FUNGAL DIVERSITY USING MOLECULAR BASED TECHNIQUES; FROM FIELD TO LABORATORY

PHOSRI C[1], SUWANNASAI N[2], SIHANONTH P[3], MARTIN MP[4], JEFFRIES P[5], WHALLEY A[6], WATLING R.[7]

[1]*Biology programme, Pibulsongkram Rajabhat University, Phitsanulok, 65000, Thailand*

[2] *Department of Biology, Srinakharinwirot University, Bangkok, 10110, Thailand*

[3]*Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, 10330,*

[4]*Departamento de Micologia, Real Jardin Botanico, CSIC, Plaza de Murillo 2, 28014, Madrid, Spain*

[5]*Deparment of Biosciences, University of Kent, Canterbury, Kent, CT2 7NJ, UK.*

[6] *School of Biomolecular Sciences, Liverpool John Moores University,Liverpool, L3 3AF, UK*

[7]*Caledonian Mycological Enterprises, Crelah, 26 Blinkbonny Avenue, Edinburgh, EH4 3HU, Scotland*

ABSTRACT

Fungi play a vital role in our everyday lives being involved with most processes in the environment as well as providing an outstanding source of important products. They can also be foes of man causing diseases of plants, animals and man himself. It is also their diversity of form, as well as function, which makes their study most challenging. Fungi represent a major source for exploitation by man and with an estimated number of over 1.5 million species must be considered to be a one of the greatest untapped living very resources today. Current knowledge of fungal diversity in Thailand is limited therefore limiting our understanding of the association between tropical forest ecosystems and fungi. Arbuscular mycorrhizal fungi (AMF), Ectomycorrhizal fungi (ECM) and Xylariaceous fungi were investigated to evaluate their taxonomic. Morphological studies in combination with molecular techniques were employed. This present study provides preliminary insights into taxonomic diversity, AMF, ECM and Xylariaceous fungi status in tropical ecosystems in Thailand.

Key words: *Annulohypoxylon, Arbuscular mycorrhizas, Ectomycorrhizas, Fungal diversity, Hypoxylon, Oil palm, Taxonomy, Xylaria*

1. Arbuscular mycorrhizal fungi in oil palm:

Arbuscular mycorrhizal fungi (AMF) are abundant and ubiquitous in almost all natural communities and form associations with 80% of vascular plants (Harley & Smith 1983, Smith & Read 1997) .This is the most ancient mycorrhizal type and their history dates back to the Ordovician era at least 450 million year ago (Redecker et al. 2000). These symbiotic soil organisms are an integral component of plant communities in both natural and agricultural ecosystem. They confer numerous benefits to host plant including improved plant growth and mineral nutrition, tolerance to diseases and stresses such as drought, temperature fluctuation, metal toxicity and salinity (Borowicz 2001; Meharg & Cairney 2000). Furthermore, AMF may play a role in the formation of stable soil aggregates, building up a macroporous structure of soil that allows penetration of water and air and prevents erosion (Miller & Jastrow 1992; Jeffries et al. 2003). From all of these beneficial effects on plant performance and soil fitness, it is evident that AMF are crucial for the functioning of terrestrial ecosystems.The benefits of inoculating a wide array of agronomic plant species with AMF have been documented in numerous studies (Harley & Smith 1983; Menge & Timmer 1982; Sylvia et al. 1993).

Oil Palm (*Elaeis guineensis* (Jacq.) is an important cash crop, valued for it edible oil, following closely behind soya and rapeseed oil, and along with its use in food, it is also used in a wide array of cosmetics and pharmaceuticals and increasingly for bio-diesel production. Well established field-grown oil palm roots are usually heavily colonized by AMF (Nadarajah 1980, Blal & Gianinazzi-Pearson 1990, Blal et al. 1990) and are probably functionally dependent on them. Our survey of oil palm trees in selected oil palm growing regions in Thailand also have found high level of oil palm root colonized by AMF. However little is known regarding the species composition of AMF colonizing oil palm. In the present study a molecular approach has been conducted for detection of the presence of AMF in oil palm rhizosphere soil which was taken from the selected regions where oil palms are planted for investigation of AMF association.

Diversity of arbuscular mycorrhizal fungi (AMF) from field of oil palm collected from different localities in Thailand was studied from both spores and roots. Due to a limitation of morphological characters to define AMF in species level, molecular methods of PCR-single strand conformation polymorphism (SSCP) and nucleotide sequences were investigated. Approximately 1-5 spores of AMF were collected under stereomicroscope and then extracted for DNA. The partial region of the large subunit (LSU) rRNA gene D2 region was amplified by using polymerase chain reaction (PCR) method. DNA fragments approximately 450 bp were then cloned and at least 50 clones of each sample were collected for SSCP analysis. The results revealed different DNA polymorphism. The representative clones of each polymorphism were selected to sequences. The analysis of sequences showed that *Acaulospora tuberculata* was frequency found in every localities studied. The percent similarity of this species compared to sequences from GenBank database varied from 97-98%. In this study, the genus *Glomus* showed high variation of species recorded. There were at least 4 different species of *G. mosseae* (varied from 97-99% similarity), *G. etunicatum*, *G. versiforme* and *G. manihotis*. Moreover, some species of genus *Gigaspora* were also noted, *G. margarita* (96% similarity) and *G. rosea* (varied from 95-96% similarity). However, this partial result was important information for AMF identification found in oil palm study.

2. Ectomycorrhizal fungi diversity in deciduous dipterocarp and pine forests, Phu Khieo wildlife sanctuary, Thailand

Fungal- forming ectomycorrhizal symbioses are accounted as many as 7,000-10,000 species spanning majority in the phyla of Basidiomycota and Ascomycota. These fungi occur naturally in association with higher plants belonging to several families of angiosperms e.g. Betulaceae, Caesalpiniaceae, Dipterocarpaceae, Fagaceae, Leguminosae, Myrtaceae, Nothofagaceae Pinaceae, Ulmaceae and Salicaceae (Taylor & Alexander, 2005). It is considered that nearly 95% of wild flora is characteristically mycorrhizal symbioses.

PhuKhieo Wildlife Sanctuary (PKWS)'s geographical is located in the western part of Northeast Thailand. The Sanctuary contributes over one third to the total area

of the forest complex as well as the bulk of its prime hill evergreen forest therefore Phu Khieo's contribution to home of biodiversity. Current knowledge of fungal diversity in PhuKhieo is very poorly documented therefore limiting our understanding of the association between tropical forest ecosystems and fungi.

Ectomycorrhizal fruiting bodies and root tips collected from deciduous dipterocarp and pine forests in Phu Khieo Wildlife Sanctuary were identified based on morphological and molecular data using nucleotide sequences analysis of internal transcribed spacers (ITS) into 14 families i.e. *Amanitaceae, Boletaceae, Cantharellaceae, Clavariaceae, Cortinariaceae, Clavulinaceae, Hydnaceae, Hygrophoraceae, Gomphaceae, Russulaceae, Sclerodermataceae, Thelephoraceae, Tricholomataceae* and *Tomentellaceae* and revealed 20 genera with up to 30 species are presented. Members of *Russulaceae,* are the most species rich taxa, consisting of 8 different species. On the contrary members of the *Boletaceae* are more prevalent in term of number of genera, containing 7 different species. Moreover, two families of ECM fungi, *Sclerodermaceae* and *Tomentellaceae,* were also noted from root tips even though their fruiting bodies were not observed during this study. In the present study, using direct sequencing of the rDNA ITS regions could help to resolve the identity of most ECM fungi, including root tips, which have remained problematic, to both species and genus level. This result showed that ECM fungi constitute a considerable proportion both above ground and underground in native forests. Nevertheless, some ECM fungi were remained as undescribed species due to high variation in species level, genetic variation, and/or a limitation of nucleotide sequences in database. Noteworthy, the high ECM fungal diversity indicated the forests richness of Phu Khieo Wildlife Sanctuary that potentially support numerous of ECM fungi.

3. Annulohypoxylon & *Hypoxylon* : in case of molecular systematic study

Hypoxylon is a large and complex genus of the family *Xylariaceae* (Ascomycota). The genus has high species diversity especially in tropics and subtropics, and high variation in morphological characteristics. Several species are difficult to identify and some are uncultured. Thailand is considered as one of the tropical areas containing a high number of unknown *Xylariaceous* fungi (Whalley 1996, Rogers 2000), but there

are only a few reports on species of *Hypoxylon* and *Annulohypoxylon* studied. In Thailand, 22 and 9 taxa belonging to *Hypoxylon* and *Annulohypoxylon*, respectively and more than 10 taxa were reported as unknown (Thienhirun 1997, Suwannasai et al. 2005). The aim of this study was to investigate genetic variation within the internal transcribed spacer (ITS) region of *Annulohypoxylon* and *Hypoxylon* species found in Thailand.

