4.7 The effective use of physical and chemical mutagen in the induction of mutation for crop improvement in Malaysia.

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Abstract

The earliest work of induced mutations breeding program in Malaysia was reported in1967. The project was carried out by Rubber Research Institute of Malaysia using x-radiation in an attempt to improve rubber trees for dwarfism and disease resistance. Subsequently, more efforts were taken up by the universities to promote the technology for genetic changes and creation of new genetic resources, particularly in crops that are not easily achievable through conventional techniques. Gamma radiation is always been used as physical mutagen, while ethyl methane sulfonate (EMS) was a popular chemical mutagen used in induced mutation breeding in the country. Gamma rays is an effective mutagen to which more than 30 potential mutants were produced up to now through mutagenesis of several important food crops and ornamental plants. Although chemical mutagen such as EMS were reported being used, the result is not so convincing as compared to gamma radiation. Malaysian Institute for Nuclear Technology Research (MINT) has initiated and promoted nuclear technique in mutation breeding for the improvement of importance food crops such as rice, legume and other potential crops for export, like fruit trees and ornamentals. Gamma radiation is the main source of mutagen used in mutation-breeding programme at MINT. The effectiveness of these two mutagens were verified with mutants derived through induced mutation breeding in the country which some mutant has shown outstanding improvement and released as new varieties and cultivars. This paper summarises and discuss the effects as well as achievement attained through the use of ionizing radiation and chemical mutagen in plant mutation breeding in Malaysia.

Introduction

In Plant Breeding, mutation induction has become an effective way of supplementing existing germplasm and improving cultivars (Micke *et al.*, 1987). Experiment with induced mutation breeding in Malaysia dates back some 30 years ago to the induce mutation research with x-irradiation of green budwood of rubber clones, carried out at RRIM between 1967 to 1974. The objectives of the investigation were to induce desirable characteristics such as dwarfism, non-wintering and also disease resistance in commercial *Hevea* clones (Ong *et al.*, 1973). Based on their findings, a few induced mutants were selected for vigor and early maturity. The first irradiation facility in the country was installed in National University of Malaysia (UKM) in 1970 and became available locally for various scientific research and also for use in mutation breeding. Later, with the establishment of Tun Ismail Atomic Research Centre (PUSPATI) under the prime Minister's Department in 1972, work on induced mutations gained its momentum. Extensive uses of ionizing radiation especially with gamma-irradiation in mutation breeding by local plant breeders were begun in 1980's. The use of mutation in research program for crop improvement has thus been regarded as a new technology in Malaysia. At present, induced mutation breeding has become a complementary or an alternative method to conventional breeding of the crop that already has an elaborate crop improvement programme. It is also regarded as a convenient and useful tool for crop improvement. Later, more efforts to use induced mutations for genetic changes and creation of new genetic resources were initiated for the improvement of important crops such as rice and legumes. Most of the experiments carried out were aimed to investigate the most effective dose for mutations and to estimate the frequency and mutation spectrum following treatment with a mutagen. Encouraging results were obtained in the form of semi-dwarf and disease resistance mutants in rice cultivars, high yielding and early maturity in soybean and adaptability to local environment and high yield in groundnut. This generated a great deal of interest in induced mutations especially for the improvement of crop plants. Therefore, the use of radiation induced mutations is becoming a new technique which is possessing great value in creating greater genetic variability, promotes genetic recombination and new valuable germplasm for breeding.

Realizing the potential applications of induced mutation in plant breeding, MINT has embarked on a mutation-breeding program since 1984 for crop improvement. The availability of irradiation facilities including a reactor for fast neutron, x-ray machine, gamma cell and multi-purpose ^{60}Cobolt at MINT, created a vast interest among plant geneticists to investigate the effects of radiation on plants. Beside research, MINT is also providing radiation services for plant mutation breeding to local plant breeders from government agencies and private companies. A large number of potential mutants have been developed in a variety of crop plants at MINT and in Malaysia as a whole over the past 9 years. Thus, mutation breeding is gaining recognition as an efficient tool for crop improvement programmes in Malaysia. This paper highlights the achievements of mutation breeding and its effectiveness future application for genetic improvement of plants.

