Background on “A Global Conservation Strategy for Cassava and Wild Manihot Species”

- Dr. Clair H. Hershey of Cornell University, acting as a consultant for the International Center for Tropical Agriculture (CIAT), facilitated the process of developing the cassava strategy “A Global Conservation Strategy for Cassava and Wild Manihot Species”.

- A survey of collections, content and status of conservation was distributed to curators in early 2008. A meeting of the key experts and curators, held at CIAT headquarters in Cali, Colombia, took place from 30 April to 2 May 2008.

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A Global Conservation Strategy for Cassava (*Manihot esculenta*) and Wild *Manihot* Species[1]

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[1] Prepared by Clair H Hershey (chh23@cornell.edu), consultant to CIAT. A summary of stakeholder deliberations and recommendations.

Contents

Disclaimer

This document, developed with the input of a large number of experts, aims to provide a framework for the efficient and effective ex situ conservation of globally important collections of cassava (Manihot esculenta) and Wild Manihot species.

The Global Crop Diversity Trust (the Trust) provided support for this initiative and considers this document to be an important framework for guiding the allocation of its resources. However, the Trust does not take responsibility for the relevance, accuracy or completeness of the information in this document and does not commit to funding any of the priorities identified.

This strategy document (dated December 2010) is expected to continue to evolve and be updated as and when circumstances change or new information becomes available.

Executive Summary

As for any crop, the future potential of cassava to contribute to the sustainable benefit of humankind will rely fundamentally on the availability and use of broad-based genetic resources. These resources are basically the landrace varieties that evolved for centuries under farmer and natural selection, and some 100 wild species of the genus *Manihot*. The genus is native to the Americas, and most of the genetic diversification has occurred here. Traders introduced cassava into Africa in the 1500s and into Asia in the 1800s. Both have become important secondary centers of genetic diversity, especially Africa.

Cassava is a vegetatively propagated crop, while all the wild species are seed-propagated in their natural environments. In order to preserve the genetic integrity of a landrace, cassava must be conserved in vegetative form. The most common forms of conservation are as field-grown plants or as plantlets started from meristem tips, cultured on sterile artificial media, under light, temperature and media conditions that induce slow growth. For either field or *in vitro* conservation, expensive periodic regeneration is required, at a much higher frequency (typically every 12-24 months) than is typical for seed conservation.

This report summarizes stakeholder input into a study whose objectives were defined jointly by the Global Crop Diversity Trust (the Trust) and the International Center for Tropical Agriculture (CIAT): “To develop a strategy for the efficient and effective conservation of *Manihot* and cassava genetic resources and identify priority collections for long-term support. The strategy will promote the rationalization of conservation at regional and global levels.”

The proposed strategy is based on the literature, personal interviews, and on two recent activities sponsored by the Trust: a detailed survey sent to some 50 genebanks around the world, and a meeting of a small group of cassava and *Manihot* genetic resources experts at CIAT from 30 April to 2 May 2008.

Thirty-four surveys were returned for cultivated cassava, and the summarized highlights follow.

- Most cassava-growing countries have established a genebank of local landraces, owned and maintained by government organizations.
- Most collections were established since the 1970s, but some as recently as a few years ago.
- Most countries note collection gaps (less so for Asia), due to lack of funding, losses from natural disasters and social conflict, difficult access to areas for collecting, and inadequate collecting techniques of the past.
- Information is generally managed manually, and even when managed electronically, is generally not available on the Internet.
- Nearly all programs rely primarily on field-grown plants, but may have part of their collection *in vitro* as well. Globally, only about one-quarter of accessions held by national programs appear to be conserved *in vitro*.

- Two international centers (CIAT and IITA) maintain regional collections for the Americas and Asia (CIAT) and for Africa (IITA).
- There are very few national genebanks that have the capacity to carry out safe international exchange in situations where viruses of quarantine significance are present. Mostly this is done via the international centers.
- Human resources development is often identified as a critical area for the future success of germplasm conservation.
- Most respondents see the value of a global network for cassava genetic resources, if it is adequately funded.

Out of the information from these surveys, and combined with information from other sources to fill in missing data, we developed a matrix of estimates, by country and region, of various parameters for cassava genetic diversity. About two-thirds of cassava is currently grown in Africa, but probably well over half the landraces occur in the Americas. This is to be expected in view of origin of the species in the Americas. This study estimates some 27,000 distinct landraces of cassava *in situ*, and about 10,000 maintained in genebanks. It is proposed that a total of about 15,000 landrace varieties should be conserved *ex situ* in order to represent the complete genetic diversity of the species.

A conservation strategy should consider security, cost and efficiency in its design. Security is a function of both the number of replications of a genebank (in different sites, or in different forms), and the management level of each. Field genebanks are the least secure, followed by *in vitro* slow growth, and finally, cryoconservation. Currently, only the IARCs have significant cryo genebanks for cassava.

The baseline for our process of thinking about conservation was a meeting held at CIAT in 1992, "the first meeting of the International Network for Cassava Genetic Resources." This group saw the need to decentralize conservation and strengthen the ability of national programs to conserve cassava germplasm, especially through training and infrastructure development for *in vitro* technologies. Unfortunately, this was near the beginning of an era of seriously declining public funds for agricultural research and development. The network did not make much progress towards its goals, and really did not become a successful forum for cassava genetic resources.

There are compelling reasons to rethink a decentralized strategy where each national program has the ability to conserve its germplasm in a highly secure system, which normally involves a field collection backed up by an *in vitro* collection. There have been some significant changes in the world of cassava genetic resources, which impact the structure of an optimum conservation strategy. First, the status of the collections maintained by the CGIAR has been clarified. These collections are now part of the Multilateral System of the International treaty under its Article 15. Secondly, international exchange has become much safer and more acceptable with advances in virus indexing.

This changed environment allows us to think in new ways about the optimum conservation system for cassava. Conservation *in vitro* (slow growth or cryopreserved) is highly non-site-specific and therefore large efficiencies can be gained by centralization. This centralization in the international centers now becomes politically viable, because ownership has been clarified, and international exchange is also clearer and more secure from a quarantine perspective. We now have an opportunity to develop a strategy that is biologically and economically rational, creates a structure of interdependence and collaboration among genebanks, and at the same time conforms to the new policy environment. We can think of this strategy as one of *collaborative centralization*.

In a nutshell, the strategy that comes out of this reality could be the following:

- Collecting in priority areas is carried out to fill gaps, with the aid of genetic diversity studies and GIS.
- National program genebanks and international center genebanks are systematically compared for matching and non-matching accessions, based on passport, morphological and molecular information. This would evolve into a *common cassava registry* at a global level.

- CIAT and IITA duplicate all the landraces of national program collections, in their respective regions of responsibility (CIAT: Americas and Asia; IITA: Africa). Currently they appear to maintain about 50-60% of these accessions.
- National programs commit to at least one working genebank that serves the purposes both of conservation at a moderate level of security, and evaluation.
- CIAT and IITA maintain at least two forms of each accession. Currently this may be an *in vitro* active genebank plus a black box duplicate kept in another center. In the future, cryopreserved accessions will be either the main or the backup genebank.
- CIAT and IITA commit to making the material they maintain available to national program genebanks, when requested.
- CIAT and IITA commit to meeting the demands and phytosanitary requirements for international exchange of cassava landrace varieties under terms of the International Treaty. Along with this, it is urgent to develop protocols for the safe movement of vegetative germplasm between the Americas and Africa.
- There is a mechanism developed for periodic interaction among stakeholders. Most notably this will be between the international centers and the national programs. Each will have a formal responsibility to periodically inform the other of the status of collections.

This collaborative centralization strategy will lead to greater overall efficiency, but at the same time, new initiatives need to be supported to get to this point of lower costs and higher security.

Information sharing is the starting point, especially to develop the common cassava registry. This will involve some standardization of information in order to succeed. The international centers should take the lead in this exercise.

Germplasm indexing and transfer between the international centers and national programs will be required, for movement in both directions. Expansion and upgrading of some facilities will be needed to allow this.

In the ongoing conservation process, there is research with a high payback in terms of greater security and efficiency. Duplicate identification, further improvements for *in vitro* slow growth techniques, improving cryopreservation, and flower induction for seed conservation are all research areas outside the funding stream for routine conservation, but which will contribute to greater conservation and use efficiencies in the long term. Having a coordinated centralized strategy will multiply these efficiencies and savings, especially in areas of safety duplication, duplicate identification, information management and international distribution. For example, the international centers and national genebanks can work jointly, on complementary tasks, for duplicate identification if there is a common cassava registry.