Three hundred and fifty six fungal specimens of *Annulohypoxylon* and *Hypoxylon* were collected from ten different locations in Thailand for taxon identification based on their morphological and cultural characteristics. There were 7 and 26 identified taxa respectively. The ITS sequences of selected fungal specimens were investigated for resolving the problematic taxa and phylogenetic relationship within the group. Thirty fungal isolates from fourteen taxa were then PCR amplified and sequenced. The size ranged between 462 to 613 bp and GC content varied from 43.60 to 53.94%. The ITS1 region showed the highest variation among taxa, while 5.8S rRNA gene was highly conserved. The phylogenetic tree constructed using the Bayesian analyses showed clearly separated each taxon including problematic taxa. Therefore, this result confirmed the distinguishing morphological and cultural characteristics of both taxa with significant support.

REFERENCES

Blal B, Morel C, Gianinazzi-Pearson V, Fardeau JC, Gianinazzi S, 1990. Influence of vesicular-arbuscular mycorrhizae on phosphate fertilizer efficiency in two tropical acid soils planted with micropropagated oil palm (*Elaeis guineensis* Jacq.). Biology and Fertility of Soils. 9, 43-48.

Blal B, Gianinazzi-Pearson V, 1990. Interest of endomycorrhizae for the production of micropropagated oil plam clones. Agriculture, Ecosystems & Environment. 29, 39-43.

Borowicz VA. 2001. Do arbuscular mycorrhiza fungi alter plant-pathogen relations? Ecology 82: 3057-3068.

Harley JL & Smith SE. 1983. *Mycorrhizal Symbiosis*. Academic Press: London.

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil

fertility. Biology and Fertility of Soils 37: 1-16.

Meharg AA, Cairney JWG. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Advance in Ecological Research. 30: 69-112.

Menge JA, Timmer LW. 1982. Procedure for Inoculation of Plants with vesicular-arbuscular mycorrhizae in the laboratory, greenhouse, and field, p 59-68. In N.C. Schenck (ed.), Methods and Principles of Mycorrhizal Research. The American Phytopatholoical Society, St.Paul, Minnesota.

Miller RM, Jastrow JD. 1992. The application of VA mycorrhizae to ecosystem restoration and reclamation, p 438-467. In M.F. Allen (ed.) Mycorrhizal functioning. Chapman & Hall, Ltd., London, England.

Nadarajah P. 1980. Species of endogonaceae and mycorrhizal association of *Elaeis guineensis* and *Theobroma cacao*. In: Mikola, P. (Ed), Tropical mycorrhiza research. Clarendon Press, Oxford, pp 232-237.

Phosri C, Rodriduez A, Sanders I, Jeffries P. 2010. The role of mycorrhizas in more sustainable oil palm cultivation. Agriculture, Ecosystems & Environment. 135; 187-193.

Redecker D, Kodner R, Graham LE. 2000. Glomalean fungi from the Ordovician. Science 289: 1920-1921.

Rogers JD. 2000. Thoughts and musings on tropical Xylariaceae. Mycological Research. 104: 1412-1420.

Smith SE, Read DJ. 1997. Mycorrhizal Symbiosis, second ed. Academic Press, London, UK.

Suwannasai N, Rodtong S, Thienhirun S & Whalley AJS. 2005. New species and phylogenetic relationships of *Hypoxylon* species from Thailand inferred on the internal transcribed spacer regions of ribosomal nucleotide sequences. Mycotaxon. 94: 303-324

Sylvia DM, Hammond LC, Bennett JM, Hass JH, Linda SB. 1993. Field response of maize to a VAM fungus and water management. Agronomic Journal. 85: 193-198.

Taylor AF, Alexander IJ. 2005. Mycologist. 19: 102-112.

Thienhirun S. 1997. A Preliminary account of the Xylariaceae of Thailand. -PhD thesis,

Liverpool John Moores University, Liverpool.

Whalley AJS. 1996. The xylariaceous way of life. Mycological Research. 100: 879-922.

PRODUCTION OF TRANSGENIC PLANTS EXPRESSING *DIOSCOREA BATATAS* TUBER LECTIN 1 TO CONFER RESISTANCE AGAINST SUP-SUCKING PESTS

KINYA TORIYAMA
Laboratory of Environmental Plant Biotechnology, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan
E-mail: torikin@bios.tohoku.ac.jp

ABSTRACT

Dioscorea batatas tuber lectin 1 (DB1) is a storage protein isolated from yam tuber, and is shown to be a mannosebinding lectin. It shows 58% amino-acid identity to insecticidal snowdrop bulb lectin GNA, and 55% identity to Garlic leaf lectin ASAL. We demonstrated that ≥1 mg/ml DB1 in an artificial diet decreased the survival and fecundity of green peach aphid, *Myzus persicae*. The number of survival aphids was reduced to 60% in transgenic tobacco expressing cDNA of DB1 under the control of Cauliflower mosaic virus 35S promoter (35S-DB1) or phloem-specific promoter of rice sucrose synthase-1 gene (RSs1-DB1). We also produced transgenic rice (cv. Taichung 65) with 35S-DB1 and RSs1-DB1, which accumulated DB1 at a level of 2.2% and 0.33%, respectively, of total soluble protein. In our preliminary small-scale test, these transgenic rice seedlings showed resistance against brown planthopper (*Nilaparvata lugens*), white back planthopper (*Sogatella fucifera*) and small brown planthopper (*Nilaparvata lugens*). Our results indicate that DB1 can be used to enhance resistance to sap-sucking pests in transgenic crops.

Keywords *: Dioscorea batatas tuber lectin 1, insect resistance, planthopper, transgenic rice*

INTRODUCTION

Dioscorea batatas tuber lectin 1, DB1, has been isolated from yam tuber, *Dioscorea batatas* Decne., as a storage protein. DB1 is a mannose-binding lectin (23 kDa) consisting of identical 12-kDa subunits. It has 58% amino-acid identity to snowdrop lectin GNA and is classified into the GNA-related lectin family (Gaidamashvili et al. 2004). The insecticidal properties of DB1 have been reported against moth larvae (*Helicoverpa armigera*). The rate of adults emerging from pupae has been reduced to 33%, when fed on 0.01% (w/v) DB1 in an artificial diet (Ohizumi et al. 2009). We also demonstrated that ≥1 mg/ml DB1 in an artificial diet significantly decreased the survival and fecundity of green peach aphid, *Myzus persicae* (Kato et al. 2010). The number of survival aphids was reduced to 60% in transgenic tobacco expressing cDNA of DB1 under the control of Cauliflower mosaic virus 35S promoter (35S-DB1) or phloem-specific promoter of rice sucrose synthase-1 gene (RSs1-DB1). Our results indicate that *DB1* can be used to enhance resistance to sap-sucking insects in transgenic crops. Here we report the production of transgenic rice expressing DB1.

MATERIALS AND METHODS

The cDNA covering full-length ORF (accession no. AB513659) was PCR cloned into pGEM T-vectors (Promega, Madison, USA) using KOD+ polymerase (Toyobo, Osaka, Japan) and primers XB/NDB1 F 5'-TCTAGAGGATCCATGGCTAACCCAGGAGCA-3' (*Xba* I and *Bam* HI sites are underlined) and S/DB1 R 5'-GAGCTCTCACTTGTTGACGACC-3' (*Sac* I site is underlined). For phloem-specific expression of DB1, the rice sucrose synthase-1 (RSs1) promoter (accession no. AJ401233) was employed as described (Shi et al. 1994). The promoter sequence (3 kb) was amplified from rice cv. Taichung 65 using KOD+ polymerase and specific primers Sal/RSs1 F 5'-GTCGACCTTTCGTGACTTGTTTTCGC-3' (Sal I site is underlined) and Bam/RSs1_R 5'-GGATCCTAGCTTGGCAGCCAT-3' (*Bam* HI site is underlined), and was subcloned into pGEM-T vector. Then RSs1 promoter was inserted into *Sal* I/*Bam* H I sites of

pBI101H, which contained the hygromycin resistance cassette. The resulting construct was named RSs1-DB1 (Kato et al. 2010). The construct was transfered into *Agrobacterium tumefaciens* strain EHA105.

