Annex 4

Summary of Country Reports

Part A. Summaries of Country Reports on Radiation Processing and Application of Chitosan

(1) Bangladesh (Ms. Salma SULTANA, BAEC)

Oligo-chitosan was applied for its potential use as plant growth promoter. Effects of oligo-chitosan on growth and productivity of Maize (*Zea mays* L) plants were investigated as a lab-scale. The morphological characters of Maize were studied randomly in different pots. The foliar spraying of oligo-chitosan (molecular weight ~ 7,000 Da) with the concentration of 25, 50 and 75 ppm was applied. The growth and productivity of these oligo-chitosan treated Maize plants were compared with those of control Maize plants. The effects of oligo-chitosan on Maize's growth and productivity were investigated in terms of plant height, weight of cob and weight of seeds. The results showed that the application of oligo-chitosan, at the concentration of 75 ppm, plays a significant role in terms of plant height, weight of cob and weight of seeds per Maize. These results suggest its potential use in agriculture purpose as growth promoter. Effects of oligo-chitosan on Fine Grain Aromatic Rice is going on field level and effects of oligo-chitosan on Tomato plants on lab-scale is also going on.

(2) China (Dr. Jing PENG, Peking University)

The recent progress in the production of oligo-chitosan by radiation degradation and key points for the industrial production of oligo-chitosan were introduced. In China, the radiation produced PGP oligo-chitosan has been used for maize, cucumber and so on. A field test indicated that the foliar spray of radiation produced oligo-chitosan and its derivatives could improve per weight, length, and diameter. In particular, the straight melon rate of cucumber could be increased by 92%, and sugar content in the cucumber could be increased. Furthermore, *Botrytis cinerea* of the cucumber could be resisted. The cost calculation identified that the cost for radiation degradation is lower than that for enzyme degradation. However, the status of commercial application of the oligo-chitosan produced by radiation as PGP is not optimistic; there are still many challenges in China.

(3) Indonesia (Dr. Darmawan DARWIS, BATAN)

Oligochitosan Plant Growth Regulator/Promoter was produced from shrimp shell using chemical methods and followed by gamma irradiation at 75 – 100 kGy. Oligochitosan was produced by this method having molecular weight of 2500 – 3500 Da. Analysis of oligochitosan plant growth regulator substances such as gibberellin, auxin and cytokinin has been done. The result showed that increasing of irradiation dose resulted in increase of gibberellin, auxin and cytokinin content in oligochitosan. Cost evaluation of oligo chitosan production for growth promoter was done based on material and processing cost. Depreciation cost of machine, overhead cost and labor cost was not included. Cost production for 1Liter Oligochitosan PGR is US$ 1.3.

During 2010, oligochitosan GP have been field tried for soybean (using Mitani and Rajabasa varieties) at Indralaya District, South Sumatra. The result showed that productivity of both soybean varieties treated with oligochitosan is higher than that of control. Improvement of productivity for Rajabasa and Mitani varieties is about 40%.

Dissemination and socialization of O-chi PGR were done to different farmer groups in Cianjur, Majalengka, Palembang and Banyuwangi. During 2009 and 2010, several activities have been conducted such as training the farmer and field trial at Cianjur, West Java on June 30, 2009. This meeting was attended by 70 participants including local farmer, Agriculture Department District staff and farmer supervisor. The lecture and discussion on processing and application method of oligochitosan as growth promoter as well as promotion of Rajabasa and Mitani soybean varieties were done. Socialization of oligochitosan GP, training the farmer and field trial were also done at Majalengka, West Java on June 19, 2010. This meeting was attended by 70 participants including local farmer, Agriculture Department District staff and farmer supervisor. Similar program was also conducted at Palembang, south Sumatra Province on August 5, 2010. The event was attended by 50 participants mostly farmer belong to soybean group farmer. Warm discussion was also conducted. The farmers were interested to used oligochitosan to their soybean plant

(4) Japan (Dr. Naotsugu NAGASAWA, JAEA)

