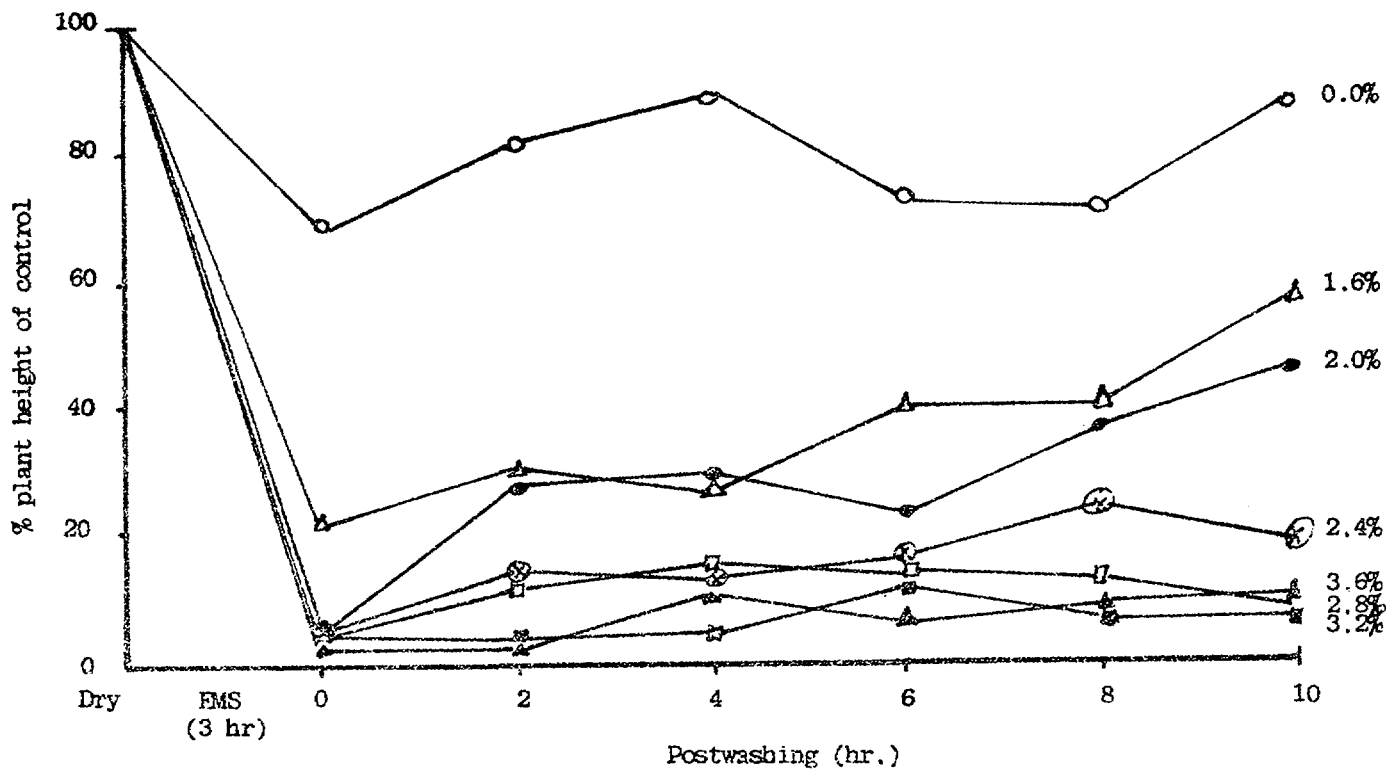


Fig. 2. Relationship between different concentrations of EMS with postwashing and mean height of cotyledon of soybean cv. Jupiter.

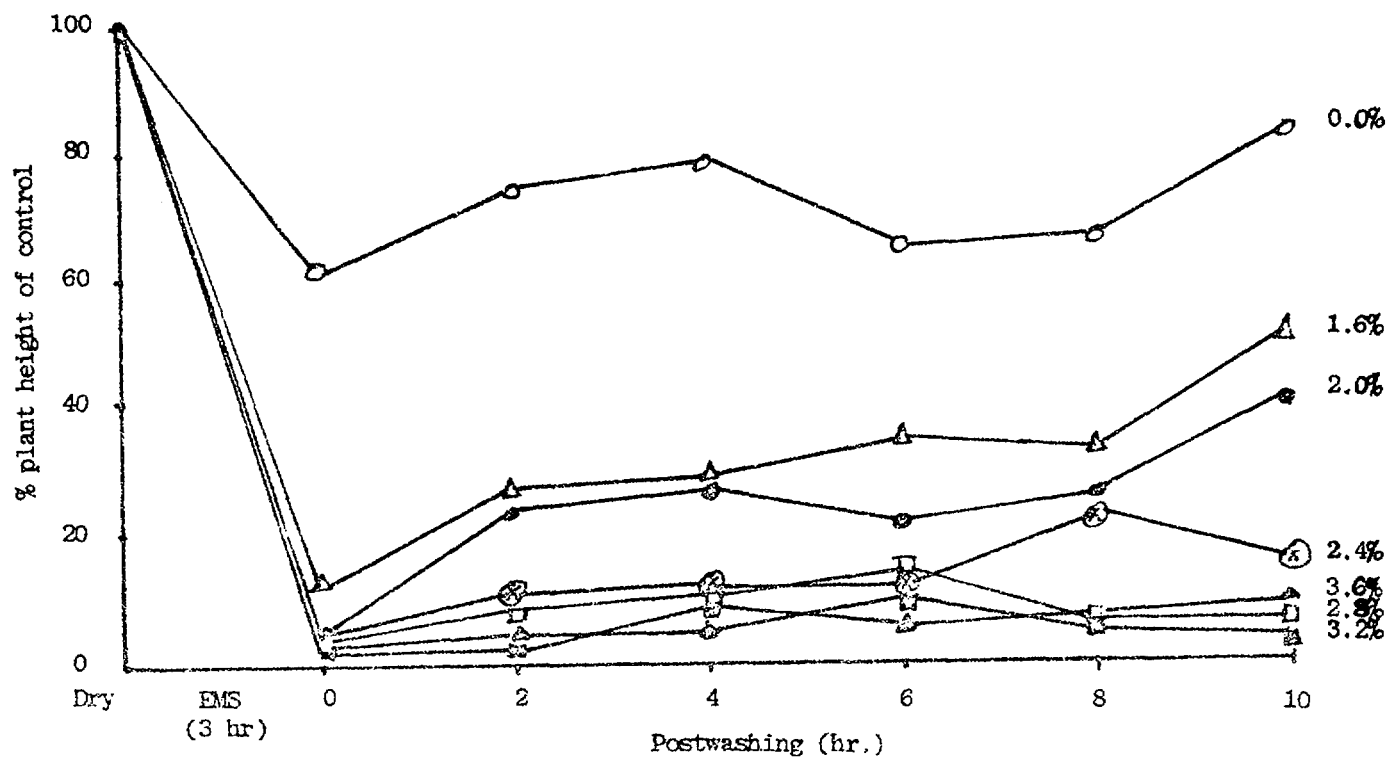


Fig. 3. Relationship between different concentrations of EMS with postwashing and mean height of first leaf of soybean cv. Jupiter.

MUTATION BREEDING FOR PARTIAL DISEASE RESISTANCE

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Abstract

Where satisfactory levels of resistance cannot be obtained, partial resistance can nevertheless be a worthwhile objective for reducing crop losses. Using wheat as experimental material, the question is studied whether improvement of partial resistance can be obtained by mutagenesis.

Introduction

For the wheat Puccinia complex four types of resistance have to be considered. These are: (i) Seedling resistance, which shows low infection type (0 to 1) at all stages of growth, (ii) Adult plant resistance, which is characterised by a susceptible infection type (4⁻ to 4) in the seedling stage and a resistant reaction (0 to 2) in the adult plant stage. The resistance in the two above mentioned categories is complete and appears to be race-specific (Green and Knott, 1962; Knutt, 1971; Russel & Hudson, 1974 etc.) (iii) Intermediate resistance, which shows infection types (2⁻ to 3) in all growth stages and (iv) Partial resistance: The susceptible infection type (3⁻ to 4) is observed in all growth stages. This type of resistance may vary as a result of differences in components that contribute to the development of the disease symptoms and severity, i.e. infection frequency, latent period, sporulation rate and the period of infection. Partial resistance is expressed as difference in the proportion of disease affected area and has the characteristics of horizontal resistance as described by Van der Plank. Horizontal resistance by definition is characterised by the absence of interaction between the genotypes of the host species and isolates of the pathogen. The presence or absence of interaction can be studied in one of the following ways:

1. Either by means of an analysis of variance which registers all deviations from additivity.
2. Or by ranking isolates in order of their pathogenicity on the various cultivars. This test registers only differential interactions.

I. Durability of resistance

It has been generally believed that the race specific resistance, which is simply inherited, is easily overcome by new virulences that develop soon after the cultivar goes into large scale planting while partial resistance is more durable because of its control by many genes, each with a small additive effect. While exceptions exist to both these assumptions, theoretical considerations favour the accumulation in the host of many genes for resistance, which makes it harder for the pathogen to develop matching multiple virulences. The real test of durability of resistance is the number of years a variety remains unaffected by disease after it has gone to large scale cultivation. The following approaches are open for experimentally achieving partial resistance effect with a reasonable hope that the resistance so achieved will be durable:

- i) Use of time tested genes for partial resistance such as are present in classical wheat varieties like Hope.
- ii) Combining genes for adult plant resistance into a single cultivar. This is easily achieved if the adult plant resistance exhibited by different donors is genetically tagged with seedling susceptibility to specific races.

- iii) Creation of new and complex loci for resistance in the host for which a matching virulence does not presently exist and will take considerable time to develop, if at all, when the cultivar carrying it is put to large scale planting.
- iv) Experimental compounding of isogenic lines with defined but different genes for race-specific resistance. Multi-lines so developed display characteristics of horizontal resistance even though the resistance is controlled by race-specific genes.

The last two approaches are amenable to mutagenesis.

II. Mutagenesis as a toll for achieving partial resistance

Mutagenesis is a workable method of generating and harnessing variability that expresses itself as partial resistance. The following considerations have important bearing on the success of efforts aimed at realizing partial resistance:

(a) Genetic background of the stock used for mutagenesis

It is generally believed that a pre-requisite to isolating partial resistance is the elimination of major genes for race specific resistance. As a result, an advocated method for selecting genotypes with partial resistance is to discard plants resistant at the seedling stage and retain only those individuals which are seedling susceptible but show low disease incidence at adult plant stage. Experiments of Simons and Frey with oats for crown rust resistance indicate that the presence of a major gene for tolerance to crown rust against races 202, 264 and 290 does not hinder the induction and isolation of tolerance to race 216. But that the genetic background is important is shown by the differential response for the degree of tolerance induced in two cultivars: Richland and CI 6665. Simons found that the most tolerant line from Richland had a tolerance ratio between 68 and 70; values which are only 11-13 points better than the parental variety. On the other hand, from treatments of CI 6665 Simons and Frey obtained lines with tolerance ratios of 99-101 which is 26-28 points better than that of the parental line.

For experiments designed to generate basic information on the mechanism of slow rusting or partial resistance, the material of choice should (a) preferably be free from major gene(s) for resistance and (b) be genetically well worked out. Although such studies would be extremely valuable with grain legumes, wheat lends itself as a more suitable experimental material. The availability of aneuploid series will greatly facilitate sound genetic probes.

(b) Inoculation density and detection of partial resistance

A heavy inoculum density for creating disease epidemic is likely to mask the identification of plant with partial resistance. Sprays of heavy spore leads or syringe inoculated spreader rows create too severe disease conditions under which can detect at best only the race specific types of resistance. It will be advisable that field inoculations are done either by uniform spraying of pathogen at low densities or by keeping diseased potted plants for relatively short periods in the fields, in this way stimulating situations of natural field spread of the disease. That the disease had been adequately introduced in the experimental plot can be monitored by planting super susceptible types of cultivars at regular intervals in the screening nursery.

(c) Handling of the mutagenised populations

For traits controlled by several genes with small additive effects it is desirable that drastic selection is not exercised during early generations. It is much better to carry an unselected sample into later generations, when the material has reached homozygosity, before consciously challenging it to the pathogen. A single seed descent method could be useful in propagating material till generations suitable for isolating mutations for partial resistance or tolerance. Recurrent mutagenesis can be another useful technique for accumulating resistance genes for achieving a greater degree of resistance.

(d) Selection for components of partial resistance

Rather than making selections on the basis of disease severity, it will be more rewarding to look for mutants for various components that finally express themselves in partial resistance. For the wheat rust, as indicated above, the components of importance are the infection frequency, the latent period, the pustule size and the sporulation index. Each one of these components can be screened in a suitably advanced mutagenised population. An encouraging aspect is that selection for the above mentioned components in our experiments with the wheat/leaf rust system has indicated a parallel response.

We have been making a study of the components contributing to slow rusting for Puccinia recondita in wheat. Based on the information gathered, the following considerations are suggested for isolation of mutants for partial resistance in wheat:

1. Differences between components of disease development like latent period, pustule number, number of spores per uridium per day at the seedling stage are not clearly evident. Where such differences do exist, they do not correspond to the behaviour during later stages of plant growth. On the other hand, measurement of these parameters at the flag leaf stage differentiates "slow rusters" from "fast rusters" much more efficiently. For screening mutagenised populations, therefore, it may be more promising to do the recordings during later stages of plant growth comparable to the flag leaf stage in cereals.
2. Our observations indicate that for uniformly spread spore showers, the pustule density on the "slow rusters" is distinctly smaller as compared to that on "fast rusters". While this information is being tested more critically in experiments with spore settling towers, we feel that the observation can be profitably used for screening mutants showing induced reduction in infection frequency. For using this criterion in mutation experiments, we plan to count the number of pustules in a fixed area in the middle of flag leaf blade following inoculation by uniform spray at low spore densities, first at the time of first pustule appearance and again after a lapse of one latent period.
3. We feel that mutants can also be isolated for increased latent period. We propose to do this by counting the number of pustules on an unit area in the middle of flag leaf. The pustule number will be recorded every day starting with the latent period of the parental variety till the pustules have appeared in this defined area. By this method we hope to detect plants with a latent period longer than that of the parental variety.
4. Pustule size can also be scored, even though with a smaller accuracy, by a visual scale.
5. Sporulation index is a more difficult parameter to score quantitatively. It can be measured by assessing the average number of spores produced per pustule either by weight or by number or through spectroscopy. The methods, however, are tedious and time consuming and have limited value for screening large populations. A rough indication can nevertheless be obtained on otherwise desirable plants by the fluffiness of the pustules.

DEVELOPMENT OF NEW PLANT TYPES IN CHICKPEA FOR HIGH YIELDS THROUGH MUTATION BREEDING¹

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Abstract

Chickpea is the most important grain legume for India. Traditional varieties are adapted to marginal soil fertility conditions and low level management. Genetic variability of the species in terms of characters suitable for modern farming is low. Mutation induction is used to obtain erect plant types with high grain productivity besides other objectives. A large number of mutants with different plant architecture has been selected.

Introduction

Grain legumes occupy an important position in world agriculture by virtue of their high protein content, low cost of production and capacity for fixing atmospheric nitrogen. The only practical means of solving the protein malnutrition in developing countries like those in the South-east Asia, where majority of the population depends for its protein requirement on plant-to-man food chain, rather than the plant-to-animal-to-man food chain, is to increase the production of grain legumes. The protein of grain legumes is nutritionally important as a supplement to cereal protein. Chickpeas contain nearly $2\frac{1}{2}$ times more protein than cereals, and have the highest Protein Efficiency Ratio (PER) among grain legumes. For India, chickpea is the most important grain legume. Its grains are more commonly consumed directly than any other legume. India also accounts for about 75 to 80 percent (5.5 million tonnes) of the world's chickpea growing area and production. It produces 5.5 million t on 8.5 million ha. In the Indian sub-continent this legume accounts for half of the area and production of grain legume crops. Pakistan, Ethiopia, Mexico, Central America, Burma, Spain, Morocco, Turkey and Iran are other important chickpea producing countries in order of the acreage.

The problems in chickpeas

The average yields in India over the past two decades were between 550 and 700 kg/ha only compared to a yield of 1667 kg/ha reported from Egypt. Because of low and unstable yields, chickpea faced competition from high yielding and fertilizer responsive cereals and by diversion of good land from chickpeas to wheat chickpea is pushed to marginal lands under a low level of management. Chickpea has been traditionally grown under varying kinds of stress conditions, with the result that survival and adaptation have been far more important than selection for high yields (Swaminathan and Jain, 1972). Under such subsistence conditions, natural selection eliminated genotypes which may respond to fertilizers and irrigation (Jain, 1975). The progress in development of high yielding varieties is slow because of the limited germplasm available. Present varieties of

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- 1) Supported by IAEA under Research Contract No. 2103/RB
 - 2) Associate and Principal Investigator respectively.

chickpea are highly reactive to daylength and temperature, susceptible to diseases and pests and take too much time till maturity (160 - 180 days in the major chickpea growing area).

Objectives of the project

The ultimate objective of chickpea improvement is to develop high yielding varieties, competitive to those in cereal crops like wheat and rice. The genetic variability in this species, however, has been greatly eroded. It is essential to regenerate genetic variability through induced mutations and hybridization.

A major objective of our project is to induce plant type mutations to develop a new productive ideotype. The desired restructured plant types should have changed physiological characteristics be earlier maturing, photo and thermo-period-insensitive and widely adaptive. Genotypes are required which can be grown under different cropping pattern and farming systems. Traditional chickpea varieties tend to be spreading, bushy, late in maturity, and form a dense foliage cover on the ground. These are obviously adaptive attributes for dry conditions and low fertility. Based on extensive studies on mutation breeding in chickpeas (Kharkwal, 1978) and conventional breeding (Bahl and Jain, 1977) we believe that the ideal plant types in chickpea would be those marked by an erect architecture, compact habit, large number of primary and secondary branches, open canopy and basal podding. In this conceptual plant ideotype of chickpea, the vertical growth will replace horizontal spread of traditional types maintaining the number of pod-forming loci. This means that we are looking for a plant type which is architecturally adapted to high plant density and narrow row spacing (Bahl, 1979). A large number of mutants carrying certain characters of the conceptual plant ideotype have already been selected (Kharkwal, 1978). Besides plant architecture mutation breeding in chickpeas may help in achieving the following objectives:

- increase in grain yield and harvest index
- resistance to diseases and pests
- reduction of vegetative growth period
- improvement in protein quantity and quality, digestibility and cooking quality
- response to higher doses of fertilizers and better nitrogen fixation over a longer period.

