ANNEX 2

Part A. Summary of Country Reports on Production and Field/Pot Test of Plant Growth Promoter (PGP) from Chitosan by Radiation Processing

(1) Bangladesh (Dr Salma Sultana, Bangladesh Atomic Energy Commission (BAEC))

With o-chitosan the yield and other parameters of spinach shows good result with compare to control. With increasing o-chitosan the yield and other parameters of spinach also increases. 100ppm o-chitosan gives three times more yield with compare to control. With 75 ppm o-chitosan the yield and other parameters of amaranth shows good result with compare to control Plant height of mustard with 2% o-chitosan shows high values with compare to control. O-chitosan shows a significant effect of ripe banana. Disease incidence (%), disease sevearity, weight loss and total soluble solid decreased with increasing the o-chitosan concentration of banana fruits. In the case of brinjal, o-chitosan applied at different concentration (from 1000-5000 ppm) extensively decreased the mycelial growth of Phomopsis vexans and maximum inhibition was obtained at the higher concentration that is 5000 ppm o-chitosan.. After 5 days average mycelial growth for control is 78.33 where as with 5000 ppm o-chitosan..no mycelial growth obtained and percent inhibition over control for 5000 ppm o-chitosan.. Is 74.89 where as for control is zero.

(2) Indonesia (Dr Darmawan Darwis, National Nuclear Energy Agency (BATAN))

Irradiated chitosan (oligochitosan) was prepared from shrimp shell by chemically extraction processes and irradiation using gamma ray. The irradiated chitosan has some benefits in plants such as plant growth promoter, plant elicitor. It is also used as plant antibiotic to reduces diseases of plant due to microorganism.

Irradiated chitosan was semi field test to several plants such as rubber tree, sapodilla, potato, cabbage and zea mays. In general, it can be said that almost all plants treated by chitosan showed increase crops yield, early maturity, more resistant to diseases, better quality of fruit compared to untreated plants (control). Potato plants treated by oligochitosan through foliar sprayed with concentration of 50 ppm once a week resulted in increasing yield up to 300% compared to control. In addition, rubber trees treated by oligochitosan resulted in increase of latex production by 2 times higher compared to control. The combination treatment of Super Water Absorbent hydrogel (SWA) and oligochitosan for shallot planted in sandy soil resulted in water used efficiency, reducing irrigation frequency and increase plant performance

(3) Japan (Dr Naotsugu NAGASAWA, Japan Atomic Energy Agency)

Oligo-chitin obtained by gamma-irradiation in fine grinded crab shell containing chitin nanofiber to reduce the preparation cost of PGP. The water-soluble oligo-chitin is effective for plant elicitor. The oligo-chitin significantly increased the plant elicitor of rice, Kinmaze and Nihonbare. To comparison with enzymatically degraded oligo-chitosan which is already well known to have plant elicitor activity, oligo-chitin obtained by gamma-irradiated at 1000 kGy is higher elicitor activity such as phytoalexin induction, defense protein production and active oxygen. In preliminary test of rice growth, oligo-chitin obtained by gamma-irradiated at 1000 kGy in swollen state has positive effects on root growth of Nihonbare in hydroponic culture controlled the essential element content.

(4) Kazakhstan (Mr Sergey Kotov, JSC "Park of Nuclear Technologies")

Since 2014 Kazakhstan has putted in operation new 5 MeV electron accelerator, which has possibility to produce such products as PGP in large scale. For PGP production in Kazakhstan the raw material should be determined because of lack of own factory of Chitosan.

(5) Malaysia (Ms Maznah MAHMUD, Malaysian Nuclear Agency (Nuclear Malaysia))

Production of oligochitosan with different Mwt using radiation-processing has been established in Nuclear Malaysia. In order to diversify the application of radiation-processed chitosan in agriculture, few projects have been done in 2014.

