IAEA

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf

Plant Mutation Reports

Vol. 1, No. 3 December 2007

http://www-naweb.iaea.org/nafa/index.html ISSN 1011-260X
http://www.fao.org/waicent/FAOINFO/AGRICULT/Default.htm

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IAEA International Atomic Energy Agency

Group photo – Participants of the TC Project RAS/5/040

To Our Readers

The IAEA provides continuous support through various regional and national technical cooperation projects to national crop improvement programs in Asia. A recently concluded IAEA-RCA project on Mutant Multi-location Trial and Mutational Enhancement of Genetic Diversity (RAS/5/040) has developed 33 mutant varieties and dozens of promising mutant breeding lines with high yield potential and/or improved agronomic characters, resistance, and end-user quality traits. Through the regional mutant multi-location trials (RMMT), six varieties were selected to be potentially released in countries other than where they originated. In this issue, papers are mainly contributed by participants of the RAS/5/040 project. We thank Dr. GSS Murty of Bhabha Atomic Research Centre, India (retired) for his help in editing those papers.

As planned, we have been trying to further improve the quality and widen the dissemination of this publication. First, an Editorial Board has been established during the past months. Second, we are planning to rename the publication *Plant Mutation Research* to broaden our appeal to contributors and readers. Third, we are planning to publish the journal on-line as a complementary media for wider dissemination.

Last but not least, we are organizing an International Symposium on Induced Mutations in Plants, 12-15 August 2008 in Vienna, Austria, in cooperation with various organizations, e.g. Chinese Society of Agricultural Biotechnology, the European Association for Research on Plant Breeding (EUCARPIA), Indian Society of Genetics, and Japan National Institute of Agrobiological Sciences, etc. I look forward to your participation.

Qingyao Shu

Announcements

International Symposium on "Induced Mutations in Plants (ISIM)", International Atomic Energy Agency, Vienna, Austria, 12-15 August 2008

Announcement and Call for Papers

1. Background

The year 2008 will mark the 80th anniversary of mutation induction in crop plants. The application of mutation techniques, i.e. gamma rays and other physical and chemical mutagens, has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies. The widespread use of induced mutants in plant breeding programmes throughout the world has led to the official release of more than 2600 mutant crop varieties. A large number of these varieties (including cereals, pulses, oil, root and tuber crops, and ornamentals) have been released in developing countries, resulting in enormous positive economic impacts.

The International Symposium on Induced Mutations in Plants (ISIM) will be the eighth in the Joint FAO/IAEA Programme's Symposium series dedicated exclusively to harnessing and disseminating information on current trends in induced mutagenesis in plants, the first of which was held in 1969 and the last in 1995. These previous symposia dealt with themes relating to the development of efficient protocols for induced mutagenesis and their role in the enhancement of quality traits, as well as resistance to biotic and abiotic stresses in crops and the integration of in vitro and molecular genetic techniques in mutation induction.

Since 1995, there has been an increased interest within the scientific community, not only in the use of induced mutations for developing improved crop varieties and for the discovery of genes controlling important traits and understanding their functions and mechanisms of actions, but also in deciphering the biological nature of DNA damage, repair and mutagenesis. A symposium that brings together the key players in basic research, as well as in the development and application of technologies relating to the efficient use of induced mutations for crop improvement and empirical genetic studies, is therefore justified and necessary.

2. Main Topics

Topics to be addressed at the symposium:

- Molecular genetics and biology of physical, chemical and transposon-induced mutagenesis
- New mutation techniques, i.e. ion beam implantation, and their integration with other molecular and biotechnological techniques
- Induced mutations in crop breeding programmes
- Mutation induction for gene discovery and functional genomics, including targeting induced local lesions in genomes (TILLING) and other reverse genetic strategies
- Mutational analysis of important crop characters (tolerance to abiotic stresses, resistance to diseases and insects, quality and nutritional characters, etc.)
- Socio-economic impact of widespread mutant varieties.

3. Target Audience

It is envisaged that this symposium will not only attract eminent basic research scientists but also active plant breeders from all over the world. Therefore, the symposium will at once provide the platform for the exposition and rigorous discourse on current research and technology development in this field and establish linkages among scientists in order to develop knowledge-based breeding strategies and mechanisms for sharing information and resources. It will also be a venue for project managers of international and national organizations, as well as multinational and private companies engaged in plant breeding activities, to gain insights into the applications of, and current trends in, mutation techniques.

4. Exhibits

Limited space will be available for commercial vendors' displays/exhibits during the symposium. Interested parties should contact Mr. Qingyao Shu, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture IAEA, at e-mail: q.shu@ iaea.org.

5. Contributed Papers and Posters

Concise papers on issues falling within the topics outlined in Section 2 above may be submitted as contributions to the symposium.

(a) Submission of synopses

Persons who wish to present a paper or poster at the symposium must submit an extended synopsis (in English) of 800 words maximum (i.e. two A4 format pages of single spaced typing or the equivalent, including any tables or diagrams and a few pertinent references) on one of the topics listed under Section 2. The extended synopsis should be submitted together with the completed Form for Submission of a Paper/Poster (Form B), and the Participation Form (Form A) to the competent national authority for official transmission to the IAEA in time for them to be received by the IAEA by **17 December 2007**. In addition, the synopsis must be sent electronically to the IAEA scientific secretariat, e-mail: plant.mutation@iaea.org.

Authors are urged to make use of the Synopsis Template in Word on the symposium web page (see Section 15).

The specifications and instructions for preparing the synopsis and how to use the synopsis template are given in the attached instructions. Also attached is a sample extended synopsis.

The synopsis should give enough information on the contents of the proposed paper to enable the selection committee to evaluate it. Introductory and general matters should not be included. The synopsis - if accepted - will be reproduced in unedited form in the Book of Extended Synopses; the original must therefore be submitted as a camera-ready copy in a form in which the author will wish to have the work presented. The general style and presentation should be as in the attached sample.

(b) Acceptance of Papers for Oral Presentation and Poster Presentation

Given the number of papers anticipated and the need to provide ample time for discussion, the number of papers that can be accepted for oral presentation is limited. Authors who would prefer to present their papers in a poster session are requested to indicate this preference on Form A with which they send the extended synopses.

Authors will be informed whether their papers/posters have been accepted for presentation on the basis of the extended synopsis. Guidelines for the preparation of the papers and the deadlines for their submission will be provided at that time.

The IAEA reserves the right to decline to present or publish any paper that does not meet expectations based on the information in the extended synopsis.

Further details about the preparation of papers and oral presentation at the symposium will be sent to the authors of the papers accepted together with notification of acceptance.

6. Expenditures

No registration fee is charged to participants.

As a general rule, the IAEA does not pay the cost of attendance, i.e. travel and living expenses, of participants. However, limited funds are available to help meet the cost of attendance of selected specialists mainly from **developing countries with low economic resources**. The grants awarded will be in the form of lump sums usually covering only part of the cost of attendance. Generally, not more than one grant will be awarded to any one country.

If governments wish to apply for a grant on behalf of one of their specialists, they should address specific requests to the IAEA to this effect. Governments should ensure that applications for grants are submitted by **17 December 2007** and are accompanied by a duly completed and signed Grant Application Form (as attached). Applications that do not comply with these conditions cannot be considered.

7. Symposium Proceedings

The proceedings of the meeting will be published by the IAEA as soon as possible after the symposium.

8. Distribution of Documents

A preliminary programme of the symposium will be sent to participants in advance. The final programme and the book of extended synopses will be distributed at registration.

9. Working Language

The working language of the symposium will be English.

10. Participation

All persons wishing to participate in the symposium are requested to **register in advance online**. In addition they must send a completed Participation Form (Form A) and if relevant, the Form for the Submission of a Paper (Form B) and the Grant Application Form (Form C) through the competent official authority (Ministry of Foreign Affairs, Ministry of Agriculture, national FAO committee, or national atomic energy authority) to the IAEA. A participant will be accepted only if the Participation Form is transmitted through the government of a Member State of the Sponsoring Organizations or by an organization invited to participate.

- Participants whose official submissions have been received by the IAEA will receive further information on the symposium approximately three months before the meeting. This information will also be posted on the symposium web page.

11. Accommodation

Detailed information on accommodation and other symposium related information will be sent to all designated participants well in advance of the symposium. This information will also be available on the symposium website.

12. Visa

Designated participants who require a visa to enter Austria (Schengen State) should submit the necessary applications to the nearest diplomatic or consular representative of Austria or any other consular authority of a Schengen partner State representing Austria as early as possible (please note that it could take up to three weeks to obtain a visa).

13. Channels of Communication

The Participation Form and as applicable, the Form for Submission of a Paper/Poster, and the Grant Application Form, should be sent to the competent national authority (Ministry of Foreign Affairs, Ministry of Agriculture, national FAO committee, or national atomic energy authority) for official transmission to the IAEA.

Subsequent correspondence on scientific matters should be sent to the Scientific Secretary and correspondence on administrative matters to the IAEA Conference Services Section.

14. Symposium Secretariat

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15. Symposium Web Page

Please visit the IAEA symposium web page regularly for new information regarding this symposium: http://www-pub.iaea.org/MTCD/Meetings/Announcements.asp?ConfID=167

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Review

Genetic Improvement of Crop Plants by Mutation Techniques in Korea

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Abstract

In Korea, mutation breeding in crops was started in the early 1960s. Although mutation breeding method has not been used heavily for the development of plant varieties and genetic resources, more than 30 varieties have been released since then. These mutant varieties released in Korea were mostly food and oilseed crops, and the major target was to improve agronomic traits such as yield, lodging tolerance, early maturity, or multi-disease resistance, etc. Additionally, horticultural crops including the rose of Sharon were improved to have a unique flower color and shape, dwarf-type height, or leaf stripes. Most of the varieties developed in the past were selected after exposing seed materials to radiation or chemical mutagens. Since the mid-1990s, the Korea Atomic Energy Research Institute (KAERI) has adopted new biotechnologies (such as tissue culture, *in vitro* selection, doubled haploids, molecular makers, sequence analysis of genes for marker development, genomics tools, etc.) which have been integrated with traditional mutation breeding methods to achieve wider mutant spectra and higher selection efficiency. Currently mutant libraries of rice, soybean, and other horticultural crops are in the processes of collecting more mutants, characterizing visual and molecular features and depositing the accumulated data, which will be a valuable resource for genetic and genomic studies. Government support was provided to construct a radiation-specific research center, Advanced Radiation Technology Institute (ARTI) in KAERI, which has essential facilities for mutation breeding and irradiation. Along with the traditional breeding approaches, a mutation breeding program, driven mostly by the ARTI, is ready to play a key role in the advancement of radiation applications to a wide range of basic and applied sciences, including the improvement of diverse plant varieties in Korea.

Keywords: Breeding, mutant, pepper, rice, soybean

Introduction

Genetic improvement of crop plants has made a major contribution to the production of food, feed, and biomaterials. Crop breeding efforts are aimed at increasing the yield and economic value by incorporating disease and insect resistance, better grain quality and a shorter growth duration. Significant achievements in the application of radiation and isotopic techniques (RT) have been made in agriculture and biotechnology (BT) fields throughout the world (Song & Kang 2003). The use of radiation induced mutations in agriculture is considered a symbol of the peaceful use of atomic energy; therefore the IAEA is promoting the use of induced mutation techniques in crop improvement. According to the FAO-IAEA Mutant Varieties Database (http://www-mvd.iaea.org), by 2007 about 2600 plant varieties were released worldwide which have been derived from the RT and chemical mutagens during the past seventy years. In Korea, mutation research in crops was begun in the early 1960s and about 30 plant cultivars have been released so far. The main objective of this report is to summarize important achievements made by the application of plant mutation breeding in Korea.

Brief history of mutation breeding in Korea

In Korea, mutation breeding in crops was begun in the early 1960s and the Radiation Agriculture Research Institute (RARI) was established for the peaceful use of atomic energy in the field of agriculture in 1966. From the mid 1960s to early 1970s, mutation breeding was recognized as one of the newest breeding techniques in Korea, and many plant breeders tried to apply the mutation techniques in their crop breeding programmes. The Rural Development Administration (RDA) and Seoul National University succeeded in the development of indica-japonica hybrid ("Tongil type" rice) cultivars with semi-dwarf and high yields by cross breeding in 1971. Thereafter, the main work in crop breeding was undertaken by the RDA due to the strong policy of the Korean government to increase rice production. RARI was incorporated into KAERI in 1973. As a result, most of the researchers related to mutation breeding moved to the RDA and to universities. Although the breeding research team was drastically reduced, the radiation breeding research team at KAERI played a major role in most of the mutation research in many crops. By means of mutation breeding, more than 30 new cultivars were developed; in rice: 17, sesame: 6, hibiscus: 5, soybean: 3, barley: 1, perilla and boxtron (= Chinese matrimony vine, *Lycium chinensis* Millor): 2 each, and distributed by KAERI singly or jointly with RDA (Table 1). Compared to other developed countries, mutation techniques in Korea have not been fully used for the development of plant cultivars and genetic resources. Since the mid-1990s, KAERI's research team has established new radiation plant breeding technologies in combination with newer ones, such as tissue culture, *in vitro* selection, molecular maker and gene analysis for developing high quality cultivars. Recently, plant mutation techniques in Korea have been gradually highlighted again in the fields of functional genomics, as well as breeding methods.

Table 1. Mutant varieties bred in Korea

Plant species	Mutant cultivars
Rice	Milyang-10, IRI-307, Wonpyongbyeo, Wonkwangbyeo, Wonmibybyeo, Huegseonchalbyeo, Woncheongbeyo, Wonpumbyeo, Wonchubyeo, Heugkwangchalbyeo, Nongwonchalbyeo, Seonong-6, Seonong-8, Seonong-9, Seolgaeng, Baegjinju, Goami-2
Barley	Bangsa-6
Soybean	KEX-2, Bangsakong, Josangseori
Sesame	Ahansankkae, Suwonkkae, Yangbaeckkae, Pungsankkae, Seodunkkae, Suwon 128
Perilla	Dasil, Kwangim
Hibiscus	Baegseol, Ggoma, Seonnyo, Daegoang, Changhae
boxtron	Cheongdae, Myoengan

Main achievement of plant mutation breeding in Korea

Rice

Rice is the most important crop grown in Korea in about 1 m ha and new cultivars have been bred mainly by cross breeding. The first rice mutant variety developed in Korea was "Milyang 10", derived by X-ray irradiation, which had a short culm, early maturity and was disease resistant in comparison with its parent cv. "Palgwaeng". Another mutant variety, IRI-307 was bred in collaboration between KAERI and the Honam Crop Experiment Station of RDA.

In 2000, KAERI released four rice mutant varieties, namely,, "Wonpyungbyeo", "Wonkwangbyeo", "Wonmibyeo" and "Heukseonchalbyeo" by irradiating with gamma rays after registering with the National Cultivar List. These new cultivars have an earlier maturity, better lodging tolerance and multi-disease resistance than their original cultivars (Shin et al. 2001 a, b, c). In 2003 another rice cultivar "Woncheongbyeo" was registered with the National Cultivar List. This cultivar had a short culm and high lodging tolerance and improved early maturity, compared to the parent "Chucheongbyeo", which has good taste and quality but was susceptible to lodging (Kang et al. 2006a). All of the newly developed varieties "Wonchubyeo"(Kang et al, 2006b), "Wonpumbyeo", "Nogwonchalbyeo", and "Huegkwangchalbyeo" were officially released to farmers in 2004. Rice seeds of the above-cited cultivars were produced by KAERI and distributed directly to farmers. Further, thirty promising mutant lines having salt tolerance, high quality and a high yield were selected by gamma ray irradiation, and conducted the evaluation of their characteristics during a regional productive trial. Rice mutation breeding is one of the major projects at KAERI. Recently, by the treating seeds with N-methyl-N-nitroso urethane (MNU), three rice cvs. "Seonong-6", "Seonong-8" and “Seonong-9” were released by Seoul National University and three rice cvs. "Baegjinju", "Seolgaeng", Goami-2 by National Crop Experiment Station (NCES) of the RDA (Table 1).

Soybean

Soybean is one of the most important crops on the Korea peninsula, where it has been known as an original genetic center for leguminous crops (Hymowitz, 2004). The soybean variety "Kwanggyo" was first released by cross breeding in 1969. At the same time, mutation breeding on soybean was started at the RARI and RDA. "KEX-2" was released in 1973 as a first mutant cv. It was developed after X-ray irradiation to "Kuemgangdaelip", which was a leading cv. in the 1970s, but had late maturity. "KEX-2" matured 11 days earlier than parent cv. and fitted well into the barley-soybean cropping system in Korea. Further, a new soybean cv., "Bangsakong" was developed by a joint work between the RDA and KAERI in 1983. When seeds of "CB-27," introduced from the USA were irradiated by 250 Gy gamma rays, "Bangsakong" was selected. It was recommended as a suitable cv. for the growing of a soy sprout with a small seed size 100-seed weight (HSW) 12g. Besides, "Bangsakong" showed higher yield (2.05 t/ha) compared to the local cvs. (1.53 t/ha) by which it was broadly cultivated in Korea (Lee, 1997).

Although mutation breeding for the soybean resulted in only two cvs., "KEX-2" and "Bangsakong" during >30 years in Korea, continuous research on the soybean has been conducted in various fields for breeding purposes using gamma ray irradiation to the seeds at KAERI. At the end of 2006 from the M_8-M_{12} generations, several promising lines were selected from the gamma ray mutated progenies, such as 6 lines with large seed size and resistance to pod and stem blight, 73 lines with small seed and higher yield, and 16 lines for cooking purpose. Besides, 16 lines with a low lipoxigenase (LX-1, LX-2, LX-3) content and 6 lines with a 20% lower phytic acid content than the parent were selected. These lines are currently subjected to confirmation at the biochemical and molecular levels before submitting for a plant variety protection in Korea. A new line for cooking with rice was selected, tested, and now applied for a plant variety protection right in 2005. The black seed-coated variety ‘Josaengseori’ had a smaller seed size (32.8 g HSW) and an early maturing (~30 days earlier) characteristic when compared to the original ‘Seoritae’ which had 40.1 g HSW. At KAERI, about 4,000 varieties/lines of native leguminous resources were collected and continuously proliferated, preserved and evaluated (Choi et al. 1999).

Mutation research by chemical mutagens in soybean has also been conducted, especially to develop root traits

such as greater nodulation by the Seoul National University and NCES-RDA.As a result, supernodulating mutants were selected on using EMS, which were characterized by a smaller plant height, greater dry nodule weight and number/plant than the parent, "Sinpaldalkong 2" (Lee, 1997).

Oil crops: sesame and perilla

Both sesame (*Sesamum indicum* L.) and perilla (*Perilla frutescens* Briton, Syn. to *Perilla. ocymoiides* L.) oil are widely used for cooking. The leaves of perilla are also commonly used as a vegetable. Mutation breeding of sesame has been mainly conducted at the NCES of RDA since the late 1970s. First a sesame mutant cv., "Ahnsankkae", was successfully developed after irradiating with 200 Gy X-rays to the seeds of a local cv., "90 days chamkkae". The mutant has desirable characteristics such as pure white seed coat, resistance to diseases, disasters and higher yield. Therefore, it was rapidly grown in more than 30% of total sesame cultivated area, 50,000 ha for a while in the late 1980s (Kang, 1997). "Ahnsankkae", "Suwon-128", "Yangbaeckkae", and "Seodnkkae" were developed following chemical mutagen, sodium azide (NaN_3) 2mM for 2.5h treatment to the seeds at NCES. "Suwon-131" and "Pungsankkae" were bred by crossing mutant DR-45 with other cultivars.

Mutation breeding of perilla was initiated at NCES in 1990. Gamma rays with 150-400 Gy and 0.5% EMS were applied to the dry perilla seeds of cvs. "Yechun", "Suwon 38," Yeupsildlkkae and Okdongdlkkae. In the M_2, 1,875 variants were selected (Park, 1997). In 2003, two new varieties of perilla were released. "Dasil" with early maturity and dwarf habit, was derived on irradiating with 350 Gy gamma rays to the seeds of "Yechun" (Park et al., 2003a). "Kwangim" with a high yield and leaf rust resistance, was induced by EMS treatment to Suwon 38 seeds (Park et al., 2003b).

In order to breed new leaf edible varieties, perilla cv., "Chukyopzaso" was irradiated with 200-350Gy gamma rays and various mutants affecting leaf shape, chlorophyll and anthocyanin were isolated in the M_2-M_3 generations. The chlorophyll content in perilla leaves ranged from 52% to 134%, compared to the parent. It had increased in 14 mutant lines and decreased in 15 lines. The anthocyanin content ranged from 74% to 157% and increased in 26 mutant lines and decreased in only 5 lines (Lee et al., 1999a). "Chukyopzaso" also gave rise to different variants in leaf flavor components. Promising mutants with high contents of limonene were obtained from 8 lines, perillaldehyde from 8 and α-pinene from one line (Lee et al., 1999b). The promising lines are under field trials for the characterization of their agronomic traits and analysis of the functional compound to register as a new variety.

Hibiscus (Rose of Sharon)

The rose of Sharon (*Hibiscus syriacus* L.) known as "Mugungwha" in Korean, is the Korean national flower. Mutation breeding on hibiscus was initiated since the late 1980s. More than 100 varieties were collected in Korea and other countries. The first induced mutant developed was "Baekseol" (meaning 'white snow'). It was derived from a bud mutation by a 50 Gy gamma ray irradiation to the cuttings of "Hwarang" (Song et al. 1999). Until now, four mutants were officially registered as new varieties and then released - "Seonnyeo" and "Daegoang" in 2003 and "Ggoma" and Changhae in 2006. "Seonnyeo" has light purple petals and white core lines on the inner petals. It was selected from a mutant line irradiated with gamma rays (100 Gy) to the seeds of "Gyewolhyang" which had dark purple petals and red core lines (Song et al, 2005a). "Daegoang", with a large flower size (12-14 cm in diameter) was derived from the mutant of "Younggwang" (8-10cm in flower diameter) (Song et al. 2002b). "Ggoma" (meaning of 'kid') is a dwarf mutant selected from the 100 Gy gamma ray irradiated seeds of "Hongdansim-2" (Song et al. 2005b). Besides shorter plant height, flower and leaf size are smaller than parent cv. In an 8-year-old shrub, the height and flower diameter are 1m and 5cm for "Ggoma" and 2.5m and 9-10cm for "Hongdansim-2", respectively. "Ggoma", can be grown as a 'bonsai' or indoor cultivation (Song et al, 2006a). Changhae was mutated from original cultivar Suminokura and shows a bluish purple color and a large flower size (Song et al., 2006b). New mutant lines of hibiscus with various plant and flower types were selected and their characteristics are continuously being evaluated.

