Agrobiodiversity Conservation: Keeping the Options Alive

The Importance of Agrobiodiversity

Biodiversity represents the very foundation of human existence. Yet by our heedless actions we are eroding this biological capital at an alarming rate. . . . The genes, species, ecosystems and human knowledge which are being lost represent a living library of options available for adapting to local and global change. Biodiversity is part of our daily lives and livelihoods and constitutes the resources upon which families, communities, nations and future generations depend.

As reflected in this statement from the 1995 Global Biodiversity Assessment of the United Nations Environment Programme (UNEP), there is a growing awareness of the profound importance of the earth's biological diversity, or biodiversity, and also of human responsibility for curbing its destruction.

Over the past decade, a series of important steps have been taken to protect agrobiodiversity in particular, which mainly includes the plant genetic resources on which agriculture depends. These and further efforts are vital for enabling countries and communities to meet their food needs, for improving rural livelihoods, and ultimately for protecting the well-being of all people now and in the future.

One major step was the creation of the *Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture.* This resulted from a conference organized by the Food and Agriculture Organization (FAO) of the United Nations in 1996 at Leipzig, Germany. Two years later, during a regional FAO-organized conference at CIAT headquarters in Cali, Colombia, Latin American countries expressed a strong commitment to research needed for effective implementation of the Global Plan.

These actions grew out of a much earlier step, the establishment in 1983 by the FAO of an intergovernmental forum called the Commission on Plant Genetic Resources (now the Commission on Genetic Resources for Food and Agriculture). The Commission has developed a global system for the conservation and sustainable use of food and agriculture plant genetic resources.

Agrobiodiversity Conservation at CIAT

As an active part of that system, CIAT conserves, studies, and uses the genetic resources of selected plant species in the American tropics. The region harbors a wide array of food crop, forage, and other species that are valuable for human and animal nutrition and provide key environmental services, not only in the Americas but in other parts of the world as well.

These plant genetic resources can be conserved through two basic approaches. First, with *in situ* methods, plants are continuously produced in their natural habitat or at locations whose climate and ecology are similar to that habitat. The second approach is *ex situ* conservation, which refers to storing seeds or other reproductive materials in climate-controlled germplasm facilities.

The main advantage of *in situ* methods is that they allow the natural evolution of plants to continue, so potentially valuable new genetic variation can be collected from time to time for *ex situ* conservation. A major disadvantage is that the rural communities responsible for *in situ* conservation may have to make difficult tradeoffs between this activity and agricultural production. A further problem is that scientists cannot always characterize plants properly in their original habitats or farm settings, and this can limit the availability of valuable information.

With *ex situ* conservation, in contrast, scientists can assess the genetic makeup and adaptation of plant species under controlled conditions in laboratories and experimental fields. This makes it possible for researchers as well as farmers and others to easily access germplasm with known characteristics, which remain relatively constant over time. A further advantage of *ex situ* conservation is that it helps restore germplasm that has been lost in the wild. Each approach, then, has strengths and weaknesses, and the two are highly complementary.

CIAT promotes closer integration of *in situ* and *ex situ* conservation by various means. First, the Center continually seeks to improve the efficiency of germplasm conservation at its own facilities in Colombia. Second, our scientists strengthen the capacity of partner organizations to conserve neotropical plant species through both *ex situ* and *in situ* methods. And third, we bring together specialists in classical germplasm evaluation and molecular genetics to characterize and evaluate *ex situ* collections. These activities help build the knowledge base and human resources needed to link *ex situ* collections with *in situ* conservation initiatives.

In connection with this work, CIAT operates a large, state-of-the-art plant gene bank—the Genetic Resources Unit (GRU)—at its headquarters in Palmira, near Cali. Three additional sites in Colombia (Santander de Quilichao and Popayán in Cauca Department and Tenerife, Valle) provide seed multiplication services to systematically replenish the germplasm collections. The main job of the GRU's 40 staff is to safeguard this material, which consists mainly of common beans, cassava, and tropical forages.