Mutagen and radiation facilities

Mutagen used for plant improvement

Mutagen involved in induced mutations research can be grouped into two, namely physical mutagen and chemical mutagen. Choice of mutagen will depend on the type of tissue to be treated, type of mutation desired, availability of mutagen and safety considerations. In the initial years of mutation breeding research in the country, chemical mutagens were the regular agents for inducing mutation for plant materials. After the first irradiation facility in the country which was available in 1970 at UKM physical mutagen was frequently used in many plant mutation breeding in Malaysia. Among physical mutagens, gamma rays is widely for mutation induction. The most widely used chemical mutagen was ethyl methane sulfonate (EMS), an alkylating agent.

Radiation facilities for induced mutation breeding

The first irradiation facility in the country was installed in 1970 and became available for various scientific research and also for use in mutation breeding. The nuclear facilities became more widely used now than chemical mutagen. Beside UKM it is also available at MINT, both in Bangi, Selangor. Many of the initial work on induced mutations were aimed at investigating the most effective dose for mutations on several important at the time.

- Facility at UKM

It was the Gammacell 220, one of the first general purpose radiators from Atomic Energy Commission of Canada. The unit consists of an annular source permanently enclosed within a lead shield, cylindrical drawer and a drive mechanism to carry samples to be irradiated from outside the shield to the source. Samples up to approximately six inches in diameter and eight inches in height can be accommodated in the chamber.

- Nuclear facilities available for scientific research and induced mutation currently available at MINT are as follow: -

1. Reactor Triga Mk II

This is a 1-megawatt (MW) thermal nuclear research reactor, which serves as a source of neutrons for radiophamaceutical and radiotracer production. Neutron have been reported to have been used to produce mutation. However, there has been no report on the use of the neutron for mutation breeding in the country.

2. Gamma Cell

Two gamma Chambers, GC4000A (10kCi) and J.L. Shepperd & Associates Model 109-68 Irradiator (24kCi) are also available for samples irradiation. These are self contained gamma irradiation units and are equipped with a mechanism to transport the material to be irradiated into and out of the radiation field. The approximate volume of the irradiation chamber is 4,000 cm^3 with a 160 mm diameter and 210 mm height. The explants normally used for irradiation are seeds, scions, buds, tissue culture materials, cuttings, and pollen.

3. Sinagama,

The irradiation plant consists of a ^{60}Co radiation source, a concrete biological shield, a product conveying mechanism, operation and safety control and auxiliary equipments. This facility offers services for medical product sterilization, food preservation, agrowaste treatment and mutation breeding. The facility normally used for big plant parts or whole plant which could not be accommodated by small chamber of gamma cell.

4. The Eldorado 8 Teletherapy (3.484 kCi)

It has a very low dose-rate i.e. 0.1 Gray min^{-1} at 85 cm distance, and is very useful for research samples including plant tissues or cells for mutagenesis at very low doses.

5. Gamma green house

This facility is future gamma facilities will be constructed in 8^{th} Malaysian Planing (will be available for service in 2003). The facility will provide service for chronic effect of irradiation in the country. This is a unique facility whereby the materials including tissue culture , whole plant or trees can be treated.

Radiation services

MINT, being the centre for application of nuclear technology in the country provides services for treating plant materials usually using gamma rays for scientists from government agencies, universities and lately increasing interest and request from private companies. At the moment irradiation, services were provided free since most of the mutations breeding programmes carried out by other institutions are in collaboration with MINT.

Radiation service statistics 1994 – 1999

No. of treated samples	240
No. of treated species	50
No. of recipients (Government and private sector)	15
Type of treated materials:	
Seed samples	45
Vegetatively propagated materials	136
"*in vitro*" materials	57
Others	none
Type of mutagen treatment	
No. of ^{60}Co gamma rays treatments	240
No. of fast neutron treatments	none
Others	none

Government agencies , universities and private company involved in induced mutation breeding and which have requested radiation services at MINT

Private company

- RMJ Managemment Sdn. Bhd.
- Everbloom Sdn. Bhd.
- Tropbio Sdn. Bhd.
- United Plantation Sdn. Bhd.

- Golden Hope Sdn. Bhd.

