FNCA Mutation Breeding project Report, November 2020

1. Administrative information

1.1 Member State: Bangladesh

1.2 National Project Leader: Dr. A. N. K. Mamun

Project progress report

To make popular to farmers, exhibition field for three mutant rice verities developed by carbon ion beam called BINA Dhan – 14, BINA Dhan – 18, BINA Dhan – 19 are going on in different location of Bangladesh. Farmer are showing their interest and start to grow these mutant rice varieties in farmers' field. There are 15-advanced promising mutant lines are selected form carbon ion beam and gamma induced population of B11, BRRI 47 and Lombur rice land races. Two of them sent for regional trials. Most of them are selected for higher yield, early & late maturity, lodging resistant, suitable for cultivation in rain feed condition, bold grain and long grain fine rice. Most of them are suitable for both cultivations in Aus and Aman seasons.

Work on nutrient use efficiency:

Experiment is conducted using 15N in rice mutant variety BINA Dhan 19 to determine the amount of 15N needed for this variety at rain-fed condition. Azola anabaena is also using in rice mutant variety BINA Dhan 14 to develop reduce amount of N is needed for cultivation of this variety. In this year 2020, 15N experiment was done for new mutant lines going to be new varieties.

Next national activities

1. We have planned to evaluate 2 advanced mutant lines for regional trail in respect to higher yield, lodging resistant and other desired traits of improvement
2. Plan to submit registration authority to release at least one mutant variety with better green traits like cultivation in rain feed condition, lodging resistant, higher yield, photoperiod insensitivity, early maturity and low cost of production.
3. Experiment conduct for molecular characterization of new mutant advance lines for green traits.
4. Experimenting with *azola anabaena* in new advance mutant lines to determine the reduction amount of N fertilizer (urea) is needed for rice cultivation.
5. To develop new mutant variety, which will uptake, more nutrient from M_2 population and resulted higher yield.

FNCA2020 Workshop on Mutation Breeding – Country Report

Breeding New Rice Varieties for Sustainable Agriculture under Climate Change

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Our rice mutation breeding project has been focused on (i) development of new rice mutants with the potential to become new rice varieties, (ii) identification of mutant genes underlying mutated traits, (iii) establishment of protocols for mutant screening, and (iv) promotion of new mutant rice varieties for commercial production. During the past year, mutation techniques have been deployed together with other molecular and biotechnological methods (anther culture, genomic editing, molecular marker assisted selection, etc.) for the development of conventional and hybrid rice varieties.

For mutation breeding, both chemical mutagens (e.g. EMS) and physical mutagens (gamma rays, ion beams) were used for mutation induction. Depending on the target trait, phenotypical selection or molecular screen techniques have been applied. For cloning of mutated genes, MutMap techniques has been deployed for fast identification of candidate gene and genomic editing for quick validation of the causal mutation, mediated by next generation sequencing and bioinformatic analysis.

The main achievement of the past year includes the following: (1) Mutant lines were developed from commercial rice varieties for enhanced tolerance to a herbicide that can kill weedy rice, one of them are now being grown in areas more than 100 ha; Jiang-liang-you 7901, a nationally registered hybrid rice, with an early maturing mutant as its female line, are being grown in several provinces with areas up to several ha in each location, and showed great promise for further expansion: excellent rice quality, superior yielding (up to 13 t/ha), and adapt to low nitrogen input (as compared with other hybrid of this type). (2) Dissection of genomic variations induced by ion beams using next generation sequencing (NGS). The characteristics of genomic variations were investigated for two indica and two japonica rice mutagenized by Ar, Ne and C ion beams, and the nature of structural variations were investigated in detail. (3) Functional analysis of genes involved in growth and stress responses in rice. By using genome editing and other molecular approaches, we have studied on 5 new rice genes for their function and working mechanisms.

FNCA REPORT 2020

SOYBEAN BREEDING USING NUCLEAR TECHNIQUE FOR ANTICIPATION OF CLIMATE CHANGE IN INDONESIA

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Summary

Soybean is one of the important plant in Indonesia, as sources of vegetable protein by making tofu, *tempe* (fermented soybean), *kecap* (soy sauce). National demand of grain soybean approximately 2.5 million ton/year, but national production only 0.9 million ton/year. To meet national needs, Indonesian government imports grain soybean reaching 1.6 million tons a year. One of the obstacles in the effort to increase soybean production is an irregular climate change. Indonesia consists of 2 seasons, namely dry season and rainy season. The problem of planting soybeans in the dry season is drought, and during the rainy season it is often flooded. To anticipate the uncertain climate change, we must prepare the superior varieties that are able to adapt to these conditions, such as drought resistance, early maturity, and so on.