Transformation of rice (*Oryza sativa* L. cv. Taichung 65) was carried out by the method of *Agrobacterium*-mediated transformation. Taichung 65 is a japonica rice cultivar that originated in Taipei, Taiwan. The transformants were selected on a medium containing 30 mg/l hygromycin and 40 mg/l meropen (Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan). The introduced DB1 was detected by PCR analysis using the following primers; DB1-F1 primer 5'-CAGAATGACTGCAACCTGGT-3', and DB1-R2 primer 5'-ACCAAAGATGGTGGCCTTAC-3' with annealing temperature at 57^0C. For southern blot analysis, genomic DNA was digested with *BamH*I, which cut once within the T-DNA, and probed with DIG-labeled DB1 cDNA. The DB1 concentration in the total soluble protein in leaves was determined by comparing the intensity of bands reacted with anti-DB1 polyclonal antiserum with those of series of known amounts of purified DB1, as described previously (Kato et al. 2010).

Evaluation of planthopper resistance was carried out at Life Science Research Institute, Kumiai Chemical Industry Co., Ltd. (Shizuoka, Japan). T1 seedlings at 5th leaf stage were infested separately with 5 female adults of small brown planthopper (*Nilaparvata lugens*), brown planthopper (*Nilaparvata lugens*), or white back planthopper (*Sogatella fucifera*). All the female adults were removed after 5 days. For brown planthopper, additional 5 females were supplemented at the 20^{th} day. The numbers of nymphs and adults were counted daily. A transgenic plant with GUS driven by RSs1 promoter (RSs1-GUS) was used as a negative control.

RESULTS

We obtained three transgenic lines for 35S-DB1 and two lines for RSs1. The accumulated DB1 in leaves was detected using western blot analysis. An approximately 12-kd band corresponding to mature DB1 monomer was detected at the same position as that of standard DB1 purified form yam tuber, indicating the proper processing of 16-kd DB1 premature protein in transgenic rice. In the case of transgenic lines with 35S-DB1, DB1 accumulated at a level of 2.2% of total soluble protein in plant no. 35S-2, 1.3% in no. 35S-6 and 0.4% in no. 35S-9. In the case of transgenic lines with

RSs1-DB1, DB1 accumulated at a level of 0.36% in plant no. RSs1-3 and 0.33% in no. RSs1-2.

A plant carrying a single copy of the integrated DB1 was selected based on the detection of a single band on Southern blot analysis. Consequently the plant nos. 35S-2 and RSs1-2 were selected. The segregation ratio of PCR positive plants and negative plants in the selfed progeny was 39:10 for 35S-2 and 5:3 for RSs1-2, which fitted to a theoretical ratio of 3:1 expected from a single copy integration.

The PCR positive plants were used for the evaluation of resistance against small brown planthopper, brown planthopper, or white back planthopper. We have tested only two plants for each, and present here the champion data in Table 1. The numbers of nymphs and adults were greatly reduced when fed on the transgenic plants with 35S-DB1 and RSs1-DB1, but not on transgenic plant with RSs1-GUS (negative control).

Table 1 Number of nymphs and adults after fed on transgenic T_1 plants. Five female adults were released at day 0 and the numbers counted on the day in parenthesis are shown.

	Number of nymphs and adults		
Line	35S-DB1	RSs1-DB1	RSs1-GUS
Amount of DB1*	2.2%	0.33%	0%
Small brown planthopper (28th day)	12	8	38
Brown planthopper (34th day)**	1	4	14
White back planthopper (34the day)	1	0	40

* Amount of DB1 protein per total soluble protein in leaves of T_0 generation.

** Five female adults were supplemented at the 20th day.

Discussion

DB1 was accumulated in 35S-DB1 and RSs1-DB1 lines at almost identical levels to those of previously reported for garlic leaf lectin ASAL accumulation in transgenic rice (Saha et al. 2006; Yarashi et 2008). Accumulation ASAL has been shown to confer substantial resistance to brown planthopper, green rice planthopper, and whitebacked planthopper, in terms of increased insect mortality, retarded development and decreasing fecundity. Recently, virus resistance has been also reported in transgenic rice expressing ASAL under the control of RSs1 promoter (Saha et al. 2006). Acquisition of resistance against planthoppers and rice tungro virus is expected in the transgenic rice plants expressing DB1. Our preliminary small scale test indicated that both 35S-DB1 and RSs1-DB1 lines exhibited high-level resistance against small brown planthopper, brown planthopper and white back planthopper, although we need to repeat the experiment in a large scale to have definite conclusion.

RSs1 promoter has also been shown to direct phloem-specific expression of beta-glucuronidase and GNA in transgenic tobacco (Shi et al. 1994). RSs1 promoter has also been successfully used to drive garlic lectin, ASAL, in transgenic rice (Saha et al. 2006). Our current study indicated that the level of planthopper resistance is the same between the RSs1-DB1 and 35S-DB1 lines, although the amount of DB1 per soluble protein extracted from leaves of the RSs1-DB1 line was approximately one-seventh of that in the 35S-DB1 line, suggesting that the amount of DB1 in phloem might be almost identical. RSs1 promoter has the advantage of maximizing expression of the insecticidal protein at the site of attack by sap-sucking insects, while minimizing it elsewhere in plants.

The plant no. RSs1-2 homozygous for the introduced RSs1-DB1 was propagated. Evaluation of planthopper resistance in a large scale is now in progress using the T3 generation of the RSs1-DB1 line (RSs1-2) in collaboration with Dr. Nono Carsono, Padjadjaran University, and Dr. M. Herman, Indonesia Centre for Agriculture Biotechnology and Genetic Resources (BB Biogen, Bogor).

ACKNOWLEDGEMENTS

I thank Drs. Masaaki Komatsu and Tsutomu Shimizu at Life Science Research Institute, Kumiai Chemical Industry Co., Ltd. for evaluating planthopper resistance.

REFERENCES

Kato T, Hori M, Ogawa T, Muramoto K, Toriyama K (2010) Expression of gene for *Dioscorea batatas* tuber lectin 1 in transgenic tobacco confers resistance to green-peach aphid. *Plant Biotech* 27: 141-145

Gaidamashvili M, Ohizumi Y, Iijima S, Takayama T, Ogawa T, Muramoto K (2004) Characterization of yam tuber storage proteins from *Dioscorea batatas* exhibiting unique lectin activities. J Biol Chem 279: 26028-26035

Ohizumi Y, Gaidamashvili M, Ohwada S, Matsuda K, Kominami J, Nakamura-Tsuruta S, Hirabayashi J, Naganuma T, Ogawa T, Muramoto K (2009) Mannose-binding lectin from yam (*Dioscorea batatas*) tubers with insecticidal properties against *Helicoverpa armigera* (Lepidoptera: Noctuidae) J Agric Food Chem 57: 2896-2902

Saha P, Majumder P, Dutta I, Ray T, Roy SC, Das S (2006) Transgenic rice expressing *Allium sativum* leaf lectin with enhanced resistance against sap-sucking insect pests. *Planta* 223: 1329-1343

Shi Y, Wang MB, Power KS, Damme EV, Hilder VA, Gahehouse AMR, Boulter D, Gatehouse JA (1994) Use of the rice sucrose synthase-1 promoter to direct phloem-specific expression of β-glucuronidase and snowdrop lectin genes in transgenic tobacco plants. J Exp Bot 45: 623-631

Yarasi B, Sadumpati V, Immanni CP, Vudem DR, Khareedu VR (2008) Transgenic rice expressing *Allium sativum* leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. *BMC Plant Biology* 8: 102

EMBRYO TRANSFER IN CATTLE

SITI DARODJAH RASAD
Animal Husbandry Faculty, Padjadjaran Universiti
Jl. Raya Bandung-Sumedang Km 21, Jatinangor

INTRODUCTION

Embryo transfer is a biotechnology in animal reproduction by which embryos are collected from a donor of female, and then it is transferred into recipient of another female, which serve as surrogate mother for the remainder of pregnancy. Embryo transfer techniques have been applied almost to every species of domestic animal and to many species of wildlife and exotic animals. Within the past decade, the degree of embryo transfer procedures has evolved to permit complete utilization of non-surgical procedures in the cows, long term embryo culturing and storage in cryopreservation, and more recent micromanipulation and many new techniques associated with genetic engineering. This is a rapid development of science with a very short lag-time between discovery and application. The potential applications continue to increase with the development of newer technology.