The international centers need to continue to evolve plans on how they are going to conserve the base collection, along with one or more systems of secure backup. A sensible starting point seems to be for the centers to have an *in vitro* base collection and an *in vitro* backup collection in another location (the current situation). The planting of field collections can be based on user demand for planting material for evaluations, and may be contracted out to breeders, for example. The question of how the international centers jointly decide to manage the collections has been based on historical regions of mandate, and the fact that there are viruses exclusive to either the Americas or Africa. This division is reasonable into the medium-term future. As the protocols for virus detection and cleaning progress, it is certainly feasible to see CIAT and IITA acting as two centers for safe conservation of the entire global collection, each acting as the safety backup for the other, and thereby eliminating the current black box system of safety duplication.

Cryopreservation is clearly an option for effective, inexpensive, secure long-term conservation, but work remains to be done on achieving an adequate recovery level for about one-third of accessions (based on results from CIAT's core collection). Research should continue on improving recovery of these recalcitrant types before committing to large-scale cryopreservation of any genebank.

As a future alternative to vegetative cassava genebanks, the seed from selfed accessions could be a less expensive and efficient conservation method, and would be equally or more effective in breeding programs. Since many cassava accessions do not readily flower, there is a need for research on the induction of flowering. There could also be collaboration with national programs that maintain collections in sites where flowering tends to be more profuse. This is another example of the efficiencies that could be introduced with a common cassava registry. Long-term, we might envision a conservation strategy that consists of a combination of cryopreserved meristem shoots, and seed maintained in conventional cold storage. This would combine the advantages of both seed and vegetative conservation in a low-cost, secure system.

The wild species present both a simpler and a more complex situation compared to *Manihot esculenta*. It is simpler in that only a handful of institutions are involved in conservation – mainly EMBRAPA and the University of Brasilia in Brazil, and CIAT. It is more complex in that:

- The taxonomy of species is still poorly defined. An ongoing taxonomic revision of the genus is stalled because of the retirement of the main scientist, Antonio Allem of EMBRAPA.
- The highest concentration of species is native to threatened habitats. This is especially true in south central Brazil, in the *campo cerrado*, where the expansion of agriculture and urbanization are rapidly encroaching on the wild species habitats.
- A secondary center of diversity, with a distinct set of species, exists in Mesoamerica. Here, and especially in Mexico, cassava is a relatively unimportant crop, and it is difficult for these governments to justify investment in *Manihot* conservation.
- Fewer than half the species are conserved *in vitro*, and very few are protected in national or regional reserves, in their native habitat.
- Wild species conservation presents many challenges, especially with regard to regeneration. Progress is being made both in seed and *in vitro* propagation, but much remains to be done.
- The value of the wild species is continually becoming more evident as new characters are identified with potential for transfer to cassava, and the techniques for efficient transfer and selection of specific genes are developed.

1 Introduction and background to the strategy

1.1 Goals and outputs

The goal of the global conservation strategy for cassava and wild *Manihot* species could be distilled to its most basic form by the question: "What are the resources and activities required to safely conserve cassava genetic resources in perpetuity?" This question encapsulates a complex set of goals established by the Global Crop Diversity Trust (hereafter the Trust) to assist in planning for the conservation of the genetic resources of many of the world's major crop species, particularly those of Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).

The goal of the Trust in this initiative is "to develop, in close consultation with representatives of the relevant networks, institutions and stakeholders, a strategy for the efficient and effective conservation of *Manihot* and cassava genetic resources and identify priority collections for long-term support and their urgent upgrading and capacity building needs. The strategy will promote the rationalization of conservation efforts at regional and global levels, e.g. through encouraging partnerships and sharing facilities and tasks and will link with the relevant regional conservation strategies."

Expected outputs are defined as:[2]

1. An evaluation and assessment, in consultation with representatives of the relevant networks and other stakeholders, of the cassava collections of most importance regionally and globally, considering its primary or secondary centers of diversity.
2. An assessment of cassava collections that are 'most important' in terms of size, extent of diversity, holdings of wild species of *Manihot* and other standards of assessment (e.g. viability, health status, availability), carried out in consultation with members of relevant regional networks.
3. A conservation strategy and recommendations for the long-term management of priority collections, promoting partnerships and sharing responsibilities, facilities and tasks.

These outputs are contained in the report that follows. It is intended to be the basis by which the Trust and others are able to follow up with resources and actions to move toward long-term, secure and comprehensive conservation of *Manihot* genetic resources.

The main goal of this initiative is secure conservation. This is understood to be the foundation of crop genetic resources management for the benefit of humankind. However, conservation activities carried out in isolation of broader goals will not bring these potential benefits. These broader interests and activities usually include the ability to utilize these resources to improve crops for specific traits. National research programs, which are by far the main owners of cassava genebanks, are generally anxious to see a balanced investment in collecting, conservation, evaluation and utilization. This report focuses on conservation, but also looks at its role in the broader context of genetic resources management in order to view the balance among all the components. There is overwhelming evidence that adequate and balanced investment in cassava improvement will result in producer and consumer benefits that far exceed the costs (see, for example, Hillocks et al. (2002) and Kawano (2003).

1.2 Building on progress, meeting new challenges

One could reasonably argue that there has been an adequate knowledge base for more than twenty years to assess what needs to be invested to secure cassava conservation. The interchange of information and germplasm by collectors, curators, plant breeders, and many others, has provided periodic input into our understanding of how best

[2] Defined in the Terms of Reference for the consultant who coordinated development of the strategy.

to manage genetic resources for future needs. The foundation of the international centers (CIAT and IITA) that took on cassava germplasm conservation on a global basis, gave considerable impetus to the interest and demand from national programs to invest in cassava and its improvement. Nevertheless, what scientists believe to be the best management of cassava and *Manihot* genetic resources has continued to evolve.

A wide genetic base is not necessarily a prerequisite to short-term or even medium-term success of a crop. Both Asia and Africa, by every measure, have a far narrower genetic base for cassava than the Americas, but the crop has succeeded extremely well on both continents. But the need for broader diversity becomes ever more evident as time goes on. For example, the challenges of evolving markets in Asia (especially for starch and ethanol), and changes in pest pressures in Africa (new races of cassava mosaic disease and recent outbreaks of brown streak disease) have been met with new genetic diversity from genebanks. We have seen many examples where weakness in a crop variety is compensated by inputs such as chemicals to protect against pests, or nutritional supplements for a sub-optimal nutritional content. This may lead to the false conclusion that broad genetic diversity is not essential for sustainable crop improvement. But genetic solutions to constraints in crop production or utilization are often overwhelmingly superior to management that requires other types of purchased or labor-intensive management inputs. The fact that there often can be a partial or complete genetic solution to problems is a strong incentive to assure access to appropriate genetic diversity.

If it were possible to define today the genetic diversity that will be needed in the distant future, we could narrowly target the type of diversity to conserve, and let the rest disappear as farmers decide to switch to new hybrids. But it is clear from history that there are no completely reliable predictors of the specific genes that will be useful in the future, and the best sources of those genes are absolutely unknown today. Pests and diseases will not stop evolving, and will always present new challenges to production practices. New markets, tastes and production practices likewise require new traits. There are many examples of needs for new genes that could not have been foreseen when the cassava collection was first established at CIAT in 1969, such as whitefly resistance or starch traits specifically suited to specific products in the market place.

This is why we preserve landrace varieties and related wild species of our crops, and why we expect to do so in perpetuity. We can have a reasonable level of confidence that greater success can be achieved when a wide genetic diversity is securely conserved, understood, accessible, and wisely utilized.

Vegetatively propagated crops are inherently more difficult to manage in genebanks than seed-propagated species, when the goal is for conservation of specific genotypes (clones). This requires continued vegetative conservation. Since all known cassava landrace varieties are highly heterozygous, the seed that results either from crossing between any two varieties, or from first generation selfing (S_1), will segregate widely. While some progeny may resemble either of the parents in some or in many traits, none will be genetically identical to them. Vegetative conservation may take several forms, including field or greenhouse-grown plants, slow-growth *in vitro* plantlets, protoplasts in culture media, undifferentiated tissue in culture media, or cryopreserved tissue. One of the key requirements is that the methodology must allow regeneration of whole plants that maintain genetic integrity, i.e. that are genetically identical to the parent clone. To date, field-grown and *in vitro* plantlets best fit this criterion.

