Annex 4 Q&A Sheet

FNCA MB Workshop Question & Answer Sheet

Reporter / Answerer	Question / Comment	Answer / Response
Bangladesh (Dr. Mamun)	1) Thank you for the nice presentation! In Slide 7, do the leaves of the mutant lines vary from one another or was it due to difference in lighting during photography? In Slide 9, the color of the grains are distinctly different from one another. Is there no picture of the control or untreated seedlings in the plot? (from Mr. Aurigue, PHL)	1) Thanks a lot Mr. Aurigue for your kind observation. In slide 7 you are right due to light duration color looks different. Another on slide 6 left side you can see the control plants photographs, Mutants are earlier in case of flowering and maturity. Thanks.
China (Prof. Shu)	1) I am just wondering whether tissue culture process in genome editing and/or doubled haploid production may induce certain amount of somaclonal variation. I hope it is a negligible level, but do you have any data on this point? (from Dr. Hase, JPN) 2) Congratulations on your accomplishments particularly the numerous publications that are useful in mutation breeding! I look forward for more relevant documentation of your works. (from Mr. Aurigue, PHL)	1) Yes, tissue culture and transformation could induce genomic variations. For genome edited plants, we had assessed the mutation frequency by sequencing 5 genomes of regenerated plants and came to the conclusion that Agrobacterium-mediated transformation (for genome editing) induced mutations at frequencies similar to routine tissue culture (Li et al 2016, Zhejiang Univ-Sci B (Biomed & Biotechnol) 2016 17(12):992-996. For anther culture, we believe the tissue culture process could also generate somaclonal variation, though we have not quantified this kind of variation. However, we did observe variations, such as male sterile plants, in a DH2 population, which suggest the existence of somaclonal variation. 2) Many thanks
Indonesia (Dr. Sobrizal)	1) What is the major determinant of high yielding in “KEMUNING 1&2”? - No. of seed pod per plant - Grain size - Biotic/abiotic tolerance	1) High yielding in Kemuning 1 and 2 was determined by combination of number of pod per plant and grain size, which were higher than their parent and checks. In addition, Kemuning 1 and 2 were developed to

	- or others? (from Dr. Hase, JPN) 2) What kind of situation is "shade tolerant"? (from Mr. Takahashi, JPN) 3) Congratulations for developing new mutant varieties of soybeans! May I know if there is any drought-tolerant variety used as control or check for Kemuning 1 and Kemuning 2? Does the parent, Pandermen, exhibit drought tolerance? Also, I wanted to confirm if Sugentan 1 and Sugentan 2, which mature very early, are NOT drought-tolerant. Thank you for your replies! (from Mr. Aurigue, PHL)	drought stress tolerance so both are suitable for drought prone area. 2) The study of shade tolerance is carried out under 50% of light intensity which is measured by lux meter. 3) Thank you! The parent of Kemuning 1 and 2 doesn't exhibit drought-tolerance. We used drought-tolerant variety as a check and the result showed both Kemuning 1 and 2 showed better performance than the check. We didn't do drought-stress test for Sugentan 1 and 2, so we cannot confirm about that.
Japan (Dr. Hase)	1) This is my first experience to join FNCA meeting and I am very happy to have this opportunity and know about the progress of several Asian countries in the use of nuclear in plant breeding. I am interested to join in the use of ion beams for mutation breeding, especially for soybean mutation, which was mentioned by Dr. Hase. How can I include genetic material in this program? (from Dr. Puspitasari, IDN) 2) Dr. Hase, have you published any of the reports that you presented in our previous FNCA Meeting Workshop? I will appreciate it very much if you could provide us the details of any publication or the link for an e-copy. (from Mr. Aurigue, PHL)	1) Thank you for your interest to use ion beams. You can do so if the purpose is in line with the FNCA project and also the PL (Dr. Sobrizal) agrees to use. Probably, I can provide a beam time for the FNCA project in April or May 2021. Details will be announced in February. Please note that the beam time is limited, and therefore, I may not be possible to irradiate all the seeds. You need to obtain phytosanitary certificate from the competent authorities of your country to export soybean seeds to JPN. I have irradiated soybean seeds from Vietnam in the last two years. Information from Dr. Thao will be helpful to determine proper dose. 2) Thank you for your interest in our work, Mr. Aurigue. Here is the link for my recent publications that I talked at the workshop. https://www.frontiersin.org/articles/10.3389/fpls.2020.00336/full https://www.nature.com/articles/s41598-018-19278-1 https://academic.oup.com/jrr/article/61/5/63

