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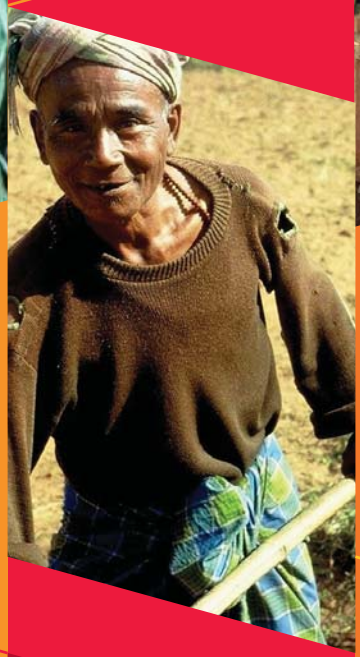

NIAS-FAO International Symposium

PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE IN ASIA AND THE PACIFIC: IMPACTS AND FUTURE DIRECTIONS

Plant Genetic Resources for Food and Agriculture in Asia and the Pacific: Impacts and Future Directions

Proceedings of a symposium held on 18th October 2011 in Tsukuba, Japan

Food and Agricultural Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, Thailand
and
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PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE: FUTURE DIRECTIONS

Foreword

In recent years, food security and food price stability have become dominant issues of international concern. FAO estimates that to feed growing populations – expected to reach 9.1 billion in 2050 – the world has to increase food production by 70 percent. In developing countries, food production has to increase by 100 percent, representing a tremendous challenge. The foundation of a secure and nutritious food supply is diversity in plant genetic resources that provide the genes to cope with the ever-changing agricultural environment. Recently, Asia has experienced massive disruption to food production and supplies because of floods in Myanmar, Pakistan and Thailand. These events remind us that agriculture will have to adapt to the conditions generated by climate change. In the Pacific an entire crop, taro, was decimated by leaf blight disease. New strains of pests and diseases, such as wheat rust strain UG99, are constantly emerging. Hence plant breeders are continuously challenged to stay one step ahead in the production of new varieties to cope with abiotic and biotic changes in the agricultural landscape.

A key to future crop production lies in the collections of crops and their wild relatives stored either *ex situ*, in gene banks, or *in situ*, growing in farmers' fields and the natural environment.

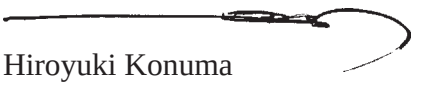
The Food and Agriculture Organization of the United Nations (FAO) has a long history of activities related to plant genetic resources for food and agriculture (PGRFA). In the 1950s, FAO helped to develop global seed systems. In the 1960s and 1970s, FAO held a series of technical conferences that led to the scientific principles on which plant genetic resource conservation is based. One outcome of these meetings was the International Board for Plant Genetic Resources (IBPGR) or Bioversity International, originally housed in FAO. In recent decades, FAO has spearheaded the effort to establish a global system with an internationally-accepted legal framework for access and benefit sharing *vis-à-vis* plant genetic resources. The first State of the World's Plant Genetic Resources was written and a Global Plan of Action was ratified by the international community in 1996. This led directly to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) that came into force in 2004. Recently, FAO has again reviewed the state of the world's plant genetic resources and in July this year a revised Global Plan of Action for the Conservation and Sustainable Use of PGRFA was agreed by the international community at FAO headquarters in Rome.

The underlying reason for the many international developments regarding plant genetic resources is to allow equitable access and sharing of their benefits. All countries benefit from germplasm that comes from another country. Central to the cuisine of Thailand and Sri Lanka is the chili pepper that evolved in Mexico. The main staple of Asia, rice, is now grown worldwide and is a staple in many countries of Latin America where 'rice and beans' is a common dish.

The importance of access to well-characterized plant genetic resources will be the key to future increases in crop productivity. Now that the era of collecting germplasm for gene banks has elapsed, the focus is shifting on to how to best use what is available in them. Hence initiatives to characterize, evaluate and promote use of PGRFA have become increasingly important. FAO and partner organizations are therefore very much involved with the Global Partnership Initiative for Plant Breeding Capacity Building (GIPB) programme that promotes the use of plant genetic resources.

The FAO Regional Office for Asia and the Pacific (FAO-RAP) has undertaken two projects to introduce a PGRFA monitoring system for national programmes, funded by the Government of Japan. As a result, 15 countries in Asia have National Information Sharing Mechanisms for monitoring the Global Plan of Action (NISM-GPA). Eight have the NISM-GPA in their own national language as well as English. These two projects have enabled the full range of plant genetic resource activities in each country to be combined in one database for the first time. The databases can also be accessed by the global community via FAO's World Information Sharing Mechanism portal.

On 18 October 2011, FAO-RAP and the National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan, conducted a symposium on Plant Genetic Resources in Asia and the Pacific: Impacts and Future Directions. It was attended by more than 100 participants and included plant genetic resource leaders from 17 countries in Asia and the Pacific. This publication presents the symposium's papers that highlight issues at the farmers' level, such as participatory plant breeding which has been essential for reintroduction of taro production in Samoa and, at the genomic level, improved productivity of crops such as soybean. It is hoped that the perspectives presented in these papers will be useful in providing information and ideas to people working on plant genetic resources in the Asia-Pacific region and beyond.



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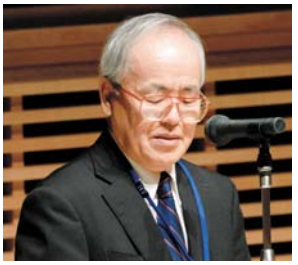

Welcome address

Teruo Ishige[1]

Good morning. It is a pleasure for me to welcome you to Tsukuba and a day that focuses on plant genetic resources for food and agriculture (PGRFA). I would like to thank FAO and Mr. Konuma for joining with NIAS to organize this symposium that reflects core activities of both my institute and FAO.

Japan, as an island country, has specific plant genetic resources. Our crop genetic resources had a special fascination for the man who has become known as the 'Father of Plant Genetic Resources Science' – Nicolai Vavilov – who visited Japan in 1929. Japan is a country that stretches about 3 000 kilometres from north to south and the land is farmed at elevations ranging from sea level to high in our mountain ranges. As a consequence, our plant genetic resources are very diverse and this is reflected in Japanese cuisine.

This morning some of you have visited the NIAS gene bank. NIAS is responsible for the central gene bank of a nationwide network devoted to conservation of plant genetic resources. In our gene bank we are not just conserving plant genetic resources we are also conducting research to improve all aspects of conservation. This has resulted in advances in cryopreservation, which is one of the topics that you will hear about today. Our scientists are also using various biotechnology techniques to develop small core collections that will enable evaluation to be conducted efficiently and, hopefully, lead to better use of conserved germplasm.

An important aspect of our gene bank activities is the international dimension. Scientists from many countries have collaborated with our gene bank in various projects. We have, over many years, conducted training in plant genetic resources; some participants present today have attended our training courses and have also been visiting scientists at our institute. So I would like to welcome back to Tsukuba Mr. Bayarsukh from Mongolia, Mr. Suu from Viet Nam and Mr. Masood from Pakistan. Our institute has benefited greatly from collaboration on collecting plant genetic resources overseas, and also in Japan, with our partners from other countries. I would like to take this opportunity, with the PGRFA leaders from so many collaborating countries present, to

[1] President, National Institute of Agrobiological Sciences (NIAS).

express my sincere thanks you for this collaboration over the years. I also look forward to that collaboration continuing in the future.

I will end by hoping that your visit to Tsukuba will be memorable and that you will share the experience of your visit with your colleagues when you return home. Thank you.

Part 1:

Symposium summary and recommendations

Symposium summary and recommendations

From 17 to 19 October 2011 plant genetic resource leaders from more than 17 Asia-Pacific countries gathered in Tsukuba, Japan. Discussions were held on progress in the development of National Information Sharing Mechanisms (NISMs) and how to further sustain this initiative in each country and through regional collaboration. On Day 2 a symposium was held on the topic Plant Genetic Resources for Food and Agriculture in Asia and the Pacific: Impacts and Future Directions. A summary of the more important points and suggestions is provided hereunder. These recommendations complement the Suwon Agrobiodiversity Framework (visit http://www.apaari.org/publications/suwon-framework.html).

a) The impacts of Plant Genetic Resources for Food and Agriculture (PGRFA) on improving food security, farm productivity and livelihoods in agriculture have been tremendous. These were described by examples of different studies that utilized wild relatives/species to improve cultivated crops exemplified by the restoration of taro production in Samoa, green revolutions in rice, adapting sugarcane to marginal lands and genetic enhancement of carnation for disease resistance and longer shelf-life. The importance of genetic exchange among countries in ensuring food security was emphasized by the examples of coping with taro blight disease and improvement in pest and disease resistance, tolerance to drought and floods, and yield in rice varieties. The impact of PGRFA in the past and their potential to help alleviate problems related to food production and adaptation to climate change in the future should be widely understood.

b) The critical linkage between conservation and use of PGRFA was underscored. However, many countries recognize that this relationship needs strengthening. While there are good examples of the success of using conserved genetic resources, all PGRFA workers would like conserved germplasm to be used more for the sustainable improvement of agriculture in the twenty-first century. Therefore, support for bridging this link by national and international organizations is recommended. Current initiatives, such as the Global Partnership Initiative for Plant Breeding Capacity Building (GIPB) (http://km.fao.org/gipb/), should be strengthened in the region.

c) The importance of new tools and approaches in exploring the use and conservation of PGRFA was also discussed in the workshop. The effectiveness of modern biotechnology like genomics and cryopreservation was discussed. However these technologies entail investment for use in developing countries. International collaboration, however, can help developing countries access and use these tools for the conservation and use of their PGRFA.

d) Information is key to the successful use of PGRFA and the symposium heard about the rapid improvement in global and national PGRFA databases. However, much more needs to be done in collecting information and maintaining these databases. The 2nd Global Plan of Action and the International Treaty on PGRFA also stressed the importance of developing these information systems. Among the areas the symposium highlighted for increased and urgent attention was ensuring that 'old data' related to PGRFA is properly available. This may require, for example, scanning handwritten documents and archival work to link conserved PGRFA with passport data that has not been done so far. A call was also made for incorporation of traditional knowledge (TK) associated with conserved PGRFA in the information systems and databases. TK is a very important component in promoting the use of PGRFA. While databases for TK do exist they are not currently linked with the large databases of conserved PGRFA.

e) The importance of periodically reviewing and reporting PGRFA status quo, by assessing threats and minimizing or, where possible, eliminating them, was stressed. Designated focal points should convey this information to FAO, and, as appropriate, to the Governing Body of the International Treaty, the Conference of the Parties to the CBD and other relevant bodies. Monitoring and reporting mechanisms for genetic erosion need to be established starting from local and national levels. They could fit within established NISMs and take advantage of NISMs' participatory structure to carry out assessments and coordinate preventive and remedial actions.

f) While it is recognized that mega-projects in crop improvement are essential to make groundbreaking progress, such as applying breakthroughs in biotechnology and other advances in science, there is also a place for many small-scale targeted crop improvement projects that have potential for good results at local and regional levels. Strong local and national programmes in the past contributed to the use of more local and introduced genetic materials resulting in broader genetic base. The

current reliance of many national breeding programmes on improved lines coming from big international breeding initiatives has narrowed the diversity of varieties popularly planted in many countries. Identifying and supporting excellent scientists and institutes that have great potential, with support from small projects, is recognized as important. The maintenance of strong national and local crop improvement initiatives that use locally-adopted germplasm and introduced materials should be encouraged to better cope with location-specific variabilities.

g) Finally, the importance of international collaboration and networking was repeatedly urged by the participants. Countries of different sizes have different strengths and weaknesses. Therefore communication and more importantly support between and among countries can benefit all. In this context, the symposium brought together workers from different PGRFA networks in Asia and the Pacific. While 'big brothers' can help smaller countries, smaller countries may well have comparative advantages in areas larger countries do not (for example rare and exotic species/ diversity). The ability of countries to meet and share experiences both in formal and informal settings, that characterized the Tsukuba meeting, was recognized as beneficial to developing the capacities of different countries and in promoting international collaboration. Therefore support for continuing such meetings was recommended.

Symposium Participants
Credit Fukuhiro Yamasaki

Part 2:

Plant Genetic Resources for Food and Agriculture: Impacts

Developing new types of sugarcane by hybridization between commercial sugarcane cultivars and wild relatives

A. Sugimoto, Y. Terajima, T. Terauchi;[1]
W. Ponragdee;[2] S. Ohara;[3] S. Tagane, T. Sansayawichai;[2] T. Ishida;[3]
S. Ando, M. Matsuoka;[1] T. Yasuhara;[3] T. Hattori;[1] S. Fukuhara;[3]
T. Sakaigaichi;[1] S. Ishikawa and Y. Tarumoto[1]

Introducing new properties such as deep rooting and high ratooning into sugarcane that has comparatively high adaptability to poor environments was attempted. First, interspecific hybridization between sugarcane for sugar production and its wild relative, Saccharum spontaneum*, was tested. Strains of sugarcane that showed marked improvements in sugar production owing to better adaptability to inadequate rainfall, high dry matter productivity and multiple rationing capacity were produced. Subsequently, back-crossing of some* F_1 *lines with sugarcane to develop promising lines with improved sugar contents was assayed. The promising line KYo1-2044 was registered in Japan and TPJ03-452, TPJ04-713, 768 will be registered in Thailand for large-scale experiments for their practical use. The promising interspecific hybrids that were created exhibited relatively high adaptability to poor environments (inadequate rainfall, etc.), but their adaptability to very poor environments was limited. However, a line of* Erianthus spp. *was shown to grow well even under such unfavourable conditions and exhibited high potential for effective growth of ratooning. Thus, intergeneric breeding between sugarcane and* Erianthus spp. *for introducing the excellent features of* Erianthus spp. *(deep roots and high ratooning potential) into sugarcane was attempted. Some of the hybrids created exhibited deep-rooting characteristics. For higher adaptability to dry condition, hybrids between* Erianthus spp. *plants and between* Erianthus *and* Miscanthus spp. *were also obtained.*

[1] Japan International Research Center for Agricultural Sciences;

[2] National Agriculture Research Center for Kyushu Okinawa Region;

[3] Khon Kaen Field Crops Research Center, Asahi Group Holdings Ltd.

Introduction

Against the background of continued population and economic growth globally, the demand for food and energy has been increasing, resulting in a growing imbalance between demand and supply. Meanwhile, owing to a number of factors, exemplified by escalating desertification, it is becoming harder to maintain agricultural land that is suitable for food crop cultivation. Under such circumstances, it is desirable to develop technology that enables increases in both food and energy production, that is, technology that enables efficient utilization of land for agriculture.

In this context, a technology is required that facilitates continuous production of crops serving as raw materials for industry (for example sugar and ethanol) even under environments that are typically unsuitable for food crop production. This includes production on land with insufficient rainfall, low-quality soil and other detrimental features.

In Japan, sugarcane is mostly cultivated in the Southwest Islands, which are located in the subtropical zone. The level of annual rainfall is relatively high, but drought often occurs during summer, which results in sugarcane yield that is lower than the global average. To continue sugarcane production as a major crop in a given region with an unfavourable environment, it is recommended that a switch be made from conventional intensified cultivation of high-sugar-content types of sugarcane (solely for the purpose of producing sugar) to cultivation of new types of sugarcane that are highly adaptable to poor environments; they should have energy- and labour-saving characteristics and capacity to preserve the environment. The goal of this switch is to achieve efficient utilization of sugarcane, such as producing not only sugar but also ethanol. This involves the conversion of conventional sugar production to an industry that can optimize the potential of sugarcane as much as possible (Sugimoto and Terajima 2006).

Tropical and subtropical zones have extensive areas that are not suitable for the cultivation of food crops or high-yield cultivation of sugarcane for sugar production because of a severe dry season, soil with low-moisture-retaining properties and low fertility, among others. Such areas include Northeast Thailand. With this in mind, attempts were made to develop sugarcane lines that are adapted to poor environments, that save labour costs and facilitate cultivation via the recycling of organic substances; this was carried out in the Southwest Islands of Japan and in Northeast Thailand, with the goal of developing commercially-viable technology related to sugarcane.

To modify sugarcane for better adaptation to poor environments, it was considered useful to develop and utilize analogous wild-type plants capable of growing in poor environments. As such, interspecific and intergeneric breeding, involving sugarcane, wild sugarcane (*S. spontaneum*), *Erianthus* spp. and *Miscanthus* spp. was assayed.

Initially, interspecific hybridization between sugarcane and *S. spontaneum* was attempted. Through evaluation of genetic resources, breeding technology (involving adjustment of the heading and blooming time, storage of pollens and other factors) and manipulations through selection in fields with poor-quality soil, many F_1 lines with desirable characteristics, such as high tillering and ratooning potentials were obtained. Juxtaposing the development of materials, methods for better utilization of the characteristics of such materials were devised. Attempts were also made to develop sugarcane for cattle feed and for raw material for the simultaneous production of sugar and ethanol.

Furthermore, efforts were made at intergeneric breeding using *Erianthus* spp., characterized by deep roots and high resprouting potential after harvesting, with the goal of utilizing areas with environmental characteristics (such as very low rainfall) unsuited to agriculture.

To date, these attempts have yielded satisfactory outcomes according to the goals and expectations presented below.

Outline of progress of development of new types of sugarcane

Promising sugarcane lines by interspecific hybridization for use in Southwest Japan

1. Nagatomi *et al.* (1988) collected wild sugarcane species distributed in Japan, such as *S. spontaneum* and closely-related species such as *Miscanthus* spp.
2. Nagatomi *et al.* (1982) attempted interspecific hybridization between sugarcane and *S. spontaneum*, as well as intergeneric hybridization between sugarcane and sweet sorghum.
3. Nagatomi *et al.* (1985) and Shimabuku *et al.* (1989) evaluated the characteristics of interspecific hybrids such as the first cross-bred generation, the first back-crossed generation and the second back-crossed generation, revealing general characteristics of these interspecific hybrids.
4. Sugimoto *et al.* (2004) attempted to develop new sugarcane lines in the context of a plan for multiple uses of sugarcane lines – created by

interspecific breeding – that are highly adapted to inadequate rainfall and that exhibit high ratooning potential and high yields.

5. Sugimoto *et al.* (2004) evaluated the characteristics of the subsequently created sugarcane lines and attempted to cultivate a commercial cultivar for use as cattle feed, focusing on high ratooning potential. This work was efficiently developed by Sakaigaichi *et al.* (2008).
6. Ohara *et al.* (2205, 2009) developed a technology and system for composite sugar and ethanol production by using interspecific hybrids characterized by low levels of sugar content and high levels of fibre content.

Promising sugarcane lines by interspecific breeding for use in Northeast Thailand

1. Nagatomi and Kassem (1980) collected *S. spontaneum* in Thailand, subsequently maintained at Kasetsart University.
2. In 1997, Sugimoto and Ponragdee (2000 in Japanese) began collection, conservation and characterization of wild sugarcane species, *S. spontaneum* and *Erianthus* spp.
3. In 2002, Sugimoto *et al.* (unpublished) began developing new lines of sugarcane, in a cooperation between the Japan International Research Centre for Agricultural Sciences (JIRCAS) and Khon Kaen Field Crops Research Centre, Department of Agriculture, Thailand (KKFCRC); this was carried out by interspecific and intergeneric hybridization between sugarcane and wild relatives of sugarcane, for use in Northeast Thailand and other regions.

Development of new sugarcane lines highly adapted to poor environments through intergeneric hybridization using Erianthus spp. plants

1. In 1997, Sugimoto *et al.* (1996) began to collect genetic resources of *Erianthus* spp. via approaches that included conducting sampling surveys of conserved species.
2. In 2008, Tagane *et al.* (2011b) began evaluation of the morphological features of *Erianthus* spp. and classification of the members of this genus.
3. Terajima *et al.* (unpublished) tried to develop technologies for adjustment of the heading and flowering time of *Erianthus* spp. and technology for short-term pollen storage.
4. Fukuhara (unpublished) introduced a technique for checking the hybridity of intergeneric hybrids using 5S ribosomal DNA.

5. Terajima *et al*. (2011) began evaluation of important traits of inter-generic hybrids between sugarcane and *Erianthus* spp.

The lines with high growth potential were evaluated in terms of root system characteristics and other features.

Results and discussion

I. Collection of wild sugarcane species and creation of optimal new lines by interspecific hybridization

Collection, conservation and evaluation of characteristics of wild sugarcane species in the Southwest Islands of Japan

Nagatomi (1988) collected many wild sugarcane and related species – *S. spontaneum,* including a small number of plants belonging to the genus *Miscanthus* and *Erianthus*. Wild sugarcane species and similar plants were also collected from the mainland (Ibaraki, Kanagawa and Chiba Prefectures). Collected genetic resources were conserved in an experimental field of Okinawa Prefectural Agriculture Research Centre. They were evaluated by Nagatomi *et al.* (1982, 1985). Many of the samples of *S. spontaneum* had excellent tillering and ratooning potential after harvesting (Nagatomi 1982, 1985, 1988). No database on the genetic resources of *S. spontaneum* has been developed yet.

Development of technology for interspecific hybridization with commercial sugarcane cultivars and the characteristics of hybrids created in this manner: Numerous lines have been produced from interspecific hybridization between sugarcane and *S. spontaneum*. However, because of difficulty in hybridization as a result of factors such as differences in heading time, the current technology has not reached a stage where it can yield any desirable combination. Among the F_1 lines created by interspecific hybridization, many lines had long stalks, many stalks and excellent postharvest ratooning potential. All of these lines had high levels of fibre content and low levels of sugar content in their juice (Nagatomi 1985; Sugimoto 2004; Terajima *et al*. 2005; Terajima *et al.* 2007).

As the generation advanced from the first generation of interspecific crossing to the first generation of back-crossing with sugarcane (BC_1) and to the second generation of back-crossing (BC_2), the prominence of characteristics that originated from the wild type decreased, while the characteristics of sugarcane became more prominent. These changes involved decrease in the number of stalks, increase in the diameter of stalks and slight decrease in the length of

stalks. The cane yield became smaller. The ratooning potential after harvesting also decreased. The sugar content, on the other hand, increased (Shimabuku and Sugimoto 1989; Sugimoto *et al.* 1989).

These results suggest that BC_1 is optimal as a line to be utilized in sugar production while retaining the most beneficial characteristics of the wild type. Also it suggests that to create ideal sugarcane lines for sugar production, it was necessary to explore species of sugarcane that exhibited a high level of sugar content and a thick stalk (for use as the maternal plant) and wild sugarcane species with a relatively high level of sugar content and a thick stalk (for use as the paternal plant).

Characteristics of promising lines yielded from interspecific hybridization and their use in agriculture: F_1 hybrids were primarily tested to select and evaluate lines showing promising characteristics; this was achieved with the participation of sugarcane farmers, regional government staff members and agricultural cooperative union employees. As a result, a first-generation hybrid with high adaptability to multiple harvesting, named S5-33 (yielded from sugarcane NCo310 x *S. spontaneum* Glagha KloeT), was selected. When the resistance of this hybrid to smut (a disease with a significant impact on sugarcane for sugar production) was evaluated, it was moderately susceptible. It was registered as a sugarcane species for cattle feed under the name KRFo93-1. Efforts to disseminate this species have been initiated. Information about the first sugarcane species for use as cattle feed (KRFo93-1) was disseminated orally among sugarcane farmers. Because the resistance of this species to smut was moderate, its dissemination in regions in which there was a risk of smut was avoided. (Sakaigaichi and Terajima 2008). This study was developed by Sakaigaichi *et al.* (2008). Another line, KR91-1003, which has resistance to smut was registered as a second commercial cultivar for cattle feed under the name ‘Simano Ushie’ (unpublished).

The possibility of using the created hybrids as raw materials for composite sugar and ethanol production was evaluated in cooperation with Asahi Group Holdings, Ltd. Most of the F_1 lines with high yields were judged to have levels of sugar that were too low. Following this finding, back-crossing was attempted using sugarcane varieties such as NiF3 and NiF8. Back-crossing allowed the level of sugar content and the stalk thickness to be improved, yielding many lines characterized by a relatively high level of sugar content, appropriate stalk thickness (not too thin), a relatively large number of stalks and excellent postharvest ratooning potential. Ohara *et al.* (2005) showed that desirable features of sugarcane for composite sugar and ethanol production are that the cane yield is twofold, total sugar yield is 1.5-fold greater than that of existing

sugarcane and fibre content is more than 16 percent (Ohara *et al.* 2005; Ohara *et al.* 2009). The promising lines selected in accordance with these criteria were evaluated in terms of productivity to identify excellent lines, which were then subjected to evaluation in terms of production processes and other factors at the pilot plant experiment station on Ie Island. Through these processes, KY01-2044 has been registered as a variety for composite sugar and ethanol production (Terajima *et al.* 2010).

Exploration, collection and evaluation of the characteristics of genetic resources from wild sugarcane species in Thailand

Nagatomi (1980) collected germplasm of *S. spontaneum* in Thailand and maintained it at Kasetsart University. Sugimoto *et al.* (1996) collected about 500 accessions of *S. spontaneum* from across Thailand and conserved them in a test field to evaluate their growth characteristics. Resembling the results from Japan, many accessions of *S. spontaneum* in Thailand had high tillering and ratooning potential. The samples collected showed wide variation in terms of ecological and morphological properties. The collected species had the following features: most of the stalks below the top of the plant were in water; the stalks growing along a river or on wetlands had long and relatively thick stalks with significant cores inside; the variation in Brix was large (about 20 percent in some cases); they could exhibit active growth even in saline soil. The variation in these species seemed to be larger than that in *S. spontaneum* in Japan. Many samples appeared to have the potential to serve as good parents for interspecific crossing. No database on *S. spontaneum* has been developed yet.

Characteristics of lines created in Thailand by interspecific hybridization with sugarcane: Although hybridization has been conducted on only a limited number of pairs synchronized in terms of the heading time, it has yielded numerous F_1 lines by crossing with existing sugarcane species exhibiting high ratooning potentials. These lines were small in terms of stalk diameter but had many stalks. The growth after harvesting by ratooning was also excellent. Growth during dry seasons was also substantial compared with that of species used for sugar production. Brix and sugar content were low. On the basis of the experience in Japan, evaluation of these hybrids as raw materials for composite sugar and ethanol production was carried out, and their usefulness as cattle feed was also evaluated with the cooperation of livestock farmers (Ponragdee *et al.* 2005; Sansayawichai *et al.* 2005).

To improve the stalk diameter and sugar content, back-crossing with sugarcane was carried out to yield numerous lines with relatively high levels of sugar

content and improved stalk diameter. From these lines, the following lines were selected as promising back-crossed lines suitable for composite sugar and ethanol production on the basis of the data on yield, ratooning potential, growth with inadequate rainfall and other features: BC03-452, BC04-713 and BC04-768. These lines will be registered as TPJ03-452, TPJ04-452 and TPJ04-768, respectively (Ponragdee unpublished).

Characteristics of promising lines from interspecific hybridization and their use in agriculture in Thailand: TPJ03-452 is characterized by high tillering and postharvest ratooning potentials enabling high yield as a ratoon crop. The yield in environments with limited rainfall is much higher with this line than with sugarcane. However, because the sugar content of this line is too low, introduction of new processing technologies, such as technology for composite sugar and ethanol production, is necessary for its commercial use.

TPJ04-713 and TPJ04-768 have many stalks and their yield in the form of ratoon crops is particularly high. Their stalks are thinner than those of sugarcane and the commercial cane sugar (CCS) rate is low, but compared with high-yield lines such as TPJ03-452, these two lines have thicker stalks and higher CSS rate. Fibre content is also high with these lines. These features are favourable for their use as raw materials for the production of sugar, ethanol and electric power currently practised in Northeast Thailand. These lines also yield large amounts of organic substances that can be reused on cultivated lands.

Although the slightly small stalk diameter, among other features, may be a shortcoming of these lines, we hope to utilize them as raw materials for continued commercial production of sugar, ethanol and electric power in Northeast Thailand.