The scientific bases for effective and efficient cassava genebank management continue to advance. We now have the ability to conserve either vegetative or seed accessions at a very high level of security. However, as will be pointed out later, achieving this high security has been more difficult than anticipated; many institutions have found it difficult to achieve conservation goals due to financial or human resources constraints. The tools and the theoretical background are also advancing rapidly for understanding the genetic structure of germplasm, and applying this information to conservation strategies. *In vitro* slow growth technologies are routine, when the infrastructure and management expertise are available. Vegetative materials can be tested for most pests and pathogens, and they can be eliminated when deemed necessary. Cryopreservation is being successfully applied to a wide range of genotypes, although more research is needed to further improve the recovery rate for broad and secure application as a

conservation strategy. Seed conservation (to conserve genes rather than genotypes) is a theoretical possibility, but cannot yet be broadly applied due to non-flowering of many accessions in important collections.

Our ability to plan collecting and conservation strategies is continually upgraded by new information on *Manihot* evolution and domestication, and genetic variability of landrace varieties. Nonetheless, we are only beginning to scratch the surface. Geographic information systems, linked with genetic resources information, can effectively guide rational and efficient collecting expeditions. There has been steady advancement in developing techniques for pathogen detection and for eliminating them from both seed and *in vitro* accessions. Conservation technologies have continued to advance, especially *in vitro* slow growth technologies and cryopreservation. While there have not been breakthrough technologies in either of these areas, the steady progress allows a greater assurance of secure conservation.

The recent implementation of black box duplicates at CIP in Peru (for the CIAT collection) and in Cotonou, Benin (IITA collection) was a major step in enhancing the security of *Manihot* genetic resources *ex situ*. This additional security should reflect back on national program conservation activities. In the major center of diversity, Brazil took steps to rationalize its cassava germplasm conservation through the establishment of regional genebanks within the EMBRAPA system, with the goal of having these accessions duplicated at the national cassava center (CNPMF) in Bahia State. National programs whose germplasm is duplicated at either of the international centers may be able to reduce their level of local duplication, and rely on the international centers for repatriation of lost materials in the event of loss. This strategy will ideally allow less investment in security backups by national programs, and greater investment in other areas of genetic resources management, such as evaluation and documentation.

Legal issues regarding germplasm exchange and utilization have been greatly clarified for countries that have ratified the International Treaty on Plant Genetic Resources for Food and Agriculture. Under the Treaty, terms of access and benefit-sharing in the case of commercial profits have been clarified and formalized. Since the Treaty is still very new for many countries, it will take some time for programs to develop the internal procedures for implementation. This transitional period has in fact created some constraints to collecting and international exchange in recent years, such as in the case of Brazil, which is of particular importance as a source of *Manihot* genetic diversity. Some national programs have put international exchange on hold as they try to understand and work out the full implications of the Treaty. At CIAT, there have been few new introductions since 1993, as a consequence of the legal uncertainties about status of cassava germplasm (Debouck, 2008).

Despite the technical and legal advances described above, there have also been some steps backward in terms of secure conservation. While a few cassava genebanks have substantially improved their status in the past two decades, many more have actually reduced their capacity for conservation and some have lost accessions. Institutions in the Americas, the primary center of diversity of the genus, seem to have experienced the greatest funding challenges, but the same is also true of some genetic resources programs in Asia and Africa. In the major center of diversity of Mesoamerica, interest and support for cassava has generally waned, with the notable exception of Cuba. In Mexico, a once-strong national cassava program, with collections of both cultivated cassava and wild *Manihot* species, lost much of its funding, and the status of its germplasm is uncertain. In Africa, support to cassava germplasm collection and conservation remains inadequate in view of the massively expanding needs for new varieties and other production and post-production technologies.

The 1970s was an era of strong growth for agricultural research. Institutions were built or strengthened, and scientists were sent for training. Many of the scientists trained at that time are reaching, or are in, retirement. In many cases, they are not being replaced with people in equivalent positions, either because training lagged in the past decade, or because funding in general has decreased. For example, in Brazil's national genetic resources and biotechnology center (CENARGEN), two long-time experts in wild *Manihot* have retired, leaving a vacuum in this area that apparently will not be filled, at least in the near future (Carvalho, 2008). It is this that has caused CENARGEN to take the decision to move the field collection of wild *Manihot* species to the national cassava center (CNPMF) near Salvador, Bahia (Alves, 2008). It also leaves incomplete a monumental work in progress on *Manihot* taxonomy and

phylogeny. Indonesia, which is Asia's largest source of cassava diversity, is also seeing the potential for losses of *Manihot* genetic resources. Retiring curators leave some of the country's principal cassava genebanks at risk of loss of materials (R. Howeler, pers. comm.). Fortunately, steps have been taken to assure duplication in CIAT's collection of those at most immediate risk (D. Debouck, pers. comm.).

1.3 Precedents and resources

The current study benefits from several global-level meetings to discuss and plan the management of *Manihot* genetic resources in the past 25 years. These have been organized jointly between national programs, international centers, and FAO.

In 1981 and again in 1982, IBPGR and CIAT jointly organized workshops on cassava genetic resources. These meetings established the importance of further collecting of both wild species and cultivated cassava, proposed collecting formats that became the standard for many subsequent expeditions, proposed evaluation criteria, and defined areas of collaboration among genebanks (Patiño and Hershey, 1981; Gulick et al., 1983).

There have been many regional meetings since the 1970s that included *Manihot* genetic resources as a discussion topic, mainly in relation to fulfilling the needs of cassava breeders. For example, the Panamerican Cassava Breeders' Network, the Asian Cassava Research Network, and the African Branch of the ISTRC have all held numerous meetings that included discussions on genetic resources. Invariably, participants have recognized the value and importance of comprehensive germplasm collections, secure conservation, and free exchange among users, as basic to the ability of national and international programs to supply material with improved genetic potential to growers.

The most recent global meeting specifically dedicated to *Manihot* genetic resources (prior to the one reported here) was held at CIAT headquarters from 18-23 August 1992, supported by IPGRI, CIAT and IITA (IPGRI, 1994). The purpose of this meeting was to develop a plan of action with three interdependent aspects:

- "The implementation of an international database on cassava genetic resources, or the implementation of regional databases, merging their files in order to create an international database;
- "a world-wide strategy for rational and safe conservation of the germplasm, using all available techniques;
- "collaborative activities for better use of cassava genetic resources and/or a research programme to solve most common problems."

This meeting was described as "the first meeting of the International Network for Cassava Genetic Resources." The participants established a steering committee with the following mission:

- Implement the recommendations of the workshop;
- Interlink with other networks;
- Seek funding for activities of the International Network for Cassava Genetic Resources beyond the commitments made by the participating institutes;
- Call outside expertise whenever required to finalize specific projects or to explore potential new activities; and
- Convene the next plenary meeting planned in four years.

The nine members of the steering committee were nominated on the basis of geographical representation and their linkages with other existing networks as well as on commitment from CGIAR institutes to develop regional databases, which would play a key role in the development of the network.

While the conclusions and recommendations of this meeting were sound, it appears that there were no follow-up meetings of the network nor of the steering committee (See Table 1). For all practical purposes, the International Network for Cassava Genetic Resources was not active after this founding meeting. Follow-up on the recommendations of the meeting was mixed -- although a few of that meeting's recommendations were carried out, many were not. This period, in the early 1990s, coincided with the beginning of a long period of generally declining financial resources for public research in agriculture. Most countries and institutions were affected. While, for some crops, the private sector stepped in to fill the gaps, this was largely not the case for cassava, with some notable exceptions, such as CLAYUCA in the Americas.