In addition, our staff test and improve conservation techniques, characterize and evaluate germplasm, make the resulting information widely available to research partners, and build local capacity for germplasm conservation by providing advice, expertise, and training as a support to national research programs in the region. The project also promotes public awareness of the need for preserving plant genetic resources.

The plant genetic resources conserved at CIAT are a component of the world "designate collection" of the FAO. Under a 1994 agreement with FAO, CIAT makes this germplasm available free of charge, upon request, to farmers, farmer associations, plant breeders, agronomists, extension agencies, universities, and biodiversity institutes with a clearly articulated need. For example, materials from the gene bank may be used for bona fide crop improvement research, field trials, seed multiplication, and training in genetic conservation. FAO designate materials are considered the collective heritage of humankind and, thus, are not patentable.

To further safeguard the plant genetic resources under its care, CIAT follows the principles and procedures listed below:

1. Long-term conservation represents a commitment to both the FAO and the countries of origin of the germplasm as a way to maintain their agricultural heritage for present and future use.
2. A set of known germplasm with specific traits is set aside for distribution on request.
3. Specific amounts of germplasm are maintained for periodic monitoring of viability.
4. In keeping with current practice among gene banks, one or two security backups are made and sent to another safe location.
5. Germplasm copies are made for the country of origin. It often happens that a country is willing to maintain a replica of its agrobiodiversity once it has established its own conservation facilities. A separate provision is made for that purpose, effectively making the GRU a gene bank for the particular country.

Every year CIAT distributes genetic material of 5,000 to 6,000 germplasm accessions in response to requests from around the world. For example, researchers in Central America recently requested 4,000 bean accessions to be screened for disease resistance.

The Germplasm Collections at CIAT

The germplasm collections stored in the GRU at CIAT encompass a total of some 60,000 samples, or accessions, of about 720 plant species, including the common bean, cassava, various wild relatives of these two crops, and a large number of tropical forage grasses and legumes. Conserving, studying, and using this material is a major part of CIAT's responsibility for research on these commodities within the Consultative Group on International Agriculture (CGIAR). In the paragraphs that follow, we briefly describe the germplasm collections and their importance.

Common bean

In many parts of Latin America and Africa, beans are considered the "meat of the poor." Their protein content is roughly double that of most cereals, and they are rich in essential micronutrients, like iron and folic acid (one of the B vitamins). Beans are also an important cash earner for poor farmers. About 40 percent of Africa's bean harvest, for example, goes to market, generating farm-gate revenues of about one-quarter of a billion US dollars per year. Latin America is the world's leading bean-growing region, accounting for nearly half of global production.

The CIAT bean collection contains over 40,000 accessions, of which 26,500 are cultivated *Phaseolus vulgaris*, or common bean. About 1,300 accessions are wild species of *P. vulgaris*. The rest are distant relatives. A subset of the overall holdings serves as a core collection for CIAT's and other organizations' bean breeding work. This subset, which is representative of the diverse environments in which beans have evolved and are grown, consists of about 1,400 accessions of domesticated common beans and 100 accessions of wild beans. Researchers at CIAT and national partners in Latin America and sub-Saharan Africa have evaluated this core collection for a range of traits, such as insect and disease resistance and tolerance to low phosphorus. Useful materials have been identified and incorporated into breeding programs at CIAT and elsewhere.

A good example of the value of preserving bean genetic diversity is the unique popping, or *ñuña*, bean. Native to Bolivia and Peru, it may be the most ancient of all beans, though it is practically unknown elsewhere in the world. Unlike other common beans, popping beans can be toasted—just like popcorn—and have a peanut-like taste.

The bean collection at CIAT includes samples of about 300 *ñuña* landraces. In 1996 the first improved popping bean variety—named Q'osco Poroto—was released by the Peruvian Ministry of Agriculture. Promoters of the new bean near Cusco are working to create new markets for it, particularly as a nutritious snack food for tourists and school children. Increased market demand would boost the incomes of the small farmers who grow *ñuñas* in poor highland areas. The *ñuñas* also have a decided ecological advantage. Since it takes far less time to toast beans than to boil them (about 10 minutes, compared to as much as 3 hours in the highlands), popping beans save firewood. CIAT is planning to evaluate *ñuñas* with bean researchers in eastern Africa, where an added-value, ecologically friendly bean could give sizable benefits.

