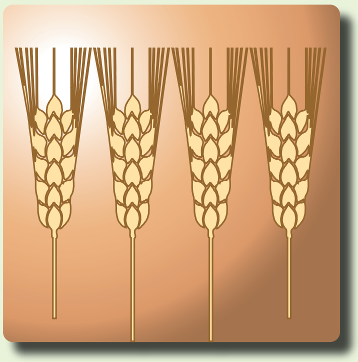
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf

Plant Mutation Reports

Vol. 1, No. 1 May 2006

http://www-naweb.iaea.org/nafa/index.html
http://www.fao.org

ISSN 1011-260X



Contents

IAEA
International Atomic Energy Agency

Participants visiting the Gamma Field of the Institute of Radiation Breeding, National Institute of Agrobiological Resources, Kamimurata, Hitachiomiya, Ibaraki, Japan

To Our Readers

Just before going to press, an administrative/technical issue prevented us from continuing to use the title, Mutation Breeding Newsletter and Review (MBN&R) for our publication. After discussions, we decided, from this issue on, to use the title, Plant Mutation Reports (PMR) to replace the MBN&R. This move was indeed in line with our planning, but occurred sooner that we expected (see To Our Readers, MBN&R No. 1). Thus, MBN&R No. 2 will be replaced by the present PMR Vol. 1, No. l. Please accept our deepest apologies for any trouble this might cause. We will continue to strive to improve the quality of Plant Mutation Reports towards a periodical of higher scientific value, as a specialized international journal on plant mutation research and its application in crop improvement. Your comments and suggestions on this subject are very much welcomed and appreciated!

“Rice is Life” At its fifty-seventh session in December 2002, the United Nations General Assembly designated 2004 as the International Year of Rice (IYR), originally proposed by the FAO. The declaration reflects the importance of rice for global food security, poverty alleviation and sustainable development. Rice is the staple food of more than half of the world’s population; rice cultivation, products and traditions attached to the crop have become an integral part of the world’s cultural heritage. In most Asian countries, the significance of rice in people’s daily life and culture is much more pronounced than on any other continents.

The Agency has long been involved in assisting Member States in enhancing capacities on genetic improvement of crop plants, including rice, using nuclear techniques. During the past 40 years, more than 20 national and regional technical cooperation projects on rice improvement using nuclear techniques were implemented in almost all of the rice growing developing countries in Asia, which has greatly advanced the development and application of nuclear techniques in rice improvement and prompted the beneficial exchange of mutant germplasm in this region.

The FAO/IAEA/RCA "Strategic Meeting on Nuclear Techniques for Rice Improvement in Asia" was organized in conjunction with the World Rice Research Conference in Japan on 6 November 2004 to fully review the economic benefits from the adoption of mutant rice varieties and the current status of induced mutations in rice germplasm enhancement, new variety development and functional genomics studies, and future perspectives of nuclear and related techniques in rice breeding and genetics. The Agency through RCA Project RAS/5/040 and National TC Projects VIE/5/014 and PAK/5/042, sponsored 15 participants. The host institute, the Institute of Radiation Breeding, National Institute of Agrobiological Resources, Japan, also invited and sponsored 5 Japanese participants. The participants presented their work on rice mutation breeding, new mutation techniques, biological basis of induced mutations, novel mutants for rice improvement and functional genomics research.

Inside this issue, you will find the complete papers of some of the presentations. You will not only have an overview of the application of mutation techniques in rice breeding in these countries, but you will also be able to envision the future perspectives of nuclear techniques in rice improvement. We also included a few papers that were not presented in the meeting but are relevant to this theme and are beneficial for you to get a broader view.

Based on my communications with these authors and my understanding of these papers, I got the following impressions on mutation techniques in rice improvement which I would like to share with you: (1) Mutation techniques can be successfully deployed even in institutes with limited infrastructure and laboratory facilities, a common situation in developing countries, which is a very important feature that made this technology widely accepted in almost all countries; (2) Mutation techniques have proven not only useful for improving agronomic traits, i.e., yield, plant height, growth duration, etc, but also for enhancing resistance to biotic stress such as disease and insect pests and tolerance to abiotic stress such as salinity and acidic soil; (3) Mutation techniques have also proven very useful in quality improvement, i.e. development of rice varieties with preferred amylose content, and probably more importantly, enhancing nutritional value of rice grains, i.e., reducing phytic acid content, with giant embryos and thus high GABA content, and increased resistant starch content; (4) Mutation techniques have been used as the sole technique for the improvement of special rice type, i.e., Basmati rice in India and Pakistan, and aromatic rice in Viet Nam; (5) Mutation induction has become an important tool in gene discovery and functional genomics studies, more and more mutant lines are being generated and analyzed worldwide; last but not least, (6) The IAEA, through collaboration with FAO, has directly (through TCPs) and indirectly (through information and knowledge dissemination) contributed to the deployment of mutation techniques in many countries, which is highly appreciated by many authors in their papers.

Qingyao Shu
Pierre J.L. Lagoda

Table of Contents

Recent Progress of Rice Mutation Breeding and Germplasm Enhancement in China

Chen[1], X., Liu[1], X., Wu[2], D. and Shu[3], Q.Y., [1]Lixiahe Agricultural Institute of Agricultrual Sciences, Yangzhou, Jiangsu, China, P.R.; [2]IAEA-ZU Collaborating Centre, Institute of Agricultural Sciences, Zhejiang University, Hangzhou 310029, China, P.R.; [3]Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Wagramer Strasse 5, P.O. Box 100, A-1400 Vienna, Austria

Abstract: The progress of mutation breeding and mutational germplasm enhancement was reviewed in this paper. From 1991 to 2004, there were 77 new mutant varieties, both of conventional and hybrid rice, officially released in China. Mutation techniques were successfully used for generating novel characteristics of direct importance in rice production and of potential quality and nutritional value. A few pilot varieties embodied with novel characteristics, i.e., leaf colour markers, giant embryos, were already officially released for commercial production.

In China, crop mutation breeding was first initiated in the early 1960's. A number of national, provincial and regional research institutes and universities have been involved in crop improvement using mutation techniques, and significant achievements were made from the early stages (Wang 1985, 1992) till very recently (Liu et al 2004). Rice is the most important food in China; tremendous efforts have been undertaken towards new variety development, mutant germplasm enhancement and preservation, and technology improvement and innovation. This paper summarized the development and achievement in rice mutation breeding and germplasm enhancement in the past 15 years in China, with emphasis on breeding of new varieties and enhancing or diversifying rice grain quality/functionality, since mutant germplasm for functional genomics studies was presented in another paper by Zhu et al in this issue.

New mutant varieties

There were 78 mutant rice varieties released from 1966 to 1990 in China (Wang 1992). During the past 15 years, the number of mutant varieties has been steadily increasing and approximately the total number of same mutant varieties developed during this time period was about the same as before 1990 (Fig. 1).

Most rice mutant varieties were released in Zhejiang (23), Hunan (15) and Jiangsu (12) Province (Fig. 2).

Among the 77 new varieties, more than half were the direct use of induced mutant lines. A significant increase of the number of mutant hybrid rice, of which either the female (CMS line) or male parent (restorer line) is a mutant line, is a new trend in rice mutation breeding during the past years. It reflected the fact that hybrid rice breeders have started to use mutation techniques in their breeding program with success.

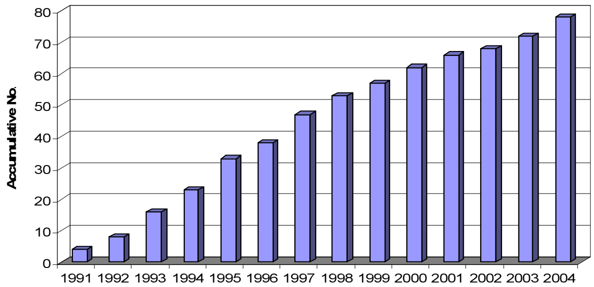


Fig.1. Accumulative number of mutant rice varieties officially released in China (1991-2004).

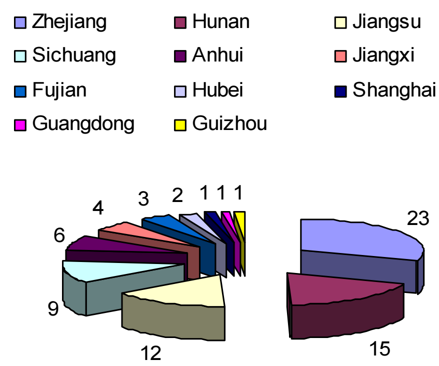


Fig.2. The number of mutant rice varieties developed in various provinces in China (1991-2004).

New techniques for mutation induction

Gamma rays are still the most frequently used mutagen, but new agents including ion beams and space condition have also been used in mutation induction and breeding (Fig. 3). Use of *in vitro* cultures for mutation induction, or use of anther culture to rapidly produce homozygous lines from irradiated progenies, has proven to be very useful in several laboratories. Ten new varieties were developed through the combined use of physical/chemical treatment and *in vitro* culture (Fig. 3).

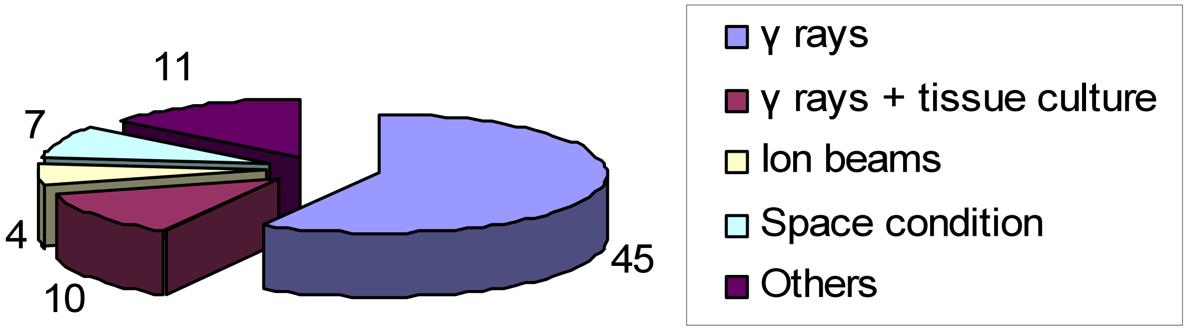


Fig.3. Number of mutant rice varieties developed using various mutagens/methods (1991-2004).

Apart from indirect use of induced mutants, heterozygous materials, e.g., F_1 and F_2 seeds, have been also used for mutagenic treatment, from which a few number of mutant varieties were developed, i.e., Yangdao # 6 was selected from the progeny of gamma rays irradiated F_1 seeds of Yangdao # 4/3021.

Novel mutant traits

In past years, mutation techniques have proven to be useful for generating novel mutant characteristics of either agronomic and grain quality or functional importance.

Leaf colour mutants. Both green-revertible albino mutation and yellow leaf mutation, controlled by a single recessive gene, were induced and proven to be useful as a visual marker for increasing and rapidly testing varietal purity of hybrid seeds (Wu et al, 2003; Zhou et al, 2006). For the green-revertible albino mutants, the first 3-4 seedling leaves appear white, which makes them visually distinguishable from normal green seedlings. Eight such CMS lines, e.g., Quanlong A (Shu et al 2001) and Baifeng A (Shen et al 2004), NHR100A, NHR103A, NHR105A, NHR107A, M98A, and three T/PGMS line, e.g., Yutu S (Wu et al 2003), NHR111S, NHR113S, have been developed. Five hybrid rice varieties, e.g., Quanyou 36 (Quanlong A/Zuhui 36), Chiyou S162 (Baifeng A/S162), Fenghuayou No.2 (Baifeng A/NHR4), Fenghuayou No.1 (NHR100 A/NHR4), and Nonghuayou808 (M98A/0208) were officially released. A xantha mutation was also introduced into a CMS line Huangyu A, and proved to be useful as a visual marker. A new CMS line embodied with this marker characteristic was already developed for hybrid rice breeding (Zhou et al 2006).

Low phytic acid mutants. Supported by the IAEA through a regional TCP and relevant local governments, a couple of low phytic acid mutant lines were developed in Zhejiang University through gamma irradiation (Wang et al 2005; Liu et al, in preparation). The high inorganic phosphorus (HIP) based indirect selection method, first developed by Larson et al (2000), was applied in screening of low phytic acid mutants (Wang et al 2005).

Three of the mutant lines, i.e., one indica mutant line HIPi1 from Xieqingzao B, two japonica lines HIPj1 and HIPj2 from Xiushui 110, have been under extensive characterization. The reduction of phytic acid content in HIPi1 and HIPj1 is as high as 50% compared to their corresponding parent lines, while it is around 20-30% in HIPj2. Changes in agronomic performance, i.e., plant height, panicle and seed size, and in some grain quality characters were observed in all mutant lines. Genetic analysis showed a single gene each was responsible for the low phytic acid phenotype in these lines, and the three genes are mutually non-allelic. Preliminarily molecular mapping using microsatellite DNA markers showed the mutation in HIPi1 happened to the same locus as the lpa reported by Larson et al (2000) in the Kaybonnet low phytic acid mutant, while the mutation in HIPj1 was found in chromosome 3 linked with the microsatellite marker RM 468 (Liu et al, in preparation).

Giant embryo mutants. Eighteen giant embryo mutants were isolated from the leading conventional and parental lines of hybrid rice, i.e., maintainer lines II-32B, Xieqingzao B, Longtefu B; restorer lines Minghui86, 7954, 527; conventional early indica rice Jinzao 47, japanica rice Xiushui63, and specialty rice Zinuo (with purple grains). Genetic analysis showed there are at least two new mutations that are not allelic to reported giant embryo mutations. Giant embryo hybrid rice varieties have also been bred directly using parents carrying the mutation. i.e., giant embryo "IIyouming86" and "IIyou7954". Gamma aminobutyric acid (GABA) is the major inhibitory neurotransmitter that helps stabilize blood pressure and reduces lipid level in the blood. The GABA content increases rapidly during pre-germination of brown rice, particularly in giant embryo rice grains (Zhang *et al.*, 2005).

High resistant starch mutants. Resistant starch (RS) is defined as starch fraction that is not digested in the small intestine. The absorption of RS in the small intestine is slower than normal rice starch, and thus can decrease the postprandial glucose and insulin responses, which is favourable for the dietary management of metabolic disorders such as diabetes and hyperlipidemia. In the colon, RS fermented by the intestinal microflora produces a range of short-chain fatty acids (SCFA), primarily acetic, propionic, and butytic acid that are greatly helpful in preventing colonic diseases.

As the staple food of over half the world's population, cooked rice with a high RS content is of particular interest, which may provide a convenient way for dietary prevention of diabetes and hyperlipidemia. Ten mutant lines, eight from a high amylose line and two from an intermediate amylose line, were identified with a high RS content in cooked rice. Mutant line RS111 is such a line, of which the RS content is as low as its wild type in the raw milled rice, but is significantly higher than that of the wild type and other varieties in cooked rice. The result is consistent with *in vitro* starch hydrolysis by porcine pancreatic alpha-amylase where incomplete hydrolysis was observed for rice mutant with high RS content in cooked rice (Fig.4)(Yang *et al.*, 2006).

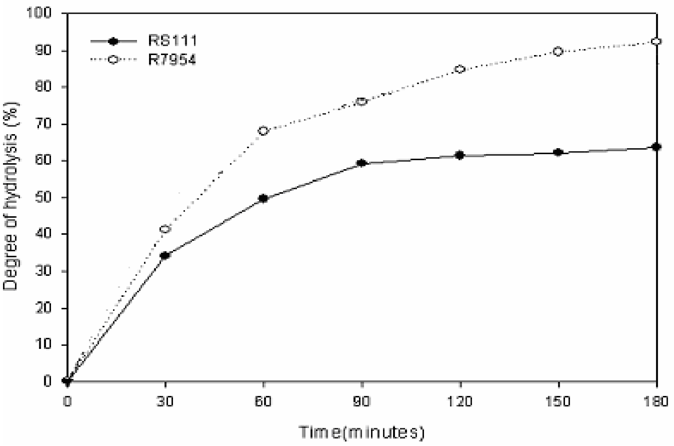


Fig.4. In vitro *hydrolysis of cooked rice crack of mutant RS111 and its wild type 'R7954'.*

Reference

Larson, S.R., Rutger, J.N., Young, K.A. and Raboy, V. Isolation and genetic mapping of a non-lethal rice (Oryza sativa L.) low phytic acid 1 Mutation. Crop Sciecne, 2000, 40:1397-1405.

Liu, L., van Zanten, L., Shu, Q.Y. and Maluszynski, M. Officially released mutant varieties in China. Mutation Breeding Review. 14, 1-62.

Shen, S.Q., Shum Q.Y., Bao, J.S., Wu, D.X., Cui, H.R. and Xia, Y.W. Studies on a green-revertible albino leaf CMS line Baifeng A. Chinese Journal of Rice Science, 2004, 18, 34–38.

Shu, Q.Y., Shen, S.Q., Wu, D.X. and Xia, Y.W. Development of a novel CMS line, Quanlong A. Scientia Agricultura Sinica 2001, 34, 349-354.

Wang, L.Q. Improved varieties of crops obtained through mutation breeding in china. Application of Atomic Energy in Agriculture, 1985, 1: 1-9.

Wang, L.Q. Advances in plant mutation breeding in China: a full analysis. Bulletin of Nuclear Agricultural Science.1992, 13: 282-295.

Wang, Y.H., Ren, X.L., Liu, Q.L. , Chen, W.Y., Shen, S.Q., Wu, D.X. and Shu, Q.Y. Studies on the induction and screening of high inorganic phosphorus mutants in rice. Chinese Journal of Rice Science. 2005, 19(1): 47-51.

Wu, D.X., Shen, S.Q., Cui, H.R., Xia, Y.W. and Shu, Q.Y. 2003. A novel thermo/photoperiod-sensitive genic male-sterile (T/PGMS) rice mutant with green-revertible albino leaf color marker induced by gamma irradiation. Field Crops Res. 81, 141–147.

Zhang, L.L., Hu, P.S., Tang, S.Q., Zhao, H.J. and Wu, D.X. Comparative studies on major nutritional components of rice with giant ermbryo. J. Food Biochem., 2005, 29, 653-661.

Zhou, X.S., Shen, S.S., Wu, D.X., Sun, J.W. and Shu, Q.Y. Introduction of a xantha mutation for testing and increasing varietal purity in hybrid rice. Field Crops Research, 2006, in press.

Yang, C.Z., Shu, X.L., Zhang, L.L., Wang, X.Y., Zhao, H.J., Ma, C.X. and Wu, D.X. Starch properties of mutant rice high in resistant starch. J. Agric. Food Chem. 2006, in press.

Nuclear Techniques for Rice Improvement and Mutant Induction in China National Rice Research Institute

Zhu, X.D., Chen, H.Q. and Shan, J.X., State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 31006, China, P.R.

Abstract: Nuclear technique is a useful tool for rice improvement and generation of mutant bank for plant functional genomics studies. About 700 morphological and physiological mutants from 2 cultivars with different mutation frequency were induced by gamma irradiations and preserved in germplasm bank. Some mutants have been successfully used for rice improvement and production.

Nuclear techniques in China have been applied widely in rice improvement [1, 2] and genomics studies [3]. Rice, as a model plant of monocotyledon, was irradiated by gamma rays or treated with chemical mutagens to induce extensive and saturated morphological or physiological mutations during plant growth and development for functional genomics research. Many induced rice mutant lines were utilized directly as new varieties in commercial production.

Development of mutant bank

Mutation induction technology has been used as a practical tool to develop and enhance mutant bank for rice functional genomics in China National Rice Research Institute (CNRRI). Two major cultivated rice (*Oryza sativa* L.) varieties, i.e., 'Zhong- Hua 11 (ZH-11)' of *japonica* subspecies, and 'Shuang-Ke-Zao (SKZ)' of *indica* subspecies, were irradiated by 60-Cobalt gamma rays with dosage of 300 ~ 350 Gy. About 700 morphological or physiological mutants were identified from ZH-11 and SKZ during growth and development. Besides, chemical mutagen N-methyl-N-nitroso urethane (NMU) has also been used to induce mutant of the *japonica* variety Nipponbare for functional genomics studies in CNRRI and other research institutes [4].

Isolated and characterized mutant lines

About 700 morphological and physiological mutant lines, 502 from ZH-11 and 172 from SKZ, respectively, were isolated and characterized in M4 and advanced generations and now deposited in the Germplasm Bank of CNRRI. According to the mutants isolated and conserved, the mutation frequency of ZH-11 and SKZ were 4.36% and 2.66%, respectively. The mutation ratio of stem, foliage, panicle and others were 18.7%, 36.9%, 30.3% and 14.1% statistically from ZH-11 sorted cursorily.

In M_2 generation from ZH-11, the chlorophyll deficiency mutation rate reached 8%; the sterility of mutation frequency was also as high as 6.65%. The frequency of other mutations, i.e., dwarfism, tillering, small or big grain, malformed and semi-dwarf mutant, etc, was around 1.78% [5]. Some mutated traits were reported for the first time, such as pith stem, broad leaf, and premature death, etc (Fig. 1).

Genetic analysis of mutations

The segregation ratio was recorded at various generations for all mutations. Some mutants were crossed to their respective parent varieties for genetic analysis. The lazy growth mutant ZM 2101 (Fig. 1e) is white in sheath, another independent mutated trait. ZM2101 was crossed to ZM 2102, another lazy growth mutant, its F_1 plant showed lazy growth habit but normal in sheath color. As expected, all plants of F_2 plants had the phenotype of lazy growth, but segregating in sheath color (1/4 plant in white sheath).

The majority of mutant phenotypes were conditioned by recessive genes, except for the premature death mutant ZM02-120, which develops a systematically death symptom 20 days after flowering. When ZM02-120 is crossed to normal growing varieties, half of its F_1 plants developed the mutant phenotype, indicating that the premature death trait was controlled by a dominant gene.

Fine mapping and cloning mutated genes

A couple of mutant genes induced above have been either mapped or cloned in CNRRI or in collaboration with Chinese Academy of Science (CAS) (Table 1). The first cloned gene was *bc-1* (brittle culm) induced from SKZ [6]. Other mutation genes have been mapped in different chromosomes of rice respectively.

Some lesion mimic mutants were obtained from SKZ and ZH-11 [7]. On the basis of initiation time of the lesion mimic phenotypes, these mutants can be divided into three categories: whole life lesion mimics, vegetative initiation lesion mimics, and reproductive initiation lesion mimics. These lesion mimic phenotypes were triggered by light, but not by wounding, and the light-mediated cell death signal was not spread to the unexposed leaf tissues. The genetic analyses of mutants Lrd39, Lrd40 and Lrd42 showed that they were controlled by one recessive gene, and Lrd39 and Lrd40 were controlled by different locus. Furthermore, Lrd37 and Lrd40 were proved to have enhanced, non-race-specific resistance to *Xanthomonas oryzae*, suggesting that Lrd37 and Lrd40 genes may be involved in defense signaling [8]. Genetic mapping and cloning of Lrd37 and Lrd40 are under way.

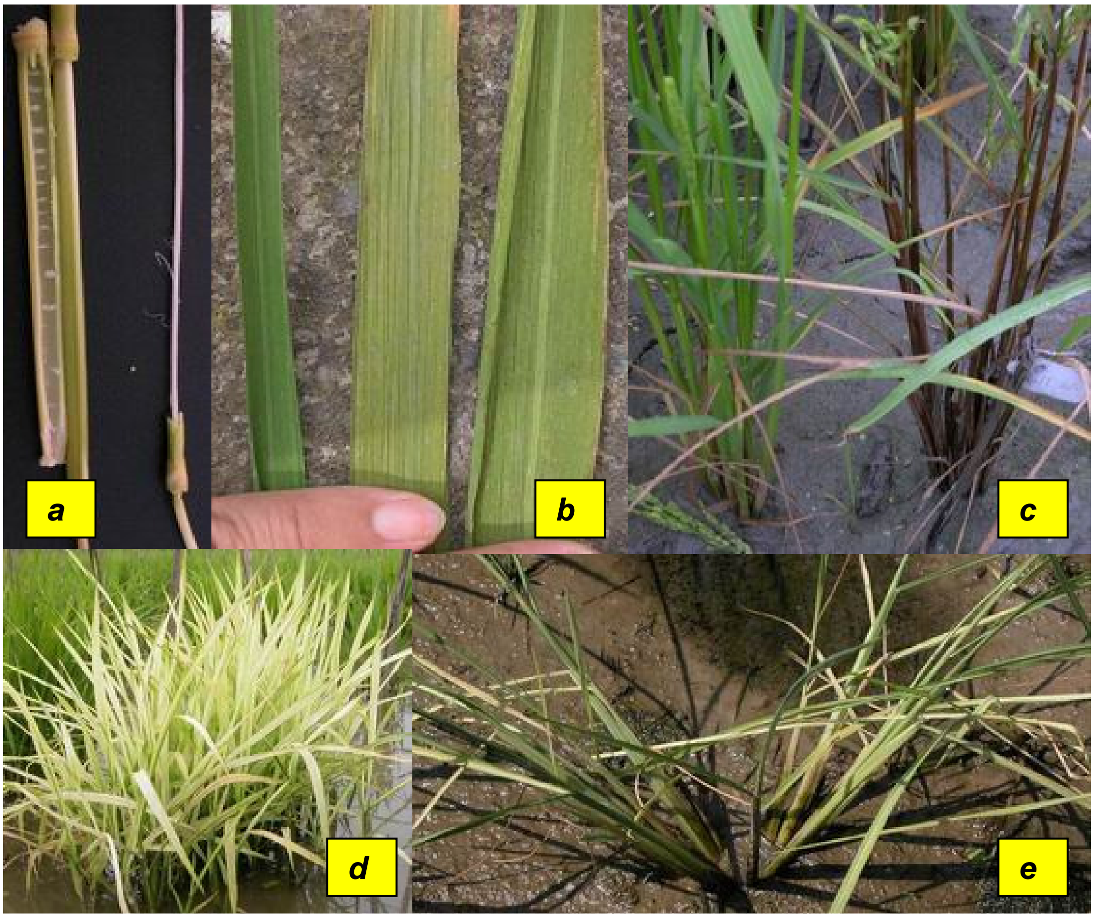


Fig.1. Unique mutants induced in rice. a: *pith stem mutant (right)and control (left);* b: *narrow (left) and broad leaf (right) mutant and normal control (central);* c: *pre-mature death mutant (right) and control;* d: *no-lethal white leaf mutant;* e: *lazy growth mutant white sheath.*

Table 1. Molecular mapping of mutated genes in induced rice mutants

Mutants	Gene	Chr.	Character
SKZ-Sui-jing	*pc*	10	pith culm (Fig. 1a)
ZH-Chuang-sui-jing	*eui*	5	elongation of upper-most internode
ZH-Cun-sui	*sp*	11	short panicle
ZH-Zhai-ye 1	*nl*-z1	12	narrow leaf
ZH-Zhai-ye 2	*nl*-z4	10	narrow leaf
ZH-Bai-hua-ye	*wl*	1	white leaf (non-lethal)
ZH-Lei-bai-ye-ku-ban	*spl*-z	2	spotted leaf (bacterial blight spot-like)
ZH-Mei-ke-hei-ban	*sph*	4	spotted hull (black spot on lemma and palea)
ZH-Xi-sui 456	*lax*-z1	4	lax panicle
ZH-Xi-sui D218	*lax*-z2	1	lax panicle

Use of Induced Mutations in Rice Improvement

Hybrid rice

Significant progress has recently been made in hybrid rice improvement using induced mutations. In China, hybrid rice occupies about 40% of the total rice area, mostly in southern rice zones. Currently, both three-line (cytoplasmic male sterile, CMS-based) and two-line (photoperiod sensitive genetic male sterile, PGMS-based) hybrid rice are grown. Nuclear techniques are frequently used in combination with anther culture and hybridization for the improvement of parent lines of hybrid rice.