Table 1. Summary of recommendations of the working group on cassava genetic diversity, of the first meeting of the International Network for Cassava Genetic Resources (IPGRI, 1994).[a]

Genetic Diversity

- Study the gene pool structure of the *Manihot* genus by way of agrobotanical characterization, ecological adaptation, combining ability and application of molecular markers
- Study phylogeny and genetic introgression among species
- Develop improved methodologies for duplicate identification
- Monitor erosion of genetic diversity
- Duplicate national germplasm collections in other locations
- Encourage and support production of catalogues, and develop databases with genetic resources information

Use of wild species

- Consistently collect wild *Manihot* species
- Develop methodologies for evaluation
- Compile and disseminate knowledge from interspecific hybridization studies

Core collections

- Study further the use of molecular and morphological markers, ecological adaptation and combining ability, for better defining a core collection
- Develop core collections in various regions
- Compile a comprehensive list of desired traits, and evaluate them in multi-location trials
- Elaborate and distribute a special catalogue
- Include the wild species in the core collection after sufficient collection and characterization

Biotechnology

- Priority 1: develop molecular markers for the more efficient selection for quarantine traits
- Priority 2: Develop improved pathogen testing methods to support germplasm exchange, including research and transfer of technologies
- Priority 3: Carry out research on transformation and regeneration technologies
- Develop techniques (such as somatic hybridization) where there is a problem in hybridization of non-compatible species or stability of the hybrids

Mechanism for safe germplasm exchange and its access

- Compile a list of quarantine regulations and restrictions for all countries
- Offer training courses on serological and biological indexing techniques
- Strengthen regional testing centers
- Develop large quantities of antisera to many pathogens
- Train regionally for in vitro and true seed
- Study the diversity of ACMV strains within Africa

Table 1. Summary of recommendations of the working group on cassava genetic diversity, of the first meeting of the International Network for Cassava Genetic Resources (IPGRI, 1994).[a]

Human resources development; train in: • Quarantine and pathology aspects • Conservation and exchange of germplasm • Pest and abiotic screening • Use of microcomputers in documentation • Analysis of genetic diversity by molecular and biochemical methods
Linkage with other network groups • Make formal contact with other groups of researchers or users whose interests and/or activities have relevance to the Cassava Genetic Resources Network • Maintain continuing communication among the networks

[a] Summarized by the author of the current report.

Clearly, the International Network for Cassava Genetic Resources was an important precedent for the current efforts of the Trust and its partners. The recommendations, follow-up, successes and remaining tasks are highly pertinent to understanding the appropriate next steps to successfully conserving *Manihot* genetic resources in perpetuity. At the same time, it is important to understand why this network had limited success, and to overcome those obstacles in the present efforts.

Both national programs and the international centers remain committed to the goals for *Manihot* genetic resources management that have largely lain dormant for 15 years. At the same time, institutions are justifiably cautious about committing energy and resources without a high level of assurance that action will follow words. Ultimately, success can only come to each program that makes a philosophical and financial commitment to this success. The current global environment has a number of features that indicate new potential for progress. With high prices for agricultural commodities in general, and the inputs to produce them, there is a broad interest in renewing investment in agriculture as the best route to sustainable development, leading to adequate food for all and improved livelihoods with minimal environmental impact. There is growing recognition that climate change is already affecting agriculture and our ecosystems, and that appropriate management of genetic resources – crops and non-crops alike – is vital to the well-being of humankind. These motivating forces, along with the commitment of the Trust to support a rational global system for genetic resources conservation, provide a renewed level of confidence in the ability of local, regional and global partnerships to succeed in these new goals for secure cassava and *Manihot* species conservation.

1.4 Sources of information

The principal sources of information for this report include a detailed survey sent by e-mail to the curators of most of the known cassava and wild *Manihot* genebanks around the world (Appendix III, IV and V; a consultation of experts held at CIAT headquarters in Cali, Colombia from 30 April to 2 May 2008 (Appendix I, II and VI), the literature, and personal contacts and personal experience of the author of this report.

As of this writing, 34 surveys were returned for cassava, and three for the wild species (see more detail on sources in Appendix IV). Those for cassava are:

AMERICAS: Bolivia, Brazil (three institutions), Costa Rica, Ecuador (two institutions), Guyana, Panama, Peru.

AFRICA: Chad, Côte d'Ivoire, D.R. Rep. of Congo, Ghana, Guinea Conakry, Malawi, Mozambique, Niger, Nigeria, Sierra Leone, South Africa, Sudan, Swaziland, Togo, Zambia.

ASIA/OCEANIA: China, Indonesia, Malaysia, Papua New Guinea, Thailand, Vanuatu, Vietnam.

INTERNATIONAL CENTERS: CIAT and IITA.

For the wild *Manihot* species, surveys were returned from Brazil (EMBRAPA and the Universidade de Brasilia) and from CIAT.

A survey is an inexpensive, but not entirely adequate, way of getting information on genebanks and their management. First, there are not many people who enjoy receiving or filling out surveys, especially extensive ones that require some research or some thought. Many people simply will decline to respond, unless there is some associated motivation, such as the possibility of getting future funding. Secondly, the person assigned to fill out the survey is not necessarily the person best qualified to answer the questions. Some people may want to provide answers that give the best public view of their program. Alternatively, some may want to exaggerate problems, with the thought that this may attract funding to improve genebank management. Thirdly, questions may be interpreted in slightly, or significantly, different ways, often because the language of the survey is not the respondent's first language. This can also introduce errors, and make it difficult to make legitimate comparisons across all the returned surveys.

2 Cassava in the global economy and agro-ecosystem

2.1 Manihot species overview and the origins of cassava

Manihot is a Neotropical genus, distributed in its natural habitat from the southern United States, through Mesoamerica, northern South America, and south through Brazil, Peru, Bolivia, Paraguay and Argentina. The latest monograph by Rogers and Appan (1973) identified 98 species. The groups of species from the northern and southern hemispheres are markedly distinct. With the exception of *M. esculenta*, none of the northern Mesoamerican species are found naturally in South America, and only *M. brachyloba* occurs in both South and Central America. In a monograph still in progress, Allem reduces the size of the genus to 70 species, with 55 in South America and 15 in Central and North America (Howeler et al., 2001).

The species of *Manihot* are perennial and vary in form from acaulescent shrubs to trees with trunks 25 cm in diameter and a height of 10 to 12 m. They are generally sporadic in their distribution and never become dominant members of the vegetation. Most are native to dry regions, with a few in rainforest ecosystems. In general, the species appear to be shade-intolerant – capable of survival only with plenty of sunlight. They are not good competitors with vigorous intercrops or with weeds. All the species are sensitive to frost, thus limiting their distribution to elevations below about 2200m. Since many of the species are found where long dry periods are common, they have evolved mechanisms of drought avoidance or drought tolerance. One of the most notable of these mechanisms is the production of storage roots where large amounts of starch are accumulated. In all species studied, these storage roots also contain the glucoside linamarin, which breaks down after cell injury to release prussic acid (HCN) (Rogers and Appan, 1973).

The genus is clearly of New World origin, but further details of its evolution and distribution within the New World have been poorly understood. Only since the 1990s, with the discovery of wild cassava, and the aid of molecular analyses to examine relationships between the crop and the wild species, has there been better progress in defining an evolutionary history. Allem (2002) describes three important questions that need to be addressed concerning the obscure origins of cassava: botanical origin (i.e. the wild species which gave rise to cassava); the geographical origin (i.e. the area where the progenitor evolved); and the agricultural origin (i.e. the area of initial cultivation of the wild ancestor by Amerindians).

Archaeological evidence of cassava in northern South America indicates that its cultivation is of great antiquity there. Radiocarbon dates are much earlier than those from the Brazil/Paraguay region. But archaeological remains are rare in humid environments, so this does not give incontrovertible support to a northern South American origin. Studies of micro-fossils, such as starch granules in the case of cassava, allow us to partly overcome this problem. Nonetheless, since cassava was broadly cultivated in the New World since several thousand years ago, it is difficult to use the sparse archaeological remains to pinpoint the origin of the crop.

There are few reliable phenotypic characters in the genus *Manihot* to indicate evolutionary relationships. Most of the species (including *M. esculenta*) show high intraspecific morphological variability. Because it was not possible to confidently narrow origins with the use of morphology or archaeological evidence, a theory of multiple origins arose, but this was based less on positive evidence than on lack of evidence for alternative hypotheses.

In what was to become the first insight into an entirely new perspective on cassava's origins, Dr. Antonio Costa Allem of CENARGEN in Brazil, discovered a putative wild population of cassava in Goias state in 1982, described as *Manihot esculenta* ssp. *flabellifolia*. Continued explorations showed that this subspecies was distributed in a zone of transitional forest between the Amazon basin and the drier savanna to the south and east, including areas of the states of Acre, Rondônia, Mato Grosso, Goiás and Tocantíns (Allem 1987; 1992; 1994).

M. e. ssp. *flabellifolia* is similar to cassava morphologically, but cassava has greater root thickening, swollen leaf scars and a stem morphology that is adapted to vegetative propagation (shortened internodes and thicker stems for more carbohydrate reserves). As with most *Manihot* species, *M. e*. ssp. *flabellifolia* is sporadic in its distribution; most populations typically comprise fewer than 15 individuals.