Government agencies

Malaysian Agriculture Research Development Institute (MARDI)
Malaysian Institute for Nuclear Tech. Research (MINT)
Department of Agriculture, Serawak.
Institut Haiwan Keluang

Universities

National University of Malaysia (UKM)
University of Malaya (UM)
University Putra Malaysia (UPM)
University Science of Malaysia (USM)

Table 1: Effective doses of several crop obtained through radiosensitivity tests conducted at MINT

Crop	Effective doses (Gray)
1. Rice	200-350
2. Groundnut	150-300
3. Chilli	100-350
4. Pepper (seed and cutting)	20-55 & 10-35
5. Guava & Mangosteen	100-200
6. Ornamental (Orchids & Roses)	20-40
7. Banana (In vitro)	30-60
8. Signal grass (*Bracharia decumbent*)	400-700
9. Markisa (Passion fruit)	100-200

Mutant Released through induced mutation breeding

Rice

High yield, good eating quality, early maturity and resistance to major pest and diseases are characters usually desired in a rice variety. The use of induced mutations for rice improvement in Malaysia started in 1972. In the project, several varieties including Jaya, Malinja and Mahsuri were treated with two mutagen, viz. Gamma rays and EMS, and screened for blast. More than 100 mutants resistant to blast were identified. Most of the mutants do not have good plant type but showed changes in many other traits. However, a more concerted effort was begun in 1979 to look for blast (BI) resistance (Arasu *et.al.*, 1983). To date, 48 varieties and elite breeding lines have been treated with ethyl methane sulfonate (1.5-2.5%) and gamma radiation (15-40 krad). Mutant line with short stature, early maturity, improved grain quality, increase BI and brown planthopper (BPH) resistances and photoperiod insensitivity were obtained (Table 2)

Mutant Muda 2 was released directly to farmers to replace the slightly taller Muda variety, which is susceptible to lodging. Another mutant from Muda, MR 90 with moderate improvement in BPH resistance, is a promising line in rainfed areas. Mahsuri mutant, which has good eating quality, is now being popularized as a high-quality rice (Hadzim *et al.*, 1988). A short-statured, early maturing mutant of Mahsuri mutant, MM 98, has lower amylose content. An aromatic hill rice, Pongsu Seribu 2, had its height halved and its yield potential improved through the mutant PS 1297 (Mohamad *et al.*, 1988). A short, early and photoperiod-insensitive mutant has been recovered from the tall, traditional, good eating variety Jarum Mas. Other mutants are being used in cross breeding.

The Internatonal Atomic Energy Agency (IAEA) sponsored a 5-year project on semidwarf rice with MINT using the variety Manik beginning in 1984. 101 semi-dwarf mutants which have the potential to be used as cultivar or as parents in cross-breeding programmes were identified. One of the mutant line MA03 and popular kown as mutant 'Tongkat Ali' which panicle remain erect even after grain filling and the stem is very strong and resistance to lodging (Ramli *et al.*,1989). Eventhough it has not been officially released, it has been planted commercially by a few farmers especially in the northern part of Malaysia. During the first season, the yield was recorded at 6,589 kg/ha and the second season between 5,715 – 7,504 kg/ha. Allelic test for semidwarfism was conducted by Farazi (1994) He discovered that singgle recessive gene was responsible for dwarf character of mutant MA03. This recessive gene was non-allelic to sd_1 (Farazi, 1994). This gene was named as sd_8. The glutinous mutant Manik 817 has shorter stature than the previously popular glutinous varieties PM I and Pulut Siding.

Soya bean mutant

Ethylmethanesulphonat and gamma irradiation were used in separate experiments to induce mutation. Observation and selection of mutant traits were carried out from M_2 generation. Individuals were selected on the basis of desirable plant architecture, and specifically for improved sink capacity in the form of a larger number of pods per plant and larger number and size of seeds. Cumulative data from replicate yield trials over the past several years have isolated two mutants, A15/10 and A5/10, which are high yielding and adaptable to the local environment. A15/10 in a large seeded mutant with a high pod number, while A5/10 is an early maturing dwarf with a good yield potential (Zakri, 1986).