Flowers

Since the 1990s, breeding of flowers has been emphasized in Korea. The National Horticulture Research Institute (NHRI) of the RDA, applied gamma radiation of 10~50 Gy to the young plants of Chrysanthemum propagated by tissue culture. Various mutants with a changed flower color and shape were obtained in the M_1V_3 generation, namely, "Bongwhang", "Herman De Boon", "Hijeong" and "Paso Doble". Pink flower color changed to yellow, orange, bronze and red. In some of the mutants, purple color changed to white, pink and red, and white changed to yellow and pink. The selected promising lines were evaluated for their characteristics (Goo, 2000). A wide range of plant materials were subjected to exposure to gamma rays for mutation induction in chrysanthemum in Korea, which include cuttings, rooted plants, and calli derived from petals or stems. Stem segments of a spray-type chrysanthemum (cv. Argus) were cultured on MS medium containing 1.0 mg•L^{-1} NAA and 1.0 mg•L^{-1} kinetin, and the regenerated plantlets on NAA and BA combination medium were treated with various doses of gamma rays. Results indicated that gamma ray radiation ranging from 30 to 50 Gy was effective in *in vitro* mutagenesis for the flower color, flower shape, and stem color of the spray-type chrysanthemum. In 50 Gy of gamma-ray irradiation, 28.2 and 15.4 % of the individuals varied from the original variety in the flower color and shape, respectively (Park et al., 2007). Development of a non-branching standard type of chrysanthemum has been investigated after irradiation with a gamma ray in several places in Korea, which is popular to many Korean

farmers due to its labor saving culturing and economic gains.

Besides the Chrysanthemun, at present many ornamental plants including rose, *Rhododendron*, lily, lawn grass, and native wild flowers are being used for mutation breeding at many national and provincial agriculture research institutes and universities. KAERI has also just started the mutation breeding of floricultural and native plants to develop new plants with desirable traits. Another increasing market in potting flower is the orchid. With collaboration with the private sector, KAERI recently released novel mutants through combining use of technologies of tissue culture and gamma irradiation. One of the new varieties, "Dongi", was derived from the imported oriental orchid Cymbidium. Another variety "Eunseol" was originated from the Korean indigenous orchid "Sokgok". Both varieties were favored for their fine and unique stripes along the leaf edge.

Pepper

In collaboration with KAERI, Nongwoo Bio Co. Ltd., one of the major Korean seed companies developed the disease tolerant lines of hot pepper (*Capsicum annuum* L) by using radiation and interspecific hybridization. Many variants were selected by irradiation with 250Gy, but most plants were recessive types and were found to be inferior. However, 12 lines from the M_6 generation were selected based on the fruit shape and fruit setting ability. Sixteen lines from the M_6 by pedigree breeding method resulted in the selection of the 95240 line, which is resistant to phytophthora root rot. This line showed diverse phenotypes and can be used as resistant sources for the phytophthora root rot, once they become bred-true.

Current trends in using mutation techniques with the RT and BT combination techniques

Application of a nuclear technique for a solution to the food problem has been useful in providing an opportunity to accelerate plant improvement. Current research programs emphasize on the development of techniques of plant tissue culture and on the induction and selection of radiation mutations. Since the mid-1990s, at KAERI new radiation plant breeding technologies have been established to breed cultivars with resistance to environmental stresses and a high quality in combination with new biotechnologies such as application of tissue culture, *in vitro* selection, doubled haploids, molecular maker, and gene analysis, etc.

Development of NaCl tolerant rice plants through the irradiation of gamma rays and tissue culture

Selection and regeneration of NaCl tolerant cell lines

Callis were treated with 30~90 Gy gamma rays on a medium containing 1.5% NaCl to obtain the NaCl tolerant cell lines. The frequency of regeneration in the irradiated (30Gy and 50Gy) calli was higher than that of the unirradiated, but decreased at over 70 Gy. Proline, phenolic compounds, sugar and protein contents in tolerant calli were 0.6, 10, 5 and 4 times more than those of non-selected ones, respectively and the peroxidase activity was 2 times more. Proline content of the plantlets derived from tolerant callus was 3 times higher than that of control. Leaves of the regenerants (M_1) derived from the salt tolerant calli were not normal in their shape compared to those of the corresponding "Dongjinbyeo" because of their frequent mutation (Lee et al., 2002a).

Double haploids have long been recognized as a valuable tool in plant breeding, since they not only offer the quickest method of advancing the heterozygous breeding lines to homozygosity, but also offer an increased selection efficiency over conventional procedures due to a better discrimination between the genotypes within any one generation. Salt tolerant mutants were obtained in the rice cv. Hwaseongbyeo, through *in vitro* mutagenesis of anther cultured calli. Various doses (30, 50, 70 and 90 Gy) of gamma rays were used to investigate the effect of radiation on the callus formation on a medium containing 1% NaCl, green plant regeneration, frequency of the selected double haploids and salt tolerance screening. It was demonstrated that 30 and 50 Gy gamma rays have a significant effect on the callus formation, regeneration and the selection of salt tolerance. No tolerant line was obtained from unirradiated cultures. From gamma ray irradiated cultures, five lines in the M_2 and 13 lines in the M_3 showed tolerance at germination and seedling stages, respectively. The frequency of the salt tolerant mutants indicates that anther culture in combination with gamma rays is an effective way to improve salt tolerance (Lee et al., 2003a).

Selection of salt tolerant lines at M_2 and M_3

To investigate the germination rate, seeds (M_2) were kept in a solution of 1.5% NaCl for 10 days. The germination rates of the M_2 lines ranged from 0 to 50% and the tolerant lines with a superior ability in growth were screened. The ratio of segregation in the tolerant line vs the sensitivity line was 1:1. The salt tolerant lines (M_3) at the seedling stage were isolated and compared to the Dongjinbyeo. The ATPase, catalase and peroxidase activity of the tolerant lines were increased in the mutants. But, the electrical conductivity, starch and Na+ content were decreased in mutants compared to the Dongjinbyeo (Lee et al., 2003 d).

Selection and test of tolerant lines in a saline field

Among the planted lines in the saline field, survival rate, plant height, panicle length, no. of hills and fertility rate of lines nos. 18, 50 and 268 had increased compared to Dongjinbyeo. Salt tolerant lines, harvested in the year 2001 were again sown at the saline field (Dobido) with two replications and designated as salt tolerant because of the increased biomass and grain yield (Lee et al., 2003b).

DNA and protein analysis of the salt tolerant lines

AFLP and RAPD techniques were used to classify the tolerant and sensitive lines. Specific bands derived from the E/ACC + M/CTG combination primer in the AFLP and the F-08 primer in the RAPD were observed in toler-

ant lines. The AFLP and RAPD bands were sequenced and analyzed for nucleotide sequence homology. AFLP band has homology with *rbcL* gene and cold stress - induced cDNA (Lee et al., 2003c).

The translation products were analyzed by two-dimensional gel electrophoresis. About 300 polypeptide spots appeared in plant leaves, 6 spots showed in only the tolerant lines and many spots showed an increase, in a relative amount. 11 polypeptides were isolated and sequenced. Among the 11 polypeptides, only 4 polypeptides could be sequenced. The homology search revealed that two peptides of 100-kD had a significant homology with phosphoribulokinase, a 35-kD protein with an oxygen evolving enhancer 1 and a 25-kD protein with a H+-ATPase (Lee et al., 2004).

Characterization of 5-methyltryprophan (5MT) resistant rice mutants with high amino acids

Selection of 5MT resistant mutants

Rice is one of the most important crops for the human dietary system. However, rice which forms the staple food of a majority for the people is very low in tryptophan and lysine in the storage proteins within the endosperm. Therefore, it is a matter of concern and also interest to know about nutritional problems in a country which lives on rice, whether there is sufficient intake of essential amino acids through rice, since they are not synthesized in humans and monogastric animals. For improvement of the grain quality in rice, 5MT resistant cell lines were selected by i*n vitro* mutagenesis using gamma rays. 5MT resistance was expressed in the regenerated plants and their progenies. Two lines, MRI and MRII, were obtained from the regenerated plants of these 5MT resistant cell lines. Both lines were successively propagated by selfing, which resulted in four homozygous lines in the M_3. Protein content of brown rice was increased by about 19% and 32%, and the total content of free essential amino acids increased to 71% and 34% over control in MRI and MRII groups, respectively.

Development of AFLP and STS markers

To develop a genetic marker for the identification of the 5MT resistant mutants in early generation, AFLP analysis was conducted with the control, homozygous MR lines, and segregating lines. Of the 3684 AFLP bands from the 45 primer combinations by eight EcoRI(+2) and MseI(+3) primers, 361 (9.8%) were polymorphic in the 5MT resistant mutants. The size of the polymorphic fragments ranged from 55 to 313 bp in length. Mutants were grouped into three clusters in a cluster analysis through the UPGMA method. Ten out of the 36 sequenced polymorphic PCR products were used for designing the primer sets for STS analysis. In the test with two homozygous M_4 5MT resistant mutants, six STS primer sets (OSMR1, OSMR2, OSMR3, OSMR4, OSMR5, and OSMR6) generated a single monomorphic PCR product identical in size to the original AFLP products. Four STS markers (OSMR1, OSMR2, OSMR4, and OSMR5) revealed polymorphic products between the control and the seven M_2 progenies phenotypically resistant to 5MT (Kim et al., 2004 b). These STS markers can serve as potentially 5MT resistance-specific markers.

Expressed sequence tag (EST) analysis

To study the gene expression in the plant vegetative tissues, a cDNA library was constructed by using the leaves and roots of the 5MT resistant mutant plants. Complementary DNA was constructed from leaves and roots of the 5MT resistant plants. Titter analysis of the secondary library indicated about a 3.55×10^{10} pfu/ml. Inserted DNA sizes were distributed from 0.35~2.5 kbp with the average value of 1.14 kbp. The expressed sequenced tags (ESTs) of 1,019 randomly selected clones evaluated by assembling 588 non-overlapping sequences. Through BLASTx search analysis against the NCBI database, 389 unigenes with significant homologies with known protein sequences and the remaining 199 unigenes were designated unidentified genes. These ESTs were grouped into 13 categories according to their putative functions. A total of 126 unigenes were considered to be genes regulated by 5MT through inference from known pathways and mechanisms related to stresses or to amino acid synthesis. Many genes that were identified tended to be related to defense and stress responses, suggesting “cross-talking” between biotic/abiotic stresses including the 5MT treatment. Therefore, 5MT resistant mutants might be of value for identifying genes related to plant defenses and stresses (Kim et al., 2007a). Further work is required for identification of the physiological response and the resistance mechanism of a plant in response to an amino acid analog related to the other stresses.

Characterization of the altered anthranilate synthase in 5-methyltryptophan resistant rice

In order to identify the 5MT resistant mechanism, the current study further investigated these mutant lines; the anthranilate synthase activity of these M_5 advanced lines was measured by a direct fluorometric detection of the formed anthranilate in the control plants and the mutant lines grown on 500 μM 5MT. The anthranilate synthase activity of the mutant plants was 2.2 - 3 times higher than that of the control. In a kinetic analysis with tryptophan, anthranilate synthase of the mutant lines was insensitive to feedback inhibition. These lines showed an enhanced accumulation of storage proteins and amino acids. The increasing rates of protein in the mutant lines, relative to the control seeds, were 17 ~ 28.5% (Kim et al. 2004). The amino acid contents were 2.4 (MRI-40-2) ~ 2.6 (MRI-110-6) times higher in the MRI lines than those in the control seeds, and 2.4 (MRII-12-5) ~ 3.5 (MRII-8-1) times higher in the MRII lines than the control seeds. Significant increases among the amino acids of the MR lines were observed in tryptophan, phenylalanine and tyrosine, which had been biosynthesized through the shikimate pathway. The transcript levels of putative OASA2, which is one of the key regulated enzyme subunits in the tryptophan biosynthesis pathway, were studied in the control and the 5MT resistant mutant lines under two tryptophan analogs (5MT and αMT) inhibition

and other abiotic stresses (ABA, NaCl, and cold). Putative OASA2 gene in the 5MT resistant mutant lines was highly expressed in a low 5MT concentration and at an early stage of the 5MT and αMT treatments. However, the mRNA accumulation of putative OASA2 gene in the mutant plants was gradually decreased by abiotic stresses such as NaCl and a cold-treatment. These results indicate that the 5MT resistance in the mutant lines was due to the altered anthranilate synthase forms (Kim et al. 2005b).

Proteomic analysis

SDS-PAGE analysis identified changes in total proteins and purified protein fractions based on their solubility properties between the control and the mutant lines. The 5MT can fit into the allosteric site of AS in the same way as tryptophan. In conclusion, it may be an inhibition reagent in a plant cell. This cytotoxic effect by 5MT may induce stresses in vivo similar to other environmental stresses. Proteins produced in elevated amounts or de novo in response to 5MT were studied by comparing the silver-stained two-dimensional gels of leaf proteins between the control and two 5MT resistant mutant lines. At least twenty proteins were produced in elevated levels or de novo following an exposure to the growth inhibitory concentrations of 5MT in MRI-40. During the investigation of the 5MT stress-mediated responses of the four antioxidant enzymes, catalase (CAT, EC 1.11.1.6), peroxidasse (POD, EC 1.11.1.7), superoxide dismutase (SOD, EC 1.15.1.1) and aspartate peroxidase (APX, EC 1.11.1.11), the activity levels of the four enzymes were increased by the 5MT treatment in the control and the 5MT resistant mutant lines. However, the mutant lines revealed higher increases of the antioxidant enzymes than the control. Differences of a significant activity increases between the control and the mutant lines were observed in the SOD and APX activity assays. Native PAGE confirmed these differences in the SOD and APX activities with the separation patterns of the isoforms of SOD and APX (Kim et al. 2005a).

Selection of azetidine-2-carboxylic acid (AZCA) resistant cell lines by in vitro mutagenesis in rice

Resistant cell lines to the AZCA were selected through rice embryo culture after callus was irradiated with 30, 50, 70, 90 and 120 Gy gamma rays. The optimum AZCA concentration for the selection of resistant cell lines was 3 or 4 mM AZCA considering the LD_{50} and the fresh weight of the callus. Survival rate of the AZCA resistant callus showed a remarkable increase in the callus irradiated with 50 and 70 Gy. Based on the fresh weight, survival rate and regeneration for the selection of the AZCA resistant cell line, 50-90 Gy was considered as the optimum dose range for gamma irradiation. Irradiated calli selected from AZCA were more tolerant to NaCl than those from the non-irradiated calli. This suggests that the elevated resistance to osmotic stress resulted from the mutagenic treatment. The level of the free proline content in the AZCA resistant cell line had increased up to 3.5 times compared to control. Proline content in the regenerant derived from the AZCA resistant cell line also increased to 1.7 times that from the control plants regenerated from the callus grown in the AZCA free medium. Selection of the proline overproducing cell lines by in vitro mutagenesis was successful and seems to be useful for the improvement of stress tolerance in rice. The selected lines were planted in a paddy field to analyze the growth characteristics (Hyun et al., 2003, Lee et al., 2002b).

Selected AZCA resistant lines that had a high proline accumulation were used as sources for the selection of NaCl resistant lines. To determine an optimum concentration for selection of NaCl resistant lines, Donganbyeo seeds were initially cultured on a media containing various NaCl concentrations (0 to 2.5%) for 40 days, and a 1.5% NaCl concentration was determined as the optimum concentration. One hundred and sixteen salt-tolerant (ST) lines were selected from 20,000 seeds of the AZCA resistant M_3 seeds in the medium containing 1.5% NaCl. The 33 putative lines (M4 generation) considered to have a salt-tolerance were further analyzed for their salt tolerance, amino acid and ion contents, and the expression patterns of the salt tolerance-related genes. Out of the 33 lines, 7 lines were confirmed to have a superior salt tolerance. Based on a growth comparison of the entries, the selected mutant lines exhibited a greater shoot length by 1.5 times, root length by 1.3 times, root numbers by 1.1 times, and fresh weight by 1.5 times that of the control. Proline contents were increased at a maximum by 20%, 100% and 20% in the leaf, seed and callus, respectively, of the selected lines. Compared to the control, amino acid contents of the mutants were 24 to 29%, 49 to 143% and 32 to 60% higher in the leaf, seed and callus, respectively. The ratios of Na^+/K^+ for most of the ST-lines were lower than that of the control, ranging from 1.0 to 3.8 for the leaf and 11.5 to 28.5 for the root, while the control had 3.5 and 32.9 in the leaf and root, respectively. The transcription patterns for the P5CS and NHX1 genes observed by RT-PCR analysis indicated that these genes were actively expressed under a salt stress. The selected mutants will be useful for the development of rice cultivar resistant to a salt stress (Song et al., 2007).

Development of herbicide-resistant cell lines using gamma ray irradiation

In joint research with KAERI, Suncheon University and Korea University teams have been trying to select cell lines with herbicide tolerance by radiation and in vitro culture methods in rice and wheat. Herbicide tolerant rice (*Oryza sativa* L. cv. Ilpumbyeo) cell lines were selected from γ-ray-irradiated anther-derived cell cultures. One line (CHB-1) showed a stable tolerance at a 10 mg/l concentration after a 6-month culture without a herbicide suspension. These results reveal that somatic hybrids were successfully obtained by fusing the cyhalofop butyl resistant cell line with a different cultivar having plant regeneration ability.

To identify the molecular markers for bentazone resistant wheat lines, wheat mutants were generated from a cultivar “Geumgangmil” by using gamma-ray irradiation.

Bentazone resistant M_2 plants were selected from seedlings and adult plants consecutively. The Bentazone resistant wheat plants were able to survive at up to 10 times (1,600ppm) the commercially recommended concentration. The four plants with the highest bentazone resistance were selected. An AFLP analysis was done to identify molecular markers for bentazone-resistant wheat mutants. Twelve polymorphic products were identified, cloned, and sequenced. Among the twelve polymorphic bands, eight Sequence-tagged Sites (STS) primer sets were designed. Only one STS primer set (HRMW-08) was converted into a "Bentazone-resistant wheat-specific" STS marker. The wheat mutants and makers could be employed in a cross-breedeing program incorporated with maker assisted selection in the early stage of population (Kim et al. 2007b).

Conclusion

The mutation breeding program in Korea has traditionally focused on major food and oilseed crops. Currently the high yield potential of the cultivated crop cultivars in the Korean field is relatively less popular than before, but the expectation of more value-added crops from the farmer's side is in high demand. Accordingly, the mutation breeding program in Korea has assigned more resources to other crop species, including some floral plants, medicinal plants, and industrial crops.

At present, besides the importance for mutation breeding, mutation techniques have been used in many germplasm enhancement programmes for functional genomics. For the use of gene tagging, some Korean research teams are producing many thousands of insertional mutant lines by T-DNA (Jeong, 2002) and transposon techniques (Han et al. 2003) in rice, *Arabidopsis* and others. However, insertional mutation techniques are very laborious and cost intensive, whereas radiation and chemical mutagenesis might be more efficient when they are combined with microarrays and other new molecular techniques such as TILLING (Targeting Induced Local Lesions IN Genomes) (Perry et al. 2003). Accordingly, the development of new crop cultivars and genetic resources is of great importance, not only for crop production to satisfy the increasing food demand, but also for functional genomic research in the post-genomic era.

In the early 2000s, the Korean government started to support fundamental and applicable research in radiation fusion technologies with BT, IT (information technology), NT (nano technology), and ST (space technology). Also, the outcome of the technologies including the agricultural varieties and gene information were eventually transferred to the related industries for a quick commercialization. The contemporary financial assistance was provided to develop essential facilities within the Advanced Radiation Technology Institute (ARTI), which include research laboratories, office buildings, irradiation facilities, breeding field of about 20 ha, parking lots, and a dormitory. Other supporting facilities designed mainly for the radiation breeding program were acquired consecutively by the ARTI, which includes glass houses, a gamma-phytotron, and a seed storage facility with low temperature. An electron beam irradiator, a cyclotron for the radioisotope production, and a training center will be operational shortly. The advanced facilities and compiled data at ARTI will significantly contribute to a radiation mutation breeding for international communities. Additionally it is expected that ARTI, with its diverse resources and governmental support can play a key role in the advancement of radiation applications for a wide range of basic and applied sciences, including the improvement of diverse plant varieties.

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Research Article

Genetic Enhancement of Groundnut through Gamma Ray Induced Mutagenesis

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Abstract

Induced mutagenesis along with recombination breeding played a vital role in the genetic improvement of groundnut **(***Arachis hypogaea* L.) at Bhabha Atomic Research Centre, India. In continuation of ongoing mutation research since the 1960s, popular cv. TAG 24 was irradiated with gamma rays in order to further generate genetic variability. As expected, higher doses of gamma rays drastically affected the seedling traits in the M_1 generation. In the M_2, in all 71 true breeding macro mutants affecting plant height, leaf colour, leaf type, leaf size and leaf shape, flower colour, pod type, seed size and seed colour were isolated, characterized and maintained.

Key words: *Arachis*, groundnut, gamma rays, mutants, heritability

Introduction

Cultivated groundnut exhibited a narrow genetic base despite having extensive morphological, physiological and agronomical variability. The scarcity of genetic variability makes groundnut vulnerable to a wide variety of biotic and abiotic stresses. For instance, groundnut cultivars grown in the Southern USA are highly susceptible to *Meloidogyne arenaria* and crop damage in heavily infested fields can be devastating (Nelson et al. 1989). Induced mutagenesis was successfully applied along with cross breeding, generating a wide spectrum of variability in groundnut affecting various traits (Gregory, 1955; Patil, 1966; Chandramouli et al., 1989; Gowda et al., 1996). Consequently, 25 mutant or mutant derivative varieties were developed and released for commercial cultivation for the benefit of Indian farmers between 1970 and 2004 (Murty et al., 2004).