Early work with molecular markers to explore evolutionary patterns of *Manihot* indicated that South American and Central American species form two distinct lineages, and cassava is more closely related to the South American group. This work included RFLPs (Bertram, 1993; Fregene et al., 1994), AFLPs (Roa et al., 1997) and DNA sequences (B. Schaal, cited in Olsen, 2004).

At the next level of molecular evolutionary studies, variations in SNPs (single nucleotide polymorphisms) and SSRs (simple sequence repeats) were used to explore cassava's relationship to *M. e*. ssp. *flabellifolia* (Olsen, 2004). These studies compared a presumed wide genetic diversity of cassava clones selected from CIAT's core collection, and samples from a range of *M. e*. ssp. *flabellifolia* genetic populations. The results appear to definitively place cassava within the range of genetic variation of the subspecies. Across the eight loci examined, the cassava clones contain an average of 18.8% of the total variation of the wild species. *M. e*. ssp. *flabellifolia* genetic variation is sufficient to account for cassava's genetic diversity, without any need to involve a hybrid origin (Olsen, 2004). The composite of evidence from molecular studies gives strong support to *M. e*. ssp. *flabellifolia* as the progenitor of cassava.

Allem (2002) also provides interesting anecdotal evidence on the possibility that domestication of cassava from wild species is not that difficult and is in fact still taking place today in parts of Brazil. He proposes a transitional link between cultivated cassava and its wild ancestor, in the form of a landrace called *manipeba* in northeast Brazil. This landrace (it is unknown how many distinct genotypes are involved) appears to be botanically and agronomically intermediate between wild and cultivated cassava, and as such gives a possible snapshot of the route to cassava's domestication.

In nature, all the wild species appear to be principally seed-propagated. As a strategy for genetic resources conservation, there is probably little need for conserving individual genotypes through vegetative propagation. In practical terms, a strategy that combines seed, field and *in vitro* conservation will increase probability of success for conservation of many difficult-to-propagate species, as well as allowing field evaluations for traits of interest, and crossing studies. CIAT has embarked on a detailed characterization of natural habitats of the wild species to better understand their adaptation, and ultimately to tailor a conservation strategy to groups of species with similar

requirements (Jarvis and Guarino, 2008). This could also be an important part of a longer-term effort to produce an inventory of all existing *Manihot* populations.

In summary, the accumulated evidence on cassava genetic diversity indicates the following[3]:

- *Manihot* apparently originated in Mexico and Central America, and rapidly radiated in South America, perhaps after formation of the Isthmus of Panama (Duputié et al. 2008).
- The genus *Manihot* appears to be recently evolved, without sharp genetic barriers among the species.
- Cassava was domesticated in the Americas, most likely along the southwestern edge of the Amazon rainforest, from the wild species progenitor *M. esculenta* ssp. *flabellifolia.*
- *M. esculenta* ssp. *flabellifolia* is distributed widely in the Americas, between the tropics of Capricorn and Cancer.
- The highest genetic diversity of cassava is in Brazil, with high diversity also noted for Central America.
- A considerable amount of recombination continues to occur in farmers' fields in some areas of Africa and Latin America.
- Studies show high genetic diversity and low differentiation in all country studied, with the exception of a group from Guatemala.
- There is little substructure in American accessions (except for the Guatemalan group), but there is a substructure in the African germplasm, explained by selection.
- Neotropical accessions can be separated from African ones using molecular markers.
- Historically, Asia received introductions from both Brazil (via Africa) and Mexico (via the Philippines).
- Asia has far less genetic diversity and less differentiation compared to the Americas or Africa.
- In modern times, massive introduction of diversity from the Americas to Asia by breeding programs has greatly enhanced diversity available for genetic improvement.
- While much of the world's *ex situ* germplasm has been evaluated for traditional major traits, there is probably a wide array of unexplored variation, such as in traits for specialty markets (e.g., sugary, amylose-free, high protein, slow post-harvest deterioration).

2.2 Production overview

Cassava is the fourth most important supplier of food calories in the tropics. The principal economic product is starchy roots, which are utilized in a wide range of end uses, most notably including human food, animal feed, and industrial products. World production in 2006 of about 225 million tons is the energy equivalent of 80 to 85 million tons of cereal grains (FAOSTAT, accessed 6/2008).

The crop is important throughout the lowland humid and seasonally dry tropics. It extends to the limits of its adaptation in the highlands of the Andean zone and East Africa (up to about 2200 masl), in the semi-arid tropics where rainfall may be as low as 400 mm annually, and into the subtropics, where its adaptation is limited by cool winters and accompanying frost. The species has not succeeded much beyond the Tropics of Cancer or Capricorn, both because of the need for a long growing season, and also the difficulty of storing planting material for extended periods (during a cold winter, for example).

In the last two decades, cassava's importance has grown much more quickly in Africa than in either Asia or Latin America. Nigeria alone plants an area 36% greater than the entire area planted in Latin America, and slightly more than all of Asia. The three top producers in Africa – Nigeria, the Democratic Republic of Congo, and Mozambique – together plant about the same area of cassava as all of Latin America and Asia combined. In the past two decades, production has approximately kept pace with population increases in producing countries. Nonetheless, there are large imbalances among regions. In most of Africa, where the crop is utilized mainly for human consumption, yields are still well below those of Asia or South America (Table 2).

[3] Information taken mainly from presentations in the CIAT workshop, 30 April – 2 May 2008, except where otherwise noted.

Table 2. Production statistics for cassava, by region.

Region/country	Area (ha)	Yield (tons/ha)	Production (tons)
Latin America and Caribbean			
Argentina	17,571	10.0	175,706
Bolivia	36,858	10.1	373,612
Brazil	1,901,561	14.0	26,713,038
Colombia	180,000	11.1	2,000,000
Costa Rica	20,000	15.0	300,000
Cuba	79,648	5.6	450,000
Dominican Rep.	15,435	6.1	93,609
Ecuador	22,677	4.4	100,229
El Salvador	1,288	12.5	16,102
French Guiana	1,608	3.5	5,582
Guatemala	6,279	2.8	17,578
Guyana	2,995	11.1	33,294
Haiti	71,270	4.6	326,821
Honduras	5,075	4.0	20,300
Jamaica	751	21.8	16,405
Mexico	1,501	13.8	20,661
Nicaragua	11,000	9.5	105,000
Panama	2,341	11.8	27,693
Paraguay	300,000	16.0	4,800,000
Peru	86,000	11.0	945,000
Puerto Rico	49	9.3	456
Suriname	225	20.0	4,495
Venezuela	41,641	11.7	489,047
Sub-total:	2,806,835	13.2	37,041,521
Africa			
Angola	757,000	11.6	8,810,000
Benin	173,450	14.6	2,524,234
Burkina Faso	1,000	2.0	2,000
Burundi	82,000	8.7	710,000
Cameroon	350,000	6.0	2,100,000
Cape Verde		11.7	3,500
Central African Republic	190,000	3.0	565,000
Chad	27,000	12.0	325,000
Congo, Republic of	110,000	9.1	1,000,000
Côte d'Ivoire	280,000	7.9	2,200,000
D.R.Congo	1,845,510	8.1	14,974,470
Gabon	45,541	5.1	231,816
Gambia	2,500	3.0	7,500
Ghana	790,000	12.2	9,638,000
Guinea-Bissau		14.8	40,000
Guinea Conakry	136,252	7.8	1,068,518
Kenya	77,502	10.9	841,196
Liberia	100,000	6.3	634,874
Madagascar	388,779	6.1	2,358,775

Table 2. Production statistics for cassava, by region.

Region/country	Area (ha)	Yield (tons/ha)	Production (tons)
Malawi	165,229	12.6	2,075,000
Mali	4,000	14.0	56,000
Mozambique	1,105,000	10.3	11,458,000
Nigeria	3,810,000	12.0	45,721,000
Rwanda	118,860	4.9	588,174
Senegal	19,464	6.2	120,841
Sierra Leone	70,000	5.0	350,000
Sudan	6,545	1.7	11,338
Togo	135,820	5.6	767,365
Uganda	379,000	13.0	4,926,000
U.R.Tanzania	670,000	9.7	6,500,000
Zambia	180,000	5.3	950,000
Zimbabwe	46,839	4.4	206,911
Sub-total:	12,110,694	10.1	122,088,128
Asia – Oceania			
Cambodia	*96,324*	22.6	2,182,043
China	265,800	16.2	4,318,000
India	242,400	31.4	7,620,200
Indonesia	1,222,814	16.3	19,927,589
Malaysia	37,719	9.9	374,679
Melanesia[a]	15,450	10.6	163,000
Micronesia	1,100	10.7	11,800
Myanmar	16,500	12.5	207,000
Philippines	204,578	8.6	1,756,860
Polynesia	1,327	13.5	17,946
Sri Lanka	23,560	9.6	226,080
Thailand	1,070,805	21.1	22,584,402
Viet Nam	474,800	16.2	7,714,000
Sub-total:	3,690,795	18.2	67,207,747
WORLD	18,608,324	12.2	226,337,396

[a] Includes Fiji, New Caledonia, Papua New Guinea, Solomon Islands. Papua New Guinea produces about 75% of the region's cassava.
Source: FAOSTAT, for 2006; accessed 5/20/2008.