To support the government's efforts in increasing national soybean production, BATAN has carried out several research topics for develop superior soybean varieties through mutation breeding program. These topics are (a) Improvement of soybean varieties for tolerant a-biotic stress (drought, acid soil); (b) Improvement of soybean varieties for very early maturity (less than 70); (c) Improvement of black soybean varieties for bigger grain size; and (d) Improvement of soybean varieties for shade-tolerant.

To develop drought tolerant soybean variety(s),Panderman variety was irradiated by gamma rays with dose of 300 Gy in 2010. After selection and purification, 8 selected pure mutant lines were obtained. These lines were subjected to various examinations such as multi-location yield trials, pests and diseases, and drought tolerant evaluations as requirement of variety release. Based on the results of these evaluations, 2 promising mutant lines were released as new varieties in 2019, with the name of Kemuning 1 and Kemuning 2. Both Kemuning 1 and Kemuning 2 varieties have high yield potential, tolerant to drought and resistant to various major pests and diseases.

To develop very early soybean variety(s) Burangrang variety was irradiated by gamma rays with dose of 300 Gy in 2012. After selection and purification 5 selected pure mutant lines were obtained. These lines were subjected to various examinations such as multi-location yield trials, pests and diseases examinations. Based on the results of these examinations, 2 promising mutant lines were confirmed to be released in 2020 as new very early mutant varieties with the name of Sugentan 1 and Sugentan 2.

Breeding of soybean varieties for shade-tolerant and breeding of black soybean for bigger grain size are under way, and it is hoped that the results will be obtained near future. With the release of 2 drought tolerant varieties and 2 very early varieties, it will be able to support the government in an efforts to increase Indonesian soybean production.

FNCA 2020 Workshop on Mutation Breeding Project
November 11th 2020, Online

Report for Mutation Breeding of Major Crops for Low-input Sustainable Agriculture under Climate Change

~ Characterization of gamma ray-induced mutations in NHEJ-deficient Arabidopsis~

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In order to understand the mechanism and to enhance the efficiency of mutagenesis, I have been working on the characterization of radiation-induced mutations in Arabidopsis. The double strand breaks (DSBs) are the most severe DNA damage and the non-homologous end joining (NHEJ) is the major repair pathway of DSBs in higher plants. However, how NHEJ influence the mutations generated by ionizing radiation remains unclear. In this context, we characterized the mutations induced by gamma rays in Arabidopsis mutants deficient in major components of NHEJ pathway.

The homozygous mutant seeds of *AtKu70* (At1g16970) and *AtLigIV* (At5g57160) were irradiated with ^{60}Co gamma rays, and the survival rates were determined. Genomic DNA was isolated from randomly chosen M_2 plants derived from M_1 seeds irradiated with 100 Gy. Whole genome sequencing was performed with an Illumina short read platform. The raw sequence reads were processed and mapped to the reference genome, and the mutations were detected and confirmed as described elsewhere (Du et al., J. Radiat. Res. 61, 639-647, 2020).

The NHEJ-deficient mutants were highly sensitive to gamma rays by around 10 times compared to the wild type. Whole genome sequencing revealed that, in NHEJ-deficient background, more than twice as many mutations with a higher fraction of Del≥2 bp and complex-type mutations were induced per unit dose. In the mutants, deletion size became longer and more complicated mutations were observed, indicating that the NHEJ pathway protects the broken DNA ends from extensive resection. Furthermore, the apparent microhomology observed at the rejoined site of deletion was longer than that observed in the wild type, suggesting the involvement of backup pathways that require longer microhomology for rejoining. These results suggest that, although NHEJ is recognized as an error-prone DNA repair pathway in plants, it is an important pathway for minimizing the deleterious effects of ionizing radiations.

