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Preserving the Future of Vegetable Improvement

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Abstract

Diverse and readily accessible genetic resources are vital for any crop improvement program oriented toward high and stable yields and specific consumer preferences to finally contribute to food security and a diverse diet for the ever increasing global population. Molecular tools to identify and use genes responsible for specific traits in gene bank accessions of wild and cultivated species have great potential to enhance germplasm utilization and to shorten breeding cycles. Complementary approaches of *in situ* and *ex situ* conservation are used to preserve germplasm for improvement programs. AVRDC – The World Vegetable Center conserves approximately 56,000 accessions and is therefore the world's most important gene bank for vegetable crops. More than 35,000 samples of regular and improved vegetable germplasm have been distributed over the last 10 years to a range of users in the public and private sectors. Another 10,000 samples have been used by the Center's scientists for their research. After extensive screening and breeding efforts, five anthracnose-resistant pepper lines have been released by the Center. Similarly, *Solanum pimpinellifolium*, a wild tomato species is being used to develop resistant varieties against bacterial wilt. Resistance against the damage from aphids was detected in *Capsicum annuum* accessions from Costa Rica. Moderate to highly resistant lines to bruchid, a destructive storage insect pest of mungbean resulted from extensive screening trials at the Asian Regional Center. The AVRDC Vegetable Genetic Resources Information System (AVGRIS) provides direct access to information pertaining to the accessions in the gene bank to all potential users through the internet. To secure the future of variety improvement programs of staple crops including fruits and vegetables, gene bank capacities for medium to long-term

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conservation, germplasm characterization and evaluation, and information exchange need to be given priority attention.

Introduction

The foundation of the current world food supply is based on thousands of years of crop selection and improvement carried out on traits of wild species (McCouch, 2004). The present-day cultivated breeds are descendants of wild species which had low yields and poor eating quality, but nevertheless provided the modern varieties with genes that can enhance crop performance. Over 60 years ago, Vavilov drew attention to the potential of crop relatives as a source of genes for improving crop performance (Vavilov, 1940, as cited by Tanksley & McCouch, 1997). This possibility motivated the setting up of gene banks which initially were focused on species that are closely related to 'exotic' crops. However, the future productivity of all agricultural crops – both exotic and local – can only be ensured if collection and preservation of genetic variation in genebanks is tied in with active utilization of the materials in the collection (Tanksley & McCouch, 1997).

There is no a single country that is self-sufficient in genetic resources for food and agriculture. All countries greatly depend on plant genetic resources for food and agriculture from other parts of the world for their food production and sustainable agricultural development. Concern over the dramatic loss of agrobiodiversity in farmers' fields and in nature has led to efforts to conserve plant germplasm in several *ex situ* collections maintained by national and international research centers throughout the world. According to FAO, approximately 6.1 million accessions of plant genetic resources for food and agriculture are currently stored in over 1400 recorded genebanks around the world (Hodgkin and Anishetty, 1998).

Genetic resources are vital for the world's future. All living things exist in a range of forms and types and this diversity must be preserved. Collecting and conserving this diversity in a form that will serve for current and future use for food and other purposes is essential for the survival of mankind. In terms of botanical diversity, there are around 400,000 known species of plants (*www.bgci.org/conservation*) and many are yet awaiting to be described. However, of this tremendous biological diversity, only a small number of species is currently preserved in *ex situ* or *in situ* collections of plant genetic resources. The Kew's Millennium Seed Bank worldwide collaboration, for example, only aims to safeguard approximately 24,000 plant species (Kew's MSB, 2004).

Issues on germplasm conservation, sharing and use

Plant genetic resources have been collected for different reasons over the last centuries. Often collections are held for specific commercial uses, for example, the collection of cocoa genetic materials maintained by the Malaysian Cocoa Board (Carrizosa, 2004) is meant to support commercial cocoa production at a national level. Other collections have been initiated for more academic or philosophical reasons such as the collections made by Linnaeus stored in London by the Linnean Society and by Darwin, housed at the Natural History Museum, London.