Rice crop application

In second quater of 2014, chitosan with Mwt 10kDa has been used together with liquid biofertilizer and liquid smoke to study the effectiveness of them on rice (MR 219) and mutant rice (MR 219-4 and MR 219-9). Liquid biofertilizer, developed by Nuclear Malaysia contains living microorganisms. These living microorganisms help plant in nutrient uptake as well as enhance the growth of plants. Besides, liquid smoke also been introduced in the package treatement as natural pesticides and insecticides. It is produced by condensation of smoke from the burning process of coconut shells. Oligochitosan, liquid biofertilizer and liquid smoke were combined in 3 different treatment packages. There were :

T1 : Liquid biofertilizer + liquid smoke

T2 : Liquid biofertilizer + Liquid smoke + oligochitosan

T4 : Liquid smoke + oligochitosan

T3 : Control

The study was done on MR 219, MR 219-4 and MR 219-9 in Perlis, the northern region of Malaysia. MR 219 is one of the most chosen variety planted by Malaysian farmers. It has good disease resistent, short maturation period and can give high number in yield. But in order to obtain high yield, it requires good water management and high fertilizer. MR 219-4 and MR 219-9 are mutant

lines which produced by irradiation of MR 219 at 300kGy. Besides, inheriting the special properties of MR 219, MR 219-4 and MR 219-9 is able to produce high yield under minimal water condition (aerobic). 3 different package treatments (as mentioned above) and 1 Control (normal practice of local farmers, T3) have been studied. Results obtained showed that MR 219-4 gives 15% higher yield compare to MR 219 (parent) and MR 219-9 after treated with T2. In the other hand, MR 219 gives 15% higher yield after treated with T1 and T4 compare to T2. T3 shows least yield. Combination of biofertilizer and oligochitosan in same treatment doesn't give great result on MR 219 yield. MR 219-4 gives 25% and 36% higher yield after treated with T2 compare to T1 and T4, respectively. But, T3 gives better yield value than package treatments. MR 219-9, gives higher yield in all package treatments compare to Control. T2 and T4 give 8% higher yield than T1. From the trial, it shows that application of package treatements can increase the yield of MR 219 as well as the mutants. For the package treatments MR 219 and MR 219-9 give positive in number of yield but not MR 219-4 after comparing to the Control (T3). But MR 219-4 still give highest yield in T2 compare to the MR 219 and MR219-9 in the same treatment. Second trial, with minimal parameters has been started at end of 2014 in order to support the first trial results.

Chili application

Application of radiation processed chitosan on Kulai chili hybrid also being done in Nuclear Malaysia using fertigation system. 3 types of chitosan with different Mwt with 1 Control which is water, are being used in this trial. As the trial was just started on last December, the results reported are only at the seedling stage. Chitosan with Mwt 6.5kDa shows the highest germination rate, 85.2% compare to chitosan 18kDa (80%), Chitosan 38.8kDa (56.3%) and Control (69.1%). The higher the Mwt the lower the germination rate achieved. The plant height and number of leaves shows no significant difference. However, chitosan with Mwt of 38.8kDa shows lower plant height and leaf number. From the results obatained, chitosan with 38.8kDa may give stress to the germination rate of chili seeds as well as seedlings growth rate. Chitosan with Mwt of 6.5kDa and 18kDa and Control has no significant difference on seedlings growth rate. Nevertheless application of chitosan (6.5kDa and 18kDa) give really higher impact on germination rate compare to Control.

Technology transfer

The techniques on production of chitosan with different Mwt using radiation technology and the application of radiation processed chitosan have been transferred to the researcher from KACST (King Abdul Aziz City for Science and Technology) under Bilateral-collaboration programme. Through the programme, the fellow was given opportunity to produce chitosan with different Mwt using various techniques (with and without H2O2, solid and liquid states). Besides, he was also given chances to participate on the field activities i.e application of chitosan on rice and chili.

Commercialization

For commercialization purpose, Nuclear Malaysia has signed 2 local companies i.e. Avid Focus Resources and Persada Agro Ent. In 2014, more than 1 tonne chitosan solution was sold.

(6) The Philippines (Ms Charito T. ARANILLA, Philippine Nuclear Research Institute (PNRI))