II. Collecting Erianthus spp. and creating intergeneric hybrids to enable stable cultivation under more severe conditions

On Minami Daito Island, which has layers of hardpan soil, tests were conducted on sugarcane lines from interspecific hybridization, genetic resources of *Erianthus* spp. and sugarcane for preliminary evaluation of its adaptability to very poor environments. Under environments like those prevailing on Minami Daito Island, which experiences severe drought during summer and has soil layers made of hardpan, the ratoon crop was markedly shorter even with the first-generation hybrid characterized by relatively deep roots (97S-41). On the other hand, *Erianthus* spp. was excellent in terms of yield following both new planting and ratooning – the yield after the latter was higher (Terajima

unpublished). These results suggest that when sugarcane is cultivated continuously in fields exposed to severe environmental conditions like those on Minami Daito Island, adaptability to the environment is not sufficiently high, even with interspecific hybrids; thus, genetic resources like *Erianthus* spp. that are highly adapted to such poor environments need to be utilized. For utilization of sugarcane in agriculture under very poor environments (such as those with insufficient annual rainfall), intergeneric hybridization with *Erianthus* spp. was attempted; *Erianthus* has remarkably deep and strong roots and thus would be expected to be better adapted to dry environments than hybrids between sugarcane and *S. spontaneum*. To date, some F_1 hybrids, produced from sugarcane and *Erianthus* spp., with root systems of intermediate length, have been created, although the number is not large.

Trials in the Southwest Islands of Japan: Accessions of *Erianthus* and *Miscanthus* growing naturally on the mainland and Southwest Islands of Japan, were collected. All of the *Erianthus* spp. plants were type I of *Erianthus arundinaceus*. They have been maintained and labelled as JW4 and JW630. *Erianthus* spp. such as IJ76-349 and 28 NG7 have been introduced into Japan from Indonesia. On the whole, *Erianthus* spp. had deep roots, high yield and high ratooning potential. The yield in the form of ratoon crops also remained high for multiple harvests.

Heading occurred earlier in *Erianthus* spp. than in sugarcane. We succeeded in synchronizing the timing of heading between *Erianthus* spp. and sugarcane by delaying the heading of *Erianthus* spp. using photoperiod treatment (Tagane *et al.* 2011). The difference in the timing of flowering within the day was also revealed. In addition to the technique for adjustment of the flowering time by dark processing, a technology for short-term storage of pollen was developed, enabling intergeneric hybridization using pollen stored in this way. Hybridity was confirmed by analysis of 5S rDNA, indicating that many genuine intergeneric hybrids had been produced. The characteristics of the F_1 hybrids obtained are now under evaluation. The interim results indicate that hybrids with relatively deep roots have been created (Terajima *et al.* 2011.)

Trials in Thailand: About 150 accessions of *Erianthus* spp. were collected from across Thailand and maintained in the KKFCRC Tha Phra test fields. Genetic resources collected to date pertain not only to *Erianthus* spp. but also to other plants that seem to be adaptable to poor environments, such as *Sclerostachya fusca* and *Miscanthus floridulus*. Some of the collected *Erianthus* spp. had deep hard roots that were capable of penetrating the hardpan layer (Sugimoto *et al.* 1996). The samples stored were divided into *Erianthus arundinaceus* and *E. procerus* according to their morphology, geographic

distribution and number of chromosomes, among other features. *E. arundinaceus* was further divided into three subtypes (Tagane *et al.* 2011). The dry matter productivity of *Erianthus* spp. was high even with ratoon crops. In Northeast Thailand, which has a severe dry season, its yield was much higher than that of Napier grass and sugarcane for sugar production. (Tagane *et al.* 2011).

We evaluated the timing of heading and blooming in Khon Kaen and have been attempting to synchronize the timing of heading and flowering between *Erianthus* spp. and sugarcane by means that include light irradiation and dark processing (Irei *et al.* 2005). We attempted intergeneric hybridization with the methods mentioned. We succeeded in improving the hybridization success rate by using modified mating methods such as strengthening of emasculation. When cultivated, most of the F_1 hybrids showed poor growth, but some hybrids grew relatively well.

Perspective for intergeneric breeding: From now on, we will focus our efforts first on creating promising hybrids with deep roots and high ratoon yield through hybridization of various combinations between sugarcane and *Erianthus*. Then we will develop technology for stable production of the lines created in this way in poor environments, by utilizing the deep root system possessed by these lines. This will be followed by the development of technologies and systems for recycling of organic substances in fields in connection with livestock farming. We will thus improve the level of organic matter in the superficial layers of soil and enhance the basic power of fields (i.e. develop a technology to improve land power for crop production).

For higher adaptability to dry conditions: Japanese scientists who have engaged in sugarcane development have a strategy to develop sugarcane as a vehicle for enhancing agricultural areas worldwide. These areas are roughly classified into four categories from good agricultural area (Category 1) to unsuitable agricultural area (Category 4). It is perceived that there can be correspondence between the type of sugarcane and area belonging to a certain category. For realizing sustainable agriculture in Category 4, utilization of *Erianthus* spp. as a raw material for energy production is useful. For this, improvement of *Erianthus* itself as an energy crop is necessary. To date some hybrids among different types of *Eriannthus* spp. and hybrids between *Erianthus* spp. and *Miscanthus* spp. have been obtained.

Conclusions

Most of the wild sugarcane, *S. spontaneum*, collected in Japan and Thailand had high tillering and postharvest ratooning potential. The samples had thin and relatively short stalks and Brix in their cane juice was low. The samples collected in Thailand varied greatly among individuals in terms of stalk length, thickness, Brix in cane juice and timing of heading. The variation in these features seemed to be greater for the materials collected in Thailand than for those collected in Japan. In both Thailand and Japan, some areas have not been explored yet. Exploration and sample collection in these areas needs to be carried out as soon as possible.

Although hybridization has been conducted on only a limited number of combinations, some of the F_1 hybrids were excellent in terms of yield, ratooning potential and adaptability to insufficient rainfall. Sophisticated control of heading and blooming and hybridization using diverse combinations are expected to facilitate efficient creation of even better hybrids. It is desirable to accelerate establishment of a database on genetic resources of wild species and to evaluate the possibility of combining various pairs of material.

Interspecific hybridization, followed by back-crossing with sugarcane, allowed creation of promising lines with relatively high yield in the form of ratoon crops. In both the Southwest Islands of Japan and Northeast Thailand, it is desirable to continue efforts to commercialize this kind of technology, evaluate its usefulness and develop systems for sugar, ethanol and electric power production, making use of interspecific hybrids in environments where the productivity of conventional sugarcane is low.

The preliminary study conducted on Minami Daito Island, which often experiences drought during summer and has hardpan layers of soil, revealed that the interspecific hybrids cannot maintain sugarcane production under severe environments. However, *Erianthus* spp. showed excellent growth under such an environment, resulting in high yield, even in the form of ratoon crops. Following this result, we attempted intergeneric hybridization with *Erianthus* spp. Although the number of trials was small, favourable outcomes such as acquisition of F1 hybrids with improved root systems were achieved. In the future, it will be desirable to establish a database on genetic resources of *Erianthus* spp. and to conduct basic evaluation of carbohydrate and fibre productivity in regions not suitable for food crop production (i.e. evaluation of the root system characteristics and ratooning potentials of hybrids) so that the perspectives for the future may be defined clearly. To realize sustainable

agriculture in very poor environments, the utilization of *Erianthus* spp. as an energy crop is deemed useful. For this purpose, improvement of *Erianthus* spp. via cross-breeding is necessary. For further improvement of *Erianthus* spp. it is important to conduct intergeneric hybridization by using plants of *Miscanthus* spp.

References

Irei, S., Ponragdee, W., Sansayawichai, T. *et al.* 2005. Improvement of hybridization procedure between sugarcane and *Erianthus* spp. *JIRCAS Working Report*, 47: 81-84.

Nagatomi, S. 1980. *JICA report on collaboration between Kasetsart University and Japan*(I): pp. 32-42.

Nagatomi, S. 1982. Method and Problems of Basic Sugarcane Breeding in Okinawa. *Bull. Okinawa Pref. Agric. Exp. Stn*., 7: 1-13.

Nagatomi, S. 1985. Evaluation on germplasm of wild sugarcane species indigenous to Japan. *Bull. Okinawa Pref. Agric. Exp. Stn*., 10(4): 25-44.

Nagatomi, S. 1988. Exploration for wild germplasm of sugarcane indigenous in Japan and their breeding use. *Research Journal of Food and Agriculture*, 11(6): 18-23.

Ohara, S., Hayano, T., Terajima, Y. *et al*. 2005. Biomass ethanol production from sugarcane for energy generation to support sugar production. *Journal of the Japan Institute of Energy*, 84(11): 923-928.

Ohara, S., Fukushima, Y., Sugimoto, A. *et al*. 2009. Reduction in greenhouse gas emissions from process retrofitting and cultivar improvement in combined sugar-ethanol production from sugarcane. *Journal of Life Cycle Assessment, Japan*, 5(4): 439-445.

Ponragdee, W., Sansayawichai, T., Takagi, H., ***et al.*** 2005. Development of sugarcane for forage use by inter-specific and inter-generic crossing. *JIRCAS Working Report*, 47: 85-88.

Sakaigaichi, T. & Terajima Y. 2008. Development and extension of sugarcane cultivar as forage crop 'KRFo93-1'. *J. Agric. Sci.*, 63: 24-29.

Sansayawichai, T., Ponragdee, W., Phaophaisal, I. ***et al.*** 2005. Investigation on cultivation conditions and yield of new breeding line for forage use, *JIRCAS Working Report*, 47: 89-92.

Shimabuku, M. & Sugimoto, A. 1989. Important factors of high yielding ability of inter-specific hybrids between sugarcane and it's wild relatives. *Bull. Okinawa Agric. Exp. Stn*, 13-21.

Sugimoto, A., Yamaguchi, I., Nagatomi, S. *et al*. 1989. Sugarcane breeding in Okinawa at present time. "Features of characters of progenies produced by inter-specific hybridization between sugarcane commercial cultivars and *S. spontaneum*. *Tropical Agriculture Extra Issue*, 1989(2): 12-13. **Sugiomoto, A., Ponragdee, W., Sansayawichai, T. *et al.*** 1996. Collecting and evaluating of wild relatives of sugarcane as breeding materials of new type sugarcane cultivars of cattle feed in Northeast Thailand. *JIRCAS Working Report*, 30: 55-60.

Sugimoto A., 2000. Report of the Kyushu Branch of the Crop Science Society of Japan, 66: 80-84 (in Japanese)

Sugimoto, A. 2004. Breeding of high-biomass sugarcane ("Monster Cane") for sugar material production under unfavorable environmental conditions. *Farming Japan*, 38(4): 38-44.

Sugimoto, A. & Terajima, Y. 2006. Breeding high yield sugarcane for new biomass industry of sugar and another product. *Journal of Agricultural Mechanization of Japan*, 68(3): 3-8.

Tagane, S., Terajima, Y., Shikina, Y. *et al.* 2011a. Effects of day-length treatment and harvesting time on the flowering of *Erianthus rundinaceus* on Ishigaki Island, Japan, *Tropical Agriculture and Development*, 55(1): 45-50.

Tagane, S., Ponragdee, W., Sansayawichai, T. *et al.* 2011b. Characterization and taxonomical note about Thai *Erianthus* germplasm collection: the morphology, flowering phenology and biogeography among *E. procerus* and three types of *E. Arundinaceus*. *Genetic Resource Crop Evol*. (in press, Online First™, 4 August 2011).

Tagane, S., Sugimoto, A., Terajima, Y. *et al.* 2011c. Evaluation and classification of *Erianthus* germplasm in Thailand. *JIRCAS Research Highlight 2011*. Available at, http://www.jircas.affrc.go.jp/kankoubutsu/highlight/highlights2010/2010_20.html

Terajima, Y., Sugimoto, A., Fukuhara, **S. *et al.*** 2005. The feature of root growth of high yielding inter-specific hybrid between *Saccharum* hybrid and *S. spontaneum* L. *Proc. Int. Cong. Sugarcane Technol*., 25: 255-258.

Terajima, Y., Matsuoka, M., Irei, S. *et al.* 2007. Breeding for high biomass sugarcane and its utilization. *Japan. Proc. Int. Cong. Sugarcane Technol*., 26: 759-763.

Terajima, Y., Terauchi, T., Sakaigaichi, T. *et al.* 2010. Productivities of a large biomass sugarcane line "KY01-2044" in Nansei Islands. *Japanese Journal of Crop Science*, 229: 124-125.

Terajima, Y., Tagane, S., Shikina, Y. *et al.* 2011. Characteristics of an inter-generic hybrid between sugarcane and *Erianthus* at the spring planting. *Tropical Agriculture and Development*, 4 (extra issue 2): 45-46.

Terajima, Y., Shikina, Y., Sugimoto, **A. *et al.*** 2011. Characteristics of F_1 populations derived from crossing between *Erianthus* accessions. *Tropical Agriculture and Development*, 4 (extra issue 2): 46-47.

The taro improvement programme in Samoa: sharing genetic resources through networking

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The outbreak of taro leaf blight (TLB) in 1993 devastated taro production in Samoa. In response, several exotic taro cultivars from Palau, Federated States of Micronesia (FSM) and the Philippines, reported to have tolerance to TLB, were introduced to meet local production demand. The cultivars were highly recommended and were instrumental in establishing the TLB horizontal resistance breeding programme in Samoa. However, despite their positive impact the need to introduce more diversity to enrich a potentially narrow gene pool was recognized.

Several exotic cultivars of Southeast Asian origin were later introduced via the Secretariat of the Pacific Communit – Centre for Pacific Crops and Trees (CePaCT) in 2004 and were incorporated into the breeding programme to support the generation of breeding lines; they were characterized by improved disease resistance, good palatability and drought tolerance. Unlike previous breeding cycles, progeny from the crosses between taro of Asian origin and those of the Pacific showed a huge diversity in types.

Regional networks, namely TaroGen and TANSAO, made it easier for the Samoan breeding programme to freely access the much-needed genetic diversity from Asia. Consequently Samoan farmers are now planting several varieties generated from the first combination of taro from Asia and the Pacific with two promising progenies favoured by farmers and highly recommended for export by the Ministry of Agriculture and Fisheries.

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Introduction

Samoa lies between latitude 13º and 15º south and longitude 170º and 173º west in the South Pacific. There are two large islands with several small islets.

The country is small and is isolated from its main industrial neighbours (the island group is located 2 500 miles east of Australia, 1 900 miles northeast of New Zealand and 5 000 miles southwest of the United States' mainland).

The climate is tropical with very little variation in annual temperature; the mean lowland daily temperatures range from 27º to 30ºC and in the uplands from 20º to 21ºC. The small range in annual temperature variation is accompanied by a variation in humidity which ranges from 80 to 90 percent (Anon 1992).

The rainy season extends from November to April, with monthly rainfall ranging from 250 millimetres in the coastal lowlands, to 700 millimetres in the upland areas of both islands. The dry season extends from May to October, and is more pronounced in the northwest of Upolu and Savai'i Islands with monthly rainfall varying from 50 to 300 millimetres.

Samoa is also vulnerable to long dry spells that coincide with the La Niña phenomenon; as a result, unexpected prolonged dry weather started early in 2011 being more pronounced in the northwest of Upolu and Savai'i. It started in February and extended beyond October, which is normally the beginning of the rainy season. The summer of 2011 is one of the longest dry periods to have affected the whole of Samoa.

Taro as a staple food

Taro, *Colocasia esculenta* var. *esculenta*, is one of the most important members of the aroid family in terms of production, utilization and commercialization in Pacific Island Countries (PICs). Generally, taro is a staple starchy food in the region. It is consumed in large quantities and is the most important traditional starch source in the daily diet of Samoans. From 1987 through to 1990 – before taro leaf blight (TLB) caused by *Phytophthora colocasiae* – hit the country an average of 32 000 tonnes was consumed annually (Galanis *et al.* 1995).

Generally taro has comprehensive nutritional quality in its edible parts (corm and leaves). Taro corm when cooked is a very good source of energy, high in carbohydrates, potassium, protein and dietary fibre (Manner and Taylor 2010).

Recent studies have shown that root tubers (including taro) with coloured flesh are very rich in betacarotene, a component of vitamin A, which is important for the body's immune system (Englberger *et al*. 2003). Foods with a high level of carotenoids have been shown to protect against chronic diseases including cardiovascular disease and diabetes. Taro leaf is rich in vitamins and minerals, and is a very popular and cheap vegetable in Samoa.

Cultural and economic importance of taro

As a crop, taro ranks first in cultural and economic importance for Samoans. Taro has a long history of social and cultural attachment in Samoan villages and rural societies and this is also evident in other PIC cultures.

Taro is a highly valued commodity for traditional social activities such as weddings, opening of new churches, schools, community halls, funerals and other traditional occasions. It is generally believed that the importance of taro economically and culturally to the Samoan people originates from its unique taste and its early association in the local culture.

In Samoa, it is the most important root crop followed by other food crops such as *Alocasia* sp., *Xanthosoma* sp., yam (*Dioscorea* sp.), banana (*Musa acuminate*) and breadfruit (*Artocarpus altilis*) in terms of consumption. According to the 1989 Agricultural Census, 96 percent of agricultural households grew taro and it was reported that there were 16 000 hectares planted to taro, compared with only 3 600 hectares planted to ta'amu (*Alocasia macrorrhizos*) and 2 500 hectares under banana, prior to the TLB outbreak in 1993.

Taro as a major export crop

Taro, as Samoa's major export crop, established slowly in the late 1970s until it became the country's largest export earner from the 1980s till the early 1990s. In 1991 production was interrupted by Cyclone Val but recovery was rapid and export volumes continued to climb until TLB was accidentally introduced, completely destroying the crop in the middle of 1993.

Prior to the TLB outbreak in 1993, Samoa's taro exports to New Zealand stood at 6 300 tonnes (f.o.b. value WST9.5 million [US$3 990 000]). This represented 60 percent of the value of Samoa's exports in that year. The largest volume of exports was 7 800 tonnes in 1989 (Central Bank of Samoa 1999).

Because of the TLB outbreak and the susceptible nature of Samoan local cultivars grown at that time, taro exports dropped dramatically and remained at

a low level until recently, when new genotypes of taro from the regional taro breeding programme based at the University of the South Pacific (USP), Alafua Campus were released, evaluated and accepted by Samoan farmers and communities. From 2002 to 2009, annual exports averaged around 153 tonnes, of which 82 percent was shipped to American Samoa. In 2010 the first trial shipments of new genotypes were sent to New Zealand with a total volume of only 330 tonnes (McGregor *et al.* 2011).

The outbreak of TLB

Taro leaf blight was first observed on the island of Upolu at Aleipata and two days later in Saanapu and adjacent districts in July 1993. The disease spread rapidly throughout the country, severely affecting all local cultivars, but it was most devastating on the *Niue* cultivar which was preferred for commercial production because of its quality and taste. Various factors contributed to the rapid spread of the disease. The area planted to *Niue* at the time was extremely large and effectively ensured a vulnerable monocrop situation. There was a continuous and abundant source of taro for the disease because farmers interplanted on old plantations and staggered their cultivation. Combined with the widespread movement of infected planting material and ideal weather conditions, the disease quickly reached epidemic proportions. Early attempts to control its spread and impact using chemical and cultural control methods proved futile (Hunter *et al.* 2001).

Taro's narrow genetic base in Samoa: the need for exotic varieties

The narrow genetic base of taro in the Pacific was made all too clear in 1993 when TLB arrived.

Molecular studies revealed that there were two distinct gene pools separating Asia and the Pacific and diversity was far greater in Southeast Asia than in the Pacific. Greater genetic diversity was found in Indonesia where the two gene pools overlap (Lebot and Aradhya 1991). The studies also showed that the genetic base of taro in the Pacific was narrow with diversity decreasing eastwards towards the Polynesian groups. This was clearly demonstrated when all eleven of the traditional cultivars of Samoa proved highly susceptible to TLB.

In 1994, the European Union-funded Pacific Regional Agricultural Programme (PRAP) embarked on a programme to screen and evaluate exotic taro varieties, mostly from Micronesia, which were available in the USP Tissue Culture Unit.

Field evaluation of varieties, originating from the Federated States of Micronesia (FSM)[5] and the Philippines[6], were more resistant to TLB. These four varieties were further multiplied and evaluated in on-farm trials during 1996 to 1998. From these trials the Philippine variety, known locally as *Fili*, proved to be the most satisfactory in terms of TLB resistance and dry matter content (Brunt *et al.* 2001; Iosefa and Rogers 1999).[7] It was promoted to farmers by the Ministry of Agriculture and Fisheries and received a high level of acceptance.

Introductions from Palau followed, which showed good levels of resistance against TLB. Prior to the commencement of the taro breeding programme, *Fili* and some of the FSM and Palauan introductions, made an important contribution to resurrecting taro production in Samoa. These introductions were instrumental in getting nutritious *palusami*[8] back onto the dinner table of Samoan households. However, the release of a few introduced varieties was not sufficient to meet the needs of the growers. Despite their success, the taste and texture were not well suited to Samoan communities, who were keen to try and produce taro with the texture and quality achieved in pre-TLB days.

A breeding programme to broaden the taro gene pool

The Samoa taro breeding approach involved the development of complementary regional and national programmes. The Australian Agency for International Development (AusAID) was a major donor and the approach was implemented by the Secretariat of the Pacific Community (SPC), Taro Genetic Resources: Conservation and Utilisation (TaroGen), the University of the South Pacific – School of Agriculture in Samoa and the Ministry of Agriculture and Fisheries.

TaroGen

The impact of TLB on Samoa, the subsequent loss of taro genetic resources and the continuing vulnerability of other PICs to the disease was the major impetus behind the development of the regional project: Taro Genetic Resources: Conservation and Utilisation (TaroGen).

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5 *Pwetepwet*, *Pastora* and *Toantal* from FSM.

6 *PSB-G2* from the Philippines Seed Board.

7 Samoans prefer dry, firm-textured taro and therefore, percent dry weight is one measure of eating quality. Dry matter content of *PSB-G2* was 37 percent (Brunt *et al*. 2001).

8 *Palusami* is traditional use of taro leaves as a vegetable.

Yet, despite the gravity of the situation, it still took five years before a coordinated regional effort would commence implementation. Initially there was a lack of understanding as to the severity of the disease and the general consensus was that the problem would be over within a year. This lack of comprehension was compounded by technical advice from other projects that were promoting cultural control methods and the use of fungicides as appropriate control measures.

The five-year AusAID-funded TaroGen project finally began in 1998. The project supported taro breeding programmes in Papua New Guinea and Samoa, the objective being to provide growers with improved varieties to overcome production constraints that had durable resistance to TLB.

The TaroGen project supported taro collection and characterization in Polynesia and Melanesia, as well as the evaluation of various *ex situ* and *in situ* taro germplasm conservation strategies. In addition the project established key positions, namely the Team Leader and the Tissue Culture Specialist at SPC, Fiji and the services of a part-time breeder/pathologist based at the USP (Alafua) Campus. These positions proved essential in establishing the successful foundations of the taro breeding programme for the work that was to follow.

TaroGen provided the structure in which several agencies, each with specific skills and expertise came together to solve two problems – that of TLB in Samoa and erosion of taro diversity in the Pacific. As well as SPC and USP, other key agencies were the International Plant Genetic Resources Institute (IPGRI now Bioversity International), the Australian Centre for International Agricultural Research (ACIAR) and the Horticulture and Food Research Institute of New Zealand Ltd. (HortResearch).

IPGRI assisted with the rationalization of collections and identification of core subsets for each country collection.[9] ACIAR, through the University of Queensland (UQ), funded DNA fingerprinting and the virus-testing components. ACIAR also funded the Queensland University of Technology (QUT) to develop methods for diagnosis and detection of taro viruses. This work enabled the safe international movement of taro germplasm, which has been key in supporting the flow of germplasm from the SPC Centre for Pacific Crops and Trees (CePaCT) to the breeding programme in Samoa.

[9] TaroGen collected more than 2 200 different accessions (or samples) of taro from across the Pacific region. This large number needed to be reduced to a more manageable core collection. A core collection contains the maximum amount of genetic diversity within the smallest number of samples. This makes long-term conservation much more feasible, particularly where resources are limited. Also with core collections well characterized, this promotes use and exchange (Mary Taylor, personal communication). The more than 2 200 accessions have been reduced to a core collection of 196 which are housed at CePaCT.

HortResearch was funded by the New Zealand Ministry of Foreign Affairs and Trade to develop methods to assess leaf blight resistance in populations of progeny for the taro breeding programme.

The TLB-resistant lines selected from the Samoa breeding programme are now available to other countries in the Pacific, as well as globally, as shown by a recent distribution to the International Institute for Tropical Agriculture in Nigeria to support farmers in Africa in their battle against TLB.

The participatory nature of Samoa's taro breeding programme

One of major factors contributing to the devastation of taro in Samoa in 1993 was the relative uniformity (lack of genetic diversity) of the crop. Increasing genetic diversity on farmers' fields was identified by researchers at the USP as an important future disease management strategy. Researchers were concerned that lessons had not been learned and that production might revert to the pre-1993 situation if only one or two improved cultivars were widely distributed and promoted.

Discussions between researchers and farmers also revealed that some of the released introduced cultivars from Micronesia had a few shortcomings that included susceptibility to the disease in wetter parts of the country, low yields and poor storability. Farmers also raised concern about the length of time it was taking to get access to resistant germplasm evaluated through formal screening programmes.

In Samoa, the lack of progress prompted efforts to link farmers to the regional gene bank, to strengthen access to exotic cultivars, and hence genetic diversity, and at the same time to support crop improvement by linking to breeding expertise through the establishment of a participatory improvement programme based at the USP. As a result, in 1999, the Taro Improvement Project (TIP) was established with support from TaroGen (Hunter *et al.* 2001).

Taro Improvement Project

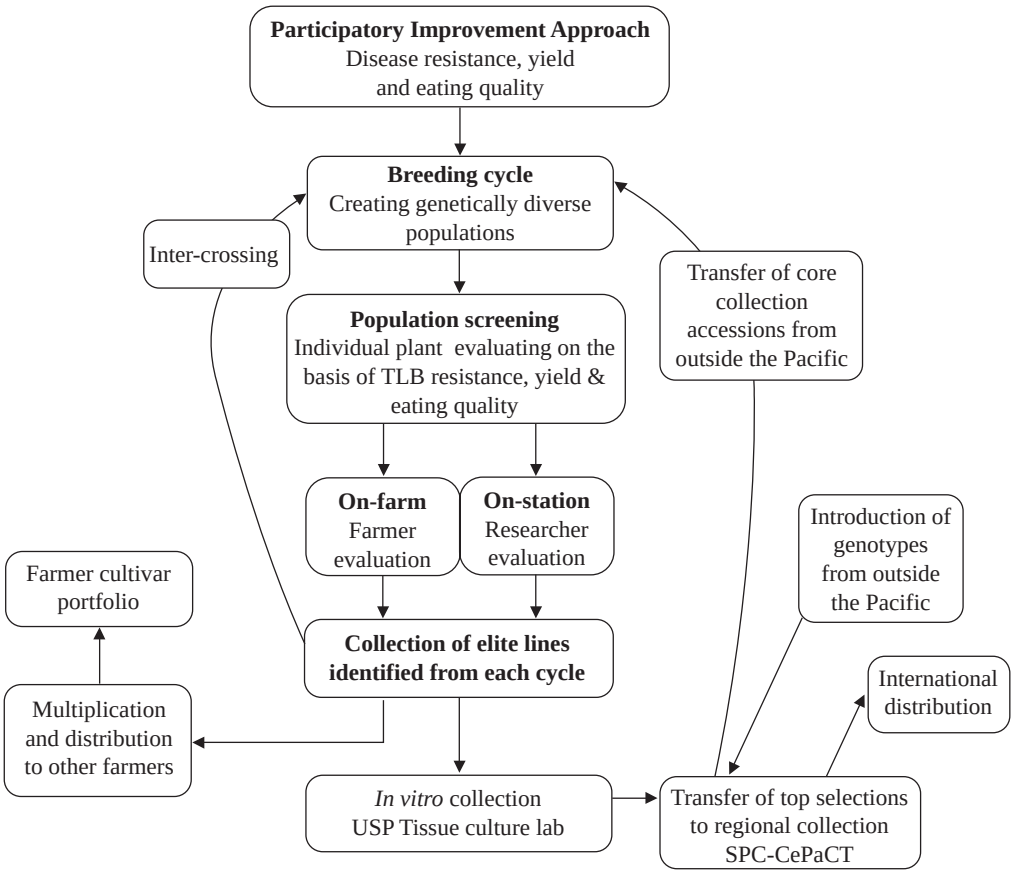
The project brought together scientists from USP and the SPC, national agricultural research and extension staff and farmers from the two islands of Upolu and Savai'i and was open to all farmers who agreed to compare taro cultivars using the participatory approach (Figure 1); this required taking part in ongoing monthly meetings, focus group discussions (FGDs) and regular farm visits to review the performance of the taro varieties. Crop-focused participatory appraisals were conducted with farmers' groups to learn about taro production

problems, farmers' perceptions of taro cultivars and key criteria important in the selection of cultivars, using various scoring and ranking exercises, FGDs and farm visits (Singh *et al.* 2010).