Our group has already developed several hybrid rice parent lines with unique characteristics. Here are a few examples.

PGMS line A7s. A pale green leaf (*pgl*) gene is built into this line as a phenotypic marker, and a stigma extrusion rate as high as 90% was obtained via *in vitro* mutagenesis. The morphological recessive gene *pgl*, induced by gamma rays, serves as an efficient marker in seed and paddy production, as all true F_1 hybrid plant has normal green leaves, and true PGMS plants have pale green leaves, which enable easy visual differentiation of true and or false plant at seedling stage in both seed production and paddy production fields. In comparison with its parent line, A7s is also earlier in heading, which was also improved through *in vitro* mutagenesis. It is highly sterile under high temperature and long-day period, and partially fertile under suitable temperature with short-day period [9].

Late maturing restorer line T461X. T461X is a late mutant line, developed from T461, an elite wide compatible CMS R-line, and using gamma rays irradiation. The growth duration of T461X is about 10 days longer than parent T461 (Fig. 2). A new hybrid Xie-he 03-8 (Lu 91 A / T461X) was already developed, with a yield of 0.56 t/ha higher than dominant commercial hybrid rice Shanyou 63 (Table 2). It performed very well in field trials (Fig. 2).

Table 2. Comparison of T461 and T461X in maturity and yield*

Year	R-line	Maturity (d)	Hybrids	Maturity (d)	Yield (t/ha)
1996	T461	120	Xieqingzao A/ T 461	130	8.21
1996	MH63	128	Shan-you 63	134	8.43
2003	T461X	130	Lu 91 A / T461X	133	8.34
2003	MH63	129	Shan-you 63	126	7.78

*The data come from the results of Trial of Zhe-jiang 8812 Collaboration

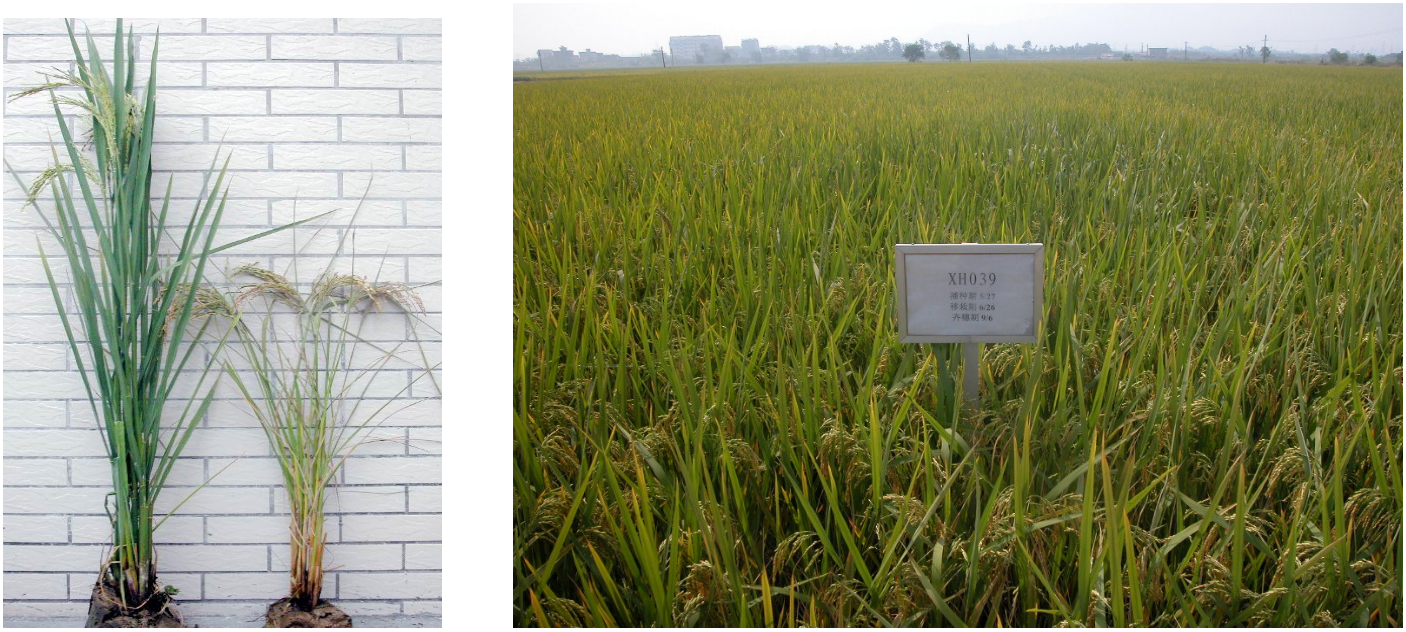


Fig.2. Left picture shows mutant R-line T461X and origin parent T461. Right one shows the demonstration of F_1 hybrids (Zhong 9A/T461X).

Enhancing abiotic stress tolerance

The abiotic stresses such as high temperature and drought have been threatening rice production. Using gamma-rays irradiation, we have developed a drought tolerant mutant line, NPB-dt. It can grow in upland fields, whereas the parent Nipponbare can only be planted in paddy fields. We have also induced several mutants, of which the water absorbing and water evaporating characters are changed, detailed analysis is being studied.

Other mutations

There are a few other mutations that may play an important role in rice improvement (Table 3). The mutant line Gh-2 has a mutation for the elongation of upper-most internode (*eui* gene) and thus is useful in hybrid rice seed production. The semi-dwarf gene (*sd*-z(t), a gene non-allelic to *sd*-1, in ZH-XHA may enable rice breeders to diversify the gene source in semidwarf breeding. In addition, the low phytic acid mutation in QZ-DZS could be of importance in enhancing nutritional value of rice grains. These mutations are either built in released new rice varieties, or are being used in various rice breeding programs (Table 3).

Several mutated genes were genetically analyzed, and mapped or cloned through the joint collaboration of CNRRI with Institute of Genetics & Developmental Biology, Institute of Plant Physiology & Ecology, CAS, and Institute of Nuclear Agricultural Science, Zhejiang University. For the time being, the nuclear technique has been useful in rice breeding, germplasm enhancement and in functional genomics studies in China.

Table 3. Rice mutants/genes in production and breeding of China

Mutants	Genes	Purpose	Released or in breeding
Gh-2, etc	*eui*	reducing the application of GA_3 in hybrid rice seed production	released
SKZ-CJ	*bc*-1	increasing culm availability after animal absorption	released
ZF-DLY	*pgl*	differentiating F_1 seeds and female parent seeds in two-line system	released
LTP-HY	*ygl*	differentiating F_1 seeds from female parent seeds in three-line system	breeding
XS-DP	*ge*	enhancing bio-availability and quantity of nutritive elements	breeding
XQZ-DZS	*hip*	decreasing the content of phytic acid in seeds	breeding
ZH-XHA	*sd*-z1	enhancing diversity of semi-dwarf gene in rice production	breeding

References

[1] Wu, D.X., Shu, Q.Y. and Xia, Y.W. A Review of Induction Breeding of Rice by Zhejiang University and Current Research Trends. *Acta Agriculturae Nucleatae Sinica*, 2000, 14(4): 251 ~ 254. (in Chinese with English Abstract).

[2] Zhu, X.D., Min, S.K. and Xiong, Z.M., Achievements of Rice Mutation Breeding in China. *Rice Review and Abstracts*, 1991, 10 (3): 1 ~ 4. (in Chinese with English Abstract).

[3] Xue, Y.B., Major State Basic Research Projects of China: Functional Genomics of Agronomic Traits in Rice. *Acta Botanica Sinica*, 2001, 43(7): 769 ~ 770. (in Chinese with English Abstract).

[4] Guo, L.B., Cheng, S.H., Qian, Q. Highlights in Sequencing and Analysis of Rice Genome. *Chinese Journal of Rice Science*, 2004, 18(6): 557 ~ 562. (in Chinese with English Abstract).

[5] Zhu, X.D., Chen, H.Q. and Luo, D. *et al.*, Screening and Characterization of Mutants Induced from Zhonghua 11 (*Oryza sativa* L. subsp. *japonica*) by Irradiation. *Chinese Journal of Rice Science*, 2003, 17 (3): 205 ~ 210. (in Chinese with English Abstract).

[6] Li, Y., Qian, Q. and Zhou, Y. *et al.*, BRETTLE CULM1, Which Encodes a COBRA-Like Protein, Affects the Mechanical Properties of Rice Plants. *Plant Cell*, 2003, 15: 2020 ~ 2031.

[7] Wang, J.J., Zhu, X.D. and Wang, L.Y. *et al.*, Genome-wide screening and physiologic resembling disease mutants (lrd) in *Oryza sativa* L. *Journal of Plant Physiology and Molecular Biology*, 2004, 30(3): 331 ~ 338. (in Chinese with English Abstract).

[8] Wang, J.J., Zhu, X.D. and Wang, L.Y. *et al.*, Disease Resistance and Cytological Analyses of Lesion Resembling Disease Mutant lrd40 in *Oryza sativa* L. *Chinese Journal of Rice Science,* 2005, 19(2): 111 ~ 116. (in Chinese with English Abstract).

[9] Zhu, X.D., Zeng, D.L. and Shi, H.M. *et al.*, Anther Culture and Inheritance of Dual-Purpose Genic Male Sterile Rice M2s with Morphological Marker. *Chinese Journal of Rice Science,* 2002, 16(2): 124 ~ 128. (in Chinese with English Abstract).

Genetic Improvement of Long Grain Aromatic Rices through Mutation Approach

Patnaik, D., Chaudhary, D. and Rao, G.J.N., Division of Crop Improvement, Central Rice Research Institute, Cuttack-753006, India

Abstract: In the export market, the price of basmati rice is two to three times higher than non-basmati rice**.** Traditional tall basmati variety Taroari Basmati, followed by Basmati 370 has supremacy over other basmati rice due to their exclusive quality. The breeding efforts of Basmati rices have resulted in the development of improved varieties, such as Pusa Basmati 1, which currently occupies a large area in the basmati zone. However, the variety has now become highly susceptible to blast, which is causing severe yield losses. The present investigation deals with the development of non-lodging, high yielding, long grain aromatic rices, employing a mutation approach of gamma rays (γ) irradiation. Mutant lines were developed from three elite basmati cultivars, i.e. Basmati 370, Pusa Basmati 1 and Pakistan Basmati. A mutant line derived from Basmati 370, CR 2007-1(IET 17276), is found to be highly promising in the basmati growing areas of Haryana and Punjab in the multi location trials. This promising mutant line consistently shows significant yield superiority over Pusa Basmati (5.36%) and Taroari Basmati (64.04%) and has all the desirable basmati grain quality traits that are closer to Taroari Basmati. It is also resistant to rice neck blast, moderately resistant to brown spot and also to biotype 1 of rice gall midge. The other mutants developed under the study have also shown higher yield potential than Pusa Basmati1, the highest yielding basmati cultivar that is being currently grown, but their quality characteristics could not reach the standard of Basmati rice.

Introduction

India has a rich genetic diversity of rice varieties reflecting the culmination of centuries of informal breeding and evolution by the farmers. India is also well known for its aromatic rices, which have a wide distribution of these special types of rices in almost all regions of the country. Among these varieties, Basmati rice is a connoisseur's delight and nature's gift to the Indian sub-continent [1]. It is cultivated on the foot hills of the Himalayas in the northwestern parts of the Indian sub-continent. It is nurtured by the waters flowing down from the snow peaked Himalayas bestowing unique fragrance, taste and texture that makes Basmati the best among the aromatic rices of the world [2].

Basmati rices are characterized by long, slender grains having a kernel length of 6.6 mm and more, length to breadth (IJB) ratio of 3 and above, and high kernel elongation rate (about 2) after cooking. The grains of Basmati cutivars are pointed at both ends, with gradual tapering at the end opposite to the germination end, and have uniform breadth between the taperings [3]. When cooked, its grains increase twice in length without an increase in breadth, do not split and remain non-sticky, soft and fluffy. In general Basmati varieties are photosensitive and thermo-insensitive and mature at low temperatures.

There are two types of Basmati rice varieties currently grown in India. One is classified as true (traditional) Basmati, and only six cultivars were identified, i.e., Basmati 370; Basmati 386; Type 3; Taroari Basmati (HBC 19); Basmati 217; and Ranbir Basmati (lET 11348). The other is recognized as evolved basmati varieties, and currently five varieties are listed in this group, i.e., Pusa Basmati (lET 10364); Punjab; Haryana; Kasturi (lET 8580); and Mahisugandha.

The classification of Basmati rices into traditional and evolved types however, has placed severe restrictions on the genetic improvement of Basmati rice. For example, Pusa Basmati 1 were improved for any character by using a non-aromatic genotype, the new improved version would not even qualify to be grouped as evolved Basmati. Developing an evolved Basmati rice variety on a par with is quite a challenging task [4]. Of the breeding options available, simple pure line selection and mutation breeding of traditional basmati rice varieties are left to meet the requirements of the seed act, for the limited low yielding varieties having Basmati's heritage qualities rule this segment of cultivation & trade. To meet the demands of a semi-dwarf, erect, non lodging type of Basmati cultivar coupled with high yield is highly needed in this agro ecological zone.

Materials and methods

Under a network program, "Improvement of Basmati rices for increased productivity and export purpose", breeding work was initiated at the Central Rice Research Institute, Cuttack during the early nineties to pursue the development of non lodging, semi dwarf basmati rices with higher productivity. Three popular aromatic cultivars i.e. Basmati 370, Pakistan Basmati and Pusa Basmati 1, were selected for the study, and mutation approach was adopted to achieve the objective. The treatments were done over a period of two years. Each population was followed separately and pooled data was presented for each cultivar. The main criteria for selection were non-lodging plant type, earliness, awnlessness and yield. All efforts were made to keep all the grain characters of basmati cultivars intact while selecting the single plants at each stage. The promising mutants were evaluated first in an observational yield trial and later in a replicated yield trial at the research station before submitting to All India Coordinated Rice Multi-location Yield Evaluation.

The selection process was initiated from M_2 generation and continued till M_6 generation and selections were made separately for wet season and dry season. This was done because the selections made in wet season from these aromatic genotypes behaved differently in the next dry season and in a similar way, the selections made in dry season behaved differently in the next wet season. As these parent cultivars tended to have irregular flowering in the dry season, panicle selections were made from the populations, and the next generation was grown on panicle to row basis for further evaluation and selection.

Results

As the objectives are clear, only the mutant selections that meet the requirements were advanced to the next generation. This has resulted in fewer mutants from the large populations grown after mutagen treatment from these three parent cultivars (Fig. 1). The performance of the selected mutant lines was detailed here.

Mutants of Basmati 370

Performance in all Indian multi-location trials

A mutant of Basmati 370, CR 2007-1(IET 17276) developed under the project was found to be promising in the basmati growing states of Haryana and Punjab by the Annual Rice Workshop of 2004. The superior performance of the mutant was noted after a multi location yield evaluation under the basmati category in the traditional basmati growing areas over a period of three years (Table 1-2). This mutant line consistently showed significant superiority in yield over Pusa Basmati (by 5.36%) and Taroari Basmati (by 64.04%).

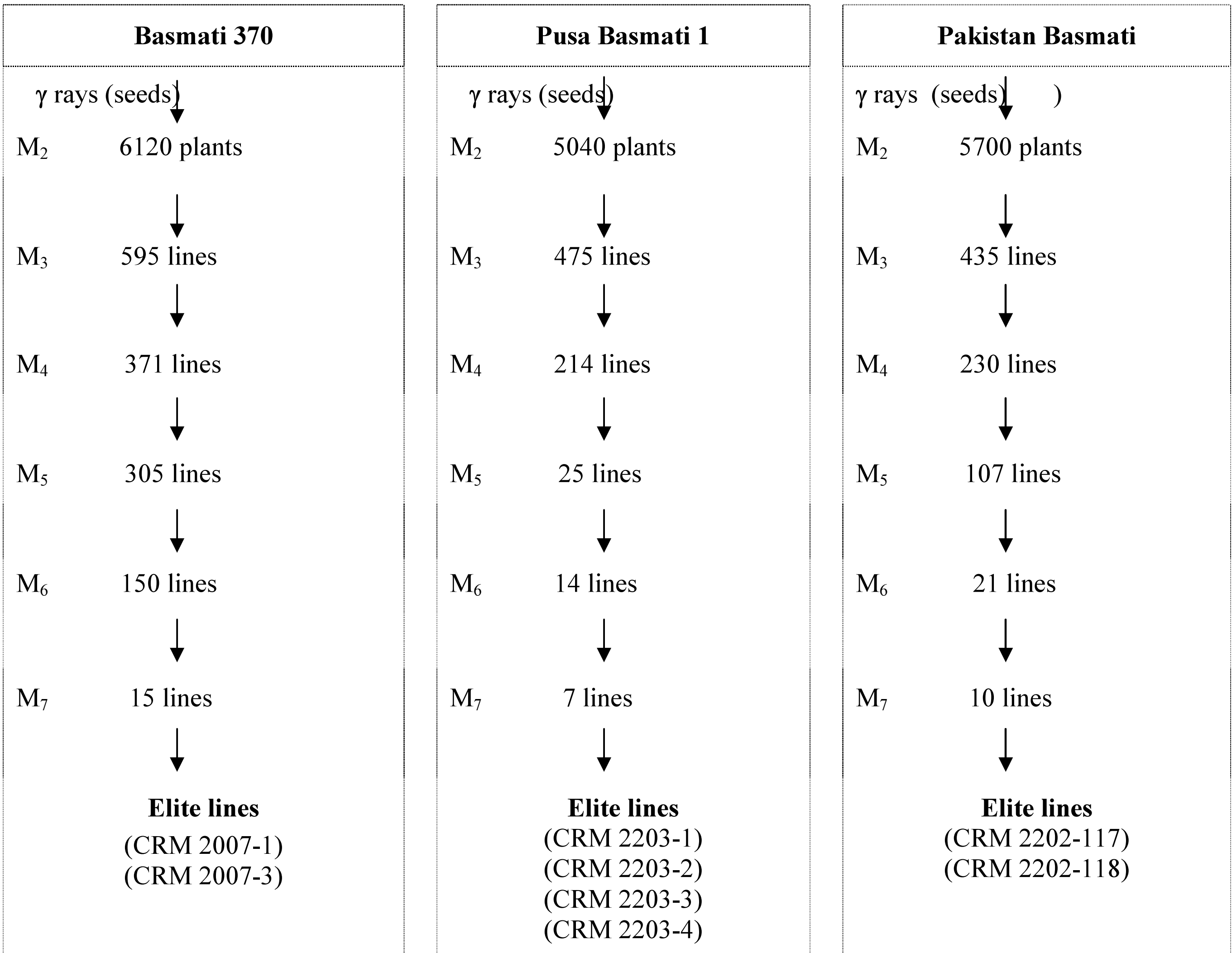


Fig.1. Flow chart on the development of promising mutant lines from three elite long grain aromatic cultivars through mutation induction of gamma rays (100, 150 and 200 Gy).

Table 1. Performance of CRM 2007-1 (IET 17276) in All India multi location yield evaluation trials over a three year period of testing

Mutant line	year	Grain yield (kg/ha)	Yield advantage (%) over controls	
			Pusa Basmati-1	Taroari Basmati
IET 17276 (CRM 2007-1)	2003	4485	5.36	64.04
	2002	3536	--	25.61
	2001	3358	8.57	31.07

(DRR reports: 2001- P.No.1.266: 2002 - P.No.1.286: 2003 P. No. 1. 260)

Table 2. Performance of CRM 2007-1 (IET 17276) in All India Co-ordinated trials of CRM 2007-1 during a three year period

Materials	Days to 50% flowering			Plant height (cm)			Number of panicles/m^2		
	2001	2002	2003	2001	2002	2003	2001	2002	2003
IET 17276	112	113	112	106	102	117	361	300	320
Pusa Basmati 1	113	113	112	110	104	110	299	321	295
Taroari Basmati	118	119	120	118	132	146	373	341	317

It has also all the desirable basmati grain quality traits, by means of both physico-chemical parameters (Table 3) and panel test (Table 4). In addition, it also showed resistant to rice neck blast and moderate resistance to brown spot and to biotype 1 of rice gall midge.

Table 3. Grain quality characters of CRM 2007-1 (IET 17276)

Materials	MILL (%)	HRR (%)	KL (mm)	KB (mm)	KLAC	L/B Ratio	ER	ASV	AC (%)	GC (mm)	Chalkiness
IET 17276	70.4	56.4	7.47	1.89	13.0	3.95	1.74	5.0	25.85	70.0	A
Pusa Basmati 1	68.7	57.3	7.62	1.76	14.1	4.32	1.85	7.0	24.35	61.0	VOC
Taroari Basmati	69.5	50.6	7.22	1.84	14.0	3.92	2.02	5.0	23.34	70.0	A

(DRR reports, 2003 p.1.262) VOC – very occasionally present

Table 4. Panel test scores of CRM 2007-1 (IET 17276)

Materials	Appearance	Cohesiveness	Tenderness on		Taste	Aroma	Elongation	Overall acceptability
			Touching	Chewing				
IET 17276	4.1	4.0	3.5	4.2	2.8	3.8	2.8	3.4
Pusa Basmati 1	4.2	4.2	4.1	4.5	3.6	3.8	3.8	4.0
Taroari Basmati	4.1	3.9	3.9	4.3	3.5	4.2	3.6	3.9

(DRR reports, 2003 p.1.265)

Performance in non-traditional basmati areas

The Performance of the mutant CRM 2007-1 was evaluated by testing it in non-traditional basmati areas in different parts of Orissa, the region which is situated in the Eastern region of India, a non traditional basmati area. The mutant performed well in some areas and has gained consumer acceptance as its rice fetches premium price in the local market, thus ensuring higher economic returns to the farmers (Table 5).

Table 5. Major agronomic performance of Basmati 370 and CRM 2007-1 in non-traditional basmati areas

Variety	Pl.ht (cm)	DFF	PBT	PL (gm)	Gr/P	P.Wt (gm)	Yield (kg/ha)	HI	1000 gr.wt (gm)
CRM 2007-1	119	92	302	29.4	116	3.2	3720	0.41	23.6
Basmati-370	135	104	344	27.3	91	2.6	2870	0.28	23.1

Summary Report on the evaluation of CRM 2007 in all India trials

IET 17276 (CRM 2007-1) a mutant of Basmati 370 nominated by CRRI is significantly superior to Pusa Basmati-1 by 5.36% and Taroari Basmati by 64.04% (1.7 tonnes) during Kharif, 2003. Quality wise IET 17276 is aromatic, possessing long slender (7.47 mm) grains without any chalkiness. It recorded 56.4% HRR. It has moderate KLAC around 13.0 mm at DRR during all years of the testing with ER being 1.74. It has typical cooking and eating quality traits as indicated by intermediate ASV and AC (25.85%) with soft GC (70 mm). In the panel test conducted during Kharif, 2003 it was rated as a desirable line on account of its aroma appearance, cohesiveness and tenderness in cooking. It has given consistently better yields over the check varieties in the states of Punjab and Haryana during Kharif, 2001 and 2002. – Promising in Punjab and Haryana of north Western Region 2. [DRR Ann. Pro. Rep. 2003, Vol 1-Varietal Improvement].

Other mutant lines

Another mutant derived from Basmati-370, CRM 2007-3 also performed well in the multi location trials under basmati category. It had out-yielded the control variety Pusa basmati 1 by 6.47% and Taroari Basmati by 29.40% on an overall mean basis computed over a six location data conducted in the traditional basmati areas. However, this mutant could not proceed further under basmati category because two of its grain characters, i.e. head rice recovery and kernel length after elongation, do not meet the standards set for basmati cultivars.

Performance of mutants of Pakistan Basmati

Two promising mutants i.e. CRM 2202-117 (IET 18007) and CRM 2202-118 (IET 18364) selected from the mutant populations were nominated for the multi location trials and the cultures performed well. The mutant CRM 2202-117 has shown excellent performance by yielding higher over both Pusa basmati 1 (14.91%) and Taroari Basmati (73.02%), the controls in the basmati category trial. Despite its superior performance, this mutant could not proceed further under basmati category because of three of its grain characters i.e. kernel length, head rice recovery and kernel length after elongation did not meet the standards set for basmati cultivars.

This happened to another mutant line, CRM 2202-118, which was evaluated for its yield potential under aromatic fine grain observational nursery (AFGON) trial. It had shown good yield potential but could not proceed further under short grain aromatic grain category

Performance of mutants of Pusa Basmati 1

Four promising mutants i.e. CRM 2203-1, CRM 2203-2, CRM 2203-3 and CRM 2203-4 were selected from the mutant populations of Pusa Basmati 1. The data on their agro morphological characters along with their yield potential is presented in Table 6.