Material and methods

Seeds of IARI chickpea variety BG-203 (Amar) with a moisture content of approximately 10-12% were irradiated with 50 and 60 Kr gamma rays using a 200 curie ⁶⁰Co Gamma Cell (available at the Division of Genetics, IARI, New Delhi) with a dose rate of 1.72 kR per minute, at ca. 25°C. Ethyl methane sulphonate (EMS) was also used to treat seeds with a concentration of 0.2% for 12 hrs with intermittent shaking at 25°C ± 2°C. Two thousand seeds were used per treatment as well as for the control. EMS treated seeds were thoroughly washed in running water for an hour to leach out the residual chemical and were dried on blotting paper. Treated and control seeds were sown on the same day in 5m rows spaced 10cm within row and 0.5m between rows. Planting was done in October 1977.

Results

I. M₁ generation

The observations recorded in M₁ included germination, pollen sterility and survival (Table 1). No dominant mutation was observed in M₁. The treatment caused relatively high sterility and lethality. All surviving plants were harvested individually and seeds of single plants from each treatment were planted separately in M₂. In case of EMS treatment, single plant yield was extremely poor because of high sterility and the seeds obtained were small and shrunken.

Table 1: Effect of mutagenic treatment on seed germination, pollen sterility and plant survival in M₁ generation

Treatment	Dose	No. of seeds planted	Germination (%)	Sterility (%)	Survival (%)
Gamma rays	50 Kr	2000	30	12	90
	60 Kr	2000	65	32	72
EMS	0.2%(12)	2000	70	60	65
CONTROL	-	2000	95	2	98

II. M₂ generation

M₂, during 1978-79 consisted of about 40,000 plants divided into 800 progeny rows (400 for each gamma ray treatment). Seeds were sown 10 cm apart in 5 m rows with spacing of 0.6 m between rows. Planting was done in October 1978. Mutations affecting morphological characters such as habit of growth, plant type, leaf morphology, flower and pod characteristics were recorded in throughout the growth period.

With a view to isolate micromutations, ten normal looking plants from each M₂ family were harvested randomly to record observations on quantitative characters such as grain yield and its components.

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IMPROVEMENT OF PLANT ARCHITECTURE IN CHICKPEA AND MUNGBEAN

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Abstract

Chickpea and mungbean are important grain legume crops of Pakistan and are mainly grown on marginal lands. Under improved cultural practices and moisture conditions these tend to make excessive vegetative growth resulting in low grain yield. A change in plant type is needed to realize higher yield under improved cultural practices. After mutagenic treatment of locally adapted cultivars of chickpea and mungbean, we selected upright compact plant type mutants in chickpea and short statured, uniformly maturing, high yielding mutants in mungbean.

The upright compact mutant of chickpea UR16 showed good yield potential when using a higher seed rate. It has a tendency to lodge but some lodging resistant recombinants have been obtained through hybridization with other cultivars. In mungbean mutant 605, 1038 and NIAB 28 are early maturing and show consistently higher yield and high harvest index. NIAB 28 is short statured and two to three secondary branches emerge near the basal node. Mutants with appropriate plant height combined with desirable pod bearing pattern have been selected after mutagenic treatment of Kabuli mung.

Introduction

Pakistan annually produces ca. eight hundred thousand tons of pulses which are the major source of protein for the larger part of the population. Pulses are generally consumed in combination with cereals. Chickpea is the most important among legume crops and accounts for more than 70% of the area and of the production, while mungbean shares only about 4% of the area and production. There is a lot of genetic variation for grain colour in chickpea and a variety of preparations exist according to consumer's taste.

Both chickpea and mungbean are mainly grown on marginal lands under rainfed conditions (67%) and acreage in the canal irrigated areas only meets local needs, however, mungbean is also cultivated in the Salaba areas where water is available through inundation along the rivers. The winter rains are erratic and scanty during chickpea growing season, while the mungbean crop receives ample precipitation during monsoon season. Chickpea grown under irrigated condition and mungbean grown during monsoon period usually make excessive vegetative growth, resulting in low grain yield. Cultivation under subsistence agriculture for a long time and conscious or unconscious selection under stress conditions have developed genotypes with poor harvest index (3). In mungbean an extended pod formation period results in irregular pod maturity. Losses may occur due to pod shedding during picking of the crop. The leafiness of the plants intercepts the sunlight penetration to the pods which otherwise could make a substantial contribution of photosynthates to the rapidly developing seeds. Pod formation should preferably occur above the main leaf level for proper exposure to sunlight. An ideal type of mungbean should have short stature, determinate habit, two or three secondary branches emerging near the basal node, pod formation above the leaves, uniform maturity and improved pod setting (4).

Chickpea plants are bushy and during favourable seasons farmers graze the crop by goats or sheep before flowering. The bushy growth habit may result in competition for sunlight, poor pod setting and inadequate grain development. The bushy thick canopy provides micro environments conducive for the development of diseases and insect pests. At maturity considerable losses due to pod dropping may occur. A change in plant type is expected to improve yield stability. Upright plants having more fruit bearing branches preferably emerging near the basal node of the main shoot, with 50-60 cm plant height even under high fertility conditions, appear to be suitable for raising the yield level of chickpea. Upright plant type is available in bold seeded cultivars of Russian origin (1), but in this type pod formation is mainly confined to the upper portion of the branches causing low grain yield compared with cultivars where pods are evenly distributed from base to top along the branches. Attempts to combine upright growth habit with yield and other desirable attributes of local cultivars through hybridization have not yielded good recombinants. There is little genetic variability available for synthesising the desired plant type of chickpea and genetic variability through mutation induction and to improve the existing well adapted cultivars or induce desirable mutations for use in cross breeding.

Materials and Methods

One thousand dry seeds of chickpea cultivar C 727 were irradiated with 10 kR of ^{60}Co gamma rays. The M_1 generation was grown in the field and seeds were collected separately from individual plants. In M_2 plant progenies were raised and selection for upright plant type was made. Confirmation of mutant plant type was made in M_3 .

Pak 13, Pak 17, 6601 and Kabuli (Bold seeded) cultivars of mungbean were exposed to 10, 20, 30, and 40 kR ^{60}Co gamma rays. The M_1 was grown in the field and at maturity four pods from the main shoot of the individual plants were harvested separately. The M_2 was raised in the field and selections were made for plant stature, uniform maturity, branching pattern and increased number of fruit bearing nodes per plant. 26 mutant lines showing improvement in various characters were confirmed in M_3 . The most promising mutant lines were further tested to evaluate their yield potential and other agronomic characteristics.

Results and Discussion

I. Chickpea

About 10,000 M_2 plants in 950 M_1 plant progenies were studied for genetic variation regarding plant type and components of yield. Mutant plants with upright compact plant type were observed in one (6). In addition 510 single plant selections made in M_2 were studied in M_3 for detecting micromutations.

The progenies of the mutant plants showing upright compact plant type were studied in M_3 generation and three progenies, UR 11, UR 15 and UR 16, were uniform for the plant type, whereas progeny UR 14 segregated for upright and parental plant type. In M_4 during 1978-79 crop season, albino plants appeared in the upright mutant line UR 15, while purple coloured plants appeared in upright mutant line UR 11. The purple coloured mutant plants were similar to upright mutant line except in pigmentation.

The tree breeding mutant line UR 16 was selected for further study on the basis of its good plant type, high fertility, and better grain weight than the parent variety C 727. In a micro yield trial the mutant UR 16 yielded 11% more (1900 kg/ha) than the parent variety (1700 kg/ha). This mutant line was also tested at different population densities viz. 7, 15, 22 cm row to row and plant to plant distance in nine possible combinations. In high plant density a general trend for increase in plant height was noticed both in the mutant line as well as in the parent variety however, the average height of the mutant UR 16 (64.4 cm) was significantly lower than the original variety (72.2 cm). Increases in number of pods per plant, grains per pod and yield per plant were observed for the upright mutant UR 16 under certain conditions of spacing (Table 1). By using high seed rate for a (determinate) mutant in Vicia faba, ten percent increase in yield were obtained by Syödin (2).

Unfortunately, mutant line UR 16 has a tendency to lodge under favourable conditions. The mutant was therefore crossed with two other cultivars 6153 and Aug 424. Some of the recombinants recovered in F_2 , particularly from cross UR 16 x Aug 424, appeared to be promising as the primary branches emerging near the basal nodes touch the soil before growing upwards, which could provide better support to the plant. These recombinants also carry the stiff stem character of Aug 424.

II. Mungbean

Mutants were selected for having short stature, uniform maturity and branching pattern of the secondary branches emerging from the basal node of the main shoot. Out of a fairly large number, the mutant lines 605, 3854, 1038 and NIAB 28 were finally chosen on the basis of desirable attributes and better yield performance (Table II and III). All the fruit bearing branches of mutant 1038 elongate to the top of the plant and attain a uniform level. Mutant NIAB 28 is a short-statured line (52 cm) with quite uniform maturity of pods. Two to three secondary branches emerge near the basal node and attain the same height as the main shoot. Two other short-statured mutant lines 4048 (52.3 cm) and 1118 (52.9 cm), derivatives of 6601 and Pak 13 respectively, showed also an improved harvest index (5).

The bold seeded kabuli mung is grown as spring crop in the vast planes of Punjab province. These types have more seeds per pod than the local small seeded cultivars and apparently a better yield potential during spring season. However, the inherent high yield potential of these types can not be realised due to restricted vegetative growth. The pods formed on the lower nodes rest on the soil and usually get damaged long before picking. Mutants with better plant height combined with desirable pod bearing pattern have been selected.

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Table I. Effect of various plant densities on plant height, yield and yield components of upright mutant UR 16 and parent variety C 727 in a trial conducted at NIAB

Plant spacing (cm)	Plant height (cm)		No. of pods/plant		No. of grains/plant		Grain yield/plant (g)	
	C 727	UR 16	C 727	UR 16	C 727	UR 16	C 727	UR 16
7 x 7	72.2	67.7	34.7	35.0	36.8	37.6	4.54	5.26
7 x 15	67.4	64.3	43.6	40.8	47.9	44.2	5.93	6.11
7 x 22	64.9	63.7	42.1	39.2	45.5	44.7	5.92	6.67
15 x 7	74.8	66.6	35.0	33.7	37.5	37.4	5.01	5.79
15 x 15	69.1	62.3	42.3	40.8	45.4	51.5	6.53	8.00
15 x 22	70.5	63.3	48.9	39.7	50.3	42.2	6.76	6.28
22 x 7	76.4	66.0	36.9	39.8	44.0	48.0	5.88	6.67
22 x 15	72.9	62.9	52.2	66.3	54.2	82.1	7.75	11.80
22 x 22	72.8	63.2	33.3	55.4	38.7	59.9	4.77	9.75
Average	72.2	64.4	41.0	43.4	44.5	49.7	5.90	7.37

Table II. Performance of four mutant lines in National Coordinated Yield Trials conducted in NIAB during 1977

Mutant line	Cultivar	Days taken to mature	Yield (kg/ha)	Harvest index (%)
605		77.5	842.00	22.77
NIAB 28		80.7	677.32	20.90
	Pak 17	92.5	622.40	15.41
3854		79.7	732.24	18.54
	6601	94.0	549.18	12.23
1038		81.2	677.32	20.53
	Pak 22	94.0	750.55	17.91

Table III. Performance of four mutant lines in National Coordinated Yield Trials conducted at NIAB during spring 1978

Mutant line	Cultivar	Days taken to mature	Yield (kg/ha)	Harvest index (%)
605		70.0	588.65	20.03
NIAB 28		71.5	756.84	24.81
	Pak 17	80.7	630.69	16.60
3854		71.5	644.71	20.52
	6601	83.5	553.61	16.16
1038		75.7	742.82	22.94
	Pak 22	82.0	609.67	17.21

USE OF INDUCED MUTATIONS FOR IMPROVING RESISTANCE AGAINST ASCOCHYTA BLIGHT IN CHICKPEA (*CICER ARIETINUM*) AND YELLOW MOSAIC VIRUS IN MUNGBEAN (*VIGNA RADIATA*)

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ABSTRACT

Heavy losses in grain yield may be caused by *Ascochyta rabiei* in chickpea and yellow mosaic virus in mung bean. Genetic resistance against these diseases is scanty in the germplasm screened so far, therefore, mutation breeding programme was initiated to induce resistance. Two cultivars of chickpea were irradiated with 10 and 20 kR of gamma rays. 20,000 M₂ single plant progenies, 208 true breeding advanced mutant lines in M₂ generation and 23 pure lines/cultivars were screened at Faisalabad under artificial and at Attock under natural epiphytotic conditions. A few mutant lines showed varying degrees of resistance against blight at one location. Only two mutant lines, CM 68 and CM 72, showed resistant reaction at both locations, however no mutant line or cultivar was immune against blight.

Eight local cultivars of mung bean were irradiated with gamma rays. Screening against yellow mosaic virus was done in M₃ generation so as to recover recessive mutations. Out of 128 single plant selections made in M₃ on the basis, six mutant lines were confirmed. The moderately resistant mutant line 3854 has good yield potential.

INTRODUCTION

I. CHICKPEA

Chickpea (*Cicer arietinum* L.) is grown in Pakistan on ca. 970,000 ha with an annual production of 541,000 t. Average yield is only 540 kg/ha. One of the limiting factors is the occurrence of blight disease caused by the fungus *Ascochyta rabiei* (Pass.) Lab. This is the most devastating disease in the larger part of chickpea growing areas of Pakistan which includes the north western rainfed area of the Punjab province and the adjoining area of the Frontier province. The incidence of disease was first reported in 1911⁽⁶⁾. The soils of the conventional chickpea growing area are usually sandy loam of uneven contour; irrigation facilities are mostly lacking and winter rains provide the only source of moisture for sowing winter crops. During epiphytotic years blight leaves little to harvest in the field.

Ascochyta rabiei is a highly virulent pathogen and is specific to chickpea. The pathogen attacks all parts of the plant except the roots, at all stages of growth. During initial stages of growth the apex of the young shoot is infected and the shoot loses turgidity and the plant gradually dries off. If the disease attacks the plant at a later stage, pods get infected and the mycelium may enter the testa as well as the cotyledons. The pathogen survives on the infected debris or seed during off-season. The development of the disease is strongly influenced by climatic factors such as rainfall, temperature, humidity, wind direction and its velocity.

Varietal resistance would be the most effective means of controlling the disease. However, genetic variability against blight is scanty. In Pakistan F₈, C 12-34, C 235 and C 727 cultivars once reported to carry resistance against blight^(10, 2, 5) are now equally affected by the disease, except C 727 which is now scored as moderately susceptible⁽⁷⁾. However, Dr. Inam Ullah Khan, Department of Plant Pathology, University of Agriculture, Faisalabd, reported variety CS 30 showing moderate resistance against blight at one location during the 1978-79 crop season.

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Induced mutations for resistance have been found in various field crops, such as wheat, (1,9) barley (8) and mung bean (11).

The present project is undertaken to obtain resistance against Ascochyta rabiei in chickpea by using physical as well as chemical mutagens.

MATERIALS AND METHODS

Seeds of two cultivars, C 727 and Punjab 6, were equilibrated over anhydrous CaCl₂ to bring the moisture content to a uniform level before treatment with 10 and 20 kR of gamma rays. The irradiated material was space planted in the field at Nuclear Institute for Agriculture and Biology, Faisalabad under disease free conditions. Seed was collected from the main shoot and 3 pods from each secondary branch of all the M₁ plants separately to raise plant progenies in M₂.

The material in the disease screening nursery comprised of

- i) about 20,000 progenies derived from two cultivars, C 727 (brown seeded) and ICG 972 (white seeded).
- ii) 208 advanced mutant lines in M₂ generation previously selected on the basis of their good plant type and yield performance from C 727, 6153, Sindhi, Thal white and Thal mankera cultivars after mutagenic treatment with gamma rays.
- iii) 23 cultivars obtained from ICRISAT, along with 8 pure lines extracted from heterogenous material collected in farmer's fields in Pakistan.

The material for screening against blight was sown at NIAB, Faisalabad as well as at the Govt. Agric. Farm, Attock. At Faisalabad in addition to the above mentioned material M₂ families were also tested under artificial epiphytotic conditions. The seeds were³ dibbled in two rod rows of 3 m length for each treatment and after every 8 rows, two rows of the highly susceptible variety Aug. 424 were planted as spreader.

Spore suspension was prepared from a 10 day old culture grown on chickpea meal medium (chickpea meal 40 gm, agar agar 20 gm, distilled water to make 1000 ml). The suspension was adjusted to 10,000 spores per ml with a haemocytometer. Mixed inoculum of Ascochyta rabiei representing the two reported races of the pathogen was first sprayed when the plants were 90 days old with the help of a fine atomizer and subsequently the crop was sprayed with three times more with weekly intervals.

Data on disease incidence were recorded 3 weeks after inoculation using the following scale:

Immune	=	No infection
Resistant	=	5% foliage infection or few small lesions on stem
Moderately susceptible	=	5 to 25% foliage infection or stem lesions 2 to 6 mm long
Susceptible	=	More than 25% foliage infection or stem lesions bigger in size than 6 mm.

RESULTS AND DISCUSSION

At Attock, heavy rains were received during February (80 mm) and March 1979 (100 mm), continuous humidity and the favourable temperature provided conducive environments for the development of disease and last years disease debris broadcast in the field before the rains provided enough spores.

At NIAB Faisalabad eight advanced mutant lines, CM 66, CM 68, CM 72, CM 73, CM 82, CM 94, CM 96, CM 202 and black seed one cultivar, 292/8 showed a certain degree of resistance against blight (Table I.), while the rest of the material provided susceptible. No mutant line showed complete immunity.

From M₂ and M₃ sixteen single plants showing resistant reaction against blight were selected. At Attock six mutant lines showed resistant reaction; but only two

of these, line CM 68 and CM 72, showed resistant reaction at both locations. However, the erratic behaviour of the other mutant lines is still an enigma and it remains to be ascertained whether there was any difference in the pathogen populations at the two locations.

II. MUNGBEAN

Mungbean (*Vigna radiata* (L.) is an important summer food legume crop of Pakistan and yellow mosaic virus disease affects its yield in warmer areas of the Punjab province. All the lines screened so far at various research institutes are susceptible to yellow mosaic virus⁽³⁾. This virus is transmitted by the white fly, *Bemisia tabaci* Genn.^(4, 12)

Yellow mosaic virus disease is characterised by broad, bright yellow patches scattered over leaflets, which later on form larger yellow areas. Sometimes the entire leaflet becomes chlorotic. Similar symptoms also appear on the pods. Losses due to disease depend upon its severity and the age of the crop at the time of infection. Out of the two distinct types of mungbean varieties grown in Pakistan (i) small seeded "Desi" type and (ii) bold seeded "Kabuli" type, the later group is more prone to this disease. Leaves showing early evidence of infection appear to recover under cool growing conditions which suggest that further spread of yellow mosaic virus requires sustained high temperature.