For several decades, the use of artificial insemination (AI) has shown the genetic progress, and it has developed quite fast with the utilization of frozen semen. In the AI program, the genetic contribution is mainly from the male because only one calf per year is resulted from a female.

As embryo transfer (ET) has been developed, a female can give more calves so that it results in more genetic progress improvement as a complement of AI program.

The success of ET technique is affected by :

1. Donor, as a producer of embryo transferable
2. Recepient, should have high pregnancy rate and consistent
3. Procedure, schedule, technique, and tools
4. Human resources, have to be competent

The major pursuit in Indonesia in term of milk consumption are to increase of milk production. Related to Embryo Transfer, that program could be increased the

milk production by upgrading of dairy cattle performance. That could be as a big chance to increase contribution of domestic milk production (current domestic production still 30%).

The first successful Embryo Transfer took place in England in the 1890's by a fellow named Walter Heap, his subjects were rabbits. Although that was a success, Embryo Transfer has not been applied commercially until the arrival of the hormone FSH, which stands for Follicle Stimulating Hormone, which occurred in the 1950's. At first the only technique was surgical to both flush and implant the embryos. While these methods were successful they were very expensive, required a large setup, and a lot of experience.

II. History of ET technique in Indonesia

Bilton was successfully applied freezing embryo early of 1980, and more than 10.000 calves from ET were born in the USA in 1989. At that time, ET technique was then applied in developing countries including Indonesia. In 1984, ET program has been applied in Indonesia based on supported of minister of Cooperation Indonesia, Mr. Bustanul Arifin. This program was located in Cicurug Cattle Farm, Sukabumi. At the time, the application of the program was under the coordination of ET team from the USA (Granada Texas) used the surgery technique on *fossa para lumbal* of Cattle.

In 1984 there was Embryo Transfer program especially for Beef Cattle, through collaboration between PT. Berdikari Unietd Livestock (PT. BULI) and Indonesian Institute of Sciences (LIPI). PT. BULI was established in 1972 as a joint venture company between local company, PT. BILA and PT. United Livestock (USA company). In 1975, the share of both companies was acquired by the government under control of BULOG (National Logistic Body); and then in 1984, was wholly acquired by PT. PP Berdikari and rename into PT. Berdikari United Livestock. The cattle breeding are done through either natural or artificial insemination with the objective to produce a high quality calves and increasing level of birth rate.

III. DESCRIPTION OF EMBRYO AND EMBRYO TRANSFER

What is the embryo ?

Embryo is egg that has already been fertilized by spermtozoa and earliest stage of development of the organism Embryo Transfer is the activities involves the removal of an embryo from a female of superior genetics as a donor animal and the placement of the embryo into the reproductive tract of a female of average genetics as a recipient animals. Basically, multiple injections of hormone to stimulate and multiply the ovulations in the cow that you want to get the embryos from. Then:

- The donor cow is inseminated at normal time but 12 hours apart and 3-4 times
- Seven (7) days later the rinsing out of the uterus to extract the embryos and ova (unfertilised, fertilised or degenerate)
- Isolation of the good embryos using a microscope and then transfer into the recipient cows or frozen.

Goal of the Embryo Transfer is to obtain the maximum number of genetically superior embryos in a minimum amount of time.

Benefit of The Embryo Transfer

Basically it multiplies the offspring of the farmers best animals. Farmers can use their best bulls over their best cow or heifer and get a good calf whereas now the farmer can run an embryo program and possibly get a life times' production with one flush.

It could be explain the benefit of the Embryo Transfer, included :

- Traditionally, cows produce only one calf per year. ET allows the production of any offspring within a year from a single cow.
- increase the genetic potential of a herd in a relatively short period of time.
- increase milk production in dairy herds.
- increase weaning weights in beef and dairy herds.
- frozen embryos can be shipped almost anywhere.
- preserves superior genetics for future generations due to embryo freezing

IV. Procedures of Embryo Transfer Technology

4.1. Donor Management

Donor Selection

There are several points should be considered in donor selection:

- Genetic value is strongly essential related the ability of transfering the good character values
- Must be based on:
    - Genetic superiority
    - Reproductive ability
    - Market or economic value of the offspring

In term of genetic superiority selection, should be based on:

- Maternal breeding value
- Yearling breeding value
- Weaning breeding value
- High milk production (for dairy cattle)
- Body conformation score

Animal donor health

The criteria of female donor candidate should be absolutely healthy because if the health condition is down, it will affect the super ovulation process negatively as the result of the decrease of reproductive condition. Therefore, female donor must be healthy. In order to assess it, some works can be done as follow:

1. Blood test
2. Vaccination
3. Rectal palpation assessment to know the normal condition of reproductive organ

Animal donor feed

There is a positive correlation between body condition and feed condition. Poor feed consumed will reduce the fertility level. Thus, there wil need good quality of feeds in order to support ptimum performance of donor.

Estrus cycle of donor

One of the key success of ET is estrus detection where this sould be done precisely. The

length of estrus cycle should be regularly normal because if it is abnormal will have a negative effect on superovulation process.

Estrus detection is better to be done on two consecutive estrus cycle and commonly it is done in the morning (06.00 a.m.) and the afternoon (06.00 p.m.). In doing estrus detection, it should be avoided abnormality of estrus cycle such as silent heat case.

4.2 Recipient management

Recipient selection

Ideal recipients are females who meet criterias as follow:

1. Diseases free, especially reproductive disease
2. Good fertility
3. Well Mothering ability
4. No distocia symptoms

Crossbreeding does not have a negative influence on fertility. Crosbreeding can improve the level of fertility to be better.

Animal recipient health

Recipient candidate must face several assessments on points as follow:

1. Health status
2. Reproduction status
3. Applied quarantine system
4. Every day routine check on diseases symptoms, body temperature condition. This should be done because they can affect the fertility, fail to do will lead to abortion case

4.3 Donor and recipient managements

Estrus detection

Estrus sinchronization should be done in both donor and recipient appropriately since this will support the ET program achievement.

Also, visual observation is the main factor that should be taken into account. This can be done through:

a. The observation of estrus detection Post AI in the morning and the afternoon as long as 30 minutes (approximately)
b. ET technique should be done on the right time in line with the appearance of estrus symptoms that will result in synchronizatio estrus grade.
c. ET technique should be done on the right time in line with the appearance of estrus symptoms that will result in synchronization estrus grade
d. The observation intensity should be done one day before and after estrus, and every day, estrus observation should be executed at 06.00 am; 10.00 am; 14.00 pm; and 22.00 pm.
e. The application of estrus detection should be done carefully and precisely since the estrus synchronization procedures between recepient and donor has a significant effect on the success of ET.
f. Research shows that the success rate of ET will be better if the recepient get estrus in one day of donor.

V. Embryo Recovery

Current ET procedures for embryo recovery or flushing is generally accomplished through non-surgical techniques at approximately seven days after breeding. The recovery process is relatively simple and can be completed in well under an hour. This process requires specific instrumentation and training.

Initially, the donor is given an epidural block at the tailhead to prevent straining. A flexible rubber tube catheter is passed through the cervix and into the body of the uterus. The cuff is inflated with saline solution to hold the catheter in place and to prevent backflow of fluids. Saline solution is flushed into the uterine horns through holes at the tip of the catheter that precede the cuff. The solution-filled uterine horn is gently massaged and the fluid containing the embryos is drawn back out through the catheter. This solution is collected through a filter and into a cylinder or dish. Embryos are then located retrieved by examination under a microscope.

VI . Embryo Evaluation and Processing

Upon collection, embryos are evaluated under a microscope for stage of development and quality of the embryo. Embryos are collected on day six to eight after breeding and are usually in the morula through blastocyst stage. It should be noted that the visual evaluation of embryos is a subjective evaluation and is not an exact science. The following standardized coding system (Table 1) is recognized by the International ET Society, Savoy, Illinois.

Table 1. Stages of Embryo Development

Stage	Description
1	Unfertilized
2	2- to 12-cell
3	Early Morula
4	Morula
5	Early Blastocyst
6	Blastocyst
7	Expanded Blastocyst
8	Hatched Blastocyst
9	Expanded Hatched Blastocyst

Obviously, the higher the stage, the more developed the embryo. A second scale of quality indicator is that of quality grades (Table 2).