Table 6. The different agro morphological characters of Pusa Basmati-1 mutant lines vis-à-vis the parent

Variety	Pl.ht (cm)	DFF	PBT	PL	Gr./P	P. Wt	Yield (kg/ha)	HI	1000 gr.wt
CRM 2203-1	118.0	118	387	31.5	155	3.7	5000	0.49	18.67
CRM 2203-2	115.0	110	306	29.6	156	3.6	4800	0.44	19.57
CRM 2203-3	114.0	112	332	30.0	185	3.8	4211	0.43	21.2
CRM 2003-4	114.2	118	354	31.0	166	3.6	5520	0.42	19.0
Pusa Basmati-1	111.6	96	308	32.8	97	2.5	3560	0.41	21.8

These mutants were also nominated for the multi location trials and all performed well. Among them, CRM 2203-1 (IET 18416), CRM 2203-3(IET18417), CRM 2203-4 (IET 18008), and CRM 2203-2 out-yielded Pusa Basmati 1 by 7.91%, 15.4%, 21.25%, and 7%, respectively. However, these mutants could not proceed further under basmati category because grain characters such as kernel length and kernel length after elongation of these mutants do not meet the standards set for basmati cultivars.

Two mutant lines, CRM 2203-4 and CRM 2202-117, were evaluated in non-traditional basmati areas (different districts of Orissa and West Bengal) for potential release as non-basmati rice in the two states of eastern region of India. The mutants had performed well in all the areas and gained consumer acceptance as the produce assured the farmers higher economic benefits (Table 7).

Table 7. Performance of aromatic mutants of Pusa Basmati-1 in non-traditional basmati areas in 2004

S. No.	Entry	Location	Yield (Kg/ha)
1.	CRM 2203-4	Bhadrak(Orissa)	3878
2.	CRM 2203-4	Puri(Orissa)	3220
3.	CRM 2203-4	Bhanjanagar(Orissa)	3947
4.	CRM 2203-4	Dinarjpur (West Bengal)	3100
5.	CRM 2202-117	Dinarjpur (West Bengal)	3127
6.	CRM 2207-117	Bhadrak(Orissa)	3789
7.	CRM 2202-117	Bhanjanagar(Orissa)	4440

Performance of grain quality characteristics of mutant lines

The grain quality characters of the mutants evaluated in the multi location trials were presented in Table 8. As can be seen from the table, the grain quality varied from mutant to mutant and the grain characters of CRM 2007-1 were found to be nearer to Taroari Basmati.

Table 8. Gain quality characters of the mutants of Basmati 370, Pusa Basmati 1 and Pakistan Basmati

IET No.	Mill (%)	HRR (%)	KL (mm)	KB (mm)	L/B Ratio	Grain Type	Grain Chalk	VER	WU (ml)	KLAC	ER	ASV	AC (%)	GC (mm)	Aroma
IET17276 CRM 2007-1	70.4	56.4	7.47	1.89	3.95	LS	A	4.9	225	13.0	1.74	5.0	25.85	70.0	MS
IET 18987 CRM 2007-3	68.9	33.2	7.20	1.78	4.04	LS	A	5.4	110	11.9	1.65	4.0	24.99	56.0	SS
IET 18416 CRM2203-1	73.8	53.5	6.62	1.79	3.69	LS	VOC	4.9	310	11.5	1.74	7.0	24.97	52.0	SS
IET 18988 CRM2203-2	72.9	42.3	6.60	1.72	3.83	LS	VOC	5.4	2.95	9.4	1.42	7.0	22.64	46.0	MS
IET 18417 CRM2203-3	73.1	49.5	6.63	1.76	3.76	LS	VOC	5.0	305	11.0	1.66	7.0	24.33	49.0	MS
IET 18008 CRM2203-4	73.0	69.6	6.31	1.75	3.61	LS	A	5.5	300	10.6	1.7	7.0	23.32	46.0	MS
IET 18007 CRM2202-117	67.0	40.3	5.95	1.77	3.36	SS	A	5.2	315	10.2	1.7	7.0	23.49	51.0	MS
IET 18364 CRM2202-118	63.0	42.1	6.25	1.76	3.55	LS	VOC	4.6	360	11.0	1.76	6.0	21.65	59.0	MS

Mill-Milling percentage (%), HRR -Head rice recovery (%), KL-Kernel length (mm), KB-Kernel Breadth (mm), LS- Long slender, SS- Short slender, A- absent, VOC- very occasionally present, VER- Volume expansion ratio, WU- Water uptake (ml), KLAC-Kernel length after cooking (mm), ER- Elongation ratio, ASV- Alkali Value, AC- Amylose content (%), GC- Gel consistency (mm), SS- Strong scent, MS-Mild Scent

Discussion

It is clear from the data that CRM 2007-1, a semi dwarf non-lodging mutant derived from Basmati 370 has the desirable quality characters (that are nearer the Taroari Basmati whose grains command a premium in the market) that meet the criteria required for export purposes. In yield wise, it has consistently outperformed Pusa Basmati 1, the cultivar that currently commands the largest area in the basmati growing regions. The advantages of CRM 2007-1 over Pusa Basmati 1 include yield and quality and can be taken as a true basmati, whereas PB 1 is classified under evolved basmati category. The consistent performance of CRM 2007- 1 in the basmati growing zone demonstrates that it is possible to employ mutation breeding to obtain high yielding mutants with desirable quality characters in the background of traditional basmati cultivars which have the potential to replace both the traditional and evolved basmati types that are currently being grown. As the international trade recognizes only the traditional basmati cultivars as true basmati, (as has been done by the European Union by waiving the entry duty to six traditional basmati cultivars), the mutant in the background of basmati 370 can also be considered as a traditional basmati and may find favour with the international market. The mutant also fits into the cropping system pattern of the basmati zone as its duration is similar to that of Pusa Basmati 1 and T. Basmati.

The other mutants generated under the program had the requisite yield potential, but could not be classified as basmati due to deficiency of in one/more quality characters that are preset for basmati. This implies that attention should be given to quality characteristics even using mutation techniques for rice improvement

Acknowledgments

The authors acknowledge the kind encouragement of Director, Central Rice Research Institute, Cuttack and Head, Division of Crop Improvement for extending the field and laboratory facilities. The authors are grateful to I.C.A.R. for the financial support in the form of network project grant.

References

[1] Thakrar, R. and Ahuja, S.C. Potential prospects for export of Basmati rice. In: Muralidharan, K. and Siddiq, E.A. New frontiers in rice research, DRR (ICAR), Hyderabad, India: 1990. 382-387.

[2] Mishra, B. Present status, issues and future strategies for increasing quality rice production and export. In: National Symposium on Strategies for enhancing export of quality rice- compilation of Contributory papers. DRR (ICAR), Hyderabad, India 2004. 1- 16.

[3] Mahindru, S.B. Manual of Basmati rice. Metropolitan Book Co. Pvt. Ltd., New Delhi, India 1995, 307p.

[4] Singh, V.P., Singh, A.K., Zaman, F.U. and Hari Prasad, A.S. Extra long slender grain aromatic rice: A new strategy. In: National Symposium on Strategies for enhancing export of quality rice- compila tion of Contributory papers. DRR (ICAR), Hyderabad, India 2004. 60-65.

Genetic Improvement of Brown – Planthopper (*Nilaparvata lugens*) Resistance through Radiation Technique in Rice

Pophaly[1], D.J., Gupta[1], A.K., Jambhulkar[1], S.J. and Awasthi[1], S.K., [1]Department of Entomology, I.G.A.U., Raipur (C.G.) India; [2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Center, Mumbai, India

The debate over genetic manipulation and its use in plant improvement for insect resistance has led to an increased demand for developing various alternative methods. Mutation induction for plant genetic improvement is one of the oldest but still relevant, economic and recognized methods. Mutations induced by radiation can alter the host parasite interaction by alteration relevant gene sequence, and thus enhance resistance to insect pests while keeping intact the original plant type [1].

Brown-planthopper (*Nilaparvata lugens*) is a major insect pest of rice in the region of Chhattisgarh State. Use of insecticide to control this insect is loosing its ground due to development of resurgence, insecticide resistance in insects and insecticide residue in crop plant at crop maturity stage. Varieties viz. Safri, Mahsuri, Dubraj and Mahamaya are most popular in this region, but are susceptible to this insect pest. Therefore, these four varieties were taken into consideration for genetic improvement through radiation techniques.

Seeds of these varieties were exposed to four doses of gamma rays viz. 150, 200, 250, & 300 Gy at Bhabha Atomic Research Center (BARC) Mumbai (M.S.) India. M_1 generations were raised in the field during 2003-2004 kharif in the experimental plot of Entomology Department, IGAU Raipur. The M_2 seeds of randomly harvested from 100 M_1 plants were tested using BPH larvae, which are throughout the year at temperature $\pm$ 28-30°C in glass house, as per the standard technique [2]. Survived seedlings from wooden screening boxes were repotted in earthen pots individually. M_3 seeds were harvested from these survived individual plants for further test.

A total of 3000 seedlings from 100 M_1 plants of each variety/treatment were evaluated in M_2 generation and 2 to 57 tentative resistant M_2 seedlings were identified for the three varieties (Table 1). In $M_{3,}$ 510,150 and 60 seedlings were grown from the seeds harvested from the resistant M_2 plants and screened for their resistance to BPH. Finally, 49, 21 and 17 seedlings were found resistant to

BPH in Safri -17, Mahsuri and Dubraj varieties, respectively (Table 1).

Except for variety Mahamaya, we identified resistant seedlings from progenies derived from plants treated with gamma rays. The segregation of resistant/susceptible seedlings in M_3 might indicate that the genetic control of BPH resistance is complicated in these lines, and the selected lines should be further tested in M_4 and advanced generations.

Acknowledgement

The financial support provided by BRNS- BARC, Mumbai, India is highly appreciated.

The authors are also grateful to Director Research IGAU, Raipur (C.G) India for providing all necessary facilities.

Table 1. Brown planthopper Rice Resistant Mutants

Variety	Treatments (Gy)	Generations					
		M_2 (2003-04)			M_3(2004-05)		
		Total seedlings tested	Reaction		Total seedlings tested	Reaction	
			R	S		R	S
Safri-17	150	3000	18	2982	150	13	137
	200	3000	18	2982	300	33	267
	250	3000	0	3000	0	---	---
	300	3000	8	2992	60	3	57
	Total	12000	44	11956	510	49	461
	Control	2400	0	2400	180	0	180
Mahsuri	150	3000	22	2978	120	19	101
	200	3000	9	2991	0	---	---
	250	3000	0	3000	0	---	---
	300	3000	26	2974	30	2	28
	Total	12000	57	11943	150	21	129
	Control	2400	0	2400	180	0	180
Dubraj	150	3000	0	3000	0	---	---
	200	3000	1	2999	30	6	24
	250	3000	0	3000	0	---	---
	300	3000	1	2999	30	11	19
	Total	12000	2	11998	60	17	43
	Control	2400	0	2400	120	0	120
Mahamaya	150	3000	0	0	0	---	---
	200	3000	0	0	0	---	---
	250	3000	0	0	0	---	---
	300	3000	0	0	0	---	---
	Total	12000	0	12000	0	---	---
	Control	2400	0	2400	0	---	---
G. Total of Seedlings (Excluding control)		48000	103	47897	720	87	633

R= Resistant S= Susceptible

References

[1] Favret, E.A., Franzone, P.M., Arias, M.C., Solari, R. and Saine, H. (1983). Disease reaction mutagenesis in barley and wheat. In: Induced Mutation for Disease Resistance in Crop Plants – II IAEA, Vienna 53.

[2] Kalode, M.B. and Krishna, T.S. (1979). Varietal resistance to brown planthopper in India. Brown planthopper: Threat to rice production in Asia.IRRI, LosBanos, Philippines 187-199.

A Significant Contribution of Mutation Techniques to Rice Breeding in Indonesia

Ismachin, M. and Sobrizal, National Nuclear Energy Agency of Indonesia, Center for the Application of Isotopes and Radiation Technologies, Jl. Cinere, Pasar Jumat, P.O. Box 7002 JKSKL, Jakarta 12070, Indonesia

Abstract: The use of mutation techniques in rice breeding was first introduced to Indonesia in 1966 by Dr. B.H. Siwi, after his fellowship training in Sweden awarded by the IAEA. However the first mutant rice variety, Atomita 1, was not officially released until 1982, though in-between several mutant lines performed very well in regional and national trials, with early maturity and high yield. The National Atomic Energy Agency (BATAN) is the only institute in the country that has the mission of research and development on mutation techniques for plant breeding. From 1982 to 2004, there have been 14 mutant rice varieties (11 lowland, 2 upland, and 1 tidal swamp rice variety) which were officially released in Indonesia, constituting more than 10% of the total number of released rice varieties during this time period. The number of variety itself cannot totally reflect the significant contribution of mutation techniques to the improvement of rice varieties in Indonesia; more importantly, are the improved traits in the new varieties, i.e. resistance to various biotypes of plant brown hoppers, tolerance to soil stresses (salinity, acidity, and high Fe concentration), in addition to other common traits such as early maturity (earlier than parent variety up to two months) and high yielding. Mutation techniques have proven particularly useful in traditional variety improvement, because no other techniques succeeded in improving their yield, disease resistance, or maturity, while keeping their quality characters unchanged. The breeding of a new mutant line, Pandanputri, and its superior performance to its parent variety is given as an example.

A brief history of rice breeding in Indonesia

Rice is the main staple food for most people in Indonesia, and is grown in lowland, upland, tidal swamp and swamp rice ecosystems. No breeding activities were reported before World War II, and farmers produced their own seeds for the next planting. After World War II, the Government initiated the official rice breeding programs, with the main objective to increase the yield potential, which is still valid until now. Before the IRRI's varieties were introduced and officially released, eighteen rice varieties were developed through cross breeding between the local varieties and germplasm introduced, mostly, from USA. These varieties were mostly of lowland type, maturing in 140 – 160 days, except Kartuna that matures in about 105 days. Seratus Malam[1] is the only upland rice variety that was officially released with the total growth duration of about 120 days. Those varieties can only be planted once per year, and are subject to lodging once high nitrogen fertilizer is applied.

IR5 and IR8 are the first IRRI's varieties that were released in Indonesia in 1967, which marked a new era in Indonesian rice production. From 1967 to 1981, 15 of the 35 rice varieties that were officially released were directly introduced from IRRI, while the others were developed through cross breeding by rice breeders in Indonesia (Table 1). Since the 1970's, IRRI's varieties have dominated rice production in Indonesia due to their high yielding potential and resistance to brown plant hopper (BPH). IR64 is still the largest rice plantation in Indonesia up till now. Pelita I/1 and Cisadane are the only two varieties that were bred by Indonesian breeders and grown as one of the leading varieties in Indonesia since 1970's.

Starting from the 1980's, the number of rice varieties that are directly introduced from IRRI and released in Indonesia has been gradually reduced, and all new varieties released after 2002 are developed in Indonesia. An important development in recent years is the rapid adoption of hybrid rice (Table 1).

Use of mutation techniques in rice breeding

Initiation of rice mutation breeding

The first commercial mutant variety in the world was produced in Indonesia. That was the tobacco mutant variety named "chlorina" bred by D. Tollenaar in 1929. He reported that a hybrid variety was developed by crossing the chlorina mutant to its parent line Kanari. In 1936, the hybrid already covered 10% of the total tobacco area in Central Jawa[3]. The mutation breeding activity was stopped due to World War II.

In 1966, at the Symposium on the Application of Radioisotopes held in Bandung, rice breeder Dr. B.H. Siwi reported his mutation work on rice after he got IAEA fellowship study in Sweden. Some work on mutation induction had been conducted in rice in the following years, but no mutant line performed well enough to be a new variety[4]. The publication of the Manual on Mutation Breeding (by the IAEA), and the IAEA/UNDP Project on the Use of Nuclear Techniques in Agriculture in the early 1970's, greatly pushed rice mutation breeding and is deemed as the turning point in crop mutation breeding in Indonesia.

The IAEA/UNDP project started in 1972, and rice mutation breeding activity was under the supervision of the IAEA expert, Dr. Knut Mikaelsen. The then new released rice variety "Pelita I/1" treated with gamma rays (0.2 and 0.3 kGy), and a wide spectrum of mutant plants, e.g., early maturity, semi dwarf, dwarf, "wheat" type, "lazy" type, many types of seed, viridis / chlorina, striata type, were isolated. Among them, three mutant lines with other promising desirable traits were submitted to the National Board for Releasing New Varieties in 1975. However, due to the outbreak of BPH (biotype 1, which caused a tremendous decrease in national rice yields in Indonesia in 1974), those three mutant lines failed to be approved by the Board, because BPH resistance was introduced as a necessary trait for any new rice variety to be officially released, which is still valid till today.

A screening lab for BPH resistance was established in BATAN in 1976, and seeds of Pelita I/1 were irradiated again with gamma rays at a dosage of 0.2 kGy. M_2 seedlings were first screened for BPH resistance before the selection of any mutants. The resistant seedlings were transplanted into rice fields, followed with selections of mutants with desirable morphological and agronomical traits in advanced generations. Since then, this protocol has been adopted as a standard procedure in rice mutation breeding program of BATAN.

Officially released mutant rice varieties

In 1982, the new variety "Atomia 1" was officially released, and became the first mutant rice variety in Indonesia (Table 2). Since then, there are a total of 14 mutant rice varieties, which have been officially released in Indonesia.

Mutant traits

It is interesting to note that there are always several traits that were simultaneously improved in the new varieties (Table 3). Indeed, only the improved traits were listed in table 2, other characteristics that may reduce the value of a variety could also be present. Lines with improved as well as detrimental traits were failed to be released as a variety, but lines with a minor "imperfect" may still be released. For example the mutant varieties Danau Atas and Situgintung, both induced from variety Seratus Malam, which has strong aromatic when its rice is cooked, are non-aromatic. Therefore, a large population is needed for the successful selection of mutants with desired target traits and minimum changes of desired characters of the parent variety.

Based on our experience, mutation techniques have the following advantages in rice improvement: (1) Almost all characters could be improved as long as they exist in nature variations; (2) Both allelic and non-allelic mutations to those already known in the germplasm collection could be induced. The dwarf character is well known and controlled by one single recessive gene: *sd*1 (semi-dwarf) allele in cv. Dee-geo-woo-gen (DGWG, and is now widely presented in modern rice varieties, while many new dwarf mutants are induced with genes known to be non-allelic to DGWG[5]. Another study also showed that the resistance to BPH biotype 2 that is non-allelic ASD-7 and Rathu Heenati could also be induced[6]; (3) Mutation techniques are useful particularly for the improvement of traditional varieties for certain traits that could not be improved using cross breeding; details will be discussed in next section.

Contribution of mutant varieties and future perspectives in rice improvement

Contribution of mutant varieties

Rice breeding could be divided into 4 periods marked with the introduction of new techniques in Indonesia. The introduction of IRRI's new variety is the end of the first period (1940–1966); and the successful use of mutation techniques marked the transition to a new period; while the rapid adoption of hybrid rice pushed rice breeding into a new stage (Table 1). Mutant variety is not only developed for lowland but also for highland and tidal-swamp rice production. Although BATAN is the only institute that is engaged in rice mutation breeding, mutant variety has contributed more than 10% of the total rice varieties after 1982, and its stake has been increasing during the past 5 years (Table 1, 2).

The induced mutant traits, i.e., resistance to BPH, and tolerance to soil stresses, are critical for rice production in certain areas, including high iron content in soil, where no other suitable varieties are available. One remarkable mutant trait frequently obtained in mutant variety is early maturity. The mutant varieties, i.e., Atomita 1 to Atomita 4, usually had significant shorter growth period, but performed better than or similar to their respective parents in other traits including yield, because otherwise they could not have been officially released. Therefore, the growing of these mutant varieties could always bring additional income to farmers.

Table 2. Rice varieties developed using mutation techniques in Indonesia

No.	Name	Year	Parent & treatment**	Improved character(s)
1	Atomita 1	1982	Pelita I/1; 0.2 kGy	Resistance to BPH biotype1 and GLH; early maturity.
2	Atomita 2	1983	Pelita I/1; 0.2 kGy	Resistance to BPH biotype1, tolerance to saline soil; early maturity.
3	Danau Atas*	1988	Seratus Malam*; 0.2 kGy	Tolerance to blast and low pH soil.
4	Atomita 3	1990	Mutant line No. 627/10-3/PsJ; 0.2 kGy	Resistance to BPH biotype1 and biotype 2; early maturity.
5	Atomita 4	1991	Cisadane; 0.2 kGy	Early maturity, tolerance to high Fe soil (very bad drainage soil).
6	Situgintung*	1992	Seratus Malam*; 0.2 kGy	Resistance to BPH biotype 1; mediate resistance to BPH biotype 2.
7	Cilosari	1996	Mutant line of Seratus Malam (SM 268/PsJ) x IR36.	Tolerance to BLB.
8	Meraoke	2001	F_1 seeds (Atomi-ta 4 x IR64); 0.2 kGy	Slender seed, tolerance to BLB IV, early maturity.
9	Woyla	2001	F_1 seeds (Atomi-ta 2 x IR64); 0.2 kGy	Slender seed, tolerance to BLB IV, early maturity.
10	Kahayan	2003	F_1 seeds (Atomi-ta 4 x IR64); 0.2 kGy	Tolerance to BLB IV
11	Winongo	2003	F_1 seeds (Atomi-ta 3 x IR64); 0.2 kGy	Big slender seed, tolerance to BLB IV.
12	Diah Suci	2003	F_1 seeds (Cilosa-ri x IR74); 0.2 kGy	Slender seed, tolerance to BLB IV
13	Mayang	2004	F_1 seeds (Cilosa-ri x IR74); 0.2 kGy	Big and slender seed, tolerance to BLB IV
14	Yuwono	2004	IR64; 0.1 kGy	Tolerance to BLB IV

*Upland rice, others without * are lowland rice
**all with ^{60}Co gamma rays

Use of mutation techniques for traditional varieties improvement

Some local traditional rice varieties are preferred in certain areas because of their quality of excellence, but are either susceptible to biotic stresses, or are very late in maturity, and consequently the yield of those varieties is very low and instable. Attempts to improve these defects through cross breeding have failed due to the inability to keep the desired quality preference. In the past years, we have been using mutation techniques to tackle this issue, and this has proven to be very promising. An example is given below.

Pandanwangi is a very famous local rice variety at Cianjur district, with excellent aroma and taste. The price of Pandanwangi milled rice is almost 3 times that of IR64 price. It is javanica rice, very late in maturity (almost 6 months) and is susceptible to BPH. Mutation techniques were used to develop early mutant lines of Pandanwangi, using the BPH screening protocol as stated above. The M_2 seedlings that survived from BPH (biotype 1) screening were transplanted into a rice field nursery, and early maturing plants with similar morphological traits Pandanwangi were selected and further evaluated in M_3 and M_4 generations. A couple of mutant lines that matured in about one month earlier were selected. However, they are still not early enough, therefore, the earliest mutant (mutant line no. PW1/PsJ) was subject to another round of treatment and selection using the same procedure as in the first round.

Four mutant lines, maturing in around 120 – 130 days, were selected and have been tested in Cianjur District and other areas since 2002. The best performing line, the “new Pandanwangi”, is now named as “Pandanputri”. Although maturing almost two months earlier than its parent, Pandanputri produces rice with the same excellent quality as Pandanwangi. In farmers’ demo-plot production, Pandanputri showed resistance to BPH, with similar maturity to other improved varieties, such as IR64. In field tests in other regions, it was reported that Pandanputri is not only matures earlier than Pandanwangi, but also is tolerant to drought and has wider adaptability. In many areas that are not suitable for Pandanwangi production, Pandanputri can grow very well. The yield is very good for a local variety. Pandanputri yields on average about 7-tons/ha of rough rice, still lower in comparison to that of other new national varieties, i.e. Ciherang (about 8 tons/ha), but the price of Pandanputri seeds is about 85% higher than Ciherang. The success of Pandanputri influenced other districts that have local rice varieties with high economical value. BATAN was already asked to do the same for their varieties. BATAN is now working on improving local rice varieties from South and West Sumatera provinces, South Sulawesi province, and East Nusa Tenggara. The preliminary results achieved so far are very promising.

Future perspectives

In Indonesia, the use of mutation techniques has been focusing on new variety development through improving characters of direct importance for a new variety, i.e., early maturity, tolerance to biotic and abiotic stresses. Little work on genetic and molecular analysis of induced mutation has been done, and many mutant lines, although owning desirable mutant traits, were discarded because they are not good enough to be a new variety due to one or more undesirable traits. Therefore, many new mutant genes /alleles of important characteristics may have already been disappeared.

In the future, more attention should be given to other "invisible" characters, such as quality related seed traits and tolerance to adverse soil and climate conditions. Also, new techniques, such as molecular marker techniques, should be introduced into projects for exploitation of mutant genes in complicated breeding programs. Last but not least, conservation of newly induced mutant germplasm and more detailed studies on mutant genes should be carried out using conventional and advanced techniques. The coordination and assistance of FAO/IAEA on this activity is urgently needed.

Acknowledgement

The authors wish to express sincerely thanks to the IAEA/RCA for its long time supports on our rice mutation breeding programs and for my attendance at this meeting. We also wish to thank Dr. Hitoshi Nakagawa and Ms. Mari Miura for their kind hospitality and help during the meeting. We are also indebted to Dr. Qingyao Shu for the improvement of our manuscript.

References

[1] Directorate of Seed Production, Department of Agriculture. Data of food crops seed production, 2002. (In Indonesian).

[2] Lesmana, O.S., Toha, H.M., Irsal Las, Suprihatno, B. Description of new released rice varieties. Indonesian Institute for Rice Research, 2004: 45-49. (In Indonesian)

[3] Tollenaar, D. Untersuchungen uber Mutation bei Tabak I. Entstehungeweise und Wesen kunstlich erzeugter Gen-Mutaten. Genetica 1934, 16: 111-152. *In*: Harten, A.M. Mutation Breeding. Theory and practical applications. Cambridge Univ. Press, 1998. 53.

[4] Siwi, B.H. Effect of gamma radiation (Co^{60}) on some rice-varieties in Indonesia. 1st Symposium on the Application of Radioisotopes, Bandung. 1973, 226-233. (In Indonesian).

[5] Clement, G. and Poisson, C. Genetic determinants of semi-dwarf characters induced in Oryza sativa for upland rice cultivations. Mutation Breeding Newsletter. 1988, 31: 5-6.

[6] Mugiono. Inheritance of resistant characters to BPH in several rice mutant lines. Doctor thesis. Yogyakarta: Gajah Mada Univ. 1990. (In Indonesian with English abstract).