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Korea (Dr. Kang)	1) In one of your slides it was mentioned that developing a new variety with high function such as rice, can you explain what is meant by high function on what character / trait you want to develop? 2) For the commercialization of this new mutated variety, what approach do you use so that it can be accepted quickly by farmers and the community? 3) For functional genomics and metabolomics study, in what generation of your mutant population was this approach / study carried out? (1)-3) from Dr. Dewi, IDN) 4) Do you have any plans to use phenomics in mutant screening? I think it is a big challenge but if it works, we could find new mutants that cannot be identified by conventional methods. (from Dr. Hase, JPN) 5) Dr. Kang, do you have any publication about the mutant varieties of ornamental plants? They are all very interesting and I would like to know more about the details. Thank you! (from Mr. Aurigue, PHL)	1) A new variety with high function means that it contains a lot of functional compounds, and the type of target compounds varies depending on the crop. Main functional compounds in recently developed rice were tocopherol, amino acids and pigments, etc. 2) New mutant varieties of rice and soybeans, which are officially registered, are usually propagated by ourselves and then distributed directly to farmers. Other crop seeds and technology developed by our research team have been transferred to the industry sector. 3) We have been mainly conducted genomics and metabolomics analysis to selected mutants or population in M2~M6 generation. 4) I think that the KAERI research team will be working on setting the conditions for the meantime. I hope to see much progress in this research in the future. 5) We published some papers about mutation breeding in ornamental or horticultural plants already. If you search my name or keyword, you can easily find it.
Malaysia (Dr. Hussein)	1) Do you have a mutation breeding program to improve local rice varieties in Malaysia to support food self-sufficiency in the region, given that local rice has specific characters, especially taste and aroma, which is maintained and developed by farmers in the area? 2) Are there any difficulties in propagating different types of mutant varieties so that they can be distributed to farmers?	1) Yes we have. Basically, the mutation breeding program is automatically generated when local verification trials (LVT) are conducted in collaboration with the industry players. Once rice mutant shows significant increase in yield, the farmers would request to be part of the mutation breeding project. Subsequently, the best mutant variety is maintained and developed by the industry players.

	(1)-2) from Dr. Dewi, IDN) 3) Sorry to ask about such minor details, but what the graph on the right side in your slide #13 represents? In that, NMR152 and NMR122 show the value of 134 and 122, respectively. (from Dr. Hase, JPN) 4) Nuklear Malaysia truly deserves the FNCA Award because of the accomplishments that have high impact to farmers and to Malaysian economy. At the same time, you are able to promote mutation breeding technology through your publications, Farmers' Field Day, and press releases. Congratulations! What is also very good about the mutants is that it helps protect the environment and the health of people due to minimal use of chemicals against pests and diseases. (from Mr. Aurigue, PHL)	2) Propagating different type of mutant is not the major problem because it can be done through research collaboration with the industry players. The major problem is the requirement to seek for approval from the national technical committee before the rice variety is allowed to be distributed to the farmers. Moreover, the approval process is tedious and time consuming. 3) The graph shows in slide no 13 is the SSR fragment analysis to differentiate between the parent and NMR152. 134 refers to the SSR analysis for NMR152 while 122 refers to the SSR analysis on the parent (MR219) control. 4) First and foremost, thank you to Mr. Aurigue for the compliments and showing great appreciation towards the output of Nuclear Malaysia mutation breeding project. Nuclear Malaysia would also like to express utmost gratitude to FNCA for the great support.
Mongolia (Dr. Noov)	1) In Slide 12, the mutant lines of wheat were classified as early maturity (81 and 83 days), medium maturity (86 and 87 days), and late maturity (92 days). Please clarify your mutation breeding objective stated in Slide 4 that short vegetation period is 80 to 100 days. Why is 92 days considered already late maturing when it still falls under short vegetation period? Thank you! (from Mr. Aurigue, PHL)	1) Thank you for question. Standard wheat maturity groups in Mongolia are: early maturity 80-84 days, medium maturity 85-90 days, and late maturity over 91 days. The slide 4 illustrates our overall objective to reduce maturity days in all maturity groups. For example early maturity not more than 80 days, late maturing lines less than 100 days. But, we understand that expression in slide 4 was not clear to explain above objective.
The Philippines (Mr. Cabusora)	1) Based on your framework, what are your considerations for screening abiotic stress in the M4 generation? (from Dr. Dewi, IDN) 2) Do you see any difference between the	1) Selection criteria is acceptable phenotype (good plant architecture, stature, early maturing, exserted panicle, etc.) at the M2 population. Since our wildtypes are abiotic tolerant landraces with poor phenotypes.