Taro diversity fairs were employed to showcase the breeding and evaluation work of the TIP. An attempt was made to establish a university taro breeders club at USP in order to integrate the elements of the breeding programme into teaching as well as to have a hands-on approach to allow students to learn about the breeding process in a practical way and to interact on a regular basis with farmers and scientists.

The TIP was supported by a regional gene bank (now CePaCT) established by the TaroGen project, which greatly improved access by farmers to exotic cultivars and at the same time provided an opportunity for any breeding lines generated by the TIP to be distributed to other countries (Figure 1).

Figure 1. TIP participatory improvement approach illustrating links to formal PGR system and information farmer network



Source: Iosefa *et al.* (2012, in press).

The TIP made some early progress and farmers evaluated and selected from clones of sexual crosses between cultivars from Samoa, Palau and the FSM (Hunter *et al.* 2001). Although selected lines from these crosses enabled farmers to start growing and eating taro again, there was concern that breeding from such Pacific cultivars alone would not significantly broaden the genetic base of taro in Samoa and might decrease heterozygosity and lead to inbreeding depression (Camus and Lebot 2010).

To broaden the genetic base it was felt necessary to provide farmers with access to virus-indexed germplasm from Asia, a bringing together of the two distinct taro gene pools.

The main objective of the TIP is to give Samoan taro farmers more options for improved taro production and management of TLB.

The USP/SPC taro breeding programme

The first cycle of the taro breeding programme was initiated in 1996/1997 prior to the start-up of the TaroGen project. Cycle 1 combined some Micronesian lines, with *PSB-G2* from the Philippines and some Samoan lines. The second cycle included varieties from Palau and combined them with selected clones from cycle 1. In cycle 3, *Niue* was reintroduced into the breeding cycle because of the importance of this variety for domestic and export markets.

Yet the progeny from the top cycle 3 clones showed few *Niue* traits, probably due to the susceptibility of TLB of lines with *Niue* parentage (SPC Land Resources News Letter April 2009). However, because of the importance of *Niue* some of these susceptible lines were still included in later crosses.

Cycle 4 consisted of crossing cycle 3 lines. Field observation showed that the progeny from these crosses were very uniform in their characteristics indicating the breeding programme had possibly reached a genetic ceiling. Further progress could only be achieved therefore with infusion of new genetic material from outside the region. It was through TaroGen and the regional gene bank that taro germplasm was introduced from outside the region.

Cycle 5 saw the introduction of TANSAO (Southeast Asian) lines from SPC. TANSAO (the Southeast Asia Taro Network) was established through European Union funding. One of the major outputs of this network was the development of a core sample of 120 varieties from Southeast Asia. This core sample was transferred to the SPC/CePaCT, and from there, virus-tested and selected varieties were made available to the Samoa breeding programme. This input of

Table 1. Taro used in the Samoa breeding programme

Region/countries	Number	Sources
Polynesia		
Samoa	4	Samoa
Micronesia		
Federated States of Micronesia	3	USP Tissue Culture Unit
Palau	7	Univ. of Hawaii/MAFF
Southeast Asia		
Philippines	1	MAFF
TANSAO Network		
Philippines	1	SPC – CePaCT
Thailand	4	SPC – CePaCT
Indonesia	3	SPC – CePaCT
Malaysia	5	SPC – CePaCT
Papua New Guinea	0	SPC – CePaCT
Total	**28**	

new diversity sufficiently broadened the genetic base to provide the opportunity for developing new varieties with such qualities as greater disease resistance, good palatability and drought tolerance.

Cycle 5 is described in the SPC Land Resources News Letter:

> Taro plants that were dwarf and TLB-susceptible to plants that were large (over 2 m) aggressive and highly tolerant to TLB, from dark to sickly plants to handsomely coloured plants, from single to multiple shoots – some up to 50 shoots and thus threatening invasiveness producing no corms to plants producing consumer preferred corms (April 2009).

With the selection of promising lines from such genetic diversity it was possible to breed back *Niue* to see if the variety's palatability could be achieved without risking disease susceptibility. Several cycle 5 (which had *Niue* genes from cycle 3 breeding) varieties were selected for the sixth breeding generation.

These clones were pollinated using pollen from the *Niue* variety to generate the *Niue* 1st back-cross generation (cycle 6 or sixth generation of breeding). Some of these cycle 6 lines were selected for the making of cycle 7 (the continuation of back-crosses).

> We crossed our preferred Samoan varieties with varieties from Indonesia and Malaysia that had leaf blight resistance. In that way we could keep the traits of our familiar and locally adapted varieties, and integrate disease resistance. It took time and a lot of work, but ultimately it was successful (ACIAR 2011).

Beginning in 2009, some 13 years after the breeding programme began, new varieties began to be widely promoted to farmers for local sale and export; these very desirable varieties appeared on the scene with the vigour of the TANSAO material and the palatability of the *Niue* cultivar – although the distribution of these varieties is still constrained by the availability of planting material.

The problem of TLB has been essentially solved and a satisfactory degree of local market consumer acceptability has been achieved. However, if exports are to be restored to their pre-blight levels, more work is required to determine overseas consumer preferences so that the breeding programme can focus on the preferences of this market, bearing in mind the need to still promote and maintain diversity (McGregor *et al.* 2011).

Emphasis is also now shifting to target drought tolerance in the breeding programme in an attempt to generate lines that can be used to combat predicted climate variables.

Outcome and benefits from sharing genetic resources through networking – the success of the TIP

The establishment of CePaCT and the availability of virus-indexed core collections have allowed farmers in the Pacific much needed access to the diversity of the Asian gene pool. Several accessions from the TANSAO core collection were first introduced into the TIP breeding programme during the creation of breeding cycle 5 in 2005 (Figure 1). To date, seven cycles of breeding including researcher and farmer selection have been completed (Tables 2 and 3). The speed at which improved taro germplasm is distributed to farmers has significantly improved. Over the course of the participatory improvement programme in Samoa farmers have proven adept at handling, evaluating and selecting for their many needs from many clones, lines and cultivars. Some research-minded farmers have managed and selected from large segregating populations.

In 2009, the Ministry of Agriculture and Fisheries made recommendations for five cultivars of which Samoa 1 and 2, identified from breeding cycle 5, are the most preferred for the export market. Further, the top selections from each breeding cycle in Samoa have been tissue-cultured and transferred to CePaCT where they have been virus-indexed, and are therefore available for wider distribution. In 2009 the Pacific region agreed that the Annex 1 collections held by CePaCT be placed in the Multilateral System of the International Treaty on

Table 2. Summary of top selections from the TIP over the past 15 years

	Year	No. of parental combinations	No. of seedlings evaluated	Top selections
Cycle 1	1996	4	2 000	10
Cycle 2	1998	5	2 000	26
Cycle 3	2000	26	2 000	30
Cycle 4	2002	45	5 000	30
Cycle 5	2005	30	5 000	42
Cycle 6	2007	33 + 9 BCF_1*	11 000	40
Cycle 7	2009	36 (17 BC_1**)	12 000	25***

Source: Iosefa *et al.* (2012, in press).

* BCF_1, first filial generation of the back-cross to taro *Niue* (most preferred local cultivar pre-TLB).

** BC_1, second generation of the taro *Niue* back-cross.

*** As of May 2011, 70 percent of these selections are being conserved at CePaCT, 18 percent of which have been virus-indexed and distributed by CePaCT.

Plant Genetic Resources for Food and Agriculture (ITPGRFA). As such these top selections are now available globally to farmers and breeders.

Securing the future of taro on a broader base of genetic diversity

The injection of 'new blood' from Asia into the Pacific taro gene pool has opened the door to improved TLB resistance, improved taste, higher yields and nutritional benefits through higher levels of carotenoids in the corm. Now with good levels of disease resistance in the taro crop the focus of participatory improvement work in Samoa has shifted to the identification of climate-ready taro. Again close links with CePaCT will ensure that the work of the programme in Samoa will have regional and global impact. The selected taro will be included in the CePaCT climate-ready collection for evaluation in the 22 SPC member countries and territories, and beyond if requested. However, TLB and other taro pests and diseases do not stand still and the genetic base of taro in other countries and regions of the world remains narrow and vulnerable as the recent outbreaks of TLB in West Africa (Bandyopadhyay *et al.* 2011) and the Caribbean demonstrate (Rao *et al.* 2010).

The participatory crop improvement approaches developed and tested under TaroGen and TANSAO and their outputs have opened up promising opportunities for taro farmers in other parts of the world which did not exist over a decade ago. TLB-resistant lines developed through the TIP coupled with advanced virus-testing technology have now made it possible for CePaCT to transfer farmer-selected resistant lines, under the auspices of the ITPGRFA, to

Table 3. Summary from preliminary trials of selected seedlings for TLB screening from each breeding cycle generation

Characteristics/cycle				
Number of functional leaves*	**No. seedlings**	**Mean value**	**Min.**	**Max.**
Cycle 3	188	3.839	2	7.4
Cycle 4	296	4.091	2	6.8
Cycle 5	156	4.378	2	8.0
Cycle 6	100	4.091	2	6.3
Cycle 7	120	4.500	3	7.0
Palatability score **				
Cycle 3	188	2.789	1	4
Cycle 4	296	2.832	1	4
Cycle 5	156	2.863	1	4
Cycle 6	100	2.896	1	4
Cycle 7	120	2.800	1	4
Corm yield (kg)				
Cycle 3	188	0.66	0.2	1.8
Cycle 4	296	0.82	0.4	1.5
Cycle 5	156	0.72	0.3	1.6
Cycle 6	100	0.46	0.2	1.2
Cycle 7	70	0.76	0.5	1.2

Source: Iosefa *et al.* (2012, in press).

*Refers to number of healthy leaves per plant.

** Palatability score: 1 = poor; 2 = adequate; 3 = good; 4 = excellent.

places where new disease outbreaks occur such as the recent TLB outbreak in West Africa. In response to this, CePaCT has recently transferred resistant lines to Nigeria for evaluation against the recent TLB outbreak there. Recognizing this global interdependence on geographically-distant taro germplasm, the European Union has contributed €3 million over five years to the newly formed International Network for Edible Aroids (INEA) which commenced in January 2011. INEA brings together scientists from 18 countries worldwide, two regional organizations: SPC and the Caribbean Agricultural Research and Development Institute (CARDI) and three international agricultural research organizations: CIRAD, IITA and Bioversity International. Through CePaCT, INEA will provide taro farmers with elite cultivars and hybrids which they will evaluate and select

from for local conditions, using participatory approaches (Iosefa *et al.* 2012, in press).

A major focus of the work of INEA will be to provide support to farmers to help their efforts to improve adaptation of taro to changing climate, especially drought tolerance. INEA is building on the work initiated by both TaroGen and TANSAO which showed the benefits that can be gained by linking farmers with scientists, and building on the practices which farmers have nurtured and developed over centuries, supporting the process of genetic adaptation and thereby enhancing the resilience of taro-based farming systems (Iosefa *et al.* 2012, in press).

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All authors were actively involved in the TaroGen project and are members of INEA.

References

Anon. 1992. *Land resource planning study Western Samoa.* Asian Development Bank T.A. No. 0165-SAM Final Report.

Bandyopadhyay, R., Sharma, K., Onyeka, T.J. *et al.* 2011. First report of taro (*Colocasia esculenta*) leaf blight caused by *Phytophthora colocasiae* in Nigeria. *Plant Disease,* 95: 618.

Brunt, J., Hunter, D. & Delp C. 2001 *Taro leaf blight – an updated. bibliography.* Suva, Fiji, Secretariat of the Pacific Community.

Camus, P. & Lebot, V. 2010. On-farm assessments of clonal production of root crop diversity in Vanuatu, Melanesia. *Experimental Agriculture*, 46(4): 541-559 Cambridge University Press, 2010.

Central Bank of Samoa. Bulletin, December 1999 Apia, Samoa.

Englberger, L., Darnton-Hill, I., Coyne, T. *et al.* 2003. Carotenoid-rich bananas: A potential food source for alleviating Vitamin A deficiency. *Food and Nutrition Bulletin,* (24: 4). The United Nations University.

Galanis Daniel J,. Chin-Hong Peter V., McGarvey Stephen T., Messer Ellen., Parkinson David., 1995. Dietary Intake Changes Associated with Post – Cyclone Food Aid in Western Samoa. Ecology of Food and Nutrition. Vol. 34. Pp. 137-147.

Hunter, D.G., Iosefa, T., Delp, C.J. *et al.* 2001. Beyond taro leaf blight: a participatory approach for plant breeding and selection for taro improvement in Samoa. *In Proceedings of the international symposium on participatory plant breeding and participatory plant genetic resource enhancement*, pp. 219-227. CGIAR Systemwide Program on Participatory Research and Gender Analysis for Technology Development and Institutional Innovation. Cali, Colombia, CIAT.

Iosefa, T. & Rogers, S. 1999. *The multiplication, growth and use of introduced taro cultivars in Samoa.* Report of an impact assessment carried out during August to November, 1998. Suva, Fiji Islands: Pacific Regional Agricultural Programme Project 1 – Farming Systems in Low Lands.

Iosefa, T., Taylor, M., Hunter, D. *et al*. 2012. in press. Linking farmers, genebanks and networks for genetic enhancement of taro. *In* W.S. De Boef, N. Peroni, A. Subedi & M.H. Thijssen, eds. *Community biodiversity management: promoting resilience and the conservation of plant genetic resources*. Earthscan publications.

Lebot, V. & Aradhya, K.M. 1991. Isozyme variation in taro *Colocasia esculenta* (L.) Schott from Asia and Oceania. *Euphytica,* 56: 55-66.

Manner, H.I. & Taylor, M. 2010. Farm and forestry production and marketing profile for taro (*Colocasia esculenta*). *In* C. Elevich. *Specialty crops for Pacific Islands Agroforestry*. Available at, http://agroforestry.net/scps.

McGregor, A., Iosefa, T., Mariner, L.T. *et al.* 2011. The Economic Impact of Samoa's Taro Germplasm Breeding Programme. Unpublished manuscript.

Rao, R.V., Hunter, D., Eyzaguirre, P.B. *et al*. 2010. Ethnobotany and global diversity of taro. *In* R.V. Rao, P.J. Matthews, P.B. Eyzaguirre & D. Hunter, eds. *The global diversity of taro: ethnobotany and conservation*, pp. 1-5. Rome, Bioversity International.

Singh, D., Hunter, D., Iosefa T., *et al.* 2010. Improving taro production in the South Pacific through breeding and selection. *In* R.V. Rao, P.J. Matthews, P.B. Eyzaguirre & D. Hunter, eds. *The global diversity of taro: ethnobotany and conservation*, pp. 168-184. Rome, Bioversity International.

The impacts of conservation of rice genetic resources

Duncan Vaughan[1]

Economic analysis suggests that the expenditure on collecting, maintaining and evaluating conserved rice germplasm, well over 100 000 different accessions, is money well spent. In this paper several examples of outstanding sources of stress resistance are given. These examples illustrate several points: (a) time from collection to evaluation and use can span many decades; (b) stress resistance may be found in areas where it might not be expected (allopatric resistance); (c) evaluation is just the first stage in fully understanding useful traits; often identifying stress resistance is not simple and several useful traits may be found in a single accession; (d) while core collection is a useful and cost-efficient approach to evaluation the potential for finding useful traits in the whole collection and in some individuals of heterozygous accessions should be remembered.

Finally four 'green revolutions' that have helped to dramatically improve rice productivity are discussed – double cropping, the rainfed lowland rice variety 'Mashuri', the semi-dwarf rice variety and hybrid rice. These green revolutions provide insights into how dramatic change in rice agriculture has occurred. Through rice breeding, the impact of rice genetic resources, now conserved in gene banks, on rice production in the last 40 years is reflected in the lack of famine in most of the rice-eating world.

Introduction

Rice is the staple food for more of humanity than any other crop. Rice has helped shape our world and human society. Its extraordinary adaptability to different growing conditions and nutritious grain wrapped in a tight protective husk (palae and lemma) are in a large measure why the population of Asia is so vast. Two different plant species in the genus *Oryza* were independently domesticated in Asia and Africa. But today Asian rice is the dominant species and is a staple in many countries all over the world.

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The challenge for rice scientists is ensuring that the genes needed for rice improvement in the future are available to complement advances in associated branches of rice agriculture. From 1961 to 1990 yields per hectare grew annually at a rate of 2 percent. However, since 1990 rates of yield increase have dropped to below 1 percent despite astonishing advances in the biological sciences (Fisher 2011).

This paper focuses on successful rice germplasms that have provided useful genes for rice improvement. These stories might pave the way for future improvements in rice productivity.

A. Economics

Policy-makers want facts and figures that provide evidence that funds are providing a good return on investment. Economists have tried to determine the real return on investment in conserving and evaluation of rice genetic resources (Evenson and Gollin 1997). Initial studies focused on an accession of the wild annual *Oryza* species *O. nivara*[2] that provided the only source of resistance to grassy stunt virus. This famous accession was heterogeneous for resistance to the virus with only three out of ten seeds tested showing resistance (Chang 1989). However, the resistant gene from those three resistant plants was transferred to elite breeding lines at IRRI. This gene transfer from wild to cultivated rice has become established in all elite rice lines. The transfer provided the basis for economic analysis and it was estimated that the initial return on the use of that gene was about US$50 million. More than enough to cover all the costs associated with conserving the rice gene pool to that point. The gene still provides resistance so its benefits continue.

It is not a simple task to estimate the return on investment of germplasm. However, the simple fact is that the annual global rice harvest has been sufficient to prevent famine despite the decline in land area devoted to rice production as a result of urban expansion and increasing population. This reflects the results of improvement in the genetics and agronomy of rice agriculture as well as infrastructure improvements such as irrigation systems.

[2] Accession number in the International Rice Research Institute's (IRRI) gene bank, 101508.

B. Characterization and evaluation – unexpected results

Silewah

Self-sufficiency in rice is the aim of all countries where rice is a staple and for most of the last 50 years Japan has been self-sufficient in this respect. However, in 1993, in part due to the effects of El Niño, a wet and cool August resulted in a much-reduced rice crop and Japan was forced to import rice. Rice grows in Japan at some of the highest latitudes for this crop and hence cold tolerance is a very important trait to incorporate into rice-breeding programmes.

In 1974 Dr. Antonio Perez (then of IRRI) and Indonesian colleagues were collecting rice on the morning of 15 February, a Friday, the Muslim day of prayer in North Sumatra. That day they visited Kabupaten Simpang Empet near Lake Toba in the highlands at 1 300 metres. This village is located just 3^{o} north of the equator. There the team of rice scientists collected *Page Silewah* (*Page* means rice). In Simpang Empat many of the varieties collected were upland (*gogo*) rice plants with poor seed quality. Perez writes in his notes about *Silewah*, "14-291 Page Silewah/gogo, highly diseased, bacterial leaf blight, neck blast, high sterility, 6.5 months duration." This does not sound like good germplasm for rice improvement.

Cold tolerance is an important but complex trait because cold tolerance at one stage in the life cycle of rice is controlled by different genes from cold tolerance at another stage. Extensive evaluation to find germplasm adapted to the tropics with cold tolerance at the seedling stage and anthesis was started in the 1970s at IRRI (Vergara *et al.* 1976). By a sequential process of elimination, the original 24 158 entries in the germplasm collection evaluated were reduced to 11 germplasm accessions tolerant to cold temperatures at the seedling, booting and anthesis stages (Vergara and Visperas 1984). These varieties were evaluated further in Japan and the Republic of Korea. The results of these tests highlighted *Silewah* as one of the most cold-tolerant varieties (Satake and Toriyama 1979). Consequently, *Silewah* was crossed with a japonica breeding line, Hokkai 241, in Japan and to IR38 at IRRI. Using the technique of rapid generation advance, these crosses were rapidly taken towards homozygosity and bulked. In 1982, four years after the original crosses were made, lines were being tested in the international rice cold-tolerant nursery (Vergara *et al.* 1982). Lines from these crosses were tested in international trials for three years in more than ten locations.

Silewah has proved to be an outstanding cold-tolerant gene source for rice breeders. In 1990, North Sumatra was again visited by an international team of

rice collectors to collect wild rice germplasm that grows on forested upland slopes in that province. The team was collecting wild *Oryza* germplasm and was close to Simpang Empat therefore it was decided to see if *Silewah* was still growing there. When the team checked Simpang Empet and surrounding villages late in the day, one farmer recalled a variety he called 'Sileu' but he said it was not a good variety and so no longer grew in the area. Tony Perez noted in 1974 that *Silewah* was susceptible to pests and diseases; however, on evaluation it was found to be very good with respect to cold tolerance.

Studies of *Silewah* and derivatives in crosses with different varieties in Japan have continued to this day. Silewah is a tropical japonica variety showing partial sterility in crosses between both indica and temperate japonica varieties when crossed (Ikehashi and Araki 1984). Based on a cross between Norin PL8 and *Silewah*, quantitative trait loci (QTLs) for cold tolerance at the booting stage were found on rice chromosomes 3 and 4 (Saito *et al.* 1995). Fine mapping of QTLs on the long arm of chromosome 4 indicated two QTLs for cold tolerance on this chromosome, designated *Ctb-1* and *Ctb-2* and these QTLs were associated with anther length (Saito *et al.* 2001). Subsequently a 56Kb region was determined as associated with *Ctb-1* and seven open reading frames (ORFs) were found in this region. Two ORFs encode receptor-like protein kinases possibly involved in signal transduction pathways. Three ORFs encode proteins that may be associated with ubiquitin-proteasome pathways and two ORFs encode F-box proteins (Saito *et al.* 2004). *Ctb-1* has now been cloned (Saito *et al.* 2010).

Recently breeding lines between *Silewah* and other Japanese varieties (Kita-ake, Yukihikari and Dohoku 50) were analysed for cold tolerance and genomic regions with chromosome segments from Silewah were found on chromosomes 3, 4 and 11. Analysis showed that the allele from *Silewah* on chromosome 3 conferred cold tolerance and was designated *qCTB3*-Silewah (Mori *et al.* 2011). This suggests that different QTLs might be exploited from the same source in different breeding programmes depending on the genetic background and environmental conditions (Mori *et al.* 2011).

Goda Heenati

Another example of a variety that is an unexpected source of a useful trait is *Goda Heenati*. In tests for submersion tolerance this variety was found to be one of a group of varieties that performed very well. The reason why this is a surprise is that it is from Sri Lanka where it is an upland rice. Although some rice varieties have been known to show tolerance to submergence since the 1950s, such as FR13A (Flood Resistant 13A), it has taken more than 50 years

to understand the mechanism and get the relevant gene into elite varieties (Bailey-Serres *et al.* 2010).

C. Pests and diseases

Brown planthopper

Brown planthopper is one of the most important rice insect pests not just for the damage it does itself but also because it transmits Grassy Stunt Virus and Ragged Stunt Virus. More than 20 genes for brown planthopper resistance are now known (Jena and Kim 2003). IR64 was developed in 1986 and this had genes for resistance to brown planthopper biotype 1 (Brar *et al.* 2009). IR64 has been one of the most successful lowland irrigated rice varieties of all time and one reason for its continuing popularity in countries like Indonesia is due to its resistance to brown planthopper based on the gene *Bph1*. However, in 2009 after more than 20 years the resistance broke down in Indonesia (Sutrisno, personal communication 2011). This has required a hunt for new sources of resistance. One particularly good resistance gene is found in the Sri Lankan variety *Rathu Heenati* a cultivar that is resistant to four brown planthopper biotypes. The gene *Bph3* from this variety has been used in breeding but one problem is that it is closely linked with the waxy locus (Jairin *et al.* 2009). However, this linkage can be broken. *Rathu Heenati* also has the gene *Bph17* that might explain the broad spectrum of resistance this variety has to different biotypes (Sun *et al.* 2005).

On Sunday, 30 November 1958, the late H.I. Oka, then of the National Institute of Genetics, Japan was in northern Thailand with Thai colleagues collecting rice germplasm. That day while walking along a forest trail at Si Samrong, Sukothai, he made the ninth collection of the trip – *Oryza officinalis*. Seeds from this population were taken back to Japan, where it was conserved (Oka 1958). In 1964, seeds of that accession (W0065) were sent to IRRI for duplicate conservation and research where it was assigned the accession number 100896.

In 1980, the Entomology Department of IRRI screened this wild rice accession along with many others for resistance to various insect pests. The *Oryza officinalis* from Si Samrong was found to be resistant to races of brown planthopper, green leafhopper and zigzag leafhopper (Heinrichs *et al.* 1985).

In 1984, K.K. Jena crossed this accession of wild rice with elite breeding lines of cultivated rice. After embryo rescue, the hybrid was back-crossed to cultivated rice. In 1987 trials, some back-crossed progeny were the highest yielding in two seasons. In 1989, rice lines with genes from the Thai wild rice

entered international yield trials (Vaughan and Sitch 1991). As a result of yield trials in Viet Nam, five of the lines with *O. officinalis* genes were released as brown planthopper-resistant varieties – MTL98, MTL103, MTL105, MTL110 and MTL114 (Brar 2005).

O. officinalis provides resistance to brown planthopper biotype 4 common in South Asia and the gene for resistance has been designated *Bph13*. *Bph13* is located on rice chromosome 3 and a co-dominant linked sequenced-tagged sites (STS) marker has been developed that is 1.3 cM from the gene to use in marker-assisted selection (Renganayaki *et al.* 2002). Other workers found a different gene for brown planthopper resistance from this wild rice on chromosome 11 that was also resistant to Indian biotypes of the pest (Jena *et al.* 2003). Japanese scientists have also studied brown planthopper resistance from *O. officinalis* and found new genes for resistance to biotype 1 on chromosome 3 linked to RFLP marker G1318 and also a gene on chromosome 4. They designated these genes *Bph11* and *Bph12* (Hirobayashi *et al.* 2003).

Today brown planthopper remains a major problem in Asia, although it is often quiescent. Indonesia in the last few years has seen an increased threat from this pest. The abundant sources of resistance available in both cultivated and wild rice genetic resources plus the new tools available to incorporate these materials into breeding lines should provide the resources needed to control the effects of this pest and its associated virus diseases.

Tungro resistance

On Friday, 6 January 1989, Dr. Songkran Chitrakon and the author visited the deep water lowlands around the ancient city of Ayutthaya, just north of Bangkok, Thailand. To get seeds of the abundant wild rice growing there (*Oryza rufipogon*) they used a small boat. During the day eight samples from different parts of the low-lying area were collected. At that time the straw of *O. rufipogon* was used to make rope by local people.

These samples are conserved in Thailand and at the IRRI gene bank. Evaluation of a wild rice core collection revealed that samples (particularly 105908 and 105910) collected on that day had resistance to rice tungro (a complex of two viruses) but susceptibility to the virus vector green leafhopper (Kobayashi *et al.* 1993a, b; Angeles *et al.* 1998). These were accessions crossed to elite germplasm, including IR24 and IR64. Based on rigorous tests of the best advanced breeding lines with tungro resistance/tolerance from different sources, the source from Thai *O. rufipogon* showed resistance to spherical tungro virus, and tolerance to bacilliform tungro virus (Angeles *et al.* 1998). Subsequently

a tungro resistance source from *O. rufipogon* (accession 105908) was released in the Philippines as Matatag 9.

More recently the 'apparent' resistance to tungro in Matatag 9 has been studied in detail (Shibata *et al.* 2007); the 'resistance' from *O. rufipogon* to tungro virus may be a combination of resistance to green leafhopper and tolerance to tungro viruses (Shibata *et al.* 2007). Evaluation of germplasm is critical for it to be used. However, evaluation for important pests and diseases is complex, particularly in the case of viruses and their vectors and requires rigorous and multiple testing to ensure that the trait of interest is properly characterized and can be appropriately deployed.

D. Problem soils

Acid sulphate soils

Oryza rufipogon grows in waters that are acidic (i.e. black water) rather than alkaline (white water). In the Sepik River of Papua New Guinea, *O. rufipogon* grows in the Black Water Lake but not in nearby Lake Chambri that has white water. There are some populations in Viet Nam that grow acid sulphate soils that can be readily recognized by the orange colour of the soil. On Thursday, 20 December 1990, a team of germplasm collectors, including Dr. B.C. Buu and the author, went by boat to the village of Hao An in the Mekong Delta, Viet Nam, to collect wild populations of *Oryza rufipogon*. From the orange colour of the soil there in which *O. rufipogon* grows it was clear that this population was resistant to acid sulphate soil conditions.