Use of Induced Mutants in Rice Breeding in Japan

Amano, E., Research Center for Bioresources Development, Faculty of Biotechnology, Fukui Prefectural University, 88-1, Futaomote, Awara-city, Fukui-ken, Japan

Abstract: Recent trends of rice breeding are reviewed with special reference to the use of artificially induced mutants. The present review discusses the novel type of mutants such as giant embryo, decreased amylose content for more sticky cooked rice and decreased protein content. All of these novel mutants had their own importance. The giant embryo mutant contained more plant oils than the wild type, and half germinated brown rice contains more GABA than those of the normal rice. The mutant with low amylose content is likely to improve the rice quality in Hokkaido, the most northern region, comparable in stickiness to other regions in Japan. In general, the objectives of the rice breeding programs including mutation breeding were to increase the protein content in the rice grain, which was also the case once in Japan. However, the induction of mutants with low protein was specifically useful for kidney troubled patients who need low levels of protein. This mutant contained a low glute-

lin level and the mutant gene showed dominant inheritance, due to RNA interference in the post transcript stage of the gene action. It also affected other very close gene families located on other chromosomes. This review also deals with retro-transposons which might be activated by callus culture, and a new physical mutagen, high LET ion beams, which is currently available for plant mutation works in Japan. The retro-transposons may throw some light on the problems of elongated callus culture, while the latter may open up a new phase of mutation breeding.

Introduction

Rice breeding was a long process, even with the use of short cut techniques like mutagenesis. Achievements of mutation breeding in rice in Japan had been reported in detail (including pedigree charts) elsewhere (Kawai and Amano 1991). Recently, general crop mutation breeding in Japan was reviewed in the Gamma-Field Symposia (Yamaguchi 2001). This present review restricts itself to presenting the latest trends in mutation breeding in rice with special reference to Japan.

Resistance to lodging

Japan is often affected by typhoons. Though the typhoons bring necessary water for living and cultivation, they also cause lodging of crops. Lodging of rice plants was a serious problem with some cultivars, especially highly valued ones like Koshihikari. This variety had survived over 50 years in Japan due to its exceptional high qualities and still continues to remain as a top variety in Japan. In the research efforts to develop a lodging resistant Koshihikari, the Fukui Agric. Exp. Station recently developed and registered a new cultivar, "Ikuhikari", which was developed through introduction of the sd_1 mutant gene of Reimei into Koshihikari.

In Japan, mutation breeding of rice has dealt with the lodging problem for some time. Due to these efforts, in 1966, "Reimei" had been registered as Norin 177. It was a semi-dwarf and a non-lodging mutant variety developed from the cold tolerant cultivar, Fujiminori. The word "Reimei" means dawn or daybreak, suggesting the opening of a new breeding method. In Japan, Reimei was a highly successful cultivar and it had been used in many crosses that resulted in the development of 60 varieties (Yamaguchi 2001), and the number is still increasing. Induction of lodging resistance was one of the most effective applications of mutagenesis in crops, as proved by the development of Calrose76 which was registered and released in the USA ten years later.

The success of the mutant variety "Reimei" strongly stimulated the mutation breeding activities in many other crop plants, and the activity was well supported by irradiation services and the information exchange through the annual Gamma Field Symposia which continued for more than 40 years.

The Pivot Institution

The Institute of Radiation Breeding, (IRB), located at Ohmiya, Ibaraki, a section of the National Institute of Agrobiological Sciences, served as the pivot Institution in these activities. The present radiation source of the Gamma Field of this Institute is ^{60}Co, 88.8TBq (2,400Ci) which will decay to half by every five years. Because in this gamma field, arboreal crops can not be moved, to maintain the constant radiation intensity, every two years the radiation source is renewed to the original level. The Gamma Field is completely circular shaped having a radius of 100m and is surrounded by an eight meter high shielding bank to shield off the direct gamma ray beam from the radiation source. One of the significant outputs from this Institute's irradiation facility is the development of a mutant cultivar "Gold-Nijisseiki", that was a clone of Japanese pear resistant against the Black Spot disease. It has other radiation sources and actually it is the pivot Institution in mutation breeding in Japan.

Giant embryo mutant

Together with waxy, sugary, and flowery mutants, the giant embryo mutant was relatively easy to detect in the M_2 rice grains on M_1 panicles. In other words, it could be detected in the same year after mutagen treatment, using a simple dehulling work of the M_2 seeds followed by visual screening. In most of the cases, the size of the giant embryo often reached to one half of the rice grain (japonica short grain). Enlarged embryo might contain more plant oils than normal wild type. The half way germinated grain of the giant embryo mutant contained much more GABA (gamma amino butyric acid) and one mutant line was officially registered in Japan as "Haiminori". Half way germinated brown rice is now commercially available in many markets in Japan.

Low amylose content

A conventionally bred rice variety, Koshihikari, which was registered in 1956, had enjoyed high consumer preference for more than 50 years and is still extending its exceptionally long cultivar life. But Koshihikari easily suffers from lodging and is susceptible to rice blast, and only through effective field management can good harvests be obtained. But the quality of the cooked rice attracted consumers because of its good taste, both in warm or cold conditions. In 1997, a surveillance report showed that, of the 45 provinces in Japan, "Koshihikari" was planted in 35 provinces as a top variety, suggesting its high adaptability in entire Japan. Based on the analysis by the breeders on its exceptional long life, the stickiness of the cooked rice seemed to be the most important factor that attracted the consumer's attention. There might be many other factors, but low amylose content was easy to alter by mutagenesis without modifying other genes of the target variety.

The first low amylose mutant variety was developed in the northern region of Japan, Hokkaido (Kikuchi 1994), where amylose content tends to increase compared to rice grown in other areas of Japan. Increased amylose reduced the stickiness of cooked rice which was not appreciated by Japanese consumers. A mutant line NM391, a mutant of Nihonmasari, was crossed to a Hokkaido variety, Ishikari, as there was a need for a very early maturing variety in the northern region of Hokkaido. Cultivars like "Aya", "Asatsuyu", "Hanabusa", and "Ayahime" were registered and released in the region and all had better taste of cooked rice.

Intermediate amylose content of about 15 to 17% seemed to be appropriate in the rice varieties. For this condition, dull series (du_1, du_2 *etc*) low amylose content seemed to be appropriate (Dung *et al*, 2000; Yano *et al*, 1988). A mutant, 74wx2N1, induced by EMS, was also used to develop two rice varieties, "Snow-Pearl" (Higashi *et al* 1999) and "Takitate". The two mutants, NM391 and 74wx2N, were provided by the Institute of Radiation Breeding. A missense or leaky mutation in the waxy locus usually leads to much lower amylose content (~ 5 to 10%), however, "Akane-Fuji" was developed from a leaky waxy mutant 74wx8N1 (Amano 1985). Stimulated by the success, and also in response to the demand of sticky rice varieties, four National Agricultural Experiment Stations used either low amylose mutants in cross breeding programs or induced new low amylose mutant lines by themselves, which resulted in the development of cultivars like "Soft-158", "Milky-Queen", "Yawara-Komachi", "Silky-Pearl" and "Milky-Princess". By November, 2004, the list of low amylose varieties reached 15, including several new local varieties, thus marking up a clear trend in rice breeding for low amylose content.

Low protein variety

Another significant trend in Japan in rice mutation breeding is to lower the protein content, which to some extend is against the world trend. Increased protein content has been one of the major breeding objectives in the world and some positive results have been reported in Japan (Tanaka, personal Comm.). However, with easy access to fish, the need for high protein content in food materials, especially in rice, declined. Instead, recent research was focused on breeding for lower protein content in rice for kidney troubled patients. In rice, there are two types of protein bodies or particles to accommodate proteins in the rice grain. One of them can be digested by human digestive organs, but the other cannot. Thus the change of harboring location of proteins may change the use by human body, thus reducing the uptake of excess protein by kidney troubled patients (Cf. Nature vol.423 (2003) p390). One of the mutant lines seemed to fit the purpose. Screening of NM67 was done at the IRB laboratory for detecting the types of changes in the major protein profiles. The task was laborious, as the work involved the crushing of rice grains separately and solubilizing each grain, running electrophoresis to separate protein components followed by staining the components rather than the well established procedure of using the micro Kjeldahl apparatus system. The mutant NM67 had a significantly lower level of glutelin by shifting the major protein content to prolamin which may not be digested by humans. The mutant line NM67 was back crossed to Nihon-Masari and was registered as "LGC-1" (Iida *et al* 1993). This mutant character was controlled by a dominant gene, an unlikely event in induced mutation in rice. Details of this mutant were reported in this Work-shop (Kusaba and Nishimura 2004 Abstract p102). Mutant gene is generally inactivation of the gene by disturbed base sequence. Therefore, usually mutant gene acts as a single recessive gene. If the normal/dominant gene was not powerful enough, the heterozygote might show gene dosage effect expressing intermediate phenotype in a F_1 hybrid, as shown in the case of complete deletion mutant (Yatou 1991, Yatou and Amano 1991) of a japonica waxy gene line, 75KURwx4. However, in the case of LGC-1, the mutant gene acts as a dominant mutant, perhaps due to the function of RNAs in the gene expression procedures (Kusaba *et al* 2003). Though the objective of lowering protein content in rice grains might be radically different from the objectives of the breeding programs for protein content in the world, the program has its own merits for lowering of the proteins for kidney patients. Lowering of rice proteins can be attained industrially by enzymatic treatment but it was much more costly. The other approach is the transgenic approach, which can be achieved through suppression of a specific protein by transformation (introduction of antisense sequence DNA). This might be effective, but the GM technology may not be of consumers' preference in Japan.

Consumers in Japan were somewhat nervous about the GM crops and local governments were reluctant to accept the planting of transgenic crops. However, the mutant derived cultivars gained acceptance by the people from its early stage, indicating that no problems were associated with the mutant varieties both for their cultivation and adoption.

New mutagenesis methodologies

Biological mutagenesis

Following the physical (ionizing radiation and UV) and chemical (e.g. EMS: ethyl methanesulfonate) mutagens, recent studies had indicated mutagenesis of ordinary plant species through biological procedures (Hirochika 1995, '96, '97). During plant tissue culture, some variant plants had been noticed as somaclonal variation. In ordinary plant tissue culture, that was designed to produce true type plants like the original parent, suppression of unwanted somaclonal variants was a necessity. But Hirochika (1995, '96, and '97) reported that callus culture seems to activate retrotransposons. When activated, they produce mRNA and the product will be reversed to a transposable DNA by a reverse transcriptase. The produced DNA will seek the transpose on a chromosome and then produce insertion mutation. The complicated procedures and product mutant gene should be studied further, but in many cases, the mutant gene was expected to have a very convenient tag with the mutant gene. After chemical and physical mutageneis systems, somaclonal variation can be treated as the third mutagenesis system, and can be be refered to as Biological Mutagenesis.

Maize transposons, e.g. *Ac* and *Ds*, introduced in other plant species, may be translocated, automatically or by the help of *Ac*. Such mutants might be unstable and not useful for practical breeding purposes. However, in case of retrotransposon activation, the original retrotransposon might be located at the original position for a long time, and even after the activation, produce a unique electrophoresis pattern. The newly induced mutant gene might also be very stable until next callus formation, and the mutant might be used in plant breeding as a stable mutant.

The rice genome sequencing had been completed, however, all the mutants, like chlorophyll deficient mutants, disease susceptible plants that are being regularly found in the mutated populations, had become invaluable materials for mapping and tagging purposes. While most mutants follow the Mendelian segregation pattern, some mutants such as rolled leaf phenotype showed strange segregation ratios, e.g. all sib-plants of the family showed mutant phenotype. If it was a dominant mutation, the segregation might be 3 rolled leaves and 1 normal, still segregating some normal plants. To explain such inheritance, other phenomena like methylation of the gene DNA were being considered. Studies should be continued further.

Ion-beam irradiation

In addition to the retrotransposon activation by callus induction and culture, recently ion-beams from accelerators in Japan could be used for biological materials as a mutation inducing agent. In the 1960's and 1970's, radiation biology presented various factors and effects of high LET radiation, followed by a period of some use of American accelerators in biology. But the use of the foreign accelerators was almost limited to scientific studies. Recently, irradiation with accelerated ions was not limited for academic studies, but also was extended for practical mutagenesis in many ornamental plants. Application to rice plant/seeds was still in the initial stage with promising results. Currently, three accelerators are in operation for plant materials, two in Kanto (near Tokyo), Riken and Takasaki (JAERI), and one in Fukui (near Kyoto).

References

Kawai, T. and E. Amano (1991) Mutation Breeding in Japan. Proc. Int'l Symp. IAEA vol. 1 pp 47-66

Yamaguchi, I. (2001) Forty Years of Mutation Breeding in Japan Research and Fruits. Gamma Field Symposia, 40: 1-11

Kikuchi, H. (1994) Improving the eating quality of rice using low amylase mutants. Gamma Field Symposia, 33: 1- 18

Dung, L., Mikami, I., Amano, E. and Sano, Y. (2000) Study on the Response of *dull endosperm 2-2, du2-2* to Two *Wx* Alleles in Rice. Breeding Science, 50: 215-219.

Yano, M., Okuno, K., Satoh, H. and Omura, T. (1988) Chromosomal location of genes conditioning low amylase content of endosperm starches in rice *Oryza sativa.* Theor. Appl Genet.76:183-189.

Higashi, T., Saitou, S., Takita, T., Yamaguchi, M., Sunohara, Y., Yokogami, N., Ikeda, R., Tamura, Y., Oyamada, Z., Kowata, H., Inoue, M., Matsumoto, S. and Kataoka, T. (1999) Breeding of a new rice cultivar withlow Amylose Content "Snow pearl". Bull Tohoku Natl. Agric. Exp. Stn 95: 1-12 (in Japanese with English abstract).

Amano, E. (1985) Genetic fine structure of induced mutant gene in cereals. Gamma-Field Symposia No. 24 81-96.

Sunridge, C. Cereal Adultery Nature vol.423 (2003) 390.

Iida, S., Takano, T., Tsubaki, K., Ikezawa, Z. and Nishio, T. (1993) Evaluation of a Rice Mutant as a Material of Hypoallergenic Rice. Japan J. Breed. 43: 389-394.

Kusaba, M., Miyahara, K., Iida, S., Fukuoka, H., Takano, T., Sassa, H., Nishimura, M.and Nishio, T. (2003) Low gluterin content 1: A dominant mutation that suppresses the glutelin multigene family via RNA silencing in rice. The Plant Cell 15: 1455-1467.

Yatou, O. (1991) DNA Modification of mutant genes. Gamma Field Symposia 30: 33-42.

Yatou, O., and Amano, E. (1991) DNA Structure of Mutant Genes in the *waxy* Locus in Rice. Proc. Int'l Symp. IAEA vol. 2 pp 385-389.

Hirochika, H. (1995) Regulation of plant retrotransposons and their use for genome analysis. Gamma Field Symposia 34: 77-91.

Hirochika, H., Sugimoto, K., Otsuki, Y., Tsugawa, H. and Kanda, M. (1996) Retrotransposons of rice involved in mutations induced by tissue culture. Proc N. A. S. 93: 7783-7788.

Hirochika, H. (1997) Retrotransposons of rice: their regulation and use for genome analysis. Plant Molecular Biology. 35: 231-240.

Iida, S. (1995) Screening and Genetic Analysis of Rice Seed Storage Protein Mutants. Gamma Field Symposia. 34: 31-48.

Effect of Carbon-ion Beams Irradiation on Mutation Induction in Rice

Yamaguchi[1], H., Morishita[1], T., Degi[1], K., Tanaka[2], A., Shikazono[2], N. and Hase[2], Y., [1]Institute of Radiation Breeding, National Institute of Agrobiological Science, 2425 Kamimurata, Hitachi-Ohmiya, Ibaraki 319-2293, Japan; [2]Department of Ion-Beam-Applied Biology, Japan Atomic Energy Research Institute, 1233 Watanuki-mashi, Takasaki, Gunma 370-1292, Japan

Abstract: The effect of ion beams on mutation induction was investigated and compared with that of gamma rays in rice. Dry seeds of rice variety 'Hitomebore' were exposed to carbon-ion beams (linear energy transfer (121 keV/μm) at a dosage range of 10-60 Gy and gamma rays at a dosage range of 100-450 Gy by irradiation at the rate 10 Gy per hour. The effects of irradiation on survival were observed at 20 Gy using carbon-ion beams and at 200 Gy using gamma rays, respectively. At higher doses, the survival rate decreased with increasing irradiation doses. The 50% lethal dose using carbon-ion beams was at 35 Gy and 300 Gy for gamma rays. At the irradiation range of 10-40 Gy for carbon-ion beams and 100-300 Gy for gamma rays, the frequency of chlorophyll mutation was investigated in the M_2 generation using the M_1-plant progeny method. The effects of mutation induction were similar for both irradiation treatments. The mutation frequency for carbon-ion beams irradiation was 9.0% and that for gamma rays was 8.4% with a 40-60% seedling survival rate. For the rate of induced mutants per irradiated seed, the highest value obtained was 6.8% at 20 Gy using carbon-ion beamss and 7.1% at 200 Gy using gamma rays. This difference was not observed among the irradiation treatments when the relative frequencies of induced albino (white), xantha (yellow) and viridis (light green) of chlorophyll mutations were compared.

Introduction

Mutation induction is a useful method for plant breeding and the type of mutagenic treatment is an important factor for successful results. Recently, many kinds of accelerators have become available for the study of living organisms, because the accelerated ion beams became available under the atmospheric conditions.

One unique characteristic of ion beams irradiation is their high level of linear energy transfer (LET) and the potential to focus that high energy on a target site. As a consequence, ion beams irradiation is likely to have a higher degree of effect on DNA, resulting in higher levels of mutagenic induction.

In addition, during ion beams irradiation treatments, a relatively small number of ion beams penetrate through the cells. Therefore, it is presumed that a higher degree of point mutations can be obtained through ion beams irradiation than through the use of gamma rays.

The level of mutation induction through the use of ion beams irradiation is known to have a higher degree of biological effect and a higher mutation induction rate when compared to gamma rays having a low level of LET (1, 2). However, the use of ion beams irradiation as a mutagen for mutation breeding has not been examined. Therefore, the present study has investigated the use of ion beamss as a treatment for mutation breeding and compared it with gamma ray irradiation treatments. The study also examines the mutagenic effects on different dosage levels on mutation frequency and the type of mutations induced.

Materials and methods

Plant material

The rice variety 'Hitomebore' was used as the experimental material.

Irradiation treatment

For the irradiation treatment with carbon-ion beams (LET 121 keV/μm), brown rice was placed on 6 cm diameter petri dishes with their embryos facing the irradiation source. The samples were irradiated with a carbon-ion beam generated by an AVF-cyclotron (Japan Atomic Energy Research Institute (JAERI), Takasaki, Japan).

Gamma rays were applied to dry seed at a dosage rate of 10 Gy per hour in a gamma-room (Institute of Radiation Breeding, National Institute of Agrobiological Sciences (NIAS), Hitachi-Ohmiya, Japan).

Effect on survival

Carbon-ion beams radiation was applied to seed at the following doses: 10, 20, 30, 40, and 50 Gy. The seeds were subjected to gamma ray treatment at the following doses: 100, 150, 200, 250, 300, 350, 400, and 450 Gy. The survival rate was investigated following three weeks after sowing.

Mutation frequency

The effect of mutation induction was investigated in the M_2 generation using the M_1-plant progeny method**.** The seeds were irradiated using doses of 10, 20, 30, and 40 Gy of carbon-ion beams and 100, 150, 200, 250, and 300 Gy of gamma rays**.** Irradiated seed were sown to the field and M_2 seeds from the primary panicle of the M_1 plants were harvested. 25 seeds in each panicle were grown individually in the greenhouse. The frequency of chlorophyll mutation was determined as the number of the M_1 spikes emerged chlorophyll mutation per M_1 spike sown.

Spectrum of chlorophyll mutations

Chlorophyll mutations were classified into four groups, i.e. albino (white), xantha (yellow), viridis (light green) and others.

Results

Effects on survival

The 50% lethal dose in carbon-ion beams treated populations was 35 Gy and that in gamma ray treatments, it was 300 Gy. Initial effect of irradiation on seed survival was observed at 20 Gy using carbon-ion beams and at 200 Gy using gamma rays. At higher irradiation dosages, the survival rate decreased with increasing irradiation doses.

Mutation frequency

The mutation frequency per irradiated seed was calculated by multiplying survival rate by the mutation frequency. The highest value for mutation rate was 6.8% obtained at 20 Gy using carbon-ion beams and 7.1% using 200 Gy for gamma rays (Table 1).

The mutation frequency per M_1 spike was observed to rapidly increase in the 90% and 100% range of seed survival. Below the 90% survival rate, mutation frequency gradually increased. The highest value for mutation induction was 9.0% using carbon-ion beams and 8.4% using gamma rays.

Spectrum of chlorophyll mutants

The relative frequency and type of chlorophyll mutations generated by each treatment (albina, xantha viridis and others), were compared (Fig.1). In both treatments, the frequency of each type was similar and no significant differences were observed. The frequency of albino was 55% using carbon-ion beams and 53.6% using gamma rays, and that of xantha was 8.7% and 8.2%, that of viridis was 25.0% and 30.6%.

Discussion

The effect of mutation induction using carbon-ion beams was similar to that of gamma rays. Comparison at a survival rate of 40-60%, the frequency of mutation per M_1 spike using carbon-ion beams ranged from 8.5 - 9.0% while that of gamma rays was about 8.4%. The highest value of mutation frequency per irradiated seed was 6.8 % using carbon-ion beams and 7.1% using gamma rays.

Table 1. Effect of carbon-ion beams and gamma rays irradiation on survival and mutation induction

	Dose(Gy)	Survival Rate (%)	Chlorophyll Mutation Frequency	
			per M_1 spike (%)*	per irradiated seed (%)
Carbon-ion beams	40	32.0	9.0	2.9
	30	68.1	8.5	5.8
	20	87.2	7.8	6.8
	10	88.8	6.0	5.3
Gamma rays	300	55.5	8.4	4.7
	250	80.8	6.8	5.5
	200	92.4	7.7	7.1
	150	95.9	5.4	5.2
	100	99.2	4.3	4.3

*The frequency of the spontaneous mutation was 0.3% estimated by examining 2,000 panicles of non-irradiated plants.

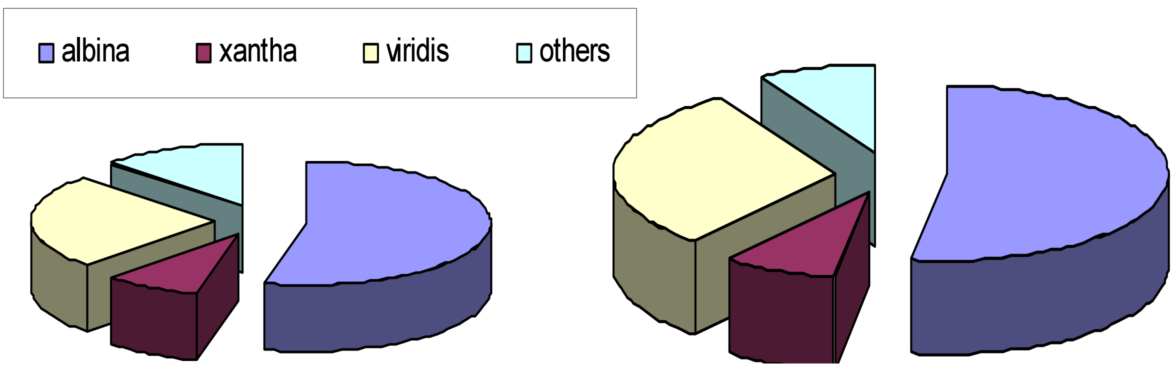


Fig.1. The relative frequency and type of chlorophyll mutants induced by carbon-ion beams (left) and gamma rays.

The spectrum of chlorophyll mutations also did not indicate significant differences in treatments. In an earlier study, Matsuo *et al.* (1958) reported that in rice, the types of chlorophyll mutants induced by X-rays and by thermal neutrons appeared similar (3). In a similar study utilizing barley, the relative frequency of chlorophyll mutations induced by neutron and by X-ray irradiation also indicated little difference in treatments. However, mutations induced by chemical mutagens, ethylene oxide etc., were different from those induced by radiation, that is to say, the frequency of viridis was higher than that of albina (4).

High dosages of irradiation resulted in a lower survival rate, and at higher irradiation levels the frequency of mutation did not increase. From these results, it is possible to calculate the optimum dosage level to obtain the highest mutation frequency per irradiated seed. These dosages were 20 Gy for carbon-ion beams irradiation and 200 Gy for gamma ray irradiation. These dosages reflect the critical point in the slope of the survival curve for both treatments and represent the optimum dosage for inducing higher mutation frequency per irradiated seed.

Reference

[1] Hirono, Y., Smith, H.H., Lyman, J.T., Thompson, K.H. and Baum, J.W. Relative biological effectiveness of heavy ions in producing mutations, tumors, and growth inhibition in the crucifer plant, Arabidopsis. Radiation Research, 1970, 44: 204-223.

[2] Mei, M., Deng, H., Lu, Y., Zhuang, C., Liu, Z., Qiu, Q., Qiu, Y. and Yang, T.C. Mutagenic effects of heavy ion radiation in plants. Adv. Space Res., 1994, 14: 363-372.

[3] Matsuo, T., Yamaguchi, H. and Ando, A. A comparison of biological effects between thermal neutrons and X-rays on rice seeds, Jap J. Breeding, 1958, 8:37-45.

[4] Harten, A.M. van. Mutation Breeding: Cambridge University Press, 1998.