In more recent times there has been increasing concern over the status of the world's plant genetic resources in relation to humanitarian, commercial, intellectual property and environmental issues. Humanitarian concerns are particularly linked to possible losses of genetic materials resulting from civil unrest (for example the civil war in Cambodia, the military conflict in Darfur, Sudan, or the religious conflict in the Mollucan islands, Indonesia). Varieties or specific, locally adapted types may be lost forever in the wake of civil unrest. In some cases it has been possible to repatriate genetic materials to their countries of origin through the use of genetic resources collections, enabling those countries to restore the natural crops and cropping patterns of the area. Some examples of these efforts are: the reintroduction of various rice varieties in Cambodia by the International Rice Research Institute (Pearce, 1998), and the repatriation of indigenous crop varieties to Afghanistan by the Future Harvest Consortium led by the International Center for Agricultural Research in the Dry Areas (*www.icarda.org*). The largest effort to repatriate local seed to date is probably the "Seeds of Hope" project, an international joint venture launched to restore agricultural biodiversity in war-torn Rwanda (CGIAR, 1996).

Commercial interests are very broad and often historically linked to patterns of human movement and needs for food, or medicinal or industrial purposes. Genetic resources have been vital in developing new and improved lines of crops and also for ensuring diversity in the human diet. High lycopene and beta carotene tomatoes, for example, provide more antioxidants and vitamin A than the regular varieties. Many of the world's medicines are derived from plants. It is reported that approximately 25% of all the world's medicines are derived from 10,000-20,000 plant species (Duke, 1993).

Ownership of traditional plant genetic resources, equitable sharing of benefits arising from their use, and emerging national plant variety protection laws are intellectual property issues affecting the way those resources are currently collected, exchanged and utilized. The United Nations' Convention on Biological Diversity and the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2001) are international agreements with the aim to ensure that plant genetic resources are conserved and remain available to mankind while recognizing sovereign rights of the countries from which the plants originated.

Environmental issues are also becoming increasingly important, as issues of climate change are discussed internationally. The impacts of climate change on natural environments could result in the loss of genetic diversity from areas which experience major environmental or climatic disturbances. Changes in seasonal variability, ambient carbon dioxide concentration, rainfall amount and distribution and air temperature will have a major impact globally. In addition, the effects of natural disasters such as the Indian Ocean's 2004 Tsunami; the 2008 earthquake in Sichuan, China, hurricane "Nargis" in Myanmar, and the monsoon flood in Bihar, India, for example, on the loss of local plant species cannot be underestimated.

Value of genetic resources in crop improvement

Genetic improvement is vital for ensuring food security in an ever increasing global population. Traditional varieties or land races originated as low yielding, relatively weedy species that had undergone natural selection and farmers' selection processes for thousands of years. New varieties have been developed through conscious efforts of plant breeders who are targeting not only yield and stabilizers of yield performance (resistance to abiotic and biotic stresses) over time but also consumer preferences, whether in terms of taste, color, storability, postharvest processing attributes or nutritional values.

To a large extent, the success of any crop improvement work depends on the genetic diversity available to the breeders. The more diverse and readily available the genetic resources are, the better the chances that the genetic improvement efforts will provide the desired plant characteristics. All genetic improvement requires an existing base of genetic material which is modified using conventional plant breeding and/or molecular genetic techniques. The latter could involve application of molecular marker aided selection and/or insertion of a specific gene to produce a genetically modified variety. Examples of the genetically-modified crop include rice with high beta-carotene content and herbicide-resistant soybeans.

The application of molecular tools to identify genes controlling specific traits in accessions of cultivated species and their wild relatives constitutes an important new role of gene banks to enhance germplasm utilization. The introgression of identified genes into genotypes with a more desirable genetic background using marker-assisted selection (MAS) has been a component of this approach (Tanksley and Nelson, 1996; Xiao *et al.*, 1996; Ortiz and Engels, 2004). As an example, the MAS scheme discussed by Causse *et al.* (2001) for the transfer of the five most important QTLs involved in the organoleptic fruit quality of tomato can be mentioned.

The analysis of genetic variation in germplasm collections and the proper documentation of the number and types of useful polymorphisms provide opportunity to estimate the value of the conserved accessions and enable the gene bank to offer specific accessions with desired characteristics to plant breeders and users in general. They can then make informed choices and select only those genotypes which best fit their objectives (Ortiz and Engels, 2004).

The rate of discovery of nucleotide variation at QTLs contributing to phenotypic variation of complex traits is expected to increase with the adoption of linkage disequilibrium and candidate gene strategies for QTLs fine mapping and cloning (Rafalski, 2002; Morgante and Salamini, 2003). This approach would eliminate the requirement for structured segregating populations and genetic studies could be directly performed on the accessions deposited in the genebank (Graner *et al.*, 2004). The feasibility of association mapping between DNA markers and agronomic traits has been successfully demonstrated in a genebank collection of 600 potato varieties. Highly significant association with QTLs for resistance to late blight and plant maturity was detected with PCR markers, specific to a major gene for resistance to late blight (Gebhardt *et al.*, 2004).