Since the ideal method of overcoming the disease would be to grow resistant varieties, the present project was started to create genetic variability in local, well adapted cultivars of mung bean and to screen segregating populations for resistance.

MATERIALS AND METHODS

Eight cultivars of mungbean, Pak 3, Pak 13, Pak 17, Pak 18, Pak 22, Pak 23, Pak 32 and 6601 were chosen for this study. 2000 seeds from each cultivar were irradiated with 10, 20, 30 and 40 kR of gamma rays. The M_1 generation was raised during summer 1974 and plant protection measures were adopted to save the crop from attack by white fly. To ensure recovery of recessive mutations, screening for resistance against yellow mosaic virus was started in M_2 . From M_2 generation single plant selections were made on the basis of disease reaction and these selections were studied during summer 1976. Disease assessment was made four times at weekly intervals during the growing period, starting when the seedlings were 20 days old.

RESULTS AND DISCUSSION

A large population of M_3 was screened against yellow mosaic virus and 128 single plant selection were made. These 128 single plant selections made from M_3 generation were again studied in M_4 to confirm their resistant behaviour to yellow mosaic virus. Six mutant lines showing consistent disease reaction were chosen. The extent of disease in these lines remained less than 30%. Five of the resistant mutant lines were relatively short-statured as compared to their parents, whereas mutant 3881 was taller (Table II). Some of the mutant lines showed a slightly higher grain yield potential as compared to the parent cultivars. During summer 1977 the mutant 3854 was finally selected as disease resistant mutant. This mutant is short-statured and possess good yield potential.

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Table I. Description of advanced mutant lines and parent cultivars of chickpea showing different disease reaction against Ascochyta rabiei at two different locations.

Mutant	Parent variety	Mutagen	Dose	Disease reaction at	
				Faisalabad	Attock
CM 46	C 727	Gamma rays	20 kR	S	R
CM 48	6153	"	5 kR	S	R
CM 48-1	"	"	5 kR	S	R
CM 57	"	"	10 kR	S	R
CM 66	"	"	15 kR	R	S
CM 68	"	"	15 kR	R	R
CM 72	"	"	15 kR	R	R
CM 73	"	"	15 kR	MS	S
CM 82	"	"	15 kR	R	S
CM 96	"	"	15 kR	R	S
CM 98	"	"	15 kR	S	S
CM 202	"	EMS	0.5% (6 hour)	MS	S
	Punjab 6	Control	-	S	MS
	6153	"	-	S	S
	292/8	"	-	S	S
	ILG 972	"	-	S	S
	C 272	"	-	S	S

Table II. Morphological characteristics of the parental cultivars of mungbean and their mutant lines showing improved resistance to yellow mosaic virus

Mutant line	Irradiation dose (krad)	Parent cultivar	Plant height (cm)	No. of pods/plant	Pod length (cm)	No. of grains/pod	Thousand grain weight(g)	Grain yield/plant (g)	Disease (%)
1429	10	Pak 18	55.9	47.0	6.6	9.8	35.0	10.0	20
3198	10	Pak 3	57.7	58.0	7.1	11.6	32.3	18.4	40
3845	30	Pak 32	63.5	98.0	6.2	8.4	33.8	27.8	40
3850	30	Pak 32	56.7	70.0	8.1	9.8	35.7	18.7	40
3854	30	Pak 32	57.0	62.0	7.2	10.2	30.3	19.8	30
3881	40	Pak 32	85.2	46.0	5.0	4.0	31.4	10.1	30
-	-	Pak 3	77.1	58.2	6.8	10.6	32.0	17.3	70
-	-	Pak 18	68.0	50.4	6.8	10.6	39.9	11.6	70
-	-	Pak 32	69.8	49.3	6.8	10.4	33.0	13.6	80
6601	-	-	78.8	52.7	6.7	11.0	29.9	11.9	100

VARIETAL IMPROVEMENT OF PULSE CROPS BY THE USE OF NUCLEAR TECHNIQUES

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Abstract

Presently grown varieties of pulses in Bangladesh are low yielding. Traditionally they are grown under marginal conditions. Restructuring of yield components might lead to more productive genotypes. A mutation breeding programme was started to create genetic variability for yield components among locally adapted and introduced cultivars of mungbean, blackgram, lentil and chickpea. Irradiation effects upon M_1 generation are reported.

Introduction

Present varieties of pulses in Bangladesh are low-yielding and were developed a long time ago, mainly through pure line selection. Apart from recognized varieties, there are many local cultivars in farmers' hands from time immemorial. The present project was initiated in the year 1978 for the purpose of (a) collecting germplasm from both within and outside Bangladesh and (b) creating variability in the existing varieties/cultivars for selecting desirable lines. Our breeding objective is to produce pulse varieties with higher yielding potential through changing the yield components.

Before embarking upon a mutation breeding programme one has to determine the sensitivity of the plant material to the mutagens to be used. Seedling growth measurement and plant survival could be used to assess the effects of irradiation. As much is known about radiosensitivity in cereals, but relatively little in pulses, a more extensive study was undertaken on 4 different grain legume species.

Materials and Methods

Air-dried seeds of two accessions of mungbean (Vigna radiata), MB-55 and MB-56 (origins: single plant selections from two local collections from Sitakundu and Jessore, respectively) and two accessions of blackgram (Vigna mungo), B-10 and B-23 (origins: single plant selections from a local collection from Rohanpur and the variety T-9 of Haryana, India, respectively) were irradiated with 50, 60, 70, 80 and 90 kR of ^{60}Co gamma-rays. Seeds of one accession of lentil (Lens culinaris), L-73, and one accession of chickpea (Cicer arietinum), G-174, (origins: recommended lentil variety L-5 and chickpea variety Faridpur-1, respectively of the Bangladesh Agricultural Research Institute, Dacca) were irradiated with 10, 20, 30, 40 and 50 kR of ^{60}Co gamma-rays.

Radiosensitivity studies were carried out by determining germination, root and shoot length, seedling and plant survival, number of days required from sowing to 50% flowering, days to maturity, plant height, number of branches, number of mature and immature pods, number of seeds/pod and seed yield/plant.

Germination was determined from 100 seeds per treatment in petridishes. For germination percentages under field conditions and survival of plants after every fortnight, 300 seeds per treatment of blackgram and 400 seeds of mungbean, lentil

and chickpea were sown in the field. 30 plants were marked out at random from each dose for collecting data on the other characters.

Daily recordings of root and shoot growth were made as follows: Wire-gauge was placed over ordinary staining jars and 20 seeds per treatment were placed on it so that half of the seed was in water. After germination, lengths of roots and shoots were measured every 24 hours. Twenty seeds per treatment were used in this experiment.

Results and Discussion

Germination and survival

Both strains of mungbean showed similar germination (Table 1). Under field condition, germination was less, but reduction was more pronounced in the treated samples. Seedling death occurred mostly within the first six weeks. There was not much difference between the two strains in plant survival.

In blackgram, both strains showed 100% germination in the petridishes (Table 2). However, germination was less under field conditions. There was a decrease in survival in both strains with increased dose. Seedlings died within the first 4-weeks, plant death was much more in the strain B-23, even in the control sample. This strain is an introduction from India to the local conditions.

In lentil, there was a decrease in germination with increase in radiation doses (Table 3). Plants died mainly during the first two months, specially following a treatment of 30 kR and above. At 50 kR dose, only 38% plants survived up to the harvest time.

In chickpea, a decrease in germination with increased doses was observed in both, petridish and field conditions (Table 3). However, much less seeds germinated in the field. Death of plants occurred at higher doses mainly during the first two months of growth.

Reduced plant viability in the M_1 generation is a common phenomenon (Borck, 1965; Rana and Swaminathan, 1967; Shaikh, 1973 and Visona *et al.*, 1966). Recording of plant survival every two weeks helped in determining for each species time during which radiation induced lethality becomes manifest.

Root and shoot length

Both strains of mungbean had a decrease in mean root and shoot length with increase in radiation doses (Table 4). After 168 hours of growth, root lengths of both strains at 90 kR dose were about one-fifth of the control whereas the shoot lengths at the same dose were about one-third. The differences of root and shoot lengths between the control and treated samples widened with passage of time, indicating a slower rate of growth in the treated material.

At blackgram, the effect of radiation treatments on root and shoot lengths (Table 5) was less deleterious. After 168 hours of growth, root lengths in 90 kR samples of both strains were about one-third of those in the control samples. B-23 was more affected than B-10. Slower rate of root growth became more acute with increasing doses. Similar results were obtained in case of shoot lengths. But the difference in shoot length between the control of 90 kR was smaller than the differences in root length.

In lentil, root and shoot growth at 50 kR was about one-half and one-third of the control, respectively (Table 6). In the chickpea strain G-174, reduction in root and shoot length with increase in radiation dose (Table 6) followed about the same pattern as in lentil but there was more reduction in root growth.

The inhibition of root and shoot growth of the various strains following in radiation indicated strain-specific differential radiosensitivity. In general, root lengths were more affected than shoot lengths.

Other plant characters

Effects of gamma-irradiation on various other characters of the M_1 plants of mungbean are presented in Table 7. Flowering and maturity were delayed in different degrees for both strains of mungbean. The delay was progressive with increase in dose.

There was a decrease of plant height in the irradiated samples, more in MB-55 than MB-56. The number of branches per plant, in general, increased with radiation dose, seemingly more in MB-56 than in MB-55.

The number of mature pods/plant decreased with increase in radiation doses. But in MB-56 there was increased pod setting in the medium dose range. There were slightly more immature pods/plant after radiation treatment.

Number of seeds/pod decreased with dose in MB-55 but not in MB-56. Seed yield/plant followed the pattern of number of pods/plant and number of seeds/pod.

In blackgram (Table 8), there was only five days delay in flowering and four to seven days delay in maturity following the highest dose of radiation. Plant height and number of branches were only little effected. A slight increase in number of branches occurred in B-10 following 50 kR treatment.

There were indications of augmented pod-setting in 50 kR of B-10 and in 60 kR of B-23. Decreased pod-setting was observed at higher doses. Number of immature pods/plant was not affected B-10, but in B-23 reduction occurred from 60 kR onward.

Number of seeds/pod increased following 50-60 kR doses and decreased at high doses. Seed yield/plant followed the pattern of number of pods per plant and number of seeds/pod.

In lentil (Table 9), number of days from sowing to 50% flowering was not virtually affected but about a week's delay was observed in chickpea (Table 9). Maturity period in lentil was delayed 13 days in 50 kR treatment, in chickpea it was only seven days. Plant height was reduced in both species. Number of branches/plant in lentil was slightly increased at 10 and 20 kR, but in all the irradiated chickpeas.

A dose dependent decrease in mature pods was observed for lentil but an increase in all doses of chickpea in accordance with the number of branches. Number of immature pods/plant increased also substantially in chickpea.

Very little variation in number of seeds/pod was observed. Seed yield/plant declined in both species but only little in chickpea.

Response in plant height to radiation doses, enhancement or reduction, have been reported in Linum (Bari, 1966), Lens (Sinha, 1967) and, Lathyrus and Vicia (Shaikh, 1972). Delay in flowering due to irradiation was reported already in Lathyrus and Cicia (Shaikh, 1972). Increase in number of branches/plant was found in Linum (Bari, 1966), Lathyrus ochrus and L. sphaericus (Shaikh, 1976).

Increase in the number of mature pods was observed before in five species of Lathyrus and one of Vicia (Shaikh, 1976).

Seed harvesting from M_1 plants and plans for M_2

Considering recommendations for optimal economy in mutation experiments (Redei, 1974; Brock, 1978) only two pods per plant of mungbean and blackgram, and 10-15 pods from lentil and chickpea were collected for growing the M_2 progeny. First formed pods were collected with the understanding that they would contain more mutations than the later formed ones since elimination of radiation induced aberrations has been found to occur with growth of the M_1 plants (Shaikh and Godward, 1974). Seeds thus collected have been bulked per radiation dose. Seeds of 30 most affected plants from each dose have been collected separately. They are expected to carry more mutations than the normal looking ones. All these seeds will be sown in the M_2 generation and mutants will be selected on the basis of agronomic characters.

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ADAPTABILITY STUDIES WITH MUNGBEAN (VIGNA RADIATA) TO IDENTIFY SUITABLE GENOTYPES FOR SUMMER CULTIVATION IN BANGLADESH

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Abstract

Time sowing experiments can provide excellent information about adaptability and yielding capacity of germ plasm, if appropriate observations are made. Such an experiment was carried out with 60 strains of mungbean with 16 sowings over the year. Productivity per day appeared to be a most useful selection criterion in this study.

Traditionally mungbean is grown in Bangladesh during the winter months with and without irrigation. It may therefore directly compete with other winter season crops namely, other legumes, wheat, oilseeds and vegetables, besides Boro (winter) rice which is usually the major crop in irrigated areas. A short duration summer type mungbean variety would be ideal to cultivate in the period between jute and wheat or between Boro and Aman (autumn) rice.

A project was initiated at INA to study the adaptability of mungbean germ plasm over various seasons of the year. A time-sowing experiment was conducted in pots starting on the 1st of June, 1977. The sowings were repeated at regular intervals of 15 days. The last sowing was done on 15th May, 1978. Sixty strains were used, including 51 introductions from Sri Lanka, India, The Philippines, Iran and Indonesia, five high yielding mutants developed at INA and four cultivars collected from farmers fields. Observations were made on various field parameters.

From the data obtained the varieties/strains could be grouped into three categories, (a) winter types, (b) summer types and (c) suitable for year-round cultivation (figures 1-5).

All three types have distinct suitability for different conditions. The following conclusions may be of interest to legume breeders in general:

1. To assess the yielding ability, "productivity/day" has been found to be more meaningful than any other mode of expression of yield. This is especially so when the selection is intended for early types, as is the case in Bangladesh.
2. Strains that showed wider adaptability over a range of environments (in time) may prove to be highly useful for the marginal farming conditions.
3. It is possible to select genotypes of mungbean which could be grown under rain-fed conditions. There has, however, to be initial moisture for good germination and sustenance of seedling for some time.

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4. The types which mature early and have a reasonable yielding capacity are those which get an early head-start and maintain this throughout the growing season.
5. There are major genetic differences in the 'hargest index' which should be subject to exploitation.
6. Screening of germ plasm, natural as well as induced for adaptation can easily and cheaply be done over a period of time when installations for artificial control of photoperiod and temperature are not available.
7. Time sowing experiments may provide valuable basic information on the material under consideration and at the same time open enormous possibilities of selection particularly if yield/day is used as a criterion for selection.

The data collected in this experiment are being analyzed in detail. This brief note is presented only to emphasize the value of time-sowing experiments in the screening methodology. It may be mentioned here that a similar experiment was conducted on rice mutants at our Institute. In the case also it was clear that correct sowing time might make a big difference to the ultimate expression of the yielding capacity (Rahman and Kaul, 1978).

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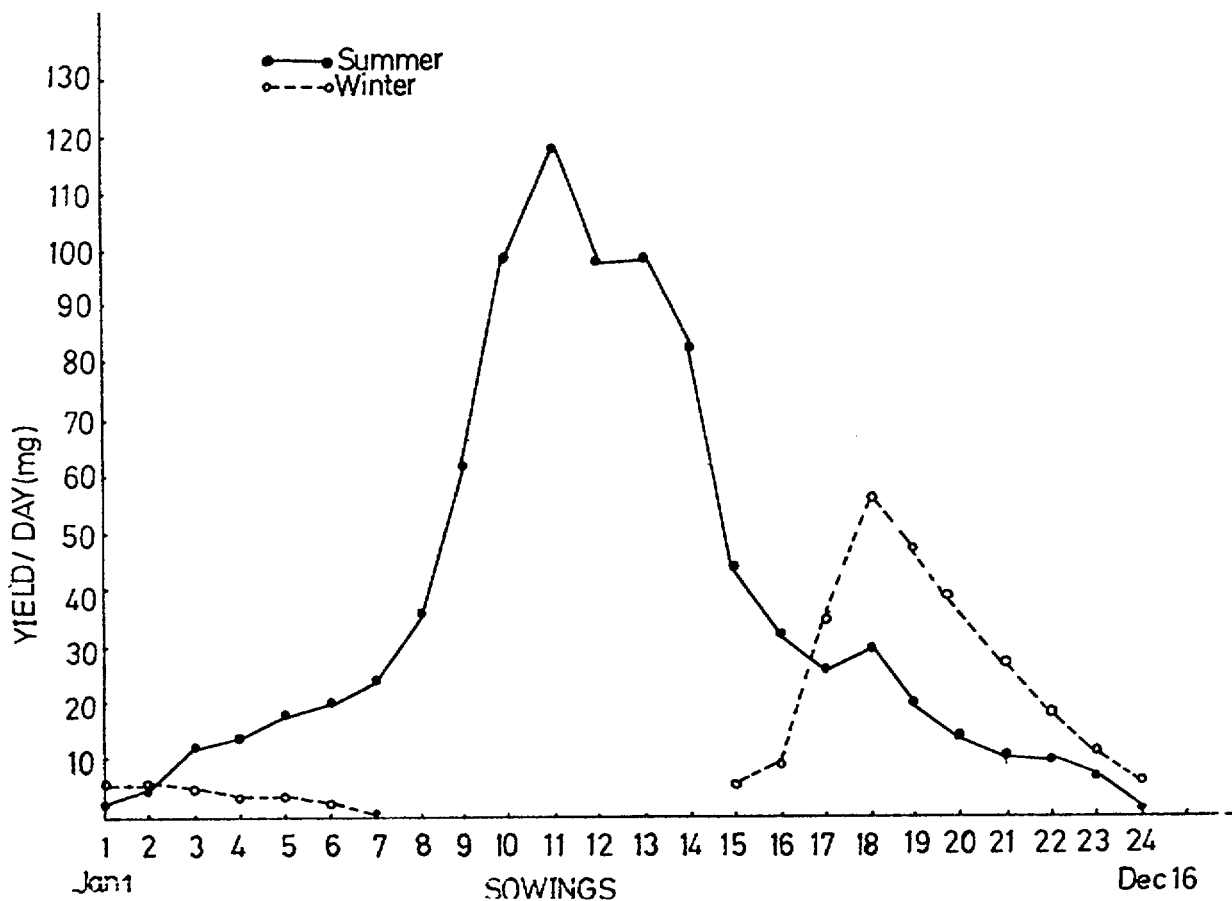


FIG. 1.