Development of Improved Rice Varieties Through the Use of Induced Mutations in Malaysia

Mohamad[1], O., Mohd. Nazir[2], B., Alias[3], I., Azlan[3], S., Abdul Rahim[2], H., Abdullah[4], M.Z., Othman[3], O., Hadzim[3], K., Saad[3], A., Habibuddin[5], H. and Golam[6], F., [1]Faculty of Science and technology, Universiti Kebangsaan Malaysia (UKM), 43600 UKM Bangi, Selangor, Malaysia; [2]Agrotechnology and Biosciences Division, Malaysian Institute for Nuclear Technology Research (MINT), Bangi, 43000 Kajang, Selangor, Malaysia; [3]Rice and Industrial Crop Research Division, MARDI Rice Research Center, Seberang Perai, 13200 Seberang Perai, Malaysia; [4]Strategic Resource Research Division (Rice Genebank), MARDI Rice Research Center, Seberang Perai, 13200 Seberang Perai, Malaysia; [5]Biotechnology Research Division, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, 50774 Kuala Lumpur, Malaysia; [6]Wheat Research Centre, Bangladesh Agricultural Research Institute (BARI), Noshipur, Dinajpur, Bangladesh

Abstract: Beginning in 1972, the use of induced mutations for rice improvement was initiated in Malaysia. The initiative became more significant when, in 1979, UKM and MARDI undertook a major and concerted effort to screen for blast resistance in Mahsuri, a popular variety at the time, which had succumbed to the disease. Mutation breeding has used both EMS and gamma irradiation, which comes from a ^{60}Co source at UKM and MINT facilities, and this approach, has now become part and parcel of the overall national rice breeding and selection programmes. Mutation breeding was aimed at developing *inter alia* disease resistance, insect resistance, photoperiod insensitivity, short stature, reduced maturity, improved plant type and good grain and eating qualities, and involved many rice genetic resources, ranging from traditional varieties to advanced breeding lines as well as commercial improved varieties. Amongst released varieties e.g. Mahsuri, Muda, Manik, MR 211 and MR 219, mutation breeding had been attempted directly on them for single trait improvements. Generally, induced mutations have yielded mutants which are either used directly or have some interesting and desirable traits for use in further cross breeding. Such mutants include Mahsuri Mutant, Muda 2, PS 1297 and Manik 817, MA03, SPM 29 and SPM 39, Q 34, MRQ 50, SPM 129, SPM 130 and SPM 142. The objectives of this paper are to describe some of these rice mutants which have been developed through mutation breeding until the present time, and also to highlight some of the current activities involving induced mutations.

Introduction

Rice production in Malaysia is concentrated in major irrigated areas to sustain the targeted self-sufficiency level of 65%, and rice breeding remains very essential for developing new improved varieties. Mutation breeding in rice is used to complement conventional breeding, since this technique is very effective in improving major traits, such as agronomic traits (e.g. plant height and maturation period, photoperiod sensitiveness, growth habit or plant type), resistance to pests and diseases (e.g. blast, BLB, BPH and GLH), and grain physical parameters and eating quality (e.g. amylose content). Apart from providing genetic stocks for the hybridization programme, the resulting mutants have occasionally been released to replace their parents [1, 2].

Rice breeding using induced mutations in Malaysia was initiated in 1972. However, a major and concerted effort only began in 1979, when a programme to screen for blast resistance in Mahsuri was started [3, 4]. This was a collaborative effort between UKM and MARDI. Since then, mutation breeding encompassing other objectives has been intensified collaboratively and independently by these and other institutions. Two approaches have been adopted. The first approach is to treat elite breeding lines or varieties which are in defect in certain important traits, and the second approach is to improve the agronomic traits of l traditional varieties preferred in certain areas. All mutagen treatments have been done in MARDI, UKM or UKM involving both chemical mutagen (mainly EMS) and physical mutagen (mainly gamma irradiation).

In general, although many mutants which had been identified usually proved to be better than their parents in terms of yield, many of them seldom exceeded commercial control varieties. This is because the target traits used in mutation breeding programmes were not directly related to yield and productivity. Yield and adaptability trials in the fields constitute an integral part of varietal evaluation before any new breeding lines are released as varieties to farmers, including mutant lines developed from mutation breeding programs. Unless they posses all the important morpho-agronomic and quality traits, mutant lines would not be released for commercial planting. In a majority of the cases, the mutant lines did not find their way to the farmers' fields; nevertheless, these lines are still very important since they continue to be utilised as donors of desirable traits in cross breeding. The objectives of this paper are to: (i) describe some of these rice mutants which have been developed through mutation breeding until the present time, and (ii) highlight some of the current activities involving induced mutations.

Development of mutant lines

Many rice mutants have made significant contributions in one way or another to the varietal development in Malaysia. Through mutation breeding, several mutant lines have been successfully produced, such as Mahsuri Mutant [5], Muda 2 [6], PS 1297 and Manik 817 [7, 8], MA03 [1], SPM 29 and SPM 39 [9], Q 34 [10], MRQ 50 [11], SPM 129, SPM 130 and SPM 142 [2], and these are described below:

Mahsuri mutant

Among the mutants that had been developed through induced mutations, the most significant is a mutant line named "Mahsuri Mutant". The mutant line is an indirect result of the collaborative research initiated by UKM and MARDI in 1979 to evaluate the use of mutation techniques in rice breeding [3]. Mutation induction by EMS and gamma irradiation was carried out on the released variety Mahsuri, which had become very susceptible to blast disease. This mutant was found to have not only blast resistance but also improved cooking and eating qualities, possessing the elongation characteristic akin to that found in Basmati and Sari [5] (Fig.1). This mutant matures 5-10 days late compared to Mahsuri, but is taller by 20 cm and its yield is lower by about 7.8% (Table 1). Varieties with milled rice grain length of more than 6.21 mm is considered long and will fetch better prices. Many released varieties have also been selected for good eating quality, with the amylose content in the range of 24-26%. Recently, high quality varieties have been bred and released, and they possesses additional special characteristics such aroma and a high elongation ratio in cooked rice. Consequently, breeders have targeted the elongation characteristic of Mahsuri Mutant as one of these important quality traits.

Muda 2

Muda 2 is a rice variety specifically developed to overcome the lodging susceptibility of its parent variety Muda, which was released in 1984 [6]. The resulting mutant had shorter plant height and stiff culms, indicating good resistance to lodging. Muda 2 produced higher yield than the original variety by 2.4% in the Yield Trial and by 1.5% in the Multi-location Verification Trial. Its highest yield was 5.9 t/ha. This variety was also found to have increased resistance to brown planthopper but reduced resistance to Penyakit Merah disease (tungro) as compared to its parent variety. This mutant line was released and distributed to the farmers under the same name "Muda", as a replacement for the original variety Muda.

PS 1297

In 1983, seeds of aromatic upland rice variety Pongsu Seribu 2 were irradiated with gamma rays with the objective of inducing short mutants and improving the yield potential. As a result, a stable semi-dwarf and aromatic mutant coded as PS 1297 was recovered. The main improvement was in the plant height, which was reduced from 121-136 cm to 64-80 cm, making it more resistant to lodging than its original variety (Table 2). Other associated changes were reduced pigmentation on the panicle and grain, shorter and erect flag leaf, more tillers and increased amylose content [7]. PS 1297 performed quite well in the yield trial averaging 3.5-4.5 t/ha. This was considered an achievement in the effort to breed aromatic rice varieties with high yield potential.

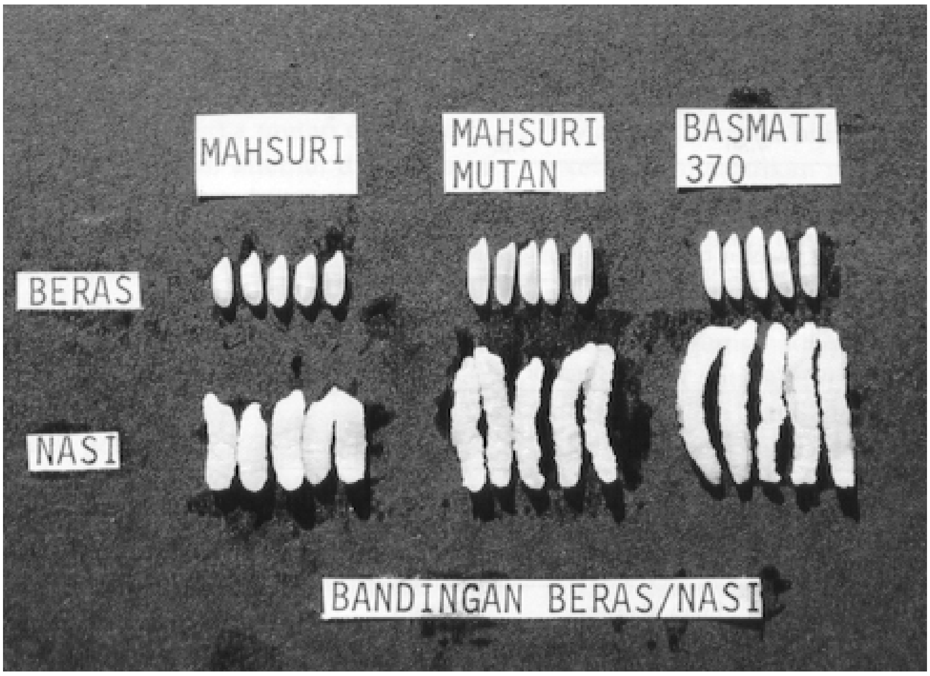


Fig.1. Elongation characteristic of Mahsuri Mutant (centre) *compared with its parent Mahsuri (*left*) and Basmati 370 (*right*).*

Table 1. Some characteristics of Mahsuri Mutant as compared to its parent Mahsuri

Characteristics		Mahsuri Mutant	Mahsuri
Yield (t/ha)	Irrigated	4.48	4.85
	Rainfed	3.66-4.35	4.18-4.60
Maturity (days)	Main season	131	125
	Off season	148	137
Culms height (cm)		105-125	100-105
Panicles per plant		11-14	10-12
Panicle length (cm)		23.8	22.9
Spikelets per panicle		194	228
1,000-grain weight (g)		19.0	16.6
Milling recovery (%)		67.5	68.0
Head rice recovery (%)		74.4	92.0
Grain length (mm)		6.50	5.38
Amylose content (%)		25.3	26.9
Alkali spread value		4.6	4.2
Gel consistency (mm)		45	51
Elongation ratio		1.7-2.2	1.5-1.8
Resistance to blast (No. of virulent isolates out of 11 tested)		2	11

Table 2. Some characteristics of PS 1297as compared to its parent Pongsu Seribu 2

Characteristics	PS 1297	Pongsu Seribu 2
Yield (t/ha)	3.5-4.5	2.0-2.5
Growth duration (d)	140-148	140-149
Culm height (cm)	64-80	121-136
Tillers per plant	13-17	10-14
Panicle length (cm)	24.8	28.0
Spikelets per panicle	137	222
Grain filling (%)	71.4	87.6
1,000-grain weight (g)	21.5	20.8
Grain length (mm)	5.75	5.63
Amylose content (%)	28.4	23.9
Gelatinization temperature	Low	Medium
Gel consistency	Hard	Soft
Elongation ratio	1.91	1.74
Aroma	Medium	Strong

Manik 817

In 1984, MINT started a coordinated research programme under RCA/IAEA/FAO entitled "Semi-dwarf mutants for rice improvement in Asia and Pacific" using the variety Manik. The main objective of the project was to generate semi-dwarfs in both native and improved cultivars for possible use as new cultivars, or as parents in cross breeding programs. Within five years, 101 semi-dwarf mutants with were recovered and evaluated. Among them, twenty-nine of these lines were reported to have grain yields of between 6.0-7.3 t/ha from experimental plots, higher than the parent, which yielded 5.7 t/ha; forty-seven lines had similar grain yields (5.0-6.0 t/ha) to the parent. Twelve mutants were resistant to brown planthopper (BPH) but had poorer yields.

Among the semi-dwarf mutants, a glutinous mutant with good yield and head rice recovery was identified from the progeny treated with 200 Gy gamma rays, and it was later designated as Manik 817 [8, 12]. The height of Manik 817 was substantially reduced, 30 cm shorter on the average than the parent variety, therefore making it less prone to lodging. The mutant had also shorter and more erect flag leaf and shorter panicles. However, the grains of the mutant were longer and more slender, but with a slightly lower 1,000-grain weight than its parent. Compared with other released glutinous varieties, Manik 817 was the shortest with an average culm height of 68 cm. It matured in 146 days in the off-season, only slightly later than Pulut Siding. Manik 817 and Pulut Siding performed similarly well (4.0 t/ha), but both yielded lower than Pulut Malaysia I. Manik 817 produced outstanding head rice recovery averaging 80.6%.

MA 03

MA 03 was another Manik mutant developed by MINT through the mutation project under the RCA/IAEA/FAO Coordinated Research Program. It was a semidwarf mutant with a very distinct feature of upright panicle and broad leaf [1]. However, this mutant matured late and produced round grains, which consequently lowered its commercial grade. Its head rice recovery was very poor, recording as low as 30%.

SPM 29 and SPM 39

Jarum Mas was a non-aromatic traditional variety but was very popular among the local consumers as a high quality variety in the past. It was tall and very susceptible to lodging. It was also photoperiod-sensitive and had a long maturation period. The variety was subject to mutation treatment using gamma rays at 200 Gy. After a series of selection, two mutants were recovered, and were named SPM 29 and SPM 39 [9]. These two mutants have a reduced culms height and maturation period when compared to their parent Jarum Mas. The average yield of SPM 29 and SPM 39 were 2.75 t/ha and 3.01 t/ha respectively, as compared to 2.02 t/ha of Jarum Mas. Other characteristics which were also altered included an increase in the number of tillers per plant, a reduced percentage of sterile grains, a reduced panicle length and an increase in 1,000-grain weight (Table 3).

Table 3. Some characteristics of mutant lines SPM 29 and SPM 39

Characteristics	SPM 2(mutant)	SPM 39 (mutant)	Jarum Mas (Parent)
Yield increase over parent (%)	30.7	39.3	-
Maturity (days):			
Off season	129	125	-
Main season	122	119	150
Culm height (cm):			
Off season	72.9	77.0	-
Main season	51.8	57.0	129
Tillers per plant	13.5	16.0	8.0
Panicle length (cm)	26.3	25.6	29.0
Spikelets/panicle	124	103	212
Grain filling (%)	80	90	68
1,000-grain weight	21.8	21.7	18.7
Milling recovery (%)	68.6	67.5	70.6
Head rice recovery (%)	61.2	63.4	69.9
Grain length (mm)	7.22	6.79	6.59
Grain width (mm)	1.84	1.85	1.78
Amylose content (%)	20.6	20.2	20.9
Alkali spread value	3.0	3.5	4.0

Q 34

Aiming to improve Mahsuri Mutant, a backcrossing effort was undertaken to reduce its culm height. As a consequence, a semi-dwarf line named Q 34 was selected [10]. Its culms height was 65 ± 5 cm, making it resistant to lodging It could be directly seeded and the average yield was similar to that of Mahsuri Mutant. Under high soil fertility, the mutant became severely susceptible to foliar leaf diseases.

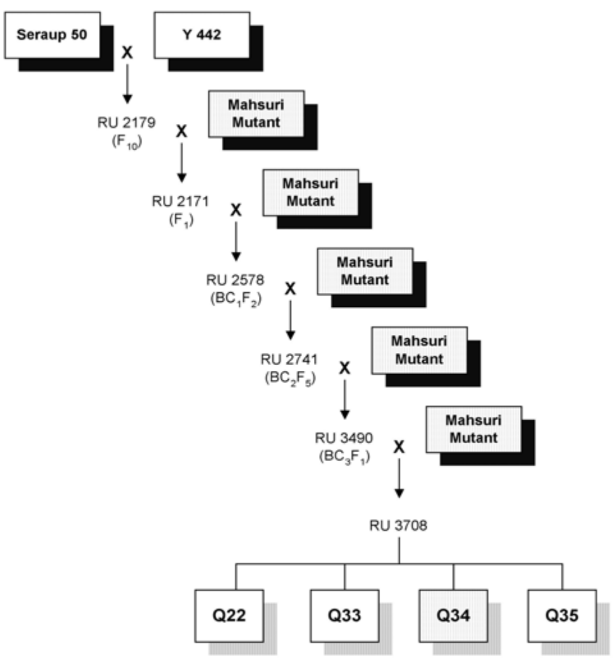


Fig.2. MARDI's breeding effort to reduce height of Mahsuri Mutant leading to the development of Q 34.

MRQ 50 (Puteri)

In the early 1990s, MARDI started a special breeding programme to develop local high quality rices. After widespread planting of an exotic fragrant variety from Taiwan brought in illegally by farmers and resulting in blast outbreaks, MARDI intensified its efforts in the development of high quality rices for commercial planting. As a result, MRQ 50 was selected and released as a specialty high quality rice variety in 1999. It was developed from the cross Q 34 x Khaw Dawk Mali (KDML), a popular high quality rice originating in Thailand. MRQ 50 has both the Basmati-like elongation characteristic derived from Mahsuri Mutant, and the jasmine rice aroma from KDML [10].

SPM 129, SPM 130 and SPM 142

MR 180 was a line introduced from IRRI, coded as IR51672-37-2. Under local conditions, it has relatively high yield, good panicle structure with medium grains, and is moderately resistant to major pests and diseases. It is a short-stature plant type of around 70-80 cm. However, it has a slightly open growth habit as well as small weak culms, making it very prone to lodging. In the off-season of 1996, the seeds of MR 180 were gamma-irradiated at 150 Gy from ^{60}Co with the objective to generate mutants with improved plant type, bigger and more erect culms, and bigger grains [2]. At the end of the off-season of 2000, three mutant lines were selected, namely SPM 129, SPM 130 and SPM 142, and promoted to the yield trial.

The three mutants, which had a short-stature and erect plant type with big, strong and compact culms, showed good potential after being evaluated for two seasons. They also showed improvement in grain length from medium to long and in increasing its head rice recovery. They out-yielded both the parents and the control variety MR 84 by 9.5-11.7% and 8.3-10.5%, respectively (Table 4). They also matured earlier, and showed the same level of resistance against blast, bacterial leaf blight and tungro as the parents did. They are currently still being evaluated under the national yield testing in Multi-location Adaptability Trial.

Table 4. Some characteristics of mutants SPM 129, SPM 130 and SPM 142 as compared to their parent MR 180 and control variety MR 84

Line	Yield (t/ha)	Maturity (d)	Culms height (cm)	Panicle length (cm)	No. of spikelets/ panicle	Grain length (mm)	Resistance to		
							Blast	BLB [a]	Tungro
SPM 129	4.71	116	71	24.8	176	6.75	R	MR	MR
SPM 130	4.70	116	72	24.5	167	6.71	R	MR	MR
SPM 142	4.62	118	72	25.4	173	6.81	R	MR	MR
MR 180	4.22	128	75	25.3	162	6.16	R	MR	MR
MR 84	4.26	128	77	-	-	-	-	-	-

[a] Bacterial leaf blight

Other activities involving induced mutations

On-going mutation breeding work by MINT and MARDI

At present, MINT and MARDI are jointly working on two mutation breeding research programs, as follows: (1) Varietal improvement of irrigated rice under minimal water conditions; (2) Mutation induction and evaluation of high yield rice mutants. Both programs have just been initiated. The objective of the first programme is to generate superior genotypes for minimal water requirements, involving three elite breeding lines namely MR 211, MR 219 and MR 220. Single plant selection will be carried out in early generations (M2 and M3) under water stress. The objective of the second programme is to screen for blast resistance using two elite breeding lines, namely MR 211 and MR 219.

Studies on inheritance of elongation characteristic of Mahsuri Mutant

Studies on the effect of artificial ageing on elongation characteristic and its inheritance pattern of Mahsuri Mutant were conducted recently [13-16]. Linear elongation of cooked grain is one of the major characteristics of fine rice [17]. Lengthwise expansion without an increase in girth is considered a highly desirable trait in high quality rice [18]. Different types of Indian and Pakistani Basmati, Afghanistan's Sadri, and Myanmar's D25-4 (Nga Kyee) possess this elongation characteristic. In all three single crosses involving Mahsuri Mutant, the frequency distribution of grain elongation ratio of segregating populations formed a bimodal curve. From the findings, it was concluded that the elongation characteristic of Mahsuri Mutant is controlled by one or two major genes, and perhaps influenced by a few modifier genes. Furthermore, it was also observed that the nature of the elongation characteristic of Mahsuri Mutant is completely different from that of Basmati type rices because Mahsuri Mutant only manifested good grain elongation after the ageing process.

According to Sood *et al.* (1980) [19], the internal anatomical structure of grain, cell shape and arrangement might have had an influence on the water uptake and the nature of swelling after cooking. Preliminary studies were conducted to determine the effects of ageing time and temperature on the change of internal physical structure of rice grain. The findings indicated that a high amount of internal cracks were found to be moderate in Mahsuri Mutant when artificially aged at 90°C for 1 and 3 hours respectively, but were found to be highest at 110°C. Under those conditions, the abundance of the internal cracks for Mahsuri Mutant was substantially increased when compared to its parent Mahsuri [20]. It was therefore concluded that the internal cracks in rice could be related to the ageing conditions and variety.

Conclusion

During the 1970s and 1980s, mutation breeding made a tremendous contribution to crop improvement throughout the world. Today, induced mutations have already been accepted by plant breeders as an additional tool for many crop improvement programmes. In Malaysia, the extent of the use of induced mutations in rice improvement is considered limited, and could have been more widely implemented. However, the improvement of important traits through mutation breeding such as shorter plant stature, earlier maturity, photoperiod insensitivity, better grain and quality traits, and resistance to pests and diseases can be regarded as successful. In the future, rice breeders should consider intensifying the use of mutation breeding as an immediate strategy, and also, quickly integrating it with other techniques, including molecular techniques, in order to enhance the usefulness and effectiveness of its applications in rice improvement in this country.

Acknowledgements

This research work is partially supported by IRPA grant 09-02-02-0077-EA205 to Universiti Kebangsaan Malaysia from Ministry of Science, Technology and Innovation Malaysia. The authors would like to thank UKM, MINT, MARDI and all individuals involved for their support in the conduct of this research.

References

[1] Mohd. Nazir, B., Mohamad, O., Affrida, A.H. and Sakinah, A. Research Highlights on the Use of Induced Mutations for Plant Improvement in Malaysia. Bangi, Malaysia: MINT, 1998.

[2] Azlan, S., Alias, I., Saad, A. and Habibuddin, H. 2004. Performance of potential mutant lines of MR 180. *In*: Sivaprasagam *et al.* (eds.). Modern rice farming. Proc. Int. Rice Conf. Serdang, Malaysia: MARDI, 2003. 293-296.

[3] Arasu, N.T., Gaul, H.P.K., Zakri, A.H., Jalani, B. S., Hadzim, K., Faizah, S., Varughese, J. and Saad, A. Breeding for blast resistance by induced mutations. *In*: Yap, T.C., Graham, K.M., Jalani, B.S. (eds). Crop Improvement Research. Proc. Fourth SABRAO International Congress. Kuala Lumpur, Malaysia: SABRAO, 1983. 167-177.

[4] Zakri, A.H. and Jalani, B.S. Mutasi aruhan - Penggunaannya dalam penyelidikan genetik dan biakbaka di Malaysia. Sains Malaysiana, 1985, 14 (3): 287-302. (in Malay with English abstract)

[5] Hadzim, K., Ajimilah, N.H., Othman, O., Arasu, N. T., Latifah, A. and Saad, A. Mutant Mahsuri: Baka untuk beras bermutu. Teknol. Padi, 1988, 4: 7-13. (in Malay with English abstract)

[6] Alias, I., Tengku Nazri, T.Z. and Azlan, S. Prestasi varieti Muda 2 di kawasan pengairan Muda. Teknol. Padi, 1988, 4: 1-6. (in Malay with English abstract)

[7] Mohamad, O., Mahmud, J., Hadzim, K., Othman, O., Ajimilah, N.H. and Abdullah. M.Z. Mutant PS 1297. Teknol. Padi, 1988, 4: 15-22.

[8] Mohamad, O., Ramli. O. and Mahmud, J. Mutant Manik 817. Teknol. Padi, 1989, 5: 39-45. (in Malay with English abstract)

[9] Mahmud, J., Alias, I., Rohimi, S. and Mohamad, O. Jarum Mas rice mutants. Teknol. Padi, 1995, 11: 7-12.

[10] Hadzim, K., Othman, O. and Ajimilah, N.H. Height reduction of mutant Mahsuri and identification of Q 34. Teknol. Padi, 1994, 10: 17-23.

[11] Phang, F.K., Wan Darman, W.A. and Othman, O. Commercial production of high quality rice - The MRQ 50 rice estate in Rompin, Pahang. *In:* Sivaprasagam *et al.* (eds.). Modern rice farming. Proc. Int. Rice Conf. Serdang, Malaysia. MARDI, 2004. 251-259.

[12] Mohamad, O., Ramli, O. and Mahmud, J. A glutinous and short-stature mutant of the rice variety Manik. MARDI Res. Journal, 1994, 22(1): 23-28.

[13] Golam, F., Mohamad, O., Hadzim, K. and Meisner, C.A. Optimization of aging time and temperature of four Malaysian rice cultivars. Pakistan J. Nutrition, 2003, 2(3): 125-131.

[14] Golam, F., Mohamad, O., Hadzim, K. and Meisner, C.A. Inheritance of gelatinisation temperature in rice. Int. J. Agri. Biol., 2004, 6(5): 810-812.

[15] Golam, F., Mohamad, O., Hadzim, K., Meisner, C. A. Inheritance of kernel elongation in rice. Int. J. Agri. Biol., 2004, 6(5): 813-815.

[16] Golam, F., Mohamad, O., Hadzim, K. and Meisner, C.A. Kernel aging: an analysis in four Malaysian rice varieties. Int. J. Agri. Biol., 2003, 2: 230-232.

[17] Sood, G.B. and Siddiq, E.A.. Geographical distribution of kernel elongation gene(s) in rice. Indian J. Genet. Plant Breed., 1979, 40: 439-442.

[18] Sood, G.B., Siddiq, E.A. and Zaman, U.F. Genetic analysis of kernel elongation in rice. Indian J. Genet. Plant Breed., 1983, 43: 40-43.

[19] Sood, G. B., E. A. Siddiq. Studies on component quality attributes of Basmati rice, *Oryza sativa* L. Z. Pflanzenziichtg, 1980, 84: 294-301.

[20] Golam, F. Physico-chemical characteristics and inheritance studies of cooking qualities and semi-dwarfism in three rice crosses involving Mahsuri Mutant. PhD thesis. Bangi: Universiti Kebangsaan Malaysia. 2004. 1-110.

Rice Mutation Breeding for Varietal Improvement in Myanmar

Khin Than New, Department of Agricultural Research, Yezin, Pyinmana, Myanmar

Abstract: Four mutant rice varieties were officially released for commercial production in Myanmar. One of the mutant varieties, Shwe War Tun, released in 1980, has been grown as the second largest variety in terms of growing area. Two new mutant varieties with desired quality characters and resistance to bacteria blight disease and white-backed plant hoppers were released in 2005.