The long growing period, like that of most non-cereal energy crops in tropical agriculture, lends it to adaptation in a wide range of production systems. Cassava may be an important component of cropping systems ranging from shifting cultivation with a long fallow phase, to intensive, continuous annual cropping (for review see Toro and Atlee (1980), Fresco (1986) and Ospina and Ceballos (2002). Small farmers planting less than one and up to a few hectares are the principal producers, although large plantations are becoming more common as the crop is industrialized, especially in Latin America (Brazil) and Asia (Indonesia).

The plant may be propagated either vegetatively (stem cuttings) or sexually (true seeds). While all commercial plantings are from cuttings, propagation from seed is important for breeding programs, and for the occasional volunteer seedling in farmers' fields. Lignified stem pieces from mature plants may be planted directly after they are cut or after storage of up to several months. Storage conditions strongly influence sprouting ability and subsequent plant vigor and yield.

Cassava production expanded broadly throughout the lowland tropics in the twentieth century, mainly on less fertile, poor quality agricultural lands. In traditional low-input cropping systems, cassava is often an end-of-cropping-phase species – the last crop before returning land to fallow. In Africa the capacity of cassava to grow and yield well on low-fertility soils, its ability to withstand locust attacks and drought, and its low cost of production, motivated farmers to use it to replace other traditional root crops such as yams, as well as other traditional cereal staples such as sorghum. In areas where population growth has caused a reduction of the rotation pattern in shifting culture and a commensurate decline in soil fertility, cassava is one of the few crops that can thrive without purchased inputs, provided some form of rotation remains. Similarly, in much of tropical Asia, cassava is relegated to lower-quality land not suited for rice production. In one of the most notable agricultural success stories of the late 20th century, the area planted in Thailand increased five-fold in the 1970s to meet an export opportunity in Europe. Most of the production continues to be on under-exploited land of the northeast, and by small landholders.

Both growers and scientists have historically considered cassava a rustic crop with few serious pest or disease problems (Purseglove, 1968). Evidence from the past 40 years, however, shows that this belief is based primarily on observations of regionally evolved and selected varieties, grown under traditional cultural practices (Lozano et al., 1980). Within these systems, the pest populations are often in balance with their natural enemies. Varieties evolved with moderate resistance to local pests. Plantings may be widely separated in space, thus limiting the rapid plant-to-plant spread of microorganisms or arthropod pests. Many times, when traditional varieties are cultivated in more intensive systems (closer spacing, monocropping), pest and disease outbreaks are common.

Because cassava is so widely cultivated throughout the tropics, and often in environments with minimal amelioration through fertilizers, irrigation, or other inputs, the crop is subject to a wide variation of environmental factors such as temperature, photoperiod, light intensity, water, relative humidity and soil characteristics. Variation is greatest across geographical areas, but can also be substantial across time within a given site. This wide range of selection environments strongly influenced the evolution of landraces.

Cassava is still widely grown as a small-farmer crop in systems with few external inputs, but farmers are increasingly adopting new varieties and new production systems. In addition, the crop is expanding into new areas as population pressures move agriculture into more marginal lands. Such changes, either in cultural practices or in variety, can result in pest outbreaks due to an imbalance in the established equilibrium. Because cassava is a long-season crop, insecticides or fungicides would have to be applied over a long period to provide satisfactory protection. Normally, this is neither economically nor environmentally sound. For many pest problems the best control strategy is through host plant resistance and/or biological control.

Most landraces, when grown under high fertility conditions, increase foliage yield proportionally more than root yield. This is a normal response in primitive varieties of many crop species that have not been genetically improved for response to more luxurious conditions. If leaves are a commercial product, this may be a desired response. However, in most cases, the principal goal will be to maximize production of high quality roots. As it has already been amply shown in breeding programs around the world, it is quite possible to breed cassava both for responsiveness to good soil fertility, and tolerance to poorer conditions.

Cassava owes part of its popularity to a wide diversity of uses for the roots: fresh or processed for human food and animal feed, specialty uses, and in various industrial products including starch and starch-derived products, alcohol and high fructose-glucose syrups. There are also instances of specialty uses such as varieties with low starch and

high sugar that are used mainly to make fermented drinks (Carvalho, 2008). Processing seems to have been an integral part of cassava culture for as long as the crop has been cultivated.

The main features of cassava that impact its form of utilization are its starch content, nutritional value, post-harvest storage characteristics, and toxicity. Cassava utilization typically performs five main roles: (1) famine reserve; (2) rural food staple; (3) urban food staple; (4) livestock feed and industrial raw material; and (5) earner of foreign exchange.

Nearly all cassava in Africa is destined for human consumption. But this is undergoing a transformation – a shift from production for home consumption to commercial production for urban consumers, and in some cases, livestock feed and industrial uses (Nweke et al., 2002).

Asia has been largely industrially-oriented. Malaysia and Indonesia were major industrial starch producers since before World War II, although these industries declined after the war. Thailand re-energized the cassava sector when it capitalized on European market opportunities for dried chips and pellets, beginning in the 1970s. In more recent years, India, Indonesia, China and Vietnam have been moving aggressively into industrialized, value-added cassava products. At the same time, cassava remains important as a basic food or feed crop of the urban and rural poor in most of these countries.

In Latin America as a whole, the main driving forces for new forms of cassava utilization are the demand for energy sources, for balanced animal feeds, industrial starch, and fermentation for ethanol production. However, there are also large areas, especially in the Amazon and Orinoco basins, where cassava cultivation has been nearly unchanged for centuries.

Of the many thousands of landrace varieties and experimental genotypes tested, all produce some level of hydrocyanic (prussic) acid (HCN), poisonous especially to warm-blooded animals. Cooking, drying and most other traditional processing methods for roots destined for human consumption reduce cyanogenic potential (CNp) to very low levels. The reasons for the evolution of a range of toxicity levels in cassava have been the subject of many years of debate. Cassava appears to be one of the few crops in the world in which there is conscious selection favoring the more toxic varieties over the less toxic ones (Wilson, 2003). The reasons for this seem to vary by region, but may involve reducing theft, repelling wild animals that would otherwise uproot plants, and factors related to starch quality. Farmers often grow high (bitter) and low (sweet or cool) CNp types as though they were two distinct crops.

There is continuing debate about the evolutionary reasons high CNp was retained in domesticated cassava. Was it a founder effect, where the wild progenitor was high in CNp, and there was no option for selection of low levels early in domestication, so farmers learned complex processing techniques to deal with it? In this scenario, only after centuries of selection, may there have been some regions and some end uses where selection for non-bitter types was a goal, and was achieved.

Table 3. Consolidated information on production, *in situ* diversity, *ex situ* holdings and proposals for genebank composition.