Table 2. Embryo Quality Grades

Grade	Description
1	Excellent or Good. Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, color, and density. This embryo is consistent with its expected stage of development. Structural Irregularities should be relatively minor, and at least 85% of the cellular material should be

	intact, viable embryonic mass.
2	Fair. Moderate irregularities in overall shape of the embryonic mass or in size, color and density of individual cells. At least 50% of the cellular material should be an intact, viable embryonic mass.
3	Poor. Major irregularities in shape of the embryonic mass or in size, color and density of individual cells. At least 25% of the cellular material should be an intact, viable embryonic mass.
4	Dead or degenerating. Degenerating embryos, oocytes or 1-cell embryos. These embryos are non-viable.

Embryos of appropriate quality (1 or 2 preferably) can be transferred directly to recipient cows or frozen for future use.

VII. Summary

The techniques involved with embryo transfer are not extremely difficult or confusing. With sufficient training and experience embryo transfer can be a very viable method for genetic improvement or manipulation within any situation. As the science behind bovine embryo transfer progresses, applications for its use will continue to become apparent in the laboratory, university, large commercial enterprise, and even the individual operation.

UNDERUTILIZED CROPS: DIVERSITY, CONSERVATION AND UTILIZATION

AGUNG KARUNIAWAN
(Plant Breeder and Plant Genetic Resources Specialist on legume and tuber crops)
Laboratory of Plant Breeding, Faculty of Agriculture
Padjadjaran University Bandung Indonesia
http://www.unpad.ac.id
Email: akaruni1@unpad.ac.id

OVERVIEW OF UNDERUTILIZED CROPS DEFINITION

In the world, there are 3 plants which are corn, wheat, and rice, which is accounted for 40% to meet the needs of the world of calories and protein. Currently, 95% of world foodstuff is only utilizing 30 species of plants, whereas for starch content is estimated there are approximately 7500 edible species. The possibility of this amount comes from Underutilized Crops, which is only use on a small scale.

Underutilized Crops are species of plants used in minority and locally as food, fiber, oil and medicine, but have not in cultivated on a large scale yet. This plant has great potential to contribute to food security, nutrition, health, as well as to improve environmental condition. One other advantage of Underutilized Crops are usually able to adapt on marginal soil and varied climatic conditions. The advantages and limitations info of underutilized crops gain worldwide attention so that the association was formed on underutilized crops named ICUC (International Centre for underutilized Crops). The organization is based in Colombo, Sri Lanka.

According to ICUC (2006), Underutilized Crops are local species locally used as food, fiber, fodder, oil or medicine in a region, but many of which are ignored by scientists and entrepreneurs to be developed. These plants have a risk of "useless" if it remains disregarded, while many of these species have an important advantage as food security resource, cultural and income for the poor. Those also have great potential for small-medium entrepreneurs' income. Unfortunately, the least attention to these species has potential to genetic erosion, until eventually lead to the extinction of species.

Therefore, ICUC mission is to promote Underutilized Crops to the world community to be developed furthermore (ICUC, 2006)

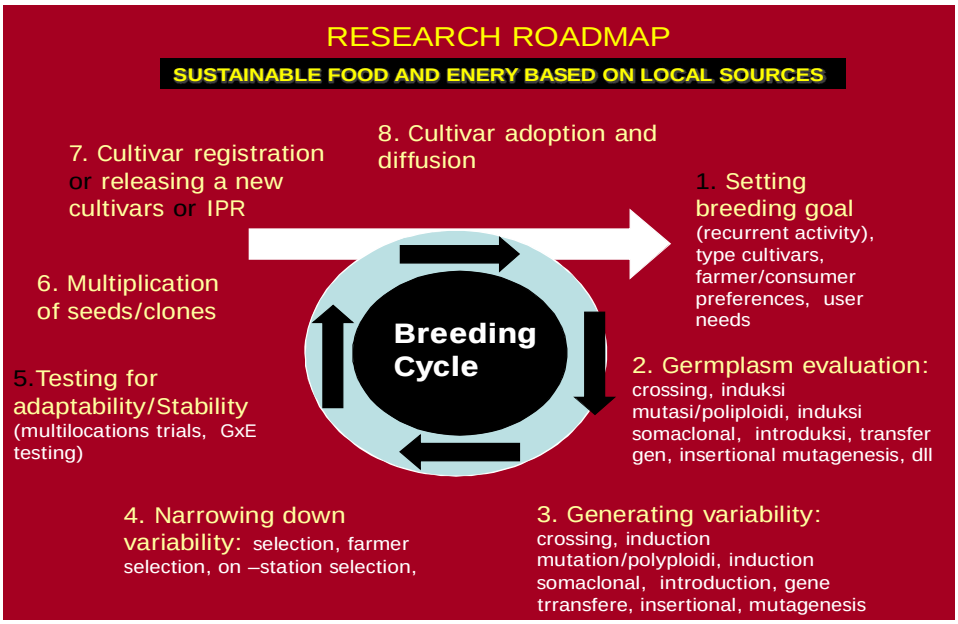
PLANT BREEDING AND THE ROLE ON UNDERUTILIZED CROPS

In accordance with the mission of ICUC, plant breeding with PGR (Plant Genetic Resources) field studies, playing a role in research towards "conservation and utilization of germplasm" is needed. Germplasm becomes particularly important as gene banks for future development of plant breeding. As cultivated plants have many limitations on human needs also limited yield, thus preserved and developed underutilized crops are expected to meet these needs. Limitations in this term are nutrition, adaptability, pest resistance, secondary metabolites as a medicine that are not owned in cultivated plants but abundantly in underutilized crops.

Plant breeding has some stages on developing a plant species to be a cultivar of superior variety. The stages include:

1. Collection
2. Characterization
3. Elders selection
4. Hybridization either conventional or biotechnology
5. Proceed with yield test and release of the variety / cultivar

then the plants commonly called neglected or Underutilized Crops will primary contribute to the assembly from the collection phase, so that the breeders will have a wide variation in the elders selection phase to get the desired character.



Adopted from Ruswandi (2010)

UNDERUTILIZED CROPS RESEARCH PROGRESS AT PADJADJARAN UNIVERSITY BANDUNG

Plant Breeding UNPAD coordinated by Dr. Agung Karuniawan as head of Plant Breeding Laboratory UNPAD have a large collection of germplasm, including the crop categorized as underutilized crops. Until 2010, it has reached approximately hundreds of accessions maintained by SEED CENTER UNPAD which is continuously studied involving undergraduate and graduate students. Collected accessions include; legumes (cowpea, green bean, red bean, chickpea, lentils sword, surly peanut, roay bean, long bean, soybean, winged bean, peanut), bulbs and tubers (sweet potato, taro, yam, yam bean, arrowroot, canna, suweg, black potatoes, dahlias) and other horticultural plants such as orchids (tiger orchids), water spinach, and the possibility for others crop germplasm to be developed. These accessions were obtained from various parts of Indonesin regions as well as from abroad. Locations of origin of the crops are also varied, some from forest, agricultural landscape, until traditional markets also modern supermarkets (as references).

Underutilized crops germplasm in UNPAD for sweet potatoes and minor tubers are: Wild yam, local sweet potato, taro family, suweg / Acung / porang / Iles-iles which are family of Araceae genus of *Amorphopallus*, there are also gembili,

Uwi, gadung from Dioscorea genus and are now being developed some black potato accessions (*Solanostemon roduntifolius*). Besides, there are canna, and arrowroot / sago which are constantly developed to achieve the nutrition information.

Researches on legume crops still being developed are Mucuna, Winged Bean, Sword Bean Koro (Cannavalia), and some time ago had been developed at prolonged period accessions of genus Vigna

Although exploration, collection, and characterization have not yet entered the core of plant breeding activities, but these are initial parts for breeding development or called pre-breeding. This activity continues until today in order to save and utilize the germplasm as well as supporting Indonesia food security which was pioneered looked like scavengers collecting "junk" but later it is going to be the future plant.