Developing these ideas further, DNA banks may evolve as a strategic component of modern gene banks providing the basis for improved gene bank management and facilitating high throughput germplasm characterization, association genetics and marker-assisted selection. DNA samples can be exchanged much easier and at lower costs than living plant materials, without the inherent risk of spreading diseases and pests (Ebert *et al.*, 2006). In addition, DNA banks may serve as reference basis for evolutionary and comparative genomic studies and may offer a complementary conservation strategy for species under threat of extinction. They can also serve as safety duplicate for the physical seed, field or in vitro collections, although it is not yet possible to regenerate plants from stored DNA.

In situ and *ex situ* conservation of genetic resources

Genetic resources include wild and domesticated species, landraces, advanced breeding lines and improved varieties. Collecting expeditions, sharing of genetic resources between gene banks, and the assembly of advanced breeding lines and improved materials are important pillars of crop germplasm collections held in gene banks.

Conservation of genetic resources can be *in situ*, at the plant's natural habitat (e.g. Yayu-Harumu region in Ethiopia to conserve diversity of Arabica coffee, *Coffea arabica* [Abebaw and Virchow, 2003]) or *ex situ*, outside their natural habitat, such as in gene banks or botanical gardens. *Ex situ* conservation consists of a number of different methods, including seed storage, *in vitro* and cryopreservation, the maintenance of living plants in field gene banks and pollen storage. *Ex situ* seed collections are usually stored at +5 °C or less than 0°C for short to medium-term storage (active collections) and at approximately –18 °C or less for long-term storage (base collections). The aim of *ex situ* collections is often long term storage including safety back-up of valuable accessions at other gene banks to reduce risks of genetic erosion or complete loss due to biotic or abiotic effects or natural disasters. All forms of *ex situ* conservation offer the possibility of reintroduction of the plants into their natural habitat.

Both types of conservation have different roles. *In situ* collections are generally used to conserve species diversity within natural habitats and ecosystems where populations of plants naturally exist, while *ex situ* conservation provides opportunities for characterization, evaluation, documentation, and supply of germplasm to users and other gene banks.

There are many other criteria which must be observed to ensure longevity of the seed collections, including appropriate physiological maturity of the seeds, absence of mechanical damage, adequate seed processing and drying, good health status and purity of the seeds, low seed water content, packaging in impermeable containers or bags, low storage temperatures, periodic viability controls and rejuvenation of seed lots when viability has declined.

Genetic resources at AVRDC – The World Vegetable Center

Vegetable genetic resources are a very important global asset, given that micronutrients and bioactive compounds which are important in human diet are found in vegetables. This increasing recognition of the important role of fruits and vegetables in nutrition and health has increased interest in the preservation of genetic resources to support vegetable improvement. It is particularly important to locate and conserve underutilized species and types, often known as indigenous vegetables. Indigenous vegetables include species that are native to a particular region or introduced historically to a region from other areas, such as nightshade, tropical violet and amaranth. Other vegetables such as carrot, onion or tomato, which have been grown and enjoyed in almost all regions in the world, are generally considered exotic vegetables.

Vegetable genetic resources are assembled by both the private and the public sectors. The private sector collections include collections of brassicas (China Long, Taiwan), tomatoes (Tomato Fest Organic Heirloom, USA), and various other vegetables (Sakata, Japan) whereas the public sector collections include among other vegetables, *Brassica* (2262 accessions) and tomato (approximately 5000 accessions) collections held by the US Department of Agriculture, the C.M. Rick Tomato Genetics Resource Center located at the University of California in Davis with approximate 3000 accessions, most of which are genetic mutants or wild genotypes (Mueller, 2007). The Tropical Agricultural Research and Higher Education Center (CATIE) at Turrialba, Costa Rica conserves a total of 4753 accessions of vegetable crops with cucurbits, chili pepper and tomatoes constituting the major crops. AVRDC – The World Vegetable Center conserves a total of 56,000 accessions and is therefore the world's most important gene bank for vegetable crops.

Vegetables are generally stored as true seed (e.g. eggplant, cabbage, cucumber, tomato, cucurbits, chili pepper), however some species must be maintained as vegetative propagules (such as onion, garlic and shallot).