A blend of mutation and recombination breeding played a significant role in the development of unique traits as seen in the development of cv. TAG 24, which has a genomic blend of five different mutants brought under the background of cv. M 13 (Patil et al., 1995). It has most of the ideal morpho-physiological traits, such as semi-dwarf habit, small, thick, dark-green leaves, determinate flowering, early maturity and enhanced dry matter partitioning (Badigannavar et al., 2002). Due to its superior performance across the country, TAG 24 is used as National Check Variety in *Rabi* (post rainy)/summer situations in the All India Coordinated Varietal Trials. In view of its worthiness, TAG 24 was chosen for gamma ray mutagenesis to induce further genetic variability.

Materials and methods

Dry seeds of TAG 24 were irradiated with 100, 200, 300, 400 and 500 Gy of gamma rays from a ^{60}Co source at the Bhabha Atomic Research Centre (BARC), Mumbai. In each dose, three replications with 20 seeds each along with un-irradiated control were germinated in laboratory on filter papers kept inside Petri plates moistened with tap water. After three days, germinated seeds were transferred to wooden boxes containing soil and sand (2:1) and kept under 24 h illumination at 22 ± 1°C. Observations were recorded on seedling height, hypocotyl length, root length, number of leaves, number of branches and germination percentage at 15 days after sowing (DAS).

For the mutation induction study, 500 seeds each of TAG 24 were treated with 150, 250 and 350 Gy gamma rays and sown in the field (M_1) along with 100 un-irradiated seeds. M_2 generation was raised as M_1 plant progenies. All throughout the crop season, M_2 plants were screened, variant plants marked at regular intervals, harvested individually and advanced to the M_3 to study their breeding behaviour. True breeding mutants were advanced to subsequent generations by growing alternately in rainy (June – October) and summer (January – May) seasons till M_8 generation with spacing of 50 X 15 cm and 30 X 10 cm, respectively. Pod beak, constriction and reticulation were recorded as per the groundnut descriptors (IBPGR and ICRISAT 1992).

Genotypic variability (V_G), genotypic coefficient of variation (GCV), heritability in broad sense and the genetic advance (GA) for plant height, number of branches, pod yield (g plant^{-1}), seed yield (g plant^{-1}), shelling outturn (%), 100 seed weight (HSW, g), oil content (%) and oil yield (g plant^{-1}) were studied from the data on five randomly selected plants in two replications among 71 mutants and parent TAG 24 from M_5 to M_8 generations.

Results and discussion

Effect of gamma ray irradiation

With an increase in dosage of gamma rays, seedling traits were affected severely (Table 1). The reduction was more pronounced in seedling height and number of branches (Fig. 1A). Based on these observations, 150, 250 and 350 Gy doses were identified as effective doses for field experimentation. In the field, the highest germination (77%) was observed in 150 Gy, while the lowest (63%) was in 350 Gy as against un-irradiated control (75%). In M_1 generation, there was a reduction in the number of surviving plants at harvest with an increase in dosage i.e., from 68% to 30% in 150 and 350 Gy, respectively.

Induction of mutants for morphological traits

M_2 generation was comprised of 785 families with a total plant population of 11,441 surviving plants at harvest. In all, 71 mutants were induced with a frequency of 0.62% affecting various characteristics which bred true in the M_3 and subsequent generations (Table 2). They were des-

ignated as Trombay groundnut mutants (TGM)s. Out of 71 mutants isolated, one mutant was obtained spontaneously from another mutant, 28 were from 150 Gy, 36 from 250 Gy and six from 350 Gy with a mutation frequency of 0.42%, 0.88% and 0.75%, respectively based on surviving M_2 population per dose. This indicated that 250 Gy appeared more effective in this study.

Table 1. Effect of gamma ray irradiation on germination % and seedling (15 days old) traits

Gamma ray treatment (Gy)	Germination (%)	Root length (cm)	Hypocotyl length (cm)	Number of leaves	Number of branches	Seedling height (cm)
0	97.7	18.7	2.2	6.0	1.5	13.8
100	95.5	18.4	2.0	6.0	1.6	15.6
200	90.9	17.9	1.8	5.5	0.7 *	13.9
300	95.5	16.9	1.8	5.5	0.5 *	8.9 *
400	88.6	15.0 *	1.7	5.0 *	0.1 *	6.5 *
500	84.1	7.2 *	1.7	3.7 *	0.0 *	3.2 *
CD 5%		2.0	NS	0.6	0.5	2.3

* Significantly different from 0 Gy at P = 0.05

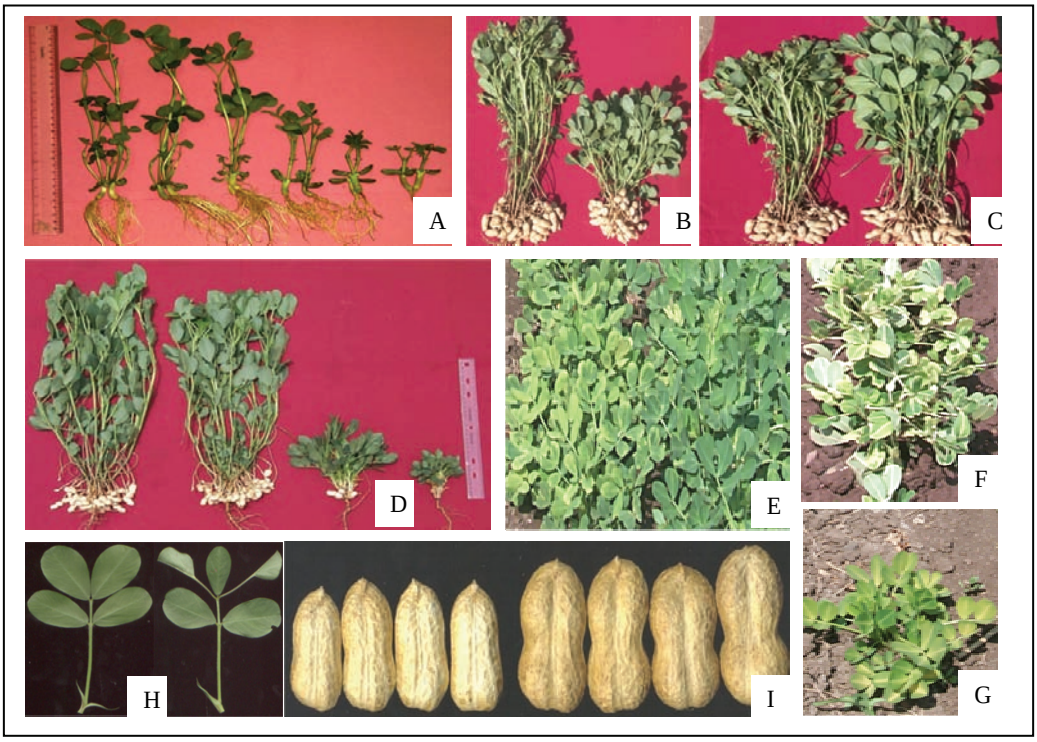

Figure 1. A: Effect of gamma rays on M_1 seedlings. Left to right: Parent, 100, 200, 300, 400 & 500Gy gamma ray treated
B: Parent (left) and dwarf mutant (right)
C: Parent (left) and tall mutant (right)
D: Parent (left) and TGM 36 for plant height segregation in the M_3 (left to right) for Phenotypes of parent, dwarf and extreme dwarf
E: Disease lesion mimic leaf mutant (left) and parent (right)
F: *Virescent* leaf mutant
G: Golden yellow coloured leaf mutant
H: Parent (left) and funnel shaped apical leaflets in TGM 51 (right)
I: Parent (left) and dumb-bell shaped pods in TGM 59 (right)

Table 2. Frequency (%) and number of mutants induced by different doses for various traits

Trait	Dose (Gy)	Mutants		
		Number	Frequency	Name
Plant height				
Dwarf	150	5	0.08	TGM 2, TGM 14, TGM 18, TGM 36, TGM 69
	250	11	0.27	TGM 1, TGM 3, TGM 4, TGM 5, TGM 8, TGM 10, TGM 19, TGM 25, TGM 42, TGM, 43, TGM 73
Tall	150	1	0.02	TGM 85
	250	1	0.02	TGM 59
	350	1	0.13	TGM 86
Leaf colour				
Disease lesion mimic	250	1	0.02	TGM 55
Virescent	250	1	0.02	TGM 58
Golden yellow		1		TGM 118 (Spontaneously occurred)
Waxy	150	1	0.02	TGM 15
	250	1	0.02	TGM 56
Leaf size				
Small	150	6	0.09	TGM 12, TGM 13, TGM 26, TGM 47, TGM 48, TGM 83
Large	250	2	0.03	TGM 59, TGM 87
	350	1	0.13	TGM 86
Leaf shape				
Funnel	150	1	0.02	TGM 50
	250	2	0.05	TGM 49, TGM 51
Long rachis	150	1	0.02	TGM 61
Suborbicular	150	1	0.02	TGM 40
	250	2	0.05	TGM 38, TGM 41
Drooping	250	1	0.02	TGM 44
Involute	150	1	0.02	TGM 46
	350	1	0.13	TGM 17
White flower	250	1	0.02	TGM 53
Pod shape				
Slight beak	150	3	0.05	TGM 7, TGM 14, TGM 21
	250	1	0.02	TGM 34
Slight constriction	150	1	0.02	TGM 7
	250	1	0.02	TGM 24
Deep constriction	250	3	0.07	TGM 70, TGM 71, TGM 72
	350	1	0.02	TGM 68
Slight reticulation	150	3	0.05	TGM 14, TGM 15, TGM 18
	250	1	0.02	TGM 51
Prominent reticulation	350	1	0.13	TGM 68
Parallel reticulation	150	1	0.02	TGM 94
Dumb-bell	150	1	0.02	TGM 117
	250	1	0.02	TGM 59
Seed size				
Small	150	1	0.02	TGM 22
	250	2	0.05	TGM 9, TGM 23
	350	1	0.13	TGM 35
Large	150	3	0.05	TGM 61, TGM 67, TGM 90
	250	6	0.14	TGM 59, TGM 64, TGM 65, TGM 66, TGM 87, TGM 92
	350	1	0.13	TGM 91
Testa colour				
Pink	150	2	0.03	TGM 62, TGM 79
	350	1	0.13	TGM 77
Purple	250	1	0.02	TGM 80
Light red	150	1	0.02	TGM 45

Trait	Dose (Gy)	Mutants		
		Number	Frequency	Name
Chocolate	250	1	0.02	TGM 78
Higher pod yield	150	1	0.02	TGM 16
	250	1	0.02	TGM 84

Mutants for plant height

Mutations for plant height included 16 for dwarf (Fig. 1B) and three for tall (Fig. 1C). Of the 16 dwarfs, five were isolated from 150 and 11 from 250 Gy. In tall mutants, one each was induced from 150, 250 and 350 Gy treatments, respectively (Table 3). Among the dwarf mutants, the plant height ranged from 21.0 to 34.0 cm in summer and 28.5 to 45.5 cm in rainy season in M_4 to M_8 generations compared to 36.0 to 41.2 cm and 52.2 to 56.8 cm in the parent, respectively. Overall mean height reduction ranged from 24.5% in TGM 42 to 41.0% in TGM 8 as compared to TAG 24.

Among the dwarf mutants, TGM 36 segregated into three phenotypes, namely, 1) parental type, 2) dwarf and 3) extreme dwarf phenotype in the M_3 (Fig. 1D). The parental and extreme dwarf types bred true in the subsequent generations, while the dwarf type continued to segregate once again for parental, dwarf and extreme dwarf types. Height of the parental type was on a par with TAG 24. Dwarf and extreme dwarf types had only 38.7% and 20.4% height of TAG 24, respectively. Although there was no reduction in the number of internodes on the main axis in the dwarf phenotype, the internodal length was considerably reduced. Similarly, among the tall mutants, the plant height ranged from 39.5 to 49.1 cm in summer and 60.3 to 74.9 cm in rainy season. The highest mean increase of 30.6% was noted in TGM 59. Plant height in groundnut mutants varied due to increased or decreased internodal length by maintaining similar number of internodes in tall or dwarf mutants, respectively (Patil 1966; Patil and Chandramouli 1978).

Mutants for leaf colour

Of the five chlorophyll mutants induced, one waxy leaf mutant was obtained from 150 Gy, one each of disease lesion mimic leaf (Fig. 1E), *virescent* leaf (Fig. 1F) and waxy leaf was from 250 Gy and one golden yellow leaf mutant occurred spontaneously. Leaves of the mutant TGM 55 mimic the symptoms of groundnut rust disease. Terminal 3-5 leaves of TGM 58 are *virescent* and characterized by yellowish light green young leaflets with white coloured rachii and midribs as compared to dark green leaflets with green rachii and midrib in parent. In the mutant, green color increased progressively with the seedling growth and was normal from the fourth leaf downwards from the top. However, colour of the rachii and midrib remained unchanged even with the age.

Mutant TGM 118 developed spontaneously from mutant TGM 16 in M_8 generation in which newly formed leaves had golden yellow colour (Fig. 1G), often referred as '*aureus'* phenotype and remaining leaves were green. The yellowness was more intense in midribs and rachii. After 80 DAS, leaf colour turned towards green and whole plant appeared normal. This mutant always segregates into mutant and parent plants with higher frequency for parent plant than mutant types. In the subsequent generations, parent plants bred true for green leaf color while mutant continued to segregate for parent and mutant phenotypes. Leaves of TGM 15 and TGM 56 on the main axis and branches are with whitish green leaflets giving a waxy appearance at all growth stages.

Mutants for leaf size and shape

Among the leaf size mutants, six small leaf mutants were induced from 150, two large leaf mutants from 250 and one from 350 Gy. A significant reduction in leaflet length, width and area in TGM 13, TGM 26 and TGM 47 and leaflet length and area in TGM 83 was noted. Reduction in leaflet area ranged from 19% in TGM 83 to 57% in TGM 47. Among three large leaf mutants, leaflet area increased from 33% in TGM 86 to 48% in TGM 87 compared to TAG 24. Reduction in leaf size was the most common phenomenon in irradiated and chemical mutagen treated populations (Patil 1966; Ashri 1970).

There were ten mutants with modified leaf shape. In mutants, TGM 49, TGM 50 and TGM 51, instead of two apical leaflets, there would be a combination of either i) one leaflet and a midrib like stalk (without lamina) or, ii) two midrib like stalks or, iii) one leaflet and stalked funnel or, iv) two stalked funnels (Fig. 1H) or v) one midrib like stalk and one stalked funnel. On an average, each plant had 15 leaves having mutant trait with frequency of 21.5%. Of these, 14.6% of the leaves had stalked funnels, 5.5% had only midrib like stalks and 1.4% had both stalked funnel and midrib like stalk.

TGM 61 mutant had almost double the rachis length and rachis length between leaflet pairs compared to parent. Further, mutant had longer stipules, larger leaflets, taller plant height and larger seed. The leaflets of TGM 38, TGM 40 and TGM 41 were near-circular with acute tip i.e., sub-orbicular leaflets as compared to oblong-elliptic in parent. This was evident from the significant reduction in leaflet length/width ratio by maintaining similar width compared to parent. These mutants were dwarf with small leaflets. TGM 44 had drooping leaves facing downwards. All the leaflets of TGM 17 and TGM 46 had involute (cup) leaflets.

Flower colour mutant

The most common flower colours in groundnut are yellow and orange. In TGM 53 the color of standard, wing, keel and central crescent area of the standard of flower

ranged from different grades of white to light-orange as compared to orange in parent. At any given time, the mutant had either all the flowers in white colour or a combination of white and light-orange flowers.

Mutants for pod traits

Wide genetic variability for pod traits was recorded in groundnut. In core germplasm maintained at ICRISAT, Patancheru, India, pod beak ranged from 1 to 9, pod constriction from 1 to 7 and pod reticulation from 1 to 9 based on 0-9 scale (IBPGR and ICRISAT 1992; Upadhyaya et al. 2002). Among the TAG 24 pod mutants, four mutants had slight beak (1-3 scale), two had slight constriction (1-3) and four had deep constriction (5-9), four had slight reticulation (1-3) and one had prominent reticulation (5-7) compared to moderate beak (5), constriction (5) and reticulation (5) in parent. Further, pods of mutant TGM 94 had parallel reticulation because of prominent reticulation along the length of pod. Mutants, TGM 59 and TGM 117 had dumb-bell shaped pods when pods are positioned with beak facing down (Fig. 1I).

Seed size mutants

Among the seed size mutants, four mutants were with smaller seeds (35.1 - 42.5 g HSW) with 9 to 25% weight reduction and ten were with larger seeds (56.1 – 72.5g HSW) with 20.0 to 54.9% increment over parent (46.8g HSW). Increased seed size was attributed to the increased cotyledonary cell volume by retaining similar cell number within unit area (Joshua and Bhatia 1983).

Seed testa colour mutants

As compared to rose testa colour in parent, one each mutant with purple (in TGM 80) and chocolate colour (in TGM 78) testa was induced at 250 Gy treatment and light red (in TGM 45) at 150 Gy. Of the three pink testa mutants (in TGM 62, TGM 77 and TGM 79), two were obtained at 150 and one was at 350 Gy.

Heritability and variability components among mutants

Consistent greater V_G was observed in different traits among induced mutants over generations despite significant seasonal influence on some of the traits (Table 3). Plant height and number of branches had greater V_G in rainy season than summer. On the contrary, pod and seed yields exhibited higher V_G in summer than rainy season. Shelling outturn, HSW, oil content and oil yield remained almost the same in both the seasons. Comparison among the traits indicated pod, seed and oil yields recorded greater GCV followed by plant height, HSW and number of branches. Oil content and shelling out turn had the least GCV among the mutants. Because of the enhanced vegetative growth in terms of increased plant height and number of branches in rainy season at Trombay, diversion of the photosynthates towards reproductive growth probably were minimized compared to summer. Hence pod and seed yields had lesser V_G in rainy season than summer.

Greater heritability coupled with higher genetic advance was observed for plant height, pod yield, seed yield, HSW and oil yield, which was due to additive genes (Table 3). On the contrary, both were lower for number of branches and shelling out turn. Oil content except in M_8 generation showed higher heritability but with lower genetic advance because of the non-additive gene action (dominance and epistasis). Furthermore, both heritability and genetic advance were consistent over seasons for most of the traits indicating that direct selection can be applied for these traits more effectively in both seasons.

Table 3. Heritability and variability components for different traits among mutants in M_5 to M_8 generations

Characters	Generation	Mean	V_G	h^2 (%)	GCV (%)	GA
Plant height (cm)	M_5	42.8	145.9	96.6	28.2	30.4
	M_6	31.2	43.1	94.9	21.0	16.4
	M_7	47.2	137.1	95.5	24.8	29.3
	M_8	30.6	53.2	97.2	23.8	18.4
Number of branches (plant^{-1})	M_5	7.8	3.4	73.9	23.6	4.1
	M_6	6.2	1.3	76.4	18.4	2.6
	M_7	6.7	1.0	50.0	14.9	1.8
	M_8	6.4	0.8	57.6	14.2	1.8
Pod yield (g plant^{-1})	M_5	22.3	57.6	95.8	34.0	19.0
	M_6	21.1	52.0	96.1	34.1	18.1
	M_7	21.9	46.7	97.7	31.2	17.3
	M_8	23.6	74.5	96.7	36.5	21.7

Seed yield (g plant^{-1})	M_5	16.0	29.8	94.6	34.1	13.6
	M_6	14.8	25.7	94.5	34.2	12.6
	M_7	15.9	28.0	99.9	33.2	13.5
	M_8	16.9	38.7	95.5	36.8	15.6
Shelling %	M_5	71.9	10.6	55.2	4.5	6.2
	M_6	69.8	18.6	70.2	6.1	9.3
	M_7	71.8	30.1	88.0	7.6	13.2
	M_8	71.7	7.7	47.2	3.8	4.9
100 seed weight (g)	M_5	50.8	108.9	98.1	20.5	26.5
	M_6	56.5	111.2	97.1	18.6	26.6
	M_7	49.5	99.6	97.4	20.1	25.2
	M_8	54.3	113.5	95.8	19.6	26.7
Oil content (%)	M_5	48.9	2.5	92.6	3.2	3.9
	M_6	46.0	1.8	85.7	2.9	3.2
	M_7	47.7	1.4	98.5	2.4	3.0
	M_8	47.4	1.5	60.0	2.5	2.4
Oil yield (g plant^{-1})	M_5	7.9	7.5	94.6	34.7	6.8
	M_6	6.8	5.9	94.6	35.5	6.0
	M_7	7.6	6.6	97.0	33.7	6.5
	M_8	8.1	9.4	95.2	37.9	7.6

GCV: Genotypic coefficient of variation (%); GA: Expected genetic advance as percent mean; h^2: Heritability in broad sense (%)

Conclusion

In the present study, gamma ray mutagenesis in groundnut was successful in the creation of several genetically diverse true breeding mutants affecting various morphological and agronomical characteristics. These new mutants will be utilized in the future as suitable genetic source material in breeding, genetic and functional genomic experiments.

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Research Article

Selection and Characterization of Gamma Ray Induced Bunchy Top Virus Resistant Mutants in Banana cv. Lakatan (*Musa* sp. AA)

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Abstract

Gamma irradiation coupled with *in vitro* technology was used to develop Banana Bunchy Top Virus (BBTV) resistance in banana cv. Lakatan (*Musa* sp. AA). A total of 6,012 plants regenerated from irradiated shoot cultures were subjected to BBTV artificial inoculation using the aphid vector (*Pentalonia nigronervosa* Coq.) in green house. From these, 64 mutant plants with varying degrees of resistance/tolerance reaction to BBTV were selected after 30 months of evaluation. The resistant mutant plants were induced from 5 to 30 Gy gamma rays. Twenty six plants showed no BBTV symptoms in both irradiated and first generation sucker plants (rated resistant). The other 38 plants showed limited BBTV symptoms (rated intermediate resistant). Yield and agronomic traits of some resistant plants were comparable to non-irradiated tissue culture plants. Shorter plant stature was also generated in some BBTV resistant mutant plants. First generation suckers from resistant plants were evaluated for second cycle stability of the resistance trait.