Oligochitosan, obtained by gamma-irradiation is effective for plant growth promoter and plant elicitor. The average molecular weight of oligochitosan is below 10 kDa by GPC. By using ultrafiltration membranes, irradiated chitosan were separated into five fractions, F1 with Mw below 1kDa, F2 with Mw: 1-3kDa, F3 with Mw: 3-10kDa, F4 with Mw: 10-30kDa and F5 with Mw more than 30kDa. The fraction F2 with Mw: 1-3kDa had the highest content in chitosan irradiated at 100kGy in 10% solution (25.9%). The chitosan fraction with Mw in range of 1-3kDa (Dp: 5-16) significantly increased the activity of phytoalexin enzyme, PAL and chitinase.

Oligo-chitosan as plant activator (trade name: oligo-glucosamine-L(OG-L)) was commercialized in Japan. To expand commercialization of oligochitosan, PGP, OG-L, we introduced OG-L in exhibition and / or seminar (8 times from April, 2010 to February, 2011).

(5) Malaysia (Dr. Kamarudin BIN BAHARI, MNA)

Pilot scale production of oligochitosan III was carried out by continuous flow gamma radiation. Total production was about 2,200 litres. Oligochitosan was characterized in terms of colour, viscosity, pH and DDA. Storage study has been carried out and oligochitosan solution degraded during storage, based on the result of viscosity and pH.

Field trial of oligochitosan as plant growth promoter and elicitor on rice was carried out in collaboration with FELCRA (M) Bhd on 26 hectares rice plantation. In this study, eight treatments (T1-T8) with triplicates and additional 2 plots for transplanting (T9) and direct seeding (T10) had been carried out. Data compilation had been done in respect to neck fungus blast, rice check, crop cutting survey, analysis on soil, nutrient analysis of paddy plant and rice yield for each plot.
Study indicated that rice yield depended on germination treatment with oligochitosan, spray treatment with oligochitosan and other factors such as soil condition, nutrient up take, insect infection and plant growth.

(6) Philippines (Ms. Charito ARANILLA TRANQUILAN)

A. Status of Research Program Proposal

The Philippine Nuclear Research Institute formulated and submitted a research program in 2009 entitled "Plant biostimulants and elicitor from radiation-modified carrageenan and chitosan" to the Department of Science and Technology. This research program is in collaboration with the Philippine Rice Research Institute and the National Crop Protection Center. It covers major research aspects to be carried out such as PGP product development, elucidation of mechanism, field test applications, and commercialization. The current status of this program is still on the evaluation stage. Due to changes in administration in the Department of Science and Technology as a consequence of national election that was held in May 2009, the processing and approval of this program was delayed affecting the workplan for conduct of field test applications using rice and bitter gourd crops.

B. Production and Cost Analysis of Oligochitosan PGP Solution

Oligochitosan solution was produced following the protocol established during the pilot-scale demonstration held in Indonesia. A pre-weighed amount of commercial-grade LMW chitosan (MW~120 kDa and 84% DDA) was dissolved in 1.5% lactic acid to a concentration of 30g chitosan per liter solution and irradiated at 10kGy. The irradiated solution was added with EtOH/NaOH preservative forming the final product of Oligochitosan PGP with a concentration of 20,000 ppm. The molecular weight of the chitosan in the final product form was less than 21 kDa.

Using the existing pilot plant facility of PNRI and the maximum capacity of irradiation facility to radiation-process oligochitosan solution as bases, a cost analysis for production of 3000 liters of PGP solution revealed a product cost of 4.80 USD per liter PGP.