Rice is the major crop and is grown throughout the country under different agro-ecosystems in Myanmar. The annual total rice growing area is 6 million hectares, with 4 million hectares of monsoon rice and 2 million hectares of summer rice. The rice yield in Myanmar doubled in a 30 year period, that is, from 8 million metric tones in the 1960's to about 16 million tons in the 1990's. Development and adoption of improved rice varieties has significantly contributed to the increase of rice production.

The national rice varietal improvement program has been undertaken at the Department of Agricultural Research, Yezin (formerly the Central Agricultural Research Institute untill January 2004). Mutation breeding is one of the most effective ways of inducing genetic variability and new mutant lines with desirable traits. In Myanmar, rice improvement using induced mutation was initiated in the early 1970's, and the first mutant rice variety Shwe War Tun (a mutant of IR 5) developed through gamma irradiation was released in 1974. Another mutant variety, Shwe Thwe Tun (a mutant of IR 24), was released in 1980. Since then, Mutant variety Shwe War Tun has been grown as the second largest rice variety in terms of occupying area in the country; it performed particularly well in rainfed lowland regions. During 1980 – 1990, induced mutation was used, attempting to improve some traditional rice varieties. However, no mutant lines with desirable traits were obtained. In this paper, breeding and performance of two new mutant varieties are reported.

Breeding of the two new mutant varieties

Manawthukha is the most popular variety, with high yield potential and wide adaptability, and Lone Thwe Hmwe is a high quality rice variety preferred by consumers and often fetches a higher price in the market. However, both varieties have limitations in rice production.

Manawthuka is highly susceptible to bacterial blight (BB) disease, which has become a serious problem in Myanmar, often resulting in significant yield reduction. It is also not suitable for summer rice cultivated areas, because it has a long growth duration (ca.135 days) which could make the harvesting time of summer rice overlap with the growing time of the raining season rice. Lone Thwe Hmwe is grown in the country as a quality rice variety with a higher price in the domestic market as well as the international market. But it is photoperiod sensitive, and cannot be grown in the summer season or in certain rice growing areas. Therefore, a mutation breeding program was established for the improvement of these two varieties.

In 1996, rice seeds of these two varieties were irradiated with different doses of gamma rays (200Gy, 250Gy and 300Gy). Eleven mutant lines were selected from Manawthuka and another 11 lines from Lone Thwe Hmwe. From M_4 generation on, those mutant lines were continuously evaluated in replicated yield trials at different locations and seasons from 2000 to 2001. Besides the yield, other agronomic and quality characters, i.e., maturity, photoperiod sensitivity, plant stature, amylose content, gel consistency, gelatinization temperature, protein content, elongation ratio, milling recovery, grain size and shape, and resistance to BB and white backed plant hopper (WBPH) were evaluated, together with respective parent varieties.

Based on the overall performance, Manawthuka mutant line MNTK M4-10 and Lone Thwe Hmwe mutant LTH M4-14 were selected as the best mutant lines and are officially released as new varieties.

Performance of the two new mutant varieties

Since 2002, the two most promising mutant lines were distributed to the farmers for field trials. According to feedback from farmers, MNTK M4-10 produced a higher yield when grown in the dry season than in wet season (monsoon), and out-yielded the parent variety Manawthuka in most trials (Table 1).

Table 1. Yield performance of Manawthuka and its mutant line MNTK4-10

Location	Year & season	Check variety	Yield (t/ha)		
			Check	Mutant	Increase (%)
Hmawbi	2001 DS	Manawthuka	4.1	4.92	20.0
Kyauktada	2001 DS	Manawthuka	4.46	4.82	8.1
Nyaungdon	2001 DS	Manawthuka	4.87	4.63	-4.9
Pyinmana	2001 WS	Manawthuka	4.16	4.92	18.3
Thegon	2001 WS	Manawthuka	4.3	4.27	-0.7
Letpadan	2001 WS	Manawthuka	3.4	3.81	12.1

The mutant variety MNTK4-10 is resistant to disease BB and medium resistant to insect pest WBPH, compared with high susceptibility in the parent variety (Table 2). With the reduction of amylose content, the mutant variety has soft gel consistency (Table 2).

Table 2. Agronomic and quality characteristics of Manawthuka and its mutant MNTK M4-10

Character	Mutant (MNTK4-10)	Parent (Manawthuka)
Growth duration (days)	116	137
Plant height (cm)	102	100
No. of panicles/hill	10.3	8.7
Grains/panicle	100	115
Filled grains (%)	89	89
1000 grain wt. (g)	19.5	19.0
Reaction to BB (score)	1 (R)	9 (HS)
WBPH (score)	4 (MR)	9 (HS)
Head rice recovery (%)	45	50
Milled rice length (mm)	6.29	6.45
Milled rice width (mm)	2.76	2.99
Kernel appearance	translucent	translucent
Amylose (%)	22.42	25.82
Gel temperature	Intermediate	Intermediate
Gel consistency	soft	Intermediate

The Lone Thwe Hmwe mutant, LTH M4-14, produced a high and stable yield in both seasons, and significantly out-yielded the parent as well as other control varieties in all tests (Table 3). The mutant LTH M4-14 has become insensitive to photoperiod; it can be grown in both the summer and monsoon season, while Lone Thwe Hmwe can only be grown in the monsoon season (Table 3).

Table 3. Yield performance of Lone Thwe Hmwe and its mutant M4-14 in various trials at different locations and seasons

Location	Year & season	Check variety	Yield(t /ha)		
			Check	Mutant	Increase (%)
Nyang don	2001 DS	Hmawbisan	4.87	5.79	18.9
Yezin	2001 WS	Manawthuka	3.81	4.57	19.9
Pyinmana	2001 WS	Lone Thwe Hmwe	4.93	5.19	5.3
Kyaukdata	2001 WS	Lone Thwe Hmwe	3.94	4.35	10.4
Kyaktada	2002 DS	Lone Thwe Hmwe	No flowering	4.50	Not applicable
Thegon	2001 WS	Lone Thwe Hmwe	3.71	4.43	19.4
Kyakse	2003 DS	Lone Thwe Hmwe	4.5	6.50	44.4
Kyakse	2004 DS	Lone Thwe Hmwe	4.16	6.44	54.8
Kyakse	2005 DS	Manawthuka	5.48	7.55	37.8

With the reduced plant height (117 cm) and thicker culm compared with Lone Thwe Hmwe, the mutant line was found to be lodging tolerant. The mutant is also shorter in growth duration and more resistant to BB and WBPH (Table 4).

Table 4. Agronomic and quality characteristics of Lone Thwe Hmwe and its mutant line LTH M4-14

Characters	Mutant (LTH M_4-14)	Parent (Lone Thwe Hmwe)
Growth duration (days)	125	155 (sowing at JUNE)
Plant height (cm)	117	167
No. of panicles/hill	7	7
Grains/panicle	145	135
Filled grains (%)	81	61
1000 grain wt. (g)	25.2	26.8
Reaction to BB (score)	3 (MR)	9 (HS)
WBPH (score)	3 (MR)	7 (S)
Head rice recovery %	49.2	51.5
Milled rice length (mm)	7.15	7.8
Milled rice width (mm)	4.16	3.9
Kernels appearance	translucent	translucent
Amylose (%)	21.6	17.9
Gel temperature	Intermediate, medium	Intermediate, medium
Gel consistency	low	soft

These 2 mutant lines were approved by the National Seed Committee for commercial release as new rice varieties in June 2005. MNTK M4-10 was named as Thukayin and LTH M4-14 is named as Yezin Lone Thwe.

Current mutation breeding program

In Myanmar rice production, about 58% of the total rice area is rain-fed lowland rice, which is subject to abiotic stresses such as drought, salinity and flood. The yield of rain-fed lowland rice is still low due to a lack of suitable improved rice varieties, especially in drought-prone and salt-affected area.

Supported by the IAEA technical cooperation project on development of improved rice with tolerance to drought and soil salinity, three local salinity tolerant rice cultivars (Let Yone Gyi, Kose, Kalei) and 2 local upland rice varieties (Kone Myint 2 and Mote Soma Kyawe Kye) were used for mutation improvement using gamma irradiation,. The objectives were to select the mutant lines with increased tolerance in salinity and drought.

A total of 58 mutant lines (39 for drought and 19 for salinity) from 5 varieties had been selected and are being evaluated in the trials. For salinity tolerance, one promising mutant M_4-6-1, of Let Yone Gyi, was selected and has already been evaluated in the farmer's fields at three different locations where salinity problems occur. Further testing is needed to confirm the increasing salinity tolerance.

Conclusion

Remarkable results have been obtained in the rice varietal improvement program through mutation breeding. This indicates that induced mutation can be profitably used for improving quality characters and tolerance to environmental and biological stresses, in addition to yield traits.

Mutation Breeding for Rice Improvement in Pakistan: Achievements and Impact

Cheema, A.A., Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Jhang Road, Faisalabad, Pakistan

Abstract: Rice occupies an important position in the economy of Pakistan. Annually, Pakistan earns some 582 million US$ from its export. The fine grain aromatic Basmati varieties of rice are considered high quality rice and fetch higher prices in the national and international trade. However, yield per unit area of Basmati rice is very low mainly due to its tall plant habit and late maturity. Mutation techniques have been used at NIA, Tando-

jam (see Baloch *et al.*, this issue) and NIAB, Faisalabad to develop short stature, short duration, and high quality rice varieties/germplasm. In NIAB, two high quality varieties, e.g., Kashmir Basmati and NIAB-IRRI-9 were developed and widely adopted. Kashmir Basmati, a short duration and cold tolerant mutant, derived from Basmati-370, has been cultivated in high mountain areas since it was first approved for cultivation in Azad Jammu and Kashmir (AJK) in 1977, and is still being cultivated on about 22% of rice area in these ecologies. NIAB-IRRI-9, a non-aromatic, fine grain, salt tolerant and high yielding mutant line derived from IR-6, occupies about 27% (57.3% in 2000) area under non-aromatic varieties in the Punjab province, where about two thirds of rice is grown in Pakistan. It has a fine and translucent grain possessing an 8% higher head rice recovery and fetches about 42% higher price than the parent variety. The export of this variety was quantified as 140 and 131 KMT amounting to 32 and 28 million US$ during the years 2001 and 2002 respectively. Another new mutant line, NIAB-2000, a short mutant derived from Basmati-370 after 250 Gy gamma rays treatment, has gone through pre-release formalities and is pending official release. Among various mutants developed in the past, an extra long grain mutant, isolated from mutagenized populations of Basmati-Pak, has shown great potential for further increasing the quality of Basmati rice.

Introduction

Rice occupies an important position in the economy of Pakistan. The total rice area under rice in Pakistan is about 2.3 million hectares, about two thirds (1.54 million hectares) is in the Punjab province [1]. The present area under aromatic and non-aromatic rice varieties is 87.7% and 10.01% respectively, in the Punjab province. The importance of rice for Pakistan is many-sided, as it is an important agricultural commodity that adds 20% foreign exchange to national foreign exchange reserves [2]. The fine grain Basmati rice varieties are considered high quality rice and fetch a high price in national and international trade. However, yield per unit area of Basmati rice is very low due to tall plant habit and late maturity. Conventional techniques have not been able to resolve these problems due to its narrow genetic base. Therefore, broadening the genetic base of rice is an essential requirement for a rice improvement program. The shortest possible method is induced mutation.

In plant breeding, mutation induction has become an effective way of supplementing existing germplasm and improving cultivars [3]. Induced mutation has been used in rice more than any other crop as confirmed by the 443 rice mutant varieties listed in the FAO/IAEA Mutant Varieties Database (www-mvd.iaea.org). Most of these are directly utilized mutants, but an increasing numbers of cultivars result from crossing with induced mutants [4]. Semidwarfism and earliness are the frequently induced mutations in the released rice mutant cultivars [5-6].

Realizing the potential role of induced mutations for the improvement of crop plants, the Pakistan Atomic Energy Commission initiated a modest research activity in 1963 at the Nuclear Institute for Agriculture, tandojam. Two other agricultural research institutes, Nuclear Institute for Agriculture and Biology (niab), Faisalabad and the Nuclear Institute for Food and Agriculture (nifa), Peshawar were established in 1972 and 1982, respectively. These institutes are now involved in a wide variety of biological and agricultural research projects using nuclear and other advanced techniques. One of their important activities is the crop improvement program, which supplements the efforts of the conventional breeders where induction of mutation provides a better, or the only approach, to evolve short stature, short duration, good quality and high yield rice germplasm/varieties. This paper presents the achievements and impact of mutation breeding made at NIAB for rice improvement in Pakistan

Breeding methods

Pure and dry dormant seeds of different varieties viz; aromatic and non-aromatic were treated with gamma rays or fast neutrons with different doses by maintaining the moisture content of the seed at about 12-14%. M_1 generation was planted at NIAB, Faisalabad with one seedling/hill spacing 10 cm apart between and within rows. The main panicle from each M_1 plant was harvested at physiological maturity and was bulked dose wise for growing M_2 population as plant progeny rows [7]. In M_2 population, desirable mutants were isolated on the basis of early flowering, short stature, good plant type and other grain characteristics. The selected mutants were further evaluated for stability of the mutant characteristics in M_3 and M_4 progeny rows, along with untreated parent rows in every 10 mutant lines.

The homozygous lines with desired grain characteristics were evaluated in micro and macro yield trials at the station along with parent and commercial varieties. The better performing lines were recommended to the National Uniform Rice Yield Trials (NURYT) for three years to test their yield performance and stability. The physico-chemical characters were also evaluated for release as commercial varieties.

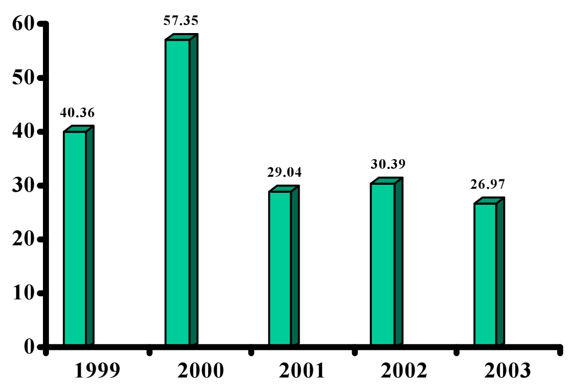


Fig.1. Area (%) of NIAB-IRRI-9 variety under non-aromatic rice varieties in the Punjab province.

Mutant varieties developed

Kashmir Basmati

Mutations were induced to reduced plant height and growth duration in a tall and long duration variety, namely Basmati370. The mutant EF-29-1 (150 Gy) was selected for early maturity (100 days), namely, three weeks earlier as compared to Basmati-370. It is ideal for northern hilly areas of the country where the summer is short and Basmati-370 does not reach maturity because of the early onset of winter. Besides its early maturity, yield and quality is at par with the parent. The mutant was approved in 1977 as a commercial variety "Kashmir Basmati". About a 22% area of Swat Valley of A. J. K. is under Kashmir Basmati. Due to its cold tolerance, it is grown at an altitude of 2000 to 5000 feet.

NIAB-IRRI-9

This variety was approved in 1999 as a commercial variety under non-aromatic group for growing in the Punjab province. Niab-Irri-9 is also salt tolerant, high yielding (7500 kgs/ha) with fine and translucent grain variety (derived from IR-6 through fast neutrons). It is fit for general cultivation in normal as well as in saline soils. It has a 10% increase in paddy length and a 10% decrease in paddy width over the parent IR6. The cultivation of 27% area of non-aromatic rice is under Niab-Irri-9 in the Punjab Province (Fig. 1).

Because of its high quality characters of NIAB-IRRI-9, farmers get 25% more price advantage for 40 Kgs than the other coarse grain varieties, whereas exporters earned 43% more price per metric tons than the other coarse grain varieties (Table 1). The total export of this variety amounted to 13.9/- Million US$ during the year 2002-03.

Table 1. Price advantage of NIAB-IRRI-9 over other non-aromatic rice varieties

Variety	Framer's gate (Pakistan Rupee per 40 kgs)	For export (US$/ton)
Niab-Irri-9	300.00	218.92
Irri-6	238.33	153.82
KS-282	245.00	65.21

Advanced mutant lines and germplasm

New variety pending for release

NIAB-2000, derived from Basmati370 through gamma rays (250 Gy) irradiation, is a candidate new variety pending release. With similar grain quality as its parent Basmati370, NIAB-2000 is about 25% shorter in height, with stiffer stems and thus more lodging resistance, is responsive to fertilizer, and has a higher yield potential than Basmati-370. In NURYTs from 2001 to 2003, NIAB-2000 out-yielded the commercial high yielding variety Super Basmati by 8.48%.

Useful Basmati mutant lines

During the past years, a large amount of mutant lines were developed from Basmati rice (Table 2), which have been widely used as parent lines in national and international breeding programs.

The extra long grain mutant, EF-6, was developed by irradiating Basmati-Pak at 350 Gy. This mutant is about 25% longer in paddy and cooked grain as compared to its parent (Fig. 2). However, the mutant EF-6 had low fertility (60%), and thus could not be directly used as a variety. Improvement of its fertility has been made through crossing to its Super Basmati, resulting in a few extra long grain lines with a fertility of up to 93%.

Conclusion

It is evident that the use of induced mutations for rice improvement in Pakistan has led to the development of improved varieties and a galaxy of useful mutants which hold the promise of either direct release as a variety, or use in cross breeding programs for the development of improved varieties.

Fig.2. Comparison of paddy, milled and cooked grains of extra long grain mutant along with other commercial varieties.

Table 2. Various useful Basmati mutant lines developed in Pakistan

Sr. No.	Mutant	Parentage	Salient Features
1	DM-16-5-1	Basmati-370	Short stature (135-145 cm), leaves erect, fine grain and moderately aromatic
2	DM-2	Basmati 370	Short stature (130-140cm), high tillering, leaves erect, fine grain and aromatic
3	DM-15-4	Basmati 370	Short stature (125-135cm), leaves erect, fine grain and aromatic
4	DM-15-11	Basmati 370	Short stature (120-130cm), leaves erect, fine grain and aromatic
5	DM-24	Basmati 370	Short stature (115-125cm), non lodging, leaves erect, fine grain and aromatic
6	DM-38	Basmati 370	Short stature (112-122cm), non lodging, leaves erect, fine grain and aromatic
7	DM-179-1	Basmati 370	Short stature (115-125cm), semi lodging, leaves semi erect, fine grain and aromatic
8	DM-28	Basmati 370	Short stature (128-130cm), lodging resistant, leaves erect, fine grain and aromatic
9	RST-24	Basmati 370	Salt tolerant, semi dwarf (100 cm)
10	EF-6	Basmati Pak	Early maturing (three weeks), extra long grain
11	EF-76-1	Basmati 198	Early maturing (three weeks), high yielding, moderate aroma
12	C-49-1-79	Recombinant (DM-107-4/ Kashmir basmati)	Early maturing, high yielding, semidwarf (100-110cm) and moderate aroma
13	C-75-8-79	Recombinant (DM-16-5-1/ Kashmir basmati)	Dwarf (135-145cm), early maturing (three weeks), high yielding
14	DM-25	Basmati-370	About 25% shoter in height as compared to parent, high yielding, grain quality and maturity period similar to Basmati 370. Responsive to fertilizers. Stiff stem. Lodging resistant.
15	DM-107-4.	Basmati 370	40% less in height, high yielding and non-allelic to DGWG, has been allotted gene symbol d59

References

[1] Anonymous 2002-03. Agricultural Statistics of Pakistan. Government of Pakistan, Ministry of Food, Agriculture and Livestock (Economic wing), Islamabad. Page: 13-14.

[2] Anonymous 2001. Pakistan Economic Key Indicators, National Bank of Pakistan, Economic Research Wing, Karachi, Pakistan, 2: 18-20.

[3] Micke, A., Donini, B. and Maluzynski, M. 1990. Induced Mutations for Crop Improvement.Muta. Breed. Rev. ISSN 1011-2618, IAEA. pp: 1-41.

[4] Wang, L.Q. 1991. Induced mutation improvement in China, A review. Plant Mutation Breeding in Crop Improvement. Proc. Int. Symp. On the contribution of plant Mut. Breed to crop improvement. IAEA, pp: 9-32.

[5] Cheema, A.A. and Awan, M.A. 1988. Performance of semidwarf mutants of Basmati-370. IRRN. 13 (3): 20-21.

[6] Awan, M.A. 1999. Mutation breeding for crop improvement: A review. Proc. Pak. Acad. Sci. 36 (1): 65-73.

[7] Rashid, M., Cheema, A.A., Ashraf, M., Qamar, Z. and Mahmood, Z. 2003. Development of Basmati rice varieties through the use of induced mutations and related techniques. Pak. J. Bot. 35(3): 811-818.

Sustainable Enhancement of Rice (*Oryza sativa* L.) Production Through the Use of Mutation Breeding

Balooch, A.W., Soomro, A.M., Naqvi, M.H., Bughio, H.R. and Bughio, M.S., Plant Genetics Division, Nuclear Institute of Agriculture (NIA), Tando Jam, Pakistan

Abstract: Four new high yield rice varieties with excellent quality were developed through mutagenesis at Nuclear Institute of Agriculture (NIA), Tando Jam, Pakistan, and released by the Provincial Seed Council, Government of Sindh for general cultivation. Both chemical and physical mutagenesis is proven to be useful in mutation induction. The first mutant rice variety "Shadab", released in 1987, is a mutant of IR6 induced using ethyl methane sulphonate (EMS 0.5%); the 2nd variety "Shua-92", released in 1993, evolved from IR8 by irradiation of 15 Gy fast neutrons; the third variety Khushboo-95, released in 1996, was selected from gamma rays (200 Gy) treated progenies of Jajai-77 (aromatic). The most recent mutant variety "Sarshar", released in 2001, was also developed from IR8 after treatment of 150 Gy gamma rays. These varieties have substantially increased the yield potential of rice production in Sindh and Balochistan provinces, and brought significant economic impact to rice farmers

Introduction

Rice has been a popular subject to mutagenesis because it is the world's leading food crop. Being diploid species, maximum genetic variability is available for selection in M_2 generation. Induced mutations have thus played a vital role for the improvement of rice by developing a large number of semi-dwarf and high yielding varieties around the world [1-5]. In Sindh province of Pakistan, rice is being cultivated near an area of 2.23 million ha of which approximately 45% is under non-aromatic varieties. After the Green Revolution in the 1960's, most of the area came under rice came under cultivation of short statured varieties IR6 and IR8. In spite of their high yield, the quality was not good enough to meet the consumer's demands. Similarly, aromatic varieties also remained under cultivation, though in lesser quantities, but have the major drawback of their long duration and lodging susceptibility. We therefore initiated our mutation breeding programme with the objectives of improving the quality of non-aromatic IRRI varieties and improving the plant architecture of locally cultivated aromatic varieties, for earliness and lodging resistance.

Material and methods

Both physical (gamma rays and fast neutrons) and chemical mutagenesis (ethyl methane sulphonate) were applied to seeds of *IR6, IR8,* and Jajai 77. Selection in M_2 was mainly done for traits of agronomic significance. Most of the mutations were deleterious and discarded.

Mutants selected for various traits were confirmed in M_3 generation and those showing phenotypic stability were evaluated in M_4. Further evaluation was carried out in subsequent generations. Only productive mutants were promoted in regional and national yield trials covering different ecological niches. Mutants with desired characters were released as new varieties, while those with useful mutant characteristics were included in the germplasm.

The released varieties were introduced into the cropping system of province over the years. There has been a gradual increase in the area and productivity of the province. In each year the estimates of additional benefits due to cropping of new varieties was worked out using the following formula.

Additional Yield = Variety Yield (t/ha) – Average Yield of province (t/ha)

Economic Impact = Additional Yield (t/ha) x Area under variety (m ha) x Rate (US$)

Varieties developed and their impact

Shadab

The rice variety Shadab evolved by treating the seeds of IR6 with a chemical mutagen, ethyl methane sulphonate (EMS 0.5%). It was approved for release in 1987 for general cultivation in the Sindh province of Pakistan [06]. The major improvement of the Shadab over IR6 were the quality characteristics: its milled rice grain is more translucent and longer (7.52 mm vs 6.91), with a length/width ratio of 4.08 (IR6: 3.5); its expansion rate during cooking is also higher (1.82) than IR6 (1.49) (Table 1). The yield potential of Shadab is similar to IR6 around 7 tones/ha. Shadab is being cultivated on more than 0.05 million hectares and has contributed 16.49 million US$ as additional income to the economy of the province.

Shua-92

Shua-92, developed from IR8 through fast neutrons treatment (15 Gy) of dried seeds, was approved for release as a new variety in 1993 for general cultivation in the Sindh province [07]. Both yield potential and quality characteristics were significantly improved (Table 1). It was later found to be good in salt tolerance: Shua-92 grew well even in soils with ECe up to 7.11 to 8.0 mmho/cm, which is above the threshold range for rice (ECe 3.0 to 3.6 mmho/cm) [08]. Shua-92 is being cultivated over an area of 0.15 million hectares in the Sindh province. The cultivation of Shua-92 has contributed an additional income of 201.27 million US$ to the economy of the province (Table 2).

Table 1. Comparative performance of mutant varieties with their parent varieties*

Characteristics	Shadab (IR6)	Shua-92*(IR8)*	Khushboo-95 (Jajai-77)	Sarshar (IR8)
Potential paddy yield (tones/ha)	7.59 (7.67)	8.5 (7.38)	5.5 (2.5)	9.5 (7.38)
Average productive tillers	20.7 (18.9)	18.3 (14.5)	14.5 (10.7)	20.2 (14.5)
Filled grain per panicle	164 (156)	168 (138)	148 (88)	156 (138)
L/B ratio of milled grain	4.08 (3.50)	3.46 (2.61)	3.24 (3.07)	3.29 (2.61)
Head rice recovery (%)	70.0 (56.0)	64.2 (33.6)	44.0 (41.5)	42.5 (33.6)
Chalkiness (%)	NA	1.9 (45.8)	20.8 (19.8)	1.62 (45.8)
Amylose content (%)	30.7 (30.1)	26.7 (26.2)	25.0 (28.8)	27.2 (26.2)
Protein content (%)	9.56 (9.11)	7.76 (7.12)	NA	7.22 (7.12)
Cooked rice elongation rate	1.82 (1.49)	1.65 (1.43)	1.55 (1.50)	1.62 (1.43)

*Parent varieties in parenthesis

Khushboo-95

Khushboo-95 is an aromatic variety developed by exposing the seeds of a local tall rice variety Jajai-77 to 200 Gy Gamma rays [09, 10]. The parent variety is tall (170 cm) and was susceptible to lodging, particularly when high doses of nitrogenous fertilizers were applied. The mutant variety Khushboo-95 has reduced plant height (130 cm) and is better in response to N fertilization, thus produces almost double the yield of parent variety (Table 1). It was approved for release as a new variety in 1996 for Sindh province. It occupies 10,000 hectares and has provided an additional income of 6.54 million US$ to farmers (Table 2).