Key words: Banana, bunchy top virus, gamma rays, *Musa*, mutation, resistance

Introduction

Banana (*Musa* sps.) is one of the most important fruit crops in the Philippines, both for domestic and export markets. More than 75% of the banana producers are small-scale farmers engaged in domestic production. The cv Lakatan is the most popular dessert banana grown for the domestic market and also exported as a novelty banana. Banana Bunchy Top Virus (BBTV) remains the most destructive virus disease of bananas in the Philippines. It causes stunting and leaf malformation that leads to the premature death of the infected plant. The spread of the disease is greatly aided by aphid vector (*Pentalonia nigrenorvosa* Coq.). While use of disease-free tissue cultured planting materials is a viable disease management option, its effectiveness is limited to where residual or alternate inoculum sources are present. Regular replanting has to be done as the initially disease-free plants get infected within one or a few growing seasons. Field re-infection of tissue cultured plants ranged from 5-75% within the first cropping cycle depending on the crop management given (Magnaye, 1998). Thus, built in BBTV resistance seems to be the most effective disease control measure.

The germplasm of commercially important banana cultivars, both dessert (AA, AAA) and cooking (ABB, BBB) bananas, are not amenable to sexual breeding because the flowers are both male and female sterile. Thus, BBTV resistance could not be introgressed into bananas by conventional breeding methods. In addition, there are no known sources of resistance to BBTV available in the banana germplasm. In other crops, it has been demonstrated that it is possible to obtain resistance to pests and diseases by inducing genetic variability by radiations, chemical mutagens, somaclonal variation or by a more direct method of *in vitro* selection in the presence of the stress factor (Roux, 2004).

Mutation and *in vitro* technologies offer opportunities to enhance genetic variability for the improvement of agronomic traits such as disease resistance, earliness in fruiting, yield and quality (Bhagwat and Duncan, 1998; Ho *et al*., 1994; Mak *et al*., 1996; Novak *et al*., 1993; Roux, 2004; Smith *et al*., 1995). In addition, the availability of tissue culture techniques aids in the induction, selection and multiplication of mutants. Plant regeneration from banana shoot tips (Damasco and Barba, 1984; Damasco *et al*., 1984) is well established.

The objective of the present report is to induce mutations in the most popular banana cv. Lakatan by gamma rays coupled with *in vitro* techniques and methodologies to obtain resistant genotypes to BBTV disease.

Materials and methods

Initial plant material

Banana cv. Lakatan was used as the initial material for mutation induction. Although it is the most popular dessert banana grown for domestic and export markets, cv. Lakatan is very susceptible to BBTV disease. Consequently, the Lakatan industry in some regions of the Philippines was wiped out due to BBTV.

In vitro culture technique

Disease free suckers of cv Lakatan collected from the field were established *in vitro* following the standard banana tissue culture procedure (Damasco and Barba, 1984). Suckers were cleaned and surface sterilized in pure bleach for 45 min. Shoot explants (1 X 1cm) were excised, cut into 4 sections and inoculated onto MS medium + 5 mg/l BAP. Shoots were regularly sub-cultured every 4 to 6 weeks onto shoot multiplication medium (SMM) containing MS basal medium + 3 mg/l BAP. Shoots were transferred onto MS basal medium + 0.1 mg/l activated charcoal for rooting and plantlet development. All irradiated shoot cultures and selected mutant lines were also micropropagated using the SMM.

Mutation induction

In 1999, shoot cultures of banana were irradiated with gamma rays from a ^{60}Co source in the Philippine Nuclear

Research Institute. The radio-sensitivity and post radiation recovery of cv. Lakatan shoot cultures were assessed by measuring the rate of shoot multiplication after four sub-culture cycles. Highly proliferating shoot tip cultures, with leaves cut almost at the base of shoot, were irradiated with 5, 10, 20, 25, 30, 40, 60, 80 and 100 Gy. After irradiation, they (M_1) were multiplied for five subculture cycles. The dose response curve for banana shoot tip cultures was established and the LD_{50} was determined by 50% reduction, expressed as percentage of un-irradiated control in growth, measured by multiplication rate of shoot cultures after five cycles. All shoots regenerated from the experiment were rooted, potted out and evaluated for BBTV resistance and plant characterization.

For bulk irradiation, a total of 670 shoot tip cultures were irradiated at 20 and 25 Gy based on LD_{50}. After irradiation, shoot cultures were multiplied for five cycles namely, M_1V_1 to M_1V_5. Regenerated shoots were rooted, potted out, and evaluated for BBTV resistance and plant characterization.

Screening for BBTV Resistance

Greenhouse Screening for BBTV resistance

Irradiated plants regenerated from radio-sensitivity experiment and bulk irradiation were screened for BBTV resistance in the greenhouse using aphid inoculation of the virus artificially. Plantlets, about three months old since potting, and with 4-5 fully expanded leaves were subjected to artificial inoculation of the virus using aphids (Fig.1). Plants were kept in the greenhouse and observed for BBTV disease symptoms expression six to nine months after inoculation. The presence or absence of BBTV infection was assessed using symptomatology, ELISA and PCR based techniques. Plants without BBTV symptoms were selected nine months after inoculation.

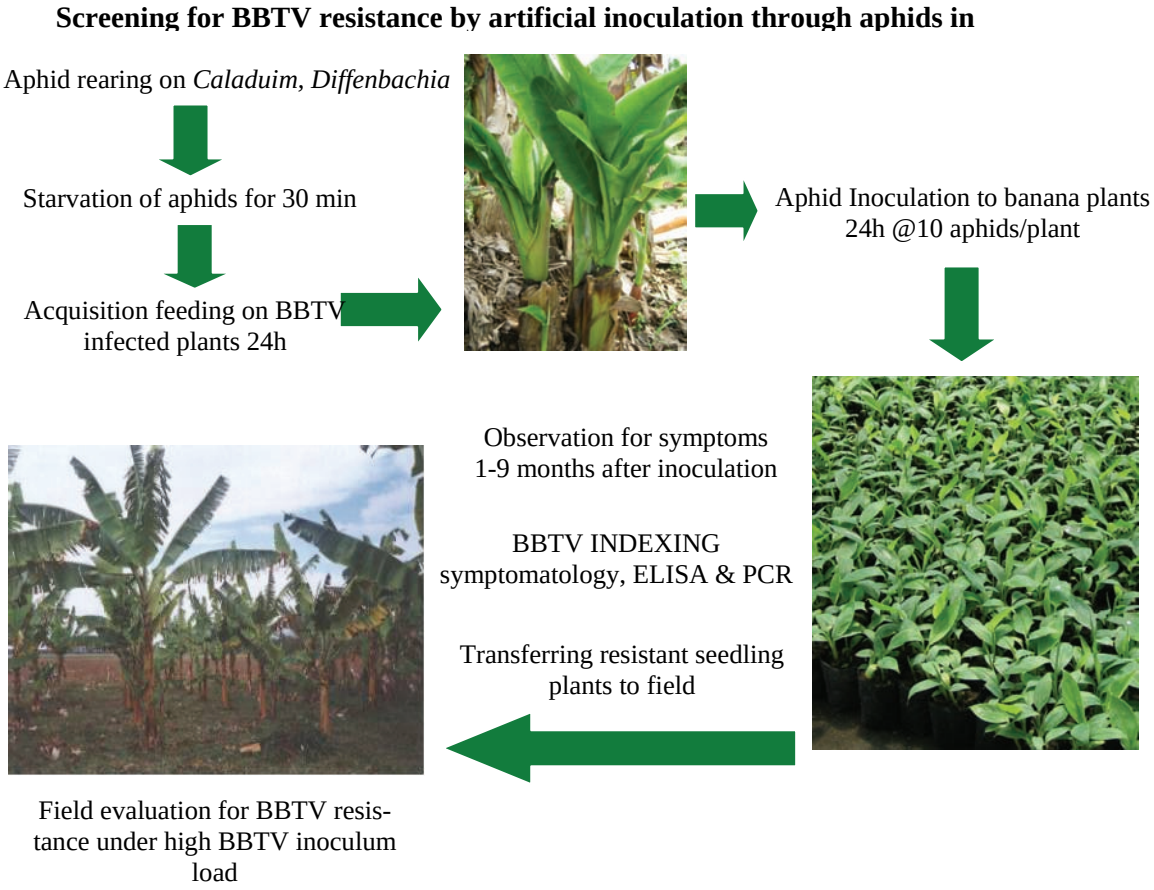

Figure 1. BBTV disease screening procedure using artificial inoculation of the virus using aphids in greenhouse followed by field evaluation under natural disease infection.

Cycle – 1 Field evaluation of selected resistant plants

Mutant plants showing resistance/tolerance to BBTV in the greenhouse were further evaluated under field conditions to study the breeding behavior for resistance to BBTV and characterization of agronomic traits. In a BBTV infected field, they were planted along with disease-free tissue culture (TC) control plants at random.

Throughout the crop growth, BBTV inoculum was kept high in the field by maintaining all infected experimental plants and planting susceptible plants. The incidence of BBTV infection in the field was monitored periodically from planting to harvest.

Cycle – 2 Field evaluation of selected resistant G1 mutant lines for stability

Generation 1 (G_1) suckers from selected M_1 plants with resistance/tolerance to BBTV and possessing good agronomic traits were selected, indexed for BBTV, micropropagated and further evaluated for a second cycle to confirm the stability of the BBTV resistance trait. Thirty two selected mutant lines were planted in experimental fields under high disease pressure. For each mutant line, a total of 30 plants were planted in a randomized complete block design with two blocks and three replications with five plants per replication. Each block was surrounded with BBTV infected plants as sources of inoculum. All infected experimental plants were also retained in the field to provide additional sources of inoculum. Observations on the incidence of BBTV infection were taken every three months until harvest. Agronomic data were taken at harvest.

Plant characterization

Experimental plants were characterized using the IPGRI Descriptors for Bananas (IPGRI/INIBAP/CIRAD, 1996).

Results and discussion

Radio-sensitivity response

The radio-sensitivity and post radiation recovery of banana cv Lakatan shoot cultures were assessed by measuring the rate of shoot multiplication after five sub-culture cycles (Fig. 2). Shoot cultures irradiated with a low dose of 5 Gy showed a higher multiplication rate while cultures irradiated with higher doses, 40-60 Gy showed more than 80% reduction in the multiplication rate. For cv. Lakatan, the LD_{50} for shoot multiplication rate was established at 20-25 Gy. Roux (2004) observed that the dose range for banana cultivars was based on genomic constitution and ploidy level. The radiation response of Lakatan, an AA cultivar, is similar to his earlier report on other AA cultivar Calcutta (Roux, 2004) and agrees with the earlier findings of Novak *et al*. (1990) who reported an LD_{50} of 25 Gy for diploid (2n) plants. For mutation induction, bulk irradiation of shoot tips was done based on LD_{50}. All plants regenerated from irradiation treatments were evaluated for BBTV resistance.

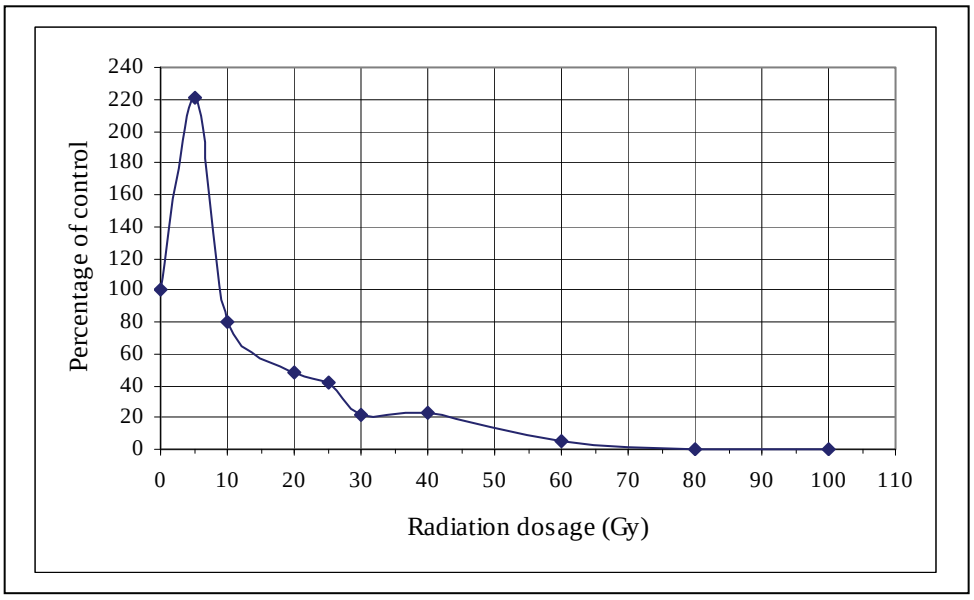

Figure 2. Effect of gamma radiation on the multiplication rate of cv. Lakatan shoot cultures after five subculture cycles.

Screening for BBTV Resistance in Greenhouse and Field

Of the 6,012 irradiated plants screened in the greenhouse, 114 plants were found without BBTV symptoms nine months after inoculation (Table 1). Disease-free tissue culture (TC) control plants, on the other hand, showed disease symptoms as early as one month after inoculation and by the sixth month all TC control plants were infected. Random samples from the 114 plants which did not show symptoms were taken and indexed for BBTV using ELISA and PCR techniques. The results indicated that 33 out of 45 (73.3%) plants indexed by ELISA, and

18 out of 25 (72%) for PCR test were negative for BBTV. The 114 selected seedling resistant M_1 plants were transferred to BBTV infected field for further evaluation of resistance and characterization of morpho-agronomic characters.

Table 1. Screening of banana mutant plants resistant to BBTV disease[1]

Radiation dosage (Gy)	Total no. of plants screened	No. of BBTV resistant plants		Frequency (%)[2]
		Greenhouse	Field	
Control	661	0	-	0.00
5	1,262	55	28	2.22
10	111	5	1	0.75
20	1,146	18	17	1.48
25	3,132	30	17	0.54
30	143	6	1	0.70
40	188	0	-	-
60	8	0	-	-
Total (treatment)	6,012	114	64	

[1] Greenhouse screening was facilitated by artificial infection using aphids and plants without BBTV symptoms were recorded as "resistant" nine months after virus inoculation; field screening was carried out for the 114 plants selected as "resistant" in greenhouse screening; plants without BBTV symptoms were recorded s resistant 30 months after natural virus infection.

[2] Based on selected plants in the field screening

The reaction of selected M_1 resistant seedlings and disease-free TC control plants to BBTV field infection from planting to harvest of all M_1 plants is shown in Fig. 3. The selected M_1 resistant plants showed 10% infection after six months, and this gradually increased and reached 42% infection at harvest. In contrast, TC control plants showed 28% BBTV infection six months after planting (MAP) and increased up to 70% at harvest. Of the initial 114 selected M_1 seedling resistant plants, only 64 plants (54.7%) remained free from BBTV infection until harvest (Table 1). The selected 64 M_1 plants showed varying degrees of resistance or tolerance to BBTV, which are rated as follows:

i) Resistant: 27 plants/lines showed no BBTV symptoms in both M_1 plants and G_1 suckers (Fig. 4A).

ii) Intermediate resistant 1: 21 plants/lines were without BBTV symptoms in the M_1, but with limited BBTV symptoms in one or two G_1 suckers (Fig. 4B).

iii) Intermediate resistant 2: 16 M_1 plants were without BBTV symptoms but all G_1 suckers infected (Fig. 4C).

iv) Susceptible: 50 M_1 plants and all G_1 suckers were infected (Fig. 4D).

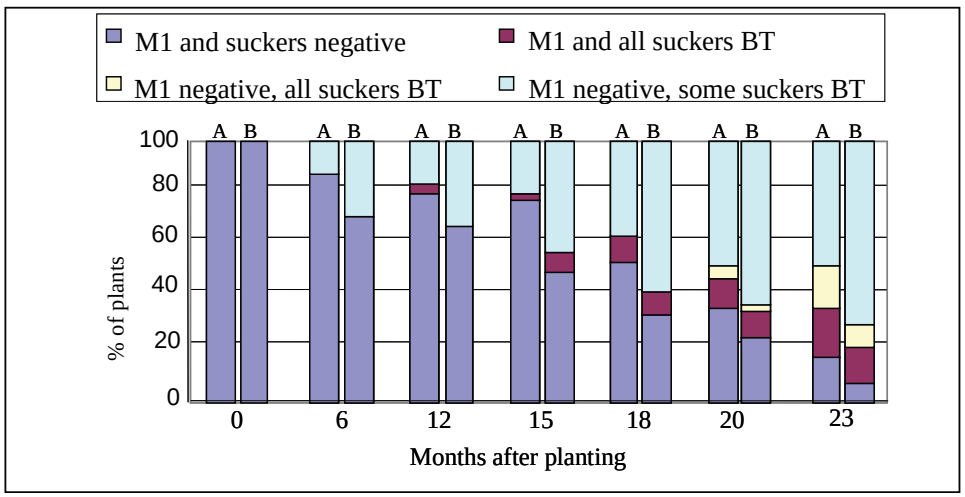

Figure 3. BBTV disease reaction of selected M_1 resistant seedlings (A-114 plants) and disease-free TC control (B-103 plants) exposed to natural disease infection from planting until harvest of all M_1 plants.

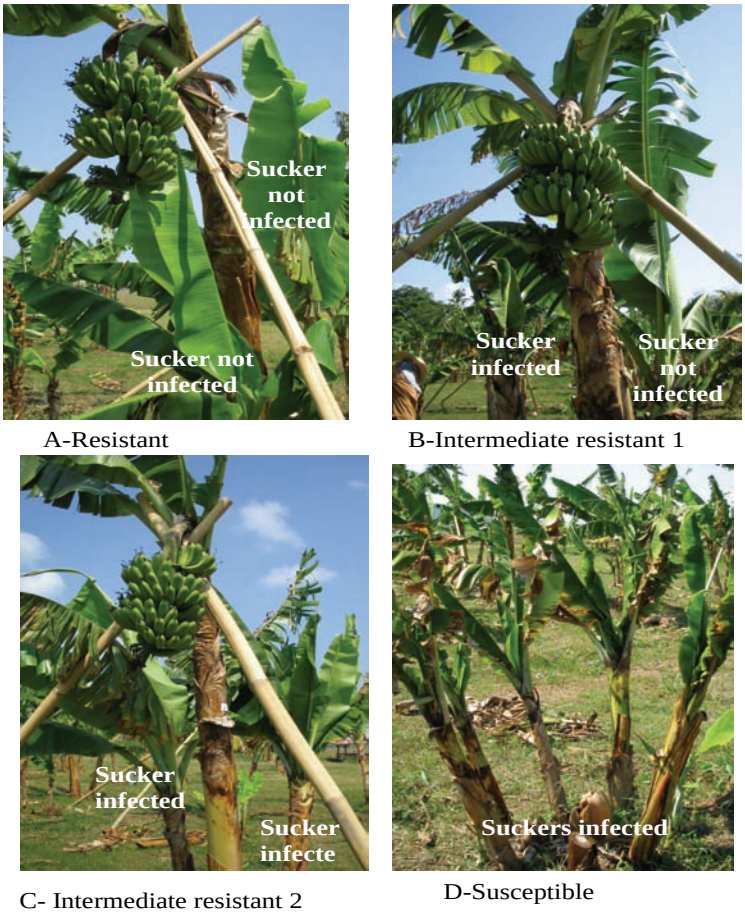

Figure 4. Rating of BBTV resistance reaction of irradiated M_1 plants:
A-Resistant: No BBTV symptoms in M_1 and G_1 plants
B- Intermediate resistant 1: No BBTV symptom in M_1 plant but with only 1 or 2 G_1 suckers infected
C-Intermediate 2: No BBTV symptom in M_1 plant but all G_1 suckers infected
D-Susceptible: M_1 plant and all suckers have BBTV symptoms

ELISA indexing showed that 49 out of 64 plants without symptoms were negative for BBTV and the remaining 15 plants, although without visible BBTV symptoms, were found positive for the virus.

Resistance in most virus diseases could be manifested in several ways either through resistance to virus inoculation, or resistance to virus spread within the plant manifested by a reduced rate of systemic disease development, or resistance to symptom expression manifested as reduced rate of disease development on systemic host or resistance to virus multiplication manifested by lower accumulation of the virus in the plant (Fauquet and Beachy, 1993). The selected M_1 resistant plants showed different forms or manifestations of resistance/ tolerance to BBTV. Some M_1 resistant plants did not show any symptoms even until harvest and showed negative reaction to BBTV on using ELISA. This response would suggest a total arrest in virus multiplication or lower accumulation of the virus. In the case of M_1 plants showing infection at a very late stage, resistance could be due to delayed spread of virus within the plant or reduced rate of disease development. In some of the resistant mutant plants the absence of visible BBTV symptoms in ELISA positive plants could be a manifestation of tolerance. The mechanism of BBTV resistance in these selected resistant mutant plants is currently being studied.

BBTV resistant plants were generated from five different doses, and with mutation frequencies ranging from 0.69 to 2.22% per dose (Table 1). From the total M_1 plants, 64 mutants were isolated from all doses, at 1.06% frequency. Results showed that mutation towards an improved trait is a random event and could be generated at any dosage. Roux (2004) pointed out that the current trend is to use relatively low dosages because they produce less chromosomal damage and other negative side effects than stronger treatments. Mak *et al* (2004) reported that the general recommendation is to use low dosage on a very large number of primary explants (*e.g.* 10,000) followed by one sub-culture *in vitro* or high dosage on a large number of explants followed by at least three subcultures.

