FNCA Mutation Breeding Project
Open Seminar

Symposium on Radiation and Nuclear Technologies for Crop Improvement and Productivity in Sustainable Agriculture

Kajang, Malaysia
February 26th – 27th, 2013

FNCA / MEXT
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Forum for Nuclear Cooperation in Asia

This report was prepared by the Nuclear Safety Research Association (NSRA) as a part of activities on Forum for Nuclear Cooperation in Asia (FNCA), organized by MEXT.

Content

Program of
FNCA Open Seminar
Symposium on Radiation and Nuclear Technologies for Crop Improvement and Productivity in Sustainable Agriculture

February 26th – 27th, 2013
Malaysian Nuclear Agency, Kajang, Malaysia

Tuesday, 26 February 2013	
Time	**Agenda**
09:00 – 09:30	Registration
09:30 – 10:30	Doa Recital
	Welcoming Remarks Dr. Sueo Machi, FNCA Coordinator of Japan
	Opening Address YBhg. Dato' Dr. Muhamad Lebai Juri, Director General, Malaysian Nuclear Agency
	Group photography
10:30 – 11:00	Coffee/tea break
Session 1	**Overview** Chairperson: Dr Muhd Noor Muhd Yunus, FNCA Coordinator of Malaysia/Deputy Director General (Research & Technology Development Programme), Malaysian Nuclear Agency
11:00 – 11:30	Strategies and Technologies in Enhancing Sustainable Agriculture YBhg. Dato' Dr. Sharif Haron, Deputy Director General (Research), Malaysian Agricultural Research and Development Institute (MARDI)
11:30 – 12:00	Nuclear Technique for Sustainable and Productive Agriculture Dr. Sueo Machi, FNCA Coordinator of Japan,
12:00 – 12:30	Use of Agricultural Chemicals and its Impact on the Environment Dr. Mohd. Khanif Yusop, Professor, Faculty of Agriculture, Universiti Putra Malaysia (UPM)

12:30 – 14:00	Lunch Break
Session 2	**Mutation Breeding I** Chairperson: Dr. Khairuddin Abdul Rahim
14:00 – 14:20	World Success and Prospect of Mutation Breeding Dr. Hirokazu Nakai, Project Leader of Japan, Prof. Emeritus of Shizuoka University
14:20 – 14:40	Success Story of Mutation Breeding in Malaysia Dr. Rusli Ibrahim, Project Leader of Malaysia, Malaysian Nuclear Agency
14:40 – 15:00	Molecular Genetic Features of Induced Mutations in Rice and Soybean Dr. Shu Qingyao, Project Leader of China Professor, Zhejiang University
15:00 – 15:20	Success Story of Development of High Yield Rice Variety Grown in Vietnam Ms. Dao Thi Thanh Bang, Institute of Agriculture Genetics (AGI), Vietnam
15:20 – 15:40	Coffee/tea Break
Session 3	**Mutation Breeding II** Chairperson: Dr. Rusli Ibrahim
15:40 – 16:00	Development of Disease Resistant Banana by Mutation Breeding in the Philippines Dr. Damasco Olivia, University of the Philippines Los Banos (UPLB), The Philippines
16:00 – 16:20	Success Story of Mutation Breeding on Sorghum for Improved Drought and Acid Soil Tolerance in Indonesia Dr. Soeranto Human, National Nuclear Energy Agency (BATAN), Indonesia
16:20 – 16:40	Radiation Breeding and Commercial Mutant Varieties in Korea Dr. Si-Yong Kang, Project Leader of Korea Korea Atomic Energy Research Institute (KAERI), Korea
16:40 – 17:00	Advanced Techniques for Mutation Breeding by Using Ion Beam Dr. Atsushi Tanaka, Japan Atomic Energy Agency (JAEA), Japan

Wednesday, 27 February 2013	
Time	**Agenda**
Session 4	**Mutation Breeding III** Chairperson: Dr. Atsushi Tanaka
09:00 – 09:20	Success Story of Mutation Breeding in Thailand Mr. Suniyom Taprab, Project Leader of Thailand Ministry of Agriculture and Cooperatives (MOAC), Thailand
09:20 – 09:40	Success Story of Mutation Breeding Research on Rice in Bangladesh Dr. A.N.K. Mamun, Project Leader of Bangladesh Bangladesh Atomic Energy Commission (BAEC), Bangladesh
09:40 – 10:00	Towards Sustainable Development of Food & Agriculture in Asia and the Pacific - Roles and Perspectives of RCA Dr. Liu Luxiang, Lead Country Coordinator of RCA Mutation Breeding Project, Chinese Academy of Agriculture Sciences (CAAS), China
10:00 – 10:30	Coffee/tea break
Session 5	**Nuclear Technique for Soil Management for Sustainable Agriculture** Chairperson: Dr. Liu Luxiang
10:30 – 11:00	Development of Plant Growth Promoter and Plant Elicitor by Radiation Processing of Natural Polymers Dr. Kamaruddin Hashim, Malaysian Nuclear Agency
11:00 – 11:30	Bio-fertilizer Production Using Radiation Technology for Sustainable Agriculture Dr. Khairuddin Abdul Rahim, Malaysian Nuclear Agency
Session 6	**Collaboration and Technology Transfer** Chairperson: Dr. Kamaruddin Hashim
11:30 – 12:00	Breeding of Roselle (*Hibiscus sabdariffa* L.) through Induced Mutations Dr. Mohamad Osman, Professor, Universiti Teknologi MARA (UiTM)
12:00 – 12:30	Radiation and Nuclear Technology for Crop Improvement and Sustainable Agriculture Mr. Ragu Ponusamy, Federal Land Consolidation and Rehabilitation Authority (FELCRA), Malaysia
12:30 – 13:00	Potential of Biofertilizer in Agroindustry: MYAGRI's Experience in Commercialization Mrs. Hayati Taib, Malaysian Agri Hi-Tech (MYAGRI)

13:00 – 14:30	Lunch break
Session 7	**Panel Discussion**
14:30 – 16:00	**Challenges of Application of Mutation Breeding and Nuclear techniques for Food Security and Sustainable Agriculture** Chairman: Dr. Muhd Noor Muhd Yunus, FNCA Coordinator of Malaysia Panel members: Invited Speakers and Experts **Points of Discussion** 1. Contamination of environment by excess use of chemical in agriculture 2. Importance of nuclear techniques for sustainable agriculture 3. Organic agriculture and acceptance of consumers
16:00 – 16:15	Coffee/tea break
16:15 – 16:30	Closing Speech Dr. Muhd Noor Muhd Yunus, FNCA Coordinator of Malaysia Dr. Sueo Machi, FNCA Coordinator of Japan

1. Overview

1.1 Strategies and Technologies in Enhancing Sustainable Agriculture

Sharif Haron
Malaysian Agricultural Research and Development Institute

Sustainable agriculture as defined by United States Department of Agriculture (USDA) is an integrated system of plant and animal production practices having a site-specific application that will, over long-term satisfy human food and fiber needs; enhance environmental quality and the natural resource base upon which the agricultural economy depends; make the most efficient use of non-renewable and integrate, where appropriate, natural biological cycles and controls; sustain the economic viability of farm operations; and enhance the quality of life for farmers and society as a whole. The four basic criteria of sustainable agriculture are (1) ensuring basic nutritional requirements, (2) providing durable employment and decent living to farmers, (3) maintaining productive capacity of natural resources and, (4) reducing vulnerability of agriculture to adverse natural and socio-economic factors. Sustainable agriculture needs to fulfill the current and future needs in economy (agriculture products, supply adequate and safe foods, economic growth), social (food security, provide employment opportunities, culture) and environment (no pollution, mitigate climate change, conserve biodiversity).

The challenges facing the Malaysian agriculture are daunting. The challenges are increase in population and cost of production to sustain food production; pollution, reduction in biodiversity and agro-waste utilization in maintaining environmental sustainability; lack of suitable land for agriculture, water scarcity and competition for use, labor unavailability; change in consumer trends such as healthy lifestyle and searching for natural products; and climate change where there will be longer dry season, shorter rainy season with intense rain, new pest and diseases and release of green-house gas (GHG).

Malaysia recognizes the challenges and the need to find solutions to ensure sustainability in its agriculture endeavor. Strategies to enhance sustainable agriculture have been formulated and these include:

a) Putting policies in place such as the Food Security, Green Technology and Renewable Energy policies,
b) Ensuring wide application of good agricultural practice (GAP),
c) Rational use of natural resources in environmentally and socially harmonious manner e.g. low water consumption,
d) Diversification of food resources e.g. encourage consumption of food availability locally, less dependency on certain staple food,
e) Enhancing R&D and use of appropriate technologies (both preventive and curative) for current and future needs e.g. agro waste for recycling, integrated pest management, adaptation and mitigation to climate change including reducing GHG gas emissions,

new crop varieties and animal breeds, precision farming for optimum inputs, optimizing land use, filling information gaps in organic farming, employing ICT tools, use of herbal alternatives in livestock production, managing peat land, highland horticultural, rice ecosystems and livestock production

f) Wide application of community practice,

g) Greater need for capacity development and extension of expertise on the ground,

h) Enhancing awareness on importance of environmental and agricultural sustainability e.g. through early education, constant and impactful campaign.

Taking into considerations the issues and challenges in ensuring agriculture sustainability, greater emphasis were given to develop technologies that could manage available natural resources efficiently and maximize productivity, in anticipation of the potential scarcity in the future. The following are some of the technologies that have been developed to enhance sustainable agriculture in Malaysia.

(1) Technologies for sustainable food production

The emphasis is to meet sufficiency level in various commodities (rice, vegetables, fruits and livestock). This will be met only through increase yield per unit area by creating new high yielding and quality varieties and breeds, efficient cropping systems and post-harvest handling as well as innovative production technology. In the case of rice, recently new paddy varieties (MR 253, MR 263, MR 269) with high yield and resistant to major pest and diseases have been developed. Two varieties under the Clearfield System, MR 220 CL1 and CL2 have been developed to overcome losses due to weedy rice infestation.

(2) Technologies for environmental sustainability

Malaysia is richly endowed with a myriad of natural fauna and flora that has been utilized as genetic resources as well as biological control agents against pests and pathogen problem in economic crops. It must also be acknowledged that abundant agricultural waste is available and this has been turned into beneficial crop booster and bio-fertilizer. Technologies that are developed to address the issue of environmental sustainability includes organic farming, zero waste production system and energy cycling, virus-based bio-pesticides, crop diversity for beneficial insects, tetra-cropping, bio-fertilizer based on microbial consortium, fungal based bio-herbicides, bacteriophage based bio-herbicides, botanical pesticide, solar drying and biogas.

Environmental-friendly virus based bio-pesticides (containing multi nucleopolyhedrosis virus or NPV) for controlling armyworm, *Spodoptera litura*, are found stable at room temperature, and have longer shelf life. Whereas, fungus based bio-herbicides are developed specifically for controlling 'rumput sambau' (Echinochloa spp.) in rice.

Tetra-cropping maize with chilli has helped reduce virus disease and its vector on chilli. Study has shown that the percentage of virus disease incidence was much lower when chilli

(C) was planted with maize (M), okra (O), eggplant (E) in the order of MCEOMCEO compared to tri-cropping, MCEMCEMC, MCOMCOMC or di-cropping, MCMCMCMC and mono-cropping, CCCCCCCC (60%, 60%, 80% and 100%, for tetra-, tri-, di- and mono-cropping, respectively).

Evaluation for potential bio-pesticide was made on selected Malaysian plants, *Andrographis paniculata* (hempedu bumi), *Cymbopogon spp.* (serai), *Pelargonium radula* (jeremin), *Annona spp.* (durian belanda), *Citrus spp.* (limau purut) and *Dioscora spp.* (ubi gadung). Oil formulations from *Pelargonium radula* and *Cymbopogon citratus* are found to be potential bio-pesticidal agents against *Plutella xylostella* (diamond-black moth).

(3) Technologies to overcome lack of resources

Food-crop agriculture productivity is basically limited due to shortage of suitable fertile land and as such it relies heavily on fertilizer input. Application of information and communication technology (ICT) via precision farming including through the use of unmanned aerial vehicle (UAV) has helped in mapping the farming areas, define the areas precisely for fertilizer application. Subsequently, it will help in increasing plant nutrient uptake and reduce fertilizer wastage and water pollution. Similarly technologies like hydroponics and fertigation whether applied in open or in green house, are also practiced.

(4) Technologies for mitigation and adaptation to climate change

Climate change has become as an emerging concern to the agricultural sector needing immediate solutions. New genotypes and breeds tolerant to multiple stresses: drought, flood, heat, pest and diseases, will help further increase in production. This would require substantial efforts through breeding and biotechnology based on collection, characterization, conservation and utilization of new genetic resources and also marker assisted technology (MAS). In Malaysia, for instance, new short maturing variety MR 211 with 100 days maturation has been developed to adapt to the climate change. In addition to that, aerobic rice production system which utilizes only half of the usual water requirement (470-650 vs. 1130-1300 mm/season) but yet giving reasonable yield (4.0 – 5.3 vs. 5-7 ton/ha) has been developed as part of a strategy to climate change.

In conclusion, R&D and technologies will continue to play a crucial role in sustainable agricultural development. There is a need for enhanced collaborative research among all parties involved to create synergies and symbiosis. New tools are required to assess sustainability, evaluate resource appropriation and production performance as well as ensure environmental wellness. The challenge is in re-designing agro-food systems in the context of promoting a holistic participatory approach, extending them to farmers for adoption, addressing the issue of self-sufficiency, and conserving our resources and environments for future generations.

1.2 Nuclear Techniques for Sustainable Agriculture

Sueo Machi
Fellow, Japan Atomic Energy Agency

1. Introduction- Nuclear technology for food security

"Food for all" is the main challenge of our society. We need to decrease number of people suffering from hunger. Nuclear technology can play an important role for addressing the challenge through soil and water management, food irradiation to reduce post-harvest loss, sterile insect technique to reduce pesticide use, and mutation breeding for better crop yields.

Sustainability of agriculture is another challenge for next generation. Nuclear techniques can also provide better technology which is more environmentally friendly.

Global warming continues because of increasing emission of CO_2 from burning fossil fuels due to rapid development of the world economy. The adverse effect of global warming on agriculture is serious concern. Mutation breeding may be able to develop new varieties which are tolerable to increased temperature.

2. Application of nuclear techniques for improving soil management for agriculture

(1) Biofertilizer:

Inoculants using microorganism such as Rhizobium for fixing nitrogen from air and Mycorrhizafor solubilizing phosphate can remarkably increase crop yield in the range of 20 to 70%. It has been proved that radiation sterilization of carriers of microorganism can produce better quality inoculants and provide effective and larger scale production process.

This development has been carried out by collaboration among Asian countries under the Forum of Nuclear Cooperation in Asia (FNCA) [1]. IAEA has a success story of Rhizobium biofertilizer application in Zimbabwe for grain legumes. Application of the biofertilizer can increase crop yield and can reduce the risk of contaminating ground water by excess use of chemical fertilizer. In Malaysia, Indonesia and the Philippines, the radiation sterilization of carriers for bio-fertilizer inoculants has recently been commercially used. Development of new mutant strains of microorganism using radiation mutation for better and multifunctional biofertilizer is being studied.

(2) Recycle of sewage sludge to soil

Sewage sludge contains nutrient and organic matter which improves quality and fertility of soil. The sewage sludge, therefore, should be recycled to soil to improve quality of soil for sustainable agriculture. Since the sewage sludge is, however, contaminated by pathogens such as salmonella and E-Coli, radiation can be effectively used to sterilize it before application. This technology is commercially used in India and used to be used in Germany in the past. There is a concern about heavy metals contained in the sludge. In many cities industrial waste water is separately treated from that of households of which sewage sludge is free of heavy metals. Recycling sewage sludge should be much more enhanced [2].

(3) Plant growth promoter (PGP) from natural polymers:
Development of production of PGP by radiation degradation of natural polymers has been achieved by collaboration of member countries under FNCA. Oligo-chitosan produced from crab/shrimp shell and oligo-carageenan from seaweed by radiation degradation show excellent effect in increasing crop yields (10-50%) for red chili, rice, tomato, potato, carrot and others by pot and field tests in Vietnam, Malaysia, Philippines, Indonesia, Japan and Thailand [3], [4]. In Japan oligo-chitosan has been recently commercially produced as elicitor for flowers. The PGP from natural polymers are environmentally friendly in terms of reducing the use of chemical fertilizer and pesticide.

(4) Super water absorbent as soil conditioner in arid region
Agriculture in arid and semi-arid region should be enhanced for better food security. Super water absorbent (SWA) produced by radiation cross-linking and/grafting of natural polymers such as starch can absorb large amount of water (SWA of 1 g. absorbs 400 grams of water). The SWA is mixed in soil to retain scarce rain and irrigation water in surface soil much longer for plant to use water efficiently in arid and semi-arid region [5]. Field tests in Vietnam prove the increase in crop yields as shown in Table 1.

Table 1: Field Tests of SWA in Vietnam

Amount of SWA: 30kg per ha Plant	Yield increase (%)
• Corn	28.8
• Peanut	21.1
• Cotton	31.1
• Coffee	23.0
• Grape	29.8
• Sugarcane	19.8

3. Sterile insect technique (SIT) by using radiation
Insect pests are reducing harvest of crops by 30%. In order to reduce number of pests, large amount of chemical pesticides is used worldwide which seriously pollutes environment and damages bio-diversity.

SIT can eradicate and/or control insect pests by the use of much reduced amount of pesticide. For SIT puppet are irradiated to be sterile. Large numbers of sterile adult flies are released to natural environment and mate with natural female, which lay eggs but these eggs can't merge. Therefore, the population of insect pest decreases generation by generation. IAEA has well known success stories, such as eradication of tsetse flies in Zanzibar Island in Tanzania.

Very large campaign of control of tsetse flies in Ethiopia is being implemented by collaboration of African Union with IAEA. Several IAEA projects have been successfully implemented for eradication or control of Mediterranean fruit flies in member states.

Japan has succeeded to eradicate melon flies in Okinawa Island around 1990 after 5 year campaign. Now, fruits and vegetable from Okinawa can be exported to the main land of Japan.

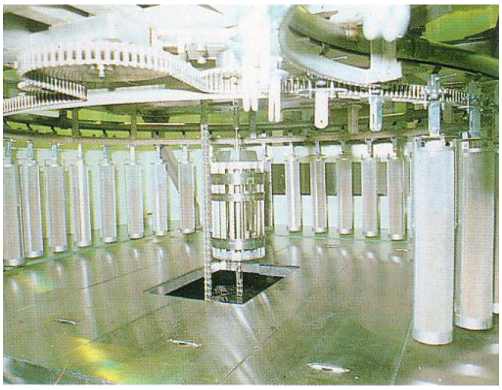

Figure1. Co-60 irradiation facility for puppet of melon flies in Okinawa

4. Mutation breeding

Mutation breeding is one of the most important nuclear technologies for improving agriculture in terms of yield increase and sustainability.

Radiation is the most efficient way to induced mutation of plant to improve character of plants. In the history more than 3000 better varieties of plants were developed and registered by using radiation mutation.

Under the FNCA project, recently, new variety of banana has been developed in the Philippines, which is better resistant against Bunch Top Virus disease. By using this variety farmers can save pesticide, which contributes to the protection of environment from pollution by pesticides. The FNCA project on mutation breeding is now focusing on the development of new rice varieties for sustainable agriculture.

5. Food irradiation

Food irradiation for food safety, quarantine for expert, and decrease in food loss by spoilage, sprouting and insect attach is expanding worldwide in 57 countries. As shown in Table 2 the world total amount of irradiated food for commercial purpose is about 500,000 tons and increasing. However, in some countries including Japan public acceptance of irradiated foods is still obstacles for expansion of food irradiation.

It should be recognized that post-harvest loss of crops is in the range of 40% in many developing countries. Food irradiation technique should be much more used to decrease post-harvest loss due to spoilage, sprouting, insect and fungus attack.

Table 2: Amount of Commercial Food Irradiation

World total of irradiated foods	ca. 500,000 ton
China: Garlic, Dried vegetables, etc.	146,000 ton
Vietnam: Frozen shrimps, etc.	14,000 ton
Japan: Potatoes	8,000 ton
USA: Spices, Ground meats, Fruits	92,000 ton
Ukraine: Wheat grain	70,000 ton
Brazil: Spices, Fruits	23,000 ton
South Africa: Spices	18,000 ton
Belgium: Spices, Frozen chicken	7,000 ton
Others:	ca.120,000 ton

Conclusion

Food security can be improved by increasing crop productivity by using nuclear techniques, such as mutation breeding, food irradiation, sterile insect technique, better soil management, and new radiation produced plant growth promoter and super water absorbent.