Ten summer vs winter types

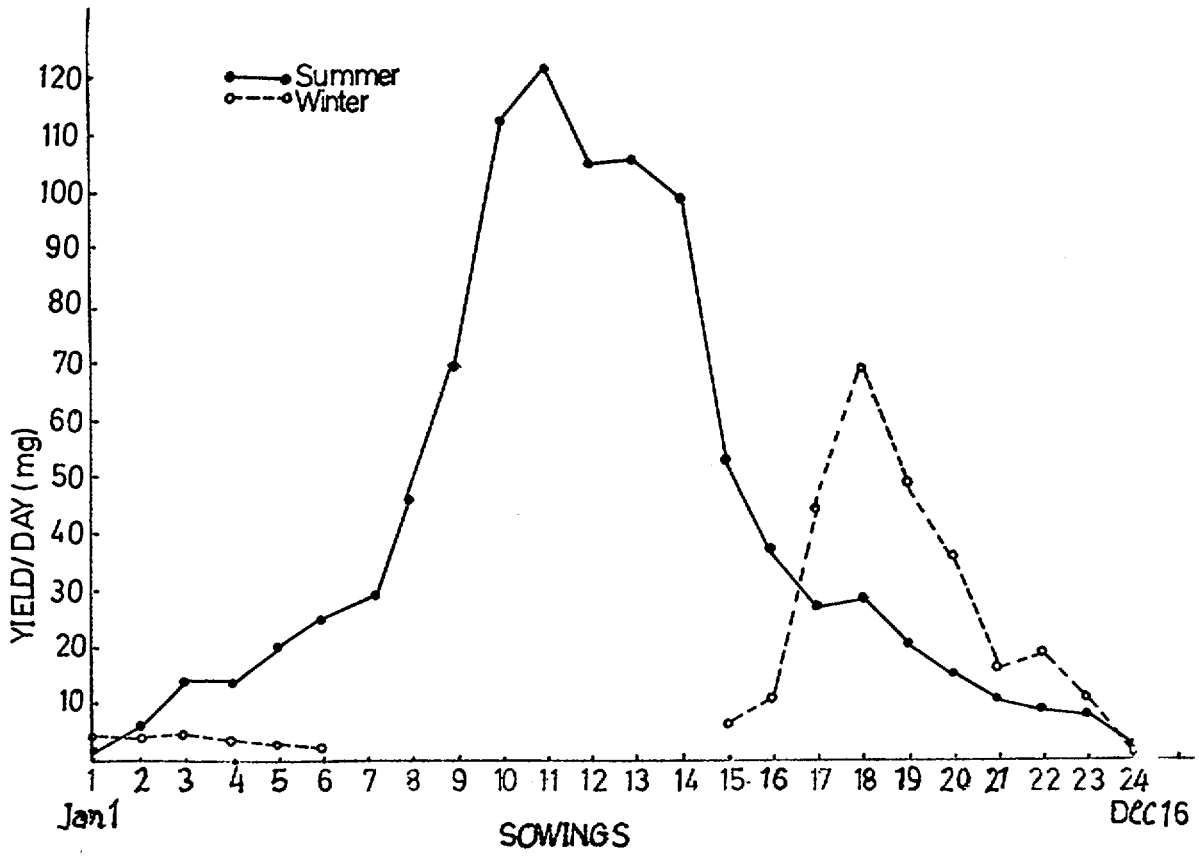


FIG. 2.

Three highest yielding summer vs. winter types

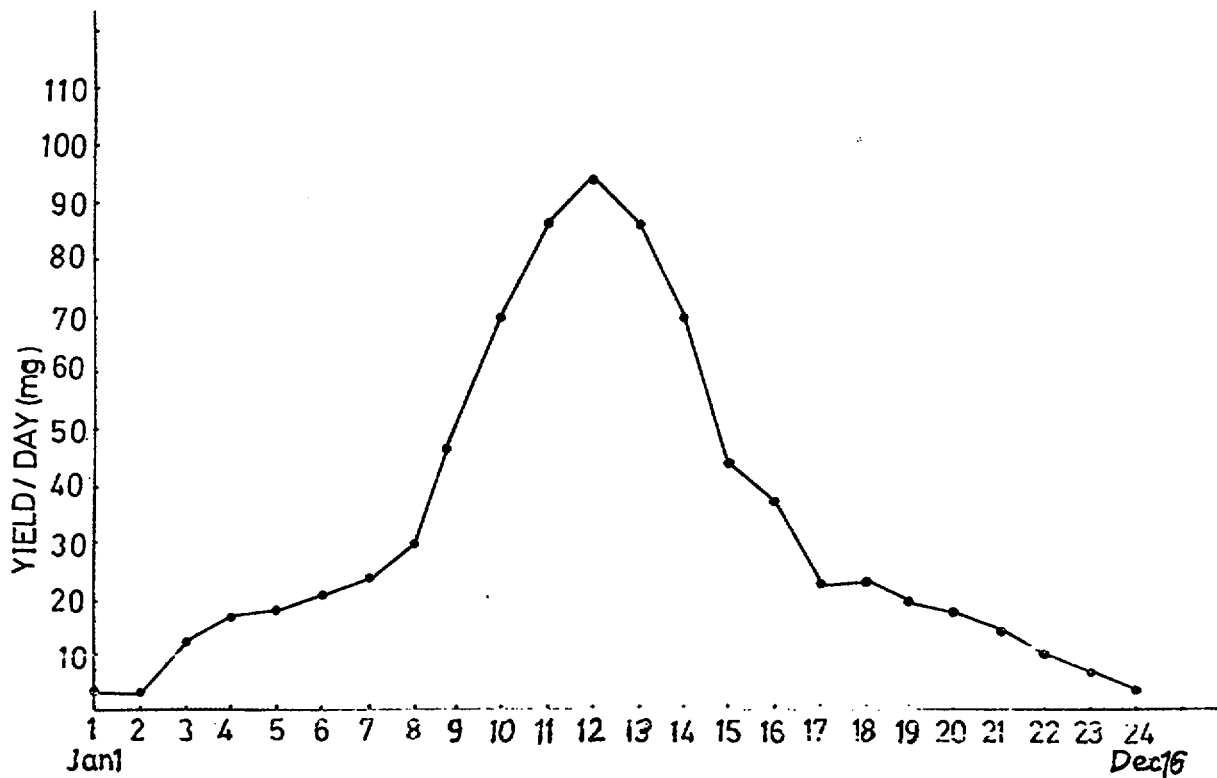


FIG. 3.

TWENTY STRAINS (Grow all over the season)

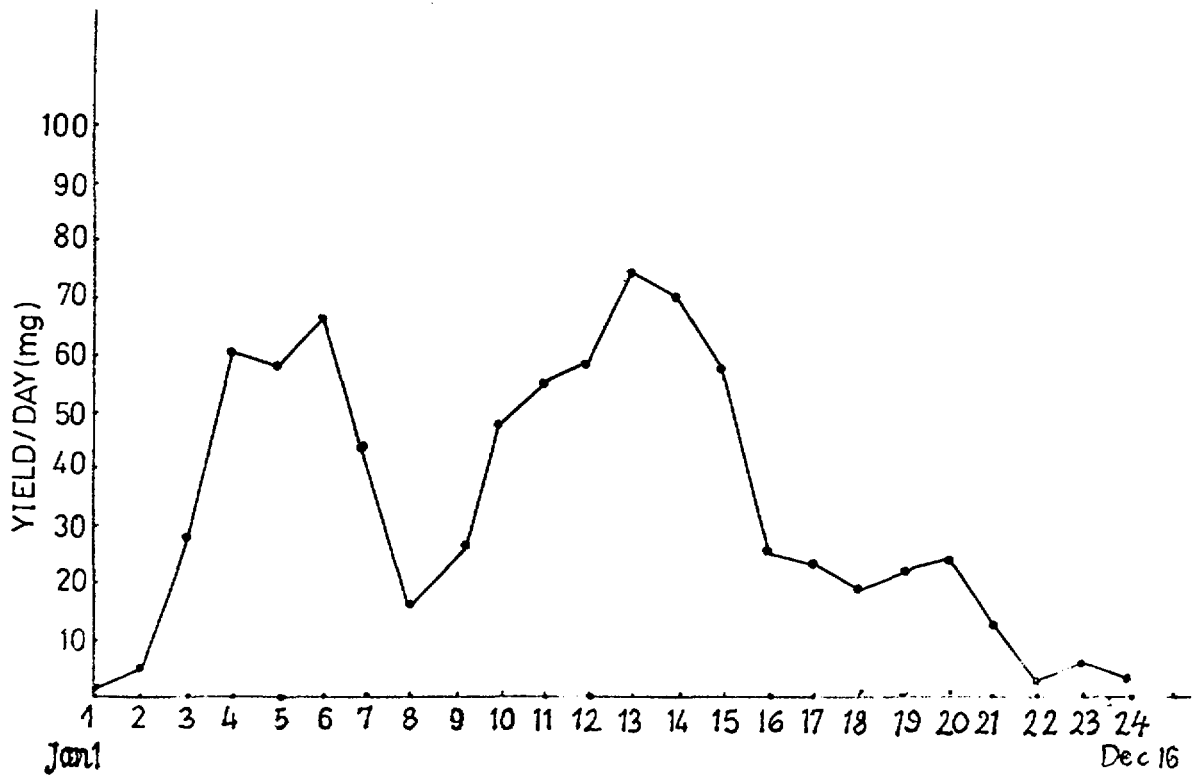


FIG. 4.

Performance of Acc.No.70 (Local collection from Bhola)

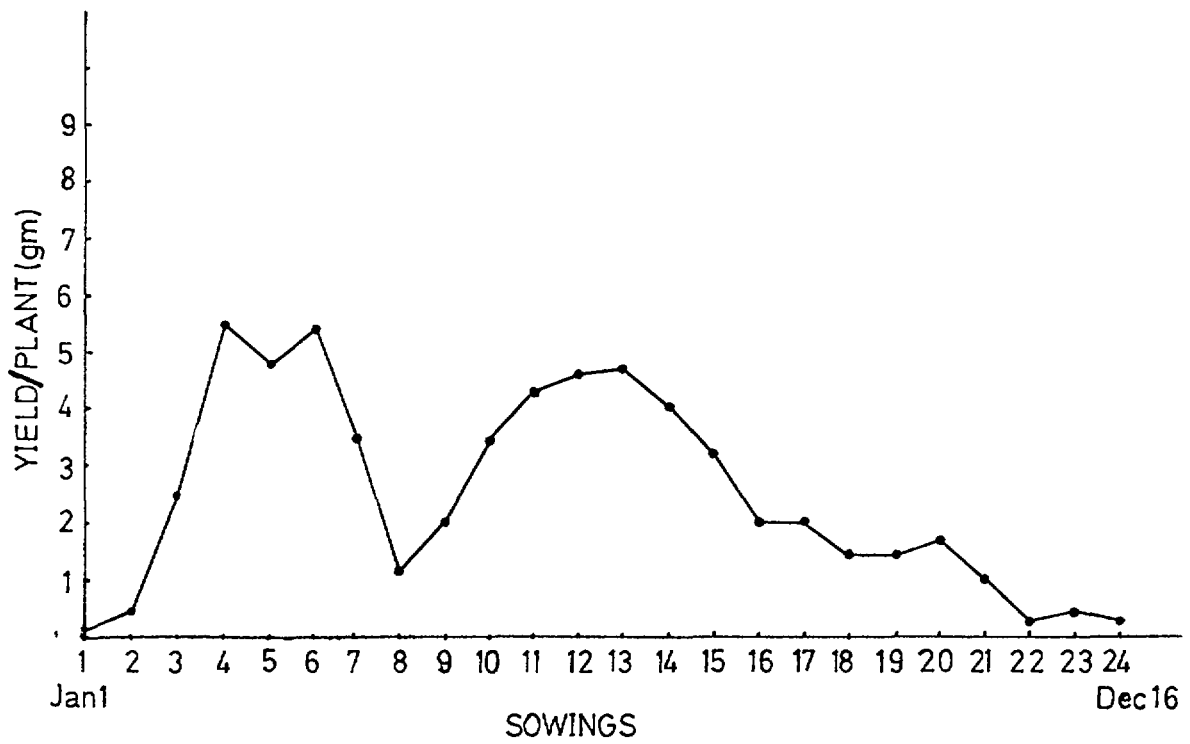


FIG. 5.

Performance of Acc.No.70 (Local collection from Bhola)

VARIETAL IMPROVEMENT OF MUNGBEAN AND BLACKGRAM THROUGH MUTATION BREEDING IN THAILAND

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Abstract

The Department of Agriculture, Bangkok, since 1972 is concerned with the improvement of mungbean and blackgram production through plant breeding. Breeders face the problem that desirable traits for variety improvement are rarely found in nature. Therefore, a mutation induction experiment was started to create additional genetic variability.

Introduction

Mungbean (*Vigna radiata* L. Wilczek.) and blackgram (*V. mungo* L. Hepper) are becoming important crops in Thailand. In 1977, the production of these two legumes was estimated about 130,000 for mung and 30,000 for blackgrams with a value of U.S.\$ 100 million. About 50,000 tons of mungbeans and 30,000 tons of blackgrams were exported. The demand for these two crops will substantially increase in both domestic and foreign markets due to their rich nutritive value, popularity in making various food products and usefulness as vegetables.

From agronomic stand points, these two legumes are well adapted to low and humid tropics, especially in Southeast Asia. Work on varietal improvement and crop management made them earlier in maturity, easier for management and suited for existing cropping systems. In addition to extra income, farmers growing these two legumes would benefit from improving soil fertility and obtaining animal feeds.

The Oil Crop Branch, Department of Agriculture, Bangkok had initiated research work on these two legumes already in 1972. A germ-plasm collection of about 2,000 entries of mungbeans and 200 of blackgrams were evaluated and documented. As result of these studies, one variety of mungbean, Uthong I and one of blackgram, Uthong II, were released and well received by farmers. Conventional breeding programmes are being conducted to obtain better varieties.

General background

Although some success had been achieved from early work on varietal improvement, several problems remain to be solved in order to make these two crops more reliable, profitable and incentive to farmers. Work on these two crops was rather limited and confined to Asian countries, where they are often treated as minor crops.

Breeders working on these two semi-domesticated crops always are confronted with the problem of narrow genetic variation within germ plasm collections. Most desirable traits necessary for superior varietal improvement were seldom found in nature. The awareness of such constraints leads to the concept of mutation induction in order to create additional genetic variation.

Scope of work

The goals of the mutation breeding project were set up as follows:

1. Plant type: Short and erect stature, more branches, resistance to lodging, loose leaf canopy, resistance to pod shattering.
2. Physiology: Photoperiod insensitivity, uniform in flowering and pod setting, early maturing, drought tolerance.

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3. Resistance to major diseases such as Cercospora leaf spot, powdery and downy mildews, and certain seedling and stem rots.
4. Seed quality: Large and uniform seed size, higher protein content, good germination and seed longevity. In the case of mungbeans, shiny and uniformly green seed coat is desirable. For blackgrams, larger hilum and evenly black seed coats are preferable.
5. Other characters: In addition to the above, other deviation and abnormalities of plant morphology resulting from radiation effects will be selected for evaluation and studies. However, agronomic characters would receive greater attention.

Materials and methods

Mungbean seed of recommended varieties were irradiated with gamma rays (from Cobalt 60) at the National Atomic Energy Authority in May 1979. Literature suggested dosages of 0, 10, 20, 30 and 40 krads as appropriate for seeds. One kilogram each of treated seeds was planted at Uthong Experiment Stations one week later. Germination, emergence, seedling performance and abnormalities will be recorded. M_2 seeds will be harvested for.

Blackgrams will receive a similar treatment in the late rainy season, in August, due to its photoperiod sensitive habit. In order to obtain good germination, harvesting should be done after rains completely stopped i.e., in November.

M_2 seeds of these two crops will be planted in January 1980 for mutant selection.

Conclusion

This is just an outline of work to be performed in 1979/80. The main purpose is to find desirable traits through the use of induce mutation to substitute or to enrich natural occurrence. Once the goal has been achieved, whether the superior mutant will be directly released to farmers or subjected to conventional breeding programme is matter of later considerations.

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IMPROVEMENT OF MUNGBEAN BY X-RAY IRRADIATION

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Abstract

Mungbeans are popular as food in Korea but in the past received little attention in agricultural research. Low yields, pod shattering and susceptibility to certain diseases appear as the main problems. Gene sources are insufficient, therefore an induced mutation project has been initiated. A productive local variety Kyungge no. 5, and a variety M-317 introduced from AVRDC (Taiwan) are used as experimental material. After appropriate radiosensitivity tests, seeds were irradiated with 30 and 40 kR X-rays.

Introduction

With a long cultivation history, mungbean has been used in Korea for many kinds of food as an excellent source of protein. Nevertheless, the crop has been regarded only as a minor crop and little is actually known about it. Nowadays, more attention is given to mungbean in our country.

The area under pulse crops is about 14 percent of the total cultivated area of 2.3 million hectare (Table 1). Among pulse crops, soybean covers about 79 per cent of the area (Table 2) and is responsible for about 83 per cent of the total production. Mungbean accounts for only 2.5 per cent of the total area of pulses and 1.7 per cent of the pulse production.

Since self-sufficiency was achieved in main cereal crops, the mungbean has attracted special attention of researchers to raise the productivity. Mungbean acreage has increased because of the nutritional value, short crop duration and drought resistance, and popularity for special preparation. Production levels are not up to demand.

At present, the major problems of mungbean production in Korea are the low yielding potential of varieties and the pod shattering which forces farmers to harvest individual pods as matured. To solve these problems, a large collection of foreign and domestic lines was evaluated by our Laboratory. (Table 3). Some varieties were promising in yield and seed size, while no gene sources were found for non-shattering and for cercospora disease and aphid resistance. Hence, our project considers to improve such main defects of this crop by means of induced mutations.

Parent material:

From careful evaluation of mungbean germ plasm, Kyunggi #5 and M-317 were adopted as the starting material for this project. Kyunggi #5, is a highly productive variety of our native collection yielding over 1300 kg per hectare with medium seed size (37 g per 1000 seed) but susceptible to shattering, Cercospora leaf spot and aphid. M-317, introduced from AVRDC, has high yielding potential (1100 kg/ha) and large seed size (60 g per 1000 seed) but also susceptible to shattering, cercospora leaf spot and aphid.