Cassava

Cassava, or *Manihot esculenta* Crantz, is grown in over 90 countries and provides a livelihood for half a billion people in the developing world. While this hardy root crop serves as a staple food for many poor farm families, it also provides a high-starch raw material for producing commercial animal feed and for other industries, including food, pharmaceuticals, paper, and textile manufacturers.

Global production of cassava is around 152 million tons per year. Half the 16 million hectares devoted to cassava cultivation is in Africa, with 30 percent in Asia, and 20 percent in Latin Amercia.

The CIAT cassava germplasm collection consists of 6,000 clones: landraces from Latin America and Asia, elite clones selected by CIAT and the International Institute of Tropical Agriculture (IITA) in Nigeria, and several wild *Manihot* species. These are stored in the form of slow-growth *in vitro* plantlets. For each accession, five test-tube plantlets are conserved to accomplish various purposes. These include long-term conservation, distribution to fill germplasm orders, security backups, and the provision of duplicates for conservation in the germplasm's country of origin. Some cassava germplasm is also conserved as seed. As with beans, Center scientists have formed a representative core collection of the cassava holdings to facilitate germplasm evaluation.

Tropical forages

Forage is any vegetative material eaten by livestock. It includes live grasses and legumes grazed directly by pastured animals as well as cut-and-carry biomass and fodder such as hay, leaves, shredded sugarcane, chopped maize cobs, and dried cassava chips.

About one-third of the earth's land surface is given over to livestock production. This enormous area consists of native and improved pastures as well as crop lands devoted to growing harvestable forage crops. As global demand for meat, milk, and other animal products grows dramatically in the coming decades, so too will the need for improved forages. Small-scale livestock producers in developing countries, faced with stiff competition from highly efficient industrial operations, both domestic and foreign, will have to look at new technical options, such as combinations of superior grasses and legumes, to replace native pasture forages, which tend to be low in nutritional value.

A number of forage species collected by CIAT over the years, and in some instances improved through our breeding work, are highly productive, have superior nutritional value, and can contribute to more environmentally sustainable land management. Some tolerate low soil fertility and can withstand the harsh dry season typical of many areas of Latin America. The African grass *Brachiaria decumbens* and the legumes *Cratylia argentea* and *Arachis pintoi* are among the promising forages being promoted by CIAT and its research partners, notably in the hillside ecosystems of South and Central America and Southeast Asia.

The Center maintains over 22,000 accessions of forage grasses and legumes in its plant genetic resources collection. The main components are 18,400 samples of 654 legume species and 1,900 samples of 178 grasses. This valuable resource serves not only the international R&D community but also CIAT's own selection and breeding efforts. Since 1970, national research programs in 14 countries have released 45 tropical forage varieties (mostly grasses) derived from germplasm selections provided by CIAT. The total area planted to CIAT-related forage varieties in Latin America is estimated at about 6.8 million hectares. There is, however, enormous potential for extending the use of improved forage germplasm since native pasture species still occupy about 90 percent of tropical America's grazing area.

Conservation Methods and Procedures

Given the collections' size and diversity in terms of plant development and reproduction, a major challenge for GRU staff is to ensure that these materials are properly preserved, using the most reliable and advanced techniques available.

Seed of common bean and forages is kept in the GRU's cold storage units, at a temperature of 5 °C for short term-storage and at –20 °C for long-term storage. Relative humidity is kept at 35 percent, at which level there is about 6 to 8 percent moisture in the seed. Under long-term storage conditions, the seed can remain viable for several decades.

The use of high-quality seed can substantially increase the productivity of tropical pastures. But little is known about seed-borne diseases that reduce the yields of tropical forages. Such is the case with the grass *Brachiaria*, one of the most important forages in tropical America. In 1999, 30 accessions were selected from the GRU germplasm collections to detect seed-borne fungal diseases. To date, 12,000 *Brachiaria* seeds have been evaluated, and eight known and 10 previously unknown fungi have been identified.