Recent research status of plant mutation breeding in Korea

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In recent, importance of seed and genetic resources has been emphasized in Korea, and many institutes, companies and private breeders are also interested in mutation breeding. New mutant varieties more than 180 were developed and registered officially in Korea and the numbers are quickly increasing and commercializing mainly by private company and breeders especially on flowers and cereal crops.

The Radiation Breeding Research Center (RBRC) was constructed at the Advanced Radiation Technology Institute of the Korea Atomic Energy Research Institute (KAERI). The RBRC is trying to develop advanced radiation breeding techniques and new genetic resources using mutation techniques combined with bio-tech to deal with loss of biodiversity due to global climate change and environmental degradation, growing global demand for food and bio-energy, and strengthened protection for new plant varieties. It has been suggested that ion beams with high liner energy transfer (LET) and high relative biological effectiveness (RBE) induce higher mutation frequency and spectrum than low LET radiations (gamma and x rays). The KOMAC (Korea Multi-Purpose Accelerator Complex) under the KAERI was constructed in Gyeongju in 2013 and then has been provide 45 MeV and 100 MeV proton beam irradiation service. The KAERI research group started wide researches for setting the irradiation condition of 100 MeV proton beam of the KOMAC for mutation breeding to 15 main seed and vegetative propagated plants as well as for development of useful new genetic resources. The heavy ion beam (200 MeV) of Rare Isotope Accelerator (RAON) has been constructed at the Institute of Basic Science in Daejeon, Korea. In near future, many applications in the plant mutation breeding will be done using the proton and heavy ion beam with high LET. In this presentation, I would like to introduce some successful research achievements and current status of plant mutation breeding by radiation technologies in Korea.

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Mutation Breeding of Major Crops for Low-input Sustainable Agriculture Under Climate Change

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Abstract

National Agriculture Policy (DPN 3) 1998-2010 and the National Agrofood Policy (NAP) 2011-2020 are the evidence of Malaysian government's efforts in transforming as well as improving the country's food quality. The National Agrofood Policy 2011-2020 was formulated with a special focus on improving the food production sector including rice. Even though Malaysia is still depending on imported rice to fulfil consumer's demand, rice industry has always been a national priority based on its strategic importance as a staple food commodity. Nevertheless, the rice industry in Malaysia is hampered by several challenges such as global climate change, lacking of new variety, insufficient certified seed, emerging of major diseases such as Bacterial Panicle Blight (BPB), Leaf Blast, drought season, flash flooding, decreasing of planting area, lost interest of the farmers due to high cost of rice production and many others. Thus, the NAP had highlighted that local rice production should be increased to ensure the country's demand in the future.

FNCA project is one of the initiatives that supported the national policy. In 2019, Malaysian Nuclear Agency has moved one step forward in the area of mutation breeding by signing the Memorandum of Understanding (MoU) with Certified Seed Company HMN (M) Sdn. Bhd and Bayer Co. (MALAYSIA) Sdn. Bhd. With this agreement, HMN (M) Sdn. Bhd. will collaborate with Malaysia Nuclear Agency to produce certified seeds, multiply and commercialize the rice mutants. Meanwhile, Bayer Co. (MALAYSIA) Sdn. Bhd. will be responsible in coating the mutant seeds with plant growth promoters. This collaboration have added value to NMR 151 (PBR 0159) and NMR 152 (PBR 0156) mutant seeds and resulting in more competitive seeds as compared to other varieties in the market. In addition, from 2017 until 2020, several local verification trails (LVT) were also conducted from the Northern part until the Southern part of Peninsular Malaysia. The data obtained from Sekinchan, Selangor revealed that NMR 152 consistently produced between 7-10 t/ha in granary area as compared to 6 t/ha produced by other varieties within the same planting areas.

Field trials also showed that the production cost was reduced by 10%, mainly due to the reduction in fertilizer and pesticide usage. At the same time, the yield could be increased between 40 - 67% depending on the planting areas. In addition, two mutant lines were successfully granted with Certificate of Registration of New Plant Variety and Grant of Breeder's Right by Department of Agriculture Malaysia in **Feb 2020** with registration number; **PBR0156 (for NMR152)** and **PBR 0159 (for NMR151).** Apart from increasing the yield and income of the

framers, the mutant lines derived from FNCA project had also greatly impacted the socio-economic status of the farmers as the mutant rice are adaptable to current global climate change conditions. Furthermore, through the MoU with the companies, around 4,200 metric tons of seeds has been produced in 2020 for the supply of 25,833ha planting areas in Peninsular Malaysia.