	mutant population generated by seed irradiation and in vitro mutagenesis, including unfavorable phenotype? (from Dr. Hase, JPN)	The advancement to two more generations (up to M4) is done only to evaluate the uniformity of the lines and the stability of the mutation. We need to be sure that the mutation is stable (genetic) and that the lines will not revert back to its original phenotype (epigenetic) on the next generations. So basically the screening for stresses, is to select mutants with good phenotype and retained abiotic stress tolerance. 2) Actually no, Sir. We don't observe any difference in terms of variability induction. The only advantage of using IVM is that at a small population, variability is high and we were able to select best lines (higher efficiency). Unlike in seed mutation we need thousands of M2, only to have small selection efficiency. Thank you for the questions, Dr. Hase. If may I request your email address. I would like to consult some matters regarding one of our mutant exhibiting novel floral mutation different from those already published. Thank you.
Thailand (Mr. Noenplab)	1) Thank you for joining the workshop. It was nice to meet you and I am looking forward to hear your research work more in detail next time. (from Dr. Hase, JPN)	1) Hello Dr. Hase. Yes, of course. Next time, we will talk about flood tolerant and drought tolerant elite lines developed from mutation breeding in our pipeline. Thank you for hosting the event.
Vietnam (Dr. Le)	1) How to use brown and black soybean? (from Mr. Takahashi, JPN) 2) The results of gamma irradiation on peanut are very good and interesting. However, could you please ascertain which chlorophyll mutation was observed? From the pictures presented in the slides, it does not look like an albina (white) at all. You may	1) Thank you for the question. In Vietnam previously only yellow soybeans were used for food. Black soybean DT215 is the first black soybean variety created and planted in Vietnam. DT215 has high levels of omega-3, omega-6, and carotene, suitable for processing functional foods. Currently, this variety has been transfer breeder copyright

	want to check if it is actually xantha or chlorina because albina hardly survives due to lack of chlorophyll. Thank you! (from Mr. Aurigue, PHL) 3-1) Responding to Dr. Hase's answer to my question, I would like to ask about the proper dosage for irradiating soybeans using ion beam. (from Dr. Puspitasari, IDN) 3-2) Could you share the data on the survival rate of soybean seeds irradiated on May 14th, 2019? On that day, I irradiated soybean seeds with the following doses. 320 MeV Carbon: 10, 20, 30, 40, 50 Gy 107 MeV Helium: 20, 40, 60, 80, 100 Gy (from Dr. Hase, JPN) 3-3) Dr. Thao, thank you for your response. Clear dose-response is seen on the survival rate of M1 seeds. Dr. Puspitasari, I think this data is helpful to check the radiation sensitivity of your materials. Let's think about it again, after becoming clear that I can hold a beam time for this project. (from Dr. Hase, JPN) 3-4) Dr. Thao and Dr. Hase, thank you very much for your cooperation. This is very useful information. We look forward to apply it to our genetic materials. (from Dr. Puspitasari, IDN)	to a food company, which processes bottled food drinks and soybean cakes rich in nutritious dini. 2) Thank you for question. In the implementation process, not only peanuts but soybeans are also often obtained albino in the M1 and M2 generations. The albino variant can be whole body or partial tree; The timing of appearance can be in the seedling or when the plant is mature. So some plants still grow and collect seeds. These variations are less in M2 and by M3 almost disappeared. In the presentation, we only give examples of the occurrence of mutants without going into further research on this mutant type, the main direction for screening in our mutants peanut breeding is to choose varieties with high lipit content. 3-1) It is true that we are having trouble determining the dose of ion beam irradiation on soybeans. We are currently evaluating the M3 generation, but the seeds numbers is very small. It seems that we determined the dose too high and the wide distance. So we are working to determine the dose closer together in next irradiate to determine the optimal dose. 3-2) Please see table 1 is the data for ion beam on soybean. Thank you for your cooperation.

Table 1

The survival rate of soybean seeds irradiated (%)

1. 320 MeV-C

	842008-1		842008-36		F3507-2		F3507-3/1	
	M1	M2	M1	M2	M1	M2	M1	M2
10 Gy	61	87,5	57	86,5	63	83,3	66	87,1
20 Gy	48	86,9	45	88,1	45	86,8	46	85,9
30 Gy	30	88,0	29	87,7	31	87,4	27	86,3
40 Gy	17	87,8	17	86,9	19	87,1	20	85,7
50 Gy	10	87,5	9	87,5	9	87,0	11	87,1
0 Gy		88,1		87,2		87,5		86,4

2. 107 MeV-He

	842008-1		842008-36		F3507-2		F3507-3/1	
	M1	M2	M1	M2	M1	M2	M1	M2
20 Gy	67	88,1	69	85,1	67	88,4	66	85,3
40 Gy	60	87,2	57	87,3	58	87,9	58	86,0
60 Gy	47	89,1	48	86,4	46	88,1	48	85,7
80 Gy	31	86,3	30	87,5	33	87,7	41	87,1
100 Gy	20	87,4	22	88,6	19	87,3	21	86,1
0 Gy		87,8		86,3		89,1		85,6