Vegetable germplasm collecting

Germplasm conserved in the Center's genebank are collected through many ways. Most of the collection came from national partners and other institutes' voluntary and requested contributions. Many friends of the Center staff became donors of traditional cultivars. While conducting research activities, scientists are not only utilizing the existing genetic resources collection, but also add to the collection from the germplasm they acquire through research projects and from results of their breeding activities. These processes of germplasm collections are invaluable, but the exact cost is difficult to estimate. In addition, there are efforts or expeditions designed specifically toward germplasm collection. An example of such is the Center's ADB-funded project in Asia which successfully collected 4326 accessions of indigenous vegetable germplasm, most of them are generously contributed by farmers from various locations in eight participating countries. Germplasm collecting activities of the Center follow international agreements and national laws.

Vegetable germplasm preservation

AVRDC – The World Vegetable Center holds the world's largest non-private sector collection of vegetable genetic resources. Genetic resources from around the world are conserved at its Genetic Resources and Seed Unit (GRSU) in Shanhua, Taiwan. In addition, the Center also has a working collection and short-term storage facilities at its Regional Center for Africa in Arusha, Tanzania.

The facilities at GRSU are suitable for short- (15 °C, 40%~45% RH), medium- (0~5 °C, 40%~45% RH) and long-term (–16 to –20 °C) storage and in-vitro conservation. In addition to seed processing facilities such as cleaning, drying and packing, the genebank also maintains regeneration and experimental facilities. The gene bank conserves 394 different species from 150 countries, making it one of the largest collections of vegetable germplasm held by a single institution. The storage facilities in Arusha, Tanzania comprise mainly +5 °C refrigerators for short-term storage of accessions and working collections. The Arusha collection comprises of 304 accessions of vegetable germplasm, most of which are species indigenous to Africa.

AVRDC – The World Vegetable Center also stores for safekeeping duplicate samples of vegetable germplasm collections of individual countries. In addition, the Center collections are partly safety duplicated at the Taiwan's National Plant Genetic Resources Center, the gene bank of the Ministry of Agriculture of Japan, the Philippine National Plant Genetic Resources Laboratory, the UK's vegetable gene bank in Wellesbourne, the US National Germplasm Resources Laboratory in Beltsville, and Norway's Svalbard Global Seed Vault, the last one being a black-box storage.

Vegetable germplasm sharing.

The processes of collecting, characterization and storage of the collections of vegetable germplasm held at AVRDC – The World Vegetable Center are a continuing activity. The Center's gene bank concentrates on the conservation of globally important vegetables, and those that are important in South Asia, Southeast Asia, and Africa. Its activities are conducted using standard protocols following the internationally recognized best practices. Although the Center's gene bank's main aim is conservation, it is also utilization-oriented: its base collection is complemented with a well utilized active collection. The Center strongly believes that while the material is stored to ensure its availability in the future, it is very important that the material is used. To enhance utilization of germplasm, the Center's gene bank regularly distributes both improved and unimproved vegetable germplasm to both public and private sectors. More than 35,000 samples have been distributed over the last 10 years to government- and non-governmental organizations, public and private seed companies, and private individuals. Another 10,000 samples have been used by the Center's scientists for their research. In 2007 alone, more than 4000 samples or 25% of the 16,279 total samples of various crops distributed by the Center worldwide were accessions from this genebank. Of this number, 29% or 1298 samples went to the regional centers and the various units at Headquarters. The rest were distributed to government and nongovernmental organizations, universities, seed companies, and private individuals in over 60 countries.

Vegetable germplasm utilization

Genetic diversity is the foundation of any varietal improvement program, with international crop centers serving as the modern centers of genetic diversity. Many wild relatives of cultivated plants possess better traits to withstand biotic and abiotic stresses than its cultivated counterpart. These wild species, however, lack the characteristics that are sought for human needs and use, such as fruit size, organoleptic traits, or shelf-life. Screening of diverse germplasm from international centers for desirable agricultural attributes is a valuable breeding endeavor. Selected materials can be used directly as varieties or parents in breeding programs.

At the Center's headquarters, resistance to Anthracnose (*Colletotrichum* spp.) in pepper (*Capsicum* spp.) was sought in an extensive survey of the Center's *Capsicum* germplasm collection, and a small number of promising accessions in *Capsicum chinense* (e.g. PBC932) and *Capsicum baccatum* (PBC80 and PBC81) were identified. The resistance was transferred into *Capsicum annuum* via interspecific hybridization and several backcrosses, utilizing a micro-injection screening protocol also developed by the Center. The gene controlling this resistance has been tagged using AFLP methods, and improved SCAR markers are currently being developed. Beginning in 2003, at least five resistant lines incorporating this resistance factor have been bred by the Center, and made available to interested researchers.