The accession collected (106412) was used in crosses with IR64. From the cross, lines were sent to the Chu Long Rice Research Institute (CLRRI) in 1995 for evaluation. Tested in both target and non-target sites three promising lines were selected and sent to CLRRI's yield-testing unit. IR73678-6-9-B (AS996) was released in 2000 as a variety for commercial cultivation in the Mekong Delta and has occupied 100 000 hectares (Brar 2005). AS996 has become an important parent for further rice improvement in Viet Nam and other countries. It is a short duration variety with qualities suitable for moderately acid sulphate soils; it is also tolerant to brown planthopper and blast disease (Brar and Khush 2002). Brown planthopper resistance was shown to be derived from *O. rufipogon*, in a recessive gene; it has been designated *Bph24*(t) (Deen *et al*. no date). The genetics of tolerance to abiotic stress were investigated for this accession and a major QTL for relative root length that is a primary parameter for aluminium tolerance was found to be located on chromosome 3 of the accession (Nguyen *et al.* 2003).

E. The green revolutions of rice – past and future

The history of Asia has been shaped by rice. Rice was domesticated originally in China and subsequently two major varietal groups evolved – indica and japonica (Vaughan *et al.* 2008; Molina *et al.* 2011). Domestication of rice can be considered 'green evolution' but since domestication of rice four 'green revolutions' of rice can be identified.

Short duration rice: Indica and japonica have been grown in China since the Neolithic Era (Bray 1984). However, it was the introduction of short duration varieties into the rice-cropping system of southern China that represented a major agricultural breakthrough. The introduced varieties, called Champa varieties, probably came from Viet Nam. These short duration varieties had two impacts. The first was that rice could be grown and harvested on marginal lands subject to inundation in the second half of the growing season. The second impact was to fit rice into various cropping systems resulting in significant yield increases. The ability to double crop is one reason why the population of China was able to grow very quickly.

Mashuri: A second green revolution was the result of a small FAO-funded project that involved indica-japonica hybridization. The hybridization was done at the Central Rice Research Institute, Cuttack, India. A group 65 crosses of which 13 were indica x japonica (F_2) lines was received by the Rice Department Malaysia in 1956. The crosses were grown in bulk until the F_7 line was achieved. In the F_7 line, progeny of each cross were put into F_8 rows. Pedigree selection was undertaken in 1959 (F_8) and subsequent selection was made after observing a line of two rows consisting of 40 hills. In 1961/1962 main season formal yield trials (F_{15}) were performed; in the F_{16} line farmers' trials were undertaken. In the F_{17} generation, nine years after the original cross was made, two varieties were released in 1965 as Malinja and Mahsuri (Van 1966). Mahsuri became popular in Malaysia and was also tested in India, but due to susceptibility to diseases it was not recommended for release. However, farmers obtain seeds of the variety and Mahsuri spread across the Terai of India and Nepal. Mahsuri was very well adapted to rainfed lowland conditions and its good quality made it popular. It became known as the green revolution variety of Nepal (Gyan Lal Shrestha, 1988, personal communication). Mahsuri spread to Myanmar and also to northern Viet Nam. In Myanmar a natural short stature mutant of Mahsuri was found in the field and selected. This mutant Mahsuri became popular in Myanmar as it was resistant to lodging. In 2010, 45 years after Mahsuri was released in Malaysia its mutant form was still the most popular variety across the rainfed areas of Myanmar (Ms. Aye Aye Myint, Myanmar Seed Bank, personal communication 2010).

The IR series: The great green revolution of the 1960s and 1970s was founded on the introduction of short stature into rice. Traditional varieties of rice are invariably tall and prone to lodging. The IRRI series of IR varieties and derivatives has dominated the irrigated lowlands of tropical Asia since then – the most famous of which have been IR8 and IR64. This green revolution was not just based on plant breeding but also on associated agronomic practices, particularly application of fertilizers.

Hybrid rice: The development of hybrid rice that began in the early 1970s required a good source of male sterility. Wild rice has been the major source of male sterility including cytoplasm male sterility. It is not uncommon to find sterile plants in wild rice populations (author's own observations). At the end of the twentieth century 90 percent of the area planted to hybrid rice in China traced its cytoplasm to wild abortive (WA) cytoplasm. This type of cytoplasm was found in a wild rice from Hainan Island, China. This wild rice was believed to be a hybrid between the red awned wild rice of the island and a late-cultivated rice cultivar. It was discovered in 1970 and the plant had very strong tillering, a slender culm, a narrow leaf and sheath, long red awns, coloured seeds and long seed dormancy. Anthers only dehisce when temperatures are above 30^{o}C for several days. Hundreds of WA type A lines have been developed by crossing rice cultivars with good maintaining ability.

In China commercial production of hybrid rice began in 1976. Production-wise there were 2.1 million hectares of hybrid rice in 1977, 10.9 million hectares in 1983 and 15.3 million hectares in 1997. From 1976 to 1997 hybrid rice enabled China to increase rice production by more than 312 million tonnes (Li and Yuan 2000). The hybrid rice green revolution of China, as with the IR series green revolution, was not based on genetics alone but required a carefully planned agronomic system to ensure its success.

Conclusions and the next green revolution

- Based on various analyses the return on investment in conservation of rice, and other crop genetic resources, has been very high.
- Systematic and rigorous characterization and evaluation is an essential interface between conservation and use of rice genetic resources. As there is now less emphasis on collecting rice genetic resources the emphasis should shift to characterization and evaluation of these genetic resources. While molecular characterization is receiving much attention, the need for more information on adaptation of genes in rice to changing environments is imperative. Initiatives, such as the Global Partnership

Initiative for Plant Breeding Capacity Building (GIPB[3]), which promote and achieve characterization and evaluation, should be a major focus of investment.

- From the examples given in this paper it is also clear that a deeper understanding of useful traits reveals their complex genetic nature. For both *Silewah* and Thai *O. officinalis* the trait of interest is not based on a single gene but several and these different genes may be exploited in different circumstances. Characterization and evaluation should not only be systematic and rigorous, but potentially useful germplasm needs to be studied in depth to fully understand traits of interest. This may mean retesting after different crosses are made or selection is made in different environments.
- It is often difficult to predict where important genes will be found. While resistance/tolerance genes are often found to be associated with biotic and abiotic stresses (sympatric resistance/tolerance) sometimes this association may not be present or at least not apparent. While core, mini-core or specialist collections may be the first place to start when looking for new characteristics, the whole collection should not be ignored if needed traits are not found.
- Time between collecting rice germplasm and use can span decades. Success in plant breeding can result in varieties remaining popular with varieties for a very long time, such as Mahsuri, IR64, Khao Dawk Mali 105 and Koshihikari.
- One of the major problems with germplasm is the difficulty that exists in tracing the route of germplasm from its collecting place to use. Germplasm only has one unique number – the passport number. This may be linked to the accession number given by the receiving gene bank but when that accession is sent to evaluators, plant breeders or other gene banks, the gene bank accession number takes precedence. Once within a breeding programme, the accession number soon becomes a breeding line number and maybe eventually become a variety. Passport numbers for accession numbers can be found for germplasm in the CGIAR[4] system on SINGER (http://singer.cgiar.org/) but for germplasm from other gene banks the importance of passport number is not always realized. Secondary transfer of germplasm, that often happens, further removes the 'unique' number for the germplasm from those using it.

[3] Available at http://km.fao.org/gipb/

[4] Consultative Group on International Agricultural Research.

- Crop improvement is not just based on genetics but has to be holistic, taking account of all aspects of the crop cycle including water and nutrient requirements as well as pest and disease mitigation practices.
- The next green revolution is less likely to come from a dramatic increase in yield from the crop because the limits to what can be achieved are known and the 'yield gap' between research station and farmer's field will be difficult to close. It is likely that a large increase in yield may come from opening new areas to rice production or increasing the number of crops from one plot of land. Theoretically, transplanted rice could give four or five crops from one parcel of land annually if water supply is available and gene rotation is actively practised. For five crops it would require 30 days in the seed bed and 60 days in the field with ten days between harvest and transplanting. Looking at the cropping system overall, crop yields in tropical countries could be boosted similarly by ensuring crop turnaround is very fast and complementary crops are planted. Hence an agronomic green revolution may be more likely and perhaps more productive than a genetic green revolution.

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References

Angeles, E.R., Cabunagan, R.C., Tiongco, E.R. *et al*., 1998. Advanced breeding lines with resistance to rice tungro viruses. *International Rice Reserch Newsletter,* 23: 17-18.

Bailey-Serres, J., Fukao, T., Ronald, P. *et al.* 2010. Submergence tolerance in rice: *SUB1*'s journey from landrace to modern cultivar. *Rice*, 3: 138-147.

Brar, D.S. & Khush, G.S. 2002 Transferring genes from wild species into rice. *In* M.S. Kang, ed. *Quantitative genetics, genomics and plant breeding*, pp. 197-217. Wallingford, CABI Publishing.

Brar, D.S., Virk, P.S. & Jena, K.K. 2009. Breeding for resistance to planthoppers in rice. *In* K.L. Heong & B. Hardy, eds. *Planthoppers: new threats to the sustainability of intensive rice production systems in Asia*, pp. 401-428, Los Baños, IRRI.

Brar, D.S. 2005. Broadening the gene pool of rice through introgression from wild species. *In* K. Toriyama, K.L. Heong & B. Hardy, eds. *Rice in life: scientific perspectives for the 21st century*, pp. 157-159. Proceedings of the World Rice Research Conference held in Tokyo and Tsukuba, Japan, 4-7 November 2004 Los Baños, IRRI and Tsukuba, Japan International Research Center for Agricultural Sciences. CD-ROM. 500 pp.

Bray, F. 1984. *Joseph Needham. Science and civilization in China.* Volume 6, part 2. UK, University of Cambridge Press.

Chang, T.T. 1989. The case for large collections. *In* A.H.D. Brown, O.H. Frankel, D.R. Marshall, J.T. Williams, eds. *The use of plant genetic resources*, pp. 123-135. Cambridge, Cambridge University Press.

Deen, R., Ramesh, K., Gautam, S.K. *et al*. No date. Identification of new gene for BPH resistance introgressed from *O. rufipogon*. *Rice Genetics Newsletter,* 25: 70-71.

Evenson, R.E. & Gollin, D. 1997. Genetic resources, international organizations and improvement of rice varieties. Economic development and cultural change. *Economic Development and Cultural Change,* 45: 471-500.

Fisher, M. 2011. The role of agronomists in maximizing the potential of biotech traits. *Crops, Soils, Agronomy News*, June 2011, pp. 4-9.

Hienrichs, E.A., Medrano, F.G. & Rapusas, H.R. 1985. *Genetic evaluation for insect resistance in rice*. Manila, Philippines, IRRI. 356 pp.

Hirobayashi, H., Kaji, R., Okamoto, M. *et al.* 2003. Mapping QTL's for brown planthopper (BPH) resistance introgressed from *Oryza officinalis* in rice. *In* G.S. Khush, D.S. Brar & B. Hardy, eds. *Advances in rice genetics. Supplement to Rice Genetics IV. Proceedings of the Fourth International Rice Genetics Symposium, 22-27 October 2000*, pp. 268-270. Los Baños, Philippines, IRRI. 642 pp.

Ikehashi, H. & Araki, H. 1984. Varietal screening of compatible types revealed in F_1 hybrids of distant crosses in rice. *Jpn. J. Breed*., 41: 359-363.

Jairin, J., Teangdeerith, S., Leelagud, P. *et al.* 2009. Development of rice introgression lines with brown planthopper resistance and KDML 105 grain quality characteristics through marker-assisted selection. *Field Crops Research,* 110: 263-271.

Jena, K.K., Pasalu, I.C., Varataxmi, Y. *et al.* 2003. Molecular mapping and marker-aided selection of a gene conferring resistance to an Indian biotype of brown planthopper in rice. *In* G.S. Khush, Brar, D.S. & B. Hardy (eds.) *Advances in rice genetics. Supplement to Rice Genetics IV. Proceedings of the Fourth International Rice Genetics Symposium, 22-27 October 2000,* pp. 265-268 Los Baños, Philippines, IRRI. 642 pp.

Kobayashi, N., Ikeda R., Domingo, I.T. *et al.* 1993a. Resistance to infection of rice tungro viruses and vector resistance in wild species of rice (*Oryza* spp.). *Jpn. J. Breed.*, 43: 377-387.

Kobayashi, N., Ikeda R. & Vaughan, D.A. 1993b. Resistance to rice tungro viruses in wild species of rice (*Oryza* spp.). *Jpn. J. Breed*., 43: 247-255.

Li, J.M. & Yuan, L.P. 2000. Hybrid rice: genetics, breeding and seed production. *Plant Breeding Reviews,* 17: 15-158.

Molina, J., Sikora, M., Garud, N. *et al.* 2011. Molecular evidence for a single evolutionary origin of domesticated rice. *Proceedings National Academy of Sciences,* 108: 8351-8356.

Mori, M., Onishi, K., Tokizono, Y. *et al.* 2011. Detection of a novel quantitative trait locus for cold tolerance at the booting stage derived from a tropical japonica rice variety Silewah. *Breeding Science,* 61: 61-68.

Nguyen, D., Brar, D.S., Buu, B.C. *et al.* 2003. Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.). *Theor. Appl. Genet*., 106: 583-593.

Oka, H.I. 1958. Report of study-tour to Thailand for investigation of rice. *In* H. Morishima (compiler). *Reports of the study-tour for investigation of wild and cultivated rice species conducted by the staff of the National Institute of Genetics, Japan and coworkers during 1957-1997*. Part 1, pp. 36-69. Mishima, Japan.

Renganayaki, K., Fritz, A.K., Sadasivam, S. *et al.* 2002. Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, *O. sativa*. *Crop Sci*., 42: 2112-2117.

Saito, K., Miura, K., Nagano, K. *et al.* 2001. Identification of two closely linked quantitative trait loci for cold tolerance on chromosome 4 of rice and their association with anther length. *Theor. Appl. Genet*., 103: 862-868.

Saito, K., Hayano-Saito, Y. Maruyama-Fumatsuki, W. *et al.* 2004. Physical mapping and putative candidate gene identification of a quantitative trait locus *Ctb1* for cold tolerance at the booting stage in rice. *Theor. Appl. Genet*., 2004: 515-522.

Saito, K., Hayano-Saito, Y. Kuroki, M. *et al.* 2010. Map-based cloning of the rice cold tolerance gene Ctb1. *Plant Science*, 179:97-102.

Satake, T. & Toriyama K. 1979. Two extremely cool-tolerant varieties. *Intl. Rice Res. Newsl.*, 4(2): 9-10.

Shibata, Y., Cabunagan, R.C., Cabunagan, P.Q. *et al.* 2007. Characterization of *Oryza rufipogon* – derived resistance to tungro disease in rice. *Plant Disease*, 91(11): 1386-1391.

Sun, L. Su, C., Wang, C. *et al.* 2005. Mapping resistance of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. *Breeding Science*, 55: 391-396.

Van, T. K. 1966. The breeding and selection of the two new hybrid varieties, Malinja and Mahsuri for double cropping in the States of Malaya. *Malaysian Agr. J.*, 45: 332-344.

Vaughan, D.A. & Sitch, L. 1991. Gene flow from the jungle to farmers. *BioScience*, 41: 22-28.

Vaughan, D.A., Lu, B.R. & Tomooka, N. 2008. The evolving story of rice evolution. *Plant Science*, 174: 394-408.

Vaughan, D.A., Lu, B.R. & Tomooka, N. 2008. Was Asian rice (*Oryza sativa* L.) domesticated more than once? *Rice*, 1: 16-24.

Vergara, B.S. 1976. Physiological and morphological adaptability of rice varieties to climate. Pages 67-83 in Climate and rice. IRRI, Los Baños, Philippines.

Vergara, B.S., Patena G. & Lopez, F.S.S. 1982. Rapid generation advance of rice at the International Rice Research Institute. *IRRI Research Paper Series* no. 84. Dec. 1982. p. 11.

Vergara, B.S. & Visperas, R.M. 1984. Adaptability and use of indica varieties in high-altitude areas. Potential productivity and yield constraints of rice in East Asia. Pages 39-52 *Proc. Intl. Crop Sci. Symp., Crop Science Society of Japan*. Pp. 39-52.

Genetic diversity of cultivated and wild soybean resources and their potential for breeding and genomic analysis

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Modern plant breeding has made enormous contributions to increased food production worldwide. On the other hand, the genetic base of recent developed varieties is extremely narrow and these varieties displace diversified indigenous cultivars and landraces. Exotic germplasm, including wild accessions, may serve as sources of foreign genes to increase genetic diversity in the improvement of agronomically important traits such as biotic and abiotic stresses. The introduction of such exotic genes, however, often brings unexpected adverse effects in addition to the target trait. Recently, an integrative genomics database for soybean has been developed based on the whole-genome sequence and many genomic, transcriptional and functional annotated sequences and molecular markers can be retrieved. These molecular and genomic resources provide a range of conclusive opportunities for genetic refinement of target traits in breeding lines by marker-assisted breeding. In addition, the advanced genomic information makes it possible to discover novel alleles and loci from the genetic resources. Thus the genetic resources of soybean, including wild soybean, are being showcased in the postgenomic era.

The importance of soybean improvement

Soybean (*Glycine max* [L.] Merrill) is one of the most important crops as a staple source for edible, feeding, industrial and pharmaceutical applications. Soybean seeds contain a high percentage of nutritious protein and oil, and are a significant component of traditional foods in many Asian countries. Soybean was planted on more than 102 million hectares worldwide in 2010, yielding 264 million tonnes; more than 80 percent of the production came from the United States, Brazil and Argentina (FAS 2010). In response to recent increases in demand, soybean production has had the highest increase compared to other major crops (Hartman *et al.* 2011). In this context soybean improvement is urgently needed to accommodate global as well as regional market demand.

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Japan is one of the world's leading consumers of soybeans, importing almost 95 percent of the annual quota. The Ministry of Agriculture, Forestry and Fisheries (MAFF) has released more than 100 accredited varieties publicly over the past five decades in Japan, but the country's average yield of soybeans still remains less than the world average. Whereas the average yield worldwide increased by a factor of 2.5 in the last two decades, reaching 2.5 tonnes/hectare in 2010, in Japan it remained at 1.6 tonnes/hectare during the same period. It is therefore imperative to develop new varieties to address the factors that cause low productivity. The goal is to achieve a stable supply for domestic human consumption that has the required seed quality.

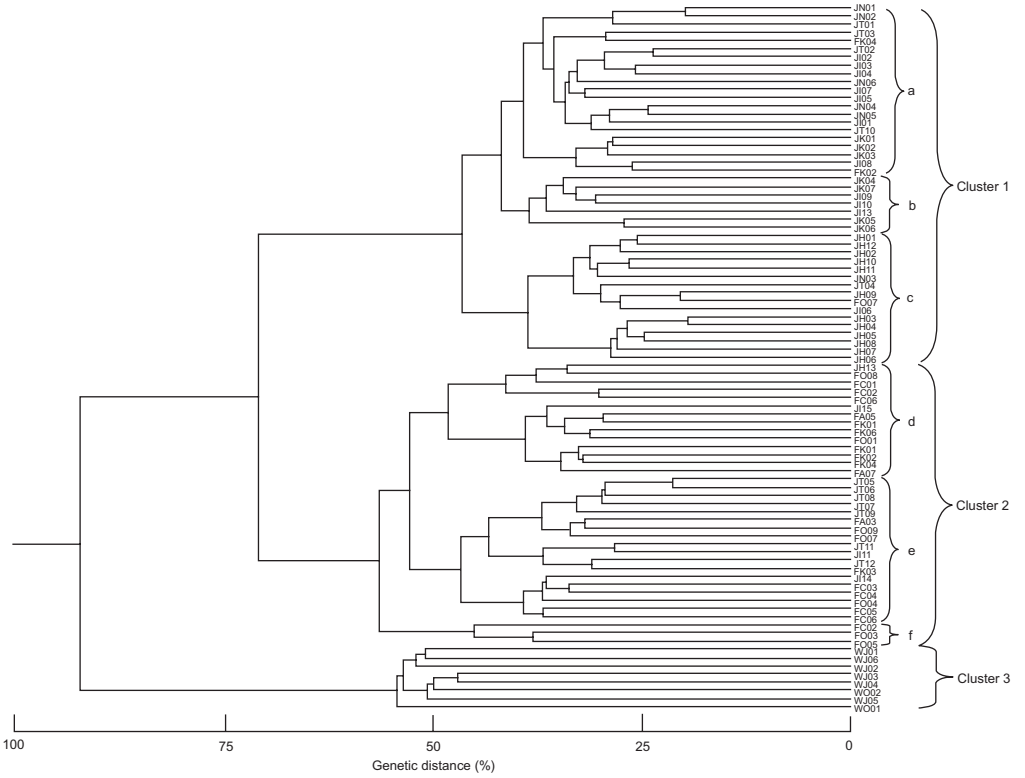
Soybean's molecular and genomic resources

The genomic era is now a reality for soybean as well as many other crops. Recently, an integrative genomics database for soybean has been developed based on the whole-genome sequence (Schmutz *et al.* 2010); numerous genomic, transcriptional and functional annotated sequences can be retrieved from Phytozome (http://www.phytozome.net/soybean.php). In addition to a large proportion of the soybean genome, several resources, including an expressed sequence tag (EST) database, full-length cDNAs and molecular markers have been developed (Stacey *et al.* 2004; Umezawa *et al.* 2008); http://soybase.org/index.php). These molecular and genomic resources provide opportunities for improving soybean by marker-assisted breeding and for understanding its gene function by map-based cloning and reverse genetic approaches.

Molecular markers are fundamental to modern plant breeding because they allow the identification of agronomic trait loci, including quantitative trait loci, and an understanding of genetic diversity and the genome structure of genetic resources. Since the 1990s, various types of molecular markers, including restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, have been developed and applied to soybean. Among them, the SSR (also known as microsatellite) marker has been a conventional tool in soybean genetics because of the high allelic diversity (Marino *et al.* 1995; Rongwen *et al.* 1995). Thousands of SSR markers have been developed in soybean over the last two decades, with more than 2 000 SSR loci integrated into a common linkage map (Cregan *et al.* 1999; Hisano *et al.* 2007; Hwang *et al.* 2009; Song *et al.* 2004; Xia *et al.* 2007). The abundant and well-documented SSR loci allow the relatively unlimited selection of SSR markers on the basis of their diverse length polymorphism and their location on the linkage and physical

maps. We selected 304 SSR markers and assembled them into 41 multiplex PCR sets to give a whole-genome SSR panel (Figure 1) (Sayama *et al.* 2011). This genome panel system has been applied to linkage analysis of more than ten segregating populations so far and it has successfully built the framework of each linkage map (Takada *et al.* 2010). The results indicate the wide applicability of the genome panel system to various combinations of soybean genotypes. The combination of multiplex PCR and the high-resolution detection system has been also applied to other genotyping studies such as marker-assisted selection and the fine mapping of qualitative and quantitative traits.

Figure 1. Dendrogram for the 87 soybean lines, comprising eight wild accessions and 79 cultivars including Japanese elite cultivars, based on clustering analysis of the 377 SSR loci by Ward's method. The information on each soybean line can be obtained from the original report (adapted from Hwang *et al.* 2008)



The re-sequencing of the soybean genome enables the identification of a huge quantity of single nucleotide polymorphisms (SNPs). Furthermore, the recent breakthrough of high multiplexing has moved the SNP into the mainstream of genotyping technology (Fan *et al.* 2006). Vast numbers of SNPs have been

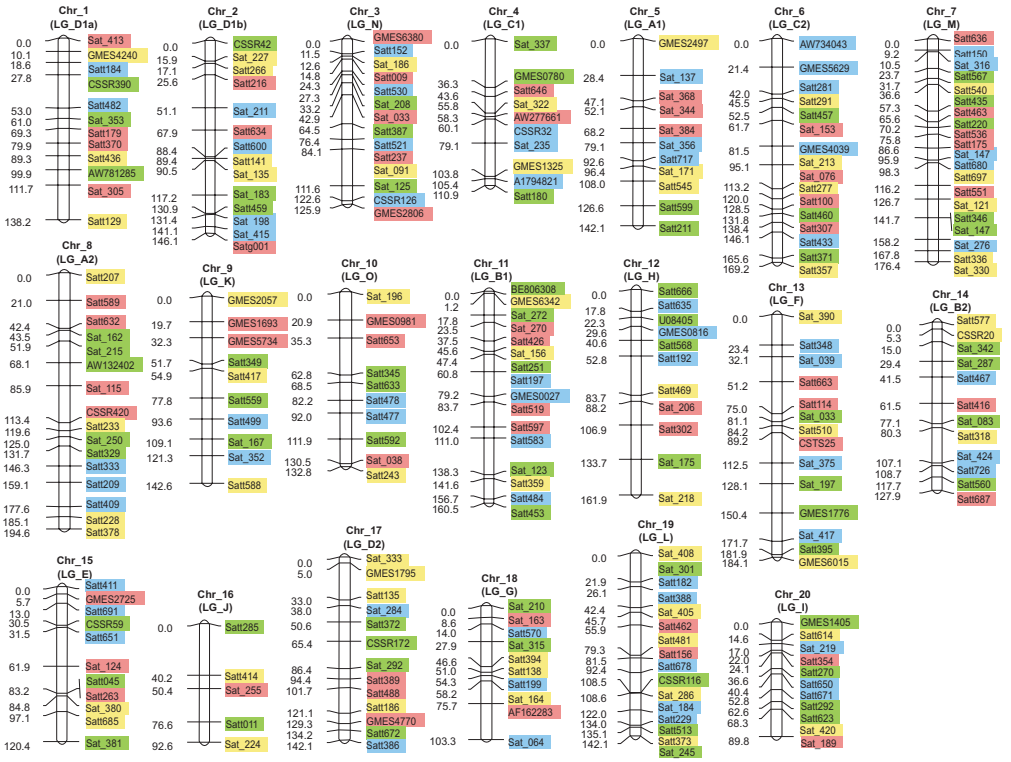
identified in soybean and have been used to design the high-throughput genotyping platforms, which discriminate up to 1 536 SNPs in one reaction (Choi *et al.* 2007; Hyten *et al.* 2010; Hyten *et al.* 2008). On the basis of its high multiplexing capacity, automation and continued improvement, the SNP array is becoming one of most reliable methods for whole-genome genotyping. However, the high-throughput genotyping platform is not flexible for modification of SNP marker selection and is expensive for each reaction set.

Genetic diversity of cultivated and wild soybean

SSR markers are used in the analysis of genetic diversity and QTLs as well as the construction of linkage maps (Marino *et al.* 1995; Wang *et al.* 2001). We selected 377 SSR marker loci distributed throughout the soybean genome and analysed them in 87 cultivated and wild soybean accessions, including Japanese elite varieties and important genetic resources (Hwang *et al.* 2008). All SSR markers showed polymorphism on agarose gel electrophoresis, which revealed a total of 1 380 alleles among all accessions. The observed genetic polymorphisms of SSR loci were subjected to cluster analysis. The analysis classified the 87 accessions into three major groups segregated almost exclusively into Japanese varieties, foreign varieties and wild accessions (Figure 2). The Japanese cultivars were grouped into three subclusters that corresponded well to their geographic origins. Such clustering may reflect the pedigree relations and commonality of important agricultural traits in cultivars from the different regions. The genetic similarities and distances of the SSR loci indicate a low level of genetic diversity in the Japanese cultivars. In particular, recently developed cultivars are closely related among the cultivars produced at the same breeding stations. Some Japanese cultivars were grouped with the foreign cultivars, indicating pedigree relations. Chinese and United States' cultivars have been used in Japanese breeding programmes, especially in Tohoku and Hokkaido, to introduce exotic genes that confer resistance to soybean mosaic virus or soybean cyst nematode (Zhou *et al.* 2002). The foreign cultivars have the potential to introduce genetic diversity into Japanese soybean breeding because the genetic base of Japanese cultivars is quite distinct from that of Chinese, United States' and Canadian cultivars (Abe *et al.* 2003; Zhou *et al.* 2000).

