Current activities of biofertilizer project in Japan

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I would like to introduce the current activities of biofertilizer project in Japan after the Workshop in Bangkok in 2001.

Biofertilizer mailing list

Biofertilizer mailing list has been established for exchanging information among researchers, producers, technicians, farmers, students and people who are interested in biofertilizer in Asia proposed in the last meeting in Bangkok. This list is managed by Project manager Dr. Tsuguo Genka JAIF.

There are 73 people in the current list.

51 Japan, 7 Thailand, 5 China, 2 the Phillipines, 2 Indonesia, 1 Malaysia, 1 Vietnam, 1 Korea, 1 Taiwan, 1 Bangladesh, 1 ICRISAT.

In order to make asian networks of biofertilizer, it is necessary to ask to resistrate more than 10 people in each country.

We send BFINFOs as follows.

0001 Start of Biofertilizer Information of FNCA (02.4.22)

0002 Draft of Bio. Newsletter from Dr. Genka (02.4.22)

0003-0006 Workshop announcement from ICRISAT (02.4.22-)

0007 Database of biofertilizer mailing group (02.5.13)

0009 The 10th New Phytologist Symposium on Functional Genomics of Plant Microbe Interactions

0010 Bio. Newsletter Issue 1

0012 Meeting about BIOTEC during 17-20 July 2003 in Thailand

0013 17th World Congress of Soil Science in 2002 Bangkok

0014 Post doctoral fellow of York Univ. England

0015 Research information about NOD facter receptor

Until now, all the informations were sent from Japanese group and ICRISAT. Please feel free to send any news, comments, questions as a BFINFO to Dr. Genka.

In near future the home page of biofertilizer project FNCA should be started based on the mailing lists.

FNCA Coordinators Meeting

The meeting was held from 6-8 March 2002 in Tokyo. In this meeting the project proposal of "production and Use of Biofertilizer" which was discussed in the workshop of August 2001 in Bangkok was officially approved. Dr. Ampan Bhromsisi of Chiang Mai University Thailand was invited to present and appeal the importance of biofertilizer in agriculture.

Biofertilizer Group News Issue no.1

Biofertilizer News Issue no.1 was published in April 2002 edited by Dr. Tsuguo Genka. The main contents are :

(1) Production and use of biofertilizer
(2) Call for the personal list for biofertilizers in Asia
(3) FNCA coordinators meeting in March 2002 in Tokyo.

Delegation of Japanese BF member to Thailand

Dr. Shotaro Ando visited Thailand from March 17 to 23, 2002 supported by MEXT Japan. He reported the current status of biofertilizer researches in Thailand (Attachment 1).

Publication of Introduction of Biofertilizer project in Japan

Two papers were published to introduce Biofertilizer project of FNCA in Japan as follows:

(1) Takuji Ohyama: Atomic cooperation in Asia, Use of radioisotopes and radiation for agriculture; Biofertilizer: Agriculture in compatible with environment. Genshiryoku eye
Vol. 48, no6, p.17 (2002) *in Japanese*
(2) Takuji Ohyama; To exchange researches and technologies of the use of biofertilizers in Asia; -Start of project in FNCA-
Isotope News no.6 pp.2-9 (2002) *in Japanese*

New project member in Japan

In the last workshop in Bangkok the importance of arbuscular mycorrhizal fungi was recognaized in the project, we asked Dr. Masanori Saito, a head of Soil Ecology Laboratory, Department of Grassland Ecology, National Institute of Livestock and Grassland Science, Japan to be a member of Japanese project member. He kindly write a country report entitled “Inoculation with arbuscular mycorrhizal fungi: the statys quo in Japan” for this workshop.

Cooperation with other groups

(1) Dr. Yoshikatsu Murooka a professor of Department of Biotechnology, Graduate school of Engeneering, Osaka University, has been managed the Joint Research Project “Evalluation of nitrogen-fixing bacteria in Southeast Asia” supported by MEXT japan. The participating scientists are from Japan, Thailand, the Phillipines, Indonesia, Malaysia, and Vietnum (Attachment 2). Prof. Murooka proposed us to cooperate FNCA biofertilizer project and his project. The meeting this year will be held Kuala Lumpur, Malaysia, from 3-5 October, 2002, and Dr. Senoo and Ohyama are invited to this meeting.

(2) Dr. Rachid Serraj ICRISAT will start BNF project. He gave us a world wide information to promote biological nitrogen fixation in agriculture. The first meeting was held at Montpellier, France on June 10-14. Japanese member could not participate in this workshop, Dr. Pham van Toan a project leader of Vietnam participated. We would like to keep contact with this project.