Groundnut mutant

Two popular local varieties, Matjan and V-13 were adopted as the starting material. 3000 seeds per dose per variety were exposed to a series of gamma rays doses ranging from 0-45 krad to induce mutation of the desired traits such as high yield and resistance to *Cercospora* leaf spot (Rusli *et al.*, 1992). Several mutant were selected resistace to *Cercospora* leaf spot but only 3 mutant (MJ40/42, V13/35/16 and MJ20/165-5) were out yielded check variety (Matjan and V13). These mutants were evaluate further to investigate N_2 fixation as well as yield performance, and as consequences Matjan mutan MJ/40/42 consistancely produced the highest pod yields, at above 4 t ha^{-1}, 14-22% higher yields than the parent (Rusli *et al.*, 1998).

Banana

Novaria is a selection made from GN-60A mutant which was originated from the Seibersdorf Laboratory of The International Atomic Energy Agency (IAEA) for its vigorous growth and early flowering. In this program, Graine Naine, a popular Cavendish banana was induced by gamma irradiation using in-vitro technique. The introduced mutant was planted and evaluated at the United Plantations Bhd since 1991 (Mak *et al.*, 1996). Early fruiting individuals with good bunch characteristics were identified, micropropagated and further selected in the field to ascertain stability and homogeneity of the mutant clone. This mutant clone specially selected for the following desirable traits :

- Early flowering
- Strong fruit pedicel or 'neck'
- Good flavour and pulp texture
- Short stature
- High yield potential

Ornamental

A multi-institutional working group was formed under the National Committee for the Use of Induced Mutation in Plant Breeding to look into the applications of induced mutations in ornamental breeding. The group consists of MINT, MARDI, UM, UPM, UKM and DBKL. The

Table 2 : Rice mutants derived from mutation breeding programmes in Malaysia

Mutants	Institution	Original or parent variety	Characteristics or traits observed in mutant
Mahsuri mutant (EMS 2.5%)	MARDI	Mahsuri	Good eating quality (high quality rice); elongation trait for cooked rice.
MM98	MARDI	Mahsuri Mutant	Early maturity; spliting of cooked rice
Q34 *	MARDI	Mahsuri mutant	Good eating quality
Muda 2	MARDI	Muda	Short stature; good grain elongation
MR90	MARDI	Muda	Moderate improvement in BPH resistance
MA03	MINT	Manik	Semidwarf; high yield and upright panicle
Manik 817	MARDI	Manik	Short stature; glutinous endosperm
PS1297	MARDI	Pongsu Seribu 2	Reduce pigmentation on the panicles and grains; shorter and erect flag leaf; more tiller per plant; shorter panicles with less spikelets; increase amylose content and spikelet sterility
SPM29 & SPM39	MARDI	Jarum mas	Reduced maturation period; increase number of tillers per plant; reduced percentage of sterile grains; reduced panicle length; increase 1,000 grain weight.
SPM 68	MARDI	Basmati	Reduced plant height; higher yield; grain quality reduced (quality lower than Basmati rice).
SPM 106 through 115	MARDI	MR162	Higher grain weight (in preliminary yield trial)
Y 1281	MARDI	Q31	Reduced maturation period; reduced plant height
Y 1290	MARDI	Mayang Bunga	Reduced maturation period; reduced plant height; higher yield potential
MR 215 & MR 216	MARDI		Better resistance to BLB and PMV.

* indirect mutant (Basiran *et al*., 1998)

Legume

Coordinated breeding programs on legume started in 1978 for the improvement of groundnut and soybean. Five institution, namely MARDI, RRIM, UKM, UM and UPM were involved to investigate and breed soybean with the use of induced mutation for cultivars with high yield and well adapted to the warm humid tropical condition. In MINT, induced mutation breeding of groundnut started in 1989, with aimed at genetic and breeding goals, specifically screening for resistance to *Cercospora* leaf spot diseases and high yield. These two project mentioned were 5 years contract sponsored by IAEA.

group is currently working on mutagenesis of orchids such as *Aranda*, *Mokara*, *Dendrobium*, *Vanda*, *Ascocenda* and *Cymbidium*. Besides orchids, the group is also developing similar techniques for ornamental plants such as Chrysanthemum, Amaryllis and Alpinia as well as other tropical flowers and foliage ornamental plants.