No	**Collections**	**Indonesian Name**	**Number of Collected Accessions**
1	Sword beans (*Canavalia* spp.)	Kacang Koro Pedang	15
2	Cowpea (*Vigna unguiculata* var unguiculata	Kacang Tunggak	61
3	Rice bean (*Vigna umbelata*)	Kacang Nasi	64
4	Kidney/Red bean (*Phaseolus vulgaris*)	Kacang Merah	23
5	Surly peanut (*Mucuna spp*)	Kacang Benguk	23
6	French Bean (*Phaseolus vulgaris*)	Kacang Buncis	35
7	Winged bean (*Psophocarpus tetragonolubus*)	Kecipir	30
8	Long bean (*Vigna unguiculata ssp. sesquipedalis*)	Kacang Panjang	23
9	Black soybean (*Glycine soja*)	Kacang Kedelai Hitam	7
10	Peanut (*Arachis hypogea*)	Kacang Tanah	68
11	Lablab beans (*Dolichos lablab*)	Kacang Roay	24

12	Sweet potato (*Ipomoea batatas*)	Ubi Jalar	41
13	Wild sweet potato (*I. trifida, I. triloba*)	Ubi Liar	200
14	'Kimpul" Taro (*Xanthosoma spp*)	Talas Kimpul	17
15	"Bentul" Taro (*Calocasia spp*)	Talas Bentul	33
16	*Canna edulis*	ganyong	25
17	Arrowroots (*Marantha arundinacea*)	Garut	12
18	Amorphophalus ("Suweg")	Suweg	51
19	Black potato (*Coleus/Solenostemon rotundifolius*)	Kentang Hitam	18
20	Yam bean (*Phachyrhizus spp*)	Bengkuang	35

RESEARCH PROGRESS OF IPOMOEA AND TARO FAMILY (AS EXAMPLES)

Research Objectives of Utilization of relatives of Wild Sweet Potatoes in Sweet Potatoes breeding:

1. Selecting the wild relatives candidates which have important traits such biotic and abiotic stress resistances, and short maturity
2. Breeding sweet potato new varieties with high yielding and appearance as consumer preferences.

Achievements up to October 2010:

1. Collection garden of 41 sweet potato accessions (elders)
2. Complete description based on the CIP / IPBGR of 41 sweet potato accessions (elders)
3. Collection garden of 245 accessions of Japan sweet potato F1 crosses
4. Collection garden of 265 accessions of Japan sweet potato F1 crosses
5. Collection garden of 200 accessions of wild sweet potatoes
6. Collection garden of 78 new Japan sweet potato with good shape based on consumer preference
7. Collection garden of 9 new Japan sweet potato for starch material
8. Collection garden of 7 new Cilembu sweet potato with good shape based on

consumer preference

9. Collection garden of 50 wild sweet potato accessions with good appearance
10. Description of 245 Japan sweet potato accessions F1 crosses
11. Description of 265 Japan sweet potato accessions F1 crosses
12. Description of 200 wild sweet potato accessions
13. Published papers at PERIPI National Seminar 2009
14. Published papers at IJJSS International Seminar 2010
15. Published papers at International Seminar in Hanoi 2010
16. Publication of local and national printed media 2010

1. ROAD MAP OF SWEET POTATO BREEDING (*Ipomoea batatas, I. trifida, dan I.triloba)*

Quarter IV	*Activities:* Cultivation and characterization planting season III	*Activities:* - Crossbreeding between cultivated sweet potato - Preliminary morphology (stems, leaves, flowers) characterization of wild sweet potato relatives	*Activities:* - Evaluation of selected sweet potato F1 - Propagation by stem cuttings-season II of wild sweetpotato relatives - Evaluation of outcrossing level	*Activities:* - Karyotype analysis of cultivated sweet-potato, F1, wild sweetpotato relatives - Selection of elders with appropriate outcrossi	*Activities:* Evaluation of F1 interspecific crosses of bacterial wilt disease resistance character	*Activities:* Registration of HKI PVT / IPR PVP

				ng level and bacterial wilt resistant		
	Output: - Complete description based CIP/IPBGR descriptor list for leaf, stem, flower and tuber - Published paper	*Output:* - Sweet potato crosses plants - Database of morphology characters for wild sweet potato relatives - Published paper	*Output:* - Obtained description - Collection garden of season III for wild sweet potato relatives - Published paper - Information on the compatibility level of wild and cultivated sweet potato relatives	*Output:* - Description database for cytology characters - Published paper	*Output:* Compatibility-parameter data (number of seeds, percentage of capsule death, etc)	*Output:* PVP certificate
Quarter III	*Activities :* Cultivation and characterization planting season II	*Activities:* - Evaluation of cultivated sweet potato - Maintaining,	*Activities:* - F1 selection - Analysis of morphological characterization (stems, flowers,	*Activities:* - Characterization of cytology (ploidi level) wild	*Activities:* - Interspecific crosses dialel model 2 according to Griffith - interspecific crosses of	*Activities:* - Test of multi-locations hybrid plants - Test of multi-locations from F1 interspecific

		fertilizing and weeding	leaves) season I - Planting seeds of wild sweetpotato relatives	relatives and F1 - Selection of elders with appropriate outcrossing level and bacterial wilt resistant	bacterial wilt disease resistance character	crosses results for bacterial wilt disease resistance character
	Output: : Complete description based CIP/IPBGR descriptor list for leaf, stem, flower and tuber	*Output:* - Obtained several accessions with superior character based on morphology, ploidi, nutrition - collection cutting garden for next plant material	*Output:* - Sweet potato with high yielding, early maturing age - Database of description, dendogram, genetic diversity - published paper	*Output:* Database of cytology character description	*Output:* Resulted F1	*Output:* - Obtained new varieties that have high tuber yield in various regions and specific areas - published paper
Quarter	*Activities:* Cultivation	*Activities:* - Nutritional	*Activities:* - F1	*Activities:* - Registrat	*Activities:* - Planting	*Activities:* - Analysis /

II	and characterization planting season I	analysis of sweet potato tuber - Collect accessions of wild sweet potato relatives	characterization - Planting seeds (I) - Harvesting seeds from the collection plot	ion of New Varieties - Evaluation and analysis of morphological characterization planting season II - Selection of elders with appropriate outcrossing level and bacterial wilt resistant	elders wild relatives and selected cultivated-sweet potato - Planting elders with appropriate outcrossing level and bacterial wilt resistant	evaluation of special characters in F1 (Crosses) - Propagation of F1 interspecific crosses of bacterial wilt disease resistance character
	Output: - Complete description based CIP/IPBGR	*Output:* - Description of complete nutritional - planting 207	*Output:* - Description of F1 sweet potato F1 - Plot experiments for planting	*Output:* - Registered new varieties of sweet-potato	*Output:* collection garden of elders as crosses material	*Output:* Obtained hybrid plants with new characters

	descriptor list for leaf, stem, flower and tuber - Published paper	accessions in experiment field	season I	UNPAD - Data base description of character for planting season II		
Quarter I	*Activitie:* Exploration and collection of sweet potatoes in West Java	*Activities:* - Analysis of Ploidi-Level of cultivated sweet potato - Exploration of wild sweet potato	*Activitie:* - F1 planting test - Propagation by stem cuttings - Study of outcrossing wild sweet potato - Evaluation of early maturating age character and resistance to diseases caused by Fusarium	*Activities*: - Propagation of F1 sweet potato - Planting seedlings II wild sweet potato relatives - Evaluation of resistance to bacterial wilt diseases for elders	*Activities:* - Selection of elders from wild relatives and cultivated sweet potato - Selection of elders elders with appropriate outcrossing level and bacterial wilt resistant character	*Activities:* - Analysis of seed variability from crosses (F1) - Analysis of interspecific crosses F1 with bacterial wilt disease resistance character
	Output: Gained 41	*Output:* - Descriptio	*Output:* - F1 field	*Output:* - F1 sweet	*Output:* Selected some	*Output:* Obtained F1

	accesions from West Java	n of ploidi character - 207 accessions of wild sweet potato relatives	experiment - Seeds of wild relatives - Information about outcrossing level of wild sweet potato - Information of early maturating age character and resistance to diseases caused by Fusarium	potato collectio n garden - Experim ent plot of wild sweet potato relatives for planting season II	accessions of sweet potato for crossing	variability value
	Year 1 (2009)	*Year 2 (2010)*	*Year 3 (2011)*	*Year 4 (2012)*	*Year 5 (2013)*	*Year 6 (2014)*

Research Objectives of “Kimpul” Taro and “Bentul” Taro:

To obtain new varieties of “kimpul” taro and “bentul” taro that have high yield and good nutrition

Achievements up to October 2010:

1. Collection garden of 50 Accessions “bentul” taro and “kimpul” taro with 3 Design Field Experiment
2. Complete description based on IPBGR for 50 accessions of “bentul” taro and “kimpul” taro
3. Tuber nutrient data of 50 accessions of “bentul” taro and “kimpul” taro

4. Published paper at PERIPI National Seminar 2009
5. Published paper at UNPAD Agriculture Cross Road International Seminar 2009
6. Published papers at IJJSS International Seminar 2010