References

[1] FNCA, http://www.fnca.mext.go.jp/english/

[2] IAEA., Irradiated Sewage Sludge for Application to Cropland, IAEA-TECDOC-1317, Vienna (2002) 238 p.

[3] http://www.fnca.mext.go.jp/english/eb/e_ws_2011_3a.pdf

[4] H. Kudo, F. Yoshii and M. Tamada, "FNCA guideline on development of hydrogel and oligosaccharides by radiation processing", *JAEA-Technology* 2009-050(2009)

[5] N. Nagasawa, T. Yagi, T. Kume and F. Yoshii, "Radiation crosslinking of carboxymethyl starch", *Carbohydrate Polymers,* **58**(2), 109-113 (2004)

2. Mutation Breeding I

2.1 World Success and Prospect of Mutation Breeding

Hirokazu Nakai
Professor Emeritus, Former Vice President, Shizuoka University

Mutation breeding has been established as one of the most important positions of breeding method, contributing to the welfare of human being in these past about 75 years. Studies on induced mutations in barley were started by Stadler in 1928, following Muller (1927) who found the usefulness of radiations to induce mutations in the experiments using fruit fly. In these years, a lot of fruits have been obtained from both practical and fundamental points of view through the studies on mutation breeding.
In the present paper, the fruits of mutation breeding were briefly reviewed and the prospect or new possibilities of mutation breeding were discussed as follows. (1) Fruits of mutation breeding in the world and Asia. (2) Development of new genes for bacterial leaf blight (BLB) resistance through the use of thermal neutrons. (3) Breeding of rice for adaptability to low input sustainable agriculture and resistance to BLB.

(1) Fruits of mutation breeding in the world and Asia

Total number of the mutant varieties released is at least 3212 according to FAO/IAEA Data base in 2011(researched by A. Tanaka)(Fig. 1). It notes that the mutation breeding would contribute to production of the most main crops in the world, e.g. rice, barley, wheat, maize, soybean. In addition, it is recognized from the table that Asia is the center of mutation breeding in the world. As for mutagens used for the mutation breeding, gamma-ray is accounted for more than 60 % of all the mutagens used. This data is reasonably thought, because gamma-ray was convenient to be treated. It is also noted that the varieties from ion-beam (high LET), are amounting to about 6 %. This data suggest that ion-beam or high LET radiations would be useful for mutation breeding in the future.
Activities of the Mutation Breeding Project promoted by FNCA (Forum for Nuclear Cooperation in Asia) are shown in Fig.2-Fig.5. The project was started in 2002, with the subject “Drought tolerance in Sorghum and Soybean (2002-2006), followed by the subjects “Insect Resistance in Orchids (2003-2009), “Disease Resistance in Banana (2004-2010) and “Composition or Quality Improvement in Rice (2007-2012). We are starting new subject “Mutation Breeding of Rice for Sustainable Agriculture” for the next five years (2013-2017) (Fig. 2). The most economically important crops for Asia were treated in the sub-project of FNCA. In orchid, mutants resistant to both mites and thrips were induced by gamma-ray and ion-beam (Fig. 3). It was noted that disease resistance was gained through the use of gamma-rays and ion-beams, respectively, against Banana Bunchy Top Virus (BBTV) and fusarium wilt disease in banana (Fig. 4). The mutants in banana and orchid were successful in technology transfer for commercial use. It was also found effective to improve quality or composition in rice (Fig. 5). It was proofed that semi-dwarf mutants could be easily obtained through induced mutations. In case of the conventional cross breeding, semi-dwarf gene

sources of rice are very limited; a majority of the genes for the semi-dwarf varieties developed in the modern breeding of rice, e.g." green evolution", have been found to be derived only from the gene of variety, Dee-geo-woo-gen. Also, a lot of varieties of crops, fruits and flowers which are economically valuable have been reported from Japan (Fig. 6).

(2) Development of new genes for bacterial leaf blight (BLB) resistance through use of Thermal neutrons

BLB caused by *Xanthomonas campestris pv. oryzae* is one of the most serious diseases in tropical Asian countries. This part is dealt with the durable or non-race-specific resistance to BLB induced by exposure of thermal neutrons to rice seeds[1, 2, 3]. It was found that the frequency of the resistant mutants was significantly higher in thermal neutrons and ion-beam than in gamma-rays and some chemical mutagens (Fig. 7, Fig. 8) [4]. This result suggests effectiveness of high LET radiations to be used for induction of mutants resistant to diseases. The mutants obtained were resistant to all the differential BLB races from Japan and the Philippines (Fig. 9) [3]. The M95 mutant was especially noted because of the high level of resistance or of complete one. The gene of M95 was estimated to be located at a part of the edge on the chromosome 1. This type of gene has never been found in the gene sources concerning BLB resistance. However, the M95 mutant carrying the durable resistance gene could not be directly used as a variety to be released, since it was of dwarf. It was confirmed through crossing tests of M95 and the original variety, Harebare, that the genes of the resistance and the dwarf were closely linked with each other. Some recombinant plants were detected in the F_2 generation derived from the cross between M95 and Harebare, indicating that the M95 mutant could be easily used for the crossing.

(3) Breeding of rice for adaptability to low input sustainable agriculture (Nature Farming) and resistance to BLB

We started breeding of rice for adaptability to nature farming of typical low input sustainable agriculture without any chemicals including chemical fertilizer at Ohito experimental farm and rice fields of farmers located at 18 districts across Japan in 2004. Grain yields of several F8 lines in the nature farming were found to be significantly higher than that of the varieties grown in the conventional farming at every district where the selection tests were conducted (Fig. 10).

In a series of the breeding, selection experiments of the lines derived from cross of Bangladesh varieties and recombinants carrying the M95 resistance gene were conducted in Ohito Experimental Farm, aiming at development of varieties adaptable to nature farming and resistant to BLB(Fig. 11). As for F_8 lines, some of them showed high values for the main yield components, e.g. culm length, panicle length, number of panicles comparing with the parental varieties (Fig. 12). This result suggests possibility to develop varieties adaptable to nature farming with durable resistance to BLB. It needs to continue the selection experiments further at later generation.

(4) Discussion and conclusion for prospect of mutation breeding

The rich fruits obtained in the long history of mutation breeding have proofed the merit of mutation breeding in comparison with the conventional cross breeding. Namely, the merits of mutation breeding are to improve easily a target character without altering the other ones, to develop new genes without existing in natural gene sources and to improve effectively plants of vegetative propagation. In the three merits listed above, the second item would be more noted for promoting breeding in future in the serious situations of lacking gene sources on a world wide scale. We must pay attention to the usefulness of mutation breeding for developing new gene sources. Taking disease resistance for instance, resistance gene for powdery mildew of barley, ml-o (Freisleben and Lein, 1942) is especially noted as typical durable resistance gene [5, 6, 7].

The function of the resistance gene has not yet been destroyed by any races of the disease for more than 70 years. M95 gene for resistance to BLB developed through use of thermal neutrons (Nakai et al., 1991) is similar to the ml-o gene. However, ml-o mutants and the M95 mutant could not be directly but indirectly used, because the gene is closely linked or pleiotropic to dwarf or to flecking of leaves [6]. Usefulness of indirect use of the induced mutants should be much more considered for the future mutation breeding.

We are proposing new theme, "Mutation Breeding of Rice for Sustainable Agriculture" for next five years in FNCA Mutation Breeding Project. There might be two approaches to complete the breeding purpose. One is to get the resistance to environmental stresses e.g. diseases, insects, drought, heat, coldness and so forth. The other is to obtain the adaptability to low input sustainable agriculture, i.e. breeding of rice varieties with ability for higher yield in the low input conditions. The results of the author's studies or practical trials presented in this paper as well as the fruits obtained in the history of mutation breeding would be promising success for these two main approaches. As for the latter approach, it was made clear in the present studies that the adaptability to low input sustainable agriculture might be genetically controlled [8, 9, 10]. Therefore, it can be stressed that there would be a great possibility to improve effectively the adaptability through mutation breeding, contributing to sustainable agriculture. However, scientific data are not enough on this matter at this time, and further fundamental studies are needed.

It is concluded that mutation breeding would be promising for contribution to solve the problems of food and environments human beings are now encountering.

Researched by A. Tanaka (FAO/IAEA Database, October 2011)

Country	No. of cv. Total	Rice	Barley	Wheat	Maize	Soybean	Chrysanthemum
All countries	3212	815	304	252	89	170	277
China	808	290	7	162	47	79	21
Japan	481	222	10	7	0	30	56
India	329	59	13	4	0	7	46
Russia	215	6	29	36	5	9	17
Netherland	176	0	1	0	0	0	80
Germany	171	0	66	2	0	1	34
USA	139	36	13	4	0	0	1

Bangladesh	44
Indonesia	29
Korea	35
Pakistan	53
Thailand	20
Viet Nam	55

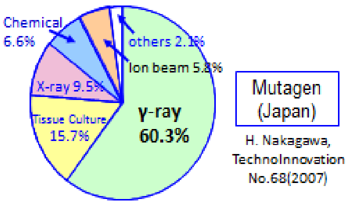

Figure 1. Mutation breeding in the world and Asia

Figure 2. FNCA mutation breeding project

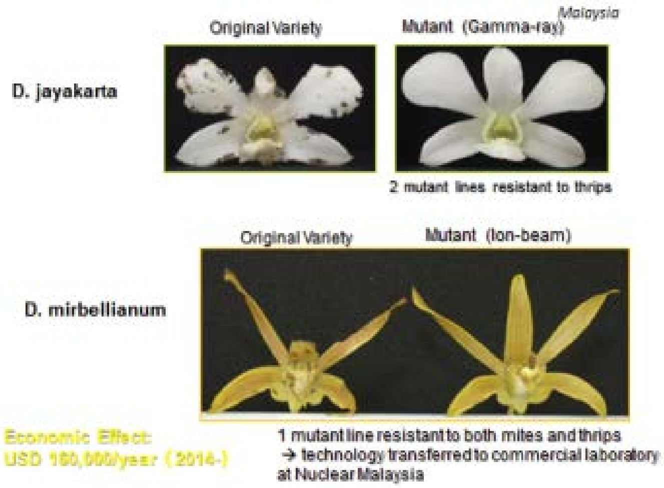

Figure 3. Insect resistance to orchid (2003-2009)

Figure 4. Disease resistance in banana (2004-2010)

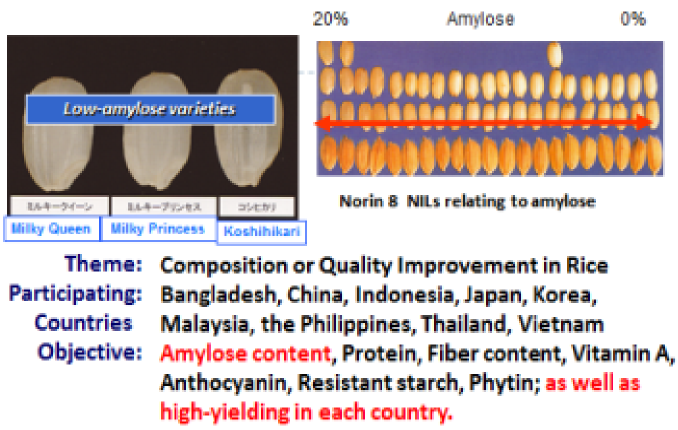

Figure 5. Composition or quality in rice (2007-2012)

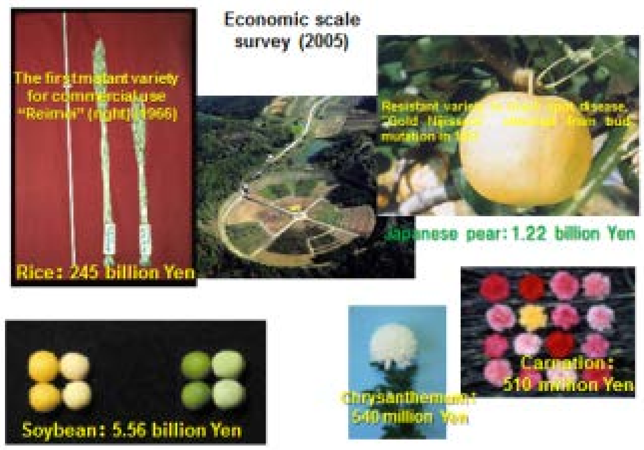

Figure 6. Composition or quality in rice (2007-2012)

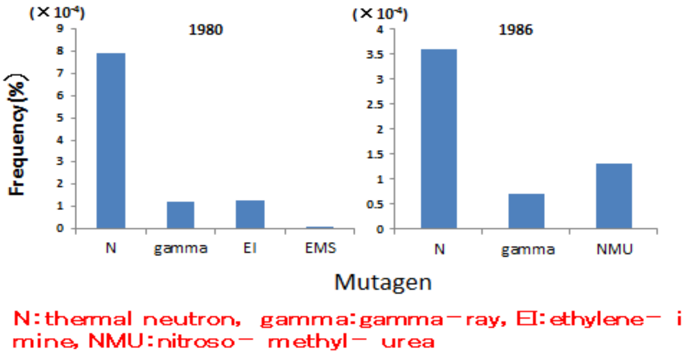

Figure 7. Effects of mutagens use for induction of BLB resistant mutants

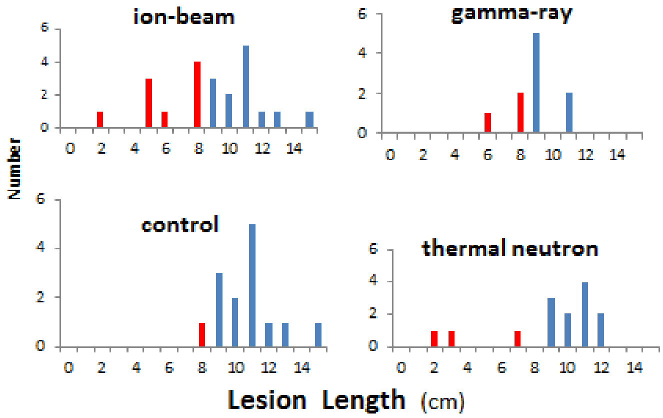

Figure 8. Frequency of BLB resistant M_3 lines of BLB resistant mutants in each mutagen

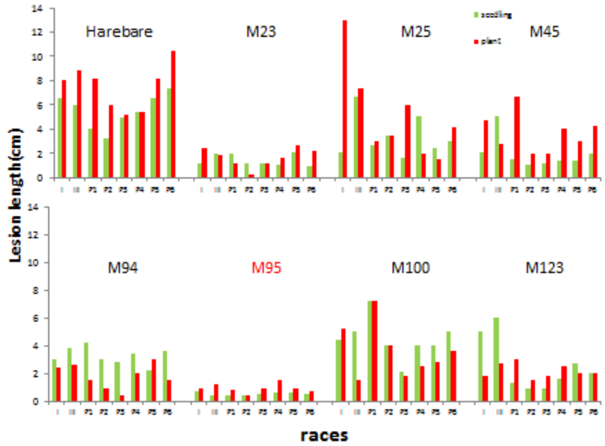

Figure 9. BLB resistance reactions of the mutants to the differential races (Okayama, 2011)

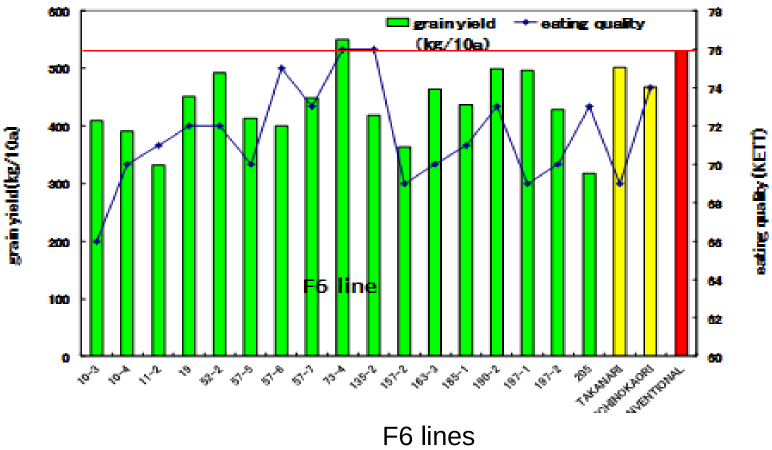

Figure 10. Grain yield of F_6 lines from the cross of Takanari x Aichinokaori

cross combination	Year of trials 2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
IRATOM24 x RS1	cross	F1	F2	F3	F4	F5	F6	F7	F8	F9
		8	1000	72	11	11	11	12	11	11
BINADAN5 x RN5		cross	F1	F2	F3	F4	F5	F6	F7	F8
			23	912	36	11	15	14	14	14
x RT3			9	1485	75	19	20	20	20	20
x RO5			5	500	21	6	10	9	9	9

IRATOM24, BINADAN5 are from Bangladesh, RS1:M95 x Sinriki, RN5:M95 x Takanari,
RO5:M95 x Kamenoo, F1 and F2 : No. of plants, F3- : No. of lines

Figure 11. Breeding of rice for adaptability to low input sustainable agriculture (Nature Farming) and for resistance to BLB (at Ohito Farm)

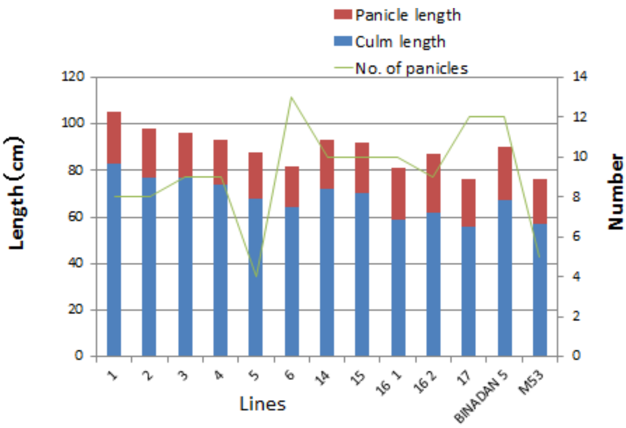

Figure 12. F_8 lines from cross of Binadan 5 x the recombinant with M95 gene for BLB resistance

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2.2 Success Story of FNCA Mutation Breeding Project in Malaysia

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Introduction

In conventional crop breeding, promising lines are identified only by their phenotypes. Apart from being time consuming, conventional breeding is also capital and labor intensive. Innovative breeding techniques, such as the use of chemical and physical mutagens for the induction of mutations and molecular marker-assisted breeding, are required to break through.

Plant breeding requires genetic variation of useful traits for crop improvement. Often, however, desired variation is lacking. Mutagenic agents, such as radiation and certain chemicals, then can be used to induce mutations and generate genetic variations from which desired mutants may be selected. Mutation induction has become a proven way of creating variation within a crop variety. It offers the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evolution. When no gene, or genes, for resistance to a particular disease, or for tolerance to stress, can be found in the available gene pool, plant breeders have no obvious alternative but to attempt mutation induction.

Identification of an efficient screening approach is essential in a mutation breeding approach since a high mutation induction rate, followed by a poor screening approach could result inefficiency and ultimately project failure. In general, selection of a trait, such as resistance to diseases, it is recommended to screen more than 1,000 plants (sometimes more than 10,000 plants) in the field or in a greenhouse to achieve the ultimate objectives. Following selection of potential genotype with desired traits, it is important to verify if the selected trait is heritable. In order to assess the heritability of a newly identified mutation, it will be necessary to conduct multi-location trials for a minimum period of 2-3 generations.

Orchid is one of the largest families of flowering plants in the world comprising of an estimated 20,000 to 25,000 species and untold number of hybrids of some 700 different genera (Zaharah & Rozlaily, 1991). They can be found growing at almost every latitude and stratosphere, where plants can normally grow. The range of different characteristics in shapes, sizes and colors have made orchids the plant for all types of interests and sought for by collectors. *Dendrobium* orchids have been the major orchid cut-flower export for Malaysia as well as for other Southeast Asian countries like Thailand and Philippines. Thailand is the major orchid producer with an export value of US$ 80 million in 2006, or approximately 80% of the country's total ornamental export value. The export value of orchids from Malaysia, which is mainly in the form of cut flowers, is estimated at RM 150 million per year, representing approximately 40% of the total floriculture production. Japan is the main importer for orchids from Malaysia (www.tiois.doae.go.th).