Wild soybean (*Glycine soja* Sieb. & Zucc.) is presumed to be the most probable ancestor of cultivated soybean (Hymowitz 1970). Wild soybeans are distributed in semi-disturbed habitats of East Asia, China, Republic of Korea, Japan and the Far East of the Russian Federation (Shimamoto *et al.* 2000). They can produce fertile offspring with cultivated soybean, and both types share

Figure 2. Molecular linkage map constructed with the whole-genome SSR panel system. The JI population, which consists of 94 F_2 individuals originating from a cross between the Japanese wild accession JP-36121 and the Japanese landrace Ibarakimame 7, was used to position fluorescently labeled SSR markers. Fluorescent material refers to Applied Biosystems; one 6-FAM (blue), VIC (green), PET (pink) and NED (yellow) was attached together with the tail of several nucleotides before the 5' end of the forward primer. A total of 304 SSR marker loci were analysed with the 41 multiplex PCR sets of six to eight primer pairs, resulting in the generation of a linkage map consisting of 249 SSR marker loci covering 2 864 cM of the soybean genome (adapted from Sayama *et al.* 2011)



a common primary gene pool and form a single biological species. Wild soybean has greater diversity than cultivated soybean. Thus wild soybeans are important genetic resources of novel alleles and loci to broaden the genetic base of cultivars.

Incorporation of potent genetic resources in soybean breeding

Breeding of soybean has improved seed quality in Japanese elite varieties for traditional food products such as *tofu* and *natto* (fermented soybean). Introduction of new genetic resources into the gene pool may improve the crop's tolerance to impediments and increase genetic variability to enable the development of high-yielding varieties (Ude *et al.* 2003). However, the introgression of desirable genes from other genetic resources can cause detrimental changes in agronomically important traits such as seed quality (Jacobsen and Schouten 2007). Many loci associated with the target traits such as disease and insect resistance were successfully integrated into the linkage map. Information on neighbouring molecular markers has been successfully applied to the analysis of qualitative and quantitative trait loci and to marker-assisted selection in soybean (Concibido *et al.* 2004; Cregan *et al.* 1999; Harada and Xia 2004). Therefore, marker-assisted selection (MAS) is a powerful tool for genetically refining the target traits of breeding lines in the process of selection (Peleman *et al.* 2005).

Advanced molecular and genomic resources make it possible to discover novel alleles and loci from the genetic resources by reverse genetics as well as forward genetics. Target induced local lesions in genomes (TILLING) is one technique for reverse genetics and induced mutants are screened to identify individuals with sequence alterations such as SNPs and In/Del (insertions/deletions) (Barkley and Wang 2008). Genetic resources have been applied to uncover natural genetic variations (Comai *et al.* 2004) and successfully identify novel disease resistance allele (Nieto *et al.* 2007). The strategy could be applied to wild and cultivated soybean germplasm maintained and operated by the National Institute of Agrobiological Sciences' (NIAS) gene bank, the National BioResource Project in Japan and the United States Department of Agriculture's (USDA) Germplasm Resources Information Network. Thus germplasm enhances their presence for soybean breeding and the discovery of novel alleles and loci in the postgenomic era.

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References

Abe, J., Xu, D., Suzuki, Y. *et al.* 2003. Soybean germplasm pools in Asia revealed by nuclear SSRs. *Theoretical and Applied Genetics,* 106: 445-453.

Barkley, N. & Wang, M. 2008. Application of TILLING and EcoTILLING as reverse genetic approaches to elucidate the function of genes in plants and animals. *Current Genomics,* 9: 212.

Choi, I.Y., Hyten, D.L., Matukumalli, **L.K. *et al.*** 2007. A soybean transcript map: gene distribution, haplotype and single-nucleotide polymorphism analysis. *Genetics,* 176: 685-696.

Comai, L., Young K., Till, B.J. *et al.* 2004. Efficient discovery of DNA polymorphisms in natural populations by ecotilling. *The Plant Journal,* 37: 778-786.

Concibido, V.C., Diers, B.W. & Arelli, P.R. 2004. A decade of QTL mapping for cyst nematode resistance in soybean. *Crop Science,* 44: 1121-1131.

Cregan, P.B., Jarvik, T., Bush, A.L. *et al.* 1999. An integrated genetic linkage map of the soybean genome. *Crop Sci.,* 39: 1464-1490.

Cregan, P.B., Mudge, J., Fickus, E.W. *et al.* 1999. Two simple sequence repeat markers to select for soybean cyst nematode resistance conditioned by rhg1 locus. *Theor Appl Genet.,* 99: 811-818.

Fan, J.B., Gunderson, K.L., Bibikova, M. *et al.* 2006. Illumina universal bead arrays. *Methods in Enzymology,* 410: 57-73.

FAS. 2010. *Oilseeds: world markets and trade, foreign agricultural service*. Washington, DC, USDA.

Harada, K. & Xia, Z. 2004. Soybean genomics: efforts to reveal the complex genome. *Breeding Science,* 54: 215-224.

Hartman, G.L., West E.D. & Herman, T.K. 2011. Crops that feed the world. 2. Soybean – worldwide production, use, and constraints caused by pathogens and pests. *Food Security,* 3: 5-17

Hisano, H., Sato, S., Isobe, S. *et al.* 2007. Characterization of the soybean genome using EST-derived microsatellite markers. *DNA Res.,* 14: 271-281.

Hwang, T.Y., Nakamoto, Y., Kono, I. *et al.* 2008. Genetic diversity of cultivated and wild soybean lines including Japanese elite cultivars as revealed by length polymorphism of SSR markers. *Breed. Sci.,* 58: 315-323.

Hwang, T.Y., Sayama, T., Takahashi, M. *et al.* 2009. High-density integrated linkage map based on SSR markers in soybean. *DNA Res.,* 16: 213-225.

Hymowitz, T. 1970. On the domestication of the soybean. *Economic Botany,* 24: 408-421.

Hyten, D., Cannon, S., Song, Q. ***et al***. 2010. High-throughput SNP discovery through deep resequencing of a reduced representation library to anchor and orient scaffolds in the soybean whole genome sequence. *BMC Genomics*, 11: 38.

Hyten, D.L., Song, Q., Choi, I. *et al.* 2008. High-throughput genotyping with the GoldenGate assay in the complex genome of soybean *Theor Appl Genet.*, 116: 945-952.

Jacobsen, E. & Schouten, H.J. 2007. Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. *Trends Biotechnol.*, 25: 219-223.

Marino, M.A., Turni, L.A., Del Rio, S.A. *et al*. 1995. The analysis of simple sequence repeat DNA in soybean by capillary gel electrophoresis. *Appl Theor Electrophor.*, 5: 1-5.

Nieto, C., Piron, F., Dalmais, M. *et al.* 2007. EcoTILLING for the identification of allelic variants of melon eIF4E, a factor that controls virus susceptibility. *BMC Plant Biology*, 7: 34.

Peleman, J.D., Sorensen, A.P. & van der Voort, J.R. 2005. Breeding by design: exploiting genetic maps and molecular markers through marker assisted selection. *In* K. Meksem & G. Kahl, eds. *The handbook of plant genome mapping: genetic and physical mapping*. Weinheim, Wiley-VCH.

Rongwen, J., Akkaya, M., Bhagwat, **A. *et al*.** 1995. The use of microsatellite DNA markers for soybean genotype identification. *Theoretical and Applied Genetics*, 90: 43-48.

Sayama, T., Hwang, T.Y., Komatsu, K. *et al.* 2011. Development and application of a whole-genome simple sequence repeat panel for high-throughput genotyping in soybean. *DNA Research*, 18: 107-115.

Schmutz, J., Cannon, S.B., Schlueter, J. *et al.* 2010. Genome sequence of the palaeopolyploid soybean. *Nature*, 463: 178-183.

Shimamoto, Y., Oono, K., Vaughan, D. *et al.* 2000. *Research on wild legumes with an emphasis on soybean germplasm*. National Institute of Agrobiological Resources (NIAR).

Song, Q.J., Marek, L.F., Shoemaker, R.C. ***et al*.** 2004. A new integrated genetic linkage map of the soybean. *Theor Appl Genet*., 109: 122-128.

Stacey, G., Vodkin, L., Parrott, W.A. *et al.* 2004. National Science Foundation-sponsored workshop report. Draft plan for soybean genomics. *Plant Physiology*, 135: 59.

Takada, Y., Sayama, T., Kikuchi, **A**. ***et al.*** 2010. Genetic analysis of variation in sugar chain composition at the C-22 position of group A saponins in soybean, *Glycine max* (L.) Merrill. *Breed. Sci*., 60: 3-8.

Ude, G.N., Kenworthy, W.J., Costa, J.M. *et al.* 2003. Genetic diversity of soybean cultivars from China, Japan, North America, and North American ancestral lines determined by amplified fragment length polymorphism. *Crop Science*, 43: 1858-1867.

Umezawa, T., Sakurai, T., Totoki, Y. *et al.* 2008. Sequencing and analysis of approximately 40 000 soybean cDNA clones from a full-length-enriched cDNA library. *DNA Research*, 15: 333-346.

Wang, D., Arelli, P., Shoemaker, R.C. *et al.* 2001. Loci underlying resistance to race 3 of soybean cyst nematode in *Glycine soja* plant introduction 468916. *Theor. Appl. Genet.*, 103: 561-566.

Xia, Z., Tsubokura, Y., Hoshi, M. *et al.* 2007. An integrated high-density linkage map of soybean with RFLP, SSR, STS, and AFLP markers using a single F2 population. *DNA Res.*, 14: 257-269.

Zhou, X., Carter, Jnr. T.E., Cui, Z. *et al.* 2002. Genetic diversity patterns in Japanese soybean cultivars based on coefficient of parentage. *Crop Science*, 42: 1331-1342.

Zhou, X.C., Cui, Z. Carter, T.E. *et al*. 2000. Genetic base of Japanese soybean cultivars released during 1950 to 1988. *Crop Science*, 40: 1794.

Carnation (*Dianthus caryophyllus* L.)

Takashi Onozaki[1]

Carnation (Dianthus caryophyllus L.) is one of the world's major floricultural crops. At present, production centres are cool highlands with suitable climates for carnation growth, such as those found in Colombia and China. The amount of cut carnations imported into Japan increases year by year. Therefore, breeding of high-value-added or distinctive cultivars is required in order to overcome the need to import them. Bacterial wilt caused by Burkholderia caryophylli *is one of the most serious carnation diseases in Japan. The first practical carnation cultivar, Karen Rouge, resistant to bacterial wilt, was developed using* D. capitatus *in 2010. The vase life of cut flowers is one of the most important breeding targets in ornamentals. Therefore, a breeding programme to improve the vase life of carnation flowers was initiated in 1992. Two carnation cultivars with long vase life – Miracle Rouge and Miracle Symphony' – were developed in 2005. These cultivars have vase lives of 17.7 to 20.7 days (about three times the vase life of normal cultivars). Interspecific hybridization between carnation lines with long vase life and* D. superbus *var.* longicalycinus*, a wild* Dianthus *species native to Japan, was carried out in order to combine early flowering, high productivity and long vase life. Several selected* BC_2 *lines with long vase life, early flowering and high productivity traits could be developed.*

Introduction

Carnation (Dianthus caryophyllus L.) is one of the world's major floricultural crops along with chrysanthemum and rose. At present, the major production areas are cool highlands with suitable climates for carnation growth, such as those found in Colombia and China (Onozaki 2006a). The amount of cut carnations imported into Japan from these two countries has increased steadily each year. Of cut carnations sold in Japan, 46.2 percent were imported in 2010 (MAFF 2010); therefore, breeding of high-value-added or distinctive cultivars is required in order to overcome the need to import them.

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Breeding for resistance to bacterial wilt in carnations

Bacterial wilt[2] is one of the most important and damaging diseases of carnations in Japan. It causes serious crop losses in carnations grown in warm districts of Japan (Yamaguchi 1994). Bacterial wilt was first found in Spokane, Washington, United States in 1941 (Jones 1941); the occurrence of this disease in Japan was first recorded in 1964 in Kanagawa Prefecture (Tsuchiya *et al*. 1965). Concerning resistance to bacterial wilt in carnation cultivars, an initial study in the United States reported differences in susceptibility among five and that Durango was immune (Thomas 1954). Subsequently, Nelson and Dickey (1963) tested 21 cultivars and found all cultivars to be highly susceptible except Elegance, Starlite and Northland. These three cultivars were somewhat resistant as less than 50 percent became infected. However, Durango, Elegance, Starlite and Northland are now not available. Recently, when Uematsu *et al*. (1991) subjected 126 commercial carnation cultivars to *B. caryophylli* in Japan, all proved to be susceptible.

To protect against this disease, cultural practices, including the use of pathogen-free cuttings, isolated bench culture and soil disinfection with steam or chemicals, were introduced in the 1970s, and the occurrence of the disease decreased. Despite these precautions, there were often widespread outbreaks, with plants displaying severe symptoms. It is very difficult to control this disease using chemicals once it has entered a crop. Breeding of resistant cultivars thus seems to be the optimal strategy to overcome this disease. Therefore, Japan's NARO Institute of Floricultural Science (NIFS) initiated a breeding programme for resistance to bacterial wilt in 1988.

Onozaki *et al.* (1999a) screened 277 carnation cultivars for their resistance to *B. caryophylli* using the cut-root soaking method, with an inoculum concentration of 10^7 cfu (colony-forming units)/mL. Of these, 207 cultivars (74.7 percent) were highly susceptible, and three (Wiko, Nocto and Sandrosa) possessed adequate resistance (Onozaki *et al.* 1999a). However, these resistant carnation cultivars seem to be unsuitable as parents for use in breeding for resistance. Wiko is difficult to exploit for carnation breeding because it produces no pollen and lacks the ability to set seeds. In addition, Wiko and Nocto are not standard carnations, but small compact types – presumably a wild species used for breeding. Although Sandrosa appears suitable based on its ability to produce pollen and set seed, it produced disappointing results in a crossing trial. We crossed three susceptible carnation cultivars with Sandrosa and tested the

[2] *Burkholderia caryophylli* [Burkholder] Yabuuchi, Kasako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa; previous scientific name: *Pseudomonas caryophylli* [Burkholder] Starr and Burkholder.

progeny for their resistance to *B. caryophylli* in 1994. No resistant seedlings were obtained from 156 F_1 plants, indicating that the resistant Sandrosa carnation cultivar is unsuitable for use as a parent in breeding for resistance.

Therefore, a study of 70 wild Dianthus accessions was undertaken to test their differences in bacterial wilt resistance. The same test protocol was used for these species that was used to screen the carnation cultivars. Based on this testing, two highly resistant wild species, *D. capitatus* Balbis ex DC. ssp. *andrzejowskianus* Zapal. and *D. henteri* Heuffel ex Griseb. & Schenk, were identified (Onozaki *et al*. 1999b). These two species showed no disease symptoms throughout the experiment.

Cultivated carnations were hybridized with *D. capitatus* ssp. *andrzejowskianus* to introduce the latter's bacterial wilt disease resistance into carnation cultivars (Onozaki *et al*. 1998). This interspecific hybridization was very difficult. When carnations were used as the seed parent and *D. capitatus* ssp. *andrzejowskianus* was used as the pollen parent, F_1 progeny were not obtained. When *D. capitatus* ssp. *andrzejowskianus* was used as the seed parent and carnations were used as the pollen parent, seed was set in only 13 out of 43 crosses (30 percent). Moreover, a large percentage of the resulting seeds was abnormal: most were smaller than the normal black carnation seeds and had a wrinkled brown surface. However, not all seeds were completely empty. The germination percentage was therefore relatively low. Only 50 F_1 seedlings were obtained in crosses conducted from 1990 to 1992.

Of these seedlings, 36 survived and were tested for resistance to bacterial wilt using the aforesaid screening method. Eleven resistant lines were selected from among the 36 F_1 plants. The resistance of the *Dianthus* wild species to *B. caryophylli* was inherited as a result of the interspecific hybridization (Onozaki *et al*. 1998). Carnation Nou No. 1 was selected from the F_1 progeny derived from a cross with the spray-type carnation cultivar Super Gold and was registered with Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF) in 1996 (Onozaki *et al*. 2002).

Carnation Nou No. 1 is a carnation breeding line that is resistant to bacterial wilt. This line has a perpetual flowering habit. In addition, the yield of this line (number of cut flowers/plant) is very high; the mean yield is 11.5 cut flowers/plant, which is higher than that of the control cultivars Super Gold, Scania and Coral. Carnation Nou No. 1 can produce resistant progeny, indicating that it is available as breeding material to introduce resistance into carnation and other *Dianthus* cultivars (Onozaki *et al*. 2002).

We conducted continuous crossings and selections for bacterial wilt resistance to develop a carnation with resistance derived from *D. capitatus* ssp. *andrzejowskianus.* Karen Rouge is the first practical carnation cultivar resistant to bacterial wilt (Yagi *et al.* 2010). The mean disease incidence of six resistance tests was 7.1 percent in Karen Rouge, which is clearly more resistant than Francesco (87 percent) and Nora (97.1 percent). The flower colour is deep yellowish red (0707: JHS colour chart), almost the same as Francesco. Karen Rouge is a standard-type cultivar with a flower diameter of 7.5 centimetres. The total number of cut flowers/plant is lower than Francesco but more than Nora. Karen Rouge has been developed by marker-assisted selection using STS-WG44 markers linked to a major QTL since 2004 (Onozaki *et al.* 2004). To our knowledge, this is the first cultivar produced by marker-assisted selection in carnations.

Improvement of carnation vase life using cross-breeding techniques

The vase life of cut flowers is an important characteristic that determines their quality and their ability to satisfy consumer preferences. Senescence of carnation flowers is normally characterized by a climacteric-like pattern of ethylene production; that is, by a surge in ethylene production followed by a decline (Mayak and Tirosh 1993). The increase in ethylene production is associated with the development of in-rolling flower petals and subsequent wilting (Halevy and Mayak 1981). Carnation flowers are highly sensitive to exogenous ethylene (Woltering and van Doorn 1988). Hence, ethylene is an important determinant of flower longevity, because it induces wilting of petals and autocatalytic ethylene production (Halevy and Mayak 1981).

Although the vase life of carnations is about five to seven days in normal Sim-type cultivars, it can be extended by means of treatment with postharvest chemicals, such as silver thiosulfate (STS) (Veen 1979). STS, which inhibits ethylene action, is widely used by commercial carnation producers to extend the vase life of cut flowers because of its outstanding effectiveness. STS is generally applied as a pretreatment solution to cut flowers. However, concerns about potential contamination of the environment from waste STS solutions have increased in recent years (Klee and Clark 2004), so alternative methods for improving the vase life of carnations must be developed. It would be desirable to genetically improve their vase life because the improved cultivars would require no chemical treatment to attain longer vase life. Therefore, a research breeding programme was started by NIFS in 1992 in this context using conventional breeding techniques.

Increased vase life of cut flowers is an important breeding target. To improve the vase life of carnation flowers, Onozaki *et al*. (2001, 2006b, 2011b) repeatedly crossed and selected promising progeny for six generations from 1992 to 2006. The research-breeding programme began in 1992 using six cultivars (Pallas, Sandrosa, Candy, Tanga, White Sim and Scania) as parental materials (Onozaki *et al*. 2001). The mean vase life of the parental generation derived from crossing these six cultivars was 7.4 days; in contrast, after six cycles of crossing and selection, vase life had improved to 15.9 days, a net increase of 8.5 days. All selected lines with long vase life showed low ethylene production at senescence. In particular, selected sixth-generation line 532-6 with an ultra-long vase life showed the longest vase life among all cultivars and lines; the mean vase life of line 532-6 was 32.7 days in 2007 and 27.8 days in 2008 (536 and 463 percent the value of White Sim, respectively) at 23ºC and 70 percent RH under a 12-hour photoperiod, without chemical treatment. Closer observation of petals during senescence showed that line 532-6 was characterized by a lack of brownish discoloration of petals, which was a senescence symptom of other selected lines with low ethylene production, when the flower lost its ornamental value (Onozaki *et al*. 2011b). Evaluation of the progeny by exposure to ethylene at a 2 μL/L concentration showed that two second-generation lines (64-13 and 64-54) were as insensitive to ethylene as Chinera, a cultivar that is known for its low ethylene sensitivity (Onozaki *et al*. 2001). Thus, many carnation lines with genetically long vase life could be developed using conventional cross-breeding techniques.

Onozaki *et al*. (2006a) developed two new carnation cultivars that had vase lives of 17.7 to 20.7 days (3.2 to 3.6 times the vase life of a standard cultivar, White Sim, under standard conditions (23ºC, 12-hour photoperiod, 70 percent RH). These cultivars were registered with MAFF and released in November 2005 as Carnation Norin No. 1 Miracle Rouge and No. 2 Miracle Symphony. Miracle Rouge, a red standard-type cultivar, was selected from the third generation of these crosses, and Miracle Symphony, a white standard-type cultivar with red stripes, was selected from the second generation. Both showed very high flower quality and adequate yields of cut flowers for commercial production, in addition to their long vase life.

Treatment with α-aminoisobutyric acid, an inhibitor of ethylene biosynthesis (Onozaki and Yamaguchi 1992), or STS, an inhibitor of ethylene action (Veen 1979), did not significantly prolong vase life in either cultivar. In addition, the petals and gynoecium of both cultivars produced only trace amounts of ethylene during natural senescence. These results indicate that their ethylene biosynthesis pathway was almost completely blocked during natural senescence, and that this change was responsible for their improved vase life.

Exploration and collection of *Dianthus superbus* var. longicalycinus and *D. superbus* in Mie Prefecture and Hokkaido

Exploration and collection of *D. superbus* var. *longicalycinus* and *D. superbus* were conducted in Mie Prefecture on 13 July 2000, and in Hokkaido from 3 to 5 October 3 2000 in order to utilize them for breeding. A total of 33 collected samples were divided into 12 samples of *D. superbus* var. *longicalycinus* from the sea coast in Mie Prefecture and Ishikari, Hokkaido, and 21 samples from the Okhotsk sea coast in Hokkaido (Onozaki 2001).

Breeding for early flowering and high yield using *D. superbus* var. *longicalycinus*

Interspecific hybridization between carnation *(Dianthus caryophyllus)* lines with long vase life and *D. superbus* var. *longicalycinus*, a wild *Dianthus* species native to Japan, was carried out in order to combine early flowering, high productivity and long vase life (Onozaki et al. 2011a). Although the interspecific hybridization of this combination was very difficult, seed was set in four out of 22 crosses. Moreover, all obtained seeds were abnormal; the seeds were imperfectly solid and had a wrinkled brown surface. However, 15 F_1 seedlings were obtained using normal cross-breeding techniques, without depending on *in vitro* culture. Selected F_1 lines had fertility in both seed and the pollen parent. SSR analysis proved that all nine selected F_1 lines were interspecific hybrids. In back-crosses between selected F_1 or BC_1 lines and carnation lines with long vase life, the percentage of seed setting was markedly increased, and the germination percentage of obtained seeds was also high. The mean vase life of F_1 generation was seven days; in contrast, the vase life of BC_2 generation, after two cycles of crossing and selection using carnation lines with long vase life, had improved to 14.7 days, a net increase of 7.7 days. Carnation breeding using *D. superbus* var. *longicalycinus* was effective for selecting early flowering progenies. F_1 generation exhibited very early flowering; the average number of days to flowering in 15 F_1 seedlings was 135. In BC_1 and BC_2 generations, segregation of seedlings with early flowering was also observed. Our results suggest that flower vase life is not linked to early flowering. Several selected BC_2 lines with long vase life, early flowering and high productivity could be developed, even when using *D. superbus* var. *longicalycinus* with very short vase life as breeding material (Onozaki et al. 2011a).

Acknowledgements

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References

Halevy, A.H. & Mayak, S. 1981. Senescence and postharvest physiology of cut flowers, part 2. *Horticultural Reviews,* 3: 59-143.

Jones, L.K. 1941. Bacterial wilt of carnations. *Phytopathology,* 31: 199.

Klee, H.J. & Clark, D.G. 2004. Ethylene signal transduction in fruits and flowers. *In* P.J. Davies, ed. *Plant hormones: biosynthesis, signal transduction,* action (3rd Edn), pp. 369-390. Dordrecht, The Netherlands, Kluwer Academic Publishers.

Ministry of Agriculture, Forestry and Fisheries (MAFF). 2010. *Statistics of agriculture, forestry and fisheries 2010.* Available at, http://www.maff.go.jp/j/tokei/pdf/sakutuke_kaki_10.pdf

Mayak, S. & Tirosh, T. 1993 Unusual ethylene-related behavior in senescing flowers of the carnation Sandrosa. *Physiologia Plantarum,* 88: 420-426.

Nelson, P.E. & Dickey, R.S. 1963. Reaction of twenty-one commercial carnation varieties to *Pseudomonas caryophylli. Phytopathology,* 53: 320-324.

Onozaki, T. 2001. Exploration and collection of *Dianthus superbus* var. *longicalycinus* and *D. superbus* in Mie prefecture and Hokkaido. *Annual Report on Exploration* and *Introduction of Plant Genetic Resources* 17, 49-54.

Onozaki, T. 2006. Carnation. *In* JSHS, ed. *Horticulture in Japan,* pp. 223-230. Kyoto, Shoukadoh Publication.

Onozaki, T. & Yamaguchi, T. 1992. Effect of -aminoisobutyric acid (AIB) application on the prolongation of the vase life of cut carnation flowers. *Bulletin of National Research Institute Vegetable Ornamental Plants and Tea Series A,* 5: 69-79 (in Japanese with English summary).

Onozaki, T, Ikeda, H., Yamaguchi, T. *et al.* 1998. Introduction of bacterial wilt *(Pseudomonas caryophylli)* resistance in *Dianthus* wild species to carnation. *Acta Horticulturae,* 454: 127-132.

Onozaki, T., Yamaguchi, T., Himeno, M. *et al.* 1999a. Evaluation of 277 carnation cultivars for resistance to bacterial wilt *(Pseudomonas caryophylli). J. Japan. Soc. Hort. Sci.,* 68: 546-550.

Onozaki, T., Yamaguchi, T., Himeno, M. *et al.* 1999b. Evaluation of wild *Dianthus* accessions for resistance to bacterial wilt *(Pseudomonas caryophylli). J. Japan. Soc. Hort. Sci.,* 68, 974-978.

Onozaki, T., Ikeda, H. & Yamaguchi, T. 2001. Genetic improvement of vase life of carnation flowers by crossing and selection. *Scientia Horticulturae,* 87: 107-120.

Onozaki, T., Ikeda, H., & Yamaguchi T. *et al.* 2002. 'Carnation Nou No. 1', a carnation breeding line resistant to bacterial wilt *(Burkholderia caryophylli). Horticultural Research,* (Japan) 1: 13-16 (in Japanese with English summary).

Onozaki, T., Tanikawa, N., Taneya, M. *et al.* 2004. A RAPD-derived STS marker is linked to a bacterial wilt *(Burkholderia caryophylli)* resistance gene in carnation. *Euphytica*, 138: 255-262.

Onozaki, T., Ikeda, H., Shibata, M. *et al.* 2006a. Breeding and characteristics of carnation Norin No. 1 'Miracle Rouge' and No. 2 'Miracle Symphony' with long vase life. *Bulletin of the National Institute of Floricultural Science,* 5: 1-16 (in Japanese with English summary).

Onozaki, T., Tanikawa, N., Yagi, M, *et al.* 2006b. Breeding of carnations *(Dianthus caryophyllus* L.) for long vase life and rapid decrease in ethylene sensitivity of flowers after anthesis. *J. Japan. Soc. Hort. Sci.*, 75: 256-263.

Onozaki, T., Yagi, M., Fujita, M. *et al.* 2011a. Characteristics of interspecific hybrids between carnation *(Dianthus caryophyllus)* lines with long vase life and *D. superbus* var. *longicalycinus*, and their backcrossing progenies. *Horticultural Research* (Japan), 10: 161-172 (in Japanese with English summary).

Onozaki, T., Yagi, M., Tanase, K. *et al.* 2011b. Crossings and selections for six generations based on flower vase life create lines with ethylene-resistance or ultra-long vase life in carnations *(Dianthus caryophyllus* L.). *J. Japan. Soc. Hort. Sci.*, 80: 486-498.

Thomas, W.D. Jr. 1954. The reaction of several carnation varieties to bacterial wilt. *Phytopathology*, 44: 713-715.

Tsuchiya, Y., Minakami, T. & Kagito, T. 1965. Bacterial wilt of carnation. *Annuals of the Phytopathological Society of Japan*, 30(5): 268 (in Japanese).

Uematsu, S., Hosoya, M. & Sekiyama, K. 1991. Reaction of 126 commercial carnation varieties to *Pseudomonas caryophylli*. *Proceedings of the Kanto-Tosan Plant Protection Society*, 38: 107-110 (in Japanese with English summary).