C. Commecialization Strategies

There are three possible strategies that can be used to commercialize PGP Oligochitosan. These are:

1. Filipinovation – a national innovation strategy of introducing a mindset of innovation in cooperation with the stake-holders from the business, academe, government and S & T sectors.
2. TECHNICOM (Technology Innovation for Commercialization - a comprehensive and unified strategy to enhance technology development for commercialization and to facilitate technological innovation enterprise spin-offs. This a DOST Program which provides funds to fast track the transfer and commercialization of research results especially those generated by member institutes of the national R & D network (RDIs, universities, SUCs). It fills the gap between the laboratory stage and commercialization stage of technology development.
3. Republic Act 10055: Technology Transfer Act of 2009 - an act providing the framework and support system for the ownership, management, use and commercialization of IP generated from Research and Development, funded by Government and for other purposes.

The Institute used TECHNICOM for the commercialization of PVP-Carrageenan hydrogel. Strategies 2 and 3 may be considered for the case of Oligochitosan PGP.

D. Workplan for 2011-12

1. PNRI (Component 1 & 2)
    a. Continue product development
    b. Conduct economic feasibilty study on oligochitosan PGP
    c. Determine the effect on the in-vitro germination of the seeds of *Spathoglottis* and *Dendrobium* orchids and their survival rate
    d. Determine the growth promoting and flower inducing effect on *Spathoglottis* and *Dendrobium* orchids
2. Philippine Rice Research Institute (Component 3)
    a. Determine the effect on growth, nutrient uptake, and nutrient availability on rice.

        -Conduct greenhouse experiments
        -Analyze nutrient uptake
        -Analyze soil and plant tissue samples

3. National Crop Protection Center-Crop Protection Cluster College of Agriculture, UPLB (Component 4)
    a. Initial laboratory screening of different dosages and timing of application of PGP on both rice and bitter gourd.
    b. Greenhouse experiment to manage all variables.

(7) Thailand (Dr. Phiriyatorn SUWANMALA, TINT)

Field test

The effect of irradiated chitosan, obtained from FNCA, on rice's growth, production and disease resistance were studied. The experiment was carried out with randomized complete block design (RCBD) with four replications. The foliar spraying with irradiated chitosan was done three times, at 20 days after sowing, at tillering phase and at panicle initiation phase, respectively. The result showed that the tiller numbers obtained from all four different treatments were not significantly different from each other. Rice plants treated with fertilizer and irradiated chitosan gave the highest number of grain yield. The differences from all four treatments were not statistically significant. Rice plants treated with fertilizer resulted in the highest seed numbers per panicle and 1,000-grain weight when compared to the control treatment but not significantly different. At booting and heading stages, rice plant treated with irradiated chitosan had lower percentage of Bacterial Blight (BB) than control. The percentage of Sheath Blight (ShB) at the heading stage and dough stage decreased with decreasing concentration of irradiated chitosan.

Lab-Scale Test: 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.

Technical Workshop: Farmers from the southern part of Thailand are interested in making chitosan from shrimp shells. They contacted TINT and requested our R&D staffs to transfer this technology to them. The project was initiated and the course was held during 14 – 16 September 2010 in Chumporn province, in the southern part of Thailand. With approximately 75 participants attending, the workshop was successful. Some participants have applied the lesson learned from the workshop for real applications. One participant has started making chitosan from shrimp shells (instead of commercial chitosan he normally purchases and uses) for treating the water in his soft-shell crab farm. Another participant has applied radiation to reduce the molecular weight of chitosan, for commercial purposes.

(8) Vie tnam (Dr. Le Hai, VAEI)

Oligochitosan with molecular weight from 5,000 to 10,000 has been produced by gamma irradiation of chitosan solution (3%) containing a small amount of hydrogen peroxide (0.5%) at a dose of about 10 kGy. The combined treatment method (gamma ray/hydrogen peroxide) is considered as an efficient method for scale-up manufacture of oligochitosan. Elicitation and plant growth promotion effect of oligochitosan for different plants such as papaya, dragon fruit, and also for rice seed treatment is being studied.