The successes of hybridization technology in producing vast number of orchid hybrids with attractive characteristics and the introduction of the *in vitro* technology to mass-propagate clonal planting materials have been a tremendous boost for the orchid growing industry. Wide range of successful cultivars with attractive combinations of spray length, bud number, flower color and form, vase life, fragrance, seasonality, and compactness have been produced through hybridization. At the same time, tissue culture has been widely used as the standard method of germinating seeds and propagating seedlings for the industry. Through the meristem cloning technique, thousands of plants can be cloned and grown in a relatively short period of time. The combinations of these two technologies have so far been very reliable in supporting the orchid industry in the region. Commercially attractive hybrids and varieties have been able to be mass propagated and supplied to the growers for the market.

Although new orchid hybrids are consistently released every year to meet the current demand by customers, the only problem that remains unsolved in this industry until today is the problem of insect infestations. Insect infestations in the orchid flowers have caused a lot of losses not only to growers but also exporters due to strict quarantine regulations. In fact, since 9th Malaysia Plan, government through Agriculture Department has put an emphasis on the production of new cut flowers which are resistant to pests and diseases (Mohd Khuzairi 2006). For export, sometimes the presence of even one insect can cause the whole export consignment to be rejected by importing countries. There were cases when the whole orchid consignment had to be shipped back to the exporting countries due to the presence of insect pests. Unfortunately, none of the commercial varieties released so far especially cut flower varieties, has been resistant to insect pests. Growers normally have to rely heavily on pesticides to curb the infestation. Based on visits and communications with local commercial growers, we found out that pesticides are applied on average twice a month, and sometimes once a week if the infestation is serious.

Within the last ten years, banana has become an important commodity in the fruit industry and popular varieties such as Cavendish and Berangan (local cultivar) had been widely planted on commercial scale for both local and export markets. Banana is one of the most important fruit crops grown in Malaysia. Its economic potential for export, especially to China is enormous. Currently, banana production covers a wide spectrum of systems ranging from the small plot subsistance farming and local market supply to plantations producing quality fruits for export. In the world market, Cavendish still dominates because of it's superiority in terms of fruit size and yield. However, a few popular local cultivars such as Berangan, Mas and Rastali which have unique taste, are potential alternative to Cavendish. One of the major set back of banana industry in Malaysia is due to low yield of local cultivars compared to

Cavendish. They are generally tall and have some other undesirable agronomic characteristics such as thick skin and small fruit size.

Banana cultivation possesses good potential for expansion due to strong demand as a table fruit and also for downstream activities. Banana cultivation very much supports the current National Agriculture Policy (NAP3 1998-2010) which stresses on maximizing land use, increased private sector involvement, increased farmer's income and export earnings. It is ideal for intercropping with plantation crops such as oil palm and coconut. This is important since more than 150,000 ha of oil palm are expected to be replaced yearly for the next few years.

In Malaysia, the most serious constraint to the production of banana and plantains is considered to be *Fusarium* wilt disease caused by *Fusarium oxysporium* f. sp. *cubense*, a soil-borne disease which affects many important cultivars of banana and plantain. The disease has caused serious crop losses in Malaysia. Most of the cultivated clones originated as spontaneous variants are highly susceptible. Chemicals can be used to control many of the pests and diseases (not effective against Fusarium), but as the costs, both economically and environmentally, continue to rise, the need for resistant cultivars as the main component of an integrated system for pest management, becomes imperative. A genetic approach to an integrated pest management scenario, is environmentally sound for long term production.

Breeding for disease resistant banana cultivars using classical breeding methods remains a difficult task because of the high sterility and polyploidy of most edible cultivars besides long generation times. Therefore, the efficiency of classical breeding may be improved by incorporating *in vitro* mutagenesis to generate genetic variability and biotechnological approaches including molecular techniques. The use of shoot-tips, male flowers and suspension cell cultures in mutation breeding of banana has offered several advantages over conventional in vivo techniques.

The use of ion beam and chronic gamma irradiation should be exploited fully in order to achieve the above objectives. Research activity had been focused mainly on screening and selection for mutants tolerance or resistance to *Fusarium* wilt disease using both popular local cultivars and Cavendish types of banana. Therefore, the main objectives of this research project is to use in vitro mutagenesis using gamma rays in order screen and select for *Fusarium* wilt disease resistance in Pisang Berangan with improved agronomic traits such as high yield, early flowering and short stature.
Rice is the staple food crop in Malaysia. In Peninsula Malaysia, rice production depends largely on the irrigated lowland production system. Currently there are 241,741 ha of irrigated rice in Peninsula Malaysia which contributes more than 85% of the national rice production. However, growing irrigated rice requires large amount of water. It was estimated that about 3,000 liters of water are required to produce 1 kg of rice. Unfortunately, there are

signs of declining water supply that threatens the sustainability of irrigated rice production.

Malaysia managed to achieve 72% self-sufficiency level in rice with the current average rice yield of 3.7ton/ha/season. In this situation, about 28% of the local demand will have to depend on rice imports. However, exporting countries were not able to fulfill our rice requirement as they also have to ensure enough supply for their own domestic use. Rice is the staple food and rice production consumes about 50% of the fresh water resources in Malaysia. In addition, drought is one of the most important constraints in rice resulting in large yield losses and limiting the average yield increase of the country.

There is an urgent need to enhance water-saving capacity or drought resistance of rice. New varieties of rice with minimal water requirement can be developed by incorporating the water-saving and drought resistance capacity mainly from the traditional upland to the commercialized paddy rice cultivars. The breeding target is a high yield potential under irrigation, an acceptable grain quality, and water consumption reduced by about 50% compared with paddy rice. In a water-limited environment, a higher level of drought resistance and reduced yield loss by drought stress are required.

Plant breeding in conjunction with optimized soil nutrient and water management practices is probably the most viable approach to stabilizing food production. Water shortage has become the bottleneck of Malaysia's food security. The development of water-saving rice varieties to decrease water consumption in rice production is inevitably a major goal in agriculture research. Thus, to achieve long-term food security and sustainable development in Malaysia, 'Water-saving or drought-resistance' (WDR) rice varieties are urgently needed.

Several rice cultivars with a yield potential of over 10 t/ha in demonstration tests were developed through conventional breeding. However, this high yield potential could not be achieved in the large-scale production in the farmer's fields. The main reason is the quality of Malaysia's paddy fields which cannot meet the growing conditions required for current high-yielding rice. Drought is one of the most important limiting factors in more than 65% of paddy fields in Malaysia where super rice varieties cannot perform well under drought stress. Therefore, the development and production of drought-tolerant rice varieties, to stabilize and improve the production levels in the low-middle-yielding fields, is needed. In recent years, the field drought-resistance screening facility was established through mutation breeding program and the evaluation standard was developed. Some advanced lines of drought tolerance rice varieties were identified and will be used in both molecular mapping and breeding programmes. The objectives of this study, therefore, were to screen for advanced mutant lines derived from gamma irradiation for high yield potential and stability under water stress conditions.

Induction of insect resistance in Orchids

This project was a collaborative FNCA project involving Malaysia, Thailand, Indonesia and Japan. The objective was to jointly produce new orchid mutants that resistant/tolerant to insect infestation. It focused on commercial hybrids or species of *Dendrobium* orchids; namely *Dendrobium* Sonia 17 Red from Thailand, *Dendrobium* jayakarta from Indonesia and *Dendrobium mirbellianum* from Malaysia. In this project, tissue culture orchid materials (protocorm-like-bodies or PLBs) were exchanged among the participated countries early in the project. The Malaysian research team had irradiated these PLBs with two ionizing mutagens; gamma rays and ion beams (JAEA). Following irradiation, regenerated plantlets were randomly selected from each dose and subjected to *in vitro* infestation with mites and thrips, to analyse their resistance towards these pests. The main aim of *in vitro* infestation was to pre-select potential mutants for secondary screening at flowering stage. Potential insect resistant orchid mutants were then selected and subsequently planted in the glasshouse. Secondary screenings were carried out at flowering stage by challenge-infestation with the target insects. This project, which was completed in December 2009, has successfully generated two *D. jayakarta* mutants tolerant to thrips, and one *D. mirbellianum* tolerant to both mite and thrips. The mutants are now being propagated to achieve large number of clones, with hope to carry out pre-commercialization studies with a private collaborator(s) in a near future.

Multiplication work is being carried out for our mutants in the glasshouse. We hope to transfer some mutant samples to a private collaborator soon. At present, we have sign a Non-Disclosure Agreement and Collaborative Agreement with the collaborator (Hexagon Green Sdn Bhd) for a project on "Pre-commercialization of Mutant Orchids for Cut Flower Industry" which was financed by Ministry of Agriculture and Agro-Based Industry, Malaysia. This project will cover selected orchid mutants developed by Nuclear Malaysia, including our potential insect tolerant mutants.

DJ35Gy03 DJ35Gy11

Figure 1. *D.jayakarta* mutants tolerant to thrips

Figure 2. *D. mirbeliannum* mutant tolerant to both mites and thrips

Improvement of Bananas for Fusarium wilt Resistance and High Fruit Quality Through Mutation Induction.

Popular local banana cultivar called Berangan (*Musa sp*p. AAA) was selected as the starting material for mutation induction using gamma rays. Radiosensitivity test was carried out by irradiating meristem tissues obtained from suckers with a series of gamma ray doses of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100Gy. Data for radiosensitivity test was determined by 3 parameters, such as 1) Plant height (shoot length), 2) Percentage of survival of irradiated explants and 3) Multiplication or Growth Rate. Based on the percentage survival of irradiated explants, LD50 and LD100 obtained for cultivar Berangan were 50Gy and 80Gy respectively. Using selected effective doses of 20, 30, and 40Gy, all in vitro shoots derived from irradiated meristems were subcultured from M1V1 upto M1V5 and rooted. These rooted in vitro plantlets were used to screen for tolerance/resistance against *Fusarium* wilt disease. Four artificial screening techniques for *Fusarium* wilt disease had been developed, such as 1) Dipping of in vitro shoots in *Fusarium oxysporioum* suspension (106 spores/ml) for 1-2 hrs and later transferred to sterile sand media in the greenhouse, 2) Double tray method by inoculating in vitro plantlets that had been planted in sand media, 3) Nursery screening method, whereby rooted in vitro plantlets that had been hardened for 4-8 weeks were transferred to media that had been pre-inoculated with *Fusarium oxysporioum* spore suspension (106 spores/ml) for 2 weeks and 4) Direct field screening, whereby rooted plantlets that had been dipped in *Fusarium oxysporioum* suspension (106 spores/ml) for 1-2 hrs were planted directly in the field. Based on the results obtained after screening of treated plants using artificial inoculation of *Fusarium oxysporioum* in the field, three potential mutants had been identified, such as 1) Tolerant/resistant mutant, 2) High yielding mutant and 3) Early flowering mutant. These mutants were further screened in the field for multi-location trials.
Memorandum of Agreement with a commercial grower, Selamat Indah Sdn. Bhd. had been successfully made for the technology transfer of tissue culture technique for micropropagation of Pisang Berangan and commercialization of selected mutants.

Mutation Breeding for Varietal Improvement of Irrigated Rice under Minimal Water Conditions.

Mutants were induced in variety MR219 using gamma rays at 300 Gy to generate superior genotypes for minimal water requirement. The mutants were screened for water stress under simulated non-flooded water regime under glasshouse and field conditions. After several series of selection and fixation, 12 potential lines with the required adaptive traits were recovered at M_4 generation. However, only two potential lines designated as MR219-4 and MR219-9 were selected. The lines were evaluated under replicated yield trial in KETARA under normal flooded conditions. In KETARA, the yield of MR219-4 and MR219-9 were 4.8 t/ha and 5.5 t/ha, respectively as compared to the 5.8 t/ha of the original variety MR219. The lines were also evaluated in MADA under saturated soil and flooded conditions. The yield of MR219-4 was 5.9 t/ha under saturated soil condition and 7.2 t/ha under flooded condition. For MR219-9, the yield was 6.8 t/ha and 6.1 t/ha under saturated and flooded condition, respectively. The lines also performed satisfactorily when grown under aerobic soil condition in MARDI Seberang Perai. Grain yield as high as 6.3 t/ha for MR219-4 and 3.4 t/ha for MR219-9 were achieved under aerobic condition. In another development, submergence screening was done to the same two lines and a new adaptive trait was discovered where both MR219-4 and MR219-9 were found to be tolerant to submergence. These findings indicated that these two mutant lines are unique because they possess “dual” adaptations ie. flooded and water stress regimes. The results also indicated that tolerance to submergence in rice is associated until 8 days submergence for MR219-4 and FR13A cultivars, but mechanism in MR219-9 cultivar is associated until 12 days submergence. These findings suggested that tolerance to submergence stress in rice may be improved by increasing the capacity of antioxidative system.

Mutant MR219-4 was unique because it performed very well under saturated conditions in irrigated areas and aerobic conditions (sprinkler assisted irrigation) under dryland regime. In addition, both mutants can also tolerate submergence and therefore can be planted in flood-prone rainfed areas. The superior adaptation and yield performance of mutant MR219-4 under aerobic condition was obviously a very interesting finding because its parent, MR219 has never been recommended for aerobic soil . Further research on the uniqueness of Mutant MR219-4 should be carried out especially on the root system and water use efficiency. Based on the overall performance, the mutants should be tested further for potential yield and adaptation under Local Verification Yield Trials in stress prone rice areas (non-granary and marginal granary rice areas).

Figure 3. Mutant Line of MR219-4

Table 1: Yield and yield components of MR219-4 and MR219-9 in Muda (direct seeding method)

Treatment	Yield (kg/ha	Yield component				Plant height (cm)
		Panicle /sq.m	Spikelets/panicle	Percent filled grain	1000-grain wt (g)	
Saturated	6480 a	503 a	107 a	68.2 a	28.4 a	97.5 a
Flooded	6799 a	500 a	104 a	70.2 a	28.3 a	95.1 b
MR 219 – 4	6542 a	447 a	107 a	73.3 a	29.0 a	97.1 a
MR 219 – 9	6460 a	512 a	99 a	77.7 a	28.9 a	98.6 a
MR 220	6815 a	517 a	109 a	61.7 b	28.1 b	95.4 a
Water Management x variety	ns	ns	ns	ns	ns	ns

Table 2. Growth and yield performance of MR219-4 and MR219-9 in KETARA (direct seeding method).

Parameters	MR219-4	MR219-9	MR219	Difference Over parent
Panicles /plant	6.5	7.4	9	lower
Plant ht	100.1	100.4	103.4	lower
No. of tiller/hill	9	8	9	lower
Percent Filled Grain	78.1	87.7	73.4	higher
1000 grain wt(gm)	30.3	30.3	28	higher
Grain Length(mm)	9.69	9.69	9.86	lower
Grain width (mm)	2.38	2.41	2.35	higher
Panicle length	n.a	n.a	n.a	n.a
Yield (kg)	5259	6000	6444	lower
Maturity	110	112	116	lower

2.3 Molecular Genetic Features of Induced Mutations in Rice and Soybean

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Summary
Identification of mutated genes and molecular characterization of mutant alleles enabled geneticists for the first time to examine the features of induced mutations, which have been widely used in crop improvement in the past century. In my presentation, the mutated traits induced by gamma rays will be first introduced together with their uses in rice and soybean breeding, followed by the description of the general processes used for mutant gene identification. The molecular features of 9 mutated genes will be elaborated together with their effect on gene function, gene splicing and gene expression.

A thorough understanding of molecular features of both induced mutants and mutations underlying a particular trait is essential for developing proper strategies for gene discovery using gamma rays induced mutants. This includes three aspects: The characteristics of induced mutant versus its parent; the overall frequency of mutations in a given mutant genome; the nature of DNA lesions caused by gamma rays. Although these issues have not been fully investigated, some features have already been reported.

1. Induced Mutants and Parental Line
Mutated genes are eventually identified by gene sequence comparing between mutant and its parent with in a targeted genomic region. Therefore, it is of paramount importance to ensure that the mutant is originated directly from the parent through induced mutagenesis. M_1 plants usually have reduced pollen fertility and hence often tend to out-crossing, which can result in genetic contamination in M_2 population. It is recommended that extreme care is taken to protect M_1 plants from pollens of other varieties through proper isolation when grown in fields, and avoid other sources of mixing. However, it is practically impossible to exclude all chances of contamination, especially when the material is grown out on a large scale in the field [4].

The selection process for mutants is indeed a process of selecting any plants that differ from its parent variety. Therefore, the probability of selecting a contaminant as 'mutant' is quite high. A selected putative 'mutant' needs to be verified through progeny test and subject to scrutiny using breeder's knowledge, expertise and experience. However, such a process cannot always lead to a scientifically correct judgment due to the potential pleiotropic effect of a mutation, which can make a mutant quite different from its parent at phenotypic level. Recently, it has recently been proven that γ rays induced mutants have microsatellite (SSR) haplotypes highly similar to their parent. Based on the finding, it is recommended to use comparative SSR analysis for discriminating false mutants from true mutants [4], which have been nicely demonstrated in rice [5].

2. Mutation frequencies

In the past, mutation frequencies have been estimated by observing phenotypic mutants, e.g. chlorophyll deficient, plant height, sterility etc, and expressed either by the percentage of individual M_2 mutants or by the percentage of mutated M_2 panicle rows, in which at least one mutant is observed. Therefore, mutation frequencies vary between different traits and among different M_2 populations derived from different mutagenic treatments.

In molecular studies, the estimation of mutation frequency is performed in a different way, that is, the frequency of induced mutation is estimated by assessing DNA lesions in selected DNA fragments of M_2 plants. If the total size of assessed DNA fragment is expressed as i kilo bases (kb), the total number of M_2 individuals as j, and the total number of DNA lesions identified as n, then the mutation frequency (f) is expressed as the number of mutations per 1,000 kb for ease of comparison:

$$f = (n \text{ x } 1000) / (i \text{ x } j)$$

This formula can be manipulated easily to determine mutation density, that is, one mutation per a set number of DNA kilo bases.

Sato et al. [6] screened a gamma-ray-irradiated rice population by mismatch cleavage analysis using Brassica petiole extracts. Analyses of 25 gene fragments (1.23 kb length on average) of 2,130 M_2 plants revealed six mutants. The rate of mutation induced by gamma rays was estimated to be one mutation per 6,190 kb [6], which is about 2% of that reported in MNU-derived rice M_2 populations [7].

3. Nature of DNA lesions in induced mutants

A large amount of data on molecular features of induced mutations have become available during the last decade; they are either from TILLING analyses of M_2 (or M_3 in a few cases) plants or discovered after a mutated gene is cloned through forward genetics schemes. In general, most mutations caused by chemical mutagens, particularly those of alkylating agents, are base pair substitutions. However, small deletions are also infrequently identified in progenies derived from chemical mutagenesis. In contrast to the large data available for chemical mutagenesis, molecular characterization of mutations induced by different types of radiation remains limited. Available data indicate that mutations induced by radiations consist of both deletions and base pair substitutions. During the past few years, we have identified about a dozen γ rays induced mutations in rice and identified that most of them are small deletions (1-6 bp), with one mutation being a ~1450 bp deletion.

Our finding in rice for γ rays induced mutations is similar to carbon ions induced mutations in M_2 plants of Arabidopsis, where majority of mutations containing 1- or 4- bp deletions [6].

Large deletions often have significant effects on gamete development or survival and hence the majority of them are not transmitted to M_2 progeny.

4. Effect of Induced Mutations

The DNA lesions caused by induced mutation can cause various effects on gene expression and function. In most cases gamma rays induced mutations are deletions, which can often cause frameshift and produce truncated non-functional proteins [8-9]; In certain cases, we also observed mutations that cause splicing changes and whole exon exclusion in cDNA [10]. We also identified a mutation that resulted from an insertion of a retrotransposon and was down-regulated in expression.

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2.4 Success Story of Development of High Yield and Quality Rice Variety Grown in Vietnam

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1. Introduction

In Vietnam, rice plays an important role for national food security and political stability. Rice also has a direct effect on social security because it is consumed by nearly 89 million of the total population and an important source of income for more than 60 million people living in agricultural and rural areas. Rice is the country's main crop, accounting for more than 90% of total cereal productivity. Rice productivity in 2009 reached 38.89 million tons, about 16.89 million tons higher than in 1995 (General Statistics Office 2009), and in 2012, it is 21.7 million tons higher than in 1995 (Out look 2013). Since the 1990s, the volume of rice exports has risen dramatically, making Viet Nam the second largest rice exporter in the world. Nevertheless, serious concerns for food security still remain. Vietnam is one of the top 5 countries that studies reveal will be severely impacted by climate change (Ministry of Natural Resources and Environment 2009). A rise in sea level of 1 meter will inundate about 5,000 square kilometers (km^2) of the Red River Delta and 15,000 – 20,000km^2 of the Mekong River Delta, reducing total rice productivity in Vietnam by about 5 million tons. Bad harvests, natural calamities, floods, and pests and diseases will also occur more often. Ensuring domestic food security has thus become a national objective that requires long-term strategies and policies, especially for the protection of the agricultural land area. Viet Nam does not only seek to achieve domestic food security but it also plays an important role in the international rice market, and consequently, in the food security of the international community.

