Annex 3

Presentation Summaries of
FNCA 2024 Workshop on Mutation Breeding Project

July 30th – August 1st, 2024
Darkhan, Mongolia

1. China (Dr. Tan Yuanyuan, Zhejiang University)

Gene editing, particularly by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) and its variants, which is highly efficient at generating targeted mutations in genome, has become a powerful technology in plant research and may become a game-changer in plant breeding. CRISPR/Cas9 has been widely applied to edit the genes involved in rice yield, appearance, quality, resistance to stresses and other important traits. *OsLCT1* and *OsNramp5* are two genes involved in Cd transport in rice. Mutation of the two genes by CRISPR/Cas9 leads to reduced accumulation of Cd in rice grains and one mutant line shows no significant difference on the yield. Different strategies are proposed to intervene to generate gene functional variations and consequently phenotype changes. The mutations on the exons and promoter region of *Wx* gene can generate various mutants featured with a reduced AC content ranging from 2.0%-16%. In addition, base-editing-library-induced high density nucleotide substitutions have been applied to screen functional mutations of *OsACC1* which can confer resistance to herbicide. Although CRISPR/Cas is powerful in rice breeding, the edited plants are still under strict regulation in China. The traditional mutagenesis is still playing important role in reverse and forward genetics and mutation breeding. New technologies are always adopted to improve the efficiency of mutation breeding. For example, next-generation sequencing (NGS) and digital PCR are also used to screen mutations with high efficiency.

2. Indonesia (Dr. Winda Puspitasari, National Research and Innovation Agency)

Sorghum and soybean mutation breeding efforts in Indonesia aim to enhance food security by developing high-yield, high-quality varieties. For sorghum, three new varieties (Pahat, Samurai 1, and Samurai 2) have been created, focusing on improving production, seed quality, and suitability as animal feed. Gamma irradiation has effectively induced beneficial traits in sorghum, leading to high heritability in important characteristics. Soybean mutation breeding has resulted in 15 varieties with traits such as high production, early maturity, and pest resistance. Current efforts aim to improve seed quality, production, and tolerance to abiotic stresses like drought and low light conditions. These breeding programs aim to produce mutant lines with high seed yields and good quality to support national food security.

3. Japan (Dr. Shimokawa Takashi, National Institutes for Quantum Science and Technology)

The Heavy Ion Medical Accelerator in Chiba (HIMAC) is the world's first medical accelerator designed for heavy ion cancer therapy and began cancer treatment from 1994. By using non-treatment hours, we have been providing this equipment to researchers for basic research, mainly for academic purposes. Currently, about 100 research projects ranging from basic physics to cancer therapy research are conducted every year. In this year, two external and one internal projects for breeding research have been performed.

In this workshop, the unique properties of the medical irradiation facility HIMAC, and ion beam breeding projects at the HIMAC, in particular, the efforts and results of J501 project, which was initiated to support breeding and mutation introduction research, were introduced.

4. Malaysia (Mr. Faiz Bin Ahmad, Malaysian Nuclear Agency)

The development of climate-resilient rice is crucial in Malaysia since our self-sufficiency ratio is still low, around 62%. Developing new varieties via induced mutation and molecular approaches can shorten the screening and selection of potential lines. Several potential climate-resilient rice lines have been developed through ion beam irradiation and crossing mutant cultivar with mega variety to improve the performance of the genotype. Application of molecular and genomic techniques such as identification of QTL and whole genome sequencing is valuable to identify genes or QTL control for specific traits. Furthermore, several potential advanced mutant lines have been planted in 3 main granary areas in Malaysia for advanced yield trial. Several advanced lines will be selected based on maturity period, yield, and good agro-morphological traits for further evaluation in the multi-location trial. Commercializing mutant cultivar NMR152 and developing new rice varieties could increase the usage and dissemination of mutant rice cultivars in Malaysia.

5. Mongolia (Ms. Uugantsetseg Battogtokh, Institute of Plant and Agricultural Science)

The mutation breeding started since 1970s at the Institute of Plant and Agricultural Science (IPAS) of Mongolia. Initially, mutation breeding mainly focused on the development of potential new mutant wheat varieties. However, in recent years the mutation breeding activity expanded to barley and rapeseed mutant variety development in Mongolia.

Mongolia successfully applied the different mutagen sources such as ion beam (He 50MeV, Carbon 320 MeV), X-ray and chemicals applied for mutation induction for wheat and barley. The seed irradiation by gamma ray mainly carried out in collaboration with Seibersdorf laboratory of IAEA in Austria and the Institute of Crop Sciences (ICS) of Chinese Academy of Sciences (CAAS). In the recent years, the seed irradiation by heavy ion beam carried out in collaboration with the National Institutes for Quantum Science and Technology (QST), Japan.

In 2023-2024, totally 2035 progenies of wheat mutant lines in M_1-M_4 have been planted in the respective breeding plots and field observation, data collection is in progress during crop duration. In the yield trial, totally 5 mutant new varieties including early variety Darkhan-225, mid maturity variety Darkhan-234, and mid late maturity variety Darkhan-243 Darkhan-245, Darkhan-246 have been tested in 4 replications and evaluated for yield performance, grain processing quality and resistance to disease and pests. In 2024, the mutant wheat variety Darkhan-225 transferred to the State variety test for further release.

