Annex 4. Parallel Session Summary on Mutation Breeding Project

FNCA 2010 Workshops on Mutation Breeding Project and Biofertilizer Project

At the beginning of Session, Dr. Artemio M. Salazar, Deputy Director of Institute of Plant Breeding, University of the Philippines Los Baños (UPLB) delivered welcome remarks.

Session 3 Achievement and Challenges of the Project

All participating countries presented their reports on each sub-project, and the brief summaries and discussion are as follows:

I. Following-up and Plan of Sub-Project on Insect Resistance in Orchid

Malaysia (Dr. Rusli Bin Ibrahim, Nuclear Malaysia)

Sub-Project on Insect Resistance in Orchid was terminated successfully in March, 2010. In addition, the research work on insect resistance in orchid was reported that it used different doses of gamma rays and ion-beam for irradiation, and they obtained promising results for ion beam. PLBs were irradiated using effective doses and mutant lines through *in vitro* cultures were regenerated, and it was successfully achieved for insect resistant *Dendrobium* orchid variety through *in vitro* mutagenesis.

II. Conclusion and Plan of Sub-Project on Disease Resistance in Banana

Each participating country reported results and conclusion since Sub-Project on Disease Resistance in Banana would be terminated this year. Most of countries, i.e. Malaysia, the Philippines and Vietnam, have succeeded in produce several promising mutants tolerant to banana diseases such as *Fusarium* and Banana Bunchy Top Virus (BBTV). Moreover, technology transfer for commercial use has been successfully carried out in Malaysia and the Philippines. As for Bangladesh, which joined to the sub-project later, the protocol and screening were established. Therefore, all FNCA participating countries recognized this project had accomplished fully and obtained promising results, indicating that the evaluation should be full marks 5. The summaries of reports are as follows.

Bangladesh (Dr. Md. Humayun Kabir, BAEC)

In vitro regeneration protocol of *Fusarium* susceptible banana cv. *Sabri* (AAB) was established on MS medium supplemented with 5.0 mg/l BA + 2% Ads. *In vitro* radiosensitivity (LD_{50}) was determined at 35Gy. More than 2,500 plantlets were transferred to the poly bags containing *Fusarium* infested soil and found heavily infested and died after 2 to 3 months. About 1,000 plants were transferred to the field with hot spot condition also showed symptoms of *Fusarium* and died within 6 to 8 months.

Malaysia (Dr. Rusli Bin Ibrahim, Nuclear Malaysia)

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, LD_{50} and LD_{100} 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 M_1V_1 upto M_1V_5 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 hours 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 hours 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 will be further screened in the field for multi-location trials

The Philippines (Dr. Olivia P. Damasco, UPLB)

10 promising BBTV resistant mutant 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 three generations of continuous evaluation under high BBTV infection. The preference of the aphids to the mutant lines was evaluated using free choice or no choice feeding method. 5 mutant lines were less preferred by aphids. The reaction of selected mutant lines to artificial inoculation of the virus using viruliferous aphids showed seven lines to consistently show low BBTV incidence compared with the Lakatan control plants. Low incidences of the BBTV were observed in four multi-location trial sites, despite the presence of infected plants and vectors within the vicinity of trial site. Mutant lines were comparable with LK control (per plant basis) in terms of plant height, girth, total number of fruits produced, and number of hands. Days to flowering were significant early in some mutant lines.

Vietnam (Dr. Dang Trong Luong, AGI)

Banana is widely grown in tropical regions throughout Asia and Vietnam. One of the most serious diseases of banana is fusarium wilt, caused by *Fusarium oxysporum f.sp. cubense*

(FOC) with several 'races' infecting plants, some being more virulent than others. In this study, we used irradiation method to generate a new mutant banana line (ABB) enhance resistant to FOC disease. Gamma treatment had some variations: change in high, the colour of bud, leaf, and frequency variation from 1.8% to 12.5% at cultured medium and from 7.5% to 15.7% at gamma mutagenic treatment formulas. At the dose of 15 or 20kGy is revealed appropriate in experiments. The evaluation of infected disease was performed with artificial disease on banana plant in greenhouse. Spore density at 10^5 spore/ml, we had 4 methods to taking disease on banana, cultured hydroponic reveals high infected sensitive. We have obtained 17 banana lines enhance resistance to *foc* disease in greenhouse (infected rates <25%). RAPD analysis showed that mutant lines were genetic variation comparison to original lines.