After 26 years innovation politics (1986- 2012), it had a remarkable achievement due to the application of advanced science and technology such as the introduction of new rice varieties, new production models, and an efficient irrigation system. Vietnam reached high speed of increasing in agriculture, especially in food production. Food production of Vietnam is not only enough for domestic consumption but also for exportation. In the year of 2012, rice production area of whole country reached around 7.76 mil.ha, increased 117,000ha compared to 2011. In 2012, rice productivity estimated 43.96 mil.tons increases 1.64 mil. tons compared to 2011, and furthermore the number of rice for exportation reached 8.0 mil tons impressively.

2. Rice Situation

Despite of a decrease in rice land area, rice productivity has been rising due to rapid yield growth. This in turn has been driven by irrigation and land development, together with technological change. In 1999, Viet Nam had nearly 4.5 million hectares (ha) of rice lands, which declined to 4 million ha in 2010. However, in 2009, the rice planted area reached 7.43 million ha because local farmers owning large tracts of land planted three rice crops. As a result, rice productivity increased from 32 million tons in 1999 to more than 38 million tons in

2009. It was a remarkable achievement due to the application of advanced science and technology such as the introduction of different rice varieties, new production models, and an efficient irrigation system. Post harvest losses have also been reduced greatly due to the mechanization of rice harvesting and drying, and soil improvement.

According to GSO (General Statistics Organization), the yields of the three crops have increased gradually from 1995 to 2009. In 2009, the yields of the summer–autumn and autumn–winter crops were about 4.3 – 4.7 tons/ha, while the yield of the winter–spring crop was more than 6.1 tons/ha. In the past years, Viet Nam has significantly increased its rice yield due to the shift from low-yield (1.5 – 2 tons/ha) to high-yield rice varieties (6.8 tons/ha), which can be grown in a shorter time (85 – 100 days/crop) and produce 2 – 3 crops/year. The rice cultivation area has also widened because of investments in irrigation and drainage, and aluminum and salt removal. The performance and productivity of rice production in Vietnam is increasingly within last decade (diagram 1 and 2).

Diagram 1. Rice productivity from 1878-2012 of Vietnam

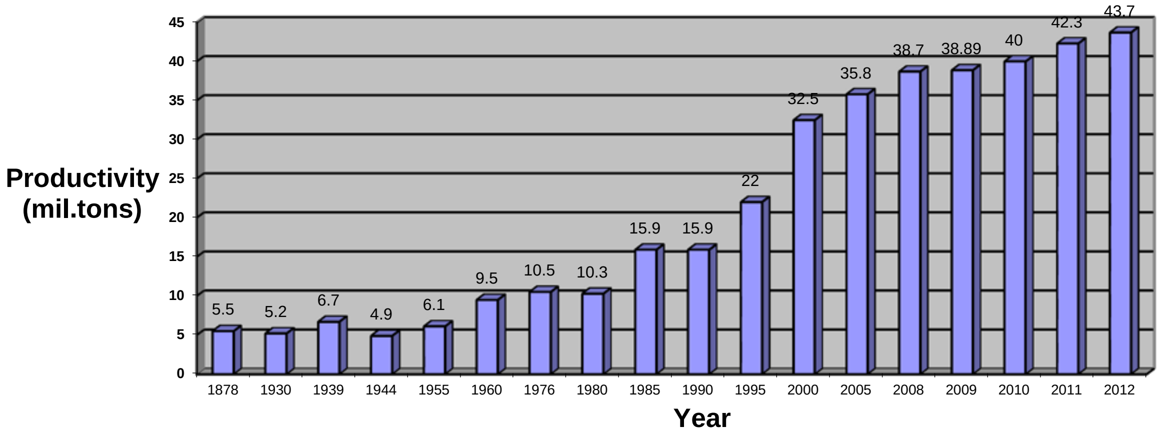


Diagram 2. Yield performance of rice production from year 1878-2012

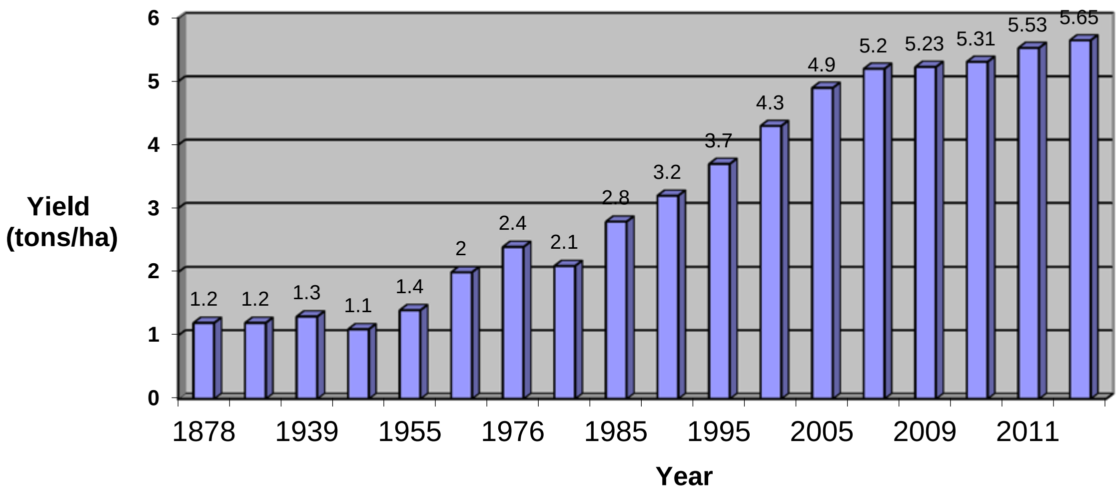
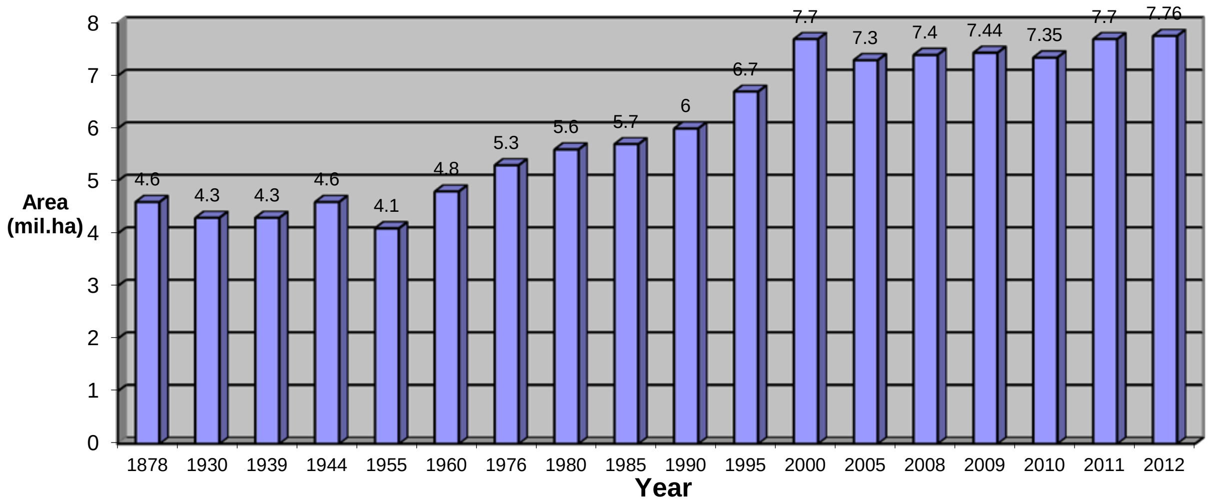


Diagram 3. Rice production area from year 1878-2012



The diagrams showed that the increasing of average yield and rice productivity in Vietnam mostly from 2008-up to now. The main reason resulting to success of Vietnam in rice production is: the water management of irrigation system is making provide water for 3.45mil ha agriculture production in which 85% rice production area, more than 90% rice production area use new varieties and applying new technology for cultivation, especially in north Vietnam in spring season.

3. Mutation Breeding in Vietnam

At present, about 15% of rice production area is covered annually by mutant varieties in Vietnam. With the great achievement by the use of nuclear techniques and related biotechniques, more than 50 mutant varieties were developed, in which most of varieties are cereal crops, especially rice. Since the 1990s, the Agricultural Genetics Institute (AGI), Vietnam Academy of Agricultural Sciences (VAAS), has been engaged in R&D activities and contributed much to the technology transfer to the agricultural sector of Vietnam. Mutation breeding is one of the major fields of institute in crop improvement, in which the biggest accomplishments have been achieved in the development of mutation varieties for crops production. Before 2000, objective of mutation breeding is often quantity of yield variety. One very interesting example from AGI is DT10 mutant variety which has been certified as national variety in 1990 due to the high performance, good tolerant to harmful condition. This variety is available in rice more than 20 years and now this is still disseminating in some specific location. This variety covers the area around 1.0 mil. ha in agriculture production.. After success of mutant variety DT10, scientists from AGI released series of mutant varieties (17 mutant varieties) and satisfied production demand (index 1). Khangdan mutant variety has been transferred copyright from AGI to the seed company since 2008 after it was certified as national variety. With some advantages, the area covered with this variety is around 300 thousands ha/year in North Vietnam. In 2012, the seed company is rewarded the national prize for Khangdan mutant variety due to the leading area for agriculture production of this variety. Recently, beside of yield varieties, scientists has been concentrating creation of new varieties having good performance on quality (aroma, protein, amylase content, component of quality), as well as tolerance to harmful condition of environment such as salinity, cold or high temperature, drought, lodging variety and so on. By the year of 2008, there are several outstanding new varieties have been certified as new varieties and ongoing projects to enlarge mutation varieties in different provinces.

Table 1. Leading mutant varieties in rice production in north of Vietnam (duration 2008-2013)

Variety	Year of certify	Dominant characters	Area of production/year (ha)
Khangdan mutant	2008	High yield, good tolerant to pest and disease	300,000
PD2	2010	Glutinous rice, photo sensitiveness, high yield, aroma	10,000
P6 mutant	2011	Short growth duration 85-90 days in summer season, high temperature tolerant	15,000
Nam dinh 5	2012	Quality, aroma, high yield	3,500
ĐB5	2008	High yield, good tolerant to pest and disease	5,000
ĐB6	2008	High yield, good tolerant to pest and disease	5,000
DT39 Quelam	2013	Quality, high protein, high yield, resistant to leaf blight	100

In the South Vietnam, mutation induction has been carried out mostly in Cuulong Rice Research Institute and Institute of Agriculture South Science (IASS). These institutes have also succeeded in the mutation breeding by the use of nuclear techniques. Over the past decade, these institutes have developed eight excellent mutant varieties with high yielding, improved quality, disease resistance, tolerance to pest and lodging resistance. Since 2008, the total cultivated area of these mutant varieties was more than 4 million ha in southern Vietnam. According to the statistics from the institutes, these mutant rice varieties have increased income by about $374 million at the end of 2008. One of these excellent mutant varieties (VND9s-20) became one of the top five varieties for rice production. It covered 300 thousand ha per year in south of Vietnam, due to its high yielding, good quality and tolerance to brown plant hopper. Not only has this variety been widely cultivated in the low lands, but also expanded to the high lands and remote mountain areas, where poor farmers are benefiting from growing it. It is very clear that the great support from the Vietnamese government makes the application of nuclear techniques in food and agriculture so successful. Due to the excellent performance of the mutant rice varieties mentioned above, some of these mutant varieties were adopted for the national strategy program of “Eradicate hunger and alleviate poverty” in different areas, particularly for central highland region, where there are many ethnic minorities living in remote mountain areas. Most of the eight mutant varieties have shown out-standing performance under local production conditions. These promising varieties were identified in the trials with improved characters, such as high yielding, increased disease and insect resistance/tolerance, short growth duration, good quality and better adaptation to environment stress. Some of mutant varieties (VND 95-20, VND 99-3, and VN121) have increased more than 30% of yield, compared to the control of the local variety. These mutant varieties have been well accepted by local farmers and will be cultivated in expanding areas.

Table 2. Economic impact of mutant rice varieties in the South Vietnam (Do KhacThinh, 2010)

Varieties	Approved in	Annual area	Value added/year Billion VND	Characters
VND 95-20	1999	300,000	600	Wide adaptation, high quality
VND 99-3	2004	20,000	40	High productivity, resistant to aluminum soil
TNDB 100	1997	30,000	60	Short growth duration, high quality
OM 2717	2004	30,000	60	Short growth duration, resistant to brown plant hoppers
OM 2718	2004	30,000	60	Short growth duration, resistant to brown plant hoppers
OM 2496	2009	3000	6	High productivity, Aroma, Resistant to Salinity
VN 121	2007	3000	6	Aroma, high production.
VN 124	2007	2000	4	Aroma, High production.
Total	-	418,000	836 = 45 mil. USD	-

Successful story of mutation plant breeding in Vietnam is contributed from political support by Vietnamese government for mutation plant breeding research and international organization such as FAO/IAEA, through project IAEA/VIE/05/13/14 from 1997-2003. On the other hand Vietnam became official member of Forum for Nuclear Cooperation in Asia (FNCA) in 1997. Scientists from different institutions have chance to go abroad and attend training course, scientific symposium and workshop, or to exchange information and experiences with scientists working in mutation breeding.

4. Conclusion

It's clear that mutation induction nowadays is a powerful tool for crops improvement in Vietnam. Mutant rice varieties from institutions contribute to increase of rice performance and productivity in recent years. This is confirmed that Vietnam Government has been concentrating research and development of the activities on nuclear techniques in agriculture. Beside of that farmers also benefit from scientific research activities.

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3. Mutation Breeding II

3.1 Development of Disease Resistant Banana by Mutation Breeding: Resistance Response of Selected Mutant Lines of Banana cv. Lakatan (AA) Against Banana Bunchy Top Virus (BBTV)

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Development of BBTV resistant Lakatan through gamma-irradiation had been successfully done as part of integrated management strategies against banana bunchy top disease (BBTD). Based on BBTV incidence in the field, % BBTV-free fruiting plants, aphid preference, and host reaction to the virus, ten mutant lines namely lines 13-30-2, 7-29-1, 22-28-2, 23-28-7, 6-30-2, 9-28-2, 9-28-3, 9-29-1, 23-30-2, and 28-30-2 were selected after four generations of selection and evaluation. Insect preference to Lakatan mutant lines was monitored by artificially inoculating viruliferous banana aphids, Pentalonia nigronervosa, and counting, at weekly interval, the number of aphid per plant. Disease resistance on virus multiplication of Lakatan mutant lines was characterized by quantifying the virus titer through Enzyme-Linked Immunosorbent Assay (ELISA). Results showed that not all lines were preferred by aphids as a host for establishing a population, and generally, disease incidence was correlated with aphid preference. Disease incidence was significantly higher (>50%) on lines that were preferred by aphids and lower (<50%) on those that were not colonized. Virus titer was also reduced on these lines, thus reduced virus multiplication. Non irradiated (control) Lakatan banana had comparably high population of aphid, high disease incidence, and high virus titer. The resistance of Lakatan mutant lines to BBTD could be attributed to non-preference of the aphid to the vector to colonize and reproduce thus attributed to lower BBTD infection. In addition, reduced virus multiplication indicative of low titer on mutant lines indicated a resistance response to the disease. The top five resistant mutant evaluated in multi-location sites showed low incidences of the BBTV in all multi-location trial sites, despite the presence of infected plants and vectors in the area and within the vicinity of trial site. The mutant lines were differentiated from the LK control using nine Simple Sequence Repeats (SSR) primers by the absence of 1 or 2 alleles detected in LK control.
Keywords: gamma-irradiation, mutant Lakatan, banana bunchy top virus (BBTV) ELISA, aphid preference, virus multiplication

Introduction

Banana (*Musa* sp), remains the country's one leading fruit crop in terms of area, volume, and value production, and export earner. The total arable land planted with banana is more than 400,000 has (BAS, 2008). National average yield reaches to 9.4 tons/ha while corporate plantations contribute to 40 tons/ha (Rivera, 2004). Lakatan is the most popular and preferred fresh table banana. Lakatan is normally grown on smallholdings in the livelihood of many growers in the country. As such, Lakatan has a significantly higher market potential given higher production (Aguiba, 2010).

Still, the most important biotic constraint in banana production is the occurrence and spread of Banana Bunchy Top Disease (BBTD) caused by *Banana bunchy top virus* (BBTV). BBTV is spread from plant to plant by the banana aphid, *Pentalonia nigronervosa,* and is transmitted in a persistent, and circulative but non-propagative manner (Hafner, 1995; Hu et. al., 1996). Initial symptoms on BBTV-infected plants include marginal chlorosis, and dark green streaks or dots on leaf veins, midrib and petiole. It later progresses into rosetting wherein emerging leaves are narrower than normal with distinct marginal chlorosis and appear as "bunchy-top." Stunted growth is observed on severely infected plants. Thus it is considered as the most devastating virus disease of banana worldwide that can result in a 100% yield loss since severely infected plants do not produce fruits.

While efforts are being done to come-up with a sound and effective management and control of BBTD including eradication of infected planting materials, control of the aphid vector and widespread use of agrochemicals; still the disease has spread dramatically over time in any place where bananas and plantains are grown. The persistent use of these agrochemicals, in addition, can pose serious threat to human health and environment.

BBTV resistance could not be introgressed into banana by conventional breeding methods because edible bananas are technically not amenable to sexual breeding since they are male and female sterile. Thus, resistance could be obtained only through biotechnology-assisted method like mutation breeding using irradiation and chemical mutagens, somaclonal variation, or through a direct method of *in-vitro* selection in the presence of stress factor.

Using gamma irradiation and *in-vitro* induced mutation, mutant lines and somaclonal variants of Lakatan with varying degree of resistance and tolerance to BBTV have been produced by the team of Damasco (2009) in IPB-CSC, CA, UPLB. The stability of BBTV resistance in selected mutant lines has been continuously expressed in succeeding generations. The mechanism and components of resistance of selected mutant lines against BBTV were determined. Molecular characterization using SSR (Simple Sequence Repeats) was also done to determine the genetic variation of irradiated lines.

Materials and Methods

(1) Selection of BBTV resistant lines

Ten (10) mutant lines were selected based on the reaction on BBTV infection, preferential studies of the insect vector, *Pentalonia nigronervosa*, and agronomic characteristics after several generations of selection and evaluation in the greenhouse and the field. These lines were further evaluated for confirmatory studies to ascertain the stability and durability of resistance or tolerance against BBTV infection.

(2) Resistance to *P.* nigronervosa: Aphid preference

Preferential studies were conducted at two different feeding procedures: by free choice feeding or by artificial inoculation using the no choice feeding (forced feeding) method. The aphids were allowed to colonize and multiply on the lines. Data on aphid population on the different lines were collected at weekly interval starting from the 1st week after artificial inoculation.

(3) Resistance to BBTV: Virus multiplication

Symptomatology, incubation period and percent incidence of BBTV on inoculated lines were observed and virus multiplication based on titer was quantified through Enzyme-linked Immunosorbent Assay (ELISA). Capture and conjugate antibodies against BBrMV (Agdia) were used in double antibody sandwich ELISA following the manufacturer's procedure and by Clark and Adams method (1977) with some modifications. Absorbance at 405nm was measured using an ELISA reader (Bio-Rad). All reactions were considered positive only if the absorbance was equal to or above the threshold value, which is two times the average absorbance of the negative control.

(4) Multi-location field evaluation for disease resistance and agronomic traits

Micropropagated plantlets from top five mutant lines and LK control were established in the farmer's field trial sites following the recommended cultural management practices for growing Lakatan. Monitoring for BBTV incidence and other diseases was conducted regularly. Agronomic and yield parameters were taken at harvest.

(5) Simple Sequence Repeats (SSR) Analysis

Banana genomic DNA from each mutant line was extracted from young leaves using the CTAB DNA extraction procedure of Doyle and Doyle (1990). A total 34 SSR banana primer pairs were tested. All PCR reactions were performed following the published procedure. Amplified fragments were scored for the presence (1) or absence (0) of bands in all mutant lines.