The stability of BBTV resistance trait in succeeding G_1 generation was observed for selected resistant M_1 plants/lines. For plants rated resistant and intermediate

resistant 1, both M_1 and G_1 plants showed resistant reaction. However, for plants rated intermediate resistant 2, only the M_1 plants were resistant and not all G_1 suckers as they were infected.

When G_1 suckers from selected resistant M_1 plants indexed negative for BBTV, they were micropropagated and subjected to another cycle of field evaluation to confirm stability for resistance. Out of a total of 1,200 plants from selected resistant lines and TC control plants planted in BBTV infected fields, ten mutant lines showed lower % of disease incidence than the TC control plants at 17 MAP (Table 2). However, only five mutant lines consistently showed lower BBTV disease incidence than the TC control plants at any given time between 3 and 17 MAP. Besides, the onset of BBTV infection in the five mutant lines was also delayed until 5 to 9 MAP (Table 2).

Table 2. The BBTV disease incidence (%) in selected BBTV resistant mutant lines 3 to 17 months after planting

Selection	3	5	7	9	11	13	15	17
PR 25-28	0	0	0	6.9	6.9	13.8	17.2	31.0
PR 23-30	0	0	0	0	3.3	13.3	13.3	33.3
PR 2-45	0	0	3.4	13.8	14.3	17.8	21.4	50.0
PR 13-30	0	0	0	3.7	3.8	7.7	7.7	50.0
PR 22-28	0	0	6.7	6.7	6.7	13.3	16.7	50.0
PR 23-28	3.4	7.1	14.3	17.8	21.4	39.3	46.4	57.1
PR 7-29	6.9	7.1	7.1	17.8	21.4	28.6	28.6	57.1
PR 23-45	3.6	3.6	3.6	3.6	3.7	11.1	14.8	66.7
PR 9-28	6.7	18.5	18.5	18.5	37	51.8	59.2	66.7
PR 4-28	0	3.7	3.7	3.7	3.7	19.4	26.9	69.2
TC Control	1.4	5.6	14.1	14.1	18.3	25.3	29.6	71.8

The ten mutant lines had higher percentage of BBTV-free fruiting plants at 12 to 17 MAP (Table 3). The percentage of such plants from selected G_1 mutant lines at 17 MAP ranged from 63 and 96% compared to 32% in TC control plants (Table 3). At present, although the only BBTV disease management strategy is to use disease-free TC planting materials, the effectiveness is limited with a high rate of re-infection in the field which could be as high as 75% within the first cropping cycle (Magnaye, 1998). The results of the present study showed that without any BBTV control measures, disease free TC control plants had 70 to 90% field re-infection within the first cropping. On the other hand BBTV resistant mutant lines had lower re-infection rates, delayed onset of infection and a greater percentage of plants that would reach fruiting stage without infection even in the presence of high inoculum in the area.

Table 3. The percentage of BBTV-free fruiting plants of selected mutant lines 12 to 17 months after planting[1]

Selection	Months after planting (MAP)		
	12	14	17
PR 25-28	6.7	53.3	96.0
PR 23-30	3.3	43.3	70.0
PR 2-45	0.0	26.7	46.7
PR 13-30	3.3	46.7	66.7
PR 22-28	0.0	60.0	76.7
PR 23-28	10.0	40.0	76.7
PR 7-29	6.7	46.7	70.0
PR 23-45	0.0	56.7	80.0
PR 9-28	43.3	50.0	63.3
PR 4-28	3.0	50.0	76.7
TC Control	0.0	14.1	32.4

[1]Combined G_1 and G_2 plants

The second cycle field evaluation further confirmed stability of BBTV resistance in succeeding generations (G_1 and G_2 plants). For most asexually propagated crops, the stability of selected traits induced from mutation is generally established after several generations of asexual reproduction.

Evaluation for agronomic characters, yield and yield components

Some of the selected resistant M_1 plants had plant height significantly shorter than the TC control plants (Table 4). The observed plant height in resistant M_1 plants ranged from 1.37 to 2.75 m compared to TC control plants with a range of 2.2 to 2.9 m. Frequency distribution (Fig. 5) showed that 20% of the resistant plants had plant height lower than 2.0 m and 3% had plant height lower than 1.5 m. For cv. Lakatan, shorter plant stature is an important trait since it is very susceptible to wind damage because of its relatively weak pseudostem. No significant differences were observed for other agronomic characters in resistant plants when compared with TC control plants (Table 4).

Table 4. Agronomic features in M_1 resistant and TC control plants

Selections	Height (m)	Girth (cm)	No. of suckers	Leaf length (cm)	Leaf width (cm)
Resistant	2.33[a]	42.73[a]	2.9[b]	191.4[a]	61.33[a]
Intermediate Resistant 1	2.17[ab]	37.38[a]	4.3[a]	174.3[a]	55.92[a]
Intermediate Resistant 2	2.30[b]	40.28[a]	4.4[a]	195.1[a]	61.31[a]
TC Control	2.4[ab]	42.00[a]	2.8[b]	191.21[a]	58.68[a]

Means in the same column with the similar superscript are not significantly different at P = 5% level.

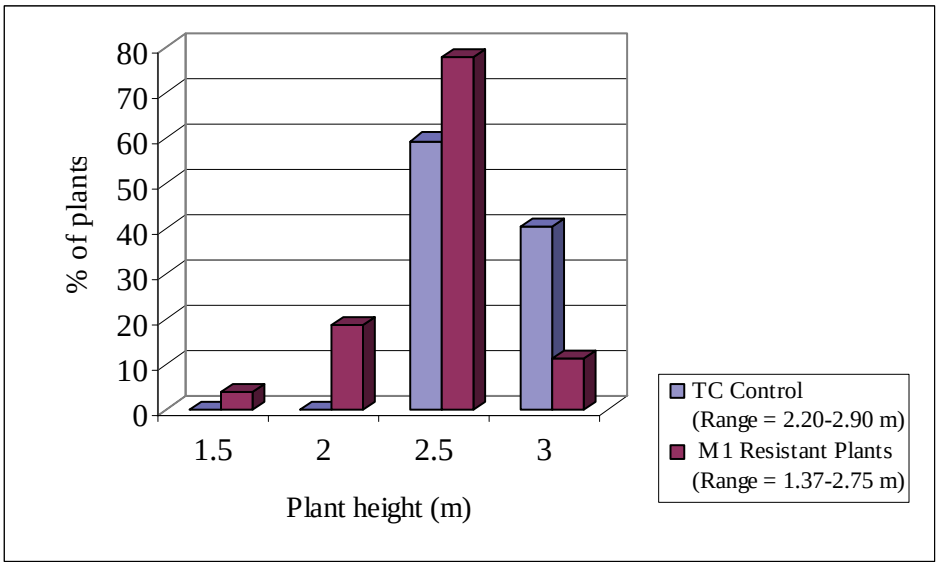

Figure 5. Frequency distribution for plant height in selected M_1 resistant and TC control plants

The yield components, in selected M_1 resistant plants were compared with TC control in Table 5 and fruit measurements in Table 6. Although significant differences were seen for number of hands and weight per hand, resistant plants showed superiority only for weight per hand over TC control. For the rest of the four traits, including weight of bunch per plant, they were on a par with TC control. This is important, since disease resistance and higher yield are usually negatively associated. There were no significant differences for fruit characters such as fruit weight, fruit length, fruit width, fruit thickness, flesh weight and total soluble solid (TSS) as resistant plants were on a par with TC control plants (Table 6).

Table 5. Agronomic features in M_1 resistant and TC control plants

Selections	No. of hands/bunch	Weight/hand (kg)	No. of fingers/hand	No. of fingers (Middle hand)	Weight of bunch/plant (kg)
Resistant	5.1[a]	1.23[a]	13.2[a]	13.6 [a]	7.81 [a]
Intermediate Resistant 1	4.9[a]	1.03[b]	12.0[a]	12.5 [a]	6.43 [a]
Intermediate Resistant 2	4.6[b]	1.11[a]	12.4[a]	12.5 [a]	6.47 [a]
TC Control	5.4[a]	1.03[b]	12.1[a]	12.6 [a]	7.53 [a]

Means in the same column with the similar superscript are not significantly different at P=5% level.

Table 5. Agronomic features in M_1 resistant and TC control plants

Selections	Fruit Wt (g)	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Flesh wt (g)	TSS (brix)
Resistant	80.7[a]	118.7 [a]	31.5 [a]	29.9 [a]	57.2 [a]	26.9 [a]
Intermediate Resistant 1	76.1[a]	114.3 [a]	30.8 [a]	29.1 [a]	53.9 [a]	26.6 [a]
Intermediate Resistant 2	74.8 [a]	114.9 [a]	30.8 [a]	29.2 [a]	53.3 [a]	26.0 [a]
TC Control	77.4 [a]	117.9 [a]	31.2 [a]	29.3 [a]	54.7 [a]	25.9 [a]

Means in the same column with the similar superscript are not significantly different at P=5% level.

Conclusion

Variability for BBTV resistance/tolerance in banana cv. Lakatan was induced through gamma irradiation coupled with *in vitro* techniques. Mutant plants with varying degrees of resistance/tolerance to BBTV were selected from irradiated plants. Twenty six mutant plants were rated resistant and 38 intermediate resistant. The preliminary yield data also indicated that although resistance could be induced in the mutants, with respect to bunch weight per plant, the mutants were on a par with controls. Other important agronomic traits such as shorter plant stature, bigger fruits could also be induced. The mechanism of resistance to BBTV in selected mutant lines is currently being studied. It seems quite possible to create further variability in other important *Musa* species like abaca (*Musa textilis*) as well as other banana cultivars by applying the similar methodologies used for cv. Lakatan in the current studies.

Acknowledgements

Funding from 1) the Philippine Council for Agriculture and Natural Resources Research and Development (PCARRD), Los Banos, Laguna, Philippines, 2) the International Atomic Energy Agency (IAEA), Vienna, Austria and 3) the Institute of Plant Breeding, University of the Philippines Los Banos, College, Laguna, Philippines is gratefully acknowledged. Thanks are due to radiation facilities extended from the Philippine Nuclear Research Institute, Diliman, Quezon City, Philippines and technical advice received from Dr. Mak Chai of Malaysia (IAEA Expert).

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Research Article

Biological Effects of High Energy ^{7}Li Ion Beams Implantation on Wheat

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Abstract

Dry seeds of four wheat cvs. LX987, XM18, ZH7 and ZY9 were implanted with ^{7}Li ion beam at the doses of 0, 10, 30, 50, 100 and 150 Gy with an initial energy level of 43MeV. The effects of damage in M_1 generation and mutagenic effects in the M_2 were analyzed. The results indicated that ^{7}Li ion beam irradiation could inhibit wheat seedling growth effectively. The inhibition effects exhibited dose dependent tendency with a peak of 50 Gy, and then decreased with further dose increase. Phenotypic variations such as chlorophyll deficiency of midrib, split leaf, curly leaf and tufted seedling etc. were observed in M_1 generation. In the M_2, mutation frequencies observed in two cvs. ZH7 and ZY9 ranged from 0.49% to 2.23%. Mutants with altered plant height, spike type etc. were selected in M_2 generation. The relationship between mutation frequency and dose in the M_2 was identical with that of inhibition and dose in M_1 generation. The optimum dose of wheat irradiation by ^{7}Li ion beams appears to be about 50Gy, which is obviously lower than that of gamma ray irradiation.

Keywords: ^{7}Li ion beam, wheat, biological effect, mutation

Introduction

Genetic manipulation in breeding by the use of mutation induction has been used as one of the most efficient means in crop breeding. So far low linear energy transfer (LET) radiations such as X- and gamma rays as well as chemical mutagens have been used to increase genetic variability in plants. Exploration and development of new mutagens is very necessary and important for significantly increasing mutagenic efficiency in crop breeding.

In respect of basic mechanism of biological effects of ion beam implantation, namely, multi-effects of implanted ions include energy deposit, momentum transmission, mass deposit and charge exchange. A previous study on the mammalian cells showed greater effects of heavy ions with high LET than those of low LET mutagens (Okayasu, et al., 1999; Rosendahl, et al., 2005). This is mainly because of an increased proportion of cells containing complex aberrations, and an increased complexity of exchanges (Virsik-Köpp et al., 2004). Heavy ion beams are very effective as a new source for mutation induction in rice, maize, *Arabidopsis*, flowers etc. (Qiu et al., 1991; Mei et al., 1995; Tanaka, 1999; Abe et al., 2002; Bae et al., 2004).

It was found that ^{7}Li ion beam implantation into crop seeds could produce the effects of not only the energy transferring, mass deposition and charge exchange similar to other ion beams, but also the inner nuclear reaction of ^{1}H(^{7}Li, ^{7}Be)n in the irradiated seeds (Yang et al., 1997). ^{7}Li-beam was the most effective in cell killing as well as inducing other nuclear damages followed by ^{12}C, ^{16}O and gamma rays in Chinese hamster cell (Rupak et al., 2007).

The objective of this work is to investigate variation induced by ^{7}Li ion beam in different varieties of wheat seedlings, evaluate the inhibition rate in M_1 generation and isolate macro mutants in M_2 generation, estimate the mutation frequency and study the bio-effects in wheat.

Materials and methods

Materials

Dry seeds of four wheat cvs. LX987, XM18, ZH7 and ZY9 were used for irradiation with ion beams. For each dose >200 seeds were used.

Seed irradiation

HI-13 Tandem Accelerator of China Institute of Atomic Energy, Beijing was used to generate ^{7}Li ions for wheat seed irradiation. The initial energy of the ions was 43MeV, and the LET was 60keV/μm. Doses used were 0, 10, 30, 50, 100 and 150Gy.

Germination test

Germination rate was recorded for both treated and untreated seeds after soaking in water and keeping them in laboratory at 21^{o}C. For each treatment, 50 seeds with two replications were used. Germination rate was calculated and seedling height and root length were measured 10 days after soaking.

Biological damage and inhibition effects in M_1 generation

Biological damage rate of each dose was estimated by the following formula:

$$\frac{\text{Mean seedling height of control} - \text{Mean seedling height of treatment}}{\text{Mean seedling height of control}} \times 100$$

After germination test, all the seedlings of untreated control and treatments were transplanted in the experimental field, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing. The field experiment was conducted in an orderly design without replication. The row length was 2m and 25cm and 10cm spacing between rows and plants, respectively. All agronomical practices were followed as required for a successful crop.

Mutant selection and mutation frequency

M_1 seeds were bulked and two M_2 populations of cv. ZH7 and ZY9 were raised, which had different irradiation sensitivity to gamma rays (Guo et al., 2007). The plant population size of each treatment varied from 1000 to 2000 based on the seed amount obtained from M_1 generation. Field management was similar to the M_1. Phenotypes with altered morphological and agronomical traits such as plant height, spike type, and heading date etc. and male sterility were marked. Variants whose mean was greater or lesser than two standard deviations over the mean of untreated control were considered as mutants. Accordingly, mutation frequency was calculated as percentage of total number of mutants divided by the total number of plants of corresponding populations. The population of 30Gy of ZY9 was not advanced to the M_2 due to limited number of M_1 plants.

Results

Phenotypic variations in M_1 generation

In M_1 generation variants such as chlorophyll deficiencies of midrib and leaf apex, split leaf, curly leaf, twin-seedling and tufted seedling were observed (Fig.1A and B). Most of the chlorophyll deficiencies observed were on leaf midrib. They were induced in each dose of all the four genotypes, and the percentage was high even up to 100% (Table 1).

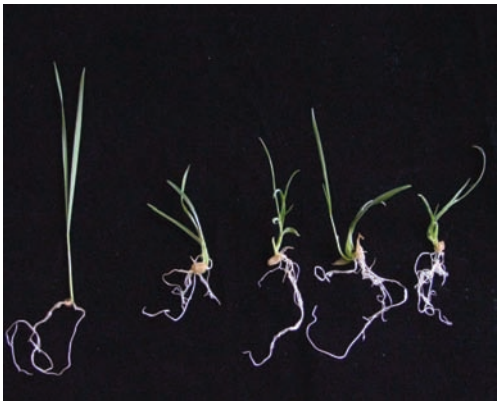

Figure 1A. Seedling variation in M_1 generation in wheat (Left: Parent, Right: M_1 variant seedlings)

Figure 1B. Left: Parent ZY, Right: M_1 twin-seedling variants)

Table 1. Percentage of chlorophyll deficiency of midrib in M_1 seedlings by different doses of ^{7}Li ion beams implantation in wheat

Genotype	10Gy	30Gy	50Gy	100Gy	150Gy
LX987	66.67	87.50	57.89	50.00	23.08
XM18	85.00	85.00	68.42	95.00	100.00
ZH7	76.47	50.00	81.82	100.00	81.82
ZY9	28.57	68.42	66.67	57.14	52.94

Variants for split leaf (Table 2) were lower than chlorophyll deficient midrib variants within the same dose. Split leaf variants were not observed in 10Gy treatment. The highest percentage variation for split leaf was observed in 50Gy for LX987, ZH7 and ZY9 and 100 Gy for XM18. Most of the seedlings showed more than one type of above-cited variations. Total variation percentage of LX987, ZH7 and ZY9 increased along with increased dosage up to 50Gy, then reduced with dose increase. XM18 showed different response with the highest percentage at 150Gy. This was because most of the seedlings in 150Gy dose showed two or three types of variations, within the same seedling leading to enhanced variation percentage (Fig. 2).

Table 2. Percentage of split leaf in M_1 seedlings by different doses of ^{7}Li ion beams implantation in wheat

Genotype	10Gy	30Gy	50Gy	100Gy	150Gy
LX987	-	6.25	15.79	-	15.38
XM18	-	15.00	21.05	70.00	55.00
ZH7	-	50.00	54.55	25.00	36.36
ZY9	-	10.53	33.33	4.76	-

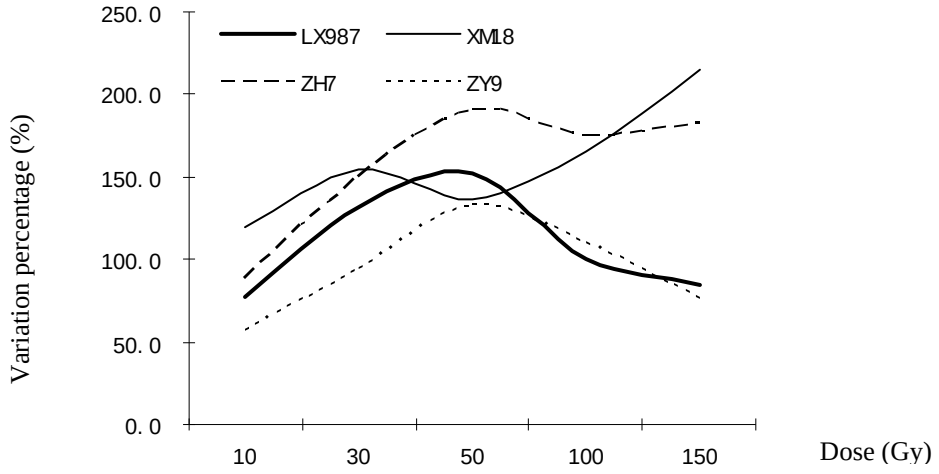

Figure 2. Total variation percentage in M_1 seedlings induced by ^{7}Li ion beams implantation

Inhibition effects of M_1 generation

^{7}Li ion beams could inhibit seedling growth in M_1 generation effectively and induce biological damage (Fig. 3). Inhibition effects on XM18, ZH7 and ZY9 were enhanced with increased dosage, and at 50Gy they were 51, 30 and 36%, respectively. This was followed by reduction with increased dosage. Coleoptile without elongation or arrested growth was found in some of the seedlings of LX987 at 100Gy and 150Gy, showing strong inhibition effects. This implied that a different and complex damage–dose relationship existed between different genotypes treated with ^{7}Li ion beam implantation.

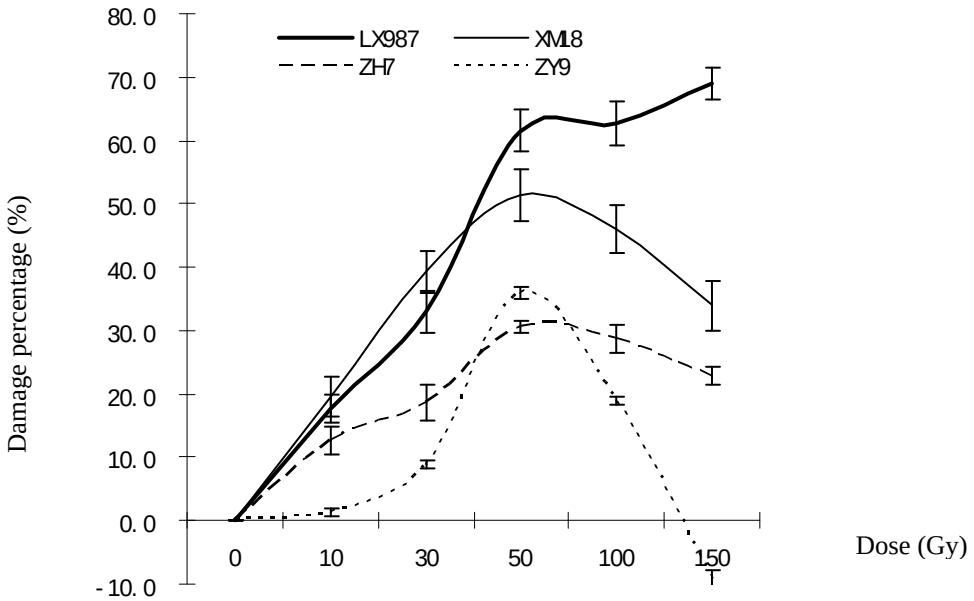

Figure 3. Biological damage percentage in M_1 seedling induced by ^{7}Li ion beams implantation

Mutation screening in M_2 generation

Variations such as, chlorophyll deficiencies of midrib and leaf apex, split leaf, curly leaf, twin-seedling and tufted seedling observed in M_1 generation did not appear in the M_2. Both morphological and agronomical mutants selected were mainly altered with plant height, heading date, spike type, seed number per spike, male sterility, thousand grain weights etc. (Table 3). Mutations occurred in both positive and negative directions compared to the respective parents. The relationship of mutation frequency and dose in the M_2 was identical with that of inhibition effect of M_1 generation, and at 50Gy the mutation frequency was highest as seen in mutagenised population of ZY9 and ZH7.