Region/country	Estimates of ex situ accessions based on different sources: Area planted (ha)[a]	IBPGR (1994)	Ng & Ng (2002)	GCDT survey[b] (2008)	In CGIAR centers[c]	Main national programs holding accessions	Consolidated estimates of unique local landrace varieties (excluding duplicates and breeding/experimental material)[d]: Ex situ	In situ	Consolidated estimated landrace density (ha per landrace)[e]: Ex situ	In situ	Estimated in situ accessions missing from CGIAR centers[f]	Proposed minimum ex situ no. of acces-sions[g]	Priority for accession duplication in CGIAR centers[h]	Priority for additional collection[i]	ITPGRFA status
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Latin America and Caribbean															
Argentina	17,571		177		122	INTA	160	250	110	70	128	200	*	*	Signature
Bolivia	36,858	18	18	30	7	IIA	18	300	2,048	123	293	200		***	
Brazil	1,901,561	4132	4,132	3075	1281	EMBRAPA, IAC, USP	1600	8,000	1,188	238	6,719	4,000	***	***	Ratification
Colombia	180,000			2000	2000	(CIAT)	1800	3,000	100	60	1,000	2,500		*	Signature
Costa Rica	20,000	71	154	72	81	CATIE	70	100	286	200	19	100		*	Ratification
Cuba	79,648	385	495		82	INIVIT	75	100	1,062	796	18	100		*	Ratification
Dominican Rep.	15,435	30	46		5	CENDA/ CESDA	25	50	617	309	45	25	*	**	Signature
Ecuador	22,677	101	101	93	116	INIAP	80	250	283	91	134	200		*	Accession
El Salvador	1,288	10					8	25	161	52	25	20	*	*	Ratification
French Guiana	1,608						0	50		32	50	50		*	Ratification
Guatemala	6,279				92		50	75	126	84	0	100		*	Ratification
Guyana	2,995			29		NARI	25	50	120	60	50	50	**	*	
Haiti	71,270						0	100		713	100	75		***	Signature
Honduras	5,075				27		20	50	254	102	23	25		*	Accession
Jamaica	751						0	50		15	50	25		*	Accession
Mexico	1,501	105	225		106	INIFAP	75	200	20	8	94	100		*	
Nicaragua	11,000	16	37		3	UNA	10	100	1,100	110	97	75	*	**	Accession
Panama	2,341	44	50	2	47	IIA	40	75	59	31	28	50		*	Accession
Paraguay	300,000	360	360		208	IAN	300	500	1,000	600	292	400	**	**	Acceptance
Peru	86,000			639	421		550	1,500	156	57	1,079	1,000	***	**	Ratification
Puerto Rico	49				17		17	25	3	2	8	15		*	
Suriname	225						0	75		3	75	25		**	
Venezuela	41,641	253	.		253	UCV	225	1,000	185	42	747	500		**	Ratification
Sub-total:	2,806,835	5,525	5,795	5940	4,851		5,148	15,925	547	176	11,074	9,835			

Region/country	Estimates of ex situ accessions based on different sources: Area planted (ha)[a]	IBPGR (1994)	Ng & Ng (2002)	GCDT survey[b] (2008)	In CGIAR centers[c]	Main national programs holding accessions	Consolidated estimates of unique local landrace varieties (excluding duplicates and breeding/experimental material)[d]: Ex situ	In situ	Consolidated estimated landrace density (ha per landrace)[e]: Ex situ	In situ	Estimated in situ accessions missing from CGIAR centers[f]	Proposed minimum ex situ no. of accessions[g]	Priority for accession duplication in CGIAR centers[h]	Priority for additional collection[i]	ITPGRFA status
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Africa															
Angola	757,000		13		3		10	300	75,700	2,523	297	100	*	***	Ratification
Benin	173,450	113	340		412	SRCV	300	400	578	434	0	100		*	Accession
Botswana			11				10	20	0	0	20	20	*	*	
Burkina Faso	1,000		14		6		10	15	100	67	9	20		*	Ratification
Burundi	82,000							50		1,640	50	25		**	Ratification
Cameroon	350,000	203	250		219		200	300	1,750	1,167	81	250		**	Ratification
Cape Verde					13		10	20	0	0	7				
Central African Republic	190,000				2		2	200	95,000	950	198	100		**	Ratification
Chad	27,000			45	3	ITRAD	40	50	675	540	47	25	**	*	Acceptance
Congo, Republic of	110,000	311			22	INERA	150	200	733	550	178	50	***	**	Accession
Côte d'Ivoire	280,000	300	300	170	23	CNRA	250	300	1,120	933	277	100	***	**	Ratification
D.R.Congo	1,845,510		250	140	22		300	1,000	6,152	1,846	978	500	***	***	Accession
Gabon	45,541	42	42				40	75	1,139	607	75	50	**	*	Ratification
Gambia	2,500				5		5	25	500	100	20	10		*	
Ghana	790,000	161	2,000	36	338	PGRC/CRI	300	400	2,633	1,975	62	100		***	Ratification
Guinea Bissau	2,700				69		20	25	135	108	0	25			
Guinea Conakry	136,252		168	50	87	IRAG	100	150	1,363	908	63	50	**	*	Approval
Kenya	77,502	213	250		10	RTCP	150	200	517	388	190	50	***	*	Accession
Liberia	100,000	50			6		75	100	1,333	1,000	94	50	**	**	Accession
Madagascar	388,779				4		4	200	97,195	1,944	196	100		***	Ratification
Malawi	165,229	200	170	192	5	RTCP	150	175	1,102	944	170	100	***	*	Ratification
Mali	4,000				1		1	25	4,000	160	24	20		*	Ratification
Mozambique	1,105,000	19	81	25		INIA	75	250	14,733	4,420	250	150	***	***	
Niger	2,595			124	10	INRAN	50	75	52	35	65	20		*	Ratification

Region/country	Estimates of ex situ accessions based on different sources: Area planted (ha)[a]	IBPGR (1994)	Ng & Ng (2002)	GCDT survey[b] (2008)	In CGIAR centers[c]	Main national programs holding accessions	Consolidated estimates of unique local landrace varieties (excluding duplicates and breeding/experimental material)[d]: Ex situ	In situ	Consolidated estimated landrace density (ha per landrace)[e]: Ex situ	In situ	Estimated in situ accessions missing from CGIAR centers[f]	Proposed minimum ex situ no. of acces-sions[g]	Priority for accession duplication in CGIAR centers[h]	Priority for additional collection[i]	ITPGRFA status
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Nigeria	3,810,000	417	435	40	547	NRCRI	500	800	7,620	4,763	253	200		**	Signature
Rwanda	118,860		280		2		125	150	951	792	148	75	***	*	
Senegal	19,464	11	57			ISRA/CDH	10	50	1,946	389	50	25	*	*	Ratification
Sierra Leone	70,000	43	134	118	110	IAR	100	200	700	350	90	100		*	Accession
South Africa			100	0		ARC	5	25	0	0	25	10		*	
Sudan	6,545			10		ARC/ SSARTO	10	10	655	655	10	10	*	*	Ratification
Swaziland				10			10	20			20	10	*	*	Signature
Togo	135,820		734	209	176	ITRA	100	200	1,358	679	24	100		*	Ratification
Uganda	379,000	200	413		14	RTCP	250	1,000	1,516	379	986	500	***	*	Accession
U.R.Tanzania	670,000	215	254		3	RTCP	250	1,000	2,680	670	997	500	***	**	Accession
Zambia	180,000		96	103		ZARI	75	200	2,400	900	200	150	**	*	Ratification
Zimbabwe	46,839		6				6	25	7,807	1,874	25	10		**	Ratification
Sub-total:	12,110,694	2,498	6,398	1,272	2,112		3,743	7,480	3,236	1,619	0 5,368	3,675			
Asia – Oceania															
Cambodia	*96,324*							25		3,853	25	10		**	Acceptance
China	265,800	28	86	4	2	SCATC/UC RI/GAAS	10	15	26,580	17,720	13	20	*	*	
Fiji Islands	2,223	6			6		5	25	445	89	19	20		*	
India	242,400	701	1,507			CTCRI	600	750	404	323	750	200	***	*	Ratification
Indonesia	1,222,814	157	251	130	136	CRIFC/MAR IF	150	1,000	8,152	1,223	864	500	**	**	Acceptance
Malaysia	37,719	55	92	52	61	MARDI	50	100	754	377	39	50		*	Acceptance
Micronesia	1,100							25		44	25	10		*	
Myanmar	16,500	7	21			ARI	7	50	2,357	330	50	20		*	Acceptance

Region/country	Estimates of ex situ accessions based on different sources: Area planted (ha)[a]	IBPGR (1994)	Ng & Ng (2002)	GCDT survey[b] (2008)	In CGIAR centers[c]	Main national programs holding accessions	Consolidated estimates of unique local landrace varieties (excluding duplicates and breeding/experimental material)[d]: Ex situ	In situ	Consolidated estimated landrace density (ha per landrace)[e]: Ex situ	In situ	Estimated in situ accessions missing from CGIAR centers[f]	Proposed minimum ex situ no. of acces-sions[g]	Priority for accession duplication in CGIAR centers[h]	Priority for additional collection[i]	ITPGRFA status
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Papua New Guinea	12,500			95		PNG NARI	75	100	167	125	100	50	**	*	
Philippines	204,578	134	384		6	PRCRTC/IPB	130	500	1,574	409	494	250	***	**	Acceptance
Polynesia	1,327						0	25	53	25	25	10	*	*	
Sri Lanka	23,560	56	128			CARI/PGRC	50	100	471	235	100	50	**	*	
Thailand	1,070,805	8	250	11	5	RFCRC	10	11	107,081	97,346	6	11	*	*	Signature
Vanuatu	281[j]			150		CTRAV-VARTC	120	50[k]	2	6	150	25			
Viet Nam	474,800	20	36	31	9	Hung Loc Agr. Center	20	50	23,740	9,496	41	25	*	*	
Sub-total:	3,690,795	1,172	2,755	473	257		1,132	2,965	3260	1245	2,708	1,170			
WORLD	18,608,324	9,195	14,948	7,685	7,205		10,068	26,986	1848	690	19,954	14,791			

[a] Source: FAOSTAT (except where otherwise indicated); data for 2006, accessed 20 May 2008.

