Annex 4. Presentation Summary

FNCA 2009 Workshop on Mutation Breeding Project

Session 3 "Sub-Project on Composition or Quality in Rice"

Member Countries presented their country report. The brief summaries of each country report are as follows:

Bangladesh (Dr. Md. Lokman Hakim, BAEC)

Irradiation of rice seeds *Ashfal* and *Morichshail* with 350 and 400 Gy resulted in delayed flowering and very high seed sterility in M_1. Some putative mutants have been selected in M_2 population of *Morichshail*. Yield trials of five long fine grains advanced rice lines including two aromatic ones at four locations showed variations in yield in different locations but the average yield suggested that three of the five lines produced identical yield which was higher compared to parent and check variety. Nineteen plants originated from irradiated seed derived calli of *Takshoyl* were raised (M_2V_1) in boro season but due to its high photo sensitiveness, a very few plants set seeds. Further regeneration of plants from irradiated calli was not possible due to non-functioning of gamma irradiator.

China (Dr. Luo Ju, CNRRI)

New mutant lines of restores (CR63, CR84 and CR1577) were selected and evaluated the restoring ability in 2008 and 2009. Low setting percentage was observed in the field to some hybrids. Six combinations were obtained between three mutants with the other restorers in 2008. F_1 and F_2 generations of these combinations were cultivated in Hainan and Hangzhou for developing good restorers with good agronomic traits in the future. In 2009, one maintainer and two restorers irradiated with 350Gy and 400Gy in 2008 are raised in order to obtain new mutants. Though screening maintainers and restorers with adaptive and similar amylose content as parents, it will be greatly helpful to reach ideal amylose content for commertial hybrid rice.

Indonesia (Dr. Sobrizal, BATAN)

To meet the Indonesian domestic demand for both rice grain quality and quantity, high variability of pure lines derived from a cross of Indica rice var. IR36 and Japonica rice var. Koshihikari were constructed. As much as 568 pure lines derived from this cross were selected, and among them two lines, KI 237 and KI 432 were irradiated by 200 Gy gamma rays to remove some undesirable characters of these lines. Both KI 237 and KI 432 have high yield potentials, but susceptible to lodging for KI 237 and late maturity for KI 432. Selections were conducted in M_2 populations with emphasize on plant height for population derived from irradiated KI 237 and days to heading for population derived from KI 432. Selections and purifications were continued in M_3 generation and finally, one dwarf M_3 line, two semi-dwarf M_3 lines, and one early maturity M_3 line were selected. One of the selected semi-dwarf Mutants is RKI 237-1. Based on the segregation analysis in M_2 and M_3 generation of RKI 237-1 mutant line, it was concluded that this semi-dwarf in this line

was controlled by a single recessive mutated gene. This gene was designated as *sd*$^{237-1}$. This mutant should be useful as a genetic resource for the improvement of KI 237 line through back-cross breeding as well as be developed further in breeding program directly to be a new high yielding mutant variety. The amylose content of 84 selected lines varied largely, ranging from 17.39 to 25.65 %. Large variations were also observed in grain size and color of lines derived from irradiated KI 237.

Japan (D.r Minoru Nishimura, IRB)

We selected several tens of new mutants with low amylose contents from Hitomebore, second largest variety in Japan. They are induced by ion beams or gamma-ray irradiations and are M_3 to M_5 generations in 2009. Amylose contents were variated from 1.9%(waxy) to 12.5.

Korea (Dr. Si-Yong Kang, KAERI)

The development of various colored kernel and glutinous characteristics is a necessary to meet the needs of rice consuming trend in Korea. In 2009, we have been cultured the about 150 mutant plants in M_4 generation that were selected by phenotypic characteristics derived from 6 rice varieties using gamma ray mutagenesis in 2007-2008. We will select some kind of useful mutant after analysis of functional compounds. And for construction of amylose content library, we are culturing M_2 plants and will select some of amylose content mutants after analysis of amylose content and seed morphology.