III. Country Report of Sub-Project on Composition and Quality in Rice

Each participating countries presented on the achievements of the Sub-Project on Composition and Quality in Rice. The main objectives of the project is to obtain good quality e.g. good amylose content, protein content, aroma etc., depend on the importance and priority for each country. For example, Malaysia had produced some potential mutant varieties, which are tolerance to minimal water condition and resistance to blast disease and they will identify potential mutant lines with improvement in amylose content and aroma. Vietnam is targeting for improved quality traits such as amylose, aroma, yield and resistance to BPH. For Thailand, main objectives are protein, low phytic acid, and amylose improvement. For the Philippines, main objectives are improvement of protein and amylose content. Japan has completed the establishment of library for amylose content of Koshihikari and Hitomebore mutant lines. For Bangladesh, main objectives are to produce mutant varieties, which are salt tolerance, aromatic and improvement in amylose. For Indonesia, main objectives are improvement of amylose content and early maturity, and for China, main objectives are improvement of amylose content for hybrid rice. It was suggested that this sub-project should be continued for another 2 years since most of the participating countries have not identified the potential mutant lines with improved target characters such as amylose, protein, aroma, etc.
The summaries of reports are as follows;

Bangladesh (Mr. Protul Kumar Roy, BAEC)
The M_3 progenies derived from carbon-ion irradiated seeds of a local salt tolerant cultivar-Ashfal were evaluated. The M_3 progenies derived from either 200Gy or 40Gy doses had been mutated as photoinsensitive, where their parent Ashfal did not flower at all. This progenies also showed early mature characters. Seeds were collected from M_3 for yield trial in the coming aman season.
BARRI-dhan 29 was irradiated with carbon-ion beam (Doses: 30, 40 and 50Gy). 3, 4 and 2 plants were selected from 30, 40 and 50Gy irradiated population for earliness by more than one week. In evaluation of long fine grain-aromatic line RC-48-1-2-3, observed that grain

quality was long fine aromatic, better production and also early mature in nature.
Screening of some mutant lines for salinity tolerance at the seedling stage in water culture was carried out under glass house condition. It was observed that Ashfal-350 mutant showed tolerant and Moinamoti-350 moderately tolerant. Both are maintaining up to reproductive stage under water culture.

China (Dr. Luo Ju, CNRRI)
Hulled seeds of Z17, local super rice cultivar, were irradiated with 7 different doses (10, 20, 40, 60, 80, 100, 120Gy) by the heavy ion beam of the TIARA, JAEA in 2010. These materials with 0 doses as control were cultivated in summer of 2010. The germination rate, survival rate, growth rate and percentage of ripening were investigated. These results suggested that the proper irradiation doses of heavy ion beam for Z17 might be between 30 to 50Gy doses.

Indonesia (Dr. Sobrizal, BATAN)
At M_6 generation, 10 semi-dwarf lines derived from irradiated KI 237 lines were selected as promising mutant lines. The result of genetic analysis of these lines indicated that the semi-dwarf character of these lines was controlled by a single recessive gene and designated as sd^{237-1}. These lines have various amylose contents, ranging from 17.39 to 25.65%, and among these lines, 7 lines and 3 lines have white and red grain color, respectively. Preliminary yield trial of these lines revealed that the highest yield is 7.15 t/ha for RKI 198 and followed by 7.12 t/ha for RKI 199, whereas the yield of Ciherang, a national leading variety, is only 6.52 ton/ha. The yields of other lines were not significantly different from that of Ciherang. These lines will be subjected to advance and multi-locations yield trials before being released as new varieties.

Japan (Dr. Minoru Nishimura, NIAS)
In addition to 12 F_4 NILs of Koshihikari with low amylose content, we obtained newly 11 mutants of Koshihikari. Amylose content of these mutants varied from 0.0% to 14.0%. We obtained 24 mutants with low amylose content from Hitomebore, second major variety in Japan by irradiation with gamma rays and an ion beam.