Part B. Summaries of country reports on Radiation Processing and Application of Starch/Polysaccharides for Super Water Absorbent (SWA)

(1) China (Dr. Jing PENG, Peking University)

In this report, the effects of parameters such as monomer content, gelatinization temperature, crosslinking content, and dose on the water absorption performance of starch based SWA was discussed. Besides the irradiation conditions, the drying and measurement conditions will affect the performance. Particularly, the presence of metal ions such as Ca^{3+}, Mg^{2+}, Fe^{3+} can reduce the water absorption ratio of SWA. Moreover, the effects of factors on the degree of remained water was discussed. Although the starch grafted SWA could be used in the growth of wheat, corn, tomato and grapes. The stability of Starch grafted SWA is not very high enough. Some modification on the starch has been carried out. In addition, UV initiation has been used recently to prepare novel salt resistant superabsorbent resin based on the starch and diatomite. The mechanism on the interaction between SWA with water and soil has not been studied clearly.

(2) Indonesia (Dr. Darmawan DARWIS, BATAN)

Super Water Absorbent Hydrogel (SWA) for soil conditioner was prepared by radiation crosslink of acrylic acid (AA) and cassava starch with addition of KOH using gamma ray with various doses ranging from 0 to 20 kGy. Several parameters such as gel fraction, swelling capability and preliminary test of SWA in soil with paddy plant were carried out. Ratio of SWA and soil used is 0, 0.3, 0.6, 1.2 and 2.4 g/kg of soil. The results showed that gel fraction of SWA hydrogel prepared from 10% acrylic acid and 12 % of cassava starch reach maximum at 5 kGy irradiation dose. Degree of swelling of Cassava-AA SWA in water at room temperature (30^{o}C) with dose of 5 to 20 kGy is 40 to 50 times of its dry weight and equilibrium swelling is reached at 5 hour immersion time. Treatment of SWA with ethanol resulting in improvement of swelling capability and reducing equilibrium degree of swelling time (EDS). EDS of SWA treated with ethanol is 80 g/g of dry gel and EDS reached at 3 hours immersion time. preliminary test of SWA in soil with paddy plant showed that concentration of SWA in soil affect the plant height and number of penicles as well. Its seem that SWA with concentration of 0.6g/kg of soil give maximum effect on plant heigh and number of pennicel. The experiment is still continuous until harvesting time.

(3) Japan (Dr. Naotsugu NAGASAWA, JAEA)

Radiation-induced crosslinking and biodegradability of carboxymethyl-starch (CMS) and carboxymethyl cellulose (CMC) were investigated for application as super water absorbent. Swelling capacities of CMC and CMS hydrogels were approximately 400 and 450 ([g] swollen gel / [g] dry gel) respectively. Biodegradability of gel is an important factor for application of carboxymethyl cellulose (CMC), carboxymethyl starch(CMS) gel to a super water-adsorbent. Biodegradability of CMS gel was about 40% for a controlled composting period of about two weeks. Biodegradation rate of CMS hydrogel was 20 times higher than that of CMC gel, therefore, the biodegradability of blend hydrogels prepared by γ-irradiation with the mixture of CMC and CMS was investigated. Radiation crosslinking of the mixture of CMC and CMS was successfully achieved to obtain crosslinked blend hydrogels and control biodegrade rates of hydrogels by composition without change in swelling property.

Radiation-crosslinking mechanism of polysaccharide derivatives such as CMC, CMS, carboxymethyl chitosan (CMCts) is not clarified yet. Radicals in CMC formed by reaction with OH radical were studied by ESR in order to elucidate the radiation-crosslinking mechanism of CMC, CMS and CMCts. ESR spectra implied that radicals were created on carboxymethyl groups linked to C2, C3 and C6 of glucose unit in CMC, CMS. The radicals of CMCts were also created on carboxymethyl groups linked to C3 and C6 of glucosamine unit.