Malaysia (Dr. Rusli Bin Ibrahim, Nuclear Malaysia)

The objective of the study was to determine the optimum doses for the production of high mutant frequency and spectrum in rice induced by carbon ion irradiation. A total number of 100 seeds per dose (0, 10, 20, 40, 60, 80, 100, 120, 160, 200 Gy) of MR219 were exposed to carbon-ion irradiation (Ion Beam) at Tasaki, Japan. The irradiated seeds were successfully planted under Controlled Environment Greenhouse for radiation sensitivity effects study. The shoulder dose was significantly obtained at 60 and 80 Gy and germination rates of M_1 irradiated seeds were above 60% for the doses ranging from 0 to 120 Gy and significantly decreased after 120 Gy.

The Philippines (Mr. Alfonso Grafia, PNRI)

4 mutant selections of rice variety IR72 irradiated with 200 and 300Gy were planted in the field to confirm whether they bred trine and possessed low amylose contents. The seeds were harvested at maturity and analysis for amylose and protein contents will follow.

Protein and amylose analysis of 97 seed samples were done on M_3 seeds previously irradiated with gamma rays. 8 mutants were found in the 300Gy of rice variety IR72 with low to inter mediate amylose contents. Only one mutant was obtained in the 200Gy treatment with low amylose contents. These mutants will be planted again for confirmation and purification.

Seeds of IR72 rice variety irradiated with ion beam were sown to determine M_1 effects on germination, seedling height, survival etc. Data on yield will be also gathered at maturity.

Thailand (Mr. Suniyom Taprab, DOR)

1. Amylose Content

200 M_4 mutants with photoperiod insensitivity were selected during dry season 2009. A half of those M_4 seeds were growing in the field, another half of seeds were going to be analyzed for amylose content in wet season 2009.

2. Protein Content

Protein extraction technique had been adapted. SDS-PAGE was used to identify protein components such as globulin-albumin, gultelin and prolamine of the varieties KOML105 and Koshihikari. Satisfied result had been obtained.

3. Low Phytic Acid

Two LPA-mutants homozygous lines derived from high yielding variety, SPR1, are going to be analyzed for Fe content and its bioavailability in 2010.

Vietnam (Ms. Dao Thi Bang, AGI)

Purpose of rice plant breeding in Vietnam is changing time to time from high yielding to full fill stomach to quality purpose. Last century, high yielding is main purpose of breeding, but now quality rice is goal of most breeder. Low amylose content, high protein, low phytate acid or photosensitiveness is main objective of rice breeder.

According to mutation plant breeding is very important work is screening method for certain character. We need to exchange experience within FNCA member especial screening protocol for mutant for example amylose content, protein content, low phytate acid. Based on this we can get homogenous result.

Session 4 "Ion Beam Irradiation to Rice Seeds"

5 countries presented their report. The brief summary of each country report is as follows:

Bangladesh (Dr. Md. Lokman Hakim, BAEC)

Hulled seeds of a local rice cultivar *Ashfal* was irradiated with 9 different doses (10-200 Gy) of carbon ion beam with 0 dose as control from Quantum Beam Science Division, JAEA, Takasaki, Japan in January, 2009 with a view to find out the effective dose for ion beam irradiation of rice seeds. From data analysis, 30 and 40 Gy appeared to be the effective dose for *Ashfal*. Five putative mutants have been selected in M_1 originated from 40,120, 160 and 200 Gy ion beam mutagenised seeds. Seeds of BRRIdhan-29 and *Ashfal* were irradiated with fixed doses of carbon ion beam in June, 2009.

Indonesia (Dr. Sobrizal, BATAN)

*Daih Suci i*s a mutant variety developed by National Nuclear Energy Agency, Center for the Application of Isotopes and Radiation Technology, and released by Indonesian government in 2003. This variety is growing largely in Indonesia because of its high yielding and good eating quality. But this variety is susceptible to lodging especially when it its grown in rainy season. To improve the lodging resistance the genetic variability will be created by exposing its seeds with ion beam irradiation. At the beginning, the preliminary experiment was conducted to obtain the suitable dose. Amount of seeds were treated by ion beam irradiation with various dose. Based on the point of seed fertility of M_1 plants, it was concluded that the dose of 20Gy is the best dose.