In the meantime, three Malaysian modern rice genotypes viz., MR84, MR219 and MR284 were examined for varietal differences in radiosensitivity to ion beam irradiations. Dry healthy seeds were exposed to variable doses of ion beam radiations i.e., 10-100 Gy with 10 Gy intervals. Result in this study demonstrated that LD50 for MR84, MR219 and MR284 were 70.9331 Gy, 69.3927 Gy and 52.78Gy, respectively. Shoulder dose for MR84, MR219, MR284 were ranged between 40Gy- 60Gy. This study also found a highly significant differences among the genotypes ($p<0.05$) for all traits studied allowing the distinction of three genotypes categories. The differences among radiation treatments were highly significant ($p<0.05$) for germination percentage, shoot and root length, chlorophyll contents (a, b), plant height and panicle fertility. Furthermore, Duncan Multiple Range Test (DMRT) was applied to compare the mean values of all genotypes and doses. MR84 and MR219 were recorded no significant differences with respect to root length. Increasing in ion beam irradiation doses caused a significant reduction in shoot length and chlorophyll a content. The genotype × dose interaction for all traits tested exhibited significant differences indicating the effect of different radiation levels in performance for characters. In general, genotypes displayed variable response towards ion beam irradiations.

On 10 August 2020, Ministry of Agriculture and Food Industries (MAFI) will certify NMR152 as national new rice variety after NMR152 undergone the technical defense with the technical committee BKKIPB (*Jawatan Kuasa Teknikal Bantuan Kerajaan Kepada Industri Padi dan Beras BIL.2/2020*). Basically, the project has addressed the national agenda and policy in generating new rice varieties and thus, increase the well-being and livelihood of the farmers.

FNCA 2020 Workshop on Mutation Breeding Project, November 11, 2020

Project Title: Mutation Breeding of Major Crops for Low-input Sustainable Agriculture under Climate Change

Member State: Mongolia

National Project Coordinator: Mr. Bayarsukh Noov, Institute of Plant and Agricultural Science, Mongolia

Background of the Project

Mongolia is experiencing dramatic climate change. Last 70 years the absolute air temperature raised by 2.1^0C and the precipitation decreased in Mongolia. By the year 2020 the average air temperature will raise by $2.2\text{-}3.0^0$C and in further 25 years warming will intensify by two times and evaporation increase by seven to ten times. Due to the above changes the yield potential of existing plant varieties reduced due to climate change, the cropping zone boundaries has been changing towards North, pest and disease distribution, frequency increased and soil erosion and degradation increased, respectively.

The wheat is dominant crop and cultivated in about 80% of agricultural land in Mongolia. In Mongolia, the breeding of high potential wheat varieties was always major subject in breeding program. During 50 years of study the over 90 cereals crop varieties developed including 72 varieties of spring wheat, nine-durum wheat, four of oats and 2 common millet varieties. Among them a new spring wheat varieties Orkhon, Khalkh Gol –1, Darkhan-34, Darkhan-74, Darkhan-144, Darkhan-131 and the barley variety Alag-Erdene, Burkhant-1 and common millet Burgaltai are officially certified and commercialized. Barley is second cultivar planting after wheat in Mongolia. Mostly used for animal feed, brewing beer and human consumption. Soybean is relatively new crop for Mongolian farmers with very rapid expansion the rotation system. However, all soybean varieties cultivated in Mongolia are from foreign origin because no local breeding activities took place before. There we need to develop short duration varieties adapted Mongolian condition.

The application of mutation breeding technique in Mongolia has conducted since 1970s at the Institute of Plant and Agricultural Science (IPAS). The mutation breeding mainly focuses on the development of new mutant wheat varieties and barley, rapeseed and rice mutant on enhancement of wheat genetic diversity for breeding.

Through mutation breeding Mongolia increased volume of wheat mutant lines with target improving traits and developed number of new mutant varieties Darkhan-172, Darkhan-173 transferred to state variety test for registry and Darkhan-141 officially registered as promising new variety.

Since 2012, we successfully initiated the mutation breeding of barley varieties. The two varieties including naked food barley Alag-Erdene and the malting barley Burkhant-1 have been selected and 87 mutant line under screening in M5.