Table 1. Area, Production and yield per hectare for food grains in 1977

	Area		Production		Yield kg/ha
	ha	%	M/T	%	
Rice	1,230,040.5	53.5	6,005,610.2	75.0	4,950
Barley	545,581.3	23.7	862,037.0	10.8	1,580
Potatoes	129,270.4	5.6	602,415.6	7.5	4,660
Pulses	319,412.4	13.9	383,321.0	4.8	1,200
Other	75,130.7	3.3	151,879.6	1.9	2,020
Total	2,299,435.3		8,005,263.4		

Source : Year Book of Agriculture and Forestry Statistics,
Ministry of Agriculture and Fisheries, Korea, 1978.

Table 2. Area, Production and yield per hectare for pulses in 1977

	Area		Production		Yield kg/ha
	ha	%	M/T	%	
Mungbean	7,963.5	2.5	6,501.0	1.7	820
Soybean	250,620.8	78.5	318,734.6	83.2	1,270
Redbean	35,217.9	11.0	34,521.4	9.0	980
Kidneybean	2,953.4	0.9	2,578.3	0.7	870
Pea	2,490.0	0.8	2,248.7	0.6	900
Peanut	7,838.2	2.5	8,572.4	2.2	1,090
Other	12,328.6	3.9	10,164.6	2.7	820

Source : Year Book of Agriculture and Forestry Statistics,
Ministry of Agriculture and Fisheries, Korea, 1978.

Table 3. Phenotypic variability for seed yield and important agronomic characters of 75 varieties

Characters	Range	Mean	S.D.	C.V. (%)
Yield (kg/ha)	210.5-1,257.5	757.9	246.60	32.54
Seed wt. (g /1000)	18.4- 69.7	46.1	13.19	28.63
Days to 1st ripe pod	69.5- 106.0	81.8	8.85	10.82
Plant ht. (cm)	32.3- 127.8	79.0	19.25	24.37
No. of pods/plt.	6.4- 33.6	16.0	5.68	35.54
No. of seeds/pod	7.2- 14.6	11.7	1.94	16.60
Pod length (cm)	5.0- 16.8	10.6	2.57	24.16
Protein (%)	19.66- 28.28	24.18	1.77	7.31
Oil (%)	0.70- 1.78	1.05	0.22	20.87

Table 4. Preparation of M2 seed materials

Variety	Radiation Source	Dosage	No. of Plants Harvested
Kyunggi #5	X-ray	30 KR	1,950
		40 KR	1,770
M-317	X-ray	30 KR	1,167
		40 KR	990
Total			5,877

Scheme to develop resistant mutant lines:

1. Determination of appropriate dose ranges. 100 seeds from each of these varieties with about 12% of water content were exposed to 5, 10, 15, 20, 25, 30, 40, 50 and 60 kR of X-rays from SHT 250 M-2 X-ray generator. All irradiated seeds were immediately planted in sand bed of a greenhouse with three replications. 15 days after seedling emergence, germination rate, seedling height, root length and survival rate were determined as criteria of radiation sensitivity of the mungbeans. 30 kR and 40 kR of X-ray were adopted as appropriate for the mutation breeding experiment.

2. Preparation of M_1 and M_2 generation:

Two thousand seeds per dose from each variety were irradiated with 30 kR and 40 kR of X-rays. M_1 generation was grown in the field with spacing of 60 cm between rows and 10 cm between plants in the end of May, 1978. All plants were harvested individually and we prepared 5877 progenies for the selection of desirable mutant characters in M_2 in 1979 (Table 4).

3. Screening for pod shattering resistance:

Non-shattering of pods is one of the most desirable traits because otherwise several pickings are required during harvest. In order to screen for shattering resistance, the M_2 generation will be grown in the field (2 m rows with 20 seeds per line) with controls in every 20th row. The degree of shattering will be scored at maturity, using a scale of 1 (highly resistance) to 5 (highly susceptible). All mutants that show less shattering than the control will be harvested individually for further progeny test with high selection pressure.

M_3 lines will be grown in single rows 3 m long with 60 cm between rows, with controls in every tenth row. All but 5 plants on both sides of the rows will be used to determine seed yield and other important agronomic characters. The plants remaining after the middle section was harvested will be scored from 1 to 5 for the degree of shattering.

In the M_4 and following generations, mutant lines selected will be tested in three⁴ rows with three replicates and the centre 2 m of the middle row will be harvested for seed yield. Other traits will be compared with the original varieties.

4. Screening for resistance to cercospora leaf spot:

The cercospora leaf spot has been a serious disease in mungbean and resistance should be improved. Screening will be conducted both under greenhouse and field conditions in collaboration with the pathologist group in our Laboratory.

- (a) Greenhouse screening

In order to screen during winter season, about 20 - 30 M_2 seeds will be planted in a nursery bed in a greenhouse and the seedlings will be inoculated at the three to four trifoliate leaf stage by spraying with spore suspension. Since infection rate is somewhat low in seedling stage, the plants will be inoculated once again in adult stage to minimize the disease escaping. The screening nursery bed is maintained in adequate humidity for disease development. Degree of resistance will be estimated by observing the number and size of lesions on the scale from 0 to 4: 0 = no infection; 1 = a few small lesions on infected; 3 = more than 50% of the leaf area infected; and 4 = more than 75% of the leaf area infected, accompanied by considerable defoliation.

- (b) Field screening

Screening will be conducted in the field without artificial inoculation since natural infection in the field is usually sufficient for severe development of the disease. Selections will be made based on the type of infection and the severity as compared with the original varieties.

5. Screening for resistance to aphid:

Screening for aphid resistance will be carried out both under greenhouse and field conditions in collaboration with the entomology group in the Laboratory. For greenhouse screening, 5 - 10 M₂ seeds and controls are sown in the nursery in a greenhouse. Aphids will be released on each seedling at the second trifoliate leaf stage. Resistance will be examined on the seedlings, and the plants which have a lower number of aphids than the original variety will be tested further under field condition.

The field tests will be conducted under natural condition without artificial infestation by aphids. Other procedures will be similar to the greenhouse screening.

Work carried out sofar:

The effects of X-ray on germination, seedling height and root length are presented in Table 5. Seed germination, seedling height and root length were gradually reduced with the increase of radiation dose and varietal differences in radiosensitivity were found. Reduction in seed germination was more in Kyunggi #5 than in M-317, while both varieties were similar in reduction of seedling height and root length as compared with the controls. However, the reduction in root length at high dose was not so remarkable than in seedling length.

Table 5. Influence of X-ray treatment on germination rate, seedling height and root length measured 15 days after sowing height

Dosage (KR)	Kyunggi #5			M - 317		
	Germination (%)	Seedling height (cm)	Root length (cm)	Germination (%)	Seedling height (cm)	Root length (cm)
Control	90.0	10.1	23.5	90.5	14.6	25.5
5	88.8	10.0	23.3	89.8	14.3	25.0
10	87.6	10.0	23.1	90.0	13.9	25.3
15	87.3	9.3	23.6	89.3	13.3	25.6
20	85.8	8.9	23.9	87.6	12.9	25.6
25	85.5	8.6	22.6	87.3	13.0	25.1
30	84.3	8.2	21.7	85.0	12.3	23.5
40	83.5	7.0	20.3	67.5	10.6	22.3
50	83.0	6.2	18.6	63.3	8.9	20.4
60	62.8	3.6	17.1	55.3	5.8	16.2
Mean	83.9	8.2	21.8	80.6	12.0	23.5
LSD.05	14.6	1.2	2.4	12.8	1.2	2.6

MECHANISMS OF RESISTANCE AGAINST UROMYCES IN PHASEOLUS VULGARIS L

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Abstract

To know potential mechanisms of resistance of crop plants against pathogens can help in developing the optimal strategy for a breeding programme. A study on mechanisms of resistance of *Phaseolus vulgaris* against *Uromyces phaseoli* is presented as an example.

Experiments were carried out to elucidate the biochemical mechanism for resistance of *Phaseolus vulgaris* to the rust fungus *Uromyces phaseoli*. All experiments were carried out with one race of the bean rust fungus and two bean varieties: The susceptible variety "Favorit" and the highly resistant, hypersensitive reacting variety "017". After rust infection phytoalexins accumulated in hypersensitive reacting tissue. Phaseollin production was detected 48 h after inoculation and reached a maximum 24 h later. As phytoalexins are highly fungitoxic they are believed to play a role in the resistance of *P. vulgaris* to *U. phaseoli*. Rust infection of the susceptible variety did not result in the production of phytoalexins. Rust infection of the hypersensitive variety did not result in the production of phytoalexins.

To get some information on the mechanism of phytoalexin accumulation, cell walls were isolated from uredospore germ tubes of *U. phaseoli* and extracted by heating under pressure for 2 h at 120°C. The cell wall extract induced protection to the rust fungus when infiltrated into susceptible bean leaves at least 2 days before the inoculation with the pathogen. However, it can not protect the plant when applied simultaneously with the pathogen (Table 1). This indicates that the cell wall extract itself has no fungicidal activity but it induces resistance in the treated tissue probably in a similar way as it is known from the elicitors of phytoalexin production.

Table 1. Density of uredosori of *U. phaseoli* on primary leaves of the susceptible bean variety "Favorit". Extract from germ tube cell walls or water were infiltrated into the leaves. Inoculation followed at different times after infiltration.

Time between infiltration and inoculation	Density of uredosori (%)	
	Water	"Elicitor"
10 h	100	108
1 day	100	72
2 days	100	9
3 days	100	12
6 days	100	1

Research carried out in association with IAEA under Research Agreement No. 2341

Biological activity of the cell wall extract was heat stable and was not digested by proteases. The extract contained carbohydrates and proteins which could not be separated by ion exchange or gel chromatography. Carbohydrates were composed of glucose. These results suggest that biological activity in the cell wall extract might be due to carbohydrates of glucane nature which were first described by ALBERSHEIM and associates as elicitors of phytoalexin production.

Cell wall extract triggered phytoalexin production in both the susceptible and hypersensitive variety. Phytoalexin accumulation started 2 days after infiltration of the cell wall extract into susceptible leaves (Fig. 1) and continued 4 and 6 days after infiltration. Thus onset and rate of phytoalexin accumulation was in agreement with the protective effect of the cell wall extract (Table 1). This indicates a possible role of phytoalexins in host resistance.

Cell wall extract could not protect the plant when applied simultaneously with the pathogen (Table 1). Under these conditions development of the fungus was not affected although phytoalexins accumulated with the same onset and to about the same concentration (Fig. 2) as in the experiment shown in Fig. 1.

These results are difficult to explain with a primary role of phytoalexins in the resistance of bean leaves to bean rust. Assuming, however, that the fungus is sensitive to phytoalexin only during the first 1-2 days of his development and becomes insensitive about 2 days after inoculation it is still possible to explain the resistance of the elicitor treated tissue with the presence of phytoalexins. On the other hand, it can not be excluded that the rust fungus is completely insensitive to phytoalexins and that the induced resistance is due to other metabolic changes occurring after elicitor treatment.

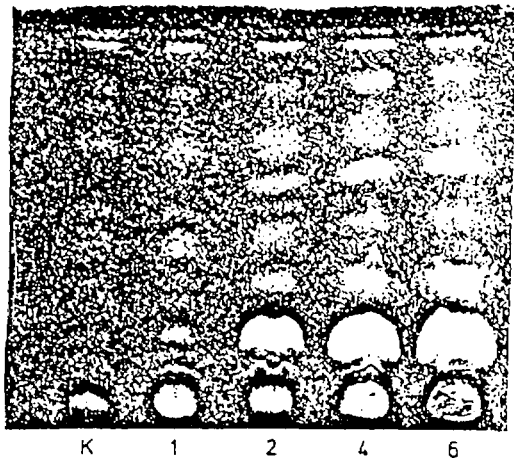


Fig. 1

Fig.1: Phytoalexins in elicitor treated, healthy tissue of the susceptible variety "Favorit".

Fig.2: Phytoalexins in elicitor treated, infected tissue of the susceptible variety "Favorit" Elicitor and inoculum were applied simultaneously. Fungal development was not affected.

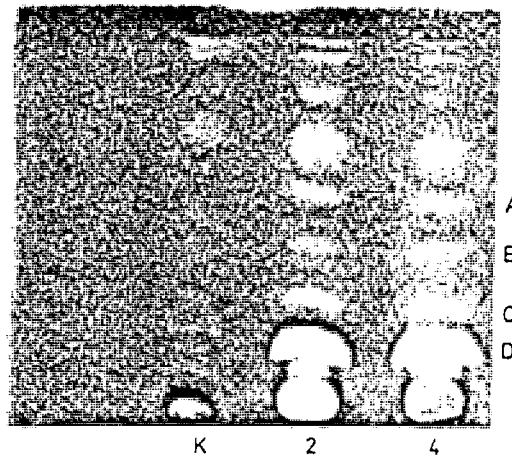


Fig. 2

A: Phaseollin, B: Phaseollidin + Phaseollinisoflavan, C,D: not identified

K: control, 6 days after water treatment
1, 2, 4, 6: 1, 2, 4, and 6 days, respectively, after elicitor treatment.

The involvement of ethylene in plant pathogenesis is well established and documented. Experiments were carried out to measure ethylene production of untreated, infected and elicitor treated bean tissue. Susceptible bean leaves showed no characteristic ethylene outburst during the time course between inoculation and formation of sori. Leaves of the hypersensitive variety O17 showed 2 clear phases of ethylene formation: one apparently connected with the penetration of the infection peg through the stomata 10 - 14 h after inoculation and a second one during expression of necrotic flecks about 40 - 70 h after inoculation with the uredospores of *U. phaseoli*. These characteristic differences in ethylene formation between susceptible and resistant tissue became also visible when hypocotyl segments cut from the 2 varieties were treated with the elicitor: Hypocotyl discs from the variety "Favorit" showed only a slight stimulation of ethylene production after elicitor treatment, whereas this increase was much more pronounced in the hypocotyl segments of the variety "O17". Ethylene production might be involved in the hypersensitive reaction of the variety "O17".

INDUCED MUTATIONS FOR DISEASE RESISTANCE IN BEANS (PHASEOLUS VULGARIS L.)

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Abstract

Susceptibility to Golden Mosaic Virus and to common bacterial blight are problems for the productive cultivation of *Phaseolus vulgaris* in Latin America. A mutant TMD-1 selected from the EMS treated variety "Carioca" shows only very few virus symptoms, but unfortunately is lower yielding than susceptible varieties even under incidence of the disease. The mutant is used in cross breeding. New mutation experiments have been started to obtain other mutants with resistance to Golden Mosaic Virus and to bacterial blight.

The research was initiated with two objectives: Resistance to Golden Mosaic Virus and to common blight diseases.

1. Induction of mutation resistant to Golden Mosaic Virus.

1.1. Description of research carried out

As reported earlier (1), one mutant named TMD-1 was obtained in the M_2 from the variety Carioca after treatment with EMS (0.04, 6 h, 20°C). After seed multiplication, work was initiated in 1978 to evaluate agronomic characteristics of this mutant. One work has the objective of evaluating yield capacity of the mutant, compared with the original variety, under field conditions with absence or presence of the disease. Thus, two yield trials (February and September 1978) with TMD-1 and Carioca (original variety) were sown out in Tiete (Sao Paulo State) where normally no Golden Mosaic disease occurs, and another (February 1978) in Londrina (Parana State) where high natural incidence of the disease is normally observed.

Other work is concerned with the possibility to transfer the virus resistance of TMD-1 to other cultivars. Crosses were made between TMD-1 and the varieties Rosinha G₂, Porrillo-1 and Turrialba-1. The varieties Rrrillo-1 and Turrialba-1 are considered to be tolerant to Golden Mosaic Virus (2). When tested under our conditions, high incidence of the disease occurred in these varieties, but despite this, relatively good plant development and good yield capacity were observed.

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Together with the practical aspect of the transfer of the resistance to these cultivars, studies on the nature of the resistance and the possible linkage or pleiotrop between the shape of primary leaves and low symptoms in the mutant, are being carried out. The segregant generations from these crossings are being inoculated in insectaries or in the field.

The last aspect of the analysis of TMD-1 is to study, if the inheritance of the resistance of TMD-1 is of the same nature as the resistance of another line recently described (3). This line, named Rosinha G₂/69 shows almost the same reaction as TMD-1 when inoculated in insectary or under field conditions. Both from a theoretical as from a practical point of view, it is interesting to determine if the gene(s) causing a similar reaction in TMD-1 and in Rosinha G₂/69 are identical, and the F₁ and F₂ will be inoculated in the insectary.

New mutagenic seed treatments are also in progress in order to screen for new mutants under field conditions. In these experiments, seeds of the varieties Aete-1-38 and Carioca were treated with gamma-rays and seeds from Carioca were treated with sodium azide. The M₁ plants will be harvested individually. The screening will be made under field conditions in February 1980.

1.2. Results obtained

Results of yield trials (February and September 1978) carried out under disease-free conditions, using randomized block design with 4 and 6 replications are shown in Table 1.

Although the trials were made at different times, the results generally coincide and some conclusions can be drawn. Under disease free conditions the mutant TMD-1 showed a productivity much lower than the original. As the number of pods per plant was similar for both materials, the lower productivity observed in the mutant was due to lower weight of seeds and number of seeds per pod. There was no difference in the quality of the seeds in September 1978 while in February the quality of the TMD-1 seeds was worst than that of Carioca. A third trial is being carried out.

To evaluate TMD-1 and Carioca productivity in fields with high disease incidence, this material was included in a trial organized by IAPAR (Agronomic Institute of Parana, Londrina, Parana) in February 1978. This trial was made on a simple 4 x 4 lattice design with two replications and besides the above mentioned, other materials were included. Natural incidence of the disease was very high and uniform within the blocks and reading of the symptoms was made 50 days after germination by Dr. Alvaro Santos Costa of the Virology Section, Agronomic Institute of Campinas (IAC). Marks 1 - 5 were given, the smallest mark corresponding to less intensity of the symptoms. Marks given are shown in Table 2., yields are shown in Table 3.

It can be noted in Table 2. that TMD-1 had the best performance, showing only few symptoms. As for the productivity, however, TMD-1 proved to be very inferior to Carioca and other genotypes that showed a higher expression of symptoms. In view of the occurrence of several unusual factors, such as weather, etc. during this trial, we will not attempt to explain the results - another trial has been set up in February 1979 and only after results are obtained, more ample conclusions can be drawn.