Sarshar

The Sarshar variety, evolved by irradiating the seed of IR8 with 150 Gy gamma rays, was approved for general cultivation in the Sindh province in 2001 [11]. This variety is not only high yielding but also characterized by resistance to insect pests and diseases, such as leaf folder, white backed plant hopper (WBPH), rice stem borer (RSB), mealy bug (MB), brown spot, narrow brown leaf spot, glume discoloration and kernel smut. It is also resistant to shattering. The quality characteristics of Sarshar are better than its parent variety IR8. It has a long slender grain with minor chalkiness of 1.62% as compared to its parent variety with 45.81% (Table 1).

The yield potential of Sarshar is 9.5 tones/ha and it occupies an area of 0.06 million hectares. It has contributed an additional income of 17.20 million US$ to farmers (Table 2).

Table 2. Economic impact and additional benefits (US$ million) of rice mutant varieties since their release*

	Shadab	Shua-92	Khushboo-95	Sarshar	Total
Area under cultivation in Sindh (m ha)	0.05	0.15	0.01	0.06	0.27
Yield (kg/ha)	3306	4008	2595	4175	
Additional Yield (%)	24	37	20	40	
Economic impact since release (US$ million)	16.49	201.27	6.54	17.20	241.50

*Area under rice cultivation in Pakistan: 2.2252 million ha, area under cultivation of NIA rice varieties: 12%. Area under rice cultivation in Sindh: 0.4883 million ha, Area under cultivation of NIA rice varieties: 54%

Source: Agriculture Statistics of Pakistan 2002-2003

Acknowledgment

We are indebted to Mr. Mohammed Afzal Arain, Nuclear Institute of Agriculture, Tando Jam, Pakistan for the critical review of manuscript and valuable comments. I sincerely acknowledge the FAO/IAEA for financial assistance and especially to Dr. Q.Y. Shu for arranging this Strategic Meeting on “Nuclear Techniques for Rice Improvement in Asia”.

References

[1] McKenzie, K.S. and Rutger J.N. A new semi dwarf mutant in a long grain rice cultivar. Crop Sci., 1986. 26: 81-84.

[2] Micke, A., Donini B. and Maluszynski M. Trop, Induced mutations for crop improvement a review. Agric. 1987. 64: 259-278.

[3] Maluszynski, M., Sigurbjornsson, B., Amano, E., Sitch, L. and Lamro, O. Mutant verities data bank, FAO/IAEA data base MBNL. 1991. 38: 16-21.

[4] Qayyoum, A., Mufti M.U. and Rabbani S.A. Evaluation of different rice genotypes for stability in yield performance. Pak. J. Sci. Ind Res. 2000. 43:188-190.

[5] Baloch, A.W., Soomro, A.M., Javed, M.A., Ahmed, M., Bughio, H.R., Bughio, M.S. and Mastoi, N.N. Optimum plant density for high yield in rice (*Oryza sativa* L.). Asia. J. Plant Sci., 2002. 2: 114 – 116.

[6] Bari, G., Mustafa, G., Soomro, A.M. and Baloch A.W. Studies on the yield performance and grain quality of mutant strains of rice. Pak. J. Bot., 1981. 13: 189-194.

[7] Mustafa, G., Soomro, A.M., Baloch, A.W. and Siddiqui, K.A. "Shua-92", a new cultivar of rice (*Oryza sativa* L) developed through fast neutrons irradiation. Mutation Breeding Newsletter, IAEA, Vienna, Austria, 1997. 43: 35-36.

[8] Baloch, A.W., Soomro, A.M., Javed, M.A., Bughio, H.R., Alam, S.M., Bughio, M.S., Mohammed, T. and Mastoi, N.N. Induction of salt tolerance in rice through mutation breeding. Asi. J. Plant Sci. 2003. 2(3):273-276.

[9] Baloch, A.W., Soomro, A.M., Mustafa, G., Bughio, M.S. and Bughio, H.R. Mutagenesis for reduced plant height and high grain yield in Jajai 77, an aromatic rice (*Oryza sativa* L.) variety. Pak. J. Bot., 1999. 31: 469-474.

[10] Baloch, A.W., Soomro, A.M., Javed, M.A. and Bughio, M.S. Use of induced mutations for yield and quality improvement in aromatic rice. Pak. J. Bio. Sci. 2001. 1: 78-79.

[11] Baloch, A.W., Soomro, A.M., Javed, M.A., Bughio, M.S. and Bughio, H.R. Genetic improvement in rice (*Oryza sativa* L.) through mutagenesis. Pak. J. Bio. Sci. 2001. 1:18-19.

Mutation Improvement of Rice Variety Bw-267-3 for Red Pericarp Grains and Lodging Resistance

Bentota, A.P., Regional Agriculture Research and Development Centre, Bombuwela, Sri Lanka

Abstract: The rice variety Bw 267-3 was released in 1981 particularly for iron toxic rice soils in the Low Country Wet Zone of Sri Lanka. It has become the most adaptable variety in the region, but it is susceptible to lodging and produces white pericarped grains, which is not preferred by consumers in the region. Mutation techniques were used for the improvement of these traits using ^{60}Co gamma rays irradiation. Non-lodging, red pericarped mutant lines, with comparable yield to Bw267-3, have been developed and are being tested for possible release as a commercial variety.

Introduction

Bw 267-3, one of the 15 rice varieties developed by the Regional Agriculture Research and Development Centre (RARDC), Bombuwela, was released in 1981, particularly for acidic (pH at 4-5.5) and iron toxic rice soils. It has become the most adaptable variety in the region, but it is also susceptible to lodging. Because consumers in this region prefer red rice, any red rice variety with the similar characteristics of Bw 267-3 combined with tolerance to lodging would be ideal for the region. The evidence from mutational rice experiments in Sri Lanka and other countries showed that the application of mutation techniques for the improvement these characters should be feasible [1]. Therefore a mutation breeding project was initiated for the improvement of rice variety Bw 267-3.

Materials and methods

In 1999, dry seeds (ca. 200 g) of Bw 267-3 were treated with ^{60}Co gamma rays at a dose of 200Gy. M_1 plants were raised in the field and were bulk harvested. The population size of M_2 to M_4 was varied from 10,000 to 6000 plants, grown in single plant per hill spacing of 15x20 cm. A section of mutants (resistant to lodging and with red pericarped grains) was first made at M_4 generation and selected plants were further evaluated in M_5 in 3-row plots (35 plants each row) at the spacing of 15x20 cm. Five promising lines, namely M27, M64, M58 M62 and M85 were selected in 2003. M27, M64, and M58 were tested in a 3.5-month maturity group with Bw 267-3 as the check variety; M62 and M85 were tested in a 3-month group with Bw272-6b as the check variety. The yield trials were undertaken in 3 x 6m plots in triplicate, following the Randomized Complete Block Design (RCBD).

M58 was further crossed with Bw 267-3 in 2001/2002 Maha, with the aim to select better non-lodging red rice lines with high yield potential. Pedigree selection method was applied with similar population size of F_2 to F_4 as in M_2 to M_5. Ten F_5 lines were selected for further evaluation in the current season, as single plant progenies in three row plots.

Results and discussion

While advancing the mutant population from M_2 to M_4, a number of mutant plants appeared either shorter in plant height, or earlier or later in maturity than the parent variety, or with small grains or red pericarped grains. However, no plants were selected until M_4 generation. In M_5, mutant lines with uniform plants were selected for evaluation of yield and other agronomic traits.

Five mutant lines with red pericarped grains showed shortened plant stature, but comparable or better yield potential in the yield trial (Table 1).

Table 1. Yield and agronomic performance of selected red pericarped mutant lines

Entry	Culms length (cm)	Days to maturity (d)	Yield (t/ha)
1. 3.5-month maturity group			
M27	52.1	102	2.37
M64	57.2	93	2.54
M58	54.9	106	3.01
Bw 267-3	80.6	102	2.43
LSD $_{0.05}$			0.75
2. 3-month maturity group			
M62	76.1	92	2.52
M85	64.5	99	1.53
Bw 272-6b	72.0	92	1.62
LSD$_{0.05}$			**0.67**

Among the tested new lines, M58 were fairly uniform compared to the other two lines, M27 and M64. This same line was short, tolerant to lodging, red in pericarp and its growth duration matched our cropping season. However, mutants M62 and M85 were not sufficiently uniform. Segregation of culms height and pericarp color still existed among plants within the line, therefore further selection is still going.

Selection of red pericarp and lodging tolerant plants from the progenies of the cross between Bw 267-3 and M58 has also made good progress; ten F_5 single plant progenies were adequately uniform in plant height, pericarp colour and heading time, and were already tested during 2004 yala season. Preliminary data showed that a few lines are very encouraging.

Conclusion

Our study showed that it is possible to induce mutations of characters such as red pericarp grain and lodging tolerance, and further improvement of characters could be made through crosses between induced mutants and parent variety.

Reference

[1] Ahloowalia, B.S, Maluszynski, M. and Nichterlein, K. (2004) Global impact of mutant-derived varieties. Euphytica, 135: 187-204.

Development and Characterization of Katy Deletion Mutant Populations for Functional Genomics of Host-Parasite Interactions and Rice Improvement

Jia[1], Y., Xie[2], J. and Rutger[1], J.N., [1]USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR 72160, USA; [2]Jiangxi Academy of Agricultural Sciences, P.R. China

Abstract: To facilitate functional genomics studies and rice improvement, the US rice cultivar Katy was mutagenized by ethyl methane sulfonate (EMS), fast neutrons and gamma irradiations in cooperation with Jiangxi Agricultural Academy of Agricultural Science. Seeds were soaked in 0.4%, 0.8% and 1.2 % EMS solutions for 6 hours and a total of 7199 M_1lines were recovered. Seven dosages of fast neutron were applied and a total of

15400 M_1 lines were recovered from treatments with 7.7 Gy, 26.3 Gy and 49.4 Gy. Preliminary analysis of M_2 seedlings revealed defects in chlorophyll synthesis in a range of 1.06% - 5.04% of M_1 derived lines. Katy containing the blast resistance gene *Pi-ta* prevents infection by *Magnaporthe grisea* races in a gene-for-gene manner. *M. grisea* races containing the avirulence gene *AVR-Pita* are being used to identify susceptible mutants and *M. grisea* races lacking *AVR-Pita* are being used to screen resistant mutants. Sekiguchi lesion mimic-like mutants of Katy were recovered from lines induced by EMS, fast neutrons and gamma irradiations. Progress in genetic analysis and advancement of these putative mutant lines are described.

Classical Mendelian forward genetics starts with a biological function that was identified through a mutation or phenotype variation and ends with characterization of the controlling gene. With abundant DNA sequence information, reverse genetics can be used to start with a predicted function of DNA sequences based on knowledge from model organisms, to identification of genes involved in biological function in an organism of interest. Routine methods to disrupt a gene have not yet been established for plants; however, plants are well adapted to random mutagenesis. Mutant seeds can be stored inexpensively to screen certain phenotypes or 'reverse screened'- i.e., screened based on gene sequences for mutants. To facilitate forward and reverse genetics studies, the elite US cultivar Katy containing a good disease resistant package including a major blast resistance gene *Pi-ta* [8, 15] was chosen for mutant population development using chemical (EMS) and physical mutagens (fast neutrons and irradiations). Progresses in mutant induction, genetic screenings for mutants in disease response and population amplification are described in this special issue.

Mutation induction using chemical and physical mutagens

We are developing a saturated mutant population using different mutagens. In 2001, 0.4%, 0.8% and 1.2% EMS was used to treat Katy following a protocol described by Hu and Rutger [6]. A total of 7,199 random M_1 panicles were collected in that fall. In 2002, 7.7 Gy, 26.3 Gy, 49.4 Gy, 63.7 Gy and 84.6 Gy fast neutrons were used, however, treatments with 63.7 Gy and 84.6 Gy resulted in no germination and M_1 panicles were collected only from 7.7 Gy, 26.3 Gy, and 49.4 Gy. In 2004, panicles from gamma irradiations 200 Gy and 250 Gy were collected and are being examined for defects in chlorophyll biosynthesis (Table 1). The range of the deficiency rates is 1.06% to 5.04%, the highest percentages of defects in chlorophyll biosynthesis were observed when plants were treated in 1.2% EMS, 49.4 Gy fast neutrons and 250 Gy irradiations, respectively. The dosage effects were also observed among all mutagens.

Table 1. Estimation of the mutation rate using deficiency in chlorophyll biosynthesis

Mutagen	Dosage*	M_1 Panicle †	M_2/M_1 # seedling ‡	Defect rate (%)¶
EMS	0.4%	3853	472/50	1.06
EMS	0.8%	1434	398/40	2.15
EMS	1.2%	1912	436/40	5.04
Fast Neutrons	7.7 Gy	7400	1296/130	1.10
Fast Neutrons	26.3 Gy	8150	8043/800	2.90
Fast Neutrons	49.4 Gy	343	343/40	3.20
^{60}Co	200 Gy	2880	30618/3000	1.75
^{60}Co	250 Gy	5184	50090/5000	5.04
Total		31156		

*% stands for the percentage of active gradient of EMS; Gy stands for gray (1 joule kg^{-1} of target specimen).
†Number of random panicles collected.
‡Number of M_2 seedlings and number of M_1 counted.
¶ Number of yellow and albino seedlings over the total M_2 seedlings counted.

Genetic screening for mutants for disease responses

Blast disease of rice caused by the filamentous fungus *Magnaporthe grisea* is one of the most serious diseases worldwide, and has been an excellent model for studying the molecular mechanisms of host-parasite interaction. In order to screen disease resistant/susceptible mutants, 7-12 seeds of each M_1 line were planted in the greenhouse, and 5 to 10 seedlings were screened for reaction to *M. grisea*. For blast susceptibility, an avirulent blast isolate IC-17 was used for inoculation, and a total of 42 susceptible mutants were identified from fast neutron treated lines. Five different blast susceptible mutants were further characterized, and three of them lost the *Pi-ta* gene. Structural and functional characterizations of these mutants are being performed. A virulent field *M. grisea* isolate TM2 was used to identify blast resistant mutants. Two putative resistant mutants were identified and are being verified.

Lesion mimic mutants produce spontaneous lesions in the absence of pathogen attack. The phenotype is similar to that induced by resistant reaction of rice to the pathogen, thus it is useful in studying plant defense mechanisms. Five such lesion mimic mutants were identified from screening of EMS treated population, and one Sakaguchi like EMS induced lesion mimic mutant was further characterized [9](Fig.1). The phenotype of the Katy Lmm1 resembles the Sekiguchi lesion mimic mutant [12] and thus allelism tests were performed. F_1 cross between Katy Lmm1 and Sekiguchi lesion mimic mutant (generously provided by Tony Marchetti) restored the wildtype phenotype indicating that Katy Lmm1 is non allelic to Sekiguchi lesion mimic mutant 1.

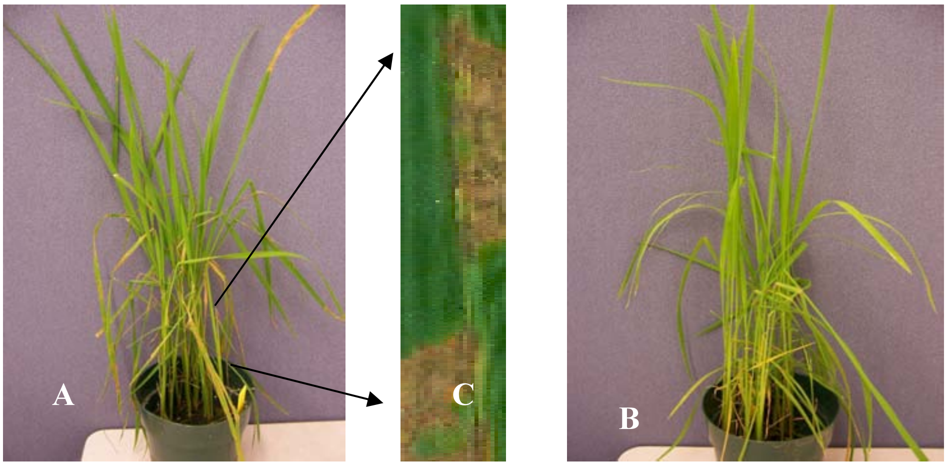


Fig.1. The lesion mimic phenotype of Katy Lmm1 intensified by a virulent M. grisea isolate (TM2) (A). Lmm1 plants with lesion mimic phenotype without intensification by the pathogen (B). Magnified lesion mimic phenotype in A (C). Pictures were taken 7 days after inoculation with the pathogen.

To determine the reaction to blast, Katy Lesion mimic mutant 1 (Lmm1) was inoculated with a wide range of US *M. grisea* isolates. Lesion mimic phenotype was rapidly induced by virulent blast isolates, and induction of the lesion mimic phenotype can only be observed when higher inocula of avirulent *grisea* isolates were used [9,10]. These results suggest that Lmm1 has enhanced resistance to blast. To identify Lmm1 suppressors that may restore the wild type retaining enhanced resistance to blast, Katy Lmm1 was treated with 200 Gy gamma irradiations. Six thousand M_1 random panicles were collected from a winter nursery in Puerto Rico in early 2005. An additional four thousand M_1 panicles will be collected from treatments with 180 Gy, 200 Gy and 220 Gy irradiations in the fall, 2005 at Stuttgart, Arkansas.

Sheath blight disease caused by the soilborne fungi *Rhizoctonia solani* is another serious disease of rice next to blast. Complete resistance to *R. solani* has not been identified from cultivated rice despite quantitative resistance sources that have been identified from some rice cultivars [7]. Three *R. solani* field isolates: RR0102, R0107, RR0113 were used for testing response of Katy LMM1 to *R. solani*. When Katy Lmm1 was inoculated with these three isolates, a consistent delayed lesion development was observed when compared with Katy (Fig. 2). These data suggest that Katy Lmm1 also has enhanced resistance to *R. solani*[9]. Phenotypically similar lesion mimic mutants were subsequently identified from fast neutron and gamma irradiation treated lines. Their allelisms to Katy Lmm1 are being tested.

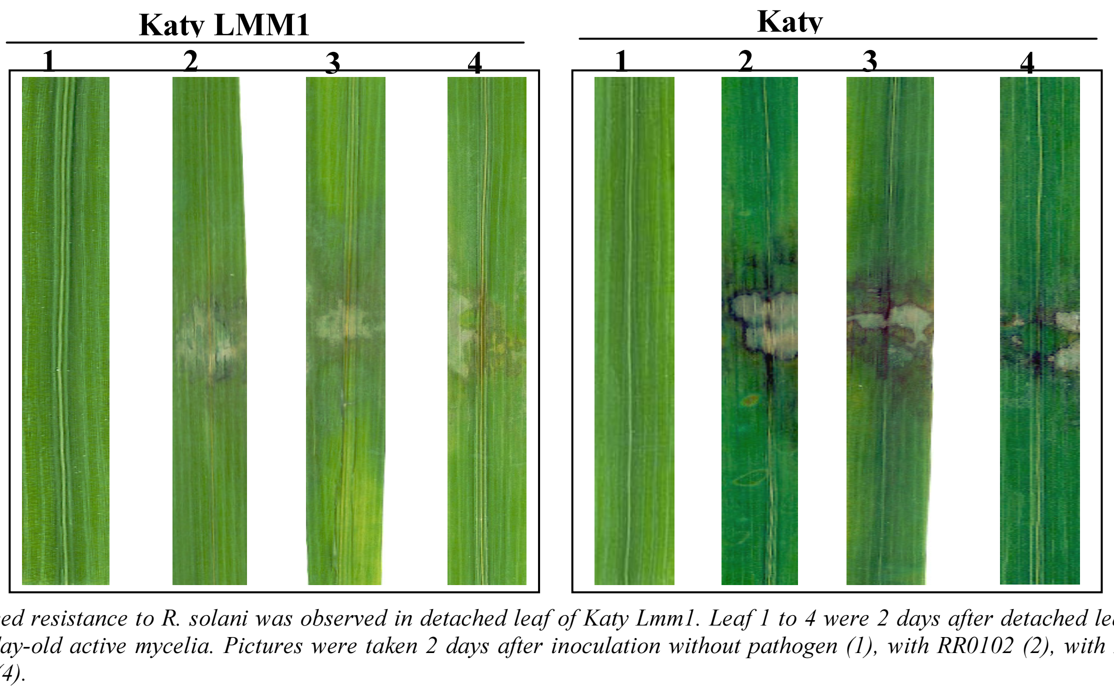


Fig.2. Enhanced resistance to R. solani was observed in detached leaf of Katy Lmm1. Leaf 1 to 4 were 2 days after detached leaves were inoculated with 3 day-old active mycelia. Pictures were taken 2 days after inoculation without pathogen (1), with RR0102 (2), with RR0107 (3), and with RR0113 (4).

Population amplification

Population amplification has progressed in segments. In 2004, five thousand EMS induced mutants were amplified to M_3, 2 panicles of each M_2 line were collected, and all 5000 lines were pooled for conditional mutant identification. These pooled putative mutants were delivered to Dr. Yinong Yang at University of Arkansas, Dr. Benildo de les Reyes at University of Maine and Dr. Rick Nelson in Noble Foundation. In 2005, an additional 10,000 fast neutron induced lines are being amplified to M_3 generation. Together, we plan to advance 20,000 lines to M_4 generation, and all of resulting lines will be deposited in the Genetic Stock *Oryza* (GSOR) collection at Dale Bumpers National Rice Research Center [16].

Future perspectives

Rice is a stable food for nearly half of the world population, and has been under intensive cultivation for several thousands years. Rapid increase of the population demands more rice. Continued increases of rice production are often limited by knowledge of rice gene function and intensified disease pressures. The genetic uniformity in new rice producing regions, such as the USA, has already created considerably favorable environments for disease epidemics; thus far, the control of some diseases still relies heavily on the use of pesticides. A long-term solution for this problem is to use more disease resistant cultivars in integrated cultural practices. Luckily, rice with a genome size of 460 Megabases is the smallest among monocots. High-density maps [14], draft sequences [4, 17], amenable transformation systems [2, 5], and a large Katy deletion mutant population are important tools in studying the function of the rice genes. Lesion mimic, blast susceptible and resistant mutants described in this study are useful in understanding the molecular mechanisms of defense pathways and the *Pi-ta* gene-mediated disease resistance. EMS induced mutants are suitable for use in targeted induced local lesions in genomes [3, 13]. Fast neutron and gamma irradiation induced mutants are suitable for genome wide oligo-chip based method for gene discovery [1]. The deletion mutants generated in this project and availability of insertional mutant populations [18] are important starting materials for the study of functional genomics of host-parasite interactions.

Acknowledgements

The authors thank China Scholarship Council for support to Jiankun Xie and University of Arkansas Adair Internship for support to Hui Lin. Technical assistance of Roger Martin, Michael J. Lin, Tony Beaty, Hui Lin, Zhonghua Wang, Lin Wu, Dianxin Wu, Lorie Imboden, Andrew Blas, Pratibha Singh, Yeshi Wamishe, Yinong Yang, Erxun Zhou, Melissa H. Jia, Shannon Pinson and Lieceng Zhu were also acknowledged. The United States Department of Agriculture Agriculture Research Service Dale Bumpers National Rice Research Center funded this research.

References

[1] Babu, M.R., Pellizzi, M., Ronald, P., Leach, J., Leung, H., Buell, R., Bruskiewich, R. and Wang, G. Characterization of rice defense mutants using whole genome expression profiling. *Plant & Animal Genomes XIII Conference*, 2005, January 15-19, San Diego, CA, p676.

[2] Christou, P. Rice transformation: bombardment. *Plant Mol. Biol.* 1997, 35:193-203.

[3] Colbert, T., Till, B.J., Tompa, R., Reynolds, S., Steine, M.N., Yeung, A.T., McCallum, C.M., Comai, L. and Benioff, S. High-throughput screening for induced point mutations. *Plant Physiol.* 2001, 126:480-484.

[4] Goff, S., *et al.* (55 authors). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *Japonica*). *Science* 2002, 296: 92-100.

[5] Hiei, Y., Komari, T. and Kubo, T. Transformation of rice by *Agrobacterium tumefaciens*. *Plant Mol. Biol.* 1997, 35: 205-218.

[6] Hu, J. and Rutger, J.N. Pollen characteristics and genetics of induced and spontaneous genetic male sterile mutants in rice. *Plant Breeding* 1992, 129: 97-107.

[7] Jia, Y., Singh, P., Eizenga, G.C., Lee, F.N. and Cartwright, R. *In vitro* identification of differential cultivar responses to *Rhizoctonia solani*. In: Norman R J and Meullenet J F. B R Wells Rice Research Studies-2002. Arkansas Agricultural Experiment Station, 2003, 504: 229-236.

[8] Jia, Y., Wang, Z., Fjellstrom, R.G., Moldenhauer, K.A.K., Adam, M.A., Correll, F.N., Xia, Y. and Rutger, J.N. Rice *Pi-ta* gene confers resistance to the major pathotypes of the rice blast fungus in the United States. *Phytopathology* 2004, 94: 296-301.

[9] Jia, Y. Registration of Katy lesion mimic mutant 1. *Crop Sci.* 2005, 45: 1675.

[10] Jia, Y., Rutger, J.N. and Xie, J. Development and characterization of rice mutant populations for functional genomics of host-parasite interactions. *Phytopathology* 2005, American Phytopathology Society, Austin, Texas (In press).

[11] Jia, Y., Wang, Z., Singh, P., Rutger, J.N. and Martin, R. Development and characterization of rice mutant populations for functional genomics of host-parasite interactions. *Proced. of the 15th International Plant Protection Congress*, Beijing, 2004, May 11-16, p.64.

[12] Marchetti, M.A., Bollich, C.N. and Uecker, F.A. Spontaneous occurrence of the Sekiguchi lesion in two American rice lines: its induction, inheritance, and utilization. *Phytopathology* 1983, 73: 603-606.