The wild tomato species *Solanum pimpinellifolium* is highly resistant against bacterial wilt caused by *Ralstonia solanacearum*, but its fruit size is very small and it has poor horticultural traits. The resistance genes from *S. pimpinellifolium* were incorporated into a cultivated recipient parent and a highly resistant commercial tomato variety, Hawaii 7997 was produced (Hanson *et al.*, 1998). *S. pimpinellifolium* is one of many wild species being used at the Center for breeding activities, aimed to produce varieties with better characteristics.

The Center recently screened over 100 lines of *Capsicum annuum* originating from Costa Rica and maintained in the GRSU collection, for resistance to damage from aphid (*Myzus* spp.). Six lines were identified that display damage levels of less than 10% the trial average. At the Center's Asian Regional Center, 611 mungbean accessions have been screened for resistance to bruchid (*Callosobruchus chinensis*), a destructive storage insect pest that bores inside and feeds on bean pods. Seven accessions with moderate to highly resistant reaction to bruchid have been identified. All these accessions can be requested from the Center by vegetable breeders.

As part of the Center's okra breeding activity in West Africa, 68 lines of okra (*Abelmochus esculentus*) from the Center's genebank together with 76 accessions introduced from the National Plant Germplasm System, Griffin, Georgia, and USA were characterized; seeds were increased and evaluated for general adaptation in Niger during the rainy season of 2008. Promising lines will be selected and crossed with small-fruited *A. manihot.*

Some other genes which have been mined and incorporated into the Center's vegetable lines include heat tolerant genes in Chinese cabbage and yellow leaf curl virus resistance genes in tomato. There are also advanced breeding lines of major vegetable crops that are tolerant to flooding and drought and are efficient in nitrogen-use.

Documentation and information sharing

AVRDC Vegetable Genetic Resources Information System (AVGRIS) is the computer-based information system developed and maintained by AVRDC's genebank staff to facilitate the daily maintenance and use activities. It links all gene bank operations associated with germplasm conservation and management. The information system is used in the recording, storing and maintenance of germplasm data, such as passport, characterization and seed inventory data. More importantly, it provides direct access to information pertaining to the accessions in the gene bank through the internet.

Information of the Center's genetic resources on AVGRIS is available through the internet (*www.avrdc.org*). These genetic resources maintained by AVRDC in-trust are considered as international public goods and are provided on agreement with the terms of the Center's Material Transfer Agreement.

Meeting the needs of the future

The availability of genetic materials in the gene bank provides a wealth of opportunities for the Center to be well positioned in tackling problems of pests and diseases as well as issues of climate change and extreme weather events in vegetable production. It underlines the importance of maintaining the Center's Genetic Resources and Seed Unit for the preservation of vegetable genetic resources diversity and to enhance its utilization for food and nutritional security.

The Center's Genetic Resources and Seed Unit is funded through contributions to the Center as an international research and development center. Funding is both from the unrestricted core funds and from designated donor-funded projects. An annual budget of approximately US$ 450,000 is needed to run and maintain the Genetic Resources and Seed Unit at the Center's Headquarters in Taiwan. The Genebank Manager supervises nine regular staff and 5~10 field laborers to manage the day to day operation of the gene bank. The storage facility in Tanzania is managed by three staff and five laborers with an annual budget of approximately US$ 150,000. The personnel cost comprises of more than 80% of the annual budget. Greenhouse and laboratory operations for regeneration, characterization, seed multiplication, etc., take up about 7%~8% of the annual cost. The infrastructure, operation cost for electricity, water, and its maintenance come next, followed by cost of packing and shipping for distribution and to backup the collection. Data base maintenance and its accessibility are another very important part of the gene bank operation involving two technical staff to keep the information up-to-date.

The establishment of international agricultural research centers dealing with important crops (AVRDC – The World Vegetable Center, International Rice Research Institute, Centro Internacional de Mejoramiento de Maiz y Trigo, International Crops Research Institute for the

Semi-arid Tropics, and Centro International de Agricultura Tropical, to mention a few) has led to worldwide efforts on genetic resources conservation, sharing and utilization for center-mandated crops. Today, the gene banks of these international centers are the primary sources of crop germplasm for valuable traits for adaptation, stability and human health. To secure the future of variety improvement programs for food and agriculture of all nations, like vegetable variety improvement, there is a need to sustain and expand the gains in crop genetic resources conservation programs of international centers that hold these resources in-trust for the world community. Germplasm collecting in less accessible areas should be done to avoid genetic erosion due to rapid urbanization, civil unrests and/or drastic global climate change. International gene bank capacities for medium to long-term conservation, germplasm characterization and evaluation, and information exchange should be given priority attention.

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