FNCA Coordinator Visited in Niigata University

Dr. Sueo Machi, Dr. Hideo Nakasugi, and Dr. Tsuguo Genaka visited Laboratory of Plant nutrition and Fertilizers in Niigata University on 5 July. Ohyama show them field experiment and hydroponic soybean. Dr. Machi proposed the exhibition of field inoculation of rhizobium in each country to understand the promotion of nitrogen fixation for legume growth and seed yield.

Research activities by Japanese members of Biofertilizer

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(2) Institution: Faculty of Agriculture, Niigata University

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(4) Main subjects: Nitrate inhibition on soybean nodulation
Nitrogen fertilization in compatible with nitrogen fixation to promote soybean yield
(5) Recent related papers and presentation in 2001-2002:
Fujikake, H., Yashima, H., Sato T., Ohtake, N., Sueyoshi, K., Ohyama,T.: Rapid and reversible nitrate inhibition of nodule growth and N2 fixation activity in soybean (Glycin max (L.) Merr.) Soil Sci. Plant Nutr.,48 211-217 (2002)
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Ohtake,N.,Sato, T., Fujikake, H., Sueyoshi, K., Ohyama, T., Ishioka, N.S., Watanabe, S., Osa,A., Sekine, T., Matsuhashi,S., Ito, T., Mizuniwa C., Kume, T., Hashimoto, S., Uchida, H., Tsuji, A.: Rapid N transport to pods and seeds in N-defiecient soybean plants, J. Exp. Botany, 52, No355, pp277-283 (2001)
6) Experimental plan for 2002-2003:
Evaluation of promotive effect of deep placement of slow release
nitrogen fertilizer using 15N and nodulating and non-nodulating isolinesof soybean in field.

(1) Name: Tamikazu KUME
(2) Institution: Takasaki Radiation Chemistry Research Establishment, Japan Atomic

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(3) Main subjects: Utilization of radiation degraded polysaccharides for plants, Radiation pasteurization of substrate

(5) Recent related papers and presentation in 2001-2002:

N. Q. Hien, N. Nagasawa, L. X. Tham, F. Yoshii, V. H. Dang, H. Mitomo, K. Makuuchi and T. Kume: Growth-promotion of plants with depolymerized alginates by irradiation, Radiat. Phys. Chem., 59, 97-101 (2000).

L. X. Tham, N. Nagasawa, S. Matsuhashi, N. S. Ishioka, T. Ito and T. Kume: Effect of radiation-degraded chitosan on plants stressed with vanadium, Radiat. Phys. Chem., 61, 171-175 (2001).

K. Watanabe, K. Rokuhira, H. Matsuda, Y. Arima, H. Hirata, T. Kume and S. Matsuhashi: Effect of Arbuscular Mycorrhizal Fungi on Growth and Phosphorus Absorption of Soybean under the Reproductive Stage -Pot Experiment Using Available Phosphorus Accumulated Upland Soil of Andosol-, Soil Sci. Plant Nutr., 72, 793-796 (2001).

T. Kume, N. Nagasawa and F. Yoshii: Utilization of carbohydrates by radiation processing, Radiat. Phys. Chem., 63, 625-627 (2002).

(6) Experimental plan for 2002-2003:

Utilization of Low Energy Electron Beam for Plants

(1) Name: Keishi Senoo

(2) Institution: Graduate School of Agricultural and Life Sciences, The University of Tokyo

(4) email address: asenoo@mail.ecc.u-tokyo.ac.jp

(4) Main subjects: Utilization of beneficial soil mocroorganisms for crop production and bioremediation.

(5) Recent related papers and presentation in 2001-2002:

Improved survival of nutrient-starved cells of Rhizobium tropici CIAT899 in acid soil associated with high Al^{3+} and Mn^{2+} contents. Choochad Santasup, Keishi Senoo, Ampan Bhromsiri, Arawan Shutsrirung, Akiyoshi Tanaka and Hitoshi Obata, Soil Sci. Plant Nutr., 47, 559-567 (2001)

Enhanced growth and nodule occupancy of red kidney bean and soybean inoculated with soil aggregate-based inoculant: Keishi Senoo, Maki Kaneko, Rikako Taguchi, Jun Murata, Choochad Santasup, Akiyoshi Tanaka and Hitoshi Obata, Soil Sci. Plant Nutr.,48, 251-259 (2002)

(6) Experimental plan for 2002-2003:

Characterization and utilization of Al-tolerant phosphate solubilizing bacteria in Al-acidic soil.