[13] McCallum, C.M., Comai, L., Greene, E.A. and Henikoff, S. Targeted screening for induced mutations. *Nat. Biotechnol.* 2000, 18: 455-457.

[14] McCouch, S.R., Kocher, G., Yu, Z., Wang, Z., Kush, G.S., Coffman, W.R. and Tanksley, S.D. Molecular mapping of rice chromosomes. *Theor Appl Genet* 1988, 76: 815-829.

[15] Moldenhauer, K.A.K., Lee, F.N., Norman, R.J., Helms, R.S., Well, R.H., Dilday, R.H., Rohman, P.C. and Marchetti, M.A.. Registration of 'Katy' rice. *Crop Sci*. 1990, 30:747-748.

[16] Rutger, J.N. and Bernhardt, L. Genetic Stocks-Oryza (GSOR) collection. *2rd International Symposium of Rice Functional Genomics*. 2004, Nov. 15-17, Tucson, University of Arizona, Arizona, p105.

[17] Yu, J., et al (88 authors). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *Indica*). *Sci.* 2002, 296: 79-92.

[18] Yung, D.-W., Cha, Y.-S., Lee, M.-C., Yi, G.-H., Won, Y.-J., Ko, J.-M., Nam, M.-H., Sun, B., Han, C.-D. and Eon, M. Establishment of Ac/Ds-mediated gene tagging systems in indica type rice. *Plant & Animal Genomes XIII Conference*, 2005, January 15-19, San Diego, CA, p269.

Rice Mutation Breeding in Institute of Agricultural Genetics, Viet Nam

Tran, D.Q., Dao, T.T.B., Nguyen, H.D., Lam, Q.D., Bui, H.T., Nguyen, V.B., Nguyen, V.X., Le, V.N., Do, H.A. and Phan, P., Institute of Agricultural Genetics, Tuliem, Hanoi, Viet Nam

Abstract: Mutation breeding was initiated in 1960 in the northern part of Viet Nam. This technology had already been systematically applied till 1980 and was considered a successful method for rice improvement. Since 1990, more than 10 mutant rice varieties have been developed. In recent years, *in vitro* mutagenesis technique has been developed by irradiating mature embryos or callus during culture. Many mutant characteristics of economic importance, i.e., high grain yield, good grain quality, short growth duration and photoperiod insensitivity, tolerance to lodging and environmental stresses, and resistance to pests and diseases, have been incorporated into these mutant varieties. The introduction of photoperiod insensitivity in some varieties enabled farmers to grow two crops of rice per year and thus dramatically increased rice production. Mutant rice varieties have been planted on over 1.0 million hectares in provinces such as Hanoi, Bacgiang, Vinhphuc, Thaibinh, Hanam, Nghean, and Hatay of northern Viet Nam, and have already produced remarkable economic and social impacts, contributing to poverty alleviation in some provinces.

A brief history of mutation breeding in northern Viet Nam

In Viet Nam, mutation breeding was initiated in 1960 by the late professor Luong Dinh Cua of Institute of Research on Food Crops. Since 1980, his work has been continued by researchers in the Institute of Agriculture Genetics (the late Dr. Phan Phai, Dr. Nguyen Huu Dong, Dr. Tran Duy Quy and Dr. Mai Quang Vinh), Hanoi No.1 Agriculture University (Dr. Tran Tu Nga), Institute of Viet Nam Agricultural Technical Science (Dr Tran Dinh Long) and Hanoi National University (Dr Le Duy Thanh).

From 1960 to 1985, mutation breeding was carried out in maize and rice. The techniques to induce mutations were mostly based on chemical agents. Irradiation equipment was not locally available. Since 1986, mutation breeding has been targeting a more diverse range of crops, including rice, maize, tomato, apple, flower and ornamental plants. New techniques for induced mutagenesis have become available. Research programs, scientific exchange and training were established with countries such as United States of America, China, Japan, Korea, Philippines, and Russia. In 1995, Viet Nam became an officially member of Pacific Asian Mutation Breeding Association.

In 1997, the IAEA began supporting the Agricultural Genetics Institute (AGI) through mutation breeding projects VIE/5/013 and VIE/5/014, providing chemical agents, the necessary equipment and training researchers. Many valuable mutant lines were induced in rice, maize, soybean, flower and ornamental plants, and some of them have been selected and developed directly or indirectly into national regional new varieties, or testing promising lines in crops other than rice (details see below), e.g. in maize (DT6, DT8), in soybean (DT84, DT95), in apple (No. 12, No.13), in tomato (No.224, No.7), and in peanut (DT332, DT329.

Recent achievements of rice mutation breeding

In the north of Viet Nam, before 1990, most of the mutant varieties were induced by chemical methods because irradiation facilities were not available in Viet Nam. After the 1990's, irradiation facilities were set up in Viet Nam for different purposes and, most of the breeders now sent seed samples to the irradiation center for inducing mutation, and, then screened and selected promising mutant lines directly or indirectly as new varieties ready for release. The irradiation technique is more convenient and is safer than chemical treatments. Most of the recent rice mutant varieties were induced through irradiation, but, in a few cases, mutants were produced by chemical treatment, or combined irradiation and chemical treatment (Table 1).

From 1990 to 2004, 10 rice mutant lines developed in the AGI have been officially recognized as new varieties (Table 1). The mutant varieties possess good characteristics, such as short growth duration, hard stem, resistance to lodging, high yield, good grain quality, break photo-sensitive, good resistance to pests and diseases (blast, brown plant hopper) as well resistance to environmental stresses (Table 1). Below are a few examples.

Traditional rice varieties usually have excellent quality characters, but are sensitive to the length of photoperiod. They usually transit from a vegetative into a reproductive stage only after exposure to a short-day period. The induction of photoperiod insensitivity in these rice varieties can greatly shorten their growth duration, which enables planting two crops per year, and thus contributes to a strong increase in rice production. Two photo-period-insensitive varieties, i.e., DT21 and Mutant Tamthom, were developed by using mutation induction, and have played an important role in rice production (Table 1).

Table 1. Rice mutant varieties developed by the Agricultural Genetics Institute of Viet Nam

Mutant varieties	Year of release[1]	Parent variety	Mutagenic Treatment	Improved Characteristics
DT10	1990 [a]	C4-63	dried seeds, 200 Gy γ rays plus 0.025% NEU[2]	Higher yield, tolerant to cold, resistant to diseases, big seeds
DT11	1995 [a]	C4-63	dried seeds, 200 Gy γ rays plus 0.025% NEU	Good grain quality, soft cooking, big seeds
A20	1993 [a]	A8	0.015% NMU[3]	Drought tolerant, salinity tolerant, grain quality
CM1	1994 [a]	Chiembau	dried seeds, 200 Gy γ rays	Salinity tolerant, seed color changed from red to white
CM6	1999 [b]	Chiembau	dried seeds, 200 Gy γ rays	Salinity tolerant, seed color change from red to white, aroma
DT33	1994 [a]	CR203	dried seeds, 200 Gy γ rays	Higher yield, resistant to blast disease
DT17	1999 [b]	DT10/OM80	cross	High yield, good quality
Mutant Tamthom	2000 [a]	Tamthom Haihau	wet seeds, 100 Gy γ rays	Photoperiod insensitivity, higher yield
DT21	2000 [a]	Mutant DV2/ Nep 415	NEU 0.025%	Photoperiod insensitivity, early maturity
DT22	2002 [b]	DT21/Dailoan	NEU 0.025%	Higher yield, good quality
DT36	2002 [c]	VN-01/BG-90-2 (F6)	Callus, 50 Gy γ rays	Higher yield, good quality
Mutant Khangdan	2003 [c]	Khangdan	wet seeds, 100 Gy γ rays	Higher yield, resistant to diseases

[1] [a]: national variety; [b]: regional variety; [c]: testing varity;
[2]NEU: Nitroso Ethyl Urea
[3]NMU: Nitroso Methyl Urea

CM1 and CM6 are also mutant varieties induced from a local variety "Chiembau". Chiembau is low in yield and its seed is in red color, which is not a preferred character. Using gamma irradiation, two mutant varieties, CM1 and CM6, were developed from mutant lines with white grain color and increased salinity tolerance. It was also found that cooked rice of CM6 is soft and aromatic attributes that give it increased commercial value.

Mutant varieties were planted in various provinces and covered approximately 1,000,000 hectares. They occupied up to 33% of the area devoted to rice production in some northern provinces of Viet Nam during the 1990's.

References

[1] Luong, L.D. Genetic Basis. P.12-13, Education Pub. House, Hanoi, 1997.

[2] Quy, T.D. New Methods for Plant Breeding P.16, Agri. Pub. House, Hanoi, Viet Nam, 1997.

[3] Dong, N.H., Bang, D.T.T., Du, L.Q. and Truc, P.D. Mutation - Theory and Practice. p.70-75. Agri. Pub. House, Hanoi, Viet Nam, 1997.

[4] Maruyama, K. A thermosensitive genic male sterile rice line induced by irradiation. Jap J Breed, 1989, 21: 19-21.

[5] Maruyama, K. Induction in tissue culture and their use for plant breeding. Jap J Breed, 1983, 33: 24-25.

[6] Maluszynski, M., Szarejko, I. and Sigurbyornsson, B. Mutation Techniques. *In:* Proc. of the Workshop on Mutation Breeding by Using Radiation Technology on Cereal Crops. Manila, Philippines, P.25, 9-15 Oct., 1995.

[7] Kume, T., Watanabe, K. and Tano, S. Use of physical/chemical mutagens in plant breeding program in Viet Nam p.45-60. *In:* Proc. of the 8th workshop on Plant Mutation Breeding - effective use of physical and chemical mutagens. Hanoi, Viet Nam, 2000.

[8] Yang, H., Tabei, Y. and Kayano, T. Detection of somaclonal variation in cultured rice cells using digoxigenin-based random applied polymorphic DNA. Plant Cell Reports, 1999, 18: 520-526.

[9] Maluzynski, M. Plant Breeding and Genetics. Newsletter, 2001, Vol.7: 4-5.

[10] Hiraiwa, S. and Takana, S. Induction of male-sterile mutation in rice. Gamma Field Symp. 1980, 19: 103-115.

[11] Shen, Y.W., Cai, Q.H. and Gao, M.W. A new thermosensitive radiation-induced male-sterile rice line. Rice Genetics Newsletter 1993, 10: 97-98.

Rice Mutation Improvement for Short Duration, High Yield and Tolerance to Adverse Conditions in Mekong Delta of Viet Nam

Do, K.T., Dao, M.S., Hung, P.Q. and Nguyen, T.C., Food Crop Research Department, Institute of Agricultural Science of Southern Viet Nam, Ho Chi Minh City, Viet Nam

Abstract: Rice plays an important role in Viet Nam, especially in the Mekong Delta (MD), where more than half of the total rice and 90% of the export rice are produced. Since rice mutation breeding was not initiated until 1992 in South Viet Nam, no mutant rice varieties were cultivated in this region until 1995. Nowadays, mutant varieties play a significant role in rice production in this area, particularly for export. In the Institute of Agricultural Science of Southern Viet Nam, dry and germinated seeds of introduced varieties (IR64, IR50404, IR59606) and local rice varieties (Nang Huong, Tam Xoan) were treated with ^{60}Co gamma rays. Populations of 10,000 – 15,000 M_1 plants were established by direct seeding, assessed according to IRRI's Standard Evaluation System (1980, 1996) through the M_7 generation, and promising lines were selected and tested in multi-location trials. The mutant characteristics identified so far consist of better resistance to lodging, disease and insect damages, improved tolerance to soil stresses such as acidic soil, drought etc, as well as early maturity and higher yield potential. Mutation techniques have shown to be very useful in rice improvement, especially for characters controlled by closely linked genes that are difficult to break by recombination. Some of the best mutant varieties: VND95-19, VND95-20, VND404, VND99-3, TNDB-100 have been released for large-scale production in South Viet Nam. Among them, VND 95-20 has become one of the 5 top varieties for export and is now grown annually on 280,000ha in MD.

Introduction

In Viet Nam, rice plays an important role as food and as an agricultural export product. This is especially true in Mekong Delta (MD), which supplies more than half of Viet Nam's total rice production and 90% of the rice that is exported. However, rice production in some areas of the MD is challenged by severe seasonal flooding, salinity, acid sulfate soils, diseases, and insect pests. These production areas require rice varieties that mature in 90 – 100 days, which allows three crops each year to be harvested and are a means of escape from seasonal flooding. Exported rice must be of high quality and all cultivars must be resistant to a variety of biotic and abiotic stresses (pests, diseases, acid sulfate, and saline soils). Before

1995, rice varieties which were cultivated in Southern Viet Nam originated from national agriculture research institutions or were introduced from IRRI. Mutation rice breeding, supported by IAEA, has been undertaken in South Viet Nam since 1992, to augment standard introduction and hybridization breeding. As a result, some mutant varieties have been developed with distinctive characters which have been significantly useful for large scale production.

Materials and methods

Materials

The breeding program used introduced varieties (IR64, IR50404, IR59606, and Jasmine 85) and local aromatic varieties (Nang Huong, Nang Thom Cho Dao, Tam Xoan).

Applied methods

Dry and germinated seeds were treated with gamma rays of ^{60}Co at the Nuclear Research Institute, Dalat City, South Viet Nam. The doses of 100, 200, 280 and 300 Gy were applied for seed treatments. The wet seeds were treated at the time after soaking and incubating of 68 –72 h. Populations of 10,000 – 15,000 M_1 plants were established and evaluated from M_2 – M_7 generations.

The segregating plants were selected using the pedigree method for 5 to 7 generations. Selected individual plants were screened and tested in greenhouse and field conditions. Twenty to twenty-five promising lines, including control varieties, were tested in yield trials in different agroecosystems of Southern Viet Nam using a Randomized Complete Block Design with 3 to 4 replications. Grain quality aspects and agronomic characters were evaluated by IRRI's standards, (IRRI, 1980, 1996).

The best lines were released, multiplied, and approved as regional and national varieties.

Results and discussion

Mutant varieties induced from introduced varieties

Agronomic characters

VND 95-20 has 90-102 days of growth duration which is 7-10 days earlier than the original variety, IR64. With early maturity, the variety can be cultivated with 2-3 crops per year and can escape drought and flooding at the early period of the rainy season in MD. Both mutant varieties (VND 95-19 and VND 95-20) have 20-120 more grains/panicle and have better plant types than IR64; greater culm strength, more erect culm angle, and better seedling vigor (Table 1).

Table 1. Agronomic characters, tolerant ability to stresses of two mutant varieties VND95 –19 & VND95 –20 in Mekong Delta of Viet Nam

Character	VND95 – 19	VND95 – 20	IR64 (original)
Growth duration (day)	98 – 108	90 – 102	98 – 110
Plant height (cm)	85 – 100	85 – 100	85 – 100
Culm strength (score)	1	1 – 3	5
Seedling vigor (score)	1	3	5
Culm angle (score)	1	1	3
Fulfilled grains/panicle	80 – 250	60 – 200	60 – 130
Wt. of 1000 grains (g)	25 – 26	25 – 26	24 – 25
Grain yield (t/ha)	5 – 11	4 – 10	4 – 8
Brown plant hopper (score)	3 – 4	3 – 5	3 – 5
Blast (score)	3 – 5	3 – 5	3 – 5
Sheath Blight (score)	5	5	5
Leaf yellow (score)	5	5	5
Iron toxicity (score)	1	2	5

Source: - National Center for Testing and Certification of Crop Cultivars of South Viet Nam, 1999

Resistance to to stresses

VND 95-19 and VND 95-20 are as tolerant to blast, leaf yellow diseases, and BPH as IR64. Both VND 95-19 and VND 95-20 are more tolerant to acid sulfate soil than IR 64. Generally, high resistance to acid sulfate soil was associated with late maturing in local varieties with low yield and poor plant type. Induced mutation breeding has demonstrated to be an advanced tool to produce cultivars having early maturity and high tolerance to acid sulfate soils; especially for iron and aluminum toxicity (Table 1).

Physiological characters

IR60819-34 and IR65619-6 are IRRI's varieties having high phenotypic acceptability and are good genetic materials to produce cultivars having an improved plant type. In comparison, physiological trait data show that the dry matter weight, chlorophyll content, harvest index and sink capacity of VND 95-19 is the same or higher than these two varieties and the control variety (Table 2). In addition, VND 95-19 has a higher total grain number (145 grain/panicle) and lower proportion of kernels that are unfilled and have intermediate grain density, as compared to the original variety (Table 3). This coupled with the higher panicle weight and harvest index (Table 2), indicates that this mutant has higher yield potential than IR64.

Table 2. Comparison of some physiological and agronomic characters of tested rice varieties

Variety	Dry matter weight (g/m^2)		Chlorophyll content (µg/mL) at growth stages		Harvest index	Sink capacity (g/m^2)
	Straw	Panicle	20 DAT	Heading		
VND 95-19	679.1	820.7	5.49	4.19	0.54	1020
IR60819 –34	646.7	816.3	5.70	4.40	0.55	1051
IR 65619-6	711.7	560.8	3.58	2.08	0.44	800
OMCS94	696.1	582.5	5.06	3.71	0.46	837
IR64 (original)	477.0	622.6	5.30	3.98	0.47	970
CV (%)	5.0	3.0	5.0		4.0	7.0
LSD 0.05	62	0.30	0.32		0.02	118

DAT: days after transplanting

Table 3. Comparison of some physiological characters of grains of tested rice varieties

Variety	Unfulfilled grains		Intermediate Specific gravity		High Specific gravity		Total grains
	/ panicle	%	/ panicle	%	/ panicle	%	/ panicle
VND 95-19	30	20.7	38	26.6	77	52.7	145
IR60819 –34	36	23.1	41	26.3	79	50.6	156
IR 65619-6	30	28.0	52	48.1	26	24.0	108
OMCS94	26	25.0	36	34.6	42	40.4	104
IR64 (original)	23	24.2	34	35.8	38	40.0	95
CV (%)	16.0				11.0		
LSD 0.05	8.2				8.9		

Mutant varieties induced from local varieties

Nang Huong is an aromatic rice variety well known in South Viet Nam for its quality. Due to its long duration (160-165 days), photoperiod sensitivity, low yield (2-4t/ha), and susceptibility to BPH and Blast disease, this variety has had limited production. Through induced mutation and selection, mutant lines have been developed that have improved traits in comparison to the original variety. The best mutant variety, VND99-3, has early maturity (93-100 days), high yield (4-8t/ha), and tolerance to diseases (blast, leaf yellow), BPH and adverse conditions (drought, iron toxicity) (Tables 4 and 5). VND99-3 has been widely accepted for production, especially in adverse conditions in Eastern South Viet Nam.

Application of mutant variety in rice production

Having improved plant type combined with iron toxicity tolerance, mutant varieties proved to possess the desirable traits from local varieties, along with new characters that were the same or better than introduced control varieties. Consequently, in 1999, VND95-19 and VND95-20 were approved as national varieties from the mutant breeding program, and are the first mutant varieties to have been grown in the 20 provinces of Southern Viet Nam.

The Ministry of Agriculture and RD certified VND 95-20 as one of 5 key varieties having good characteristics suitable for export. In 2003, VND 95-20 occupied about 280.000ha and accounted for 20.3% of the area that is cultivated for export in MD (Table 6). After 5 years of cultivation in large scale production, these mutant varieties remain stable in yield and quality aspects. In addition, VND95-19 and VND99-3 have good tolerance to adverse conditions like acid sulfate soils and drought that occur in Southern Viet Nam.

Table 4. Agronomic characters, tolerant ability to stresses of the mutant variety VND 99-3

Character	VND99-3	Nang Huong (original)	IR64 (control)
Growth duration (day)	93 -100	160 - 165	98 – 110
Plant height (cm)	85 – 100	150 -155	85 – 100
Culm strength (score)	1	9	5
Seedling vigor (score)	1	3	5
Fulfilled grains/panicle	70 -150	150 - 220	60 – 130
Wt. of 1000 grains (g)	26 – 27	20-21	25-26
Grain yield (t/ha)	4-8	2-4	4 – 7
BPH (score)	3	7-9	5
Blast (score)	3 – 5	3 – 5	3 – 5
Sheath blast (score)	3	1	3
Leaf yellow (score)	3	3	5
Drought tolerance (score)	1-3	3	5
Iron toxicity (score)	1 - 2	2	5

Source: National Center for Testing and Certification of Crop Cultivars, 2004; Institute of Agricultural Science of South Viet Nam, 2004.

Table 5. Tolerance to some adverse conditions of mutant lines of Nand Huong variety

Materials	BPH (score) [1]	Blast (score) [1]	Sheath blast (score) [2]	Culm Strength (score) [2]	Iron toxicity (score) [3]
NH 1 (Mutant)	3	4	5	1	2
NH 2 (Mutant)	3	4	5	1	2
NH 3 (Mutant)	**3**	**3**	**3**	**3**	**2**
IR64 (control)	5	4	3	4	4
Nang Huong (parent)[4]	9	2	1	9	2

[1]Evaluation in green house;
[2]Evaluation in the field condition;
[3]Evaluation at acid sulfate soil condition in rainy season;
[4] Nang Huong: local original variety (radiation treated material).

Table 6. Cultivated area of rice varieties for export in Mekong Delta of Viet Nam in 2003

Variety	Dry season		Wet season		Whole year	
	Ha	%	Ha	%	Ha	%
IR64	120,994	22.73	163,944	29.90	284,938	26.28
OM1490	154,067	28.94	144,786	26.42	298,854	27.56
VND95-20	136,043	25.55	84,060	15.33	220,103	20.30
OMCS2000	57,721	10.84	102,494	18.69	160,215	14.78
MTL250	19,547	3.00	24,215	4.42	43,761	4.04
OM2031	47,651	8.94	28,740	5.24	76,391	7.05
Total	536,023	100.00	548,238	100.00	1,084,261	100.00

Source: Ministry of Agriculture & Rural Development, Viet Nam, 2003

VND95-19, VND95-20, VND404, VND99-3, TNDB-100, and THDB-128 are some of the best mutant varieties that have been released, developed and approved as national and regional varieties for large-scale production in Southern Viet Nam.

Conclusions

- VND95-19 has a high yield potential of more than 10t/ha, with better plant type, more resistance to lodging and acid sulfate soils than its parent variety, IR64.
- VND95-20 can produce 15-20% higher yield as compared to its parent variety IR64. It is widely adaptable, has good grain quality, and shorter growth duration, which allows it to be cultivated as 3 crops per year and escape early flooding common to the MD. VND95-20 has been approved as a key variety for the export market from Viet Nam.
- TNDB-100, VND99-3 and VND404 are good varieties with high yield, short duration and tolerance to BPH, blast diseases, and iron toxicity and are suitable for cultivation in both wet and dry seasons.

- Induced mutation using radiation is an effective tool for producing rice varieties, within a relatively short time period, that have better plant types and good physiological and agronomic characters. Mutant varieties are highly improved for tolerance to adverse conditions such as iron toxicity and drought, as well as some important insects and diseases that are difficult to be obtained through traditional breeding.

References

IRRI, *Standard Evaluation System for Rice*. International Rice Research Institute, 1996, P.O. Box 933, 1099 Manila, Philippines.

National Center for Testing & Certification of Crop Varieties. *Report on results of Testing & Certification for Promising Crop Varieties in South Viet Nam 2001*, 2002 (in Vietnamese).

Ro Pham Van. Two Promising Mutant Rice Varieties in *Results of Scientific Research, 1977-1997, Institute of Cuu Long Delta Rice Research*, Omon – Cantho, Viet Nam, Pub. House of Agriculture 1998.

Phuc Phan Van. *Assessement of Characters of Promising New Rice Lines and Varieties* , Msc.thesis, University of Agricuture & Forestry, Ho Chi Minh city, 1999.

Thinh Do Khac, Hung Phi Oanh, Nguyen Thi Cuc *et al. Improvement of introduced rice varieties through radiation induced mutation for cultivation in South Viet Nam*, reported at FAO/IAEA seminar on Mutation Techniques and Molecular Genetics for Tropical & Sub- tropical Plant Improvement in Asia / The Pacific region, Philippines, 11-15 Oct. 1999.

Thinh Do Khac, Dao Minh So, Truong Quoc Anh *et al. Breeding & Development of Mutant Variety VND99-3* reported in National Scientific Conference, March 1-3, 2004 in Ho Chi Minh City.

Development of Photoperiod Insensitive Mutant Lines Using Gamma Irradiation of Tradiational Aromatic Rice

Le, X.T., Center for Nuclear Techniques, Ho Chi Minh City, Viet Nam Atomic Energy Commission (VAEC), 217 Nguyen Trai St., Dist. 1, Ho Chi Minh City, Viet Nam

Traditional high quality rice vareities are very often photoperiod sensitive and thus have long growth duration. The aromatic rice varieties, Aromatic Tam Rice (ATR) grown in North Viet Nam, and Khaodowmali 105 (KDML 105) grown in Thailand, are representative of this kind. ATR has been grown in the Red River Delta of North Viet Nam for thousands of years. It is a strictly photoperiod sensitive variety, and has a long growth duration of more than 160 days, therefore, it can be grown only once a year. It has also other non-prefered characteristics, such as high plant type (>160 cm), small and short grains (19 g per1000 grains), and low yield (<3.0 t/ha), although it has a high quality for domestic consumption mainly due to the special aroma. For the improvement of ATR, germinated seeds (48 hrs incubation in water) were treated with gamma rays of ^{60}Co at the dosage of 60 Gy. Three novel mutant lines were selected at M_4 and became bred-true at $M_{6\text{-}7}$. The mutant characteristics included photoperiod insensitivity, shortened plant type (<100 cm), early maturity (90 - 100 d), and extra long and large grain type (35-65% increase of 1000 grains weight). Subsequently, the mutant lines all had higher yields (over 5.5 t/ha) than ATR, while their grain quality remained high. In addition, a mutant line with red pericarped grains was also isolated from the mutant population. Similar results had been obtained from the irradiated KDML105 progeny. Two photoperiod insensitive mutant lines were identified and have already been demonstrated in large scale trials in Soctrang Province.

The Plant Mutation Reports Vol. 1, No. 1 is the continuation of Mutation Breeding Newsletter & Reviews No. 1.

IAEA
International Atomic Energy Agency

Plant Mutation Reports
Vol.1, No. 1
May 2006

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,
May 2006

06-17471