(4) Philippines (Ms. Charito ARANILLA TRANQUILAN, PNRI)

The Institute is developing a superwater absorbent material based on carboxymethyl-kappa-carrageenan. Previous study conducted has clearly shown the potential of these hydrogel as a superwater absorbent. Carboxymethyl-k-carrageena (CMkC) was synthesized using two-step carboxymethylation. Paste CMkC of concentration 25-30% was irradiated and crosslinked using gamma radiation at adose of 30 kGy. The hydrogels were characterized in terms of gel fraction, equilibrium degree of swelling and water retention. The dry CMkC gels were then tested for water retention in sand and in

soil and compared with the performance of commercially available SWA based on grafted starch (CSWA).

CMkC hydrogel exhibited an EDS of 100g/g while CSWA showed an EDS of 80g/g. In sand and soil system, the two hydrogels were comparable. About 10-20% water remain in the system. Preliminary investigation on pot test application on wheat grass showed a higher value on number of leaf harvest, leaf height, weight of roots and yield, compared to system without the hydrogel and system with CSWA. Repeat tests must be conducted to confirm results. Previous studies on the biodegradability of CMkC hydrogels revealed that it is 40% degraded in 2 months period.

Future plans include continuation developing the CMkC SWA, conduct more pot tests using sandy soil and optimize concentration of application and conduct actual biodegradation test.

(5) Thailand (Dr. Phiriyatorn SUWANMALA, TINT)

Lab scale

Superabsorbent was synthesized by radiation-induced graft polymerization of acrylic acid onto cassava starch. The synthetic parameters such as absorbed dose and the amount of monomer were investigated in order to determine the optimum conditions for the grafting polymerization. The criteria are emphasized by the optimum conditions of important parameters to give a maximum amount of water absorption. In addition, water retention, germination percentage and germination energy were determined in order to evaluate the possibility of superabsorbent in agricultural applications, especially in arid regions. The graft copolymer was characterized by FTIR. The results indicated that the sand mixed with 0.1%wt superabsorbent can absorb more water than the sand without superabsorbent. The germination energy of corn seeds mixed with 0.5% superabsorbent was obviously higher than those without superabsorbent. This is attributed to the fact that superabsorbent can not only absorb large amount of water but also have good water retention capacity. The experimental results showed that the superabsorbent have considerable effect on seed germination and the growth of young plant. The pot tests of *Tagetes erecta L.* with use of the superabsorbent were positive.

Field Test: Currently, preparation of superabsorbent in large scale is being done for a field test.

Future Plan: The future plan for this project is to establish cooperation with TINT's Business Development Unit in order to carry out a case study for a business potential to commercialize the super water absorbent for agricultural purposes

(6) Vietnam (Dr. Le Hai, VAEI)

Floods and droughts are the major losses to the agriculture development of many countries around the world and especially the countries in the Asia – Pacific. Today the problems of drought for crops need to apply the technical and scientific products and technologies. The super water-absorbing product produced from natural sources with polymer processing methods of radiation treatment has contributed in no small part to the target application. SWA can be prepared from cassava starch/ or other natural polysaccharides such as wooden, coir powders, carboxymethyl cellulose (CMC) with sodium/ or potassium acylate. The major characteristics of SWA are swelling degree, using time, bio-gradable, absorbability and release rate. Nowadays a large number of Vietnamese farmers have progressive ideas; they always want to apply scientific and

technological products in the agricultural sector of them. SWA needs to use very large but must be provided low cost and high quality. The selection of technological procedure and raw materials are very necessary and should be considered.

The research and application direction of super water absorbing polymers is one of the research and development program on radiation processing in Vietnam. Studies on the SWP production have been carried out in success at VINAGAMMA and at NRI. Two types of the products as GAMMA-Sorbs and Agro-gel have been produced from alkalized cassava starch grafted with acrylic acid in which have been being applied and commercialized. To meet the requirements of users on SWA products from coconut flour graft polymerization with neutralized acrylic have been studied successfully put into production line in semi-industrial scale at the Nuclear Research Institute. The SWA products in which are produced by these materials having water swelling degree are higher 200 times and dry rapidly. The cost of product is relatively low, and it is easily accepted by users.

The report presented the parameters on production technology of super water polymers, their properties, the cost and using demand of SWA products.