(1) Name: Tadashi Yokoyama

(2) Institution: Faculty of Agriculture, Tokyo University of Agriculture and Technology

(3) email address: tadashiy@cc.tuat.ac.jp

(4) Main subjects: Molecular mechanism between soybean-*Bradyrhizobium* symbiosis. Classification of *Bradyrhizobium* isolated from genus *Vigna* and *Glycine* in Asia.

(5) Recent related papers and presentation in 2001-2002:

Y. Yokoyama, T. Hakoyama, Y. Shibuya, Y. Shibata, Y. Arima. Earliest response of soybean suspension-cultured cells to a lipochito-oligosaccharide(Nod factor) produced by *Bradyrhizobium japonicum*. In “Recognition and Signal Transduction in Plant-Microbe interactions.” PSJ Plant-Microbe interactions Symposium Report Vol..37, p25-34, 2001

M. Iizuka, Y. Arima, T. Yokoyama, K. Watanabe. Positive correlation between the Number of root nodule primordial and seed sugar secretion in soybean(*Glycine max* L.) seedlings inoculated with a low density of *Bradyrhizobium japonicum*. Soil Sci. Plant Nutr. Vol. 48, p219-225 2002

Transcriptional response of soybean suspension cultured cells induced by Nod factors obtained from *Bradyrhizobium japonicum* USDA110. Plant cell Physiology. 2002(submitted)

Y. Yokoyama, S. Ando. Flavonoid-specificity of NodD1 proteins in phylogenetical ly different *Bradyrhizobium* strains. Microbiology. 2002(submitted)

T. Yokoyama, T. Hakoyama, Y. Shibuya, Y. Shibata, Y. Arima. A large scale of down-regulation of mRNA transcription in soybean suspension-cultured cells triggered by Nod factor and its molecular mechanism. Plant J. 2002(submitted)

(6) Experimental plan for 2002-2003:

Classification of *Bradyrhizobium* isolated from wild-species of genus *Vigna* in Sri Lanka and Myanmar.

Isolation and characterization of genes which are specifically up-regulated in soybean cells with Nod factor.

(1) Name: Masanori SAITO
(2) Institution: National Institute of Livestock and Grassland Science
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(4) Main subjects:
Physiology and molecular biology of nutrient exchange between host plant and arbuscular mycorrhizal fungi.
Ecology of arbuscular mycorrhizal fungi in grassland and in degraded soils.
Culture collection of arbuscular mycorrhizal fungi.
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Sawaki, H. and M.Saito (2001) Expressed genes in extraradical hyphae of an arbuscular mycorrhizal fungus, Glomus intraradices in symbiotic phase. FEMS Microb. Lett. 195: 109-113.
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Saito, M. & Marumoto, T. : Inoculation with arbuscular mycorrhizal fungi: the status quo and the future prospects in Japan. Plant Soil (in press)
(6) Experimental plan for 2002-2003:
Biochemical and molecular biological analysis of phosphate related metabolism in arbuscularl mycorrhizal fungi.
Ecology of arbuscular mycorrhizal fungi with emphasis on revegetation process in degraded soils.

Inoculation with arbuscular mycorrhizal fungi: the status quo in Japan*

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*This report was prepared based upon the following article.
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Introduction

Inoculation with arbuscular mycorrhizal (AM) fungi has potential value for improved crop production, and numerous trials have been conducted since the 1970's. However, the difficulty in inoculum production due to the obligate biotrophic nature of AM fungi has been the biggest obstacle to putting inoculation into practice. At present, nevertheless, several companies all over the world have commercialized the inoculum of AM fungi (http://dmsylvia.ifas.ufl.edu/commercial.htm).

Inoculum production in Japan

In the 1940's in Japan, the growth promoting effect of endomycorrhizal fungi on plant growth was established by a pioneering scientist, T. Asai (Asai, 1943; Asai, 1944). However, his work was neglected until research on inoculum production of AM fungi boomed in the 1980's in Japan (Ogawa, 1987). In the early 1990's some companies started commercial production of inoculum of AM fungi.