Veen, H. 1979. Effects of silver on ethylene synthesis and action in cut carnations. *Planta 145*, 467-470.

Woltering, E.J. & van Doorn, W.G. 1988 Role of ethylene in senescence of petals – morphological and taxonomical relationships. *Journal of Experimental Botany*, 39: 1605-1616.

Yagi, M., Onozaki, T., Ikeda, H. *et al.* 2010. The breeding process and characteristics of carnation 'Karen Rouge' with resistance to bacterial wilt. *Bulletin of the National Institute of Floricultural Science*, 10: 1-10 (in Japanese with English summary).

Yamaguchi, T. 1994. Carnation. *In* K. Konishi, S. Iwahori, H. Kitagawa & T. Yakuwa, eds. *Horticulture in Japan*, pp. 139-147. Tokyo, Japan, Asakura Publishing Co. Ltd.

Part 3:

Plant Genetic Resources for Food and Agriculture: Future directions

Cryostorage facilitation by the NIAS Genebank

Shin-ichi Yamamoto, Kuniaki Fukui and Takao Niino[1]

A safe and reliable long-term storage system is essential for a gene bank. Cryopreservation is an ideal method for long-term preservation of plant germplasm because it requires minimum storage space, labour and maintenance. Although still limited, cryopreservation techniques are now being adopted in an increasing number of institutes around the world. The construction of a cryobank will contribute significantly to the cost-effective long-term preservation of vegetatively-propagated crops in a stable manner under safe and disease-free conditions. In the NIAS Genebank, dormant buds of 1 283 mulberry accessions have been cryopreserved. But this method cannot be employed for plants that do not form dormant buds. Although existing cryopreservation methods such as vitrification and droplet methods for in vitro *shoots can be applicable for these plants, a more systematical protocol is desirable for facilitating an appropriate cryobank. In this context, an efficient and simple cryopreservation method, the V-cryo-plate procedure, was developed. The aluminium cryo-plate is 7 x 37 x 0.5 millimetres in size with ten micro-wells. The successive steps of the V-cryo-plate procedure are preculture, adhesion of shoot tips on the cryo-plate, osmoprotection, dehydration, storage and regeneration. Using this procedure, considerably high regrowth of cryopreserved shoot tips was achieved. The V-cryo-plate method appears to be very systematic and time saving and highly promising to facilitate large-scale cryobanking in gene banks.*

Introduction

Conservation of plant genetic resources (PGRs) can be divided into two – *in situ* and *ex situ* conservation. Gene banks worldwide have important roles for *ex situ* conservation of PGRs so a safe and reliable long-term storage system is essential. The PGRs with orthodox seeds can be stored easily in a cold chamber with low temperature and low humidity, while those with recalcitrant seeds or vegetatively-propagated PGRs have to be kept as living plants.

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The National Institute of Agrobiological Sciences (NIAS) Genebank is the central coordinating centre in Japan for the conservation of plants, micro-organisms, animals and DNA materials related to agriculture. It coordinates this activity in collaboration with a network of institutes throughout Japan.[2] The gene bank houses approximately 216 000 registered items. Genetic resources of rice and other cereals (such as various millets) are among them .

Approximately 35 000 vegetatively-propagated clonal germplasms come mainly from root and tuber crops, fruit trees, flowers, mulberry and tea, comprising 16 percent of the general stock. This rate is relatively high; in other foreign institutions, the figure is about 10 percent. The genetic resources are basically conserved in the field or greenhouses. However, there are some problems related to their safe and reliable storage. Field conservation costs are high owing to expenses for maintaining the huge space involved and concomitant labour needs. There is also the risk of natural disasters, pests and diseases. Some germplasms are partially conserved through *in vitro* cultures but costs for subculture and the possibility of somatic mutations or the risk of contamination have to be considered

As a partial solution, cryopreservation procedures have been developed over the last two decades and cryopreservation is becoming a very important tool for the long-term storage of PGRs for future generations, requiring only minimal space and maintenance. Cryopreservation also has some important advantages. The need for space is not an issue – approximately 1 000 accessions can be stored in a 450-litre liquid nitrogen (LN) tank. Manipulation is only warranted when the germplasms are stored. If the materials are kept at ultra low temperature, all the metabolic activities of the cells are at a standstill and they can be preserved in such a state for extended periods. Also, management is easier than other conservation methods. Because of these characteristics, cryopreservation can be suitable for long-term conservation as an alternative to the field gene bank and *in vitro*-cultured plants.

The demand for long-term storage is expanding to the preservation of cultured cells, somatic embryos, enhancing the preservation of endangered and rare plants, remnant crops or underutilized crops and newly developed crops including genetically modified organisms. The development of a simple and reliable method for cryopreservation should be undertaken to allow transfer of materials directly from room temperature into LN and allow the widespread use of materials that have been cryopreserved. Recently, vitrification, encapsulation-

[2] For further information visit http://www.gene.affrc.go.jp/

vitrification and droplet vitrification techniques have been reported, and the number of cryopreserved species has increased markedly over the last 10 to 15 years (Reed 2007). Vitrification-based protocols are potentially valuable cryogenic procedures for cryopreserving meristems and somatic embryos grown *in vitro*. Under optimized conditions, vitrification protocol produces higher levels of growth after LN recovery.

This paper describes the present status of our cryopreservation activities and our new cryopreservation technique – the V-cryo-plate method.

Overview of developed cryopreservation protocols

It is essential to avoid the lethal intracellular freezing that occurs during cooling in LN to maintain the viability of hydrated cells and tissues. Cells and tissues that are to be cryopreserved in LN need to be sufficiently dehydrated before being immersed in LN. There are two types of liquid-solid phase transitions in aqueous solutions. Ice formation is the phase transition from liquid to ice crystal, and vitrification is the phase transition from a liquid to an amorphous glass that avoids crystallization. Vitrification refers to the physical process by which a highly concentrated cryoprotective solution supercools to very low temperatures and finally solidifies into a metastable glass without undergoing crystallization at a practical cooling rate (Fahy *et al*. 1984). Because glass is exceedingly viscous and stops all chemical reactions that require molecular diffusion, its formation leads to dormancy and stability over time (Burke 1986).

In conventional freezing methods, slow freezing to about -30ºC results in sufficient concentration of the unfrozen fraction of the suspending solution and cytosol to enable vitrification upon rapid cooling in LN (partial vitrification, where only the cytosol vitrifies). Vitrification can also be achieved by direct immersion in LN without the freeze-concentration step by exposing cells and tissues to an extremely concentrated solution (7-8 M) of cryoprotectants. This technique is referred to as complete vitrification (as both cytosol and the suspension solution vitrify), and is distinct from conventional slow-freezing methods.

Successful cryogenic procedures applying practical cooling rates by either partial or complete vitrification can be divided into four categories based on the dehydration method used before the immersion into LN (Sakai 1995):

1. Slow prefreezing (freeze-dehydration).
2. Vitrification (osmotic dehydration) with or without encapsulation.

3. Encapsulation/dehydration (osmotic dehydration combined with air drying).
4. Air desiccation.

In these procedures, cryopreservation using dormant buds and cryopreservation of *in vitro*-grown shoot tips by vitrification have been used in the NIAS Genebank.

Cryopreservation using dormant buds

In hardy woody plants, using dormant buds is one way of achieving successful cryopreservation. The protocol for their cryopreservation is as follows (Niino *et al*. 1995). First, the branches of woody plants are collected in winter when their buds are still in a state of quiescence. Axillary buds, with about 10 millimetres of vascular tissue, are removed from the branches. The dormant buds are then put into a polyethylene bag or polyethylene cryotube and prefrozen, with the temperature being decreased by 5ºC each day to -20ºC (mulberry) or -30ºC (pear). Subsequently, they are transferred to a deep-freezer maintained at below -135ºC or a vapour phase of the LN tank maintained at below -150ºC. After storage, the buds are rapidly thawed in a water bath at 37ºC and sterilized. The shoot tips, consisting of the meristem and five to eight leaf primordia, are then dissected and cultured on a Murashige and Skoog (MS) medium. If it is necessary to recover flowering plants faster, micrografting of cryopreserved dormant buds is feasible. The thawed and excised buds with vascular tissue are grafted directly onto one-year-old seedlings.

It is very important that cryopreserved shoot tips maintain viability during long-term storage. We examined changes in the survival rates of dormant buds of the mulberry stored at -135ºC for one to eight years using the aforesaid procedure and found no significant changes. Another step in successful cryopreservation is to adapt this method to many mulberry varieties. The survival rates of 376 varieties cryopreserved for five years in a deep freezer at -135ºC were tested, and 279 varieties (about 74 percent) showed survival rates of more than 50 percent. Only 24 varieties (about 6 percent) had survival rates of less than 30 percent. In the case of these low rate varieties, we obtained at least one regenerated shoot (Niino *et al*. 1995). These results clearly show that cryopreservation using dormant buds is a reliable and safe method for long-term storage. In the NIAS Genebank, 1 283 accessions have been stored already. These methods, with slight changes, have been successfully applied to dormant buds of deciduous woody trees such as apple (Tyler and Stushnoff

1998a, b), pear (Suzuki *et al*. 1997), blueberry and raspberry (Niino *et al*. 1990) and persimmon (Matsumoto *et al*. 2004).

Cryopreservation by vitrification and the keys for success

For the crops without forming dormant buds and *in vitro*-cultured materials, shoot tips have to be used for cryopreservation. In the NIAS Genebank, *in vitro*-grown shoot tips of several plants such as strawberry and mat rush have been cryopreserved experimentally in the LN tank by vitrification.

In general, for successful vitrification, cells and meristems must be sufficiently dehydrated with plant vitrification solution (PVS), without causing injury, to be capable of vitrifying upon rapid cooling into LN in the vitrification method. We used glycerol-based, low toxic vitrification solutions designated PVS2 (Sakai *et al*. 1990, 1991). PVS2 solution contains 30 percent (w/v) glycerol, 15 percent (w/v) ethylene glycol and 15 percent (w/v) dimethyl sulfoxide in a basal culture medium (without growth hormones) containing 0.4 M sucrose (pH 5.8). The key for successful cryopreservation by vitrification is to develop the dehydration tolerance to PVS2 solution. Many papers have demonstrated that the cells and meristems acquired dehydration tolerance to PVS2 solution (the treated control without cooling to -196ºC) by preconditioning and optimizing each step of the procedure. They survived subsequent rapid cooling and rewarming in the excursion of vitrification procedure with little or no additional loss in survival (Yamada *et al*. 1991; Niino *et al*. 1992a,b; Matsumoto *et al*. 1994; Reinhoud 1996). Thus it can be hypothesized that meristems with acquired dehydration tolerance to PVS2 solution can survive during cryopreservation by vitrification. It is still not clear whether the constituent of the PVS2 solution penetrates into the cells during the dehydration process or not.

In the vitrification method, the following procedures such as preconditioning, preculture, osmoprotection (loading treatment), dehydration in PVS2 solution and postLN handlings are vital for successful cryopreservation. Several factors enhance post-thaw survival in cryopreservation by vitrification. High survival of *in vitro*-grown materials is determined not only by the cryogenic protocol itself, but also by the physiological conditions of the materials to be cryopreserved. These include growth stage, preconditioning and the post-thaw medium. To increase the chances of a positive and uniform response to treatment with loading solution (LS) and PVS2, specimens homogeneous in terms of size, cellular composition, physiological state and growth response are used for vitrification.

Cold-hardening and preculture of shoot tips with sucrose-enriched media are effective for improving the post-thaw survival of some temperate and tropical species (Takagi 2000). During preculture on a sucrose-enriched medium, concentrations of sugar, starch and proline are greatly increased in the shoot tips and may enhance the stability of membranes under conditions of severe dehydration (Matsumoto 2002). In addition, a cryoprotective or osmoprotective treatment with LS appears promising as a means of enhancing the dehydration tolerance of shoot tips of several species (Matsumoto 2002). The protective effect of this solution in cellular periprotoplasmic spaces may be due to mitigation of the large osmotic stress from exposure to PVS2, as well as to some mechanisms that minimize the injurious membrane changes from severe dehydration (Crowe *et al*. 1988; Steponkus *et al*. 1992).

The optimal dehydration time with PVS2 is also a key factor. Careful control of the procedures for dehydration and prevention of injury by chemical toxicity or excess osmotic stresses during treatment with PVS2 are important for successful cryopreservation. To determine the optimal exposure time with PVS2 is the first step to develop a cryopreservation protocol of *in vitro*-grown shoot tips by vitrification. In *in vitro*-grown apple shoot cryopreservation, the survival rate of vitrified shoot tips increased gradually with increasing time of exposure to PVS2, and reached a maximum at around 80 minutes after exposure (Niino *et al*. 1992a). Optimal exposure time was species-specific. The exposure time in PVS2 (dehydration time) might vary with the size, stage and morphological state of the shoot tips.

Development of the cryopreservation technique using an aluminium cryo-plate (V-cryo-plate procedure)

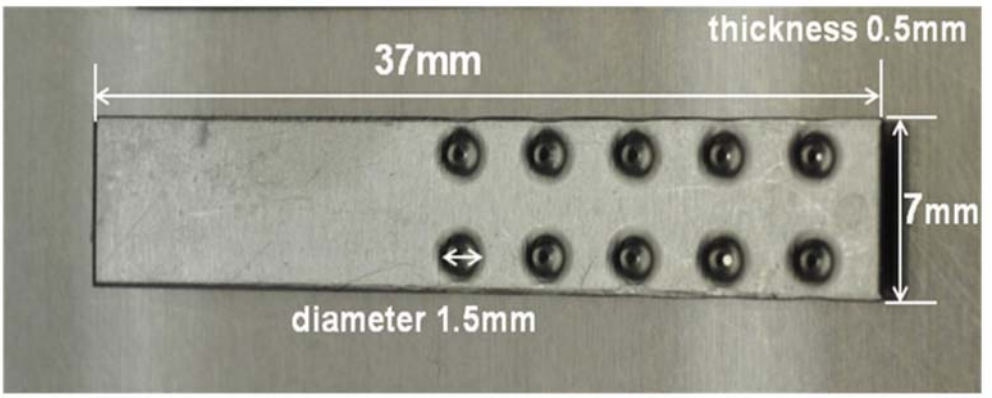
Cryopreservation techniques for shoot tips are now well developed. However, practical application to preservation at gene banks for large-scale operation is still quite limited. Cryopreservation of shoot tips of vegetatively-propagated species currently operated at gene banks still tends to be limited to temperate species: potato (International Potato Center [CIP], Dutch-German Potato Collection), cassava (International Center for Tropical Agriculture [CIAT]), banana (Belgium), garlic (Republic of Korea), Japanese wasabi (Japan) and pear (United States). It is probable that this is attributable to problems in storing the large scale of samples, which inhibits the wide utilization of cryopreservation. These problems are: 1) the need for skilful techniques for the appropriate treatment of shoot tips; 2) the possibility of injuring and losing shoot tips during manipulations; and 3) difficulty in treating many shoot tips at the same time. Thus, standard techniques for tissue culture and cryopreservation

are needed to enhance cryostorage reliability. Therefore, we try to develop more efficient and systematic vitrification procedure.

V-cryo-plate procedure

We contrived and made to order aluminium plates (7 x 37 millimetres and 0.5 millimetres in thickness with ten micro-wells [Plate 1]). The diameter of the wells was 1.5 millimetres. These plates were conformed to fit to a 2-millilitre cryotube. The experimental procedure of the vitrification method using aluminium plates has several steps: establishment of *in vitro* culture, preconditioning, excision of shoot tips, preculture, adhesion to the cryo-plate, osmoprotection in LS, dehydration in PVS, immersion into LN and storage, rapid rewarming for regeneration and plating on the regrowth medium. This method is based on a combination of droplet vitrification and encapsulation vitrification protocols. We called this vitrification procedure using an aluminium cryo-plate the 'V-cryo-plate procedure'.

Plate 1. Aluminium cryo-plate developed in the NIAS Genebank



The case of carnation

Carnation is one of the most economically-important flowers cultivated for the market in Japan. According to the aforesaid experimental procedure, practical cryopreservation of *in vitro*-grown carnation shoot tips by vitrification was conducted (Sekizawa *et al*. 2011).

The different steps of the V-cryo-plate procedure are:

1) Cut shoots (5 millimetres) with a lateral bud and plate on solidified MS medium and culture for two weeks at 25°C with a 16-hour photoperiod under white fluorescent light (52 μmol/m^{2}s: standard

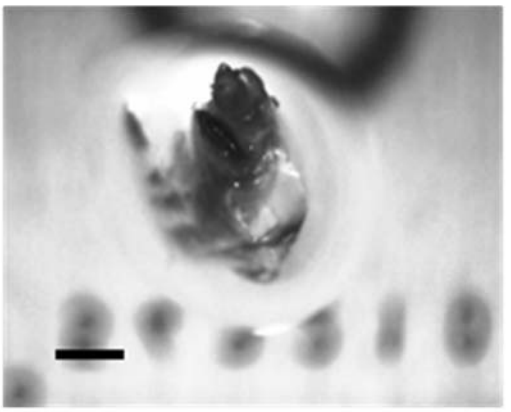
condition). Then dissect shoot tips with basal plate (1-1.5 millimetres long x 1 millimetres wide) from the shoots and preculture for two days at 25ºC in the MS medium with 0.3 M sucrose.

2) Place an aluminium cryo-plate in a petri dish and pour a 2.5 µl 2 percent (w/v) Na-alginate solution with 0.4 M sucrose in a calcium-free MS basal medium on a well.
3) Place the precultured shoot tips in the well, one by one, with the tip of a scalpel blade and slightly press the shoot tips to make them fit into the plate's well.
4) Pour a calcium solution drop (about 0.3 millilitres in total) on the section of the aluminium plate where the shoot tips are located until they are covered and leave for 15 minutes to achieve complete polymerization. The calcium solution contains 0.1 M calcium chloride in the MS basal medium with 0.4 M sucrose.
5) Remove the calcium solution from the cryo-plate by sucking it gently up with a micropipette. Shoot tips adhere to the cryo-plate by the alginate gel.
6) Place the cryo-plate with shoot tips in a 25-millilitre pipetting reservoir filled with about 20 millilitres of LS which contains 2 M glycerol + 1.4 M sucrose in a liquid MS basal medium. Shoot tips are thus osmoprotected at 25ºC for 30 to 90 minutes.
7) Remove the cryo-plate from the LS and place it in a 25-millilitre pipetting reservoir filled with about 20 millilitres of PVS2 (Sakai *et al*. 1990). Shoot tips are dehydrated at 25ºC for 15 to 35 minutes.
8) After dehydration, transfer the cryo-plate to an uncapped 2-millitre plastic cryotube, which is held on a cryo-cane, and directly plunge into LN where it is kept for at least 30 minutes. For long-term storage, the cryotube containing the cryo-plate and LN is capped and stored in the LN tank.
9) For regeneration, retrieve the cryotube from the LN, take the cryo-plate with shoot tips out of the cryotube and immerse it in a 2-millilitre 1 M sucrose solution in a 2-millilitre cryotube. Shoot tips are incubated in this solution for 15 minutes at room temperature and then transferred onto gellan gum-solidified MS medium.

Post-thaw regrowth was evaluated after four weeks of culture at 25ºC under standard conditions. In this experimental procedure, osmoprotection and dehydration steps which were decisive for success were optimized.

The optimal protocol for carnation is as follows. Shoots grown for two weeks from lateral buds were used. The shoot tips (1-1.5 x 1 millimetre) were dissected from the shoot and precultured at 25ºC for two days on MS medium containing 0.3 M sucrose. The precultured shoot tips were placed on the aluminium cryo-plate containing ten micro-wells embedded with alginate gel. Osmoprotection was performed by immersing the cryo-plates in LS (2 M glycerol and 1.4 M sucrose) for 90 minutes at 25ºC. Then dehydration was performed by immersing the cryo-plates in PVS2 for 25 minutes at 25ºC. After storage in LN, shoot tips attached to the cryo-plate were directly immersed into 1 M sucrose solution for regeneration. Using the optimal procedure, we tested the regrowth levels of vitrified shoot tips excised from four carnation cultivars. Regrowth was very high for all cultivars, ranging from 93 to 97 percent, with an average of 95 percent for the four cultivars. The treated shoot tips resumed growth within three days of plating and developed normal shoots without any intermediary callus formation (Plate 2).

Plate 2. Regenerated plantlet of carnation three days after plating. Bar = 1 mm



Key factors for successful cryopreservation

In conventional vitrification procedures, small shoot tips are usually treated in a cryotube floating or suspending in a solution. Treatments, like pipetting, result sometimes in the damage and/or loss of shoot tips. In the droplet vitrification procedure, osmoprotected and dehydrated shoot tips have to be transferred onto aluminium strips with a droplet of PVS2 or PVS3 just before immersion into LN, which is cumbersome (Kim *et al*. 2009). The V-cryo-plate method can overcome these disadvantages because all treatments can be carried out only by moving and transferring the cryo-plate (with attached shoot tips) from one solution to another. The hard cryo-plate makes the manipulation of fragile shoot tips easy.

For successful cryopreservation, key factors are preconditioning, hardening, preculture, osmoprotection by LS, dehydration by PVS treatments and post-thaw handling (Sakai *et al*. 2007). In any of the cryogenic protocols, the cells and tissues to be cryopreserved must be in a physiologically optimal status for the acquisition of dehydration tolerance and to produce vigorous recovery of growth (Dereuddre *et al*. 1988; Withers 1979). Preconditioning of shoot tips is necessary to obtain uniform and vital shoot tips to start with. For this, carnation shoots grown for two weeks from lateral buds were used for shoot tips to be cryopreserved. As such, there is no need for cold acclimation in carnation. Secondly, preculture on MS medium with high sucrose concentrations and osmoprotection was effective for the induction of osmotolerance towards PVS2 (Niino *et al*. 2003; Niino *et al*. 2007). In potato cryopreservation by encapsulation vitrification, the LS solution (a mixture of 2 M glycerol plus 0.6 M sucrose) was effective in increasing osmotolerance towards PVS2 (Hirai and Sakai 1999). Also, Hirai and Sakai (2003) showed that in sweet potato cryopreservation, both a higher concentration of sucrose (1.6 M) in the LS and a longer period of osmoprotection (three hours at 25ºC) were necessary to increase the osmotolerance. Kim *et al.* (2009) indicated for developing a new LS solution for the droplet vitrification procedure, that the loading treatment may act as an osmotic stress neutralizer and/or induce a physiological adaptation of tissues and cells prior to both dehydration and vitrification. Also, they pointed out that appropriate LS should be selected for plant species which are highly sensitive to the cryotoxicity of the PVS solutions. In the case of carnation, 90 minutes' osmoprotection by the LS solution containing 1.4 M sucrose was most effective. The last key parameter for successful cryopreservation by vitrification, are the carefully controlled procedures for dehydration and prevention of injury by chemical toxicity or excessive osmotic stresses during treatment with PVS solutions. With the V-cryo-plate method, it is possible to control these procedures more easily. Using this procedure, in the case of carnation, optimal exposure time to PVS2 has showed a wide spectrum of efficiency (from 15 to 35 minutes). This was also the case when applying the droplet method to shoot tips of black chokeberry (Tanaka *et al*. 2011). This proves that the cooling and warming rates of the V-cryo-plate method are probably at the same level as that of the droplet method (Yamamoto *et al*. 2011a).

Adaptation to other crops

Currently the method is being adapted to other plant species such as Dalmatian chrysanthemum, mulberry, strawberry and mint. Under optimal conditions for each crop, quite high regrowth averages were obtained: 77 percent for six

cultivars of Dalmatian chrysanthemum (Yamamoto *et al*. 2011a); 87 percent for 12 cultivars of mulberry (Yamamoto *et al*. 2011c); 81 percent for 15 cultivars of strawberry (Yamamoto 2011d); and 88 percent for 16 cultivars of mint (Yamamoto *et al*. 2011b). Each condition was crop-specific, but the conditions of preconditioning, osmoprotection and dehydration were most important.

Key factors for facilitating cryostorage

Keller *et al*. (2008) reported that variation in cryopreservation responses may arise from differences in staff skills and competence as well as differences in equipment and minor technical details. Similarly, Reed *et al*. (2004) reported that such differences in cryopreservation from laboratories arise from the culture system, technical procedure and operator skill. This procedure was developed to reduce the problems of operator skill and technical difficulty and can be performed by semi-skilled staff with a little expertise in mounting shoot tips (Yamamoto *et al*. 2011b). We tested the regrowth variations of mint by comparing the results by four operators who performed the experiment with this procedure from excision of shoot tips to plating cryopreserved shoot tips on the medium. The resulting regrowth level of cryopreserved shoot tips showed no significant difference by operators and achieved high regrowth level, indicating wide application and easy adoption of this protocol by semi-skilled persons.

Also, practical cryostorage of 29 mint lines has already been stored in the liquid phase of the LN tank. In this trial, the V-cryo-plate method was demonstrated to be very systematic and time saving. For example, for mint, 15 cryo-plates (150 shoot tips) can be treated within two hours from mounting of Na-alginate gel on the plate to storage in LN. Severt-seven germplasms have been cryopreserved in the LN tank using the V-cryo-plate method since April 2011. This method appears to be promising for facilitating large-scale cryobanking in gene banks.

Practical applications of cryopreservation at gene banks

In the NIAS Genebank, field collections are maintained using the duplicated conservation system in principle, however, there are some exceptions. These include crops such as mulberry, mat rush and taro, which are maintained in only one sub-bank. Cryopreservation should be considered as a backup to field collections to insure against loss (Reed 2001). In this, we mean the priority of collections to be cryopreserved should be given to the ‘at risk’ plants that have an increased chance of being lost from a collection. At the same time, we should determine the most practical steps for the crops to be cryopreserved in

setting up a storage protocol (including material form), a long-term monitoring system and a database.

Regarding the cost of cryopreservation, Hummer and Reed (2000) estimated that the annual maintenance cost of one temperate fruit accession at the Corvallis Repository was US$77 in the field, US$23 under *in vitro* slow-growth storage and US$1 under cryopreservation, to which US$50-65 per accession should be added as an initial start-up cost for cryopreservation. Although prices of LN, LN tanks and other equipment vary among different countries, undoubtedly, the management cost under cryopreservation for the long-term is nil.

Future research needs in cryopreservation

Although remarkable progress in cryobiological studies of plant materials and technology development was made in the last decade, cryopreservation as a preservation method for PGRs is still limited in comparison with microbial and mammalian culture collections. Cryopreservation protocols for plant materials were mostly developed following an empirical, trial-and-error approach. Even if a good protocol is established, it cannot be adopted to all other materials, because different species, varieties and tissue types tend to show different responses to cryopreservation protocol.

To further improve widely applicable protocol for large-scale utilization of cryopreservation for germplasm conservation, basic research is needed to understand the biophysical and metabolic processes underlying the resistance and sensitivity of plant tissues to cryopreservation. Especially, understanding of cryo- and dehydration tolerance and the basic studies on preconditioning for induction of dehydration tolerance are necessary for further development of cryopreservation of tropical plants. Further research is also needed on 1) genetic integrity of cryopreserved materials and 2) development of techniques for recalcitrant seeds and tropical plant species for which cryopreservation research is much less advanced.

Conclusion

The V-cryo-plate method has the following advantages:

1. Handling of shoot tips during the procedure is very easy and quick because the handling is carried out only by moving the cryo-plate with shoot tips.
2. The possibility of injury and omission of shoot tips is much lower than other methods.

3. The shoot tips can be treated with LS and PVSs without floating and/or clinging to the cryotube inside.
4. Cooling and warming procedures also can be done very easily by only dipping the cryo-plate into LN and 1.0 M sucrose solution.
5. A high or higher regrowth rate might be obtained by the V-cryo-plate method.
6. The method is much less laborious than others.
7. Anyone can do this procedure with a modicum of training in mounting shoot tips.

These advantages make the V-cryo-plate procedure a very practical cryopreservation method for several crop germplasms such as carnation, Dalmatian chrysanthemum, mulberry, strawberry and mint. The V-cryo-plate method also appears to be promising for the cryopreservation of other plants. We believe that our cryopreservation method should contribute to and advance the establishment of national cryo-gene banks in each country for storing plant germplasm according to the priority of collections to be cryopreserved.

References

Burke, M.J. 1986. The glassy state and survival of anhydrous biological systems. *In* A.C. Leopold, ed. *Membrane, metabolism and dry organisms*, pp. 358-364. Ithaca, NY, Cornell Univ. Press.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Rudolph, A.S., Wistrom, C.A., Spargo, B.J. & Anchordoguy, T.J. 1988. Interaction of sugars with membranes. *Biochem. Biophys. Acta*, 947: 367-384.