Ornamental mutant produced at MINT

Collaboration in research between MINT and private company in ornamentals were undertaken in order to produce mutant varieties of tropical ornamental plants to meet the demands of the local market. The joint research projects with a private company began in 1994 when a MoU was signed between MINT and RMJ Management Sdn. Bhd. The private company will provides the materials to be irradiated with facilities available at MINT. MINT provides technical assistance such as handling of materials and selection. Gamma radiation was used as mutagen for induced mutation breeding of ornamental plants. Many mutants were produced in this collaboration. 12 Mutants of Ornamental derived from mutation breeding program at MINT were launched in August 2000 (Table 3).

Table 3 : Ornamental mutant released through induced mutation breeding at MINT

Crop/species	Mutant	Original or parent	Characteristic or traits observed in mutant
Hisbiscus	MINT- hrs 20001 'Siti Hasmah PinkBeauty' MINT – hrs 20002 'Siti Hasmah RedShine'	Common Hibiscus	Flower color change to light pink and dark red.
Dendranthema	MINT -dt 10001 MINT -dt 10002	Taihe	Color change, short and strong stem and heavy branching.
Orchid	MINT-ds 35005 MINT -ds 35003	Dendrobium Ekapol	Flower color and size changes
Cordyline terminalis Cordyline fruticosa 'compacta'	MINT – cfa 20001 MINT – cfa 20002 MINT – cfa 200016 MINT – cfc 45001	Cordyline	Leaf color change
Duranta repen	MINT – dr 45001 MINT – dr 15002	Duranta gold	Leaf variegation (mosaic & marginal)

Table 4 : Experiment on Induction and use of Mutation in Plant Breeding carried out at MINT and in Collaboration with other Institutions.

Crop	Institute	Dose (Gray)	Objective	Result	Year of project started
Rice Manik	MINT	150-400	To Induce Semidwarfism	101 mutant lines were semidwarf and high yielding [4]. Tongkat Ali (MA03) yields 5,700 – 7500 kg/ha (Rusli et al 1991).	1984
2. Groundnut Matjan V13	MINT	 0-400 0-450	1. To Induced mutant with high yielding and resistance to Cercospora leaf Spor 2. 2. To induce mutant with high Biological Nitrogen Fixation (BNF)	3 mutant lines were high yielding and high in BNF in associtioin with tolerance to Cercospora Leaf Spot (Rusli *et al.*, 1991)	1986
3.Black pepper (*Pipper nigrum* L)	Agriculture Research Centre, Semonggok, Serawak	0-100 (seed) 0-50 (cutting)	1. To create genetic variability 2. To produce mutants which are tolerant or resistant to *Phytopthora capsisi*	11 mutant line were had long and abundant fruit spike and good fruit setting (Paulus, 1993)	1987
5. Banana	MINT, UM, USM and UPB	60 (IAEA material) 35 (Fusarium Wilt)	1. Evaluation of mutant material from IAEA 2. To induce mutant which are resistant to *Fusarium* wilt 3. Improvement of pisang Berangan CV. Intan	NOVARIA, mutant released from IAEA in vitro culture material which were Early flowering Strong fruit pedicel or 'neck' Good flavour and pulp texture Short stature High yield potential (Tan *et al.*, 1993)	1990

CONCLUSION

The use of physical and chemical mutagen have shown to be very effective in inducing some traits in plant Reduction in duration and plant height, disease and pest resistance, and improved eating quality are attainable objectives in mutation breeding without reducing grain yield, as experienced with rice. Therefore, mutation breeding has been regarded as a complementary or an alternative method to conventional breeding using physical and chemical mutagens. This has proved to be an efficient tool for enhancing genetics variability, thus creating greater chances for selection. Radiation induced mutagenesis, in combination with the emerging technologies of in vitro culture, offer the possibility to create new germplasm.. It is evident that induced mutation breeding plays an important role in crop improvement and its application has led to the development of high yielding, new and improved germplasm, early maturity, disease resistance, short stature, erect and lodging resistance mutant varieties of rice, groundnut, ornamental, banana and many other crops. The productions of this improved mutant variety have somehow contributed to the vital impact and significant role in mutation breeding in the country.

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