The program on "Plant Bio-Stimulants and Elicitor from Radiation-Modified Natural Polymers" was approved for continued implementation by three collaborating agencies (PNRI, PRRI, NCPC-UPLB) for 2014-2015 by Department of Science and Technology. The accomplishments for year 1 of the program include the following (studies conducted in screenhouse and field): (1) Project 1 (screenhouse conditions), oligo-carageenan at concentration of 60 ppm, increased yield of mungbean from 0.72 g/plant to 3.74 g/plant (419%) while oligochitosan at concentration of 40 ppm increased mungbean yield from 0.72 g/plant to 2.81 g/plant (290%). In the case of peanut, oligo-carageenan at concentration of 60 ppm increased yield by 346% (3.87 g/plant to17.28 g/plant) while oligo-chitosan at concentration of 80 ppm increased yield by 278% (3.87 g/plant to 14.64 g/plant); (2) Project 2 (screenhouse conditions), improved agronomic traits such as plant height, tiller number, and chlorophyll content (N content of the leaves) due to oligocarrageenan and oligochitosan treatments and increased yield by 32% due to oligochitosan application at 25 ppm concentration in addition to the fertilizer recommended rate in granular form (RRG);(3) Project 3 (screenhouse and field conditions), reduced blight severity regardless of k-carrageenan and chitosan concentration (50, 100, and 150 ppm) used. Tungro virus infection was not present in the areas treated with k-carrageenan and chitosan despite the presence of green leafhoppers and infected field nearby. For aggregate beneficial insects, count is highest at 20 DAT with RRG plus 100 ppm chitosan but decreased at 35 and 45DAT; (4) Technology promotion at the 58th IAEA General Conference in Vienna, IRAP Conference in Korea and National Science and Technology Celebration in different places in the Philippines.

Workplan for 2015-2018 include comparative field experiments on oligocarrageenan PGP versus commercial product; field experiment for effect on physiology of rice; field experiments on induced pest resistance in rice in farmer’s field and conduct economical analysis.

(7) Thailand (Dr Phiriyatorn SUWANMALA, Thailand Institute of Nuclear Technology)

Chitin was prepared from local shrimp shells. The prepared chitin was changed into chitosan, by chemical reactions. Radiation-induced degradation was used to reduce the molecular weight of the prepared chitosan, yielding oligochitosan. The obtained oligochitosan was tested for its potential use as plant growth promoter. Effects of oligochitosan on growth and productivity of Thai chili plants were investigated. The experiment was carried out with randomized complete block design (RCBD) with ten replications. The foliar spraying of oligochitosan (molecular weight ~ 15,000 Da) with the

concentration of 20, 30, 40 and 80 ppm mixed with fertilizer was applied. The growth and productivity of these oligochitosan-treated chili plants were compared with those of untreated chili plants. The effects of oligochitosan on Thai chili's growth and productivity were investigated in term of plant height, total number of chilies, total weight of chili, total number of green chilies, total number of red chilies, harvest time and weight per chili. The results showed that the application of oligochitosan, at the concentration of 80 ppm, mixed with the fertilizer displayed significant effects, statistically, on chili height, total weight of chili, total number of chilies, total number of green chilies, total number of red chilies and weight per chili. The results showed that productivity was increased up to 34%. The oligochitosan exhibited the ability to protect not only aphid inflection but also the ability to shorten the harvest time of chili plants. The treatment of chili plants by oligochitosan clearly displayed positive effects on chili's growth and productivity. These results suggest its potential use in agriculture purposes as growth promoter for Thai chili plants. After positive results with chili, a field test of PGP with Mariam Plum was done at Nakon-Nayok Province. The results have shown that applying PGP increased Mariam Plum production up to 23%. A pilot plant for the production of PGP with the capacity of 15,000 liters/month was set up at Thai Irradiation Center, Phathumthanee.

(8) Vietnam (Dr Nguyen Quoc HIEN, Vietnam Atomic Energy Institute)

Oligo beta glucans were prepared by gamma Co-60 irradiation of 10% beta glucan/0.5% H2O2 solution mixture in the dose range of 10-15 kGy. The obtained oligo beta glucan exhibited strongly growth promotion effect for mustard green with optimal concentration of 75 mg/L and fresh weight increase of about 30% compared with the control. Oligoalginates were prepared by gamma Co-60 irradiation of 4% alginate/0.5% H2O2 solution mixture at dose of 8 kGy. The resultant oligoalginate showed highly growth promotion effect for lettuce with optimal concentration of 75 mg/L and fresh weight increase of about 45% compared with the control. The registration of oligoalginate as liquid fertilizer is in progress. Oligochitosans were also prepared by gamma Co-60 irradiation of chitosan/H2O2 solution mixture. The elicitation/growth promotion effect of oligochitosan with different molecular weight namely: 9000, 5000 and 2.500 g/mol on chili plant in green house at Hi-Tech Agriculture Center HoChiMinh city has been being studied. In addition, oligo beta glucan supplement with 500 mg/kg feed for chicken the increase of weight gain of about 24% compared with the control.