Dereuddre, J., Fabre, J. & Bassaglia, C. 1988. Resistance to freezing in liquid nitrogen of carnation (*Dianthus cryophyllus* L. var. Eolo) apical and axillary shoot tips excised from different-aged *in vitro* plantlets. *Plant Cell Rep.*, 7: 170-173.

Fahy, **G.M.**, **MacFarelane, D.R., Angell, C.A. & Meryman, H.T.** 1984. Vitrification as an approach to Cryopreservation. *Cryobiol.*, 21: 407-426.

Hirai, D. & Sakai, A. 1999. Cryopreservation of in vitro grown meristems of potato (*Solanum tuberosum* L.) by encapsulation-vitrification. *Potato Res.*, 42: 153-160.

Hirai, D. & Sakai, A. 2003. Simplified cryopreservation of sweet potato (*Ipomoea batatas* (L.) Lam.) by optimizing conditions for osmoprotection. *Plant Cell Rep.*, 21: 961-966.

Hummer, K.E. & Reed, B.M. 2000. Establishment and operation of a temperate clonal field genebank. *In* F. Engelmann, ed. *Management of field and* in vitro *germplasm collections*. pp. 29-31. Rome, IPGRI.

Keller, E.R.J., Kaczmarczyk, A. & Senula, A. 2008. Cryopreservation for genebanks – a matter between high expectations and cautious reservation. *CryoLetters*, 29: 53-62.

Kim, H.H., Yoon, J.W., Pak, S.U., Lee, S.C., Baek, H.J., Cho, E.G. & Engelmann F. 2009. Development of alternative loading solutions in droplet-vitrification procedures. *CryoLetters*, 30: 291-299.

Matsumoto, T., Sakai, A. & Yamada, K. 1994. Cryopreservation of in vitro grown apical meristems of wasabi (*Wasabia japonica*) by vitrification and subsequent high plant regeneration. *Plant Cell Rep.*, 13: 442-446.

Matsumoto, T., Niino, T., Shirata, K., Kurahashi, T., Matsumoto, S., Maki, S. & Itamura, H. 2004. Long-term conservation of Diospyros germplasm using dormant buds by a prefreezing method. *Plant Biotechnology*, 21: 229-232.

Matsumoto, T. 2002. Cryopreservation of in vitro-grown apical shoot tips of wasabi (*Wasabia japonica*) by different procedures. *In* L.E. Towill, & Y.P.S. Bajaj, eds. *Biotechnology in agriculture and forestry 50. Cryopreservation of Plant Germplasm II*, pp. 180-195. Berlin, Heidelberg. Springer-Verlag.

Niino, T., Oka, S. & Ishihara, A. 1990. Survival of shoot formation *in vitro* in raspberry and blueberry winter buds cryopreserved in liquid nitrogen. *2nd Plant Tiss. Cult. Colloquium Proc. Jap. Plant Tiss. Cult. Soc.* pp. 78-79.

Niino, T., Sakai, A., Yakuwa, H. & Nojiri, K. 1992a. Cryopreservation of in vitro-grown shoot tips of apple and pear by vitrification. *Plant Cell Tiss. Org. Cul.* 28: 261-266.

Niino, T., Sakai, A., Enomoto, S., Magoshi, J. & Kato, S. 1992b. Cryopreservation of in vitro-grown shoot tips of mulberry by vitrification. *CryoLetters*, 13: 303-312.

Niino, T., Shirata, K. & Oka, S. 1995. Viability of mulberry winter buds cryopreserved for 5 years at -135ºC. *J. Seric. Sci. Jpn*., 64: 370-374.

Niino, T., Tanaka, D., Ichikawa, S., Takano, J., Ivette, S., Shirata, K. & Uemura, M. 2003. Cryopreservation of *in vitro*-grown apical shoot tips of strawberry by vitrification. *Plant Biotech.*, 20: 75-80.

Niino, T., Tanaka, D., Tantely, R.R., Fukui, K. & Shirata, K. 2007. Cryopreservation of basal stem buds of *in vitro*-grown mat rush (*Juncus* spp.) by vitrification. *CryoLetters*, 28: 197-206.

Reed, B.M. 2001. Implementing cryogenic storage of clonally propagated plants. *CryoLetters*, 22: 97-104.

Reed, B.M., Kovalchuk, I., Kushnarenko, S., Meier-Dinkel, A., Schoenweiss, K., Pluta, S., Straczynska, K. & Benson, E.E. 2004. Evaluation of critical points in technology transfer of cryopreservation protocols to international plant conservation laboratories. *CryoLetters*, 25: 341-352.

Reed, B.M. 2007. Cryopreservation – practical considerations. *In* B.M. Reed, *Plant Cryopreservation, A Practical Guide*, pp. 3-14. New York, NY, Springer.

Reinhoud, P. 1996. *Cryopreservation of tobacco suspension cells by vitrification*. Rijks University, Leiden. (Ph.D. thesis)

Sakai, A., Kobayashi, S. & Oiyama, I. 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *brasiliensis* Tanaka) by vitrification. *Plant Cell Rep.*, 9: 30-33.

Sakai, A., Kobayashi, S. & Oiyama, I. 1991. Survival by vitrification of nucellar cells of navel orange (*Citrus sinensis* var. *brasiliensis* Tanaka) cooled to -196ºC. *J. Plant Physiol.*, 137: 465-470.

Sakai, A. 1995. Cryopreservation of germplasm of woody plants. *In* Y.P.S. Bajaj, ed. *Biotechnology in agriculture and forestry, vol. 32, cryopreservation of plant germplasm I*, pp. 53-69. Berlin, Heidelberg, Springer-Verlag.

Sakai, A., Hirai, D. & Niino, T. 2007. Development of PVS-based vitrification and encapsulation-vitrification protocols. *In* B.M. Reed, *Plant cryopreservation, a practical guide*, pp. 33-58. New York, NY, Springer.

Sekizawa, K., Yamamoto, S., Rafique, T., Fukui, K. & Niino, T. 2011. Cryopreservation of in vitro-grown shoot tips of carnation (*Dianthus caryophyllus* L.) by vitrification method using aluminum cryo-plates. *Plant Biotechnol.*, 28: 401-405.

Steponkus, P.L., Langis, R. & Fujikawa, S. 1992. Cryopreservation of plant tissues by vitrification. *In* P.L. Steponkus, ed. *Advances in low temperature biology V. 1.* pp. 1-61. Hanpton Hill, UK, JAI Press.

Suzuki, M., Niino, T., Akihama, T. & Oka, S. 1997. Shoot formation and plant regeneration of vegetative pear buds cryopreserved at -150ºC. *J. Jpn. Soc. Hort. Sci*., 66: 29-34.

Takagi, H. 2000. Recent developments in cryopreservation of shoot apices of tropical species. *In* F. Engelmann & H. Takagi, eds. *Cryopreservation of tropical plant germplasm – current research progress and applications,* pp. 178-193. Tsukuba, JIRCAS and Rome, IPGRI.

Tanaka, D., Matsumoto, T., Nishiuchi, A. & Niino, T. 2011. Cryopreservation of *in vitro* grown black chokeberry shoot tips by droplet vitrification. *Acta Hort.*, (in press).

Tyler, N.J. & Stushnoff, C. 1988a. The effect of prefreezing and controlled dehydration on cryopreservation of dormant vegetative apple buds. *Can. J. Plant Sci.*, 68: 1163-1167.

Tyler, N.J. & Stushnoff, C. 1988b. Dehydration of dormant apple buds at different stages of cold acclimation to induce cryopreservability in different cultivars. *Can. J. Plant Sci.,* 68: 1169-1176.

Withers, L.A. 1979. Freeze preservation of somatic embryos and clonal plantlets of carrot (*Daucus carota* L.). *Plant Physiol.,* 63: 460-467.

Yamada, T., Sakai, A., Matsumura, T. & Higuchi, S. 1991. Cryopreservation of apical meristems of white clover (*Trifolium repens* L.) by vitrification. *Plant Sci.*, 78: 81-87.

Yamamoto, S., Rafique, T., Priyantha, W.S., Fukui, K., Matsumoto, T. & Niino, T. 2011a. Development of a cryopreservation procedure using aluminum cryo-plates. *CryoLetters,* 32: 256-265.

Yamamoto, S., Rafique, T., Fukui, K., Sekizawa, K. & Niino, T. 2011b. V-Cryo-plate procedure as an effective protocol for cryobanks. Case study of mint cryopreservation. *CryoLetters* (in press).

Yamamoto, S., Rafique, T., Sekizawa, K. Koyama, A., Ichihashi, T. & Niino, T. 2011c. Development of an effective cryopreservation protocol using aluminum cryo-plates for *in vitro*-grown shoot tips of mulberries (Morus spp.) originated from the tropics and subtropics. *Sanshi-Konchu Biotec* (in press)

Yamamoto, S., Fukui, K., Matsumoto, T., Rafique, T., Khan, N.I., Sekizawa, K. & Niino, T. 2011d. Cryopreservation of *in vitro* grown shoot tips of strawberry by vitrification method using aluminum cryo-plates. *Proc. 1st International Symposium on Cryopreservation of Horticultural Crops in China,* p. 20.

The importance of information in the context of the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture

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The Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture (PGRFA) was endorsed by the Commission on Genetic Resources for Food and Agriculture at its 13th Regular Session in July 2011.[2] It is in the agenda of the 143rd Session of the FAO Council for its approval on behalf of the FAO Conference in November 2011.

The Second Global Plan of Action was prepared on the basis of the gaps and needs identified in the Second report on the state of the world's PGRFA, *which was published a year earlier, and took into account contributions from regional consultations as well as other inputs from governments and international organizations.*

The Second Global Plan of Action is an agreed strategy to improve the conservation and use of PGRFA around the world, starting from national programmes up to regional and global coordinated efforts. It follows the first Global Plan of Action which was adopted in 1996.

During the past 15 years information management and exchange technologies, together with molecular biology, were among the fields relevant to PGRFA conservation and use that have experienced the most rapid advances. Important progress has also occurred in the policy framework at the international level with the entry into force of the International Treaty on PGRFA and the establishment of the Multilateral System for Access and Benefit Sharing which covers a set of crops and species.[3]

Projected demographic changes both in terms of world population size[4] and its distribution in rural and urban areas and the growing impact of climate change

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2 http://www.fao.org/nr/cgrfa/cgrfa-meetings/cgrfa-comm/en/

3 See Annex I of the International Treaty on PGRFA.

4 The world population in 2050 is forecast to be 9 billion; more than 70 percent will live in cities compared to approximately 50 percent today.

on agricultural production are increasingly emphasizing the need to conserve PGRFA efficiently and to intensify their sustainable use.

The Second Global Plan of Action recognizes that effective information management and sharing, by taking full advantage of advanced information technologies, will be an important prerequisite to achieving this objective.

Progress and constraints in PGRFA information management

Juxtaposing the revolution that communication and information management systems have undergone over the past 15 years, there have been important improvements in the availability and accessibility of PGRFA information.

A growing number of gene banks have published part of their germplasm data through searchable web systems. These include *inter alia* EURISCO,[5] NIAS[6] and GENESYS[7].

Despite these improvements, according to the *Second report on the state of the world's PGRFA*, the documentation of much of the world's *ex situ* PGR collections is insufficient. This continues to be a major impediment to the increased use of germplasm in crop improvement and research. In addition, where germplasm data do exist, there are frequent problems in standardization and accessibility, even for basic passport information.

The lack of a freely available, flexible and up-to-date gene bank information system that is user-friendly and multi-lingual has constrained documentation improvement in many countries, although in some cases, regional and/or bilateral collaboration has helped to meet information management needs through the sharing of experiences and tools.

To fill this gap, a major global project focusing on PGRFA accession-level information management was initiated a few years ago with support from the Global Crop Diversity Trust. The first tested release of an advanced gene bank information management system, GRIN Global,[8] derived from that in use by the Germplasm Resources Information Network (GRIN) of the United States Department of Agriculture, will be made freely available in the coming months. Crop descriptor lists for a number of crops have been developed under the coordination of Bioversity International.[9]

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5 http://eurisco.ecpgr.org/home_page/home.php

6 http://www.gene.affrc.go.jp/index_en.php

7 http://www.genesys-pgr.org/

8 http://www.grin-global.org/

9 http://www.bioversityinternational.org/?id=3737

The World Information and Early Warning System (WIEWS[10]) on PGRFA has continued to update its database on germplasm holdings from more than 1 750 gene banks around the world and has provided relevant information for the preparation of the *Second report on the state of the world's PGRFA*. Starting in 2004 a number of National Information Sharing Mechanisms (NISMs) on PGRFA[11] has been established with technical assistance from the WIEWS network of officially-appointed national focal points. NISMs allowed countries to document efforts on PGRFA conservation and use and to monitor, with the participation of key stakeholders, the degree of implementation of the Global Plan of Action. The WIEWS platform hosts NISM portals and databases.[12]

Second Global Plan of Action for PGRA: priority activities

In situ *conservation and management*

1. Surveying and inventorying PGRFA.
2. Supporting on-farm management and improvement of PGRFA.
3. Assisting farmers in disaster situations to restore crop systems.
4. Promoting *in situ* management of crop wild relatives and wild food plants.

Ex situ *conservation*

5. Supporting targeted collecting of PGRFA.
6. Sustaining and expanding *ex situ* conservation of germplasm.
7. Regenerating and multiplying *ex situ* accessions.

Sustainable use

8. Expanding the characterization, evaluation and further development of specific subsets of collections to facilitate use.
9. Supporting plant breeding, genetic enhancement and base-broadening efforts.
10. Promoting diversification of crop production and broadening crop diversity for sustainable agriculture.

[10] http://apps3.fao.org/wiews

[11] More than 70 countries around the world, about 20 percent of which was supported through the Japanese-funded FAO projects GCP/RAS/186/JPN and GCP/RAS/240/JPN.

[12] http://www.pgrfa.org

11. Promoting development and commercialization of all varieties, primarily farmers' varieties/landraces and underutilized species.
12. Supporting seed production and distribution.

Building sustainable institutional and human capacities

13. Building and strengthening national programmes.
14. Promoting and strengthening networks for PGRFA.
15. Constructing and strengthening comprehensive information systems for PGRFA.
16. Developing and strengthening systems for monitoring and safeguarding genetic diversity and minimizing genetic erosion of PGRFA.
17. Building and strengthening human resource capacity.
18. Promoting and strengthening public awareness of the importance of PGRFA.

Global priorities for improved information management and exchange

Almost every agreed priority of the Second Global Plan of Action, if not all, stresses the importance of information gathering and exchange as well as the use of improved methods and technologies for achieving the priority objectives.

Priority activity 1 *Surveying and inventorying PGRFA* promotes the improvement and application of methods – such as GPS, remote sensing and molecular markers – for assessing distribution of and threats to PGRFA. Indicators are particularly needed to monitor changes in the distribution of diversity and to aggregate information on individual species and populations.

The goal of priority activity 5 *Supporting targeted collecting of PGRFA* is to ensure adequate collecting of PGRFA, together with associated information, focusing on diversity that is missing from *ex situ* collections, under threat or expected to contain useful traits. Collected material should be conserved in the country of origin whenever possible and a duplicate sample deposited elsewhere for safety purposes.

Priority activity 8 *Expanding the characterization, evaluation and further development of specific subsets of collections to facilitate use* directly focuses on PGRFA documentation. Information on morpho-agronomic traits as well as on reaction to biotic and abiotic stresses is as important as the material itself.

This information enables breeders and other users of PGRFA to identify which accessions they can deploy directly in the field or use in research and crop improvement. Nowadays it is increasingly important to assess gene bank accessions and breeding materials for traits associated with mitigation and adaptation to climate change and to make this information widely available. The goal is to make gene bank collections as useful as possible. This activity may involve improving methods for gathering characterization and evaluation data and creating subsets and trait-specific collections, particularly for crops of global importance.

Priority activity 15 is probably the most explicit with regard to information management and exchange. It calls for *Constructing and strengthening comprehensive information systems for PGRFA*. Information exchange is also accorded high importance throughout the International Treaty. In particular, it is recognized as one of the supporting components of the International Treaty in Article 17, the Global Information System. Information sharing is also one of the main mechanisms for equitable sharing of the benefits derived from the use of PGRFA under the Treaty's Multilateral System.

Emphasis is put on the need to have efficient and accessible national information systems including, but not limited to, gene bank accession-level information systems, to better manage PGRFA data and to support the participation of countries in global information systems.

Another top-information priority is activity 16 *Developing and strengthening systems for monitoring and safeguarding genetic diversity and minimizing genetic erosion of PGRFA*. This priority activity is also particularly relevant in the context of the Convention on Biological Diversity's (CBD) adopted Strategic Plan for Biodiversity for the period 2011-2020 and its 20 'Aichi Biodiversity Targets'. Target 13 focuses on genetic diversity for food and agriculture: "By 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity."

The work ahead

Information systems are often tools for specialists to collaborate on exchanging data, knowledge and experiences. However they can also became tools for communicating and spanning specialist groups such as gene bank managers,

breeders, seed producers, farmers and policy-makers, in other words, serving PGRFA national programmes and promoting integration and mutual support.

At the international level a number of important developments relevant to PGRFA information management are expected to take place before the XIV Regular Session of the Commission and 6th Governing Body meeting (scheduled in 2013) with regard to PGRFA information management and exchange and to the implementation of Article 17 on the Global Information System. These include:

Indicators and reporting format for monitoring the implementation of the Global Plan of Action will be revised in the light of the Second Global Plan of Action. The Second Global Plan of Action emphasizes that indicators and methods for assessing genetic diversity over time and minimizing genetic erosion and its drivers are required in order to be able to establish national, regional and global baselines for monitoring diversity and developing effective early warning systems. Higher order indicators, in particular addressing genetic diversity and erosion, will be proposed for the attention of the Intergovernmental Technical Working Group on PGRFA (ITWG-PGRFA) which will meet in November 2012.

An improved system for monitoring Global Plan of Action implementation and for supporting NISMs will be developed and released in 2012. It will take into consideration the priority activities of the Second Global Plan of Action and allow comparisons with the first Plan.

Revised standards for gene bank management of germplasm, including orthodox and recalcitrant seeded plants as well as vegetatively-propagated species will be made available at the next session of the ITWG-PGRFA.

Descriptors based on international standards for new and underutilized crops and crop wild relatives (CWR) need to be developed.

The Second Global Plan of Action calls for countries to take the lead in the implementation of all its 18 priority activities. A number of relevant information-related tasks/policies could boost progress in the conservation and sustainable use of PGRFA at the national level, if pushed forward. These include:

- In all gene banks an efficient database management system should be in place and used. Ideally it should allow a careful monitoring of routine activities, allow curators access to records of standardized passport and characterization data but also provide for germplasm evaluators to record genotype *x* environment interaction data.

- A number of significant data sets and knowledge still exists in vulnerable and hard to access forms or systems. The effort required to rescue and share this information is in many cases negligible compared to the benefits that would derive from it.
- Common standards for passport, characterization and evaluation data need to be applied systematically for fostering data exchange and access, but also to support technology transfer and global, regional and national assessments of PGRFA.
- Governments should periodically review and report on the situation of PGRFA, assessing threats and minimizing or, where possible, eliminating them. Designated focal points should convey this information to FAO, and, as appropriate, to the Governing Body of the International Treaty, the Conference of the Parties to the CBD and other relevant bodies.
- Monitoring and reporting mechanisms for genetic erosion need to be established. They could fit within established NISMs and take advantage of NISM participatory structure to carry out assessments and coordinate preventive and remedial actions.
- Efforts should be made to ensure that relevant information generated by extension services, local non-governmental organizations, the seed sector and farming communities can be linked to early warning systems at the national and higher levels. Novel tools of information and communication technologies, including mobile phones, can greatly facilitate reporting and collating information from such disparate sources.
- Special efforts are needed to identify the species and populations that are most at risk and are most likely to harbour traits that will be important in the future; this is particularly important with regard to farmers' varieties/landraces and CWR.
- Considerable efforts are needed to raise awareness of the importance of documentation and information management and to boost capacity at all levels within the national programmes.

Finally, the global-level effort on research should be supported *inter alia* to:

- Develop appropriate and low-cost methodologies and technologies for compiling and exchanging data;
- Develop improved molecular methods to monitor genetic diversity/erosion over time, together with
- Improving the use of GIS technology to survey PGRFA and monitor genetic diversity.

Conclusions

PGRFA information management and exchange are top priorities in the Second Global Plan of Action as through them a more rational system for PGRFA conservation and sustainable use can be set up. Progress in this area can have a multiplier effect and benefit the entire PGRFA chain from conservation to seed delivery to farmers.

The approval of the Second Global Plan of Action by the FAO Council calls for action at national, regional and international levels and this should involve all relevant stakeholders. Its full implementation will require significant acceleration in the current activities for PGRFA conservation and use, and will also contribute to International Treaty implementation, as the Plan is a supporting component of the Treaty.

Countries should make every possible effort to provide, in accordance with their capacities, financial support with respect to national activities to achieve the objectives of the Second Global Plan of Action. The Funding Strategy of the International Treaty, including the Benefit Sharing Fund and the Global Crop Diversity Trust, will be major contributors for its implementation at the international level.

However every effort should also be made to seek new, additional and innovative sources of funding to support the implementation of the Second Global Plan of Action. International cooperation for conservation and sustainable use of PGRFA should be strengthened, in particular to support and complement the efforts of developing countries and countries with economies in transition.

Challenges for conservation and utilization of plant genetic resources

Kazuo Watanabe[1]

Plant genetic resources (PGR) should be regarded as essential components of the biosphere. They are vital for human survival, betterment of livelihoods and sustainable development. Their conservation should be promoted as they can deteriorate or be lost easily. However paradigm shifts in ownership have been drastic in the past three decades.

Research and development/science and technology can facilitate PGR conservation and utilization but in this context no substantial progress has been realized in backstopping the genetic resources of locally important species; moreover there is differentiation in resource allocation for a few dozen significant crop species and many of the underutilized groups have been orphaned. Related political, economic, social and technological issues are highlighted in this paper.

Paradigm shifts in ownership and recognition of PGR

Plant genetic resources (PGR) have contributed to the daily lives of the people worldwide through their domestication and cultivation. They should be regarded as essential components, just as water, air and soil, of the biosphere. They have been recognized in United Nations' forums such as the World Sustainable Summit on Development (WSSD) in 2002 and Millennium Ecosystem Assessment (MEA) report in 2005 as vital inputs for human survival and sustainable development. But the paradigm shifts in ownership have been drastic in the last three decades, and especially swift in the last 20 years. PGR were regarded as a common heritage in the past. Now there are issues pertaining to their ownership, intellectual property rights (IPR, equitable accessibility and benefit sharing.

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The 10th Conference of Parties (COP-10) of the Convention on Biological Diversity (CBD) had vigorous discussions with regard to an international protocol for fair and equitable access and benefit sharing (ABS) for PGR[2] besides other outstanding issues.

ABS issues have been under negotiation since 2000 starting with the Bonn Guidelines[3]; COP-7 in 2004 that strongly recommended further exploration into development of an international protocol, which would potentially be legally binding. The major issue has been how biodiversity-rich countries can protect their ownership of PGR and how they can assure benefit sharing with users. Of particular concern has been pharmaceutical product development and profits from intellectual property rights, which hint at vast cash opportunities. On the other hand, ABS discussions in the international arena have often dodged the concomitant risk to users' investment in research and development (R&D) after legal acquisition of the genetic resources and whether they receive any profits generated. Indeed there is a low probability of substantial benefit from a natural substance-based product such as an anti-cancer remedy. However, biodiversity-rich developing countries have insisted on a substantial and mutual benefit-sharing agreement prior to any actual R&D success to protect their ownership against other IPR issues that might arise.

Crop genetic resources have been discussed at FAO-based forums; to protect food security and further sustainable development, FAO's International Treaty on Plant Genetic Resources for Food and Agriculture was ratified in 2001 and came into effect in 2004. This international legal instrument has a different spirit concerning genetic resources compared with other forums. With more focus on the human heritage aspect of sharing plant genetic resources, the Treaty supports the many users of PGR. However there has been stagnancy in implementation and ABS is generally a cumbersome issue. Indeed, different forums have been discussing IPR and/or ABS on genetic resources as shown in Figure 1, and complications related to understanding ownerships and rights could impede a healthy and smooth ABS vehicle.

The World Intellectual Property Organization (WIPO)[4] has carried out intergovernmental consultation on genetic resources with 17 meetings so far. Meanwhile the CBD-ABS has had nine working group meetings with two conferences plus the ongoing Informal Consultation Group meetings at COP events.

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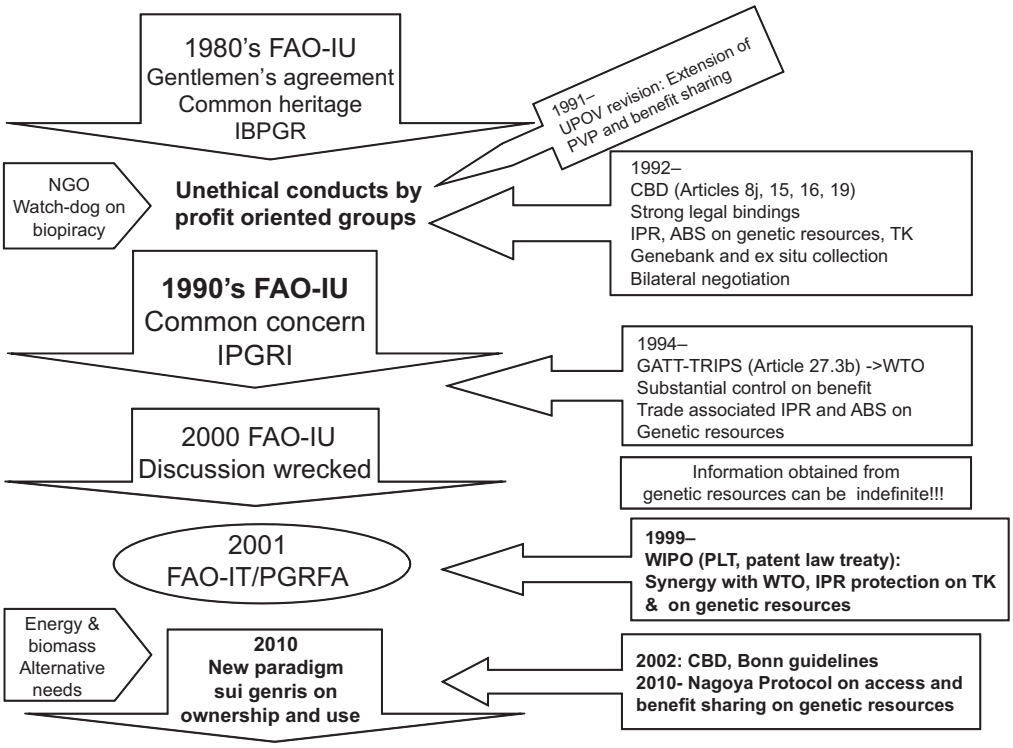
2 See http://www.cbd.int/abs/ir/

3 See http://www.cbd.int/abs/bonn.shtml

4 Visit http://www.wipo.int/portal/index.html.en

Figure 1. History of PGR-related forums. Revised from Watanabe (2004) and Watanabe and Hirano (2011)



The international Union for the Protection of New Varieties of Plants (UPOV)[5], whose members come from developed countries mainly, has developed variety protection models. Article 27 of the World Trade Organization's Trade-Related Aspects of Intellectual Property Rights (WTO-TRIPS) Agreement is also relevant in the PGR context.

One difficulty is parallel discussions at different forums, split in governments sector-wise; for example the CBD addressing environment and/or science and technology, FAO and UPV information technology and agriculture, WIPO and WTO trade and industry. Intersector discussion and integration needs to be streamlined and possible coherence of national policy may be one way to make some progress on ABS (Raman and Watanabe 2000).

While laws are regulated by the governments, negotiations on PGR *vis-à-vis* mutually-agreed terms, transfer conditions and follow-up deals could involve public and commercial mediators such as bioprospecting consultants (Watanabe and Teh 2011).

[5] Visit http://www.upov.int/index_en.html

Discussion on regulating ABS is often theoretical, and lessons from different cases by alternative sectors should be promoted to further modify and support ABS implementation plans by each nation.

All stakeholders should study the history of the PGR movement to promote, sustain and develop agricultural activities worldwide. No country or territory can survive solely on its own natural genetic resources, so the need for mutual dependency among nations should also be understood with regard to the global sharing of PGR.