2. ROAD MAP OF KIMPUL TARO BREEDING (*Xanthosoma* spp.)

Quarter IV	*Activities:* Harvest and characterization of tuber quality	*Activities:* Harvest and evaluate yield	*Activities:* Harvest and evaluate bulb yield	*Activities:* Harvest and evaluation of purification result	*Activities:* - Harvest and verification of yield character on purified accession - Submission of PVT (PVP)
	Output: - Description of the entire morpho-agro characters of 17 accessions - Published paper	*Output:* - Description of the 17 accessions on 2 planting seasons - Published paper	*Output:* - Description - Published paper	*Output:* - Description - Published paper	*Output:* - Description and new varieties - PVP certificate
Quarter III	*Activities:* Maintenance	*Activities:* Maintenance	*Activities:* Maintenance and evaluation of morphology	*Activities:* Maintenance	*Activities:* Maintenance
	Output: Obtained 17	*Output:* 17 accessions	*Output:* description	*Output:* - Accession in	*Output:* - Accession

	accessions which have good condition	which have good condition on 2 design method		good condition. - Description	in good condition. - Description
Quarter II	*Activities:* Characterization of leaf and stem morphology	*Activities:* morphological characterization of leaf and stem on 2 design methods	*Activities:* Planting selected accessions that have good nutrition and morphology	*Activities:* Evaluation of accessions which was purified based on morphological characters of leaf and stem	*Activities:* Verify the characters of leaf and stem
	Output: Description of leaf and stem morphology of 17 accessions	*Output:* Description of morphology 17 of accessions on 2 planting seasons	*Output:* Collection garden	*Output:* Purified accessions description	*Output:* Standard description
Quarter I	*Activities:* Exploration and Collection of "Kimpul" taro	*Activities:* - Nutritional analysis of taro tuber - Planting season 2 with 2 experiment field methods	*Activities:* Registration of 17 Local "Kimpul" taro accessions varieties	*Activities:* Purification of the selected accessions	*Activities:* Selection of purification and propagation
	Output: - Formed collection garden consisting of 17 accessions - Published paper	*Output:* - Nutrient data of 17 "kimpul" accessions - 17 "kimpul" accessions with augmented and Block	*Output:* Pre HKI (IPR) of 17 "kimpul" accessions	*Output:* Accession garden toward uniformity	*Output:* new variety candidates

		Randomized Design			
	Year 1 (2009)	*Year 2 (2010)*	*Year 3 (2011)*	*Year 4 (2012)*	*Year 5 (2013)*

3. ROAD MAP OF BENTUL TARO BREEDING

Quarter IV	*Activities:* Harvest and characterization of tuber quality	*Activities:* Harvest and yield evaluation	*Activities:* Harvest and tuber yield evaluation	*Activities:* Harvest and evaluation of purification result	*Activities:* - Harvest and verification of yield character on purified accession - Submission of KHI-PVT (IPR-PVP)
	Output: - Description of the entire morpho-agro characters of 33 accessions - Published paper	*Output:* - Description of the 33 accessions on 2 planting seasons - Published paper	*Output:* - Description - Published paper	*Output:* - Description - Published paper	*Output:* - Description and new varieties - PVP certificate
Quarter III	*Activities:* Maintenance	*Activities:* Maintenance	*Activities:* Maintenance and evaluation of morphology	*Activities:* Maintenance	*Activities:* Maintenance
	Output: Obtained 33 accessions which have good condition	*Output:* 33 accessions which have good condition on 2 design method	*Output:* description	*Output:* - Accession in good condition. - Description	*Output:* - Accession in good condition. - Description

Quarter II	*Activities:* Characterization of leaf and stem morphology	*Activities:* morphological characterization of leaf and stem on 2 design methods	*Activities:* Planting selected accessions that have good nutrition and morphology	*Activities:* Evaluation of accessions which was purified based on morphological characters of leaf and stem	*Activities:* Verify the characters of leaf and stem
	Output: Description of leaf and stem morphology of 33 accessions	*Output:* Description of morphology 33 of accessions on 2 planting seasons	*Output:* Collection garden	*Output:* Purified accessions description	*Output:* Standard description
Quarter I	*Activities:* Exploration and Collection of "Bentul" taro	*Activities:* - Nutritional analysis of taro tuber - Planting season 2 with 2 experiment field methods	*Activities:* Registration of 17 Local "Bentul" taro accessions varieties	*Activities:* Purification of the selected accessions	*Activities:* Selection of purification and propagation
	Output: - Formed collection garden consisting of 33 accessions - Published paper	*Output:* - Nutrient data of 33 "bentul" accessions - 33 "bentul" accessions with augmented and Block Randomized Design	*Output:* Pre HKI (IPR) of 33 "bentul" accessions	*Output:* Accession garden toward uniformity	*Output:* new variety candidates
	Year 1 (2009)	*Year 2 (2010)*	*Year 3 (2011)*	*Year 4 (2012)*	*Year 5 (2013)*

NEW HOPE ON UNDERUTILIZED CROPS

Exploration is continuing, and preservation of biological resources, particularly in Indonesia and also all over world expected to improve food quality and human life in the future. The existence of collections and studies of Underutilized Crops which began to appear, and also organization charged with promoting and motivating continuity of Underutilized Crops development like ICUC, which has already had representatives in many countries in Asia, is expected will continue to assist the development of Underutilized Crops in the future. Therefore the Underutilized Crops will be able to be cultivated in large quantities such as other current food crops. Besides, it also will slowly eliminate the dependence on certain food (rice for an example), so that it will be formed a balance in supply of food and ecosystems.

Part of the results of new varieties developed have been recently applied for an IPR through Plat Variety Protection (20 new sweet potatoes clones of cv Cilembu dan Japan, 4 accessions of yard long bean, 20 accessions of morning glory,6 accessions of black soya, and 'local variety registration' (200 accessions of various crops and 200 accessions of wild Ipomoea as breeding materials) at the ministry of Agriculture Rep. of Indonesia.

ACFKNOWLEDGEMENTS

The research is funded by Padjadjaran University Bandung under a scheme of "Riset Andalan UNPAD" fiscal year 2009/2010

REFERENCES

Herbs. 2007. *Amorphopallus campanulatus*. Agriculture Department

Ian K Dawson, Luigi Guarino and Hannah Jaenicke. 2007. *Underutilized Plant Species: Impacts of Promotion on Biodiversity*. ICUC. Colombo. Srilangka

ICUC. 2006. Under-Utilized Crops Can Play A Crucial Role in Food and Livelihood Security for The Poor. Press Release. Colombo. Srilangka

Made Sri Prana. 2008. *Artificial Pollination on "Acung"* (*Amorphophallus decus-silvae*Back. & v.A.v.R.). Biodiversitas Vol 8 No.4

Plant Breeding Laboratory of Padjadjaran University. 2006-2010. List of Collected Accessions. Padjadjaran University. Unpublished.

MICROBIOLOGY FOR SOIL HEALTH

PETER MCGEE
School of Biological Sciences
University of Sydney, NSW,
Australia.

Scientists have a clear understanding of the importance of biodiversity to humanity and the planet. However, legislative support for conserving biodiversity is globally limited. Analyses of the economic benefits of biodiversity are all too frequently overlooked and social and cultural consequences of reduced biodiversity ignored. This paper briefly examines one way in which the economic and cultural importance of biodiversity may be used to enhance support for political decisions to more fully conserve biodiversity.

Global climate change is the consequence of an increased and recent movement of carbon into the atmosphere, directly and indirectly associated with human activities. One of these activities is land use. Some 30% of the additional CO_2 in air comes from soil (IPCC 2007). The loss of carbon from soil also has profound consequences for agricultural production. Productivity of land declines with loss of organic carbon, and increases the pressure on the land that is less degraded. This ongoing spiral of degradation and increasing pressure on land, especially with increasing global population, cannot be allowed to continue. My talk will focus on soil, and the potential role of fungi in restoring productivity of soils. While I will be talking about soil, the principles apply much more broadly.

The first question we asked is whether fungi can increase the store of stable organic carbon in soil. The model my group is working on argues that organic carbon is held in aggregates where it is protected from oxidation and enzymic attack: the finer the aggregate, the greater the protection. In this first experiment, we grew mycorrhizal plants for 6 months in mine spoil (which lacks organic carbon) amended with 6% compost.