Results and Discussion

(1) Selection of BBTV resistant lines

Ten mutant lines of Lakatan (namely 28-30-2, 13-30-2, 9-28-2, 22-28-2, 9-28-3, 6-30-2, 7-29-1, 9-29-1, 23-28-7, and 23-30-2).with the best reaction on BBTV infection and aphid preference were selected after four generations of evaluation and selection. These lines, generally, had high percentage of fruiting plants free from BBTD at 11MAP compared to the control Lakatan. In terms of aphid preference, initial studies on free choice feeding showed that all the lines tested had low aphid colony count. On the other hand, BBTV incidence was comparably lower in mutant lines ranging from 3 to 67%, in contrast to high BBTV incidence in control Lakatan (81%).

(2) Insect vector – host relationship: Aphid preference

The aphid population taken at the peak of colonization, 3 weeks after artificial inoculation, in a free choice feeding method was significantly lower in some mutant lines (9-28-2, 9-28-3, 9-29-1 and 13-30-2) compared with control Lakatan, indicating that these lines were not preferred by the aphids as host plants (Table 1). A very high population of aphid was observed on control Lakatan when no choice (forced) feeding method was utilized (Table 1). The result of the no choice (forced) feeding experiment confirmed that mutant lines13-30-2, 9-28-2, and 9-28-3 were not preferred by the aphids. The aphid colony counts on these lines were statistically lower than control Lakatan. In addition, lines 22-28-2 and 23-28-7 which were not tested with free choice feeding were likewise significantly less preferred by the aphid vector.

(3) Virus – host relationship: Symptomatology and Virus Multiplication

Table 2 presents the reaction of ten selected lines to BBTV infection under greenhouse condition. Generally, symptoms started to appear at 4 weeks after inoculation as dark green streaks or dots on petiole extending to midrib. Marginal chlorosis and "bunchy top" appearance having narrow and stiff leaves appeared during symptom progression. All lines, except 7-29-1 (58%) and 23-30-2 (71%), had low incidence of BBTV compared to the Lakatan control plants (58%) (Table 1). Line 28-30-2 (20%) had the lowest incidence of the disease in contrast to 23-20-2 (71%) with the highest incidence. In terms of incubation period, 28-30-2 and 13-30-2 had the longest period (6 weeks after inoculation) for symptom expression to visibly express. Likewise, 13-30-2 and 22-28-2 were observed to express a delay of symptom development which started at 5 weeks after inoculation. In virus multiplication, ELISA result showed that all lines had the minimum OD reading lower than that of the control plants, except line 9-29-1. On the other hand, only 13-30-2, 9-28-3, and 7-29-1 were observed to have low virus concentration at maximum OD. Line 13-30-2 had the lowest value of 0.956. This implies that the virus titer is lower on mutant lines compared with control Lakatan, suggesting a degree of resistance.

(4) Vector – Virus – Host Relationship

In a free choice feeding of the vector (Table 2), lines 6-30-2, 13-30-2, 23-28-7, 28-30-2 showed low BBTD incidence (<50%) despite the preference of the vector to the host (high aphid colony count). These results suggest that the mechanism of resistance could be due to the resistance of the host to the virus. On the other hand, lines 9-28-2 and 7-29-1 which were less preferred by the aphids also had correspondingly low disease incidence which suggests that the resistance mechanism could be due to the less preference of aphids to the host.

Table 1. Aphid colony count on selected mutant lines 3 weeks after inoculation.

Mat/Line selection	Aphid colony count		Symptomatology	Incubation period (WAI)[3]	BBTV incidence (%)[2]	ELISA (OD405nm) range[4]	
	Free choice feeding[1]	No choice (forced) feeding[2]				MIN	MAX
28-30-2	40	62*	bunchy top	4 - 6	20	0.189	1.276
13-30-2	22*	65*	bunchy top, rosette	5 - 6	25	0.174	0.956
9-28-2	12*	36*	bunchy top	4 - 5	50	0.189	1.605
22-28-2	not tested	65*	rosette	5	38	0.188	1.570
9-28-3	12*	49*	bunchy top	4 - 5	50	0.179	1.267
6-30-2	27[ns]	59*	bunchy top	4 - 5	56	0.184	2.292
7-29-1	14[ns]	110[ns]	bunchy top	4 - 5	58	0.182	1.128
9-29-1	1*	83[ns]	bunchy top	4 - 5	33	0.203	1.545
23-28-7	not tested	16*	bunchy top	4 - 5	45	0.171	1.336
23-20-2	40**	76*	bunchy top	4 - 5	71	0.179	1.680
LKD control	23	127	bunchy top	4 - 5	58	0.194	1.287

[1] Data analysis using t-test at α=0.05; SD* significantly less preferred, ** more preferred, [ns] not significant

[2] Mean values obtained from two separate trials

[3]Weeks after inoculation

[4]OD – optical density. Mean values obtained from two separate experiments given the no choice feeding method

In a no choice/forced feeding of the vector, some lines (28-30-2, 13-30-2, 22-28-2, and 23-28-7) which were significantly less preferred by the vector (aphid colony count significantly lower than the control) likewise showed correspondingly low BBTD incidence (<50%). On the other hand, line 9-29-1 which was preferred by the aphid had also low BBTD incidence (32.8%). In no choice feeding set-up, mutant lines likewise showed resistance to BBTV either through less preference of the vector to the host or resistance of the host to the virus despite the preference of the vector to the host.

(5) Multi-location field evaluation for disease resistance and agronomic traits

For all trial sites, low incidences of the BBTD (were observed from planting up to 19 MAP despite the presence of the virus and vector within the trial site and also within the vicinity of the sites. The highest percentage incidence of the BBTD was recorded at 13.3%; the diseases did not spread extensively within the trials sites and the occurrences of the disease infection were random. The low incidence BBTD in trial sites could be due to greater number of resistant plants (approx. 70%) in comparison with the susceptible plants (30%) planted within the area. Furthermore, the random manner by which resistant mutant lines (with varying resistance mechanisms) were planted could have also contributed to the low disease incidence in the field. The use of mutant lines coupled with the farmer's practice of regular rouging of infected plants at least once a month provided the most effective control against BBTV.

The BBTV resistant mutant lines were comparable with LK control in terms of plant height, girth, total number of fruits per bunch, number of hands per bunch and number of fingers per hand (Table 3). The number of days to flowering was significantly different for lines 13-30-2 (26 says earlier), 22-28-2 (21 days earlier) and 28-30-2 (17 days earlier). Mutant lines had a mean of 6-7 hands/ bunch and 17-18 fingers/hand.

(6) Simple Sequence Repeats (SSR) Analysis

The mutant lines were differentiated from the LK control using nine SSR primers (CIR 13, CIR1113, Ma 3-90, Mb 142-2, CIR 07, CIR 09, PR 27, PR 108, PR 260 and 631a). For most primers (CIR 13, CIR1113, PR 27, 631a), the mutant lines were differentiated from LK control by the absence of 1 or 2 alleles detected in LK control.

Table2. Relationship of aphid colony count and BBTD incidence as influenced by vector feeding method in selected mutant lines.

Mat/Line selection	Free choice feeding of the vector		No choice/forced feeding of the vector	
	Aphid Colony Count	% BBTV incidence	Aphid Colony Count	% BBTV incidence
28-30-2	40	3.1	62	19.6
13-30-2	22	40.0	65	25.0
9-28-2	12	40.0	36	50.0
22-28-2	NT	NT	65	38.4
9-28-3	12	63.6	49	50.0
6-30-2	27	50.0	59	55.6
7-29-1	14	50.0	110	57.5
9-29-1	1	66.7	83	32.8
23-28-7	NT	10.00	16	45.0
23-20-2	40	41.7	76	70.7
LKD control	23	81.2	127	58.3

Table 3. Agronomic and yield parameters taken from BBTV resistant mutant lines (mean from multi-location trials).

Mat/line selection	Plant height* (cm)	Girth (cm)**	No. of days to flowering	Total No. of fruits per bunch	No. hands/ bunch	No, of fingers per hand
9.28.2	342.6	56.1	408	123	6.6.	17
9.28.3	340.8	54.8	408	128	6.5	17
13.30.2	334.5	54.1	397***	119	6.3	17
22.28.2	347.0	55.5	402***	130	6.6	18
28.30.2	342.3	55.3	406***	130	6.5	18
LK control	319.6	52.3	423	108	6.3	19
t- test P=0.05	NS	NS	SD	NS	NS	NS

*Measurements taken at flowering ** Measurements taken 1m from the base of the pseudostem at flowering

***Significantly different from LK control using t test at P=0.05

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3.2 Success Story of Mutation Breeding on Sorghum for Improved Drought and Acid Soil Tolerance in Indonesia

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Abstract

The limited factors in developing dryland farming agriculture in Indonesia are mostly related to drought problem in the east and soil acidity in the west part of the country. The development of dryland farming agriculture may be directed to crops that require less agricultural inputs, have wide adaptability and with good economic values such as sorghum. Globally, sorghum is recognized as sources of *Food, Feed, Fuel* and *Fiber* (4Fs). In Indonesia, sorghum is still regarded as a minor cop and its cultivation is limited, mostly grown by local farmers in a specific region. Sorghum is not Indonesian origin so that the available plant genetic variability is low. Attempts to increase sorghum genetic variation was achieved through introducing plant materials from ICRISAT, China and Australia. These materials were used in breeding programs by conventional, mutation and biotechnological approaches. Through mutation breeding, a number of sorghum mutant lines were identified to have highly tolerance to drought and soil acidity with improved yield and quality. In collaboration with the Ministry of Agriculture, universities and private companies, some promising mutant lines were investigated under intensive multi-location trials in several Provinces in Indonesia. In 2011, one sorghum mutant variety was released by the name of "Pahat", an acronym of "*Pangan Sehat*" meaning healthy food. Meanwhile, some sorghum mutant lines showed improved agronomic performances such as lodging resistance, early maturity, higher sugar (brix) content and biomass production in later generations. These promising mutant lines were kept at our sorghum germplasm collections for further breeding program towards improvement of sorghum utilization for 4Fs. The on-going research is currently being done for evaluating the sorghum mutant lines and released variety in farmers field in different regions for increasing marginal land productivity. Sorghum mutation breeding was included in the JSPS Core University Program (1999-2008), part of FNCA projects (2000-2004), and currently supported by IAEA through the CRP Contract No. 16947 and RAS/5/056 project.

Introduction

For agricultural purposes, arable land in Indonesia is actually dominated by dryland farming areas. Meanwhile, wetland (paddy field) areas tend to decrease due to several reasons such as intensive expanding of land transformation to non-agricultural purposes happened recently. The fact that water scarcity still becomes a problem in some agricultural areas makes dryland farming agriculture be more important and reliable for supporting sustainable agriculture development in the country. The most significant limited factors in developing dryland farming agriculture in Indonesia are related to adverse conditions i.e.

drought in the eastern part and soil acidity in the western part of the country [1]. The development of dryland farming agriculture may be directed to include potential crops that require less agricultural inputs (water, fertilizer etc.) with high economic values such as sorghum.

Sorghum (*Sorghum bicolor*) is thought of being suitable crop for dry land farming agriculture in Indonesia owing to its wide adaptability, high yielding and more tolerant to adverse conditions than the other food crops [2]. Sorghum is a cereal crop that is usually grown under hot and dry conditions, and globally it is used as sources of 4Fs namely *Food* (grain sorghum), *Feed* (forage sorghum), *Fuel* (sweet sorghum) and *Fiber* (sorghum biomass). As food, sorghum was reported to have good nutritive values with low glutein and glycemic index, and contained the phytochemical (phenolics) that were associated with protection from and/or treatment of chronic diseases such as heart disease, cancer, hypertension, diabetes and other medical conditions [3].

Sorghum has a high yield potential, comparable of rice, wheat, and maize. On a field basis, yields have exceeded 11 ton/ha, with above average yields ranging from 7-9 ton/ha where moisture is not a limiting factor. In those areas where sorghum is commonly grown, yields of 3-4 ton/ha are obtained under normal condition [4]. Sorghum is also known to have wide adaptability, ranging from lowland, medium and highland altitude. Highest yields are usually obtained from varieties maturing in 100-120 days. Late-maturing varieties tend to be appropriate for forage crop. Available sorghum genotypes have been introduced from abroad e.g. from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). Through plant breeding programs, some local varieties have also been released by the Ministry of Agriculture. Further sorghum breeding, however, is needed especially to search for and to develop genotypes tolerant to adverse conditions such as drought and soil acidity which become the most limiting factors in dryland agricultural development in Indonesia [5]. Beside food security, Indonesia is also currently looking for alternative renewable energy resources for ensuring energy security. Sorghum is one source of bioenergy (bioethanol) with high productivity. The productivity of bioethanol from sweet sorghum reached 8.419 liter/ha/year i.e. twice that of cassava (3.835 liter/ha/year) since sorghum can be planted twice a year in Indonesia [6]. From a total of 99.50 million ha of dryland agricultural area in Indonesia, about 68.75 ha (69.1%) consisted of acid soil (*ultisols*) which is mainly found in Sumatera and Kalimantan [7]. The main constraint of crop production in acid soils is phosphor (P) deficiency and aluminium (Al) toxicity [8, 9]. Therefore, development of sorghum cultivation in dryland farming areas requires varietes which are tolerant to such conditions. The objective of our sorghum mutation breeding was to search for sorghum genotypes tolerant to drought and soil acidity, with improved yield and quality for 4Fs purposes.

Sorghum Mutation Breeding

Sorghum mutation breeding was conducted at the Center for Research and Development of Isotope and Radiation Technology, National Nuclear Energy Agency (BATAN) in Jakarta, Indonesia. Beside from ICRISAT, our sorghum breeding materials were also introduced from India, China and Australia. Sorghum mutation breeding was included in the JSPS Core University Program (1999-2008) and also as part of FNCA projects (2000-2004), specifically for screening mutants for drought tolerance.

Sorghum Durra and Zhengzu varieties from ICRISAT and China, respectively, were used as starting breeding material in mutation breeding. The dry seeds of these sorghum varieties were irradiated with Gamma rays emitted from Cobalt-60 source in the Gamma Chamber 4000A. The dose levels of 0-1000Gy, with increment of 100Gy, were used in the experiment of estimating appropriate radiation doses for sorghum breeding purposes. Response of sorghum growth in the M1 generation was studied by best-fitting curve software, and the LD-20 and LD-50 values were determined for estimating optimal irradiation doses for sorghum breeding purposes [10].

Some M1 plants were bulkly harvested to generate of about 4000 M_2 plants. Individual plant selections based upon phenotypic performances (variations) were started in the segregating M2 population, focusing on agronomic and yield characters which are significantly different from the control plants. The two parental varieties and two local varieties (UPCA and Higari) were used as control plants in the experiment. Selected plants which showed "better" traits than the control plants were separated and registered as putative mutants for further screening for drought and acid soil tolerance in the M_3 generation.

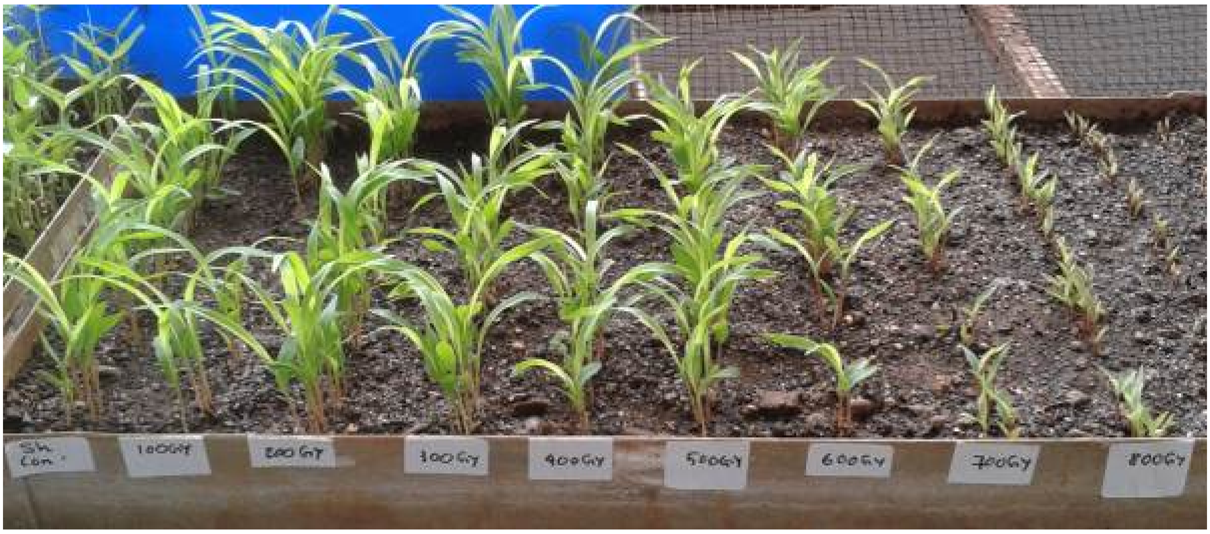

Fig 1. Sorghum response to gamma irradiation at seedling stage (M_1).

Screening for Drought Tolerance

Screening for drought tolerance used combination of indirect selection (PEG method) and direct selection in the field. For the PEG method, concentration of 25 % polyethylene glycol was used and applied in the seedling stages. According to Singh and Chaudhary, PEG could reduce water potential equivalent to natural drought condition so that water absorption by roots could be affected [11]. A total number of 170 selected tolerant M_3 plants using the PEG method were obtained and then transplanted to the field for their seed multiplication.

Direct drought tolerance screening in the field for these selected plants was conducted in drought prone areas of Gunungkidul District, Yogyakarta Special Province during dry season in 2002 (M_4), 2003 (M_5), and 2004 (M_6), respectively. Sowing time was adjusted to early dry season i.e. around March or April. Artificial irrigation was given only in early growth stage to stimulate seed germination. One month after sowing, the irrigation was stopped and the plants were entirely exposed to the natural drought condition. The growth and yield of the selected drought tolerant plants were also evaluated in the field condition under normal season using randomized block design.

Production of total biomass, yield and its components and drought index were measured and used as criteria for selection of the tolerant mutant plants. Drought index was calculated by dividing the grain yields under drought to that of normal condition. It measured relative sensitivity of genotypes to water stress response with regard to grain yield production. Through field screening and selection processes in successive generations, 10 mutant lines were finally found and registered as highly drought tolerant. These mutant lines were B-69, B-72, B-75, B-76, B-90, B-92, B-94, B-95, B-100 and ZH-30. Statistically, these mutant lines had agronomic and yield performances significantly better than that of the original variety (Durra) and the control national check variety (UPCA and Higari). Some of these mutant lines had been proposed for official reslease to the Ministry of Agriculture. In 2011 the mutant line ZH-30 was released by the name of "Pahat", an acronym of "*Pangan Sehat*" meaning healthy food.

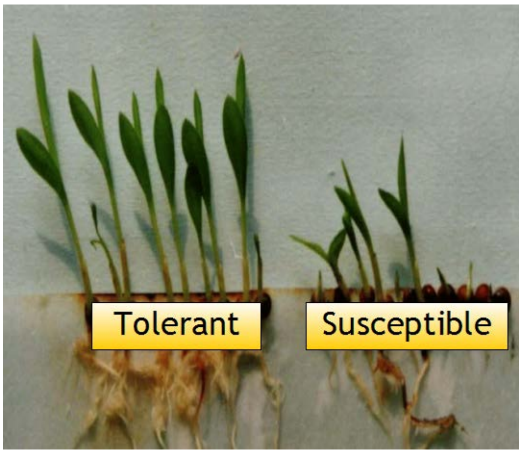

Figure 2. Seedling screening for drought tolerance (PEG method).

Figure 3. Tolerant plant grown in the field (B-100 mutant line).

Screening for Acid Soil Tolerance

Screening for acid soil tolerance was conducted indirectly in Laboratory using AlCl3 method and also directly in the field at lands with acid soil condition. A total number of 59 selected tolerant M_3 plants using the AlCl3 method were obtained and then transplanted to the field for their seed multiplication. Field screening and evaluation of selected M_4 plants were conducted at Taman Bogo in Lampung Province where soil condition was classified as very acid with soil pH of 4.2 and 39% Al saturation. The parental varieties (Durra and Zhengzu) and national varieties (Numbu and Kawali) were used as control plants.