Arrangements for conserving cassava germplasm are considerably more complex than those for beans and forages. The standard farmer method of propagating cassava is to plant cuttings called stakes. Although stakes have some practical advantages as a germplasm storage and propagation medium, they are sources of plant disease and may not be transported across international borders. It is therefore vital that the GRU at CIAT develop and employ alternative methods that permit convenient distribution of disease disease-free cassava germplasm to meet growing demand from major cassava-growing regions of Africa, Asia, and Latin America.

The cassava germplasm stored at CIAT is currently being conserved *in vitro*. With this method plants are kept in test tubes in an agar solution containing minerals and vitamins that can sustain the plant for 12-18 months. As explained below, efforts are under way to increase the efficiency of this method and to develop superior alternatives.

The GRU's procedure for handling cassava germplasm is outlined below:

Introduction—Material is introduced in the form of seed, *in vitro* shoot tips, or as cassava stakes.

Plant quarantine—To ensure that exchanged germplasm is free of pests and pathogens, CIAT follows regulatory measures and safeguards under the supervision of the Colombian Institute of Agriculture (ICA). ICA has established quarantine procedures to regulate the introduction of plant germplasm and the issuing of phytosanitary certificates that accompany germplasm for export.

Pathogen eradication—The extreme tip of the cassava shoot, or meristem, is extracted. This part of the plant, measuring about 0.5 cm, continuously produces leaves and internodes. Meristems are cultured for about 12 days in a propagation medium and then undergo three cycles of thermotherapy, or heat treatment, at temperatures ranging from 35 to 40 °C to ensure they are virus free. A total of 4,054 cassava clones have been processed to date through *in vitro* thermotherapy techniques, that is, 70 percent of the FAO designate collection. Of these, 1,308 clones have proven to be free of major diseases. The entire collection is expected to have undergone thermotherapy and indexing by 2001.

Indexing of clones—The materials are next subcultured, and a sample of each cassava clone is subjected to standard disease indexing techniques, either ELISA or PCR. Plants free of disease are micropropagated and preserved; those that are not are subjected to further thermotherapy.

Conservation—As mentioned above, the best option available currently is *in vitro* storage.

Repatriation—Part of the germplasm is sent back to the country of origin, usually in a foil package, which is immediately put in cold storage (-20 °C).

Field genebank—The plant is grown in the field for ongoing agronomic evaluation under normal growing conditions, and samples are taken for conservation and characterization. Plant multiplication is also conducted in the field.

Distribution—Germplasm can be requested through national agricultural research systems (NARS), the FAO, or directly from CIAT.

This cassava germplasm management procedure provides invaluable information for improving crop productivity. For example, the use of micrografting to detect disease in cassava has advanced our understanding of frog skin disease, which can cause losses up to 90 percent in some cassava-growing areas.

Research on Agrobiodiversity

The germplasm collections at CIAT are the focus of intensive research aimed at determining how this material can be better conserved and used. For example, such work has led to a better understanding of reproduction in the important grass species *Brachiaria*, and this knowledge could help farmers increase the quantity and quality of fodder for livestock. Other lines of research are described in the sections that follow.

Slow **in-vitro** ***growth of cassava***

With the current *in vitro* method, cassava plantlets have an effective life span of only 12-18 months (in contrast to the decades-long viability of frozen seeds). After that, tissues must be removed from the aging plantlets and recultured in fresh growth medium.

To improve the efficiency of this procedure, CIAT scientists are experimenting with two methods for reducing the growth and thus extending the life of cassava *in vitro* plantlets. The two alternatives, water deficit through osmotic substances and ethylene control, should at least double the time before each clone has to be subcultured—thus greatly reducing the costs involved.

With the water deficit method, researchers lower the osmotic pressure of the tissue's cells by sugar alcohols, growing the plant in simulated desert conditions. This essentially slows down the plant's metabolism. The trick is determining the correct amount and combination of the compounds; too much will kill the plant.