Table 3. Mutation spectrum and frequency (%) of wheat cvs. ZY9 and ZH7 induced by ^{7}Li ion beams in M_2 generation

Genotype	Dose	Plant height	Waxy leaf	Heading date	Male sterility	Spike type	Grains/spike	1000-grain weight	Grain color	Total mutation
ZY9	50Gy	0.63	0.09	0.09	0.09	0.45	0.36	0.54	0.09	2.23
	100Gy	0.45	-	0.09	0.06	0.12	0.18	0.09	-	0.97
	150Gy	0.21	-	-	-	0.08	0.03	0.18	-	0.49
ZH7	30Gy	0.42	-	-	0.17	0.17	0.17	-	-	0.92
	50Gy	1.00	-	-	-	0.25	0.50	-	-	1.75
	100Gy	0.56	-	-	-	-	-	-	-	0.56
	150Gy	0.67	-	-	-	0.50	0.33	-	-	1.50

Among induced mutations, frequency of altered plant height was the highest, varying from 0.21% to 1.00%. Mutations affecting spike type included speltoid, semi-speltoid, sub-compactoid, and compactoid spikes.

Mutation spectrum of ZY9 and ZH7 was different. More mutations occurred with ZY9 than ZH7, including thousand-grain weight, heading date, grain color and leaf wax. Mutation frequency for this type of agronomical traits was relatively high even up to 0.54% in ZY9.

Discussion

The biological effects and dose is nearly in positive linear relationships for low-LET radiation such as gamma rays. The differences between high- and low-LET radiations may be due to many factors, almost all of which are related to radiation track structure in one way or other and some can in principle lead to qualitative as well as quantitative differences between the radiations (Dudley, 1999). The relative biological effectiveness (RBE) and dose relationships of high LET irradiation are different from low-LET rays. The RBE values for wing-hair mutations peak of *Drosophila* is near 150keV/μm and decrease with further increase in LET (Yoshikawa et al., 1999). RBE values in a number of mammalian cells also perform to increase with LET up to about 100keV/μm (Kiefer, 2002). The frequency of chromatin breaks are obtained at higher LET values due to the large number of ionizing events being concentrated in a small volume of the cell nucleus (Ewa et al., 2004), and this maybe the reasons that variation effects and inhibition effects decreased with further increase dose after 50Gy peak, and showed part of the characteristics of high LET heavy ion beam irradiation.

High LET heavy ion beam implantation could induce higher RBE than low LET rays (Qiu et al., 1991; Mei et al., 1995; Sakashita et al., 2002; Rupak et al., 2007). Valuable mutant lines of rice have been obtained at 90-100Gy by Argon ions treatment (Mei et al., 1995). A high rate of chlorophyll deficient mutants in *Arabidopsis* were obtained at 40Gy and 20Gy by C and Ne ion beams' implantation, respectively (Abe et al., 2002). Cell killing and other nuclear damages induced by ^{7}Li ion beams in Chinese hamster cells were more effective than that of ^{12}C and ^{16}O (Rupak et al., 2007). Damage and inhibition effects in M_1 generation and mutation frequency in M_2 generation all peaked at 50Gy. Hence, it was presumed that the optimum dose of wheat irradiation by ^{7}Li ion beams was about 50Gy, and it is obviously lower than that of gamma rays irradiation (Xu, 1996). Breeding behavior of selected mutants will be studied in M_3 generation. Based on the current study, it may be possible to create variability using ^{7}Li ion beams implantation in wheat as well as other crop plants.

Acknowledgements

Research was partially supported by the International Atomic Energy Agency, Vienna through the Projects IAEA/RAS5040, RC12610 and RC14195, and China NSFC project (30670488).

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Research Article

Genetic Improvement of Soybean Variety JS 80-21 through Induced Mutations

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Abstract

Seeds of soybean cv. JS 80-21 was irradiated with 250 Gy gamma rays to induce genetic variability for earliness, improved plant architecture, and higher seed yield. A large number of mutants affecting the morphological characters were identified and characterized. They include dwarf, crinkled leaf, pink flower colour and good pod bearing mutants. Seven mutants were studied for yield, morphological and quality characters like oil and protein in M_5 generation. Mutant M-107 showed higher mean number of branches, higher number of pods per plant and higher harvest index compared to the parent JS 80-21. Cluster analysis based on dissimilarity values grouped the mutants into three clusters and mutant M-59 grouped in Cluster III showed maximum dissimilarity of 26% from the parent JS 80-21. Four high yielding mutants M-21, M-76, M-91 and M-107 gave 15 to 20% higher yield than the best checks in station trials at Trombay. One of the mutants, TAMS 98-21 produced 21% higher seed yield (2243kg/ha) over the best check variety JS-335 (1853kg/ha) in Maharashtra State multilocation trials. It also showed multiple disease and pest resistance. TAMS 98-21 was released and gazette notified for commercial cultivation by Central Varietal Release Committee, Government of India in January 2007.

Key Words: Gamma rays, *Glycine max*, Mutant, Soybean

Introduction

Soybean [*Glycine max* (L.) Merrill] has been recognized as valuable source of high quality protein and oil. In India it has emerged as an important oilseed crop and occupies a parallel place to groundnut and rape seed mustard. At present it is cultivated over an estimated area of 8.0 million ha with a production of about 0.75 million t. It shares nearly 32 percent of the total oilseeds produced in India and contributes about 13 per cent to the domestic edible oil pool (Dupare et al., 2005). In addition, India earns substantial foreign exchange to the tune of US$ 733 million through export of defatted soy meal.

In India, the productivity of soybean is 0.8 – 1.0 t/ha which is very low as compared to the world average. The major constraints for low productivity are poor seed viability, non-availability of early maturing, high yielding cultivars with resistance to biotic and abiotic stresses. Secondly, the narrow genetic base of the released soybean varieties is also responsible for low average yields (Satyavathi et al., 2003). The degree of genetic variability available for selection can play an important role in overcoming yield barriers. Mutations are important source for inducing variability. Improvement in either single or few economic traits and quality characters can be achieved with the help of induced mutations within the shortest possible time. In India, so far four soybean varieties, namely, Birsa Soy 1, VLS-1, NRC-2 and NRC-12 have been developed using induced mutations. The objective of present report is to create variability for earliness, improved plant architecture, and higher seed yield in cv. JS 80-21.

Materials and Methods

Plant material and mutation studies

Five hundred seeds of the soybean variety JS 80-21 were exposed to 250 Gy gamma rays from a ^{60}Co source at BARC. The treated seeds along with the un-irradiated control were sown in the experimental field at Trombay to raise M_1 generation. The data on germination was recorded at three to twelve days after sowing. A total of 258 M_1 plants were harvested individually and the seeds obtained were used to raise the M_2 generation as plant to row progenies. In the M_2, 4,104 plant population was carefully screened for morphological and agronomical mutations. Plants which appeared different from the parent for one or more traits were marked and harvested separately. Mutation frequency was calculated based on number of mutants scored against total M_2 plant population. Breeding behaviour and salient features of the mutants were studied through M_3 - M_5 generations. In the M_5, seven mutants with superior agronomic traits were evaluated for various quantitative characters in comparison with the parent cv. JS 80-21. Initially promising mutants were evaluated for yield in station trials at BARC, Trombay in a randomized block design with three replications. Subsequently, one of the mutants (M-21) was evaluated in Maharashtra State multi-location trials under the name Trombay Amravati Soybean (TAMS) 98-21.

Estimation of oil & protein contents

Oil content of seed samples was estimated by solvent extraction method using Soxhlet apparatus [Soxtec system – HT (1043)]. The nitrogen content of the defatted seed meal was determined by the micro-Kjeldahl method (A.O.A.C., 1984) and the amount of total protein was calculated from percent nitrogen using a conversion factor of 6.25.

Statistical methods

Observed differences were subjected to various standard statistical methods (Panse and Sukhatme, 1978) to know the significance. For morphological characters of mutants Numerical Taxonomy Analysis System, Version 2.00 (Rohlf, 1990) program was used.

Results and Discussion

Studies in M_1 and M_2 generations

In soybean, various studies have been carried out to understand the biological effects of mutagen. Different parameters like reduction in germination percentage, seed-

ling height and survival at harvest in M_1 generation have been extensively used in soybean to measure the mutagenic effect (Mehetre et al., 1994). In current studies, the percent germination observed in the M_1 generation was 51.6%. In the M_2, chlorophyll and viable mutants affecting morphological and physiological characters were identified and selected. Chlorophyll mutants including albina, xantha, virescent and chlorina were observed. The frequency and the spectrum of mutants in the M_2 are given in Table 1. The total morphological mutants observed were 55 with a mutation frequency of 1.34%. The other morphological mutants were those affecting early or late maturity, plant height, leaf shape, flower colour, sterility, number of pods per plant and seed colour. Constantin et al. (1976) observed that decrease in survival, plant height and seed yield with increase in dose rate of mutagen and found 200 to 300 Gy of gamma radiation would be useful to induce genetic variability in soybean. In soybean 100 to 300 Gy doses of gamma rays were reported very effective (Mehetre et al., 1994; Mehetre et al., 1996; Geetha and Vaidyanathan, 1998; Wakode et al., 2000). Accordingly, in the present study 250 Gy gamma ray dose was chosen and found effective for inducing genetic variability in cv. JS 80-21. Two pink flower mutants (Fig. 1A) were observed and both the flower mutants showed normal plant growth similar to the parent. Two crinkled leaf mutants (Fig. 1B) were also observed. Four mutants M-21, M-76, M-91 and M-107 had good pod bearing and mutant M-107 exhibited pleiotropic effect having alteration in more than one character. The mutant was dwarf with small leaves, more branches and dark green foliage with late maturity (Fig. 1C).

Table 1. Frequency and spectrum of mutants in M_2 generation in the cultivar JS 80-21

Character of the mutant	No. of plants selected	Frequency (%)
Cholorphyll		
Albina	2	0.05
Xantha	3	0.07
Virescent	4	0.10
Chlorina	6	0.15
Leaf size and shape		
Round	1	0.02
Narrow	1	0.02
Crinkle	2	0.05
Small	2	0.05
Thick	1	0.02
Flower colour	2	0.05
Sterile	1	0.02
Late maturity	1	0.02
Plant characters		
Dwarf	8	0.20
Tall	1	0.02
Bushy	1	0.02
Good bearing	19	0.48
Total	**55**	**1.34**

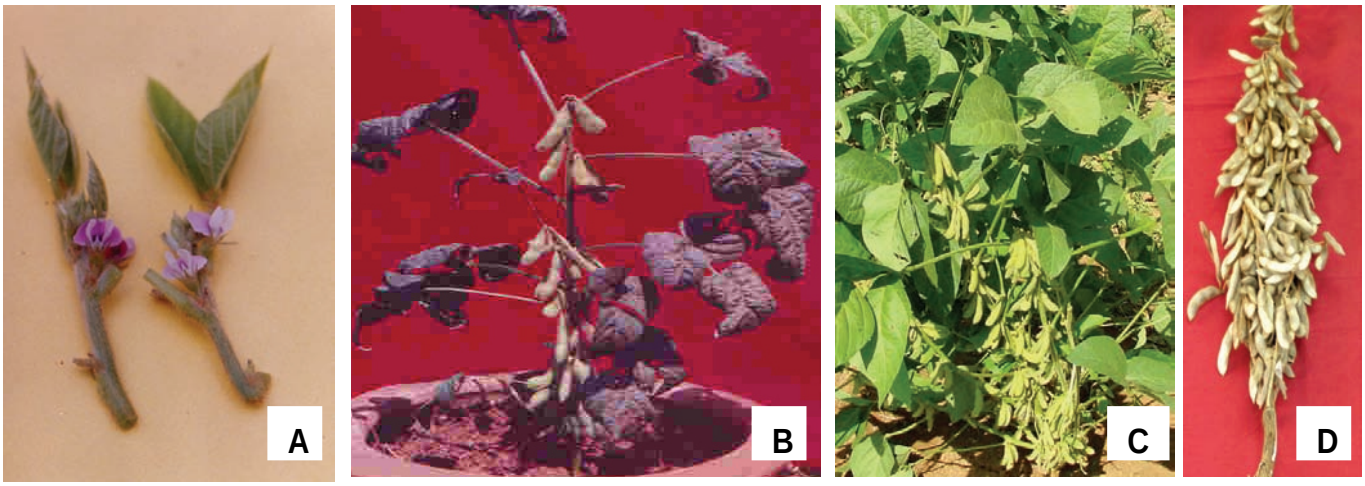

Figure 1. Soybean mutants and mutant variety
A. Parent (left) and Pink flower mutant M-91 (right); B. Crinkled leaf mutant M-39; C. Mutant M-107; D. New mutant variety TAMS 98-21

Evaluation of mutants

Seven mutants in the M_5 were evaluated for various quantitative characters in comparison with cv. JS 80-21. Mean values for important morphological characters are shown in Table 2. In all the mutants, days to flowering ranged from 35 to 36 days except one mutant M-107 that flowered in 39 days. Mutant M-107 matured in 110 days as compared to parent cv. (105 days) and the rest all showed similar maturity as the parent. The mean plant height of cv. JS 80-21 was 38.1 cm, Chlorina mutant (M-59) showed extreme reduction in plant height. Crinkled leaf mutant (M-20) and M-107 were shorter than the parent and others were on a par with parent. Compared to parent (2.6), mean number of primary branches were higher in M-107 (3.5). Number of pods per plant in cv. JS 80-21 was 32.5 while mutant M-59, M-20 and M-39 showed lower number of pods. On the other hand, mutant M-107 had higher number of pods (38.0). The 100-seed weight ranged from 7.5 to 11.0 g in the mutants. Mutant M-91 showed significantly higher 100- seed weight (11.0g) than the parent (9.2g). Three mutants M-20, M-39, M-59 and M-107 had lower 100 seed weight. Mutant M-107 showed significantly higher harvest index compared to parent. Mutants M-20 and M-59 recorded lower harvest index.

Seed oil content in the parent was 18.9% while it ranged between 16.7 to 19.1% in mutants (Table 2). Mutants M-20, M-39 and M-107 showed significantly lower oil content. Similarly, mean seed protein content in JS 80-21 was 40.8%. Although there is variation for protein content in the mutants between 38.4 to 41.2%, they were not significantly different from the parent (Table 2). Mean yield per plant in cv. JS 80-21 was 19.5g (Table 2) and mutants M-107, M-21 and M-91 showed higher seed yield over the parent. Chlorina (M-59), crinkled leaf (M-20) and M-39 gave a lower seed yield. Induced mutations for quantitative traits (Kundi et al., 1997; Geetha and Vaidyanathan, 1998), leaf and floral modifications (Singh and Jha, 1978, Wakode et al., 2000; Smutkupt, 1996) have been reported in soybean.

Table 2. Morphological characters of JS 80-21 mutants in M_5 generation

Mutants	Days to		Plant height (cm)	No. of branches	No. of pods/ plant	100 seed weight (g)	Oil %	Protein %	Harvest index (%)	Yield/plant (g)
	Flowering	Maturity								
M-20	36	103	**24.4**	1.3	29.2	7.8	16.7	40.7	29.8	13.1
M-39	36	103	38.6	2.1	25.7	7.6	16.8	41.0	33.0	13.4
M-59	36	103	**14.1**	2.3	15.5	7.5	17.6	38.4	28.5	12.7
M-21	35	104	39.3	2.9	34.5	8.6	18.6	40.8	34.7	**23.0**
M-76	35	105	41.0	2.7	34.7	8.8	18.2	39.6	31.8	18.1
M-91	36	106	39.3	2.4	31.0	**11.0**	19.1	39.1	34.5	**22.2**
M-107	**39**	**110**	**26.5**	**3.5**	**38.0**	7.8	16.7	41.2	**38.5**	**23.0**
JS 80-21(C)	35	105	38.1	2.6	32.5	9.2	18.9	40.8	34.9	19.5
C.D. 5%			2.2	0.7	5.6	1.0	0.8	1.2	2.32	1.8
CD 1%			3.3	1.0	8.3	1.5	1.2	1.8	3.4	2.7

Genetic diversity studies

The mutants and parent were distributed into three clusters (Fig. 2). Cluster I included crinkled leaf mutant (M-20) and small leaf, dwarf and late maturing mutant (M-107). Cluster II showed two sub clusters, having crinkled leaf mutant (M-39) in one sub cluster and high yielding mutants (M-21, M-76, M-91) and parent JS 80-21 in another. Cluster III is formed by only one mutant M-59 and showed maximum dissimilarity of 26% from the parent JS 80-21. Cluster I also showed 12% dissimilarity from the parent. Improvement in yield is normally attained through exploitation of the genetically diverse genotypes in breeding programmes. Based on the present study the mutant M-59 was found to be distinct and diverse and can be utilised in the breeding programme for developing better varieties of soybean.

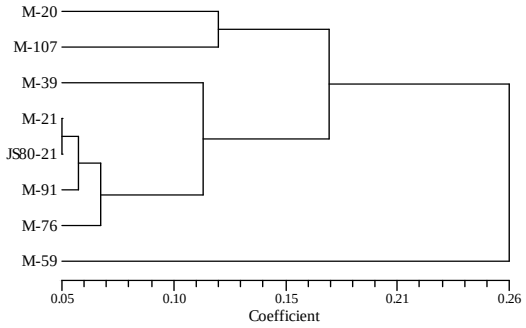

Figure 2. UPGMA dendrograms obtained from dissimilarity index values

Yield evaluation

Four mutants were initially evaluated along with three checks for yield at Trombay (Table 3). Mutants M-21 (1653 kg/ha) and M-91 (1533 kg/ha) gave significantly higher yield over the parent JS 80-21 (1463 kg/ha). The other mutants M-107 and M-76 were on a par with JS 80-21. These two mutants M-21 and M-91 were further evaluated by including in the multi-location trials conducted by Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola in the Maharashtra State (Table 4). Based on performance in five years, pooled mean for seed yield across twenty locations was 2243 kg/ha for M-21 which was renamed as Trombay Amravati Soybean (TAMS) 98-21 (Fig. 1D). M-91 gave a mean yield of 2106 kg/ha, in comparison to the best national check JS 335 (1853 kg/ha). Both the mutants proved their superiority over the best check by recording 21.0% and 13.7% higher yield. TAMS 98-21 not only showed high seed yields but also showed multiple disease and pest resistance.

Table 3. Seed yield (kg/ha) of JS 80-21 mutants at Trombay

Progeny No.	Yield (kg/ha)
M-21	1653**
M-76	1407
M-91	1533*
M-107	1518
JS 335 (Check)	1458
PK 472 (Check)	1351
JS 80-21(Check)	1463
SE	21.8
CD 5%	67 kg
CD 1%	94 kg
CV %	7.71

* and ** indicate significant superiority over the best check cv. at 5% and 1% levels, respectively

Table 4. Performance of TAMS 98-21 in Multi-location Varietal Trials in Maharashtra State in India

S. No.	Genotype	Mean Seed Yield (kg/ha)					
		1999-00 (3 Locations)	2000-01 (4 Locations)	2001-02 (4 Locations)	2002-03 (4 Locations)	2003-04 (5 Locations)	Pooled Mean (20 Locations)
1	TAMS-98-21	2636	2108	2266	1795	2409	2243
2	TAMS-98-91	2559	2104	2027	1495	2345	2106
3	TAMS-38	2854	1966	1947	1351	2078	2039
4	JS-335 (Check)	2175	1940	1665	1630	1853	1853
5	JS-7105 (Check)	1569	1670	1522	1524	1556	1568
	C.D.at 5 %	525	170	304	195	415	Not available

Based on the superior performance in the multi-location trials and other desirable agronomical traits, TAMS 98-21 was released for commercial cultivation by the Maharashtra State Sub-Committee during the year 2006 and subsequently gazette notified by the Central Varietal Release Committee, Government of India in 2007.

In breeding programme, hybridisation provides unlimited possibilities of generating new combinations of characters, which can be selected in the segregating populations. In contrast, with induced mutations it is possible to improve a single trait without causing extensive disruption in the genome. The use of induced mutations technique for crop improvement over the past few decades has shown that it is an effective plant breeding method to improve yield, quality and resistance to biotic and abiotic stresses (Nichterlein et al., 2000). More than 100 soybean mutant varieties were developed and released for commercial cultivation (http://www-mvd.iaea.org). Thus induced mutations can play a vital role as a supplementary approach in the crop improvement programme. In the present studies the mutant variety TAMS 98-21 was a direct mutant developed by irradiating with gamma rays. The results also indicated that a dose of 250Gy gamma rays is a useful dose to induce broad genetic variability in soybean. The other variability generated would be utilized in near future in the applied and basic research.

Acknowledgement

The authors thank Dr. S.F. D'Souza, Head, NABTD, BARC for providing the facilities to carry out this work and the International Atomic Energy Agency, Vienna for partial financial support under the RAS 5/040 project.

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Short Communication

Inter Simple Sequence Repeat (ISSR) Markers for Detecting Radiation Induced Polymorphisms and its Application as Genetic Marker System in *Sesbania rostrata* (Bremek. & Obrem.)

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Abstract

A comparative study was conducted to detect the polymorphism between a radiation induced *Sesbania rostrata* mutant and the parental genotype using three different marker systems, namely, random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP). Of the 200 RAPD primers used, only 3% produced a polymorphism between the mutant and the parental genotype, whereas 12.5% of the AFLP primers and 15.7% of the ISSR primers produced polymorphisms. Hypervariable region based ISSR markers were found to be better in detecting variations. This study indicates that in addition to the widely known larger deletions, the radiation-induced mutations could also result from point mutations or small insertions or deletions in or around the repeat region. The molecular events leading to such mutations involving the tandem repeats are not fully understood. There is a need for the mechanistic evaluation of such variations. The application of ISSR technique for detection of radiation-induced variations is discussed.