Plant growth, grain yield, biomass, stem juice, brix content and ethanol production were measured and used as criteria of selection for acid soil tolerance. Analysis of bioethanol was conducted at the laboratory of Center for Starch Technology (B2TP-BPPT) at Sulusuban in Central Lampung. Ratooning ability was also measured with regard to bioethanol production. The experiment used randomized block design with 10 samples taken from each genotype treatments.

Among 59 mutant lines tested in the field, a number of 15 mutants were found and identified as highly tolerant to acid soil condition. Three of these tolerant mutants i.e. Patir-1, Patir-4 and Patir-9 are now being proposed for official release as new sweet sorghum varieties to the Ministry of Agriculture.

Figure 4. Seedling screening for tolerance to Al toxicity in media containing 148 μM $AlCl_3$.

Figure 5. Field screening on acid soil with soil pH of 4.2 and 39% Al saturation.

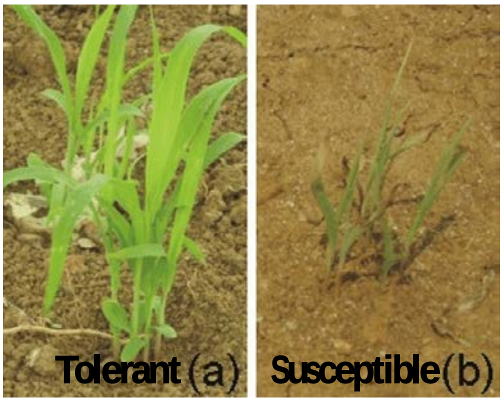

Figure 6. Tolerat vs susceptible plants.

Figure 7. Released mutan variety "Pahat".

Conclusion

Mutation breeding of sorghum has successfully produced some mutant lines that are potential to be developed further in drought prone and acid soil areas in Indonesia. Since sorghum can be used for food, feed, fuel and fiber sources, thus, sorghum development will increase land productivity for sustainable agriculture and ensure food and energy security in the country. Sorghum technology transfer to private industries has showed significant achievements in sorghum development in Indonesia.

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3.3 Radiation Breeding and Commercial Mutant Varieties in Korea

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Radiation technology

In the early 2000s, the Korean government decided to support fundamental and applicable researches of radiation technologies. The Advanced Radiation Technology Institute (ARTI) of KAERI was established to promote the research and development in the fields of radiation applications including agricultural, industrial, environmental, food and biotechnology, radio isotope and medicinal sciences, etc. We believe that the ARTI will significantly contribute to the researches on radiation mutation breeding and other industry fields in the world as well as in domestic.

Current status of mutation breeding in Korea

The mutation breeding has been achieved for new variety development, the creation of new genetic resources, and the use of mutants for a genomics study. Before 2000 year, mutation derived varieties in Korea was not higher than other competition countries. More than about 50 varieties have been developed by using the mutation breeding method since the mid-1960s, and the released varieties were mostly developed (76%) by exposing to radiations. Most of the released mutant cultivars (74%) in Korea were food and oil seed crops, especially for improving agronomic traits such as yield, lodging tolerance, maturity, or functional compounds. Currently a high yield potential of cultivated crop cultivars is relatively less popular than before, but the expectation of value-added crops, from the farmer's side, is in high demand. Accordingly, the mutation breeding program in Korea has assigned more resources to other crop species, including some flowering and ornamental plants. These flowering and ornamental plants are ideal systems for a mutation breeding because their favored traits such as flower color or shape and plant architecture can be visually monitored after a mutagenic treatment. Additionally, these plant species are genetically heterozygous and often propagated vegetatively, which allows for an isolation and selection of mutants within few generations. In recent, many researchers and breeders have been interesting on mutation breeding techniques on major flowering and ornamental crops in Korea such as rose, chrysanthemum, lily, carnation, orchids, and clover. The potential outcomes from the program will be new highly valued-added cultivars which will provide greater money gains to Korean farmers and lots of valued mutants used for a gene isolation of interest and reverse genetics or functional genomics. From 2012, a big research project, "Golden Seed Project' was launched supported from Korean Ministry of Food, Agriculture, Forest and Fisheries (MIFAFF). The main aim of the project is for the development of potential new varieties of 20 items including food and vegetable crops, animals and fishes, etc., so that rising domestic seed industry for reducing royalty payments and seeking greater opportunities in global seed markets. Mutation techniques also can be contributed many research and breeding works of the Golden Seed Project. Appropriate strategies should be implemented to complete its goals successfully, which includes a) induction of a wide mutant spectrum, b) applications of new irradiation techniques, c) unraveling the complex genetic phenomena controlling mutant traits, and d) development of a mass production and an intensive export system for the developed cultivars.

Mutation breeding researches in KAERI

The mutation breeding in Korea has been conducted by the KAERI and some other researchers since mid of 1960's. The ARTI is only one institute with essential facilities for mutation breeding and irradiation in Korea. With financial support from the government (MIFAFF), a new Radiation Breeding Research Center with advanced research facilities is schedule to be completed by 2013 in ARTI, and it will play a key role in research and extension of mutation breeding technology.

Since 2000, KAERI has released 13 rice mutant varieties and distributed rice seeds to farmers directly each year. Among them, a green-kerneled glutinous rice mutant, "Nongwonchalbyeo" and an aromatic and anthocyanin pigmented rice mutant, "Huegseonchalbyeo", have become very popular with farmers and consumers. In 2011, two new rice varieties, "GoldAmi-1" and "Wonmyoung" were officially registered. "GoldAmi-1" was developed by radiation fusion technology combining with plant tissue culture, and had characters of 70% increased amino acid compared with the original variety "Dongan" and check variety "Youngan", high lysine accumulating variety. "GoldAmi-1" will be applied in baby food and powdered milk industry. In addition, a new rice variety "Wonmyoung" with high quality was mutated from the original variety "Koshihikari", the most famous variety in Japan, and had characters of lodging resistance and mid-late maturing. In recent, new lines with high tocopherol content has been selected and it will be released after official registration.

As the outcome of recent researches at KAERI, an early mature and high-yielding mutant soybean, "Josaengseori," was released and two new soybean varieties ("Wonyul", Wonhyeon") were applied for a variety protection right to the Korea Seed & Variety Service. Also sixteen mutant lines lacking seed lipoxygenases and six mutant lines with a low phytic acid content were selected and will be applied for plant variety copyright protection. In hibiscus (rose of Sharon), six mutant varieties ("Baekseol", "Seonnyeo", "Daegoang", "Ggoma", "Changhae" and "Dasom") were developed through gamma ray irradiation. In particular, "Ggoma" (meaning 'kid') is a dwarf mutant and has received considerable attention as a 'bonsai' or indoor cultivation. Recently, mutation techniques have been applied to many crop species, including some flowering and ornamental plants, medicinal and industrial plants, and vegetable plants. In recent, KAERI singly and jointly with other research organizations and private companies had released new mutant varieties of kenaf, orchid, rose and blackberry, etc.

A completion of genome sequencing of some model plant species left challengeable assignment of understanding the gene functions predicted by the sequence information. A direct approach of discovering the function of a novel gene is to use a mutant which has altered or eliminated function as a result of a mutagenesis. Mutants that have favored traits for the cultivar development or distinct features for studying gene functions, therefore, are equally important. Experimental effort to apply diverse ionizing energy and irradiation facility to induce a broad spectrum of plant mutants are now underway at KAERI. Irradiation with an electron beam, ion beam, chronic gamma ray, and space radiation loaded in a spaceship are of interest research topics. We assume that there will be various types of mutants induced because of difference in ionizing ability, penetrating power, irradiation duration, and kinds of radiation. However, only a limited information is available in our hands so that we are planning to collect empirical data from the different radiation or ionizing particle sources. In order to identify gene function using mutant population, TILLING (Targeting Induced Local Lesions IN Genomes) projects were promoted in rice. This technique is high-throughput and reverse genetic approach and contributes in the identification of mutation and efficient application of mutant resources. The populations of 3,000 rice lines were constructed from the embryogenic culture system and gamma-irradiation.

3.4 Advanced Techniques for Mutation Breeding by Using Ion Beams

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1. Introduction

A great deal of studies on plant mutagenesis by ionizing radiation has been carried out since mutation was induced in maize and barley with X-rays by Stadler in 1928[1). Biological effect of ion beams has been also investigated and found that ion beams show high relative biological effectiveness (RBE) of lethality, mutation, and so on compared to low linear energy transfer (LET) radiation such as gamma-rays, X-rays and electrons[2)]. As ion beams deposit high energy on a target densely and locally, it is suggested that ion beams predominantly induce single- or double-strand DNA breaks with damaged end groups whose reparability would be low[3)]. Therefore, it seems plausible that ion beams can frequently produce large DNA alteration such as inversion, translocation and large deletion rather than point mutation, resulting in producing characteristic mutants induced by ion beams. However, the characteristics of ion beams on the mutation induction had not been clearly elucidated yet. On the base of the Consultative Committee for Advanced Radiation Technology in Japan, the Takasaki Ion Accelerator Advanced Radiation Application (TIARA) was established and basic research on plant mutation by ion beams was started in 1991. For over twenty years the biological effects of ion beams have been studied and novel mutants and varieties of crops have been consistently and efficiently produced using mutations induced by ion beams[4)]. At present, plenty of research activities in Japan utilize the ion beam irradiation in several irradiation facilities such as RIKEN RI beam factory, the Wakasa Wan Energy Research Center Multi-purpose Accelerator System with Synchrotron and Tandem (W-MAST), and the Heavy Ion Medical Accelerator in Chiba (HIMAC) of National Institute of Radiological Sciences.

2. Irradiation methods

In TIARA, characteristics of ion beams for biological endpoints have been investigated using Arabidopsis, rice, barley, tobacco, chrysanthemum, etc. Several kinds of energies and ions such as carbon (C), helium (He) and neon (Ne) were utilised in this study[5)]. All ions were generated from the AVF-cyclotron in TIARA. In general, ion beams are scanned at around 70 X 70 mm, and exit the vacuum chamber through a beam window of a 30 μm titanium foil. The sample is placed under the beam window and irradiated in the atmosphere. For example, 100-3,000 Arabidopsis or tobacco seeds are sandwiched between kapton films (8 μm thickness) to make a seed monolayer for homogeneous irradiation. In the case of relatively large seeds such as rice, barley or soybeam seeds, the embryo side faces the ion beams. Tissue culture such as ornamental explants, calluses, shoot primordial in a aseptic petri dish is directly irradiated except that the lid of dish is exchanged to a thin film in order to decrease the loss of ion beam energy. An irradiation sample is irradiated within 2 min.

3. Characteristics of mutations induced by ion beams

Mutation frequency: Mutation frequency was investigated on a gene locus basis using visible known Arabidopsis mutant phenotypes, such as transparent testa (*tt*), in which the seed coat is transparent because of the lack of pigments, and glaborous (*gl*), in which no trichomes are produced on leaves and stems. The average mutation frequencies of *tt* and *gl* loci induced by C ions showed a frequency of 0.07 X 10^{-3} mutants/ locus, which was 20-fold higher than those induced by electrons as a low LET radiation[6]. Koornneef *et al.* [7] investigated mutations induced by EMS with a frequency of about 0.20 X 10^{-3} mutants/ locus. Thus, carbon ions seemed to be about three-fold less mutagenic than EMS treatment. It is noteworthy that the high mutation rate or frequency by carbon ion irradiation was observed at a relatively low dose of 150 Gy, at which virtually all plants survive.

Mutation spectrum: In order to elucidate the features of ion beams as a new mutagen, the mutation spectrum induced by ion beams was compared with that induced by low LET radiation. Nagatomi *et al.* [8] investigated the spectrum of mutations in flower color using chrysanthemum cv. Taihei with pink-color petals. Most flower color mutants induced by gamma rays were light pink, and a few were dark pink in color. By contrast, the color spectrum of the ion beam-induced mutants shifted from pink to white, yellow and orange. Furthermore, flower mutants induced by C ions showed complex patterns of colouration, and striped color types, that have never been obtained by gamma-ray irradiation of this cultivar. The mutation spectrum of flower color and flower shape of carnation was investigated by Okamura *et al.* [9] investigated with carnation variety Vital, whose phenotype is spray type with cherry pink flowers and frilly petals. Flower color mutants such as pink, white and red were obtained by X-ray irradiation, whereas the color spectrum of the mutants obtained by carbon ion irradiation was far wider and included pink, light pink, salmon, red, yellow, complex and striped types (Table 1). In addition, many kinds of round shaped petals were induced. It was suggested, therefore, that the mutation spectrum of flower color and shape induced by ion beams is broad and that novel mutation phenotypes can be obtained.

Table 1. Mutation spectrum of flower color in carnation

Mutation frequency (x 10^{-1} %)										
Mutagen	Light pink	Pink	Dark pink	Red	Salmon	Cream	Yellow	Minute striped	Complex	Stripe
EMS	0	5.2	0	1.0	0	0	0	0	0	3.1
Soft X-rays	1.7	8.4	0	3.4	0	0	0	0	0	0
Gamma-rays	1.7	2.6	0	1.7	0	0	0	11.3	0	0
Carbon ions	2.4	4.7	2.4	3.5	2.4	1.2	2.4	0	2.4	3.5

Molecular mechanisms of mutation: C ion- and electron-induced Arabidopsis mutants were compared at molecular (DNA) level [6),10)]. In the case of C ions, fourteen loci out of 29 possessed intragenic point-like mutations, such as base substitutions, or deletions of several to a hundred of bases. Fifteen out of 29 loci, however, possessed intergenic DNA rearrangement ('large mutations') such as chromosomal inversions, translocations, and deletions. In the case of electrons, nine alleles out of 12 loci had point-like mutations and three out of 12 loci had DNA rearrangements. Sequence analysis revealed that C ion-induced small mutations were mostly short deletions. Furthermore, analysis of chromosome breakpoints in large mutations revealed that C ions frequently deleted small regions around the breakpoints, whereas electron-irradiation often duplicated these regions. These results could imply that different types of non-homologous end joining pathways operate in response to the mutations induced by the two radiation types and that C ion-inducedmutations are mostly likely to result in nulls.

4. New varieties and mutants

Model plants: Several new Arabidopsis mutants, and the gene responsible for these mutations, were identified following ion beam mutagenesis[11)]. Ultraviolet light-B (UV-B) resistant or sensitive mutants were obtained, and the genes responsible have been identified. New anthocyanin-accumulating or anthocyanin-defective mutants were also obtained and some of the genes responsible have been identified. A novel flower mutant, *frill1*, which has serrated petals and sepals, and the gene responsible for this phenotype have been found. A novel auxin mutant, the *aar1-1*, was also obtained. In *Lotus japonicus*, which is used as a model leguminous plant, a novel hypernodulation mutant, named *klavier* (*klv*), was isolated following irradiation with helium ions.

Crops: Ion beams have been used for inducing mutants resistant to major diseases. Mutants resistant to bacterial leaf blight and blast disease were induced in rice[12)]. Higher mutation frequency was found in the ion beam treatment compared to gamma-rays or thermal neutrons. Two mutant lines of yellow mosaic virus resistant barley were found in a screen of ca. 50,000 M2 families. By exposure of tobacco anthers to ion beams, mutants resistant to potato virus Y have been obtained. Banana mutants tolerant to black Sigatoka *in vitro* were induced by carbon ions. Recently, low-nitrogen-fertilizer grown rice mutants were induced for a solution to eutrophication. Moreover, low-cadmium rice mutants were obtained from the most popular Japanese rice cultivar, Koshihikari[13)].

Ornamentals: Complex and stripe types of flower color have been obtained in chrysanthemum[8)] as described above. On the other hand, a reduced axillary flower bud mutant that shows excellent character for labor-saving, named Aladdin, was induced by carbon ions for the first time[14)]. By the use of re-irradiation of this mutant using carbon ions, the ideal characters of not only a few axillary flower buds but also low temperature flowering were also obtained. In addition to the carnation varieties[9)] described above, mutants of petunia with altered flower color and form have been induced by ion beams. In rose, mutants with more intense flower colors or mutants in the number of petals, flower size and shape have been obtained. Mutants induced by nitrogen or neon ions in *Torenia* include two groups of flower color mutants, ones that lack genes required for color or pigment production and others in which their expression is altered. Taking advantage of ion-beam irradiation and the newest information of genomics in flavonoid biosynthesis, breeding glittering carnations and fragrant cyclamen with novel flower color have been succeeded [15), 16), 17)].

Trees: Ion beams have also been used for generating mutants for tree breeding. Wax mutants and chlorophyll mutants such as Xanta and Albino were obtained in the forest tree, Hinoki cypress. For phytoremediation, shoot explants of *Ficus thunbergii* were irradiated with several kinds of ion beams in order to produce novel mutants with a high ability to mitigate pollutants. A mutant variety, KNOX, with 40-80% greater capability to assimilate atomospheric nitrogen dioxide has been induced[18)].

Microbes: Ion-beam irradiation is also effective to obtained desired mutants of microorganisms. One of these mutants, a new yeast variety superior in improvement of alcohol fermentation property has been induced to produce Japanese sake.

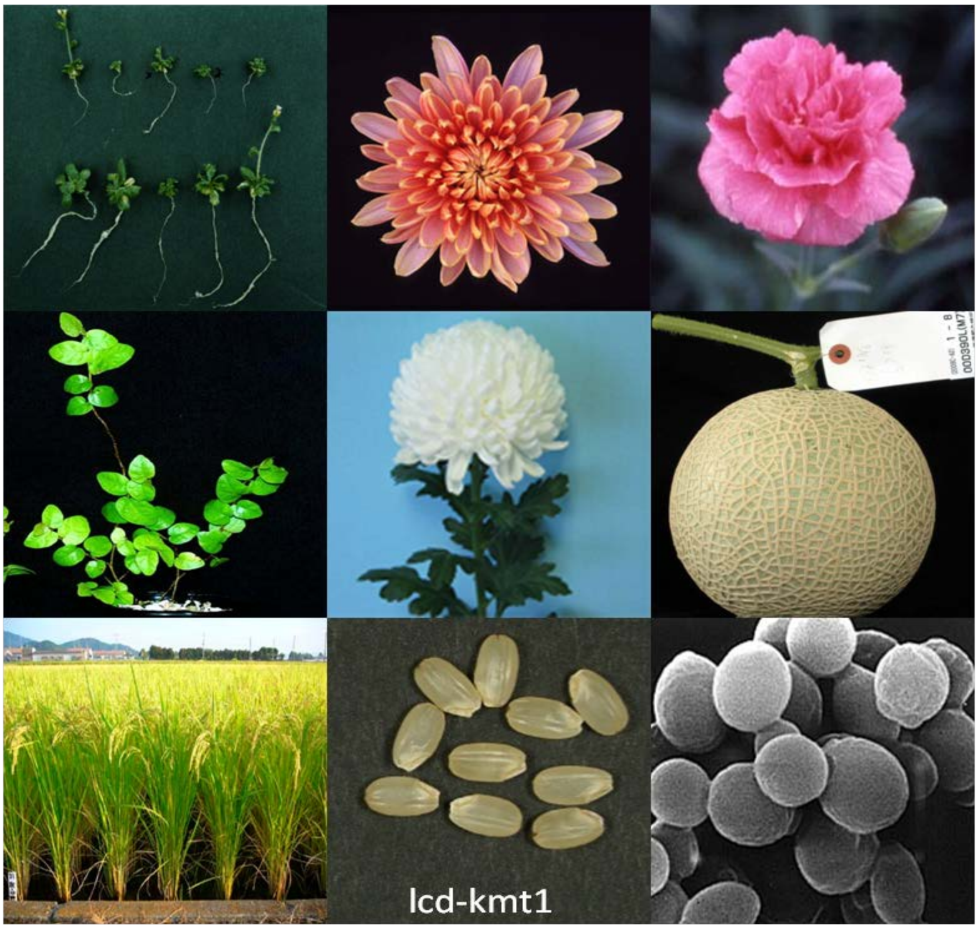


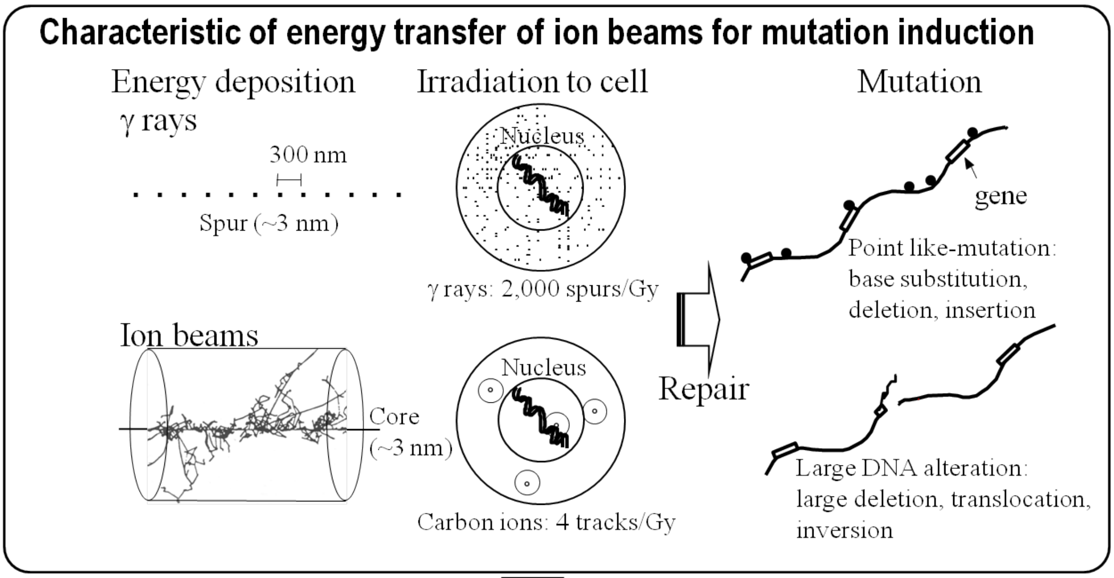
Figure 1. Novel varieties and mutants induced by ion beams

From leaning to the left to the lower right: Arabidopsis UV-B resistant mutants (lower rank) ; chrysanthemum complex-color variety, "Ion-no-Seiko"; new rose-flower type carnation variety; 'KNOX' of *Ficus thunbergii*; chrysanthemum variety 'Aladdin'; low-temperature growing variety, Shizuiku 1 gou, of Musk melon; low-nitrogen-fertilizer grown rice mutant variety from 'Aki-no-uta'; low-cadmium rice mutant from 'Koshihikari'; new yeast variety improved alcohol fermentation that produces a higher amount of ethyl caproate.