Malaysia (Dr. Rusli Bin Ibrahim, Nuclear Malaysia)
Mutants were induced in variety MR219 using gamma rays at 300Gy 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. Two potential lines designated as MR219-4 and MR219-9 were selected tolerance to saturated condition in irrigated areas and aerobic conditions with improved yield.

The Philippines (Ms. Adelaida C. Barrida, PNRI)

Development of rice mutants with desirable agronomic traits, good eating quality (low to intermediate amylose content) and high protein using mutation breeding was initiated at the Philippine Nuclear Research Institute (PNRI) as part of cooperative project with the Forum for Nuclear Cooperation in Asia entitled "Composition or Quality Crop Breeding in Rice: Grain Quality Improvement in Rice (*Oryza sativa L.*)".

In M_4 generation, results of amylose content analysis using iodine staining method showed that only few lines belong to low to intermediate amylose in plants irradiated with 200 and 300Gy, while the control had intermediate to high. Slight increased in the protein content of irradiated plants were obtained in comparison with the control.

Initial result using ion beam irradiation revealed that the best dose was 20Gy and the lethal doses were 160 and 200Gy. Earliness in the number of days to heading was observed in the M_1 plants irradiated with 10 and 20Gy, and the control, however, this result needs to be confirmed on the succeeding generations.

Thailand (Mr. Suniyom Taprab, DOR)

Seeds had been irradiated and M_1 obtained in wet season 2007. M_2 plants had been selected on photoperiod-insensitive mutants. Then, M_3 seed of 200 mutant lines had been separately harvested as single hill per line. Those 200 photoperiod-insensitive mutants were continuously screened for important agronomic traits and to be analysed for amylose content. They were again tested for photoperiodism, tillering ability and maturity in dry season 2009 and had been analysed for amylose content. Selected M_5 mutants had been grown in dry season 2010 and are being presently grown in wet season 2010. In 2008, we developed simple technique for amylose content determination on breeding lines and distributed to FNCA members. While amylose content was being analysed, we developed a technique for protein analysis. Protein extraction technique reported by Iida et al.(1993) and Tanaka et al. (2004) had been modified and tested for indica rice protein analysis. Extracted proteins such as globulin, albumin, prolamine and glutelin from KDML105 and Koshihikari had been identified using SDS-PAGE technique (Laemmli, 1970). Low amylose mutant lines had been achieved from KTH17 which is originally high amylose content local variety. Various amylose content mutant lines from original low amylose content varieties KDML105 and RD15 had been obtained. Some of those mutants contained lower and higher amylose content than did of their wild types. Protein and its components are being analyzed. In case of low phytic acid mutants, 2 mutant lines from SPR1 are going to be tested for bioavailability in artificial intestine digestion. New irradiated populations from CNT1 had been screened for low phytic acid mutants.

Vietnam (Ms. Dao Thi Thanh Bang, AGI)

Ion beam irradiation treatment was conducted with a certain number of the seed to determine the optimal dose for experiment. From the optimal dose, 6,000 seeds of Khang dan variety was irradiated in 40Gy and 60Gy of ion beam in Japan last May 2009. Beside of that 3,000 seeds of each dose for gamma ray at 100, 150 and 200Gy for Khang dan and Bacthom varieties. The reason to use Khang dan and Bac thom varieties for experiment is Khang dan is normal rice for common use, but Bacthom is quality rice this variety is always give higher price for milled rice. Quality rice usually gives low yield, problem is how to increase of yielding but still keep quality at the reasonable level. At M_1 generation, 5 seeds per plant were taken in random for making M_2 generation. In M_2 generation, from gamma ray as well as ion beam irradiation treatment of both Khang dan and bac thom varieties range of segregating were observed in plant height, grain weight, maturity or sterile. M_3 generation, some interesting characteristics related to yield were chosen for example: grain weight, short grow duration, short plant and tolerant to disease. According to Khang dan variety, higher yield is most important then lodging character and disease resistant. But for Bacthom variety first is also higher yield, then quality in acceptable level (still aroma) and disease resistant. Most of those characteristics will be evaluated in M_4 generation. Through ion beam irradiation of Khang dan variety we recognized that the grain weight of the seed is sensitive with ion beam.