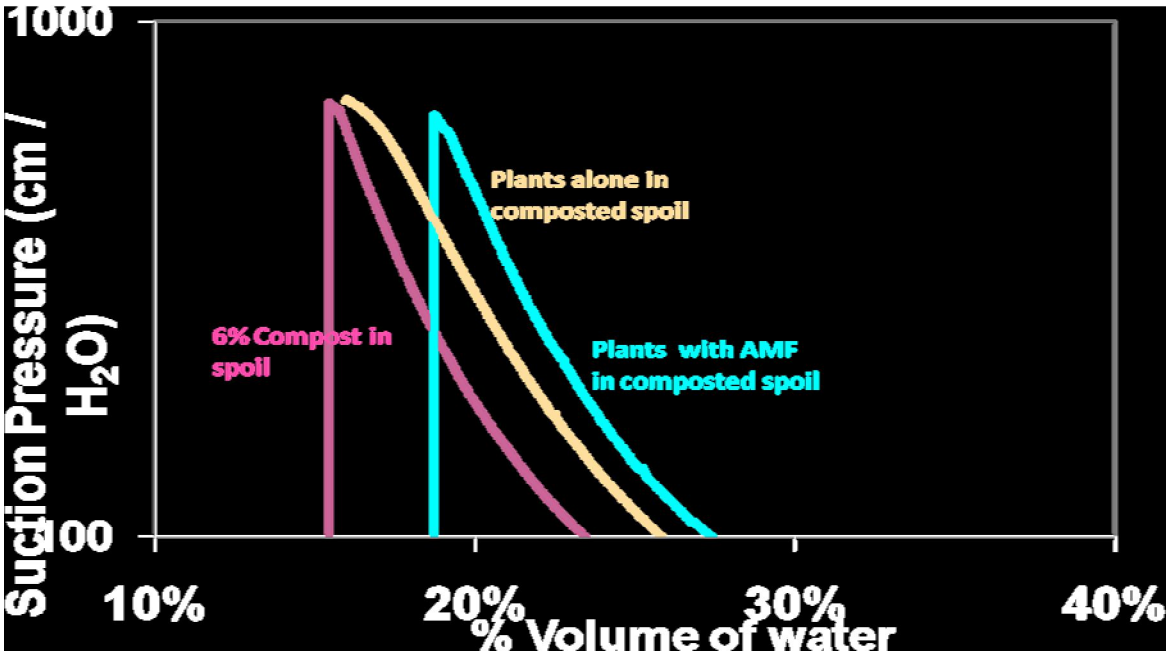
Table 1. Mean proportional organic carbon (OC) in aggregates from the 50 – 250 µm, and the 250 – 700 µm grids, and mean weight diameter of compost amended mine spoil. * indicates statistically significant differences from the control.

	% OC (50 – 250 µm)	% OC (250 – 700 µm)	Aggregation MWD
Plant 1	4*	5.4	863
2	3.5*	4.3	940
3	3.5*	4.8	824
All	3.2*	4.6	950
Nil	2.7	4	925

While overall aggregation remained unchanged in this experiment, the storage of carbon increased under mycorrhizal plants, significantly so in the fine fraction (Table 1) where aggregates are better protected. Further data from this experiment indicates that the presence of mycorrhizal fungi is crucial to the process of developing aggregation.

In another experiment, spoil was amended with varying amounts of compost, and then either planted using plants with or without mycorrhizal fungi, or left unplanted. After 6 months we measured aggregation, and water retention. Aggregation and water retention are inversely related in that water is held in the pore space remaining around aggregates. The pressure to remove water indicates the water available to plants.

Fig 1. Water retention in mine spoil amended with 6% compost after 6 months either without plants (green curve on left), with plants (dark curve in centre), or with mycorrhizal plants (red curve on right).



Without compost, the water retained was similar in unplanted, planted and mycorrhizal planted treatments. The addition of compost increased water retention by about 25%. In the spoil amended with 6% compost, plants alone increased water retention, and mycorrhizal plants increased it further (Fig 1). While compost increased

water storage, mycorrhizal plants increased water storage the most.

The importance of mycorrhizal fungi to the sustainable use of soil cannot be overstated. However, do different species of mycorrhizal fungi have the same effect, and is that effect additive? Preliminary evidence shows that different species of AM fungi increase aggregation and water retention differently over time and space. Some fungi have a negligible overall impact and some have a significant effect. The addition of further mycorrhizal fungi increases aggregation and water storage, but the interaction between mycorrhizal fungi is complex.

We have also looked at the impact of culturable fungi on aggregation. Among the Trichocomaceae, some 4% of 80 different isolates significantly aggregated soil by enmeshment (Daynes and McGee unpublished data). A different 4% of isolates released mucilage that also enhanced aggregation. As Trichocomaceae have only a short active life span in soil, we are now examining fungi that form endophytic associations with the roots of plants. Endophytes are assumed to have access to an ongoing source of energy and will thus continue to function so long as the host is alive. We are interested in those fungi that are dark, believing that melanin will degrade more slowly in aggregates. Though we isolated the genera *Phoma* and *Diaporthe* we found that these melanitic endophytic fungi are mostly undescribed species. Of the 120 species of melanitic endophyte we tested, some 60% had no effect on plant growth. Of those, some 75% aggregated a field soil. We have tested aggregation with fungi in both the petri dish and *in planta* (Mukasa Mugerwa and McGee unpublished data). In summary, a range of soil fungi have the potential to increase soil aggregation and presumably storage of organic

carbon. Many of these fungi remain undescribed.

In addition to their impact on aggregation and water retention, we examined the role of melanitic endophytic fungi in modifying soil pH. Some 30% of the isolates increased the pH of acid soil (pH 5 → 5.5), 2% decreased the pH and the rest had no significant impact. As soil acidity is an important aspect of soil degradation in many countries, the potential importance of fungal amelioration of soil pH cannot be overstated. In terms of biodiversity, we now have data showing some 60 unidentified fungi from soil have a potentially profound influence of soil function. We do not even know the identity of most of the few hundred isolates we have tested.

I will examine one further aspect of biodiversity. Entomopathogenic fungi cause disease of insects. Entomopathogenic fungi can be used to modify the populations of pest insects. At least five species of entomopathogen are widely used commercially. What is less well known is that many of these species are also endophytes. We have inoculated soil with several commercial species of fungi and subsequently isolated the fungi from leaves, stems and roots of the host plant. The fungi influence rates of growth and behaviour of two important insect pests (Gurulingappa et al 2010). It turns out that several mycologists have isolated these fungi from soil and/or plants, but ignored the data because the fungi were categorised as entomopathogens and could not therefore also be inhabitants of the soil. Our ignorance of the functions of soil fungi goes beyond our ignorance of their diversity. As scientists some of us have been guilty of limited vision.

Soil supports a huge array of important functions, including production of

food and storage of organic carbon. We have known for a long time that soil also contains a huge diversity of microbes, including fungi. Most of these fungi will, at any one time, be dormant or quiescent. However, pretending that they do not exist or worse, deliberately degrading this enormously important resource will lead to a catastrophic decline in the sustainable use of soil and the food it supplies. Ignoring the potential of using soil to store carbon removes one important and readily available process to help resolve a global challenge.

Why should we be concerned about diversity? Each microbe has a specific suite of environmental conditions (soil pH, temperature, water availability, mineral etc) under which it ideally functions. Different microbes contribute to each function, under each environmental condition and host plant. A single microbe contributes to only a few functions, and often for only short periods of time. We cannot remove one fungus and hope another will take its place: if continued loss of biodiversity will reduce the environment under which the specific functions will be maintained. Maintenance of microbial diversity is essential for maintaining soil function and the sustainable use of soil.

More broadly, the various biological components of the environment play interlocking roles in the environment. We can define the functions of only a few microbes, and then in only a limited way. Conservation of these enormously valuable organisms, and especially conservation of their diversity, is absolutely essential for the sustainable use of soil and all other parts of our environment. The century of greed and ignorance cannot be allowed to continue. As scientists we must ensure that politicians,

and society more generally, be made aware of the role these various groups play, and then support moves to ensure their diversity is sustained. Lack of action now will have profound consequences as humans place more pressure on the resources of the earth.

REFERENCES

Gurulingappa P, Sword GA, Murdoch G, McGee PA (2010) Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when *in planta*. Biol. Control 55: 34-41.

Intergovernmental Panel on Climate Change (IPCC) (2007) Climate Change 2007: The Physical Science Basis. Summary for Policy Makers. Geneva, Switzerland.