First studies on the genetic nature of TMD-1 resistance were made through crosses between TMD-1 and the cultivar Rosinha G2. Plantlets of F₁ and F₂ generations of this cross were inoculated in the insectary of the Virology Section of the IAC and Dr. Alvaro Santos Costa gave marks and made the selection. It was noted that the F₁ showed the same susceptibility as Rosinha G2 suggesting the recessive nature of the resistance. F₁ also presented

normal primary leaves (auricular format) similar to those of Rosinha G2, while the primary leaves of TMD-1 were more straight at the base (triangular format). This also proves the recessive genetic nature of the primary leaves of the mutant. With regard to F_2 , of a total of 207 plants, 22 showing low incidence of the symptoms which were similar to those observed for TMD-1, which indicated an association between resistance and primary leaf shape. Of the plants selected, two that showed pink colour similar to cultivar Rosinha G2, named R1-TMD-1 and R2-TMD-1, were multiplied. In February 1978 these lines were sown in Londrina, under high disease incidence conditions, together with TMD-1, Carioca and Rosinha G2. The evaluation of the symptoms was made as described for the previous trial. The results of this evaluation are shown in Table 4. With these results it can be confirmed that it was possible to transfer TMD-1 resistance to Rosinha G2 cultivar since the two lines selected resemble Rosinha, but in the insectary showed lower incidence of the disease (1.4 and 1.3) than the original cultivar (2.5).

The transfer of resistance from TMD-1 to Turrialba-1 cultivar could result in a line of great interest. Tables 2 and 3 show that in spite of the high incidence of the disease, Turrialba-1 was the material with the highest yield. Therefore, crosses between TMD-1 and Turrialba-1 had already been done with the objective of selecting better TMD-1 lines with higher yield or Turrialba-1 lines with better resistance. F_1 and F_2 of this cross were made in the greenhouse of CENA, Piracicaba, 195 progenies being obtained. Such progenies (F_2 generation) were sown in the field in Londrina in February 1978 under high disease incidence conditions, together with the original material as controls. A large variation has been noted both between and within the progenies tested, in relation to virus symptoms. Some progenies received a mark 1.0 (little expression of symptoms) and others received a mark 4.0 (high expression of symptoms). However, also within progenies with high expression of symptoms, it was possible to select individual plants with low symptoms. Of the progenies observed, a total of 100 plants which showed high vigour and low symptoms were selected. Seeds of these plants were multiplied in the greenhouse in September 1978 and were sown in the field in Londrina in February 1979, where a high incidence of the disease is expected to aid further selection. Some of the selected plants from TMD-1 x Turrialba-1 cross, were crossed with other materials selected by IAPAR. F_2 from these crosses were also sown in the field in Londrina in February 1979.

Identity test is been made with the cooperation of Dr. Pompeu (IAC). With regard to low incidence of the disease, Rosinha G2/69 selected by IAPAR, was the line closest to TMD-1 as shown in Table 2. It was therefore decided to make crosses between TMD-1 and Rosinha G2/69 to check, through F_1 and F_2 analysis, if the resistance is based upon the same gene(s). Generations F_1 and F_2 of this cross were sown in pots in insectaries in the Virology Section of the IAC. No definite results have been obtained yet.

1.3. Conclusions

1.3.1. A trial was carried out under disease conditions, and which included the resistant mutant and other resistant material together with the original cultivar. It revealed that although the mutant showed the least expression of symptoms, its yield was also lower.

1.3.2. Trials carried out under absence of disease conditions, including the TMD-1 and the original cultivar, showed that the mutant produced on average 38.63% less due to less weight of the seeds and less number of seeds per pod.

1.3.3. Genetic studies on mutant resistance, indicated a recessive nature of the resistance. An association between the form of the primary leaves of the mutant and less incidence of symptoms was observed, but it has not been possible so far to determine if this is due to pleiotropy or a linkage between two genes.

1.3.4. The resistance of the mutant was transferred to other materials, and plants of segregating generations from crosses with other materials were selected and will be observed in the field in 1979, in an attempt to obtain higher productivity with low incidence of symptoms.

2. Induction of mutation for resistance to *Xanthomonas phaseoli* (bacterial Common Blight)

2.1. Work carried out

Efforts to obtain induced mutants resistant to the causal agent of bacterial Common Blight are being made. After June 1978, the necessary preparations for this research work begun. The construction of a special greenhouse where generations M_2 and/or M_3 will be inoculated is not yet completed. It may be ready for use soon as the interior coolers and heaters have been received. Also being developed is a way to maintain high relative humidity in the greenhouse for the purpose of creating suitable conditions for the screening of resistant mutants.

By literature screening and through good contacts with other institutions in Brazil, the appropriate isolates to be used in the screening, the methods of inoculation, and the stage of the plant to be inoculated have been identified. Seeds of several varieties have been treated with gamma radiation or EMS and planted in the field.

2.2. Results obtained

The question which inoculum to use was discussed with research-workers of other institutions involved in bean diseases. Recent publications (4, 5) show that no interaction between cultivars and isolates of the pathogen exists. Races occur with different degrees of aggressiveness but not with different virulence. CNPAF No. 15, the most aggressive isolate of the common bacterial blight (4), will be used in the selection for resistance in the greenhouse.

For field inoculation, the most aggressive isolate present in the local population of the pathogen will be used.

Other points discussed and adopted refer to the method of inoculation and the stage of the growth of the plant to be inoculated. Incision of primary leaves is considered to be appropriate for discriminating different levels of resistance. Although there are some references to the effect that the plants showing leaf resistance may show pod susceptibility (6), the selection of only a leaf resistant mutant is interesting because it reduces the inoculum available for pod infection.

During greenhouse construction, the search for the materials to be used in the screening was initiated. As there is evidence of a resistance to *X. phaseoli* of quantitative nature (7), it was decided that selection should begin with the M_2 generation. In February 1978, seeds of the Carioca cultivar were treated with gamma radiation of 20 and 24 krad and with 0.02, 0.03 and 0.04 M of EMS for 6 hours at 20°C. 800 seeds submitted to each one of the treatments above were planted and the surviving plants collected individually; 300 progenies of M_2 were planted in September 1978 and the plants collected individually. About 3000 progenies of cultivar Carioca (M_2) are available to be inoculated with *X. phaseoli* at the beginning of March 1979.

In September 1978, seeds of varieties Costa Rica L-1095 and Roxo 749 had also been treated with 24 krad of gamma radiation; the surviving plants were collected individually and the M_2 progenies were planted in the field in February 1979. Seeds of the variety Costa Rica L-1095 were also treated with 0.04 M of EMS for 6 hours at 20°C and then planted in the field during September 1978; seeds from surviving plants were planted in the field during February 1979.

Therefore, beginning in May 1979 there will be about 3000 progenies (M_3) to be screened for mutants with resistance to X. phaseoli.

2.3. Conclusion

M_2 and M_3 generations of different varieties obtained by physical and chemical mutagens will be screened under greenhouse conditions by inoculation with a more aggressive isolate of X. phaseoli using the primary leaf incision method.

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Table 1. Results of trials with the mutant TMD-1 and Carioca (original variety)*

Time and Material	Yield Kg/ha	weight of 100 seeds g	Nº of pods/plant	Nº seeds/pod	Seed quality**
February 1978					
TMD-1	1096 ^a	23.24 ^a	17.23 ^a	3.17 ^a	2.73 ^a
Carioca	1712 ^b	27.72 ^b	17.13 ^a	4.66 ^b	4.17 ^b
September 1978					
TMD-1	985 ^a	21.12 ^a	19.48 ^a	3.12 ^a	3.38 ^a
Carioca	1679 ^b	24.12 ^b	18.73 ^a	4.73 ^b	3.50 ^a

* The same letter indicates no statistical significance

**Quality varying from 1 (worst quality) to 5 (best quality)

Table 2. Incidence of golden mosaic virus disease under field conditions (1 = lower expression of symptoms; 5 = maximum expression of symptoms)

Material	Replication		Average
	I	II	
Aeté 1/38	1.5	2.0	1.75
Rosinha G ₂ /69	1.5	1.0	1.25
TMD-1	1.0	1.0	1.00
Carioca	2.5	2.5	2.50
Turrialba	2.5	3.5	3.00

Table 3. Yield (Kg/ha) of several field materials with high incidence of golden mosaic virus disease.

Material	Replication		Average
	I	II	
Aeté 1/38	1200	980	1090
Rosinha G ₂ /69	870	880	875
TMD-1	370	280	325
Carioca	1350	1030	1190
Turrialba-1	1320	1340	1430

Table 4. Symptoms of golden mosaic in lines (R1-TMD-1 and R2-TMD-1) selected from the cross TMD-1 x Rosinha G₂ and in original material under field conditions (1 = lower expression of symptoms, 5 = maximum expression of symptoms)

Material	Replication					Average
	1	2	3	4	5	
R1-TMD-1	1.0	1.5	1.5	1.0	2.0	1.4
R2-TMD-1	1.0	1.5	1.5	1.0	1.5	1.3
TMD-1	1.0	1.0	1.5	1.0	1.0	1.1
Rosinha G ₂	2.5	2.5	3.0	2.0	2.5	2.5
Carioca	3.0	2.0	3.0	2.5	2.5	2.6

MUTATION INDUCTION FOR IMPROVING RESISTANCE OF VEGETABLE LEGUMES AGAINST UROMYCES PHASEOLI AND UROMYCES PISI

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Abstract

Beans (Phaseolus vulgaris) and peas (Pisum sativum) are important leguminous vegetable crops in Egypt. They are attacked by several diseases, especially rusts caused by Uromyces phaseoli and Uromyces pisi. These diseases are widespread in northern Egypt. A mutation induction programme has been initiated to obtain resistant mutants.

As a first step, the effects of mutagens on the M_1 generation have been studied using gamma rays and ethyl methanesulphonate (EMS).

Introduction

Beans (Phaseolus vulgaris) and peas (Pisum sativum) are important leguminous vegetable crops in Egypt. The area planted with beans in 1976 was about 24000 ha and for peas about 6000 ha. These crops are attacked by Uromyces phaseoli and Uromyces pisi respectively. These diseases are particularly widespread in the northern parts of Egypt.

Control of these diseases can be achieved to some extent by agricultural practices which help disease escape or reduce the damage. Chemical protection is possible but laborious, costly and dangerous.

The most effective method of control would be resistant varieties. Mutation breeding may be of value in obtaining new types of resistance or tolerance or by breaking undesirable linkages involving existing genes for disease resistance. Radiation as well as mutagenic chemicals can be used to separate by chromosome breaking and translocations useful disease resistance genes from associated genes that cause deleterious effects on other traits.

Review and Literature

In mutation induction programmes, the effectiveness of mutagens to be applied can be tested by their effects on M_1 performance. The criteria for measuring the degree of damage in the M_1 generation are: the percentage of germination, seedling growth, survival and fertility.

Magri Allegra and Zannone (1963) carried out a study on Vicia sativa and reported varietal differences in mutagen sensitivity. Among several mutagens, EMS induced the highest mutation rate. Enken and Sidorova (1964) treated two different pea varieties (with 0.10% EMS solution for 12 hours) and obtained promising mutants. Heringa (1964) found that EMS caused greater disturbance in the germination capacity of pea seeds and the fertility of later generations than irradiation with gamma rays. Wellensiek (1964) found that EMS was most promising in producing mutations in pea seeds, but with a high degree of sterility carried into the M_2 . EMS treatment yielded approximately 7 times as many chlorophyll mutants and 5 times as many other mutants than the irradiation. Sharma (1965), in peas, showed that gamma-ray doses up to 4000 r had little effect on growth of the M_1 plants, while chemical mutagens (Nitrosomethyl urea NMU, EI, Diethyl sulphate DES) were more effective. Sharma and Rapoport (1965), later on reported from the same experiment that they obtained

29 different mutants. Ten of which were selected from the progenies of M_1 material treated with chemical mutagens, and eight mutants obtained from irradiation. Sharma (1966a, 1966b) in another study stated that chemical mutagens gave more mutations than irradiation and that also a larger number of multiple mutations were produced. Further mutations often appeared in later generations even in the M_6 and later. Higher mutagen doses caused a higher degree of M_1 sterility.

Sutka (1966) treated pea seeds with EMS and increased the range of variation in plant height and number of seeds per pod.

Blixt (1967) studied the differences in radiosensitivity of 34 different genotypes of Pisum sativum and later (Blixt 1969, 1970) extended his study to include 62 different genotypes. Applying the criteria germination and growth inhibition for measuring the effects of M_1 generation, he reported differences in radiosensitivity between genotypes.

Pipie (1968) treated two different varieties of Pisum sativum, with various doses of diethyl sulphate (DS) and/or EMS. He found varietal differences regarding the M_2 mutation frequency and concluded that EMS was relatively more effective.

Magaudda and Donini (1969), with acute gamma irradiation of several pea genotypes, found that germination and survival responded differently to the various radiation doses employed.

Rukmanski (1969) irradiated seeds of three different varieties of garden French beans with various gamma ray doses. He reported greater variability in plant height, stem length, growth period and seed maturation in the M_1 .

Kalloor (1971) treated different varieties of Pisum with EMS and DS. He obtained reduced germination and survival in both cases.

Purpose and Investigation

The purpose of this study is to induce mutations in peas and beans resistant to rust.

Materials and Methods

Peas: In the field:

Pea cultivars for this study were chosen according to their commercial value and their behaviour to rust disease. The following pea varieties are used:

a) Little Marvel:

High yielding variety, short plant type, early flowering, and moderately resistant to rust.

b) Lincoln:

High yielding variety, tall plant type, late in flowering, susceptible to rust.

Irradiation:

750 g of each of these varieties were subjected to 8, 10 and 12 krad of ^{60}Co gamma rays at the Arab Centre of Radiation in Dokky, Giza, Egypt. Seeds were soaked in water for two hours before their exposure to radiation. 5000 seeds from each variety/treatment were sown on November 30, 1978 at Kafr Elzayat, Garbia (about 120 kilometers from Cairo) and the same number of untreated seeds soaked in water for 2 hours as control. Four replicates were used in randomized blocks.

Chemical treatment:

500 g of each of these varieties were treated with EMS. Seeds were soaked in water for eight hours before treatments, then were soaked in 0.5% and 1.5% EMS for four hours and were planted on the same day of November 20, 1978 at Kanater Research Station, Kalubia (about 20 kilometers from Cairo).

3000 seeds from each variety/treatment untreated seeds, soaked in water for 12 hours as control were planted in four replicates in randomized blocks.

Greenhouse tests:

Test for seed germination were carried out by sowing 100 seeds of each treatment in pots in the greenhouse at Dokky, Giza. Untreated seeds were also cultivated as control.

Beans: In the field:

Beans cultivars chosen for this study on the basis of their commercial value and behaviour to rust disease were as follows:

- a) Giza 3: High yielding variety but susceptible to rust.
- b) Giza 4: Moderately yielding variety and moderately susceptible to rust.

Irradiation:

750 g of each of these varieties were subjected to 8, 10 and 12 krad of ^{60}Co gamma rays at the Arab Centre of Radiation in Dokky, Giza, Egypt. Seeds were soaked in water for two hours before their exposure to radiation. 5000 seeds from each variety/treatment and the same number of untreated seeds, soaked in water for 2 hours as control were sown on April 15, 1979 at Kafr Elzayat, Garbia (about 120 kilometers from Cairo). Four replicates were used in randomized blocks.

Chemical treatment:

5000 g of each of these varieties were soaked in water for eight hours before chemical treatments, then treated in 0.5% and 1.5% EMS for four hours, and planted on the same day of March 19, 1979 at Kanater Research Station, Kalubia (about 20 kilometers from Cairo).

3000 seeds from each variety/treatment and the same number of untreated seeds, soaked in water for 12 hours as control were planted in four replicates in randomized blocks.

Greenhouse tests:

Tests for seed germination were carried out by planting 100 seeds of each treatment in pots in the greenhouse at Dokky, Giza. Untreated seeds were also cultivated as control.

Results and Discussion

Peas: Germination and survival in the field:

1. Radiation

Seedlings were counted at 30 and 60 days after sowing in the field. The data obtained are shown in Table 1.

Table 1. Percentage of germination and plant length of peas in the field after exposure to different ^{60}Co gamma doses

Variety	Treatment ^{60}Co γ	Germination %		Plant Length (cm)	
		After 30 days	After 60 days	After 30 days	After 60 days
Little	8 krad	75.4	56.18	18.9	23.5
Marvel	10 krad	48.3	40.31	12.2	26.5
	12 krad	36.7	32.31	8.5	22.9
	Control	80.5	77.62	20.8	34.6
Lincoln	8 krad	32.5	23.37	10.8	14.9
	10 krad	24.7	15.00	7.5	14.2
	12 krad	20.3	8.06	5.5	13.2
	Control	45.8	38.37	15.8	18.1

High doses of radiation reduced the percentage of germination and also it has been noticed that a number of plants died between 30 and 60 days after planting. Dwarf, malformed and abnormal plants were noticed and they gave small grains or unmaturing grains.

2. EMS treatment

Seedlings were counted in the field. The data obtained are shown in Table 2.

Table 2. Percentage of germination and plant length of peas in the field, treated with EMS 0.5% and 1.5%

Variety	EMS % Treatment	Germination %		Plant Length (cm)	
		After 30 days	After 60 days	After 30 days	After 60 days
Little	0.5	71.50	64.67	10.2	50.4
Marvel	1.5	20.83	10.20	7.5	40.2
	Control	80.83	64.83	18.8	55.9
Lincoln	0.5	32.33	8.83	7.5	32.2
	1.5	10.67	2.00	5.3	21.9
	Control	46.83	38.71	10.5	54.4

EMS treatment reduced the percentage of germination and plant length. Great damage of plants occurred after germination. Dwarf, malformed and abnormal plants were noticed. They gave small and unmaturing grains. No difference of reaction to rust disease has been noticed in M_1 following irradiation or EMS treatment.

Beans:

1. Radiation treatment

Seedling counts were in the field. Data are shown in Table 4.

Table 4. Percentage of germination and plant length of beans varieties after 30 days exposed to different gamma doses

Variety	Treatment ⁶⁰ Co gamma	Germination %	Plant length (cm)
Giza	8 krad	27.11	18
	10 krad	27.11	17]
	12 krad	15.40	14
	Control	45.40	20
Giza 4	8 krad	22.60	21
	10 krad	23.10	20
	12 krad	15.50	18
	Control	38.15	25

The percentage of germination in the treated plants decreased, compared with the control. Generally the surviving treated plants were somewhat smaller than the controls.

2. EMS treatment

Germination and survivals

Seedling counts have been made in the field 60 days after sowing (Table 5).

Table 5. Percentage of germination and plant length of beans varieties treated with EMS 0.5% and 1.5% concentrations

Variety	Treatment of EMS	Germination %	Plant length (cm)
Giza 3	0.5%	29.00	34
	1.5%	35.33	30
	Control	53.00	40
Giza 4	0.5%	29.16	38
	1.5%	29.50	35
	Control	48.50	45

The treatments of EMS at 0.5 and 1.5% concentrations decreased the percentage of germination and the plant length.