Keywords: AFLP, ISSR, Radiation, RAPD, *Sesbania rostrata*

Introduction

Radiation induced mutagenesis, used mainly to broaden the genetic variation, forms the core of mutation breeding research. After screening and selection, the mutants are used directly as well as in crossing programs for varietal improvement (http://www-mvd.iaea.org/MVD/default.htm). Such mutants constitute an important resource not only for breeding programs, but also as tools to study various genetic pathways. In addition, these mutants can also be used for studying the molecular nature of radiation-induced mutations.

The eucaryotic genomes have a large number of repeat sequences, like minisatellites, microsatellites and expanded simple tandem repeats, collectively called the tandemly repeated DNA loci (TRDLs). They are inherently unstable and also, have shown an increased frequency of induced mutations (Dubrova et al 1996, Ellegren et al 1997, Kovalchuk et al 2000, Bridges 2001). The high frequencies of radiation-induced mutations have indicated that these mutations are not directly linked to the site of DNA damage and hence are "untargeted". In addition, the radiation induced genomic instability has another feature of "delayed mutations", where mutations are caused long after the radiation exposure has ended (Niwa 2006). The mechanisms of such non targeted and delayed effects of radiation are not understood, but it is assumed that some radiation induced event triggers these sequences to become genetically less stable (Bouffler et al 2006). Several studies have demonstrated the use of tandem repeats as reporters of mutation (Yauk 2004, Armour 2006).

In this communication molecular markers were used as a tool to study the polymorphism between *Sesbania rostrata*, a tropical, non-grain legume and its radiation induced late flowering mutant Trombay Sesbania rostrata-1 (TSR-1). The use of inter simple sequence repeat (ISSR) assay to detect radiation-induced variations and its further use in studying the molecular nature of the mutations using plants as model system is discussed below.

Materials and methods

Plant material: The gamma ray induced mutant Trombay Sesbania rostrata-1 (TSR-1) (accession no.: INGR-01014) and its parental genotype *Sesbania rostrata* were used for assessing the radiation induced polymorphism. TSR-1 is a flowering time mutant that flowers later than the parental genotype, irrespective of the time of sowing. The radiation induced mutation shows a strong effect on flowering time, with no other visible pleiotropic effects on gross morphology or development. An F_2 population of 93 plants raised from a cross between TSR-1 and *S. rostrata* was used for studying the inheritance of the polymorphism. Two additional accessions of *S. rostrata* collected from West Bengal and TamilNadu were used for confirming the radiation-induced nature of mutations.

Genomic DNA extraction: DNA was extracted from a leaf tissue sample as previously described (Dellaporta et al 1983). The DNA was quantified using spectrophotometric analysis.

RAPD analysis: RAPD amplifications were performed using 200 random decamer primers from Operon Technologies, Inc, Almeda, USA (Kits: OPA 1-20, OPC 5-7, OPC 10-11, OPC 13, OPD 1-4, OPD 9, OPD 11, OPD 13-20, OPH 1-16, OPI 1-20, OPJ 1-8, OPJ 15-20, OPK 1-20, OPL 1-20, OPN 1-20, OPT 1-4, OPU 1-17, OPV 1-19, OPW 1-10). Amplifications were performed in an Eppendorf Master-cycler gradient (Eppendorf Netheler-Hinz GMBH, Hamburg) in a 25 µl reaction volume containing 50 ng of template DNA, 0.5 U of *Taq* DNA polymerase (Bangalore Genei Pvt Ltd.), 0.4 mM each of dNTPs (Bangalore Genei Pvt Ltd.), 0.2 µM random primer in a 1 X PCR buffer (Bangalore Genei Pvt Ltd.) containing 10 mM Tris- HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin. Amplification conditions were an initial denaturation at 94°C for 4 min and 45 cycles at 94°C for 1 min (denaturation), 37°C for 1 min (annealing) and 72°C for 2 min (extension). A final extension for 7 min at 72°C was performed.

ISSR analysis: One hundred ISSR primers (UBC#9 series, The University of British Columbia, Vancouver, Canada) were screened for polymorphisms between the parental genotypes *S. rostrata* and TSR-1. ISSR-PCR reactions were performed in 25µl volume containing 50 ng of template DNA, 0.5 units of *Taq* DNA polymerase, 0.4 mM each of dNTPs, 0.2 µM primer in a 1X PCR buffer containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin. Amplification conditions were an initial denaturation at 94°C for 4 min and 1 cycle of 94°C for 30 sec, 55°C for 45 sec and 72°C for 2 min. The touchdown PCR was performed by stepwise reduction of 1°C in annealing temperature from 55°C to 50°C in the first 5 cycles. In the subsequent 35 cycles annealing temperature was maintained at 50°C followed by 1 cycle of 7 min at 72°C.

For visualization of RAPD and ISSR profiles the amplified products were separated by electrophoresis in a 1.5% agarose gel in 1X TBE buffer at 75V. The gels were stained with ethidium bromide (1µg ml^{-1}) and photographed with a digital gel documentation and image analysis system (Syngene, U.K.).

AFLP analysis: AFLP analysis was carried out as per the protocol previously described for medium sized genomes (Vos and Kuiper 1998). Prior to loading the amplified products, 15 µl of formamide loading dye (98% formamide, 10 mM EDTA pH 8.0, 0.25% bromophenol blue and 0.25% xylene cyanol) was added and denatured for 5 min at 95°C followed by quick chilling on ice. A total volume of 5.5 µl was separated on 5% denaturing polyacrylamide gel (containing 8 M urea in 1X TBE buffer). The separated PCR products were visualized by silver staining (Bassam et al 1991), with slight modifications as follows: the gels were fixed in 40% methanol for 30 minutes, followed by pretreatment with 2% nitric acid for 10 minutes. The staining reaction was stopped using 0.1 M citric acid.

Results and Discussion

Three widely utilized marker techniques were assessed for their efficacy in detecting polymorphism between radiation-induced mutant (TSR-1) and the parental genotype. The results are summarized in Table 1. Very few differences in markers were observed between the radiation-induced mutant and the parental genotype. For RAPD analysis 200 random primers were screened to detect polymorphism, of which only 6 primers (3%) detected polymorphism with only one polymorphic amplicon per primer. In the case of AFLP only 8 out of 64 (12.5%) primer pair combination produced polymorphisms. In addition, each of these 64 different combinations of AFLP primers produced on average 50-60 fragments, of which the 8 polymorphic primer combinations produced only one polymorphic amplicon per primer pair (approximately 2% polymorphism). Similar observations were reported previously where analysis of 10 radiomutants of Chrysanthemum using RAPD technique revealed a very low genetic diversity among the mutants (Lema-Rumińska et al. 2004). In another study using AFLP technique no polymorphism was detected between a closed capsule mutant of *Sesamum indicum* and the cultivar, except for the AFLP marker identified for the trait (Uzun et al 2003). In case of ISSR technique, 57 primers produced amplification and 9 primers (15.7%) produced polymorphism between TSR-1 and *S. rostrata*. A representative gel profile using primer UBC 842 is shown in Fig. 1. More than one polymorphic amplicon per primer were also observed in the case of some ISSR primers (UBC 868, UBC 845, UBC 848). Nine polymorphic primers produced a total of 66 amplicons, of which 14 were polymorphic amounting to approximately 21.2% polymorphism. Thus, the polymorphism observed by ISSR primers is higher as compared to the other two techniques. In order to be sure that the observed polymorphism by ISSR markers is due to radiation induced mutations and not due to spontaneous natural variation, the polymorphic ISSR primers were also tested on 6 randomly chosen individual plants of each genotype. The analysis revealed the same polymorphic markers with no variation in the amplicon profiles. Additionally, two more accessions of *S. rostrata* collected from diverse geographical locations were also tested for polymorphism. No polymorphism was observed between the *S. rostrata* accessions; whereas reproducible polymorphisms were produced between the radiation-induced mutant and all the *S. rostrata* accessions using the same set of ISSR primers. This indicated the existence of increased microsatellite variations in case of radiation-induced mutant.

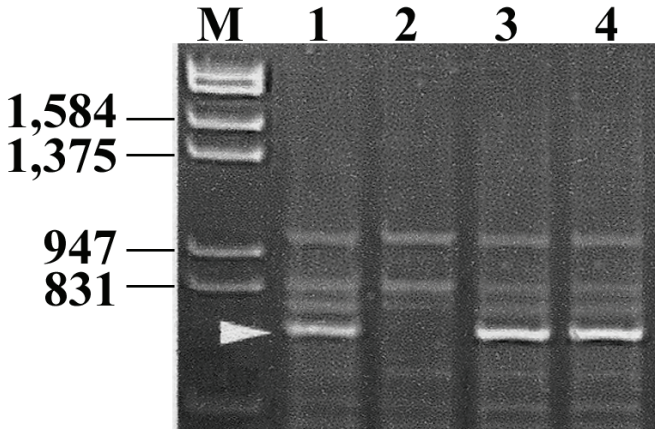

Figure 1. A representative ISSR profile using primer UBC 842. Lanes 1-4 represents the genomic DNA from S. rostrata, TSR-1, S. rostrata (West Bengal), S. rostrata (Tamilnadu) as template. Lane M is the standard molecular size marker (lambda DNA digested with Hind III and EcoR I). The polymorphic marker fragment is marked with a white arrowhead. The molecular sizes are depicted in basepairs (bp).

The stability of the polymorphic ISSR markers over generations was also studied in a genetic cross between TSR-1 and *S. rostrata*. The polymorphisms were found to be stably inherited in the F_2 progeny resulting from this cross. Hence these polymorphisms can be effectively used as a genetic marker system. Work done in our laboratory indicates that one such ISSR amplicon generated

by a pentanucleotide repeat primer (UBC 881) is linked to the flowering time trait in *S. rostrata* (Joshi-Saha and Gopalakrishna 2007). The amplicon was cloned and sequenced (Genbank Accession no. DQ 431666). The internal primers designed from this sequence failed to detect the polymorphism between TSR-1 and *S. rostrata*, and produced monomorphic bands. This indicated that the mutation leading to the polymorphism could be point mutations or small insertions/deletions in or around the primer-binding site.

The genomes of all organisms have a large number of repeat sequences that vary in complexity, number and distribution within the genome. One of the important characteristics of these repeat-sequences is that they tend to have higher mutation rates that mostly lead to a change of the array length (Udupa and Baum 2001). A comparative study done of a population of wheat plants grown for one generation in a heavily contaminated zone around Chernobyl nuclear power plant with a control population grown elsewhere has indicated an increased frequency of germline mutations at microsatellite regions in the exposed population (Kovalchuk et al. 2000). So far, the mechanisms of radiation induced mutation at plant microsatellite loci are not clearly known. Microsatellites have an inherent spontaneous instability due to susceptibility of either or both of the two processes viz. replication slippage or unequal recombination, and the elevated rates of these two processes are presumably involved in the susceptibility of these loci to radiation induction (Bridges 2001). It is also assumed that some radiation-induced untargeted event triggers the repeat sequences to become genetically less stable, but the exact nature of the events taking place is as yet unknown (Kovalchuk et al. 2000; Bridges 2001; Bouffler et al. 2006).

Table 1. comparative summary of three marker techniques in detecting polymorphism between radiation-induced mutant and parental genotype

Sr. No.	Primer	Sequence[a] (5'-3')	NPB[b]
1	RAPD - No. of primers: producing amplification (200), producing polymorphism (6)		
	OPI 11		1
	OPK 2		1
	OPK 16		1
	OPN 1		1
	OPU 2		1
	OPW 2		1
2	ISSR - No. of primers: producing amplification (57), producing polymorphism (9)		
	UBC 841		1
	UBC 842		1
	UBC 845		3
	UBC 848		3
	UBC 864		1
	UBC 866		1
	UBC 868		2
	UBC 876		1
	UBC 881		1
3	AFLP - No. of primers: producing amplification (64 primer pairs), producing polymorphism (8 primer pairs)		
	EACC/MCAT		1
	EAGG/MCAA		1
	EAGC/MCTG		1
	EACG/MCAG		1
	EACG/MCAA		1
	EACA/MCAG		1
	EACA/MCTA		1
	EACC/MCAA		1

[a]: AFLP primers contain three selective bases after 'E' and 'M' as indicated.
Y=(C,T); R=(A,G); E(5'-3'): GACTGCGTACCAATTC; M(5'-3'): GATGAGTCCTGAGTAA
[b]: NPB: Number of polymorphic bands

The studies on molecular characterization of ionizing radiation-induced lesions in higher plants are rather limiting. Ionizing radiations have been classified into two types based on linear energy transfer (LET): low LET radiations (like γ and X rays) and high LET (such as α particles and heavy ion particles). The molecular nature of mutations caused by ionizing radiations can be different depending on their LET. High LET radiations are reported to contain more DNA rearrangements with smaller sizes of deletions/ inversions as compared to those produced by low LET radiations. This is mainly attributed to the dense and localized ionization produced by high LET radiation in the irradiated cells (Goodhead 1995, Shikazono et al. 2001). Earlier studies showed that the mutations caused by fast neutrons as well as gamma rays were mainly deletions larger than 5 Kbp (Shirley et al. 1992;

Bruggemann et al. 1996; Cecchini et al. 1998). But recent reports have revealed a much more complex pattern of germline mutations that not only included complete deletion of loci but also showed a bias towards mutations with gains and losses of multiple repeat units. In addition, frequent insertions of DNA of unknown origin were also observed (Kovalchuk et al. 2003). In the present study, a possibility of point mutations and/or small insertions/deletions in or around the microsatellite region is suggested. Shikazono et al. (2005) reported an increased frequency of point mutations in *A. thaliana* after electron irradiation. The point mutations were shown to be small deletions and insertions with sizes of 1-2 bp within a run of repeated sequences.

Plants can be a good system to study the molecular nature of radiation-induced mutations, as they can be easily irradiated at different growth stages including seeds or callus. They can be bred easily and a number of population types and sizes can be raised to study the molecular genetics of the radiation-induced lesions. In addition the technique of pollen irradiation can be used to study the radiation induced germline mutations, which are otherwise difficult to study (Naito et al. 2005).

Radiation induced mutations are random events. It was suggested that in addition to the type of radiation used for mutagenesis, the loci flanking the analyzed locus are also important for various type of mutations observed (Shikazono et al. 2005). In plants, most of the radiation-induced mutation events studied to date have been using the specific gene loci as reporters. These genetic loci are mostly coding for functions that are dispensable for e.g. seed coat colour mutations. In addition to the coding regions there are tandemly repeated regions in the genome that may be mostly functionally neutral but can be attractive targets as reporters of mutation events. Repeat-based assays have been effectively used in mice as well as humans to measure induced mutations in somatic as well as germline cells. The one disadvantage of this approach is that it is very specific and there is a lack of understanding of the mechanisms causing such mutations in comparison to mutations at other non-repetitive loci (Singer et al. 2006). The present study indicates a possibility of ISSR based assay to detect radiation-induced variation in plants pertaining to the microsatellite repeats. The sequence analysis of such polymorphisms can also give an insight to the molecular nature of mutations. The technique also offers the advantage of being simple and a number of primers can be designed to target different types of repeats. Anchoring of bases at 5′ end of the primer would be useful in detecting the variations in repeat lengths.

Although repeat variations may not confer any observed phenotype, such microsatellite repeats are sometimes found to be associated with the coding regions of the genome (Morgante et al. 2002) and instabilities within the repeats are reported to influence gene function in some cases (Armour 2006). Hence, there is also a need to make a mechanistic evaluation of such genomic instability at the repeats.

Acknowledgement

We are thankful to Drs. S.E. Pawar and D.C. Joshua for seeds of *S. rostrata* and TSR-1. We are grateful to Dr. G. Sarkar, Bidhan Chandra Krishi Vishwavidyalaya, West Bengal, India and Dr. K. Mohana Sundaram, Tamil Nadu Agricultural University, Coimbatore, India for seeds of *Sesbania rostrata* from their collections.

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Mutant Variety

Evolution of Trombay Groundnut Varieties through Mutation and Recombination Breeding

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Abstract

Genetic improvement of groundnut has been in progress since the late 1950s at BARC, Trombay. The mutation breeding programme was initiated with X-ray irradiation to cv. Spanish Improved. This was followed by the use of gamma rays periodically to further enhance variability. As a result, a wide spectrum of mutants affecting both vegetative and reproductive plant parts was induced. Mutants directly, or in cross breeding with other mutants, mutant derivatives or cultivars resulted in the evolution of 12 Trombay groundnut (TG) varieties. They were released for commercial cultivation after evaluating in multi-location trials in the national and/or state agricultural universities. Some of the mutant varieties helped increase groundnut productivity and farmers' income in major groundnut growing states. Besides, four more varieties were developed by different agricultural universities by using TG germplasm as source material in their breeding programmes. Thus, a blend of mutation and recombination breeding played a very significant role for groundnut improvement in India.

Key words: *Arachis*, groundnut, induced mutagenesis, mutant

Introduction

Groundnut (*Arachis hypogaea* L.) is an important edible oilseed, food and feed crop grown in India, grown in more than 6.5 m ha. Genetic improvement of groundnut by using mutation and recombination breeding has been in progress since late fifties at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. Sustained research efforts resulted in the the isolation of several mutants with unique and desirable traits followed by the release of 12 TG varieties for commercial cultivation in different states across the country. Besides, more than 300 induced groundnut mutants and breeding lines were developed and maintained at BARC.

X-ray irradiation of the seeds of cv. Spanish Improved (*ssp. fastigiata*), led to mutations altering sub-specific traits in TG 1, from ssp. *fastigiata* to ssp. *hypogaea* and an increased number of branches in TG 3 (Patil 1966). TG 1 was the first induced mutant variety released in 1973 in India which was followed by the release of TG 3 in 1985. Further, sub-specific traits were altered from ssp. *hypogaea* to ssp. *fastigiata* in TG 18A and seed size in BARCG 1 due to gamma ray treatment to TG 18 and 'JL 24', respectively (Chandramouli and Kale 1982; Chandramouli et al. 1987) (Fig. 1). Other variability derived due to interaction of two mutant genomes, hitherto unknown in our breeding material include, high oil trait in TG 9 and extreme *fastigiata* phenotype in TG 17 (Patil 1973, Patil 1977) which was released for commercial cultivation in 1985. Hybridizing TG 17 with mutant variety TG 1 resulted in development of the first large seed variety, TKG 19A under ssp. *fastigiata* habit with 20 days fresh seed dormancy. A genomic blend of three mutants of Spanish Improved led to early maturing culture, TGE 1 and mutant genome in the background of Gujarat dwarf led to TGE 2 (Fig. 1). Under the genomic background of cvs. Robut 33-1 and M 13, mutant and mutant derivatives of Spanish Improved generated two varieties, TG 22 and Somnath, respectively. Somnath was released for Gujarat state in 1991 and TG 22 for Bihar state in 1992. A genomic blend of five different mutants of Spanish Improved was brought under the background of M 13 to develop TAG 24 (Patil et al. 1995) and TLG 45.

TAG 24 was initially released for Vidharba region, Maharashtra state in 1992 and later it was adopted by Andhra Pradesh, Karnataka, Gujarat, Rajashtan and West Bengal. TAG 24 is a semi-dwarf, early maturing cultivar with superior harvest index, partitioning efficiency and water use efficiency. Similarly, five mutants of 'Spanish Improved' and one mutant of cv. JL 24 with cv. M 13 background resulted in TG 26 (Kale et al. 1996). TG 26 was initially released for Gujarat, Maharashtra and Madhya Pradesh in 1996 and became popular in Karnataka state. It is similar to TAG 24 with improved traits viz., compact pod setting, smooth pods, high shelling out turn and fresh dormancy. The genomic combination of TG 26 was diversified with Gujarat dwarf to evolve TG 37A (Kale et al. 2004a) and with cv. Girnar 1 to evolve TG 38 (Kale et al. 2007). Similarly, TPG 41 had resulted from five mutants of Spanish Improved and one mutant of JL 24 under M 13 and Robut 33-1 genetic backgrounds (Kale et al. 2004b).

The genetic contribution of an ancestor to a groundnut variety was determined as the fraction of genes in the variety that could be traced to an ancestor through pedigree analysis, namely by coefficient of parentage (CP) (Badigannavar et al., 2002). Entire genetic base of TG varieties was contributed by apparently unrelated six ancestors namely, Spanish Improved, JL 24, Robut 33-1, M 13, Girnar 1 and Gujarat dwarf (Fig. 1). Among them, Gujarat dwarf is a natural mutant and all others are either selections or cross derivatives, which were released for commercial cultivation in India. Mutants of Spanish Improved chiefly contributed to evolution of TG varieties as evidenced from their genomic involvement in all the 12 varieties with a mean CP of 67.7%. This high CP value of cv. Spanish Improved was attributed to inter-mutant crosses and mutant-derivatives. In the later period, breeding efforts were oriented to enhance genetic diversity by involving other genomes. Consequently, M 13 was involved in seven varieties (CP = 10.4%), JL 24 in four

(CP = 10.4%), Robut 33-1 in two (CP = 6.3%), Girnar 1 in one (CP = 4.2%) and Gujarat dwarf in one (CP = 1.0%). Spanish Improved and JL 24 made TG genomic contribution through their respective mutants only, while the rest of ancestors by their involvement in cross breeding (Badigannavar et al., 2002). This diverse background within an individual cultivar would serve as a buffer against the possible genetic vulnerability associated with a narrow genetic base.