Purpose of the Project:

Improvement of major crops productivity and drought tolerance through application of mutation technique combined biotechnology and marker assisted selection following tasks identified:

- Enhancement of genetic diversity in wheat, barley through application of mutation techniques
- Development of high yielding, drought tolerance, disease resistant wheat and barley varieties

PROJECT PROGRESS REPORT

Objective in 2020

- Planted M1 generation of wheat, which has treated by ion beam treatment in 2-3 different doses.
- Evaluation and screening M2 generation in the field condition and using mutant progenies for hybridization on the desired traits
- Screening and selection of advanced mutant lines of wheat and barley with improved mutant traits through field on the yield performance and drought tolerance and disease resistance.

Project progress

Totally, 2834 wheat mutants in M1-M4 have planted in the respective breeding plots and field observation, data collection is in progress during crop vegetation crop growing season.

The field observations on plant growth, screening of field germination, viability tests are going on. The mutants with desirable green traits selected by plant maturity, spike shape, productivity, resistance to disease and lodging.

In the yield trial, totally 4 mutant new varieties including early variety Darkhan-225, mid maturity variety Darkhan-229, Darkhan-234 and mid late maturity variety Darkhan-222 have been tested in 3 replications and evaluated for green traits, quality and resistance to disease and pests.

Mutant Darkhan-222 advanced lines matured 88-92 days and gave grain yield of 1.21-2.22 t/ha during four years study. A thousand kernel weight of Darkahn-222 mutant line reached 36.6g, protein content was 12.7% and gluten content was 32.4%. The mutant wheat variety Drakhan-222 to transfer to the Dtate variety test end of 2020.

In 2020, planted barley mutant progeny five different doses of gamma ray to improve productivity and lodging. Totally, 145 progenies of barley mutant lines in M2-M5 have planted in the respective breeding plots. The field observations on plant growth, screening of field germination, viability tests are going on during growth duration. The improved barley mutants with desirable green traits selected by plant maturity, shattering, spike shape, seed color, productivity, resistance to disease and lodging. Food purposed barley mutants gave 0.6-1.2 t/ha over yield than parent Alagerdene varieties. MB-40 mutant line matured 2 days short than parent variety. Malting barley mutant lines gave 0.2-1.2 t/ha over yield than parents and MB-593 mutant lines matured 5 days before parents

FUTURE WORKPLAN IN 2021

- Induction of selected wheat and barley varieties by gamma ray and ion beam treatment in 2-3 different doses and cultivation of M_1 generation
- Continue screening and selection of advanced mutant lines with improved mutant traits through field test and evaluation the promising mutants for improved traits and drought tolerance
- Continue regional multi-location trial for the state variety test for new variety release
- Drought screening of advanced mutant lines using PEG6000 by index of some germinating and root traits

DEVELOPMENT OF RICE VARIETIES ADAPTED TO STRESS-PRONE ECOSYSTEMS THROUGH INDUCED MUTATION

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SUMMARY

In vitro mutagenesis (IVM) is a breeding strategy combining tissue culture technique and irradiation by gamma ray. This strategy is one of the approaches adopted by the Philippine Rice Research Institute's Rainfed-Drought Prone and Adverse Environments Rice Breeding Program to develop new rice varieties that can survive, adapt and perform well under rice ecosystems experiencing abiotic stresses such as drought, submergence and salinity brought by climate change. The breeding program started the utilization of IVM in 2009 in traditional rice varieties, such as *Apo* (NSIC Rc9), *FR13A* and *Salumpikit*, with known tolerance to abiotic stress but possessing undesirable phenotype. After a series of evaluation for pest and disease resistance, grain yield under stress and non-stress condition and grain quality, promising mutant lines were selected from these genotypes. In 2019, one line from *Apo* was released as new variety for cultivation and adoption, and was given the National Seed Industry Council (NSIC) registered name and local name of NSIC 2019 Rc572 and *Sahod Ulan 28*, respectively. On the other hand, promising IVM lines from *FR13A* and *Salumpikit* have been entered and currently being evaluated in the National Cooperative Testing (NCT).