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THE MUTATION BREEDING PROGRAMME IN GRAIN LEGUMES IN KENYA

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INTRODUCTION

Bean (Phaseolus vulgaris), cowpea (Vigna unguiculata) and pigeon pea (Cajanus cajan) are the most important grain legumes of Kenya. Beans are grown in medium and high potential area whereas cowpea and pigeon pea are adapted to arid and semi-arid areas. Besides grain, cowpea leaves are widely eaten as green vegetable throughout Kenya. Considerable yield losses are caused in these grain legumes due to diseases and insect pests. Many cultivars have poor plant architecture. In some cases the cultivars have high yield potential and possess high degree of resistance to diseases but their seed colour is not acceptable. Research projects for improvement of these grain legumes are in progress at the Faculty of Agriculture, University of Nairobi in cooperation with the Ministry of Agriculture. The mutation breeding programmes was initiated with a view to improve one or a few specific characteristics of promising cultivars.

As the project has just started with effect from 1st March 1979 and funds became available on 11th May 1979 an outline of the work planned is presented for discussion.

PLANT MATERIAL

A brief description of the plant material to be improved is given below:

Beans: The important cultivars are: Mwezi moja (GLP24), Canadian Wonder (GLPIO) and Black bean (NB16) in that order (Table 1).

Table 1. Characteristics of bean cultivars

Cultivar	Yield (kg/ha)	Maturity	Seed	Diseases
Mwezi moja	1800	early	variegated lever colour	anthracnose (<u>Colletotrichum lindemuthianum</u>) halo blight (<u>Pseudomonas phaseolicola</u>)
Canadian Wonder	2500	medium	red	anthracnose, halo blight
Black bean	3000	late	black	Resistant to common diseases

Cowpeas: There are no improved commercial cultivars. Various cultivar mixtures are grown by farmers. Cowpea Improvement Project was initiated in April 1977 and screening of cultivars is now in progress. Table 2 presents some indicative results.

Table 2. Characteristics of cowpea cultivars

Cultivar	Origin	Plant type	Maturity	Diseases/Pests	Other characters
Katuli-108	Kenya	erect	early	Target leaf spot (<u>Ascochyta</u>)	loose seed coat
KCIP-200	Kenya	Semi-spreading	medium	<u>Ascochyta</u> leaf spot	black seed coat
Emma-60	Kenya	erect	early	<u>Septoria</u> leaf spot <u>Heliothis</u> pod borer	pigmented pods
Machakos-74	Kenya	semi-spreading	medium	<u>Ascochyta</u> leaf spot, <u>Heliothis</u> pod borer	Pods covered within foliage
VITA-4	IITA	erect	medium	<u>Cercospora</u> leaf spot, yellow mottle.	wide adaptation
TVX337-3F	IITA	erect	medium	<u>Septoria</u> leaf spot, yellow mottle	-
TVX 1193-9F	IITA	erect	early	<u>Ascochyta</u> leaf spot	15-20% shattering

Pigeon pea: There is no improved pigeon pea cultivar. Locally adapted cultivars are tall and late in maturity. They take about 10 months from planting to harvesting. An early maturity (5-6 months) determinate cultivar NPP199/10 has been introduced. It has green stem, shrunken

Pods, dirty khaki seed coat colour and small seed size. Both types are susceptible to leaf spot disease caused by Mycovellosiella cajani. This disease causes severe yield losses due to defoliation and shedding of flowers and young buds. Another important disease is the Fusarium wilt.

OBJECTIVES

- Beans:
- (1) To improve resistance to diseases such as anthracnose and halo blight in Mwezi moja and Canadian Wonder.
 - (2) To induce white or light seed coat colour in Black bean.
 - (3) To improve plant architecture - bushy plants, strong primary stem with pods above ground.
- Cowpeas:
- (1) To improve resistance to diseases such as Ascochyta leaf spot, Septoria leaf spot, yellow mottle and cowpea mosaic and pests such as aphids, Taeniothrips and pod borers (Heliothis and Maruca).
 - (2) To induce white or light seed coat colour in black seeded cultivar KCIP 200.
 - (3) To improve shattering resistance in TVX 1193-9F.
 - (4) To induce tight seed coat in cultivar kaluli 108.
 - (5) To improve plant architecture - high peduncles above canopy in Machakos 74.
- Pigeon pea:
- (1) To improve resistance to leaf spot caused by Micovellosiella cajani.
 - (2) To improve plant architecture - spreading branches and less determinate habit in NPP 199/10.

Nutritional and Quality factors

- (1) To improve total crude protein and amino acid composition.
- (2) Reduction in anti-nutrient and flatulence factors in seed.
- (3) Development of acceptability and nutrient qualities of cowpea leaves.

MUTAGENIC TREATMENT

Air dried seeds of three bean, seven cowpea and one pigeon pea cultivars were treated with five different doses of 60.Co gamma radiation (dose rate 2430 rad/min) at the Scheibersdorf Laboratory, IAEA, Vienna and seeds returned to Nairobi. Five different doses given to seed samples as proposed by IAEA were:

- Beans: 7 Krad, 11 Krad, 14 Krad, 17 Krad, 21 Krad.
Cowpeas: 15 Krad, 18 Krad, 22 Krad, 25 Krad, 28 Krad.
Pigeon pea: 8 Krad, 10 Krad, 12 Krad, 14 Krad, 17 Krad.

HANDLING OF M₁ MATERIAL

100 seeds per dose were treated. Because the dose response for these grain legumes is not known it was intended first to test the range of radio-sensitivity of these cultivars. This study of radio-sensitivity was performed in the greenhouse. The greenhouse tests for the seedling growth response to five mutagenic doses in M₁ will be used as a basis for selecting the optimum dose levels for the field planting. The dose resulting in approximately 50% seed set in M₁ plants (LD 50) will be noted. Treatments at LD 50 and at dose below and above it will be used for field planting. It is intended to plant at least 10,000 irradiated seeds per variety.

For greenhouse tests treated seeds together with their control (untreated) will be grown in 4 replicates in plastic pots size 10 filled with sterile soil mixture. Treatment effects on germination, seedling height, root length, survival, M₁ injury and sterility will be recorded. M₁ sterility will be measured in terms of pollen abortion and seed setting per pod.

One seed from each pod (modified single-seed bulk method) of all M₁ plants will be taken and grown in M₂ as bulk.

HANDLING OF M₂ AND SUBSEQUENT GENERATIONS

M₂ population will be grown in the field in spaced plating. The single-seed bulk harvest of M₁ will be followed by selection in M₂ of single plants to progeny test in M₃. About 50% of the M₂ populations will be saved for progeny tests in M₃. Selection criteria of M₂ plants will be based on resistance to diseases and pests, plant architecture, seed yield, maturity, seed colour and adaptability to arid and semi-arid conditions.

It would not be possible to screen all M₂ plants for protein content and their amino acid composition it is proposed that checking on protein content will start on M₄ and M₅ populations with desirable agronomic attributes. Similarly tests on acceptability and nutritional attributes of seed and leaves (cowpeas) will be conducted on a few promising mutants.

- GENERAL
- (1) The coordination will be beneficial
 - (2) Methods for determining optimum dose to be applied to the grain legume seeds, screening for diseases and protein content and quality should be discussed.

MUTATION BREEDING OF GROUNDNUT AT TROMBAY

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Groundnut is well-known as the king of oilseeds in India because it contributes about 60 per cent to the total oilseed production in the country. Most households in southern and western parts of the country use groundnut oil for cooking. Besides, a large quantity of groundnut is consumed as roasted kernels. The annual production of oilseeds in India cannot meet the requirement. There is always a deficit which is covered by imports.

A research programme for improving the productivity of groundnut was initiated during 1957-58 at the Bhabha Atomic Research Centre (BARC), Bombay. Using radiations to induce genetic changes in the characters contributing to yield, several mutants with changes of pod size, plant height, leaf colour, number of branches, pods, etc. were isolated in a popular groundnut variety, 'Spanish Improved'. After valuating these mutants, 6 Trombay Groundnut (TG) varieties were selected for agronomic trials in 1965. 'TG-1' with large seed (0.85 gm/seed) and 'TG-3' with normal seed (0.50 gm) size proved to be high-yielding. After further testing in the yield trials of the All India Co-ordinated Research Project of the Indian Council of Agricultural Research (ICAR), these two varieties were officially released for cultivation in 1973. In recognition of this work the "Dr. R.D. Asana Endowment Prize" for the triennium 1974-77 was awarded by the ICAR in December 1977.

Agronomic Improvements

Meanwhile, research efforts continued for further agronomic improvements. Intercrossing radiation induced mutants was found to be useful. New strains viz., 'TG-7' to 'TG-20' were selected during 1969-75 having improvements in oil content, seed size, yield and maturity. After field trials, six strains viz., 'TG-1', 3, 14, 16, 17 and 19 were found consistently superior.

In a recent crop competition for groundnut production organised by the Department of Agriculture, Karnataka, two farmers growing 'TG-3' and 14 have won first prizes by producing 6000 and 5000 kg/ha yield compared to less than 4700 kg from the local varieties in Chitradurga and Davangere talukas respectively.

'TG-1' and 19 are not only superior in yield, but also in seed size. It may be emphasized that the seed weight of 'TG-19' (1.25 gm/seed) is two and a half times more than that of the parent. Large sized kernels are preferred for "table purpose". The exporters of HPS (Hand Picked Selection grade) groundnut are very much interested in 'TG-1' and 19 as these kernels would fetch a premium of US\$ 150 per tonne in the international market.

Farmer's Interest

Demonstration-cum-seed multiplication trials with TG-varieties attracted the attention of farmers. 100 farmers from Rajkot district in Gujarat and Dhar district in Madhya Pradesh visited the Experimental Field Station of BARC at Trombay during September 1976 and 1977. Demand for TG-seeds is increasing. Seed distributions by BARC, VMA and some progressive farmers indicated that approximately 6000 to 8000 ha would be under the improved TG-varieties in Kharif 1978.

Exports of about 60,000 tonnes of present grade HPS groundnut earn approximately 45-50 million US dollars annually. If TG-1 and 19 kernels replace the present grade, additional 10-12 million dollars could be realised without increasing the quantity of exports. Similarly the high-yielding TG-varieties would produce additionally approximately 3-4 lakh tonnes, if grown even on 30 per cent of the groundnut area in the country. This improved production could reduce the edible oil import by one lakh tonnes, thereby, saving at least 60 million dollars. Thus, the mutation research leading to development of improved TG-varieties at the Biology and Agriculture Division is an illustration of research benefits to the nation.

INDUCTION, EVALUATION AND UTILIZATION OF BENEFICIAL MUTATIONS IN THE WINGED BEAN (PSOPHOCARPUS TETRAGONOLOBUS)

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Abstract

Winged bean appears to be a promising future legume crop for the tropics due to its nutritional value. For larger scale cultivation however non-winding types would be necessary. These are aimed at by mutation induction with gamma rays, fast neutrons, EMS and sodium azide. Mutants with altered photoperiodic reaction will also be looked for as these could be useful for cultivation of winged bean in March/April.

The winged bean seed has about the same chemical composition as the very nutritious soybean and, therefore, has the potential of becoming the tropical alternative for soybean (which is basically a temperate crop). For crop production, winged bean has the disadvantage of being winding. Trellises which are needed to grow the crop successfully are expensive to put up. In addition, harvesting becomes a tedious operation because of the intertwining vines.

Since there is no close relative of the winged bean from which to obtain the bushy character, mutation induction is the only way of obtaining a bushy-type winged bean. In addition, a mutant with altered photoperiodic reaction would be welcome since this may be planted even in March or April without having to wait for about 150 days for the first flower.

We have decided to use native varieties of the winged bean which usually have short pods of about 15 cm. for two reasons, namely: (a) Reduction of plant height would need to be accompanied by shorter pod length, and this could be problematic if one starts from a winged bean variety with long pods, and (b) The native varieties are having general tolerance or resistance to local pests and diseases.

Two physical mutagens (gamma rays or fast neutrons) and two chemical mutagens (ethyl methane sulfonate or sodium azide) will be utilized. For gamma rays, I will be using the following doses: 10, 20 and 40 kr to reduce the possibility of missing out on the mutation spectrum of any particular dose level. On an assumption of an RBE of 20 for fast neutrons, the equivalent fast neutron dose levels would be 500, 1000 and 2000 kr, respectively.

For ethyl methane sulfonate, the following concentrations will be used: .01, .02, .03 and .04 M which is based on the observation that the more radioresistant mungbean (80 kr maximum dose for the mungbean vs. 40 kr gamma rays for the winged bean) can tolerate up to .06 M for 24 to 26 hours soaking at about 25°C room temperature. Regarding sodium azide, the appropriate concentration levels and duration of treatment to use will have to be determined.

For each dose of physical or chemical mutagen treatment, I will initially aim at, at least, 100 surviving seedlings. It will be desirable to have as many series, of course, as can be handled. Production of the M_2 seeds will be done at the Philippine Atomic Energy Centre. Growing of the M_2 plants will be done at various agricultural schools and colleges.

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The pedigree method will be used until the M_3 generation. M_2 seeds from each M_1 plant will be kept separate. Up to thirty M_2 seeds per M_1 plant will be sown. Plants with putative change in growth habit will be confirmed in the M_3 . Twenty seeds each from such plants should be sufficient for confirmatory purpose.

The second objective is more difficult to screen for as this would require planting in March or April. Unfortunately, this is not the best time to screen for changes in growth habit. Problems in M_2 seed supply, land area available for planting and availability of manpower to screen for either the bushy type or the photoperiod-insensitive type are anticipated. In case of such difficulties, priority for screening for the bushy type will be given. Therefore, planting for such screening will be done mainly in May or June and in November or December or during the two regular planting seasons in the year.

No attempt will be made to prevent cross-pollination among the plants within each treatment and between plants in different treatments in the M_1 generation as this would be too laborious and does not appear to be necessary in relation to the objective of inducing a bushy-type and/or a photoperiod-insensitive mutant.

It is hoped that a bushy-type mutant obtained can be used directly as a commercial field-crop variety. Other, some crossing might have to be done.

Irradiation of the seed with its multicellular embryo is a very convenient approach. However, somatic competition wherein the mutated cells lose out to normal cells reduces the number of mutations recovered in the M_2 generation. Therefore, two single-cell approaches will be considered. These are: (a) Irradiation of the winged bean plant at flowering time so as to mutate the pollen and/or the egg or the zygote and (b) Single-cell in vitro culture. This last one may be as difficult as it is in soybean. We have obtained callus by growing the cotyledon (without the embryo) in culture medium. The breakthrough that is needed is to get the callus to differentiate into roots and shoots. But this single-cell in vitro culture is worth trying since it could tremendously increase the number of mutations recovered in the M_2 generation.

PEA IMPROVEMENT BY POLLEN IRRADIATION

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Abstract

In the course of mutation induction experiments aiming at genetic improvements of economic value, seed and pollen irradiation were compared. It was found that the latter produced a larger proportion of mutants with practical interest than seed irradiation. In another set of experiments mutants carrying duplicated chromosome segments were selected which may represent valuable new genetic material.

Introduction

Peas are mainly cultivated in the north and south of Italy for industrial processing (freezing and canning).

At Casaccia, a breeding programme is underway by cross breeding and mutagenic methods, to improve earliness, the yield with mechanical harvesting, and the technological quality of the fresh and processed products.

Several experiments performed with physical and chemical mutagens on different ontogenetic stages in diploid and polyploid species (Monti *et al.*, 1969; Scarascia Mugnozza *et al.*, 1969; Contant *et al.*, 1971), showed the advantage of pollen irradiation: Because of the absence of chimeras in M_1 plants, the M_1 material is much easier to handle.

In peas, seed and pollen treatments were performed in several varieties and this paper reports the results of pollen treatments with regard to 1) the induction of mutants with agronomic value and 2) the induction of chromosome duplications.

1. Mutations of agronomic value

Comparing the effects of seed and pollen treatments performed on the canning variety "Sprinter" by grouping all the mutations found in M_2 into chlorophyll and morphological mutations, a higher proportion of the latter types was found after pollen treatments (82.5%) than after seed treatments.

The progenies of the morphological mutants isolated in M_2 after seed and pollen treatments, were analyzed in the following generations. The same selection procedure was followed from M_2 on and in M_3 only mutants which could have a practical interest were carried on, i.e. having mutations for height, early flowering time, fertility, pod length, seed weight and seed size. From M_4 on, only lines with good agronomic performances were continued. In M_8 , only two lines showed superiority for outyielding the control variety: Both lines derived from pollen irradiated material.

Comparative agronomic and technological trials, performed in different Italian districts, confirmed the progressive value of the two mutant lines, for some parameters concerning the processed product, such as taste and colour. The certification procedure already started to release the two lines as new varieties.

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The comparison of the results of the two treatments indicates that pollen treatments may induce a higher rate of point mutations than seed treatments: this assumption is based on 1) the lower rate of chlorophyll mutations, which are mainly attributed to chromosomal mutations, 2) the higher number of out yielding lines selected from M_2 on. The higher rate of point mutations induced by pollen treatments in comparison with seed treatments is probably due to the sieve action of the haplontic selection at the moment of the M_1 zygote formation.

2. Induction of chromosome duplications

It is generally assumed among geneticists that duplications may provide an important source of new genetic material. For this reason the production of duplications may be considered as a useful plant breeding technique. The method followed by Hagberg (1962) to induce duplications in barley was the use of translocation lines. In our experiments we obtained pea lines with duplicated chromosome segments, by using lines with dicentric chromosomes.

After pollen irradiation of the fodder pea "Parvus", some variegated plants were obtained in M_1 , which segregated in M_2 for different phenotypes. After cytological characterization of the variegated mutants (Monti *et al.*, 1969; Saccardo, 1971), this abnormal segregation was explained on the basis of the behaviour of some unstable chromosomes which were found to be present in this material.

Variegated phenotype is characterized by leaves with irregular margins and with small light-green spots and variable thickness of the veins; lighter coloured areas are due to lack of palisade tissue and of chloroplasts.

Cytological analysis of such variegated plants, has shown a mixochimeric structure due to the presence of dicentric chromosomes which can go through a "breakage-fusion-bridge" cycle. Dicentric chromosomes were demonstrated to be persistent and transmissible also through male gametes. At meiosis the dicentric chromosomes associate with their homologues. From the disjunction of such associations, normal gametes, aneuploid gametes and gametes with dicentrics are formed, by breakage of dicentric chromosomes in anaphase I and II, deleted or duplicated chromosomes can also result.