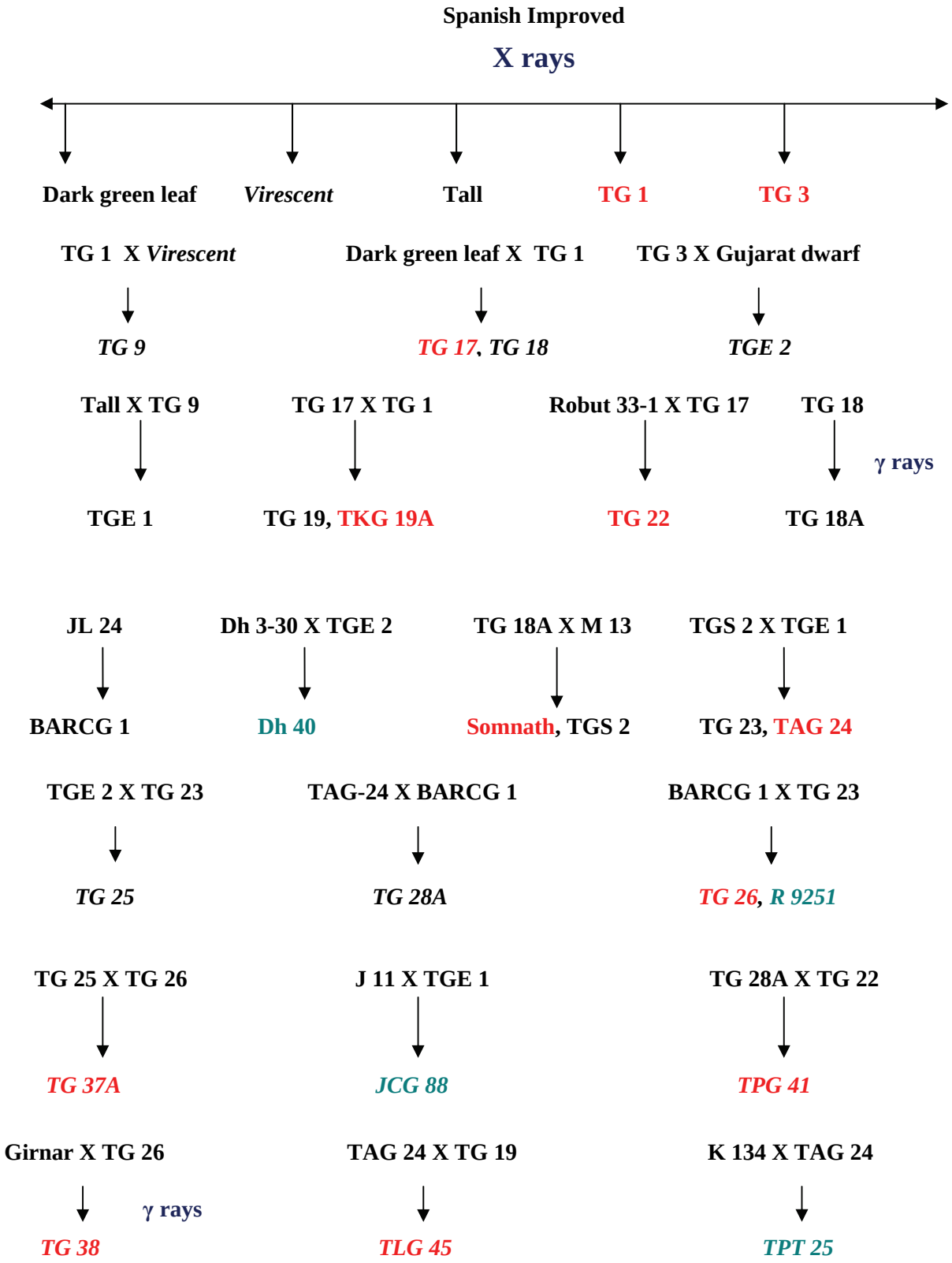

Figure 1. Evolution of 12 groundnut varieties developed by BARC and four by other institutes using TG germplasm as source (varietal names shown in red and green colours, respectively).

Among the TG varieties TG 37A (Kale et al. 2004a), TG 38 (Kale et al. 2007), TPG 41 (Kale et al. 2004b) and TLG 45 are recently developed. TG 37A and TPG 41 were released for commercial cultivation and gazette notified by the Ministry of Agriculture, Government of India during 2004, while TG 38 and TLG 45 in 2006 and 2007, respectively. Salient agronomical and other features of these new varieties are described below:

TG 37ATG 37A (Fig. 2A) is a high yielding Spanish bunch (ssp. *fastigiata* var. *vulgaris*) variety derived from the cross between TG 25 and TG 26 (Fig. 1). It was released for Haryana, North Rajasthan, Punjab, Uttar Pradesh States (Zone I) for rainy season (June to October) in 2004 and Southern Rajasthan and Gujarat (Zone II) for rainy season in 2006 and Orissa, West Bengal, Bihar and North Eastern states (Zone IV) for post-rainy (September-January) /summer (January-May) seasons in 2006. This variety is an improvement over TG 26 with respect to seed size, plant height and wider adaptability. In the All India Coordinated Research Project on Groundnut (AICRPG) Trials for Zone I, TG 37A recorded a mean pod yield of 1963 kg/ha and seed yield of 1246 kg/ha with a superiority of 26% and 40% over best check variety. In these trials, it matured in 114 days with a shelling out turn of 64 %, 100-seed weight (HSW) 39g and oil content 51%. Similarly, in Zone II TG 37A performed well with superior pod yield of 3048 kg/ha and seed yield of 2173 kg/ha (22% increase) and in Zone IV it had recorded 3186 kg/ha pod yield and 2231 kg/ha seed yield (20% increase). Thus, TG 37A not only showed superior agronomic traits but also showed wider adaptability in different agro-climatic zones. It has an erect growth habit with sequential flowering, semi-dwarf habit, medium-size leaflets, compact pod setting, smooth pod surface, spherical seed shape and rose testa colour, containing 48.0% oil, 23.0% protein, 19.3% carbohydrate, 4.5% sucrose and 2.8% crude fibre. Its oil contains 40.7% oleic, 39.8% linoleic and 12.3% palmitic acid. TG 37A has tolerance to collar rot (*Aspergillus niger* van Tieghem) and peanut bud necrosis (peanut bud necrosis virus) diseases and moisture stress.

Figure 2. New groundnut mutant varieties

TG 38

TG 38 (Fig. 2B) is a high yielding, Spanish bunch (ssp. *fastigiata* var. *vulgaris*) variety originated by irradiating F_1 seeds of the cross Girnar 1 X TG 26 with 300 Gy gamma rays (Fig. 1). It was released during 2006 for Orissa, West Bengal, Bihar and North Eastern states (Zone IV) for post-rainy/summer. This variety is an improvement over TG 26 for seed size and shelling out turn. In the AICRPG trials for Zone IV, TG 38 recorded mean pod yield of 2768 kg/ha and seed yield of 1984 kg/ha with a superiority of 19% and 21%, respectively over the best check variety. It matures in 100-110 days with 75% shelling and HSW 45g. TG 38 has an erect growth habit with sequential branching, semi-dwarf height and medium-size dark green leaflets. It has a compact pod setting with a smooth pod surface. Seeds are more spherical in shape with rose colour testa and contain 48.0% oil, 22.6% protein, 20.4% carbohydrate, 5.0% sucrose and 2.7% crude fibre. TG 38 oil contains 39.6% oleic, 39.6% linoleic and 12.1% palmitic acid. It has shown a tolerance to stem rot (*Sclerotium rolfsii* Sacc.) and dry root (*Macrophomina phaseolina* (Maub.) Ashby.) incidence under natural field conditions.

TPG 41TPG 41 (Fig. 2C) is a Spanish bunch (ssp. *fastigiata* var. *vulgaris*) large seed variety derived from a cross between TG 28A and TG 22 (Fig. 1) and released for the entire country for post-rainy/summer situation. It is an improvement over TKG 19A with respect to higher productivity, larger seed size and higher proportion of large seeds. In AICRPG large seed trials, TPG 41 produced mean pod yield of 2313 kg/ha and seed yield of 1586 kg/ha registering 19% and 29% superiority over the best check variety.

It also recorded 49% increased pod yield in 26 farm trials spread over Maharashtra. It matures in 120 days with HSW 70g and 70% shelling. TPG 41 is erect with semi-dwarf plant height and sequential flowering. Seeds are cylindrical in shape with pinkish rose testa colour containing 48.6% oil, 25.0% protein, 20.3% carbohydrate and 3.9% sucrose. Its oil contains 62.4% oleic and 19.3% linoleic acid. It has a fresh seed dormancy of 25 days, an important trait, which prevents *in situ* seed germination due to end-season rains or pre monsoon showers, when the crop is ready for harvest.

TLG 45

TLG 45 (Fig. 2D) is another Spanish bunch (ssp. *fastigiata* var. *vulgaris*) large seed variety derived from the cross between TG 19 and TAG 24 (Fig. 1) and released for Marathwada region of Maharashtra in 2007. It is similar to TPG 41 with an improved seed size and a lesser maturity period. In multi-location large seed varietal trial in Marathwada during the rainy season, TLG 45 produced mean pod yield of 1506 kg/ha and seed yield of 1031 kg/ha registering 28% and 37% increase over the best check variety. It also recorded 23% and 33% pod and seed yield superiority in inter-institutional large seeded varietal trial spread over Maharashtra. It matures in 115 days with an average 100-seed weight of 75g and 70% shelling out turn. TLG 45 is erect with semi-dwarf height and sequential flowering. Seeds are cylindrical in shape with rose testa colour and contain 49.6% oil, 26.9% protein, 54.3% oleic acid, 27.5% linoleic acid and 12.1% carbohydrate and 4.5% sucrose.

Groundnut mutant/mutant derivatives developed at this centre also contributed as a source material in the release of varieties at the other agricultural research centers in the country (Fig. 1). Towards this, University of Agricultural Sciences, Dharwad and Raichur released Dh 40 from the cross Dh 3-30 X TGE 2 and R 9251 from the cross BARCG 1 X TG 23, respectively for Northern Karnataka state. Similarly, Acharya N.G. Ranga Agricultural University, Jagatial and Tirupati released an early maturing variety JCG 88 from the cross J 11 X TGE 1 and drought tolerant variety TPT 25 from the cross K 134 X TAG 24, respectively for Andhra Pradesh state. Development of improved groundnut varieties was possible due to the successful isolation of mutants and bringing mutant genes into ideal genetic backgrounds. Thus, a judicious use of mutation and recombination breeding has a high potential in the genetic enhancement of groundnut.

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Mutant Variety

A Salt Tolerant Mutant Wheat Cultivar 'H6756'

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Wheat (*Triticum aestivum* L.) is the second most important crop and has been making a great contribution to food security, health and agricultural sustainability in China. Abiotic stresses such as drought and salinity have become one of the main bottlenecks in upgrading the productivity and production of wheat by a big margin in China. Improvement of stress tolerant trait of wheat depends on the availability of sufficient variability which is lacking in wheat cultivars and adapted germplasm. Mutation breeding technique by using nuclear radiations and related biotechnology could provide possibilities to tackle the difficult breeding problem effectively. This report is on the development of a new salt tolerant wheat mutant variety by using irradiating with gamma rays, double haploids and *in vitro* screening techniques.

Winter wheat cv. 'H6756' is developed at the Institute of Crop Science (ICS), Chinese Academy of Agricultural Sciences, Beijing, China, and received registration No.2004-31 from the Shandong Provincial Committee of Crop Variety Examination and Approval, on 1 September 2004. It was released due to its good salt tolerance and superior agronomic performance.

Cv. H6756 was derived from the cross "(Laizhou 953 X 90γ4-85L) X Ji87-5108)", made at ICS in 1994. Cv. Laizhou953 was released in Shandong province in 1994 by Agricultural Institute of Laizhou City, Shandong Province for its erect flag leaf, large spike and high grain yield (Wen, 1996). 90γ4-85L is an advanced mutant line induced following 250Gy gamma ray irradiation at ICS in 1989. It has good resistance to stripe rust. Ji87-5108 is the original code of a mutant cultivar'Jimai37' released in Hebei province in 1996 by the Institute of Cereal and Oil Crops; Hebei Academy of Agricultural and Forestry Sciences for its early maturity, wider adaptation and high grain yield (Zhang et al, 1998).

The F_1 seeds from the above-cited cross leading to the selection of H6756, were grown in the field at Beijing in 1995. The anthers from F_1 plants were irradiated by 1.5 Gy gamma rays and cultured in the medium containing 0.4% NaCl for the callus induction and first *in vitro* screening. Callus differentiation was then conducted on a medium containing the same concentration of NaCl for the second *in vitro* screening (Liu et al, 2003, 2006). Regenerated pollen plants were then transplanted into the field to obtain the naturally doubled haploid individuals in 1996. Plants with a normal seed setting were grown for propagation in the soil without salt-stress during1997-1998 to remove the possibility of the existence of physiological adaptation. Among them, two lines coded as H94 (6)-675-6-2 and H94 (6)-675-6-4 performed well and had identical phenotypes. These two lines were then bulk harvested and named as H6756 in 1999. Seedling and adult plant screening tests conducted in both laboratory and field conditions at the same time of yield trials conducted during 1999 and 2000 showed that H6756 exhibited very good salt tolerance. Results of salt tolerance identification in the field by Dezhou Agricultural Institute of Shandong Province in 2000 also revealed that H6756 had a salt tolerating ability similar to the local best salt tolerant cv. DK961. It was able to grow in soil containing NaCl as high as 0.3-0.5%, with an average grain yield of 5205 kg ha^{-1}.

H6756 yielded 6292 kg ha^{-1} on an average in the Shandong provincial regional multi-location salt tolerant trials of wheat, during 2001-2003, with a yield advantage of 17.3% over the check cv. DK961. In the demonstration trials organized in large areas during 2003-2004, H6756 yielded 5314 kg ha^{-1}, an increase of 4.7% than the check cv. DK961.

H6756 is an early maturing and semi-dwarf winter wheat. It has mid-dense and spindle shaped heads with white awns and glumes. The plant height is 83 cm. The spike is 10-12 cm long. The average grain number per spike is 40. Kernels are white, hard and plump (Fig.1 A, B, C), with the thousand-grain weight 40-42g, test weight 825 g/l, grain protein content 15.5% and wet gluten content 36.8%. The SDS-sedimentation value is 21.6 ml, dough development time 2.0 min and dough stability time 2.1 min. The noodle quality assessment score is 83. H6756 is resistant to stripe rust and leaf rust, but is moderately susceptible to powdery mildew disease.

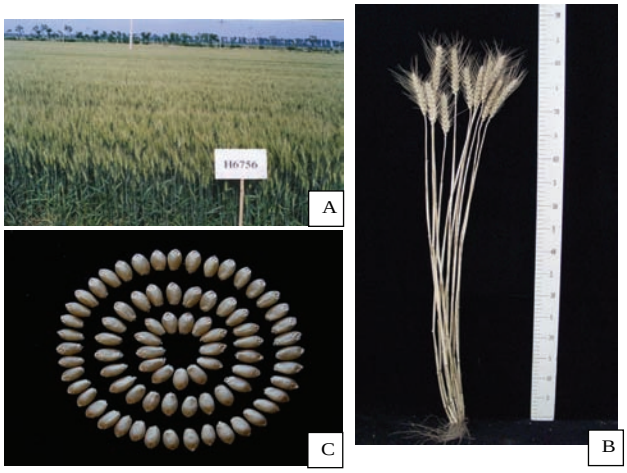

Figure 1. Salt tolerant wheat cv. H6756 – A: Field growth, B: Plant type and C: Grain

Acknowledgements

Research was partially supported by the International Atomic Energy Agency through the Projects IAEA/RAS/5/040, IAEA/INT/5/147 and RC12987.

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FAO/IAEA Mutant Variety Database

The FAO/IAEA Mutant Variety Database (http://www-mvd.iaea.org/MVD/default.htm) provides information on plant varieties developed by using induced mutations. The database has recorded more than 2500 mutant varieties of about 180 plant species, together with information about the mutagen and dose used in mutation induction; the main improved character(s), release time, amongst other parameters. You can search individual mutant varieties, number of mutant varieties in each crop species, or crop varieties developed in the country you are interested in. The submission of mutant varieties information is on volunteer basis, so that the actual number of mutant varieties must be much larger than the number in the database. If you have information that is not yet in the database, we welcome you to submit it to our database; please contact us through email: plant.mutation@iaea.org.

Mutant Variety

A Rice Mutant Variety with Lodging Tolerance and High Yield

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Abstract

Rice (*Oryza sativa*) is the most important staple food crop in Korea. Chucheongbyeo is a major rice cultivar especially in the central west plain area of Korea because it is of good eating quality for Koreans. The cultivar has been regarded as a mid-late maturing and the panicle number type rice with high tillering. And one of the weakest points in the cultivation of Chucheongbyeo is that it is susceptible to lodging, therefore severe lodging damage sometimes occurs. We have tried to improve the weak characteristics of the cultivar by mutation techniques.

Keywords: Rice, *Oryza*, gamma rays, mutant, Woncheongbyeo

"Woncheongbyeo" is a new *japonica* rice mutant developed by the Korea Atomic Energy Research Institute (KAERI) and was released in 2003. In order to select the mutants with good traits, 300g dry seeds of Chucheongbyeo were irradiated with 300 Gy gamma rays (0.833 Gy/min) from a ^{60}Co source at the KAERI. In 1992, the irradiated (M_1) seeds were directly seeded in high density in a paddy field. At maturity, bulk seeds were harvested from the upper part of spikes from more than 2,000 M_1 plants (Fig. 1). In 1993, 6,000 seedlings of M_2 plants were transplanted in a field and 217 variants affecting various characteristics were selected. During 1995, 52 useful mutant lines were re-selected. Among them, 12 useful mutant lines showing short plant height (Fig. 2A) and high yield were selected and performance test was conducted during 1996 and 1997. One of the promising lines (Wonnong-6) was entered in a regional adaptability trial during 1998 to 2000, and then applied for registration to the National Seed Management Office (NSMO) as a new national rice variety. After two years of registration and field tests by the NSMO, the new rice variety "Woncheongbyeo" was officially registered in 2003 and then released (Fig. 1) to farmers.

Figure 1. Pedigree of new variety "Woncheongbyeo" isolate from "Chucheongbyeo"

Year	`92	`93	`94	`95	`96	`97	`98 ~ `00	`01 ~ `02
Generation	M1	M2	M3	M4	M5	M_6	M_7 ~ M_{10}	M_{11} ~ M_{12}
	Chucheong-byeo irradiated with gamma ray	Direct seeding cultivation and bulk seeds harvesting ⇨ 1, 2, ., ., ., 6,000	⇨ Field test of 217 lines	Field test of 52 lines	⇨ CCM1, ., (CCM6), ., CCM12 (Performance test of 12 lines)	⇨ CCM6-1, (-2), CCM6-5	⇨ Wonnong- 6	Woncheong-byeo
No. of lines (plants)	Bulk seeds	6,000	217	52	12	4	1	1
Remarks					PYT†	PYT†	RAT	VRT

† PYT = Preliminary yield trial; RAT = Regional adaptability trial; VRT = Variety registration test.

According to the regional adaptability trial, "Woncheongbyeo" has a short stature (Fig. 2A) due to 64 cm culm length, a lower degree of lodging and a 3-day early heading date compared to cv. Chucheongbyeo (Table 1). It has considerably erect pubescent leaf blades and a tough culm with good canopy architecture. "Woncheongbyeo" has similar tillers per hill, panicle length, and a slightly higher ripened grain ratio, but more spikelets per panicle than its parent (Table 1). It is resistant to blast and leaf blight diseases, but susceptible to major insect pests (Table 2). "Woncheongbyeo" has a medium to small grain size with clear non-glutinous endosperm without a white center and white belly, and its palatability of cooked rice is excellent. The new cultivar showed 72.0 in taste value by Toyo-midometer, 9.4% and 19.4% in protein and amylose content (Table 3), respectively. The mean grain yield of "Woncheongbyeo" was about 5.62 t/ha in a multi-location trial during 1999 and 2000 (Table 4). It is highly adaptable to the northern plain areas and to the middle and southern mid-mountainous areas of Korea.

Figure 2. A Chucheongbyeo (left) and Woncheongbyeo (right) at the heading stage B Wonnong-6=Woncheongbyeo (left) and Chucheongbyeo (right) at the ripening stage.

Table 1. Major agronomic traits and yield components of "Woncheongbyeo"

Cultivar	Heading date	Culm length (cm)	Panicle length (cm)	No. of panicles per hill	No. of grains per panicle	Ratio of ripened grain (%)	Brown rice 1,000 grain weight (g)	Brown/ rough rice ratio (%)
Woncheong-byeo (Mutant)	Aug. 12	64	19	20	80	86.9	20.4	83.4
Chucheong-byeo (Parent)	Aug. 15	88	19	21	73	87.9	20.3	82.8

Seeding date: April 25, transplanting date: May 25.

Table 2. Ranking for disease, pest and lodging resistance in "Woncheongbyeo"

Cultivar	Leaf blast	Neck blast	White leaf blight	Sheath blight	Main insect injury	Field lodging degree
Woncheongbyeo (Mutant)	0	0	0	0	3	0
Chucheongbyeo (Parent)	2	0	0	0	3	5

*The scale of damage of pest and lodging degree; 0= Susceptible and 9= Resistant

Table 3. Grain quality and taste of "Woncheongbyeo"

Cultivar	Taste value (Toyo-midometer	Protein (%)	Amylose (%)	Fatty acid (mgKOH/100g)
Woncheongbyeo (Mutant)	72.0	9.4	19.4	14.5
Chucheongbyeo (Parent)	77.3	7.8	19.4	15.1

Table 4. Yield performance of "Woncheongbyeo" in a regional yield trial

Location	Milled rice yield (t/ha)					
	Chucheongbyeo (Parent)			Woncheongbyeo (Mutant)		
	1999	2000	Mean	1999	2000	Mean
Cheongwon	5.33	5.12	5.23	5.65	5.82	5.74
Iksan	5.40	4.83	5.12	5.35	5.01	5.18
Nonsan	5.46	5.77	5.62	5.91	5.98	5.95
Mean	5.40	5.24	5.32	5.64	5.60	5.62

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Acknowledgements

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Plant Mutation Reports

Vol.1, No. 3 December 2007

The Plant Mutation Reports is issued twice per year by the Plant Breeding and Genetics Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf.

International Atomic Energy Agency
Wagramer Strasse 5, P.O. Box 100,
A-1400 Wien, Austria

Printed by the IAEA in Austria,
December 2007

07-48741