The ethylene control method makes use of the fact that the substance ethylene (the same natural chemical that causes tomatoes and bananas to ripen) is produced within tissue culture test tubes as a product of respiration. Its action can be altered by ethylene-inhibiting chemicals that prolong the viability of the cultures. After 9 months one of the test media has reduced the length of plantlets by a third, compared to the control. Various concentrations of another ethylene-inhibiting chemical also reduced growth and kept the plants viable with multiple sprouts. These preliminary results have opened the way for a new experiment with selected treatments and larger number of cassava varieties.

Cryopreservation

Another promising option for safe, long-term storage of cassava germplasm, called cryopreservation, involves making and freezing “artificial seeds.” Cassava shoot tips (meristems) are given a protective coating of sodium alginate and then made to coagulate into tiny beads by immersion in a calcium chloride solution. After having their water content reduced in a two-step process, the beads are stored in liquid nitrogen. In this extreme environment of –196 °C, all biological activity is effectively halted. The technique allows the cassava to be conserved for 30 years or more with no maintenance other than periodic monitoring. Furthermore, it is less labor-intensive than *in vitro* culturing, requires less storage space, and allows for easy duplication and transport of the collection to other sites. The main disadvantage is that generating whole cassava plants from frozen beads is much more difficult than from test-tube plantlets, and the success rate is still rather low.

This technique is being tested under a pilot project at CIAT involving 60 clones. If successful, the project will be expanded to include the entire cassava core collection of 600 clones. Forty full plants have been regenerated from frozen shoot tips. The plants are thawed, rehydrated, reestablished *in vitro*, and are eventually planted in glass houses. Special cryoprotectants are applied to ensure that cells survive and recover for safe duplication.

Generic conservation techniques

Some of the new conservation techniques being developed at CIAT can be adapted and applied to other crops, including tropical fruits and forest species. Experience in Colombia has shown that with slow-growth techniques the period between periodic subculturing of vegetatively propagated tropical fruit species can be extended from just 3-4 months to 12 months or more.

National institutes in other countries as well, particularly Ecuador, Peru, and Venezuela, have requested assistance from CIAT in adapting such techniques for conservation of species such as passion fruit and highland papaya. The GRU has also been asked by private industries to share information on cryopreservation protocols for use in preserving germplasm of food crops as well as forestry and agroforestry species.

Germplasm characterization and evaluation

In order for the germplasm collections at CIAT to be a truly useful genetic resource, researchers and other users need reliable data about key traits of particular materials. To provide such information, CIAT scientists routinely characterize and evaluate germplasm from the GRU in the field. In the case of beans and cassava, they focus particularly on the core collections to simplify somewhat the huge task of generating useful information about many thousands of accessions.

The tropical forage collection is particularly diverse, with more than 700 species, ranging from small herbs to multipurpose trees. Evaluating this collection presents a special challenge but also provides a unique opportunity for researchers and students. In 1999, 867 new forage accessions were characterized.

Partnerships

Partnerships are an essential part of CIAT's strategy to better integrate *ex situ* and *in situ* conservation of plant genetic resources. Listed below are some of the Center's key partners in this work:

- International Plant Genetic Resources Institute (IPGRI), Italy. The Institute operates a global program of research and support for the conservation of plant genetic resources, and its office for the Americas is located at CIAT headquarters.
- Crop networks. The Bean Advanced Research Network (BARN), Cassava Biotechnology Network (CBN), and International Network for Evaluating Tropical Pastures (RIEPT) have promoted biotechnology applications and other steps that facilitate the use of genetic resources for crop improvement.
- Gene banks and biodiversity institutes in the developing world. Facilities in Brazil, Costa Rica, Colombia, Mexico, Peru, and other countries are our natural allies in germplasm conservation. The project also has close ties with organizations responsible for research on biological diversity.
- Universities, research institutes, and gene banks in the industrialized countries. The project counts on support from many such organizations in a number of areas requiring specialized expertise.