IV. Ion-beam Induced Mutation Breeding of Rice

This research activity was started in January 2009 and most of FNCA participating countries had produced promising results with irradiation of ion beam, which depended on their materials and experimental conditions. Some countries have problem with the seed germination after treatment, especially for Bangladesh and Malaysia. As for Vietnam, they achieved good result in germination but not so promising result at field condition. Most of the members suggested to repeat the experiment by irradiating the seeds with ion beam for next year in 2011. Dr. Atsushi Tanaka, Japan Atomic Energy Agency (JAEA) discussed the appropriate population size for irradiation treatment and suggested the minimal number of initial cells to be irradiated at least 5,000 in order to achieve high frequency of mutation. The protocol for selection of seeds after irradiation was also discussed and it was suggested to harvest 5 seeds from 5 panicles from each M_1 plant.

Most of participating countries had completed the experiment at M_2- M_3 generation and for the year 2011, potential mutant lines with improved quality traits such as amylose will be evaluated. Finally, it was suggested that this activity should be continued at least for another 2 years in order to produce promising results.

Session 4 Possible Cooperation with IAEA/RCA

Dr Luxiang Liu, Project Lead Country Coordinator of RCA/IAEA project RAS5045, China, Chinese Academy of Agricultural Sciences (CAAS), reported on the regional activities and main achievements of the RCA project. The summary of report is as follows ;

There are 14 member states participating in this RCA Project. The overall objectives are to develop and transfer methodologies for induction and identification of mutated genes contributing to improved quality and stress tolerance and develop improved breeding materials using molecular marker-assisted selection. For 2007-2010, RCA had conducted 2 regional meetings and 3 regional training courses and published training manuals and working document. For 2010, the major achievements of RCA include new techniques/methods established and improved including mutation detection platform using EMAIL and TILLING methods, development of new mutated populations and mutant lines. 33 new mutant varieties were officially released under this project by the end of September 2010.

Possible Synergy FNCA/RCA

Dr. Sueo Machi, FNCA Coordinator of Japan, suggested the possible FNCA/RCA synergy on mutation breeding. All participants agreed following points ;

a. FNCA Project of Mutation Breeding to be continued to develop mutant varieties of rice for quality improvement. The project studies could be supported by molecular techniques that have been focused in the RCA project RAS 5045 in the Member States.
b. FNCA project results on ion-beam induced mutation breeding could be used by the new RCA project on Mutation Breeding for the development of new mutant varieties by sharing of experience and knowledge.
c. In near future, workshop of Mutation Breeding Project of FNCA should be held in parallel with workshop of Mutation Breeding project of RCA in order to have joint session for one or two days to share views, information and experience to enhance synergy of the two projects.

It was also agreed that FNCA and RCA would go as planned and communicate with scientists within each country.

Session 5　Future Plan and Meeting Report

I. Activity Plan for Mutation Breeding of Rice : Quality, Dwarf, Selection Method of Targeted Mutants;

Dr. Nishimura presented an example of future activities of Mutation Breeding Project in Japan. Japan needs 2 more years to complete the project. For other members, Bangladesh joined the project, so they need 2 more years to complete. All members need to do analysis on grain quality in 2011 and probably finalize the report in 2012. The members agreed to continue mutation breeding by sharing of facilities of ion beam and other mutagenesis in other aspects. For this project, members suggest two more years to finalize the report.

II. Possible Application of Mutation Breeding for Sustainable Agriculture in Asia

The 3 points to be discussed for the breeding purposes of rice and mutagenesis to be used were proposed by Dr. Hirokazu Nakai, Project Leader of Japan, as follows:

a. Resistance to various environmental stresses e.g. soil salinity, minimal water input, various diseases (bacterial leaf blight/sheath etc.), drought, high and low temperature, heavy fluctuation of weather, etc.

b. Adaptability to the conditions of the organic farming (or low input sustainable agriculture)
c. Mutagenic effect, including that of ion-beam.

Referring to the above proposals, the future plan of Mutation Breeding Project was discussed. The direction of the above proposals was generally accepted. It was concluded as follows ;

1) The projects already started, e.g. quality of rice, content of protein, etc. may be continued for another one year.
2) Trials of mutation breeding for resistance to environmental stresses need to be challenged depending on the situations of each country.
3) As for mutagens to be used, every country should share irradiation facilities for mutagenesis treatments of plant materials.