5. Conclusion & Future prospect

From the number of investigations indicated above, it is plausible that the characteristics of ion beams for mutation induction induce mutants with high frequency, show a broad spectrum of mutation phenotype, and therefore produce novel mutants. Another characteristic of ion beams for mutation induction would be to induce a minimum number of mutations compared to other mutagens(Fig.2). As the main reason to explain these characteristics of ion beams, we hypothesize that chemical mutagens, such as EMS, and low LET ionizing radiation, such as gamma-rays and electrons, will predominantly induce many but small modifications or DNA damage to DNA strands, resulting in several point-like mutations on the genome. In contrast, ion beams as high LET ionizing radiation will efficiently cause not so many but large and irreparable DNA damage locally, resulting in a limited number of null mutations. Therefore, novel mutants were efficiently obtained, and most showed distinct characteristics without detrimental characteristics, and mendelian traits without backcrossing.

The most important question is whether mutation is randamly occurred or directed. Nagatomi *et al.* [19] showed that the mutation rates of flower color of plants regenerated from petals were higher than from leaves with both ion beams and gamma-rays. This is considered to be because the genes for flower color in petal cells are more active than in leaf cells, which this may lead to a higher mutation rate of regenerated plants from petals. Recently, the increase of flower color mutation was shown by combining ion beams and high sucrose content as pre-treatment, suggesting the possibility of directed mutation.[20] If directed mutation could be controlled in higher plants, combination of the best ion type for energy and the best plant organ or tissue will enable the induction of objective mutation.



Characteristics of ion-beam induced mutation for plant breeding

1. High mutation rate : small samples/spaces for mutation breeding
2. Broad mutation spectrum : producing new varieties and mutants
3. Minimum No. of mutation : pinpoint-breeding without bad characters

Figure 2. Characteristics of ion-beam induced mutation in higher plants

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4. Mutation Breeding III

4.1 Success Story of Mutation Breeding in Thailand

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Mutation breeding through irradiation and chemical application had been continuously done in Thailand for long period of time. Various species of plants such as ornamental plants, vegetables, field crops and rice were induced mutation for various purposes. In Thailand, small nuclear reactor supplying irradiation from Cobalt 60 and Cesium 137 sources had been utilized for mutation breeding. With restricted amount of budget, nuclear scientists and mutation breeders, mutation breeding in Thailand was not widely supported but it created great impact on rice breeding. Three rice varieties derived from mutation breeding in the years 1977 1978 and 1981 are still widely being cultivating at present. So, this paper will mostly tell success story of mutation breeding on rice.

In 1959, Thai government by Rice Department released the best aromatic rice, namely Khao Dawk Mali 105 (KDML105), derived from local variety Khao Dawk Mali via pure line selection. KDML105 is non-glutinous rice, photoperiod sensitive with medium duration of maturity. It has very good grain quality in according to long grain quality standard. It has long grain (7.5 mm long brown rice) with slender shape, transparent (without chalkiness) and shiny polished grain and also yielded high milling quality (Figure 1). Its cooking quality had been widely accepted by consumers in various countries. Its distinct aroma (odor) with soft and slightly sticky cooked rice had been well known in world rice market. So, more than a half of rice cultivated area in Thailand had been occupied by KDML105 (Figure 2) since it had advantages in tolerance to acidity, salinity and moderately tolerant to drought. Its disadvantages are susceptible to diseases and insect pests, low yield and could be cultivated only one crop per year. With high grain quality, KDML105 is premier exported rice of Thailand and domestically widely used to develop high grain quality varieties and abiotic stresses tolerant varieties.

Figure 1. KDML105 milled rice

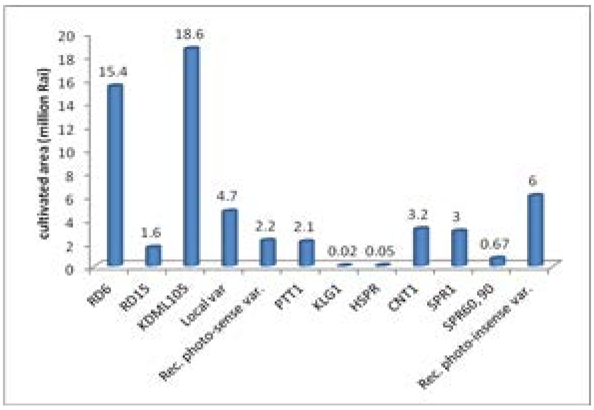

Figure 2. Rice cultivated area in wet season 2011

In 1965, KDML105 had been induced mutation by 20 Kilorad gamma irradiation of Cobalt 60 source. Its mutant had been tested and selected by conventional pedigree selection method. Many derivatives obtained from this program. One promising glutinous mutant had been released in 1977 namely RD6. One year later, 1978, an earlier matured mutant was also released namely RD15.

RD6 had very good grain physical properties similar to its original KDML105 (Figure 3). It also gives very cooking quality with soft sticky without paste-like and aromatic cooked rice. More than 80 percent of glutinous rice cultivated area in Thailand is being occupied by RD6. RD15 is very similar to KDML105 in all characteristics except its earlier matured (Figure 4). RD15 has 7-10 days earlier in maturity as compared to KDML105, it is more suitable for semi-arid area and some area with water shortage. Since it has good grain quality with aroma, RD15 is another premier exported aromatic rice of Thailand.

Figure 3. Grains of RD6

Figure4. Grains of RD15

In 1969, Thailand released the first semi-dwarf and photoperiod insensitive variety namely RD1. This variety caused very big change in rice cultivation in Thailand. Since it is photoperiod insensitive with semi-dwarf plant height, it has good response to high productive inputs and provides high yield in all growing seasons through year round. Thailand can produced more paddy and extend exporting since then. Many modern varieties had been developed after introduction of RD1. This is another green evolution in Thailand. When RD1 was being recommended to the farmers, it had also been induced mutation in 1969. It had been irradiated with fast neutron of 1 Kilo Rad dosage. Mutants had been tested and selected by conventional pedigree selection method until 1981. New glutinous semi-dwarf type and photoperiod insensitive variety, namely RD10 had been released (Figure 5). RD10 had been a variety utilized to increase glutinous rice production in Thailand until present and had also been exported.

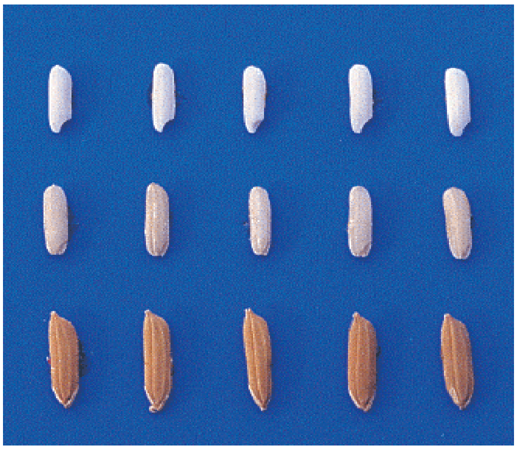

Figure 5. Grains of RD10

Even though Thailand has limited resources, but mutation rice breeding is still being conducted. Good grain quality mutants are expected to achieve from local photoperiod sensitive varieties. Photoperiod insensitive aromatic mutant is particular breeding goal. Meanwhile, other properties of grain quality are being induced in high yielding varieties. For example, less chalkiness is being screened from mutants of high chalky variety, RD25. Some mutants of high yielding varieties are being screened for low phytic acid content. From collaborated project of FNCA, mutants of KDML105 and RD15 are being screened for various amylose content and low protein content. Isogenic lines of photoperiod insensitive mutants with various amylose content that retain aroma were obtained (Fig 6a, 6b). This is a big advantage for genetic study on aromatic rice and new opportunity for Thailand to produce various products of aromatic rice to the world market.

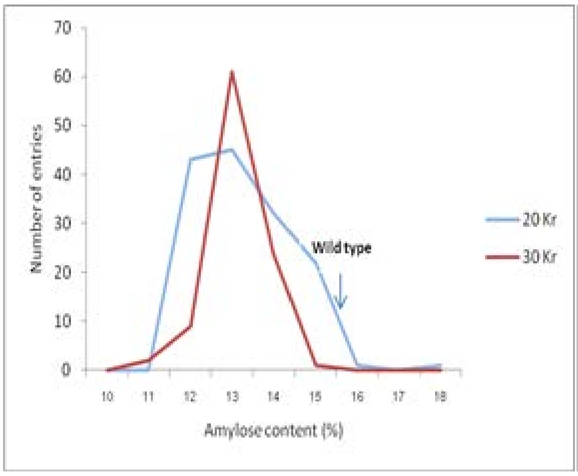

Figure 6a. Amylose content of KDML105 mutants

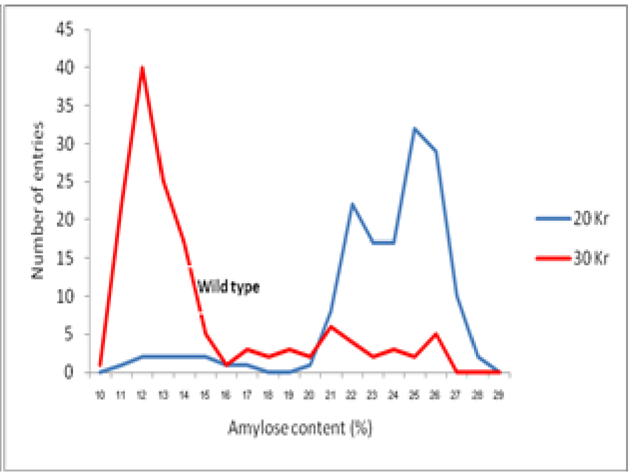

Figure 6b. Amylose content of RD15 mutants

4.2 Success Story of Mutation Breeding Research on Rice in Bangladesh

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Bangladesh has so far release nine improved varieties of rice using induced mutation technique. Bangladesh Institute of Nuclear Agriculture has play a vital role to produce and release these new varieties. Two varieties called Iratom-24 and Iratom-38 were developed by irradiating the seeds of 'IR 8' and released by national seed board of Bangladesh (NSB) in 1974 for Boro (December-May) and Aus (March-June) seasons. The mutated characteristics of these varieties are short crop duration, resistant against bacterial leaf blight (BLB) and medium fine grain size. 'Binasail' is also developed by irradiating the seeds of 'Naizersail' and released in 1987 for Aman (July-December) season with mutated characteristics like long panicles, more number of grains, medium fine grains with higher weight, early maturity with photo-insensitivity and higher grain and straw yields. 'Binadhan-4', 'Binadhan-5' and 'Binadhan-6' were developed by irradiating F_2 seeds of parents 'BR-4'and 'Iratom-38' and released 1998, 'Binadhan-4' for Aman and 'Binadhan-5' and 'Binadhan-6' for Boro season. 'Binadhan-7' was developed from M_3 seeds of the cv 'Tai Nguen' of Vietnum. This variety was release in 2007 for Aman season with earliness, long fine grain and high yield. Early mature character of this variety helps to escape drought and insect attach during flowering and drought stages. Moreover it helps increase cropping intensity by facilitating timely cultivation of following winter crops. 'Binadhan-8' was released in 2010, Crop duration 130-135 days, Salinity tolerance, Seedling stage: 12-14 dS/m, Mature stage: 10-12 dS/m, Suitable for cultivation in both Boro and Aman season. 'Binadhan-9 was developed by hybridizing between the local cv. 'Kalozira' and an exotic mutant line Y-1281. It was released in 2012 for Aman season, and also can be grown in Boro season. Unlike the parent 'Kalozira' it has short duration, long and slender grains with slight aroma. Using recent ion bean irradiation technique we has already developed two mutant lines that can be grown after harvest mustard or rapeseed during the first week of February to first week of March and can be harvested after 121-134 days. These new developed mutant lines produce high yield and relatively fine grains.

4.3 Towards Sustainable Development of Food & Agriculture in Asia and the Pacific − Significant Roles of IAEA / RCA Projects

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1. Overview of RCA

The Regional Cooperative Agreement for Research, Development and Training (RCA) is an intergovernmental agreement established in 1972 under the auspices of the International Atomic Energy Agency (IAEA). It is open to the participation of any Member State of the IAEA in the area of South Asia, South East Asia and the Pacific or the Far East. A Member State from one of these regions may become a party to this Agreement by notifying its acceptance thereof to the Director General of IAEA. There are now eighteen Government Parties to the RCA, i.e., Australia, Bangladesh, China, India, Indonesia, Japan, Korea, Malaysia, Mongolia, Myanmar, Nepal, New Zealand, Pakistan, Philippines, Singapore, Sri Lanka, Thailand and Vietnam.

The vision of RCA is to strive to become a respected regional resource community in nuclear science and technology, competent and cost effective in providing high impact solutions on a sustainable basis to socio-economic development problems for identified end-users within the Region and the Member States. During the past 40 years, more than 100 RCA technical cooperation projects in Agriculture including the field of plant mutation breeding were successfully organized and implemented through the appropriate national institutions in the RCA Member States.

2. Global Food & Agriculture Situation

There are at least 1 billion hungry people worldwide in 2009, and the Asia and the Pacific region leads the biggest estimated regional distribution of hunger. Price volatility makes both smallholder farmers and poor consumers increasingly vulnerable to poverty. The developing world's poor are experiencing the effects of higher commodity prices, and declining agricultural productivity growth is exacerbating the problem.

Lifting a billion people out of poverty and feeding an extra 2.3 billion by 2050 will require increasing cereal production by 70%, doubling the output of developing countries. Accelerating development of agriculture to continually increase productivity should be the final approach to end the poverty.

3. Inputs for Nuclear Agriculture through RCA

Since the establishment of the RCA, many problems in the agricultural sector have been effectively addressed using nuclear technologies. There are totally seventeen RCA projects in the agricultural sector have been implemented by far with focus on four main areas, i.e., plant mutation breeding, animal health &production, food irradiation and soils and land use. Through these projects, more than 780 young scientists were trained, more than 640 senior scientists were appointed to participate in the coordinating meeting or workshop, more than 110 expert missions or scientific visit were implemented for specific technique issue.

4. The Impacts of RCA Projects on Plant Mutation Breeding

Mutation breeding techniques have played very significant roles in combating poverty and food security problems by developing new mutant germplasm and mutant varieties. By the end of 2009, 1877 mutant varieties, which accounts for more than 60% of the total number of mutant varieties in the world, have been released or approved for cultivation in the Asian and Pacific region. Such great success of mutation breeding is partially contributable to the previous RCA projects on mutation breeding, which have provided training and techniques in the Member States and organized research and development activities in enhancing crop genetic diversity, development of new techniques and germplasm, and facilitated the exchange of information and genetic materials.

(1) Increased Mutant Germplasm Exchange and Utilization

In the Projects RAS5037 and RAS5045, one of the major task is to exploit direct or indirect use of mutant varieties/lines in other countries than its origin, through germplasm exchange and evaluation among participating countries. This has been carried out through RMMTs for rice, soybean, mungbean, sesame, groundnut, wheat and sorghum. Mutant lines were further demonstrated in farmers' fields and national yield trials when they have the potential to be released as a new variety in testing countries, or were used in breeding programs as a donor variety if they had useful characteristics. There are totally 135 mutant varieties/lines of rice, wheat, soybean, mungbean, groundnut and sesame were exchanged and evaluated in more than 80 locations among the 14 participating Member States. At least 15 non-native mutant varieties were identified for further yield trial for commercial release.

Through the systematic trials in Thailand, one soybean mutant variety from Vietnam (DT84) and one from Korea (Bangsakong) consistently outperformed (higher yield or early maturity) local check varieties. The counterparts have thus proposed to officially release these two lines as a new variety for farmers in rice based cropping systems in the upper northern part and lower northern part of Thailand, respectively. Ten groundnut varieties plus local controls were tested in Indonesia, Philippines, Sri Lanka and Vietnam. All five countries except Vietnam have identified promising lines which have been recommended for either variety release or inclusion in national new variety tests. In Sri Lanka, for example, after demonstration trials on farmers' land, four varieties, i.e. B/30/12/10, Kidang, Binschinabadam 2 and Karisma Serene appeared to be very promising and will be recommended for official release. It was reported that some of the exchanged mutant germplasm in the RCA Member States have already been used as parents in their respective breeding programs, which potentially can lead to the development of even more new varieties in the coming years.

Based on the mutant germplasm exchange network, an autonomous non-governmental professional association on mutagenesis study was established with the name of Asia and Oceania Association of Plant Mutagenesis (AOAPM).

(2) New Mutant Germplasm Developed

Hundreds of well characterized stable mutants of wide range of crops and for important agronomic traits such as improved yield, resistance to diseases, protein, oil, starch and sugar content Here are a few examples of significance in future crop improvement: Mutants resistant to soybean crinkle leaf disease (Thailand); soybean mutants with enhanced tolerance to salinity (India); soybean mutant lines with reduced phytic acid content (China); rice mutant MR219-4 with drought tolerance(Malaysia).

In other crops, various mutants have also been developed, e.g. a large collection of mungbean and groundnut mutants have been developed in Thailand and India, respectively. Individual mutant lines are developed in all participating institutes, e.g. drought tolerant mutant lines were developed in China and Indonesia, respectively for wheat and sorghum; no-shattering sesame lines and easy-cooking soybean lines in Korea; early maturing groundnut in Sri Lanka; and virus resistant mungbean lines in Pakistan.

(3) New Mutant Varieties Released

70 more new mutant varieties have been developed and disseminated in the RCA Member States through RCA projects. Taking an example of the number of released mutant varieties under the RCA project RAS5045:

- In 2007 a total of 15 mutant varieties where released in: Bangladesh 1 rice variety; China 1 rice and 4 wheat varieties, India 1 each of groundnut, sunflower, soybean, mustard, pigeon pea and cowpea and 2 mungbean varieties; Indonesia 1 rice variety.
- In 2008, 9 mutant varieties were released in: China 1 wheat variety; India 1 groundnut variety; Indonesia 1 each of rice, soybean & cotton varieties; and Vietnam 4 rice varieties.
- In 2009, 6 mutant varieties in: China 1 rice variety; India 1 variety each of groundnut and pigeon pea; Indonesia 2 rice varieties; and Vietnam 1 rice variety.
- In 2010, 7 mutant varieties where released in: Australia 1 barley variety; China 2 wheat varieties; India 1 mungbean variety; Indonesia 1 each of rice soybean varieties; and Sri Lanka 1 tomato variety.
- In 2011, 2 mutant varieties of sorghum and rice had been released in Indonesia as

at the time of the meeting.

The dissemination areas of officially released mutant verities under the RCA Project RAS5045 were about 118 mha, with a benefit increase of about 227 millions USD for farmers in the participating MSs.