Korea (Dr. Si-Yong Kang)

In 2009, the dry seeds of japonica rice cultivar, "Ilpum", were irradiated by carbon ion of the TIARA, JAEA by the doses of 10, 20, 40, 60, 80, 100, 120, 160 and 200 Gy. Survival rate of the ion beam irradiated seeds was decreased upper than 20 Gy dose and almost did not appeared at 60 Gy. Shoot and root growth at the 5 weeks after also decreased upper than 20 Gy dose. These results suggest that the proper irradiation doses of heavy ion beam for rice cultivar "Ilpum" might be between 20 to 30 Gy doses. We would like to irradiate more Korean rice seeds during this winter season using the heavy ion beam to start some kinds of mutation breeding researches under the FNCA cooperation project from 2010.

Malaysia (Dr. Rusli Bin Ibrahim)

This Project is focused on developing new rice cultivars with high yield under minimal water requirement. Seeds of selected elite genotypes were irradiated with gamma rays (300, 400Gy) and exposed to preliminary selection pressure using different levels of PEG. A total of 38 potential mutants families from MR211 and MR219 were successfully evaluated in the field with minimal water requirement and screened for low amylose content. However, only two potential lines designated as MR219-4 and MR219-9 were selected for further field trials under saturated soil and flooded conditions based on high yields.

Vietnam (Ms. Dao Thi Tanh Bang)

Thanks to good protocol for screening sensitive dose from Takasaki (TIARA), we found that the

dose of 40Gy and 60 Gy are optimum for irradiation treatment of *Khang dan* variety. The result is almost the same in different varieties from different countries. Based on this optimum dose we have irradiated 3000 seeds/dose. The purpose of breeding should be high yield and good quality in term of low amylose content or high protein content and another character should also be consider for example dwarf and semi dwarf. In my opinion, we should follow one protocol for screening mutant. After one crop season, we should send data to Dr.Tanaka for result evaluation and comment. Based on this exchange comments and experiences, we can adjust for each member if you have problem or difficult, so the end of project we can get good result for all.

Session 5 "Sub-Project on Disease Resistance in Banana"

Dr. Rusli Bin Ibrahim, Malaysian Nuclear Agency, presented a progress report. The brief summary is as follows:

Main objectives of the project are to produce new mutant varieties of banana resistance to *Fusarium* wilt disease with improved fruit quality. Meristem cultures of local banana cultivar called Berangan (*Musa sp*p. AAA) were irradiated with gamma ray doses of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100Gy. Based on the percentage survival of irradiated explants, LD50 and LD100 obtained were 50Gy and 80Gy. Using selected effective doses of 20, 30, and 40Gy, cultures were micropropagated from M1V1 to M1V5 stage for field screening. Four artificial disease screening techniques had also been developed which can be used effectively for nursery and field screening. At present 3 potential mutant lines resistance to *Fusarium* wilt disease had been selected with the following improved traits such as high yield, early fruiting and short stature.

Session 6 "Sub-Project on Insect Resistance in Orchid"

Dr. Rusli Bin Ibrahim, Malaysian Nuclear Agency, presented a progress report. The brief summary is as follows:

She summarized overall activities and achievements of the project from year 2003-2009. In this project, collaborating countries (Indonesia, Thailand and Malaysia) had exchanged tissue culture materials for irradiations (D. Sonia Red 17: Thailand; D.jayakarta: Indonesia; D.mirbellianum: Malaysia), with an aim to produce insect resistant clones. The target pests were mites and thrips. For Malaysia, in vitro mite infestation technique has been established. Overall, for mutant development, 50 D. mirbellianum plantlets tolerant to mites were selected at tissue culture stage, and of these, one promising mutant plant was identified to be tolerant to thrips at flowering stage. For D. jayakarta, screening works at flowering stage have identified 2 promising mutants for thrips tolerance. These promising mutants are being propagated to achieve high number of clones for further trait stability tests. Details of the report are in the attachment.