Sustainable Uses of Mutant Rice Varieties in Thailand

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RD15 was invented by mutation from a rice elite line, KDML105, using gamma radiation at 15 Krad. It is characterized by low amylose content, fragrant aroma, height of 140 cm, yield of 3,500 kg/ha, resistance to leaf brown spots, and drought resistance.

Currently, Rice Department is working with Top Organic Products and Supplies Co., Ltd. from the company's request in the Thung Lo Organic Farming Project. The aim of the project is to produce organic Hom Mali rice with RD has KDML 105 and RD15 varieties. The project consists of 3 sectors: namely private sector, governmental sector, and the farmer groups. The Chiang Rai Rice Research Center under Rice Department provides knowledge to four groups of farmers in the area with total area of approximately 2,100-3,500 ha. Private sector representatives consist of Top Organic Products and Supply Co., Ltd., Riseria Monferrato, Italy, and BioAgriCert, Italy. The private sector takes care of the market side. They procure products for sale to the EU market as needed. The government sector is Phrae and Chiang Rai Rice Research Centers, which are under Rice Research and Development Division in the Rice Department. The Rice Research Center also analyzes seed quality, soil chemical and physical properties. The representatives of the farmers group consisted of 4 groups in proximate regions of Chiang Rai and Phayao.

Operations took place during 2008-2019. The Chiang Rai Rice Research Center advised all four groups of farmers on the production system of proper use of organic seeds, rice seed sowing with the seed rate of 93.75-125 kg per ha, rice seedling transplantation with the seed rate of 43.25-62.5 kg per ha. Manure application at the rate of 625-1,563 kg per ha was used before planting. Compost was applied with tilling the cover crop, sunn hemp, at the rate of 18.25-31.25 kg / ha; tilling of the cover crop was done while the flower was blooming and left for one month before rice plantation. Also, rice plowing without burning was applied. In conclusion, a group of 121-231 farmers planted on land of 791-1148 hectares. Production was between 1,624,524- 3,440,166 kg, and the production was worth between 32,500,613-75,269,316 baht with the selling price between 12.00- 20.62 baht per kg.

MUTATION BREEDING OF MAJOR CROPS FOR LOW-INPUT SUSTAINABLE AGRICULTURE UNDER CLIMATE CHANGE

Le Duc Thao, Agricultural Genetics Institute

The orientation in breeding of new mutant varieties (rice and soybean) in Vietnam are high yield and quality; wide adaptation, easily cultivation; short growth duration; tolerant to most important biotic and abiotic stresses in climate change condition. From 2008 to 2020, by gamma rays, Vietnam has released 14 new mutant varieties (12 rice and 02 soybean). In this, rice outstanding variety is Khang dan Mutant, released in 2008 with characters high yield, good tolerant to pest and disease cover about 400,00ha/year. DT39 Quelam released in 2013 has quality, high protein, yield and resistant to leaf blight. DT80 released in 2017 is the quality, BLB resistance variety. On soybean, the DT2008 is outstanding variety with the highest yield and tolerance ability in soybean breeding history, special in drought and diseases tolerance. Beside that, the first time, by the mutation method, Vietnam have a black soybean variety DT2008DB have almost characteristics the same DT2008 and content high nutrion and omega,... excellent in food use.

Besides that, within Forum Nuclear Cooperation of Asia (FNCA) framework, rice seeds were sent to Japan for ion beam irradiation; and base on the fellowship from The Fukui International Human Resources Development Center for Atomic Energy (Fukui HRDC), research in topic of DNA marker development was carried out with materials induced by ion beam irradiation. From ion beam irradiation obtained promising lines for further evaluation: 6 (M7); 6 (M6); 31 (M5); 39 (M4); 145 (M2). In 2020, we released the new variety DT99 from ion beam irradiation.

In addition, peanuts are a valuable food crop in Vietnam, which we initially studied breeding mutant varieties towards high lipit content. Until M2 has obtained some beneficial mutations and is continue screening in the next growing seasons.

In Future Work Plan, we need continuing testing promising lines in different ecological zones, continue screening mutation lines to select the good and stable lines and repeat irradiate with range of doses to performed the most effective dose for mutation breeding using ion beam. In addition, to really improve the effectiveness of the project, we are very desirable in the framework of the project, especially the Japanese managers and scientists support us to irradiate by ion beam on others crops such as soybean, peanut,..