5. Plant Mutation Breeding In CAAS

Mutation technique has become one of the most fruitful and widely used methods for crop improvement in China, which have played a very important role in China's crop production, especially food crop production. The total number of mutant varieties officially released, by December 2011, accumulated to 810 including 45 crops and ornamental species. With the best fit cultivation practices, the maximum area in total per year for mutant varieties cultivation was over 9 million hectares, which accounted for about 1/10 of the total planting area for relevant species in the past 20 years in China.

Protocols for crop mutation induction by spaceflight environment, energetic heavy ion beams, fast neutrons and gamma rays irradiation have been established and applied for mutation induction. Doubled haploids based in vitro mutagenesis and screening on salt tolerance were optimized. A TILLING platform for wheat mutant gene discovery and lab screening methodologies for rice quality characters were set up. More than 20 mutant varieties in rice, wheat, corn and vegetables were developed and officially released by the national or provincial authority in CAAS in the past ten years. The total extension areas of officially released mutant verities since 2007 were about 700,000ha, with a benefit increase of about 75 millions USD for farmers in China.

6. Conclusions

Nuclear techniques can enhance biodiversity, improve crop cultivars and increase farmer's income. Routine utilization of induced mutations for crop improvement have made great achievements especially through RCA. Induced mutants are not GMOs, as the mutant genes have been changed from their own exist genes.

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5. Nuclear Technique for Soil Management for Sustainable Agriculture

5.1 Development of Plant Growth Promoter and Plant Elicitor by Radiation Processing of Natural Polymers

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1. Pilot Scale Production of Oligochitosan

Pilot plant production of oligochitosan was carry out using the existing facility of gamma vulcanize rubber latex available at MINT Technology Park of Malaysian Nuclear Agency. Firstly, chitosan powder was irradiated by gamma for 50 – 75kGy irradiation dose prior to dissolution in lactic acid to reduce it molecular weight. Pre-irradiated chitosan powder (3%) was dissolved in 2%lactic acid solution and 0.1% to 0.3% hydrogen peroxide prior to irradiation at 12kGy by continuous flow gamma irradiation. The solution is then neutralized with sodium hydroxide to pH 6-8. The molecular weight of oligochitosan produce will be in range of 10kDalton to 15kDalton depends on the initial molecular weight of chitosan powder. Minimum production of oligochitosan by this system is 2.2 ton per run with concentration 20,000ppm. This process is more efficient and cost effective in comparison with other technique such as enzymatic and acidic hydrolysis degradation. Radiation degradation oligochitosan has a potential to be used as plant elicitor and plant growth promoter for many type of plants – vegetables, perennial crops and others. Protocol on the production of oligochitosan has been established.

2. Application of oligochitosan as plant growth promoter and elicitor

2.1 Field test on rice

Field test using oligochitosan produce by pilot plant was performed on rice as plant growth promoter and elicitor in collaboration with FELCRA Bhd, government owned company in agriculture business, Sg. Burung Agriculture Department and Malaysian Agriculture Research Development Institution (MARDI). Field test with FELCRA Bhd was implemented form August 2008 until January 2011 for 4 season rice plantation. However, field test with the agriculture department is only for 2 seasons from January 2011 until December 2011.

The germination study on rice seed indicate that seedling period is shortened to 10-12 days compare to normal practice 15-17 days using commercial chemical germination product. Where, rice seed was soaked with 100ppm oligochitosan and the plant sprayed 3 times with 40ppm oligochitosan during the seedling period. During the germination period, rice seed of oligochitosan treatment does not required nutrient compare to chemicals based treatment need additional nutrient to support the growth. Result of field test at FELCRA Bhd show that seed treatment and spray with 100ppm oligochitosan increase rice yield up to 26% compare to control without oligochitosan. Field test at agriculture department give only up to 15% increase in yield by spray 40ppm oligochitosan. Blast study at MARDI shown that oligochitosan has elicitation effects on paddy plants against blast attack. Laboratory study effect of oligochitosan on growth of Pyricularia oryzae has shown that oligochitosan 40ppm enough to prevent the growth. The field trial results also shown that oligochitosan which is environmental friendly product, can replace chemical fungicide commonly use by the farmers and at the same time give significant increase in yield. Protocol on application of oligochitosan on rice has being established.

2.2 Field test on agarwood plant

Interest on Agarwood plant is due to its resin which being commercially explore for perfume production. Oligochitosan is used to replace using chemical pesticide in grown the plant. Study on oligochitosan as plant elicitor, the result from field trial on agarwood plant shown less disease infection and the survival rate of the plant also increase. Early stage of growth up to 2 years for agarwood plant, the plant can easily infected by fungus and insect. By spraying oligochitosan every 1-2 week with minimum concentration 80 – 100pmm, the plant is being protected from infection. Result reveal that about 58% increase in height and diameter of the plant when foliar spray with oligochitosan. Field test on agarwood plant shown that oligochitosan improve growth and has elicitor effect on the plant.

2.3 Other application of oligochitosan

Tissue culture study on banana and pineapple indicate that the existence of oligochitosan in the media increase the growth of the tissue plant. In the case of banana, 2ml of 10000g/mol oligochitosan increase the weight more than 3 times compare to control without oligochitosan. For the pineapple, 40ppm oligochitosan in the media is enough to triple the growth of the pineapple tissue culture plant. Testimonial of farmers on the application of oligochitosan as plant growth promoter shown that oligochitosan sprayed on the trunk of jack fruit plant increase fruit production.

5.2 Biofertilizer production using radiation technology for sustainable agriculture

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Division of Agrotechnology and Biosciences, Malaysian Nuclear Agency

The landscape of Malaysian agriculture is dominated by plantation crops of oil palm and rubber, in particular, and followed by rice, fruit trees, vegetables and herbal plants. Malaysia has a history of heavy usage of chemical fertilizers (220 kg/ha), which was normally associated with high productivity as compared to average Asian countries (80 kg/ha). About 90% of the chemical fertilizers are imported, thus making the country's agroindustry vulnerable, generating an issue of national security. Nitrogenous fertilizers are the most used; for example, urea is used at 70 Mt/yr @ RM 1500/t. 450,000 MT are N-based Fertilizers (urea & ammonium sulphate/chloride/nitrate). About 60% of oil palm cost of production is on fertilizers. For comparison purposes FIVE kg of Bioorganic fertilizer is able to supply the same amount of nutrients as ONE kg of N-based fertilizer. Thus, if we were to totally replace N-based fertilizers, a total of 7,250,000 MT Bioorganic fertilizers needs to be produced and readily available. Currently that capacity is far from attainable. Government subsidised chemical fertilizers for farmers also contribute to the favourable adoption of chemical fertilizers in normal agricultural practice.

With the present concerns on crop quality, food safety, and environmental safety there is increasing awareness on biofertilizers among the agricultural industry, even the large plantation companies. Several of them are beginning to be actively pursuing the 'green" approach to complement their usage of chemical fertilizer. For example Felda, Sime Darby and Felcra are embarking on their own biofertilizer production, especially for oil palm. Their interest in biofertilizer is partly due to increasing cost of chemical fertilizers, particularly urea, and partly to awareness on green technology for crop production. It is estimated that the market size for bioorganic fertilizer in Malaysia is RM 638.87 million/yr.

The main issue is sustainable agriculture. Malaysia is facing infertile soil due to loss of top soil and years of planting on same soil in addition to increasing pest and diseases. The oil palm industry, for example, faces pressure to become more sustainable, especially to achieve the Sustainable Oil Certificate and Roundtable of Sustainable Oil Palm. An important element to consider is mitigating climate changes, where the climate pattern suitable for planting, weeding and fertilizing hardly exist, hence providing greater stress to overall plantation management.

Nuclear technology is central to the activities, through the use of gamma irradiation for sterilisation of various carriers and the use of isotopic tracers to evaluate the efficiency of uptake of certain major nutrients, especially N, through the usage of biofertilizers. For the future, the use of ionising radiation for mutagenesis of biofertilizer microorganisms is proposed. Figure 1 the flow chart of a typical biofertilizer production utilising gamma irradaiation for sterilisation of carrier or substrate.

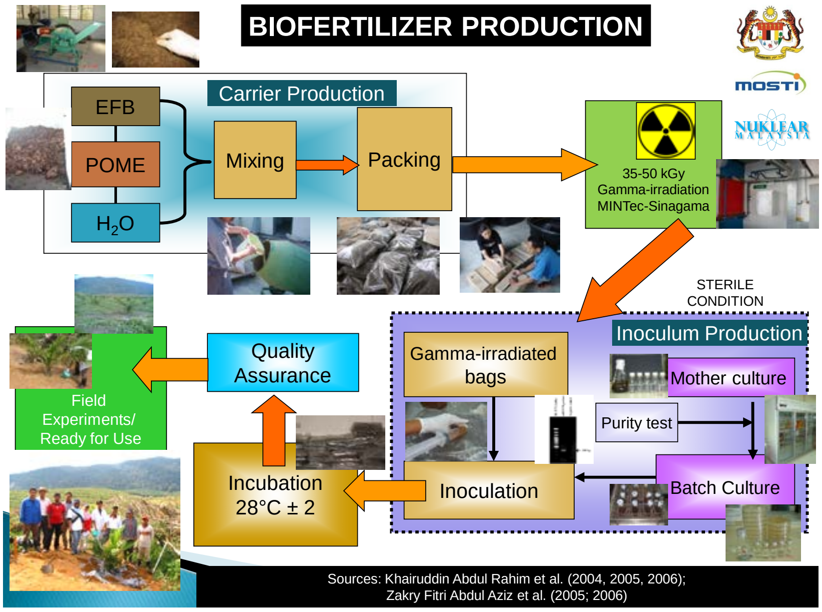

Figure 1. Flow Chart of Biofertilizer Production

Most recent research at the university and research institutions utilised the gamma irradiation facility at MINTec-SINAGAMA, Malaysian Nuclear Agency for sterilisation of substrates or carriers. These substrates from compost of empty fruit bunches of oil palm, peat and vermiculite can be packed in plastic bags and sterilised in bulk. MYAGRI, a biofertilizer company in Malaysia has been sending their vermiculite carriers for sterilisation for several years now, and indicated the suitability of the technology for it commercial activities, which include close vicinity to MINTec-SINAGAMA. Figure 2 shows the gamma irradiation facilities at Nuclear Malaysia.

Figure 2. Gamma Irradiation facilities at Nuclear Malaysia

The opportunities lie ahead of the Malaysian biofertilizer industry.

- Less reliance on imported chemical fertilizers (many from fossil fuel based sources) – cost savings
- Protection of biological assets from plant diseases– healthier plants result in higher yield, longer lifespan and higher productivity
- Established high value green and biotechnology products and services have good potential in other palm oil producing countries (Indonesia, PNG, Western Africa) – Malaysia as hub for 'bio and green' technologies
- Large fertilizer production and customized blends allows for niche product development – good potential for development of organic food industry
- Demand for treated organic fertilizers in Japan, Korea, Europe and the USA

The is much need on INNOVATION in formulation (e.g. multifunctionality); exploration of biodiversity; mutagenesis of microorganisms; on methods of application e.g. Biofertilizer + Oligochitosan), Biofertilizer + Biochar or without the use of expensive or cumbersome carriers at all. We have to be innovative in the 'positioning" of biofertilizer in Malaysia agroindustry, for example thorugh collaboration with companies for transfer of technology and commercialization; providing incentives to biofertilizer companies in support of 'Green Technology' for food safety and societal wellbeing; proposal for government subsidy of biofertilizers to farmers, as given to chemical fertilizers; and enhance promotional activities. There for policy support, for instance on gulation on QC/standards of biofertilizer products. As well as continued scientific and technical support from groups like the Forum for Nuclear Cooperation in Asia (FNCA) – Biofertilizer Project (www.fnca.mext.go.jp).

There has been inroads of biofertilizer in the Malaysian agroindustry. However there is still much to be done, to ensure the small successes be escalated to bigger ones in the future. The following need to be considered:

- Awareness in agroindustry; market needs; demand driven; issues of food safety and security.
- Vibrant R&D in research institutes, universities and agribioproduct companies
- Avenues of commercialisation of R&D products.
- Supportive government policies – green technology, bioeconomy initiatives
- Availability of advanced manufacturing technologies, including biotechnology and irradiation technology; this will lead to production of quality products
- Receptiveness of agroindustry to advanced technologies.
- Effective promotional programmes.

For biofertilizers to have good reputation versatility is important, especially in terms of function, effectiveness, ease of application and affordability.

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1. MOSTI
2. Malaysian Nuclear Agency
3. FNCA
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5. MYAGRI Group of Companies
6. Inno Integrasi
7. Fellow researchers and proponents of biofertilizer.

6. Collaboration & Technology Transfer

6.1 Induced Mutation Breeding of Roselle (*Hibiscus sabdariffa* L.)§

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Extended Abstract

Roselle (*Hibiscus sabdariffa* L.) is a tropical annual plant species in Malvaceae family, which originated from West Africa, predominantly from Sudan. It is a multi-purpose plant species used for a myriad of purposes, from health to other uses in different countries spanning from Sudan, West Indies, Mexico, Germany, India, Malaysia, Indonesia, Thailand to China. It has a naturally satisfying sour taste and considerably unique nutritional characteristics, in particular its high contents of vitamin C and anthocyanins. Roselle was introduced into Malaysia in early 1990s. Then its commercial planting was first promoted by the Department of Agriculture in Terengganu in 1993 in collaboration with Universiti Malaya and MARDI. The present total planted area is still small; however, its planting has now spread to many states with total planted area of about 100 hectares annually. At present, there are two main varieties available to growers, namely UMKL-1 (aka Terengganu variety) and Acc. 21 (aka Arab variety). The latter variety yields higher, both in terms of fruits and calyces. These two varieties are mainly cultivated to produce pro-health juice for their high contents of vitamin C and anthocyanins from freshly harvested calyces. The calyces are also processed into sweet pickle, tea, and jam to some extent.

The roselle industry has faced many ups and downs like a yo-yo. Today, there are about 18 companies still involved in the production, processing and marketing of roselle products for the local markets. It is estimated the total market value of the Malaysian roselle industry to be approximately RM10-15 million, with 65-80% staying with the processors. The domestic market consumes 500 tons of roselle calyces annually, of which over 80% are processed as concentrate and drink. The current annual export value of fresh calyces is estimated at RM2.5 million, mainly to Australia. Currently, it is estimated there are 25,000 domestic consumers.

Worldwide, crop improvement efforts through conventional breeding have been little attempted since hybridization is very difficult due its cleistogamous nature of reproduction, although there have been reports of considerably low natural outcrossing. Based on cytological observations (2n=72) and trait segregation patterns, roselle is believed to be a tetraploid species. Therefore, its segregating populations would need longer time for fixation if compared to diploid species. To overcome this reproductive obstacle, mutation breeding programme was initiated in UKM in 1999 in collaboration with Nuclear Malaysia (then known as MINT). In 2006, new work on mutation breeding was applied on two main varieties;

however, only Arab variety responded favourably to radiosensitivity tests. These tests, based on height of 2-week-old seedlings, determined the LD_{50} value of 80 Gy. Seeds of Arab variety were then treated with gamma radiation from ^{60}Co source using LD_{50} dose at UKM to produce M_1 generation. Selection and evaluation were done from M_2 through M_6 generations, alternating and overlapping between UKM, Bangi, Selangor and TFirdauce, Tasek Gelugor, Penang in order to achieve rapid generation advance. Six potential selections identified and further evaluated. As a result of this mutation breeding programme, UKM launched in 2009 three of these stable selections as new varieties. The three new varieties are named UKMR-1, UKMR-2 and UKMR-3. They are easily differentiated by their calyx colour, namely bright red, dark red and light green, respectively. To date, UKMR-3 has been approved for registration by Department of Agriculture under the Plant Protection of New Plant Varieties Act 2004.

From then onwards, the development of the three new roselle varieties has enabled the roselle industry to move forward, particularly in terms of providing additional choices for growers and the industry. Together with the two main varieties, they have pushed roselle to gain wider acceptance by the growers and consumers. Research done in UKM had also discovered that some roselle selections contain high contents of hydroxycitric acid (HCA), a potent anti-obesity compound found in many nutritional supplements for managing body weight. To date, three patent applications related with roselle have been filed.

The three new varieties exhibited significant differences for plant characteristics compared to its parent Arab variety and also the control Terengganu variety, showing better performance for four parameters. These varieties have a number of their own special characteristics but in general, they have overall features of having shorter maturity, medium plant size and reduced plant height compared to their parent Arab variety. The leaf colour is generally green except for UKMR-2 which possesses purplish-red pigmentation in its stems and leaves. The fruit shape is attractive and somewhat different from their parent variety, but the fruits of UKMR-2 closely resemble that of the parent. These three new varieties are superior to both Arab and Terengganu varieties in terms of plant height (shorter and more lodging resistant) and shorter crop cycles of approximately 4 - 4½ months (i.e. these three varieties mature earlier by more than one month). To ensure their genetic uniformity, the mother plants of these varieties are maintained vegetatively such as through microcutting in soilless polythene mist-propagation chamber, while field planting of these varieties will use seeds harvested from the uniform mother plants.

While roselle has been known to have been introduced into the country many decades ago, the actual crop improvement work on roselle in Malaysia is considered quite recent and has taken quite a 'long' journey, where it was first introduced by Universiti Malaya in 1990s and afterwards promoted by Department of Agriculture and MARDI, its breeding work was initiated and accomplished in UKM, Bangi in 1999-2010, later continued in IIUM, Kuantan 2010-2012, and presently it will continue its journey further at Universiti Teknologi MARA (UiTM).

6.2 R&D Activities in FELCRA

Ragu Ponusamy
Head of R & D, Federal Land Consolidation and Rehabilitation Authority, Malaysia

FELCRA, (Federal Land Consolidation and Rehabilitation Authority) was established IN 1966 with the objective of poverty eradication and improving the living standards of the rural poor. FELCRA achieves by developing land and cultivating with oil palm, rubber, rice and other profitable crops. To date FELCRA has successfully developed 220,000 ha. of land with a record of 95,000 families as beneficiaries. FELCRA R&D activities are currently focused on yield improvement strategies such as Breeding and Tissue culture techniques for production of superior planting materials besides conducting trials on other crops of commercial value.

Radiation and Nuclear technology has great potential particularly in FELCRA's Rice sector and short term crop like banana, stevia etc. As the Research and Development Department in FELCRA is still in its infancy, FELCRA is open to new technologies which would ultimately result in better yields and improved living conditions of the rural poor.

FELCRA is willing to participate in collaborative research efforts particularly in the area of Agronomy, Biotechnology, Soil fertility and Green technology.

Keywords: Poverty eradication, collaboration, yield improvement.

6.3 Potential of Bio-fertilizer in Agro-industry: MYAGRI'S Experience in Commercialization

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MYAGRI is a group of biotechnology companies dedicated to providing more efficient, effective and environmentally friendly solutions to challenges faced by today's agricultural and environmental management. MYAGRI principal activities consist of identifying, isolating, and propagating beneficial micro-organisms which are then formulated into ready-to-use products with specific and efficient delivery systems. These products are aimed at improving crop productivity, land rehabilitation, bio-remediation of wastes and long term sustainability of agriculture, environment and living.

MYAGRI's range of premium biotechnology products include MYCOgold® - a root enhancer, TRICHOgold® - a plant defence booster, MYCOblend® - a semi-organic fertilizer, aGricare® BIO-ORGANIK - premium grade compost, D'Comp™ Bio-plus – a bio-organic decomposer and METAXORB® - a bio-pesticide.

MYAGRI's Bio-Organic program is a green waste management solution that allows the Plantation and Agriculture Industry to seriously look at Nutrient Recycling for all of their bio-resources, and reduce or complement the application of chemical or inorganic products.

The program assists the Oil Palm Plantation to convert all Green Wastes available at the Oil Palm Mill such as Empty Fruit Bunch (EFB), Mesocarp Fiber, Decanter Cake and Palm Oil Mill Effluent (POME) Sludge. These materials are treated and composted until they become mature organic material. They are later enriched with MYAGRI's beneficial microbes to produce Bio-Organic fertilizer. One of the microbes used in this program is a bacillus based formulation, trade named aGricare® Organic N, that has been developed in collaboration with Malaysian Nuclear Agency in 2010.

MYAGRI is committed to continuing R&D and commercialization of beneficial microbes and green biosciences that will improve the current agricultural practices; and welcomes collaboration with local and international organizations.

Keywords: Bio-fertilizer, Biowaste conversion, beneficial microbes, yield improvement