The Ministry of Agriculture, Forestry and Fisheries (MAFF) of the Japanese Government has promoted the introduction of various technologies to reduce agrochemical inputs to arable lands for sustainable agriculture. MAFF recognized that AM inocula are useful to reduce phosphate fertilizer application and in 1996 approved

AM inocula as soil amendments by an ordinance of the Soil Productivity Improvement Law. The ordinance regulates the quality of 12 types of amendments such as zeolite, peat, and other organic/inorganic materials that are effective for improvement of soil productivity. The ordinance specifies that a quality guarantee be labeled on the outside of the product container. In the case of AM fungi, the following items are required; i) name and address of producer, ii) raw materials, iii) symbiotic efficiency, iv) efficacy, v) recommended application rate, vi) storage conditions, vii) expiration date. In item ii), carrier material, such as peat or zeolite, is indicated. In item iii), the symbiotic efficiency is expressed as percentage of colonization by the inoculum of a specific test plant such as Welsh onion under standard conditions. In item iv), the producer should note that the inoculum is not effective for some crops species belonging to the Brassicaceae and Chenopodiaceae, and that the inoculum may not be effective in soils rich in available phosphate. This quality guarantee is important to expel poor quality microbial inocula from the market place.

At present, three companies, Central Glass Co., Idemitsu Kosan Co., and Osaka Gas Co., produce AM inoculum. The MAFF statistics indicate that 28 to 83 tons of the inocula were supplied per year from 1997 to 1999. Much of inoculum was supplied for non-agricultural applications such as rehabilitation of degraded or devegetated landscapes. Because enduse of the inocula is not within the framework of the ordinance, the above statistics do not include the supply for such non-agricultural objectives.

Although the detailed procedure for inoculum production is proprietary, these inocula are produced under glasshouse conditions based upon the pot culture technique. One company uses expanded clay as potting medium. Others extract and concentrate the propagules of AM fungi from potting media, and carriers such as peat are mixed with the propagules. The inocula are mainly sold to horticulture farmers. Some formulations are specific for each crop species by taking into account factors such as host plant species, AM fungal species, and the handling of inoculum into horticultural practices.

The cost of inoculum production is a serious problem because the inocula are not competitive in price with phosphorus fertilizer. Even if farmers understand the significance of sustainable agricultural systems, the reduction of phosphorus inputs by using AM fungal inocula alone cannot justify the use of the inocula except in the case of high value crops. Another serious problem is control of phytopathogenic microorganisms. At present, the inoculum produced is not completely free from pathogens, even though the producers attempt to control pathogens with various agrochemicals. Farmers are very aware of the risk of pathogens, so they do not accept inoculum containing host root residues. Although pieces of root colonized with AM fungi, especially *Glomus intraradices* and related species, function well as propagules, the companies remove such residue of host roots from their products.

Rehabilitation of a volcanic deposit-affected area

Inocula of AM fungi are expected to be substantially beneficial in the establishment of vegetation in degraded or bare landscapes. Currently, in Japan, AM fungal inocula has been applied most successfully in revegetation of land by devastated by volcanic activities (Marumoto et al., 1999).

Improvement in inoculation performance

The effectiveness of AM fungal inoculation is affected by various environmental and biological factors, especially the phosphorus availability in soil and the inoculum potential of indigenous AM fungi. Field trials in Japan with a commercial inoculum indicate that the efficacy is generally highest in Andisols which show high phosphate fixing capacity (Ueda and Kubo, personal comm.). On the other hand, in soils rich in available phosphorus, the effectiveness is reduced low and the inoculation might even reduce the crop performance.

Although the effectiveness of inoculation is primarily limited by inoculum potential and P availability, there are possible ways to improve inoculation performance. The inoculation process can be divided into three stages: i) spore germination, ii) colonization, and iii) growth of extraradical hyphae and sporulation. The potential improvement of inoculum performance is discussed for each stage of this process.

Spore germination: The propagules (mainly spores) in the inoculum should be active and should immediately initiate growth after inoculation. Many chemical compounds and unidentified fractions of various extracts have been found to stimulate spore germination and hyphal extension (Ishii et al.,1997). Addition of these compounds to the inoculum may improve the germination rate and increase the colonization potential of the inoculum (Tawaraya et al., 1998).

Root colonization: *Arbuscular mycorrhizal* colonization is comprised of a series of complicated processes from recognition between the fungi and host plant to arbuscule formation. Root exudates contain compounds that promote colonization by AM fungi (Tawaraya et al., 1998). Application of such compounds at transplanting may increase inoculation performance.