Many instruments and forums are oriented towards modern industry apropos of IPR-related laws; however, considering the nature of mutual dependency on PGR for supporting the livelihoods of many subsistence users, crop genetic resources may not be dealt with appropriately by legislation (Chapman and Watanabe 2007).

Plant genetic resources should have unique IPR protection and ABS based on a comprehensive protection system rather than existing IPR protection by patent. PGR must have a system that is in a class of its own such as plant variety protection under UPOV; a joint effort should be made with major international instruments among CBD, WIPO and others, to form a common forum or commission for users and providers of genetic resources, and benefit-sharing mechanisms for people 'on the ground' such as subsistence farmers.

Balancing conservation and utilization of PGR

PGR are not only important natural resources, but they also have strong links with environmental conservation issues. For example, tropical forests consist of a complex of plant species, which are essential for the conservation of ecosystems and self-remediation. Thus, the value of PGR cannot be accurately measured now or in the future (Altman and Watanabe 1995).

Conservation of PGR, therefore, should be promoted as these resources can be degraded or lost without good stewardship. In the past, more plant species were exploited for various uses compared to the present. Genetic diversity can be expanded or maintained well with different management methods: 1) tomato landrace diversity in Myanmar is very broad with very actively selected and has various uses by local people while tomatoes comes from the New World (San San Yi *et al.* 2008) and 2) gene bank management approaches alter the genetic diversity in its collections such as in ginger and wheat (Jatoi *et al.* 2008, Hirano *et al.* 2010).

Advances in R&D/science and technology can support conservation methods, especially *ex situ* conservation. However legal, social and cultural aspects, especially with regard to ethical, legal and social implications (ELSI) may be separate issues for *in situ* conservation and/or on-farm management. The following section draws on the assessments of Watanabe and Iwanaga (1999).

Review of science and technology for conservation

Conservation of PGR in gene banks

Conventional conservation of plant species has been conducted *in situ* by local communities and small-scale traditional farmers. However such efforts are vulnerable to various factors such as climate change, disease and pest epidemics, and arguably the effects of globalization. On the other hand, it is vital to maintain the dynamics of genetic variation by cultivating many genetically-different landraces and by enhancing participatory crop improvement by farmers. On-farm management could complement and reduce the expenses of *ex situ* conservation (Watanabe and Pehu 1997).

Many perennial species, especially trees, lack relevant genetic information so field research favours economically-important species such as *Eucalyptus* spp. (Mimura *et al.* 2009).

Gene banks have also been engaged in preserving collections *ex situ*. In this context the focus is on extracting basic PGR data for gene banks.

True seed-propagated species

The following subjects are unresolved in many species and need further research:

(1) Physiology of flowering and seed propagation;
(2) Physiology of seeds for long-term storage;
(3) Population size and change in allelic frequencies on rejuvenation of seeds;
(4) Increasing opportunities for recalcitrant species, especially in the tropics;

To determine the amount of seeds to be preserved, two issues are important: (a) duration of the period of seed storage in each seed generation and statistical probability of viability and (b) genome-wide genetic diversity and preservation of

specific alleles of interest but with simple and fail-safe genetic markers (Yamanaka *et al.* 2003).

(5) Polyploidy is another important aspect of germplasm conservation, especially with regard to seed propagation. Polysomic polyploid species such as sweet potato and strawberries need more research on their genetics such as allelic diversity (Anwar *et al.* 2009) that supports conservation and breeding strategies, especially the application of a whole-genome study with genomics tools; and

(6) Overall revision of collecting strategies in conjunction with gene bank conservation, even for well-represented species (Hirano *et al.* 2008, 2009, 2011a).

Under common recommendations, many seeds for each accession should be maintained, bearing in mind that an enormous number of accessions are preserved at a gene bank. Scientific rigour should support cost-related aspects; core collections and population size determination are some of the key issues in gene bank management and user access, besides seed availability with relevant germination capacity.

Vegetatively-propagated and/or perennial species

The following subjects need further research for more effective management of the clonally-maintained accessions:

(1) Rooting capacity in woody species and epiphytes;
(2) Identification of the clonal variation *in situ* to determine the extent of *ex situ* conservation (Hirano-Machida *et al.* 2011b);
(3) Reduction of somaclonal variation in *in vitro* preservation;
(4) Duplicate identification;
(5) Genotype independent tissue culture methods;
(6) Cryopreservation, especially for tropical species; and
(7) Germplasm health associated with quarantine.

Critical number of seeds/propagules for the maintenance of each accession should be another factor for PGR preservation at a gene bank. Unfortunately, the reality of gene bank management is far from being desirable due to lack of funding in many countries. At many gene banks genetic erosion could be taking place due to poor management practices resulting from insufficient funding. Now many gene banks are facing political and financial crises; consequently

there is the danger of losing precious PGR collections while strong national sovereignty rights are demanded at international forums.

Facilitating conservation and use of PGR via technology

Industrial crops assist with the production of basic materials. Planned production to maintain PGR in plantations and agricultural fields would be less damaging than collection/harvest from natural reserves, although serious environmental concerns about pesticide use and erosion exist. As well as agricultural plant species, it should be recognized that a gradual shift from slash-and-burn of tropical forests to scheduled and systematic production has been taking place. This is based on mass production of propagules through the application of micropropagation of industrially-interesting species and extensive public education of the *in situ* conservation and use of natural forests. On the other hand, only a limited number of species can be employed by such a system due to the unavailability of specific technology relating to particular species; there is differentiation of resource allocation to a few dozen crop species and many underutilized groups (Altman and Watanabe 1995; Watanabe and Pehu 1997; Watanabe and Iwanaga 1999). Also the extreme bias towards selection or monoculture of cultivars/species results in genetic erosion; thus strategic use of technologies should be accompanied by sustainable use of PGR harmonized with genetic conservation. With the advancement of basic R&D in the plant sciences and technologies, the following thrusts should enhance the conservation and use of PGR:

(1) Mass propagation by tissue culture for conservation.

(2) Photo-autotrophic micropropagation on a large scale for industrial propagule production.

(3) Immunological or molecular biological tools for detection of plant pathogens and pests in plant quarantine and gene banks, accompanied by measures to maintain the quality of commercial propagules.

(4) Detection of environmental toxins/pesticides in *in situ* conservation.

(5) Genome-wide use of molecular markers for the evaluation of genetic diversity and genetic erosion.

(6) Molecular biological approaches to isolate and utilize plant genes in genetic engineering.

These approaches are being used in specific cases, however, there is a need to accelerate basic research although some criteria were identified decades ago.

As information on tropical plant and heterozygous perennial species is incomplete further research and application of technology application is warranted. These needs, noted in the list below, for systematic industrial utilization of PGR were identified years ago (Watanabe and Iwanaga 1999):

(1) Extensive survey of the genetic diversity and specific phytochemical substances of industrial interest other than major crop species;

(2) Survey of the relationships between pesticide residues and conservation of PGR, and development of agrochemicals with low residue and low toxicity; landraces and wild species are often more sensitive to agrochemicals than modern cultivars;

(3) Low cost and low profile tissue culture systems for small-scale industry;

(4) Mass production methods for elite clones for large-scale industrial purposes that avoid slash-and-burn practices;

(5) Preservation methods for recalcitrant seeds in true seed-propagated species;

(6) Strategies for seed collection and propagation; conservation of genetic diversity, especially on out-crossing polyploids;

(7) Databases for plant pathogens and pests including biology, epidemics, diagnostics and protection methods, a molecular plant *x* pathogen/pest interaction, chemical ecology and integrated pest management should be emphasized on the basis of sustainable industrial production based on resistant cultivars and management methods;

(8) Screening methods for chemical components, particularly for pharmacognotic uses; and

(9) Transformation processes and cost reduction for new industrial materials such as biodegradable plastics derived from plants.

Beyond science and technology

ELSI aspects need to be revisited with regard to stakeholder ethical issues in the industrial application of biotechnology (Okada and Watanabe 2008a,b) and legal and regulatory aspects of genetic engineering (Watanabe *et al.* 2005). Biotechnology applications need regional harmonization (Okusu and Watanabe 2005), and feedback is required to stimulate decision-making on technology use (Sinebo and Watanabe 2005).

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References

Altman, D.W. & Watanabe, K.N., eds. 1995. *Plant biotechnology transfer to developing countries*. Georgetown, Texas, R.G. Landes Co. 300 pp.

Anwar, N., Kikuchi, A., Kumagai, T. *et al.* 2009. Nucleotide sequence variation associated with β-amylase deficiency in the sweet potato *Ipomoea batatas* (L.) Lam. *Breed. Sci.*, 59: 209-216.

Chapman, J. & Watanabe, K.N. 2007. Current issues on IP management for health and agriculture in Japan. *In* A. Krattiger *et al.*, eds. *MIHR-pipra handbook of best practices for management of intellectual property in health and agriculture*, pp. 1621-1650. Chapter 17. Univ. California, Davis.

Hirano, R., Kikuchi, A. Kawase, M. *et al*. 2008. Evaluation of genetic diversity of bread wheat landraces from Pakistan by AFLP and collection strategy. *Genetic Resources and Crop Evolution*, 55: 1007-1015.

Hirano, R., Jatoi, S.A., Kawase, M. *et al*. 2009. Consequence of ex situ conservation on the genetic integrity of germplasm held at different gene banks: a case study of bread wheat collected in Pakistan. *Crop Science*, 49: 2160-2166.

Hirano, R., Than Htun Oo & Watanabe, K.N. 2010. Myanmar mango landraces reveal genetic uniqueness over common cultivars from Florida, India and Southeast Asia, *Genome*, 53: 321-330.

Hirano-Machida, R., Naito, K., Fukunaga, K. *et al*. 2011a. Genetic structure of landraces in foxtail millet [*Setaria italica* (L.) P. Beauv.] revealed with transposon display and its interpretation to crop evolution of foxtail millet. *Genome*, 54: 498-506.

Hirano-Machida, R., Ishii, H., Than Htun Oo *et al.* 2011b. Propagation management methods have altered the genetic variability of two traditional mango varieties in Myanmar, as revealed by SSR. *Plant Genetic Resources*. Date of issue: 10.1017/S1479262111000049. Published online: 01 2 2011.

Jatoi, S.A., Kikuchi, A., Mimura, A. *et al.* 2008. Relationships of Zingiber species, and genetic variability assessment in ginger (*Zingiber officinale*) accessions from ex-situ genebank, on-farm and rural markets. *Breeding Sci.*, 58: 261-270.

Mimura, M., Barbour, R., Potts, R.C. *et al.* 2009. Comparison of contemporary mating patterns incontinuous and fragmented *Eucalyptus globulus* native forests. *Molecular Ecology*, 18: 4180-4192.

Okada, Y. & Watanabe, K.N. 2008a. Social responsibility for the use of genes, genomes and biotechnology in biotechnology companies: A commentary from the bioethical viewpoint. *J. of Commercial Biotechnology*, 14: 149-167.

Okada, Y. & Watanabe, K.N. 2008b. Rationalization of the genes as a corporate stakeholders based on international laws. *J. of International Biotechnology Laws,* 5: 103-112.

Okusu, H. & Watanabe, K.N. 2005. Regional focus on GM crop regulation. *Science,* 308: 1409.

Raman, K.V. & Watanabe, K.N. 2000. Contribution of plant genetic resources for plant breeding. *In* K.N. Watanabe & A. Komamine, eds. *Plant sciences, food, environment and the 21st century. challenge of plant and agricultural sciences to the crisis of biosphere on the earth in the 21st century*, pp. 159-170. Georgetown, Texas, R.G. Landes Co.

San San Yi, Jatoi, S.A., Fujimura, T. *et al.* 2008. Potential loss of unique genetic diversity in tomato landraces by genetic colonization of modern cultivars at a non-center of origin. *Plant Breeding,* 127: 189-196.

Sinebo, W. & Watanabe, K.N. 2005. The fate of politically modified crops in Africa. *Plant Biotechnology,* 22: 185-193.

Watanabe, K. & Pehu, E. eds. 1997. *Plant biotechnology and plant genetic resources for sustainability and productivity*. Georgetown, Texas, R.G. Landes Co. 247 pp.

Watanabe, K.N. & Iwanaga, M. 1999. Plant genetic resources and its global contribution. *Plant Biotechnology,* 16: 7-13.

Watanabe, K.N. & Hirano, H. 2011. *Access and benefit-sharing on plant genetic resources for sustainable development under modern paradigm.* Proceedings of International Symposium on Genetic Resources at the VIth National Meeting on Agricultural Innovation. INIFAP, 13 October 2011. Leon, Gunajuato, Mexico.

Watanabe, K.N. & Teh, G.H. 2011. Wanted: bioprospecting consultants. *Nature Biotechnology,* 27: 873-875.

Watanabe, K.N., Sassa, Y., Suda, E. *et al.* 2005. Global political, economic social and technical issues on transgenic crops. *Plant Biotechnology,* 22: 515-522.

Yamanaka, Y., Suzuki, E., Tanaka, M. *et al.* 2003. Assessment of cytochorome P450 sequences offers a useful tool for determining genetic diversity in higher plant species. *Theor. Appl. Genet.,* 108: 1-9.

Considering the future: conservation of crop diversity

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Crop diversity is represented in ex situ *conservation by large numbers of accessions according to the Second Report of the State of the World's Plant Genetic Resources for Food and Agriculture. As the numbers of accessions conserved increases, the conservation technologies and facilities likewise have improved. Faced with the figures, it may be hard for the observer to understand why the objective to safeguard the world's crop diversity still demands urgent support.*

The Global Crop Diversity Trust has been working with national and international institutes around the world for the past three years to support the regeneration, characterisation and safety duplication of seed and vegetatively propagated crop collections. We have worked with partners in many countries in the Asia and Pacific Region, including Bangladesh, India, Indonesia, Lao PDR, Malaysia, Mongolia, Myanmar, Nepal, numerous Pacific Island States, Pakistan, Papua New Guinea, Philippines, Thailand and Viet Nam. The implementation of the project has made evident the willingness of national and international institutes to collaborate and share and receive expertise and germplasm. The varying capacities and experiences of institutes involved suggest that there is room for much deeper collaboration and sharing of roles.

This paper summarizes some of the main outcomes and findings of the Project and the implications for the future.

Introduction

Ex situ conservation of plant genetic resources is a notoriously difficult area of work for which to seek funding. Operations are generally routine and generate relatively little immediate impact in terms of improving food security and saving lives. Any gaps in funding lead to backlogs in routine operations such as viability testing and regeneration, which can radically reduce the value of the collection. It is hard to escape the fact that keeping such collections adequately

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financed from year to year poses a considerable challenge. Yet if crop diversity is not safely conserved, the long-term consequences in terms of food security are potentially catastrophic.

The Global Crop Diversity Trust was conceived to address this very challenge. The Trust is an independent international organization, which was established by the Food and Agriculture Organization of the United Nations (FAO) and the Consultative Group on International Agricultural Research (CGIAR) in 2004. The Trust aims to build a rational, effective, efficient and sustainable global system for conserving crop diversity and making it available to breeders, farmers and other users. One of the main objectives of the Trust is to raise funds for and manage an endowment fund, the annual income from which is dedicated to supporting the maintenance of international, globally important crop collections. The endowment, which presently stands at more than US$120 million, is a unique mechanism for providing funding in perpetuity. Once a contract is signed for the support of an eligible collection, the Trust is then committed to providing 'non-stop' funding into the future. At present, the Trust provides this long-term support to contribute to the management costs of 20 crop collections in ten institutes and the Svalbard Global Seed Vault.

The Trust also manages project-based work to address critical issues in *ex situ* conservation of crop diversity. Since 2007 the project work has been financed by grants from the UN Foundation/Bill & Melinda Gates Foundation and the Grains Research and Development Corporation of Australia (see Box 1) and, in 2011, a major grant from the Government of Norway was secured to work on wild relatives of major food security crops. This paper describes some of these project activities and their outcomes.

Box 1. The activities of the Global System Project (2007-2012)

- Regeneration, characterization and safety duplication of regionally or globally important collections managed by national institutes.
- Competitive grants for collecting.
- Competitive grants for evaluation of collections.
- Conservation research for difficult-to-conserve crops.
- Development of information tools for accession-level data management, GRIN-Global, and a web portal for accession-level information, GENESYS.
- Pre-breeding through the Global [Partnership] Initiative for Plant Breeding Capacity (GIPB).

Regeneration and safety duplication of threatened collections

One of the main focuses of the Trust's project work, to date, is the regeneration of threatened collections and their duplication in international gene banks. The initiative comprises 56 projects that target 94 996 accessions in 246 collections of 22 crops held by 86 institutes in 77 countries worldwide. The projects are contracted to individual national institutes, and in a few cases multilateral partnerships where a crop or regional network can provide the coordination.

So far 67 percent of the target has been achieved, with a total of 63 995 accessions (51 868 seed and 12 127 vegetative accessions) successfully regenerated. Some 26 institutes have also received support to put 5 872 accessions *in vitro*.

Table 1. Regeneration, characterization and safety duplication projects in the Asia-Pacific region

Crops	Institute	Country	Accessions
Chickpea, grasspea, lentil	BARI	Bangladesh	1 455
Maize, rice	CGRI	DPR Korea	5 700
Banana	NRCB & NBPGR	India	200
Banana, maize, rice, sweet potato, taro	ICABIOGRAD	Indonesia	1 850
Rice, sweet potato	RCCRC & NAFRI	Lao PDR	244
Cassava, rice, sweet potato	MARDI	Malaysia	1 243
Barley, wheat	PSARTI	Mongolia	835
Banana, bean, rice	DAR	Myanmar	737
Barley, bean, chickpea, finger millet, grasspea, lentil, maize, rice, wheat	NARC	Nepal	5 475
Banana, breadfruit, coconut, edible yams, sweet potato, taro, yam	FSM, French Polynesia, Fiji, Kiribati, New Caledonia, Samoa, Solomon Islands, Vanuatu & SPC		900
Banana, sweet potato, taro, yam	NARI	Papua New Guinea	1 527
Banana, cowpea, maize, pigeon pea, rice, sweet potato, taro, yam	BPI/UPLB & PhilRootCrops	Philippines	4 059
Vigna	FCRI	Thailand	750
Banana, rice, taro, yam	FAVRI & PRC	Viet Nam	2 366

The priority collections for regeneration and safety duplication were identified in large part through the processes of developing crop and regional conservation strategies. The collections that received support in the Asia-Pacific region are listed in Table 1.

Research to improve conservation

The conservation research activities are aimed at overcoming the conservation problems of coconut and vegetatively-propagated crops. Cryopreservation promises to be a more effective long-term conservation technique for such challenging crops. Six interlinked projects are developing robust protocols for the cryopreservation of sweet potato, yams, cassava and aroids involving two research institutes (KULeuven and IRD-France) and four institutes holding major collections (CIAT, CIP, IITA and SPC). The protocols for yam and edible aroids have provided sufficiently satisfactory results for the institutes involved to initiate steps towards cryobanking these crops.

The Trust also contributed to solving the frustrating problem of finding a safe and effective way of moving coconut germplasm around for safety duplication or conservation. Collaborative work led by the COGENT network is under way to test and improve the implementation of a protocol for the transfer of coconut embryos.

Evaluating collections for key traits

As a result of three competitive calls for evaluation proposals, 43 projects are underway to evaluate selected accessions of 59 collections for 143 traits of significance to climate change. They involve 58 different national/regional research institutes and eight CGIAR centres in 43 countries.

There are nine evaluation projects in the Asian region (Table 2). One such project is being carried out by the Field Crops Research Institute (FCRI) in Viet Nam. FCRI evaluated 200 local rice varieties for drought tolerance, salinity tolerance, brown plant-hopper resistance and bacteria blight resistance. Salinity and drought tolerance were measured at the germination, seedling and early vegetative growth stages using standard protocols in pot experiments. About 45 accessions were identified as of potential use in breeding for drought tolerance, and 40 for salinity. These datasets will be made available through the institute's Web site after publication in the journal of the Vietnam Academy of Agricultural Sciences.

Table 2. Evaluation projects undertaken in the Asia-Pacific region

Crop	Institute/country	Trait
Grasspea	BARI-Bangladesh	General evaluation
Banana	NRCB-India	Drought tolerance
Pearl millet	ICRISAT-India	Heat tolerance
Wild rice	Punjab University-India	Water-use efficiency
Barley & rice	NARC-Nepal	Stress tolerance
Banana	Philippines, China, Indonesia, South Africa	*Foc* Tropical Race 4
Rice	IRRI-Philippines	Heat tolerance
Coconut	CRI-Sri Lanka	Characterization
Rice	Pham Quang Duy-Viet Nam	Drought tolerance, salinity, brown plant-hopper & bacterial leaf blight

Information systems for improved management and use of collections

The Trust takes very seriously the role of good quality information in an easy-to-use form in promoting the use of crop diversity. We are investing in two global systems for managing accession-level data. Firstly, GENESYS, developed by Bioversity International on behalf of the CGIAR system, is a web portal that has the capacity to bring together the world's accession data into one easy-to-search Web site – an 'Amazon.com' for the gene bank world. Data from CGIAR gene banks (SINGER), the European network (EURISCO) and USDA (GRIN) are now accessible from GENESYS, contributing information on 2.3 million accessions held in some 356 gene banks, including evaluation data from USDA and some CGIAR centres. Work to add to the evaluation data, and bring new gene banks on board, continues and comments are welcome from any potential users (visit www.genesys-pgr.org).

GRIN-Global is designed for gene banks to use for the management of accession-level data. It is a significant update of the system deployed by the USDA National Plant Germplasm System. A version of the latest software is being tested by a number of gene banks around the world. More on the database and its development can be found on the GRIN-Global wiki at www.grin-global.org.

The state of gene banks

Most of the project partners have received visits from Trust or CGIAR staff, and these visits have helped illustrate the constraints faced by many gene banks in their efforts to store seeds in conditions suitable for long-term conservation according to recommended standards (see Box 2).

Of the 86 national institutes with whom the Trust is working, we estimate that only around a third of them had the capacity to dry seeds satisfactorily before the initiation of the project. One-third of the institutes do not have any staff dedicated to conservation operations. Less than half can store seed in long-term conditions with a reliable electricity supply. Nearly half of these national gene banks have found that more than one in ten of the accessions selected for regeneration were non-viable, and a quarter of the institutes have found that more than 25 percent of the accessions for regenerationok were non-viable.

In numerous cases, stock has had to be replanted more than once in order to attain sufficient seed for conservation and safety duplication. More than 10 000 of the originally targeted accessions are reported by partners to be dead. Some additional equipment and supplies such as silica gel and sealable bags have been provided by the project to ensure seeds are dried and packed adequately from now on.

The safety duplication of part of the freshly regenerated stock to international gene banks nominated by the national partners has significantly improved the security of these valuable national collections. So far, some 20 048 accessions have been sent for safety duplication at CGIAR centres and other international gene banks.

Ex situ conservation worldwide

The number of accessions conserved in the world's gene banks has increased continuously since 1984 and continues to rise. The total reached 7.4 million accessions in 2009 according to the *State of the world report*, of which perhaps only 2.2 million are unique (FAO 2010). Studies of the costs of gene bank operations undertaken on CGIAR centre gene banks (Koo 2004; Shands *et al.* 2010) indicate that the annual cost of conserving a single accession varies (from US$3.00 to US$174) depending upon the size of the collection, efficiencies of the gene bank but most importantly the type of crop (whether a clonal crop, a self-pollinator or out-crosser, or wild relative). Very roughly speaking, to conserve correctly a 'low-maintenance' accession – including periodic viability-testing, disease-cleaning and regenerating – it will cost, at

Box 2. Issues affecting national gene banks

- Major problem of sustainable funding and capacity to support basic, routine operations.
- Infrastructure is great! Several new gene banks have been built in Asia, but equipment, supplies and secure staff positions are lacking.
- Staff turnover can be frequent. There is considerable need for capacity building and transfer of expertise and information.
- Severe challenges exist in achieving standards of drying, long-term storage and *in vitro* conservation.
- Passport data are poor and, in many cases, cannot be easily improved.
- Poorly-stored seeds and field conditions resulted in germination and replanting.

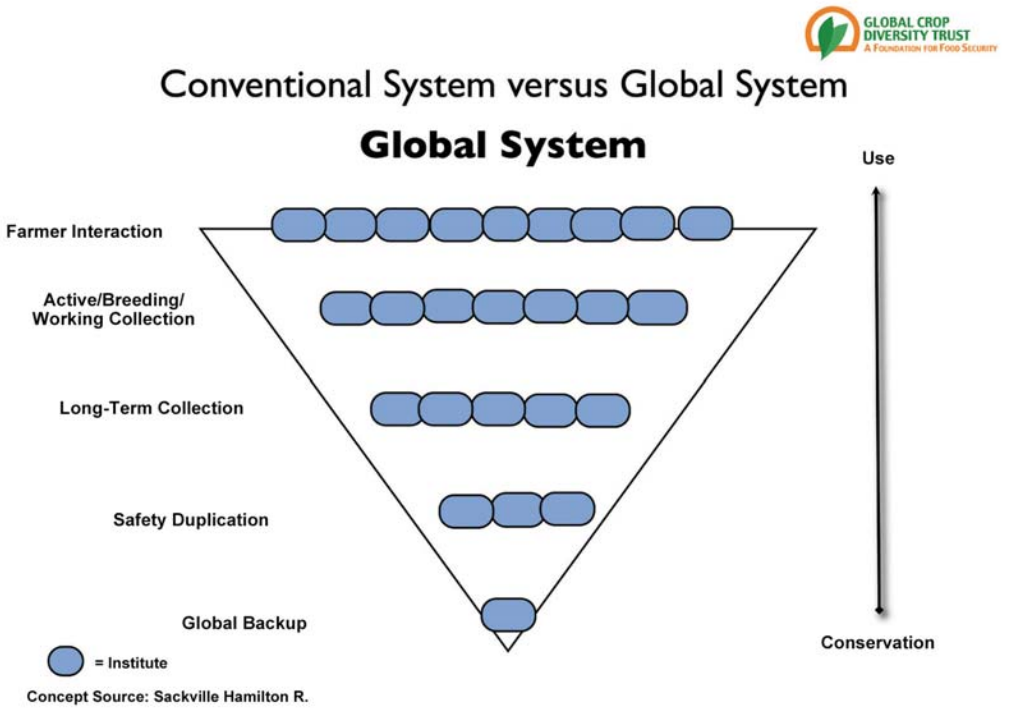
minimum, US$10 per accession per year and probably a great deal more. This suggests that if the global community is to conserve 7.4 million accessions, then it should be investing at least US$74 million in the management of gene banks every year. This, almost certainly, is not the case. As a consequence we can be fairly certain that of these 7.4 million accessions currently documented only a fraction will actually be alive in ten or 20 years.

The Trust's vision is to develop a more rationalized approach where roles can be shared; fewer accessions may be conserved in the system (i.e. eliminating duplicates) but much more efficiently. Such a global system is an extension of the international links, connections and interdependency that have driven agriculture for the past 10 000 years. The simplest way to depict this vision is to consider the levels of interaction required for each of the potential roles of a gene bank from conservation to use. Many gene banks (or institutes) and individuals need to interact with farmers at a local level, but fewer institutes need to develop breeding or working collections, which have relevance at a wider local or regional levels. When it comes to conservation, given present-day technologies, communications and transport there is no need to have more than a small number of institutes conserving seed or tissue culture, especially in long-term conditions. Even fewer interactions or institutes are needed to ensure that accessions are safety duplicated or backed up.

An example of the opposite extreme to the active and busy interface between farmer and supplier institute is the Svalbard Global Seed Vault (SGSV). The SGSV is alone in the world in providing an extremely low-maintenance,

efficient facility for long-term backup. The door of the facility is literally kept closed for most of the year. In fact since its inauguration in February 2008, the Vault has opened for deposits only 13 times. However, the total samples deposited in the Vault to date amount to 665 422 from more than 30 countries. The SGSV is a bright illustration of the global system in action.

Figure 1. Levels of interaction for each of the potential roles of a genebank



References

Food and Agriculture Organization of the United Nations (FAO). 2010. *Second report on the state of the world's plant genetic resources for food and agriculture.* Rome, FAO.

Koo, B, Pardey, P.G., Wright, B.D. *et al.* 2004. *Saving seeds: the economics of conservation crop genetic resources ex situ in the future harvest centres of the CGIAR.*

Shands, H., Hawtin, G. & MacNeil, G. 2010. *The cost to the CGIAR centres of maintaining and distributing germplasm.*

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