6. The Philippines (Mr. Christopher C. Cabusora, Philippine Rice Research Institute)

Development of Rice Varieties Adapted to Adverse Rice Environments Through Induced Mutation

- **Variety Development:** In 2019 and 2023, 2 new mutant varieties, from *in vitro* mutagenesis, were approved for cultivation and commercialization, by the National Seed Industry Council, for saline-prone and irrigated lowland. Though they are released in single environment, these varieties have multiple tolerance to drought, saline and submergence. The varieties were issued with NSIC names, NSIC Rc 686 and NSIC Rc 740.
- Prior to their nomination to the National Cooperative Testing (NCT) for rice, the corresponding breeding lines of these varieties, were evaluated for field performance under irrigated lowland, managed drought stress, simulated rainfed, and submergence stress growing conditions. The results of the evaluation showed that these varieties can out yield the corresponding check varieties in each growing conditions.
- Evaluation for drought and submergence tolerance at seedling, reproductive and vegetative stage, and salinity tolerance at seedling stage showed that NSIC Rc 686 and NSIC Rc 740 were tolerant to all of the abiotic stresses.
- Multi-location and multi-season evaluation of the varieties, in the NCT showed that the varieties have yield advantage over the check varieties, across location and season.
- **Technology Demonstration**: Pilot technology demonstration of 3 saline tolerant varieties, in a 10ha field demonstration area, in Tiwi, Albay incurred a 46% increase in rice production, compared to 2022.

7. Thailand (Ms. Nila Rasidee, Rice Department)

Thai rice improvement for flood tolerance through electron beam-induced mutation was performed. Five parents/varieties (Pathum Thani 1 Chai Nat 1 Suphan Buri 60 Phitsanulok 2 and RD49) were used as a breeding material to be mutated for submergence tolerance.

Five hundred grams of seeds were irradiated with 0.30 kGy electron-beam. M_1 populations were pre-germinated and broadcasted, then collected 500 panicles from the main tiller to obtain M_2 seeds. Five hundred rows of M_2 generation were planted by panicle/row. Twenty-six plants were selected from each row as M_3 seeds and evaluated for submergence tolerance. Unfortunately, the experiment was affected by salinity due to the rising sea water level. The remaining materials were collected for submergence tolerance screening during M_4 to M_7 generations. The result shows the plant survival was 10-98% comparable to tolerant check FR13A (95-100%) and significantly higher than susceptible check IR42 (0%). 5 elite lines were selected to conduct On-station Yield Trial. Intra-station trials during 2019 - 2021 at Prachin Buri Rice Research Center, PSL2'14E1-PCR-B-428-7B has 3,238 kg/ha close to RD49 (3,381 kg/ha). Inter-station trials at Prachinburi Rice Research and Phra Nakhon Si Ayutthaya Rice Research Center in wet season 2021, PTT1'14E1-PCR-B-190-7B has 3,381 kg/ha close to RD49 (3,416 kg/ha). In 2023 five mutant lines were tested in the farmer field yield trials experiments at Phra Nakhon Si Ayutthaya province, RD49'14E1-PCR-B-375-7B and PSL2'14E1-PCR-B-428-7B has 4,300 and 4,056 kg/ha more than IR64-Sub1 and RD41 (2,968 and 4,037 kg/ha) The reaction of five mutant lines to blast disease conducted upland short row in 2023 at Prachinburi Rice Research Center showed highly resistant. In the future these five elite mutant lines will be tested in the farmer field yield trials in the Central, Eastern, Northeast, and Southern regions in Thailand to confirm for submergence tolerance and studied for agronomic traits. To release a submergence tolerance rice varieties for farmers in flood-prone areas, this will lead to the sustainability of rice cultivation.

8. Vietnam (Dr. Le Duc Thao, Agricultural Genetics Institute)

In 2023-2024, Vietnam continuous testing ecology areas and develop the mutant soybean varieties (DT2010, DT215, DT218, DT219), rice (DT80) and peanuts LDT3 to production; screening new mutant crop lines in soybeans and peanuts in M_4; mutation induction on soybeans and peanuts to create starting materials for selecting and breeding new varieties. In 2023, a new peanut variety LDT3 and soybeans variety DT219 have been released as national varieties. In addition, AGI has created a number of mutant peanut, soybean and rice lines with one or several improved traits compared to the original varieties such as early maturity, high yield and quality, improved plant hardness. Regarding the development and expansion of the area of mutant varieties that have been released, AGI to expand the area of rice varieties DT80, soybeans DT215, DT2010, DT215, DT218, DT219, etc. However, due to limited funding, the area is still limited.

[Summaries shared by member countries]

Bangladesh (Dr. A.N.K Mamun, Bangladesh Atomic Energy Commission)

BINA dhan 25 is a mutant of BRRI dhan 29 becoming more and more popular to farmer for its premium quality, higher market price early mature, day neutral, higher yield (7.14 to 8.50 t/ha) and long grain fine texture and also it can cultivate relatively low fertilizer and low irrigation system. Other 23 advance mutant lines are selected for further research.

Korea (Prof. Si-Yong Kang, Kongju National University)

The main content of my presentation is an introduction to the current status of ion beam breeding in Korea and the results of my recent research using proton beams. In Korea, a proton accelerator (KOMAC) was completed in 2012, and the Radiation Breeding Research Team of the Korea Atomic Energy Research Institute has been conducting research on setting conditions for proton beam irradiation on plants and verifying mutagenic effects. And I have recently irradiated the newly constructed proton beam TR-102 and the existing TR-103 on RC-Br (rapid cycle Brassica), a Brassica model crop, and Arabidopsis thaliana, a model plant, to verify their mutagenic effects. In the future, RC-BR plans to conduct research to select useful mutant genetic resources such as disease resistance and introduce them into cultivated cabbage.