Growth of extraradical hyphae and sporulation: Application of charcoal to soil stimulated the colonization of crops by indigenous AM fungi (Nishio and Okano, 1991; Ogawa et al., 1983; Saito,1990). The effect of charcoal was ascribed to its physioco-chemical properties. Charcoal is porous, weakly alkaline, and does not serve as a substrate for saprophytes. AM fungi sensitive to competition from saprophytes can easily extend their extraradical hyphae into charcoal buried in soil and sporulate in the particles (Ogawa, 1987). Charcoal particles act as a micro-habitat for AM fungi to survive and later grow into the soil, which makes charcoal suitable as a carrier of AM fungal inoculum (Ogawa, 1989). Charcoal can be applied in a large scale in infertile soils rich in indigenous AM fungi, but its cost is not competitive with inorganic fertilizers at least in Japan.

Cropping system: In arable lands, various crops are cultivated in sequence. Absence of a host plant, cultivation of non-host crops, or long fallow negatively affect the population of AM fungi in soil. On the other hand, some mycotrophic crops increase the growth of the succeeding mycotrophic crop (Arihara and Karasawa, 2000; Karasawa, et al.,2001). Irrespective of inoculation, therefore, crop sequence should be taken account into when predicting the responses of AM fungi in a sustainable agricultural system.

Future prospects

In the field application of any microbial inoculum, it is essential to verify that the inoculated microorganisms caused the plant response in field. Various molecular techniques have been developed to distinguish the inoculated strain from other indigenous strains. However, these techniques to identify AM fungi are still not routinely used because of genetic heterogeneity in AM fungi. Currently we developed a molecular marker to detect a specific isolate of *Gigaspora margarita* (Yokoyama et al. 2002). Therefore, from the standpoint of not only basic biological interest but also application, molecular genetics of these multinucleate fungi is of high research priority.

It is now well recognized that inoculation of AM fungi has a potential significance in not only sustainable crop production but also environmental conservation. However, the status quo of inoculation is far from practical technology that can be widely used in the field. Together, a basic understanding of the biology of AM fungi (i.e. Senoo et al. 2000; Solaiman and Saito 2001; Uetake et al. 2002) and an improvement in inoculum production and inoculation technology are required to advance management of these fungi.

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Tawaraya K, Watanabe S, Yoshida E, Wagatsuma T 1996 Effect of onion (*Allium cepa*) root exudates on the hyphal growth of *Gigaspora margarita*. Mycorrhiza 6, 57-59.

Tawaraya K, Hashimoto K, Wagatsuma T 1998 Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonisation by the arbuscular mycorrhizal fungus *Gigaspora margarita*. Mycorrhiza 8, 67-70.

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Attachment

Attachment 1:

Biofertilizer Researches in Thailand

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I visited Thailand from March 17 to 23, 2002 to exchange information and discuss the future collaboration using stable isotopes to develop biofertilizer researches. I would like to thank the FNCA (Forum for Nuclear Coorporation in Asia) for sending me to Thailand. In this article, I report the current status of biofertilizer researches in Thailand.

At first, I would like to introduce Soil Microbiology Research Group. This group belongs to Soil Science Division, Department of Agriculture (DOA) and it is one of the most important research groups, which work on subjects relating on biofertilizer in Thailand. Scientists in this group study on rhizobia, mycorrhizal fungi, free-living nitrogen-fixing bacteria, organic matter decomposing bacteria, and so on. They produce inoculants of rhizobia with peat for soybean, groundnut and mungbean. Recently, they developed the liquid inoculant of rhizobia. Rhizobia can be preserved more than one year without decreasing the number of bacteria at room temperature.

Discussion with researchers of DOA was very fruitful. They interested in researches using stable isotopes to develop biofertilizer researches. But, they also pointed out the difficulty of budget. Chemicals and analysis of stable isotopes are very expensive. DOA has a mass spectrometer, but they said that each researcher did not have enough budget to use it without a special project. Stable isotope is a powerful tool to study on nitrogen fixation. It can use for the estimation of nitrogen input by nitrogen fixation (Fig. 1). Thai researchers also interested in the evaluation of efficiency of nitrogen fertilizer and the estimation of nitrogen input from plant residues using stable isotope in order to know the nitrogen cycle in the field.

Recently, many kinds of new products of organic and microbial fertilizer are sold in Thailand. Some of them are popular in some region, but effectiveness of them has not been clear even though relatively high price. There are so many new products that farmer are confused. Thai researchers were stressed the importance of transfer appropriate knowledge and information of organic and microbial fertilizer to the farmers.

Fig. 1 Nitrogen fixation in sugarcane is attractive subject to study using stable isotope.

Attachment 2: A member list of Prof. Murooka’s research project.

Attachment 2: A member list of Prof. Murooka's research project

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