On the basis of such findings and considerations, the progenies coming from four M_1 variegated plants ($2n = 12+1 \text{ dic.} +1 \text{ telo.}$) isolated after X-irradiation of pollen in the canning varieties "Sprinter", were examined more carefully. Three different phenotypes were found in M_2 , corresponding to three different genomes: normal phenotype with 14 chromosomes, variegated phenotype with dicentric chromosomes, and abnormal types which were found to be aneuploids (partial trisomics). The progenies of normal plants were carried on for a number of generations with the same selection procedure. In M_6 , two lines coming from such material, showed good yield and a better technological quality than the mother line.

Two longer chromosomes were found at the mitotic metaphase in both lines. No translocations were found in meiotic analysis of F_1 plants obtained after crossing the two lines with the mother variety and the pollen fertility in F_1 was normal. So it was concluded that the longer chromosomes are the result of duplicated chromosome segments.

The caryotype analysis revealed that the duplications were present on the chromosome belonging to group 6.

The two lines with chromosome duplication, will be studied for various parameters (e.g., disease resistance and chemical characters of the seeds), in order to check whether there are other effects of these chromosome aberrations. These effects will first be studied in the "Sprinter" background but after crosses also other genetic backgrounds.

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BREEDING FOR DISEASE RESISTANCE IN PEA AND OTHER VEGETABLES

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In our Laboratory a breeding programme for disease resistance has been started which includes cross breeding and mutagenic techniques. The aim is to develop pea varieties resistant to powdery mildew (Erysiphe polygoni) and nematodes (Heterodera goettingiana), induction of resistance in pepper to wilt disease (Verticillium dahliae) and in tomato to late blight (Phytophthora infestans). The programme involves the following aspects:

1. World collections of Pisum, Capsicum and Lycopersicon species are being examined for disease resistance;
2. A mutagenesis programme is being performed to induce resistance against pathogens for which no suitable sources of resistance are available where reproductive barriers between species prevent the transfer of resistance genes to the cultivars, and where a complicated backcross programme would be necessary in order to break undesirable linkages;
3. Development of rapid screening methods is attempted in order to identify partial and other types of resistance.

IN VITRO STUDIES FOR VIRUS RESISTANCE OF LEGUMES

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Tissue culture techniques are now being used as tools in studying various basic problems not only in plant physiology, cell biology and genetics, but also in agriculture, horticulture and forestry. These techniques have important applications in plant pathology, too. They provide excellent possibilities for investigating the infection by viruses, the processes of recognition and replication, and other aspects of host-parasite interaction. Tissue culture techniques have an increasingly important role in practical plant protection as well. Examples include the production of pathogen-free plants using meristem tip and callus culture, and regeneration of novel disease-resistant plants from cells which have been genetically manipulated. Introducing these techniques in improvement of leguminous species could be extremely important because of the role legumes play in food and feed.

Main lines of the projects are:

- to establish tissue and organ cultures from different legume varieties
- to regenerate plants from tissue or shoot tip cultures
- to optimize the culture conditions for studying the host-virus relationship
- to investigate the effect of irradiation by Cobalt 60 gamma rays on the different parameters of our experimental system.

In vitro virus-host interaction study:

- to control the virus replication in tissue culture: chemical treatments, e.g. thiouracil/Cobalt 60 or Cesium 137 irradiation effect of different plant hormones
- to obtain virus resistant plants using different mutagenic agents
- to find selective inhibitors of virus biosynthesis
- to compare phenomena of susceptibility and resistance in intact plants and in tissue cultures.

CONCLUSIONS AND RECOMMENDATIONS

Following the presentation of research project reports and extensive discussions of specific aspects and general concepts of research plans strategy and methods, a number of committees were formed to summarize results, to draw relevant conclusions and to make recommendations for future work. The conclusions and recommendations of the Regional Seminar on Induced Mutations for the Improvement of Grain Legumes in South East Asia, 1975 (IAEA-203, 1977) were considered and generally endorsed. Additional aspects came up and some clarification was necessary.

1. Germplasm for breeding and starting material for mutation

Starting material for mutation breeding experiments should have good agronomic performance and should be optimally adapted to the environmental conditions, under which an improved variety is intended to be used. It would be ideal to resort to mutation induction, if the genotype in question needs improvement only in one or a few well defined and easily selectable traits. In certain specific instances, however, mutation induction might be employed on primitive or wild genotypes to make them more suitable for use in cross breeding. Often it would be advisable to treat several different genotypes. In any case, an evaluation of available germplasm must precede the implementation of a mutation breeding project.

It became clear that by far not all the desirable genetic variation exists among the germplasm currently held at various institutes and genebanks. Previous views on this subject tended to be more optimistic. Statements as expressed at the Seminar in 1975 about cowpea (IAEA No. 203 p. 177) have to be revised (following research by Pathak in Kenya).

2. Objectives of mutation breeding

Like in any plant breeding project, clear and precisely defined objectives are the most essential prerequisite for success. For the plant breeder this may require prior studies on the genetic and environmental factors influencing the character in question as well as various components involved. The means of selection, the time scale and the amount of material to be handled in various generations will have to be taken into account, when defining a programme's objective, and economic considerations cannot be neglected either. As for specifically desired characteristics a number of observations were noted:

a) Climatic adaptation

There is a need for alteration of photoperiodic reaction, for obtaining more uniform flowering, for drought tolerance and for earlier maturity in many of the legume crops. Practicable selection methods for some of these characters have to be developed. For evaluating yielding capacity in relation to earliness "productivity per day" should be used as criterion. In soybean, adaptation to higher altitudes is desirable. Sowing time experiments may provide a good means for selecting both, for photoperiodic and temperature response, as well as for stability of production, specially under rainfed and marginal conditions.

b) Plant architecture

Crop plant's architecture is intimately related to the purpose and system of cultivation as well as the way of harvesting. Therefore, if the cultivation system or the kind of use is to be changed substantially, the plant architecture has to be changed as well. This should be even more obvious, when wild species, evolved under natural selection pressure and in competition with other species, are to be domesticated for utilization as crop plants in present day agriculture or horticulture. The breeder, having in mind such evolutionary changes, must have a clear concept of the utilization envisaged, the

cropping system, the season and all the environmental factors, the genotype of the future crop variety has to interact with. He of course also has to be familiar with the biological peculiarities of the species. Being aware of Vavilov's law of homologous genetic variation, the breeder should nevertheless stay away from unwarranted generalization. Whether dwarf or tall, determinate or indeterminate, winding or non-winding, spreading or erect types are desirable, has to be examined in each case. Varieties for intercropping of course require different plant architecture than varieties for monoculture.

Existing types of chickpea for example have apparently too much a tendency for spreading, bushy and excessive vegetative growth over a long period of the crop growing season. Upright habit with a large number of branches is aimed at in India, Pakistan and by ICRISAT. Still some of such types have an insufficient number of pods and consequently a low "harvest index". An upright mutant reported by Shakoor (Pakistan) shows promise but needs further improvement, which is being attempted through cross breeding and repeated mutagen treatment. It is evident that an improvement in plant architecture towards the conceptual ideal type does not automatically bring the desired yield improvement. In evaluating such improved genotypes, an adjustment of agronomic conditions such as sowing time and sowing density may be required.

In mungbean, short stature mutants with good branching pattern were selected (Shakoor, Pakistan). There exists certainly a need for restructuring the plant architecture of other grain legumes like pigeon pea, black gram, cowpea and lentil, keeping in mind a great variety of farming conditions, including also intercropping. The position of pods, their size and distribution are important factors for high grain yield and minimal yield losses. The location of pods above the leaf canopy in case of cowpea and mungbean may offer advantages in terms of easier harvesting and reduced disease incidence but may favour insect pest damage to pods and seeds.

c) Production physiology

Grain yield is a complex deriving from a number of yield components which as such are subject to various environmental factors and to mutual compensation. The relationship of yield components to the plant architecture, the root system and the canopy structure makes it even more complicated for the plant breeder to effectively advance grain yield. The problems are augmented by the necessity to deal with it under nursery conditions (spaced plants, small plots).

There appears a general consensus that most leguminous crops have more leaf area than required for the production of a good grain yield (e.g., in cowpea, mungbean, pigeon pea). In groundnut mutant cultivars, 30% higher grain yield was obtained in spite of 50% reduction of the leaf area. Too little is known about the contribution of particular leaves to the pod filling. Studies on light penetration, photosynthesis, source/sink relationships in leguminous species are urgently needed to guide the plant breeder in developing genotypes with higher grain production.

Nitrogen fixation is a most important aspect of legume cultivation. It should be attempted to improve onset, duration and intensity of the process. The relevance of excess leaf area for providing energy towards nitrogen fixation should be clarified.

Flower and pod abortion are high in several legumes. The extent of loss varies from cultivar to cultivar as does the initial number of flowers. Physiological studies should clarify whether flower and pod abortion are due to restricted assimilate supply. If plants produce excess flowers, the breeder may be able to improve yield by genetically restricting the number of flowers, thus adjusting the "sink" to the "source".

Where grain legumes are cultivated without irrigation, periodical lack of water or continuous water stress may set limit to grain production. Different forms of drought tolerance are required for different types of water shortage. The relationship of leaf characters and root characters and of transpiration with drought susceptibility must be studied.

Shattering of seeds from mature pods or mature pod shedding is a characteristic of wild plants that has been gradually eliminated from many cultivated species during domestication. Selection is simple, mutation induction has been effective (e.g., in lupins). The degree of shattering and shedding resistance that is required, depends upon the harvesting technique. An alteration of the plant architecture may often be helpful. The magnitude of losses encountered depends of course to a large extent upon the time from maturity till harvest and also upon the weather conditions.

d) Seed and grain quality

Seed quality appears to be a critical factor in many leguminous crop plants. There exists variation for impermeable seed coat and for seed germinability among cultivars. Small seeds were found to germinate better than large seeds in some instances (e.g., soybean). There are also differences in longevity during storage, particularly under less than optimal conditions. Breeders will have to pay attention to these problems but take also into account the quality demand from the consumer.

Quality from the consumers point of view refers to chemical composition (e.g., protein, oil, alkaloids, glucosides, nutritional inhibitors), attractiveness (colour, size, shape), cooking quality, digestibility and taste. Advances have been made, e.g., in peanuts by developing bold seeded and high oil content mutants with good yield (Patil, India). Consumers preferences concerning seed colour and shape are quite specific. In some cases attractiveness of a good introduced variety may be improved by a mutation changing seed coat colour. In some legumes, dark seed colour is assumed to be associated with insect resistance. Mutational colour changes can be used to prove this assumption or dissociate colour from resistance. Required duration of cooking is quite an important variety characteristic for areas with a shortage of firewood or other forms of energy. Its improvement by breeding is certainly possible, but care should be exercised not to spoil seed traits affecting germination and longevity. Antinutritional and flatulence factors should be examined in advanced generations. For soybean and mungbean, various food preparations have specific requirements, but little is known about the genetic aspects. The removal of toxic or bitter substances from the grain, which was achieved in most legume species during domestication, seems to be a promising domaine for mutation breeding projects. Where leaves are used as a vegetable, (e.g., cowpeas in Africa), leaf quality characters must be considered too.

e) Resistance to pathogens and pests

The continued evolution of pathogens requires continued efforts by plant breeders. Breeders results in terms of resistant varieties often have accelerated the evolution of pathogens. This can and should be avoided by proper management of available resistances and by using more durable types of resistance, even if their protective effect is only partial. Mutation induction must be considered as a means to create genetic variation with regard to host/pathogen interaction. The screening method applied will play a crucial role concerning the results of a mutation induction experiment, perhaps more than for any other breeding objective.

Before starting efforts for improving resistance by cross breeding or mutation induction, the breeder must know the biology of the pathogen, the epidemiological situation, and the genetic variation among existing germplasm as far as can be judged from tests with available pathogen

types. He must establish methodological procedures and facilities for collecting, maintaining and dispersing inoculum. He must be trained in assessing qualitative and quantitative differences in host reaction. He would be well advised to seek the close cooperation of experienced plant pathologists.

There were many open questions concerning the methodology of breeding for disease resistance in general and of mutation breeding in particular. Should one use artificial inoculation and if so, what about the amount of inoculum (natural spore density or more) and its genetic composition (one genotype or mixture)? Should one allow the plant to use all kinds of defense mechanisms or should one force the pathogen into the tissue? Would it be useful and practicable to screen for components of resistance by assessing time required for the pathogens establishment and till reproduction, the amount of spores produced, and infectiveness of the spores following normal spread in the field?

Resistance against virus diseases may be complicated by involvement of a third organism, the virus vector. Furthermore, virus infection may remain unnoticed in some genotypes or under particular environmental conditions, thus simulating resistance. Tolerance against a pathogen in terms of ability to produce satisfactory yields in spite of infection must be looked at as a very valuable character. However, tolerant varieties may contribute to the development and spread of an epidemic and therefore one should attempt to combine tolerance with some kind of resistance.

Resistance against insect pests and nematodes has been largely neglected, although it appears of mounting importance. While considering breeding efforts for establishing inherited resistance, one should not forget to exploit all other agronomic means for reducing the incidence of pests and the amount of damage (rotation, tillage, seasonal variety change, intercropping etc.). Actual problems identified were lack of resistance against aphids in mungbean (Korea), and against aphids and pod borers in cowpea (Kenya). Screening for "non-preference" under open field or greenhouse condition is being carried out. However, "non-preference" type of insect resistance might not be effective enough, once a variety of such kind is grown on a larger scale. More effective types of resistance may be screened for by releasing insects in cages. In case of insect transmitted virus, resistance against both the pathogen as well as the vector are desirable. Resistance against the vector alone may not be effective enough. When insects are only probing on "resistant" plants, the virus may already be transmitted.

Examples of positive results encourage to pursue further the potential of mutation induction for improving resistance. Such examples are *Verticillium* resistance in peppermint, mildew resistance in pearl millet and barley, rust resistance in wheat, leaf hopper resistance in rice. In grain legumes, promising results have been reported with regard to golden mosaic virus of *Phaseolus vulgaris* (Brazil), mildew of *Pisum sativum* (Italy), yellow mosaic virus of mungbean (Pakistan), *Ascochyta* blight resistance in chickpea (Pakistan), and even for soybean rust (Indonesia, Thailand).

f) Mutation breeding methodology

Although the essential principles of mutation breeding are spelled out in the Manual on Mutation Breeding (IAEA 1977), a number of points have not been taken satisfactorily into account in some of the projects presented at the coordination meeting.

Following the choice of appropriate parent material, all depends next upon an effective mutagenic treatment. (ref. Manual on Mutation Breeding, chapters 2,3 and 7). For a widest possible spectrum of mutations, the use of different mutagens is recommendable. In the case of seed treatment for practical purposes with ionizing radiation, a dose causing 30 - 50% lethality can be called effective. To account for unpredictable variation in mutagen sensitivity, 2 - 3 doses may be chosen

and only the best one carried forward into the next generation. The optimal dose range has to be determined by a pre-test. When carried out in a greenhouse one has to keep in mind that survival under field conditions may be less, due to additional stress factors. Seedling height measurements are being employed successfully in cereal mutation experiments for assessing mutagen dose effects and choosing the optimal dose range (Manual, chapter 5). In dicotyledonous plants, such tests are more complicated due to the different morphology and growth pattern of seedlings. Primary leaf diameter or epicotyl length appear to be usable criteria, but the best time for such measurements and the correlations towards M_1 survival and M_2 mutation rates need to be still established in most species. For chemical mutagens, experience with grain legumes is rather limited. Careful studies concerning mutagen uptake, post-wash, dry-back and storage effects are needed. Some grain legumes exhibit sensitivity against presoaking in water. Under certain circumstances, pollen treatment may be profitable, giving rise to a non-chimeric M_1 -generation.

The size of the surviving M_1 -population giving M_2 seeds is very crucial for the prospects of success of a mutation induction project (Manual, chapter 13). In view of expected mutation rates in the order of 10^{-3} to 10^{-4} , a surviving M_1 population of 10 000 plants must be looked at as a minimum. The seeding rate should take into account the expected lethality, so that close enough spacing is achieved, which would not only save experimental field but may also reduce "diplontic selection". This phenomenon is known to reduce the number of recoverable mutations in cereals (ref. Manual, chapter 7.2.2). To what extent it has the same effect in various leguminous species would be important to know.

Outcross between M_1 plants and other germplasm should be avoided by appropriate isolation (Manual, chapter 7.1.4). Outcrossing with the control or mutagen treated material of the same origin can be tolerated in a practical mutation breeding project.

The recommendable ways of handling the M_1 harvest, growing the M_2 and selecting mutants are spelled out in detail in the Manual on Mutation Breeding (Chapter 7.1).

All mutants selected in M_2 must be confirmed in the next generations. Many of them will be heterozygous for one or more other mutations and therefore segregate in M_3 . This requires reselection. The higher the mutagen dose applied the more mutations were induced in cells of M_1 -plants and the more segregation of multiple mutations will be observed in M_3 and M_4 -generation. Polyploids tolerate more chromosomal aberrations and therefore may show longer lasting instability and segregation. Genetic changes with small phenotypic expression may not be selectable in M_2 but rather on a plot basis in M_3 or later generations. In this case, M_2^c plants may be selected randomly.

g) Use of induced mutants

Induced mutants may be used directly, after appropriate evaluation and propagation, as new varieties, or they may be used in cross breeding. From experience with peanuts (Patil, India) one must conclude that cross-breeding among mutants offers an enormous potential of creating additional useful genetic variation from recombination, not predictable from the phenotype of the originally selected mutants. In general, one may, however, use mutants of proven value as gene source in crossing with other valuable varieties. Linkage with undesirable mutations can be removed by crossing, but disturbing pleiotropy may likewise be modified through transfer of a mutated locus into another genetic background.

Mutant testing and evaluation should not be restricted to conditions optimal to the original variety. Often different agronomic or environmental conditions have proven to be better for a mutant.

More emphasis should be given to making available promising mutant lines to other plant breeders. The use of mutants in commercial plant breeding can often be promoted by offering the same or similar mutant traits in different genetic backgrounds of agronomically attractive value. This may require a certain amount of mutant crossing, and mutation breeders are encouraged to keep such practical aspects in mind when doing crosses for elucidating the genetic behaviour of promising mutants.

Mutant lines are often developed by institutes not officially responsible for variety development. Efforts should be made to establish links and cooperation with main plant breeding programmes at an early stage for the mutual benefit of the different programmes and in order to ensure that any positive achievement from mutation breeding will reach the farmer in the shortest possible time.

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