[b] Information provided by survey respondents indicated in Appendix III.

[c] Based on reports from CIAT and IITA, in the 30 April – 2 May 2008 workshop at CIAT.

[d] These are approximations based on region (primary or secondary center of diversity), area planted, ex situ accessions reported, and personal knowledge of the author about diversity in individual countries.

[e] Total area planted to cassava in the country (column 2), divided by estimates of landrace varieties (columns 8 and 9).

[f] Estimates of in situ unique landrace varieties (column 9) minus number of accessions in CGIAR centers (column 6).

[g] Estimated number of accessions that would be required to fully represent a country's cassava genetic diversity. More accurate estimates will be possible as more molecular information becomes available on genetic variation.

[h] *=low; **=intermediate; ***=high; based on current availability in CGIAR centers, risk of loss in national program centers, and importance of diversity for cassava improvement programs.

[I] ***=low; **=intermediate; ***=high; based on expected importance of a country's genetic variability available in situ, the amount of that variability already collected, and the risk of losses of landraces in situ that are not already collected.**

[j] Source: *CTRAV-VARTC.*

[k] There appear to be a large number of landrace varieties lost from farmers' fields and home gardens in the past two decades due to declining cassava production.

2.3 Evolution and farmer selection

The genetic diversity that exists today in cassava germplasm is the consequence of natural selection over millennia, added to more recent farmer selection, and progress from breeding programs. One can only speculate as to the total number of cassava clones cultivated worldwide. All current germplasm collections are sub-samples of the total diversity, albeit some more complete than others. Probably in only a few cases, with the greatest likelihood in some Asian countries, is it likely that all the landraces grown in a country have been collected and conserved in genebanks. In Latin America and the Caribbean, where the crop originated, there are probably on the order of 16,000 clones, based on numbers in existing *ex situ* collections, on reports from collectors and ethnobotanists from field observations, and on more recent molecular information (see Table 3). In Africa, diversification seems to have occurred rather quickly, probably in response to the broad array of growing environments and market uses, but was also enabled by continuing small-scale introductions since the original introductions in the 16th century. Because of the extent of cassava cultivation in Africa, the range of environments in which it is cultivated, and the known diversification through natural intercrossing of landraces in farmers' fields, there has been an explosion of genetically distinct genotypes cultivated in farmers' fields. It is still unclear, however, whether there are any genes in African germplasm which do not exist in Latin American landraces.

The Collaborative Study of Cassava in Africa (COSCA) identified some 1,200 local varieties in 281 villages in countries representing 70 percent of the continent's cassava. There are probably more than 7,000 distinct clones cultivated by farmers in Africa, excluding the minor variations that seem to have appeared through seedling propagation, which were later incorporated into variety complexes (see Table 3). In Asia and Oceania, the number of landraces appears to be much more limited than in Latin America or Africa, perhaps on the order of 3,000 distinct genotypes (see Table 3). Indonesia and India seem to be the main repositories of distinct landraces in Asia. Although India has a larger *ex situ* germplasm collection, many accessions are the product of breeding programs.

2.4 Modern genetic improvement

Involvement by trained plant breeders in cassava improvement began early in the twentieth century. It appears that the earliest programs were established in Brazil, India, Madagascar, Nigeria and Tanzania. Most were directed toward starch industry interest in higher productivity. Some of these continue to modern times, but others were discontinued after World War II. A renewed interest in cassava breeding followed establishment of root and tuber crop programs in two newly developed international centers in the late 1960s and early 1970s – CIAT in Cali, Colombia and IITA in Ibadan, Nigeria. Many national breeding programs were established or strengthened as a result of support from these centers.

Breeding goals across countries, as documented in regional workshops or symposia, appear to be remarkably similar. Nearly all programs include among their goals: high yield, high dry matter or starch, early maturity, tolerance to local pests and diseases, and adaptation to local environmental conditions. The widespread adoption of goals for stress tolerance and pest resistance reflects a recognition that most farmers apply few inputs to alleviate factors causing yield and quality variations. In Africa, yield and resistance to the African cassava mosaic disease have been the primary breeding goals for many years, but after widespread success in developing resistance, goals have broadened to include other traits, such as root quality. In Asia, there are few pest or disease constraints and attention focused on yield and starch content for industrial markets. The Americas present a broad range of major and minor constraints, and goals have been more regionalized and more diverse.

The establishment of the Cassava Biotechnology Network in 1988 was the first step in a long-term strategy to bring the benefits of biotechnology to the most relevant research areas. This has now evolved into the Global Cassava Partnership, which held its first scientific meeting in Ghent, Belgium in July 2008. There are now a number of genetically transformed varieties in preliminary field trials, and it is expected that farm-level benefits from these new technologies will begin to become evident within a few years.

Cassava breeding has been a very successful enterprise, especially considering the relatively low support it has received, in comparison to several other major world crops. While there are no detailed studies on the global impact of the benefits from breeding, it is known to be in the billions of US dollars (Kawano, 2003). Thailand is perhaps the most remarkable success, where nearly all the cassava area is planted to high-yielding improved varieties. Likewise, in the Americas and Africa, there are many examples of successful adoption and impact. But there are continual new challenges and new needs as farming conditions, pest and disease pressures, and the market demands evolve.

Breeders continue to rely heavily on landraces as sources of genes in improvement programs. As goals have evolved more toward industrial uses of cassava (especially in Asia and Latin America), there is new interest in exploring the germplasm base for novel traits such as starch quality, micronutrient content, and rate of postharvest deterioration. Cassava breeders universally appreciate and support comprehensive, well-managed genebanks as an essential part of their work, long into the future.

2.5 Landraces and wild species at risk

Cassava may represent a unique situation compared to the major cereal crop species with regard to the relationship between modern and traditional varieties (landraces). This uniqueness relates to the relatively recent, and low level of, emphasis on cassava genetic improvement, as compared to other major crops. Most cassava production is still based on the planting of landraces, although this is changing quickly, especially in the past decade, and in selected countries like Thailand, Brazil, Colombia and Nigeria. There are no comprehensive global statistics on the adoption of improved varieties of cassava, but this is probably currently on the order of 20-25% of area where local landraces have been replaced by selections out of breeding programs. Twenty-five years ago it was near zero, although there were some notable early successes in extending improved varieties to farmers, such as from the Instituto Agronómico de Campinas in São Paulo, Brazil, one of the world's oldest continuing operating breeding programs (since the 1930s).

The spread of new varieties (and displacement of local landraces) is fastest outside the center of diversity for the species – the Americas. Asia and Africa are seeing the highest rates of varietal replacement. Reasons for this are varied. In the case of Asia, expansive industrial demand, beginning in the 1970s, along with the limited potential of local landraces, drove development of successful breeding programs and replacement of landraces by new hybrids. In Thailand, Asia's largest producer, nearly all the current cultivated area is planted to the products of breeding programs (Kawano, 2003). In Africa, the change from landraces to improved varieties was, and continues to be, driven by the devastations brought by diseases (especially cassava mosaic disease, and more recently cassava brown streak disease), and the better resistance and yield potential offered by new varieties. Replacement of landraces still appears to be low in Africa, but can become serious with progressive success of breeding and extension programs. One antidote to such genetic erosion, of course, is to have secure *ex situ* collections established. There is time, but perhaps not much, to assure the conservation of the world's remaining cassava landraces that are not yet in *ex situ* collections. This is a matter that merits close observation, documentation and action. It is among the more important criteria for prioritizing further collecting.

Landraces continue to be used extensively in breeding programs (i.e., used directly as parents in crossing nurseries), in contrast to several others of the major crop species, in which most breeding is done among advanced breeding material, and landraces play little direct role. This means that cassava breeders in general have a very strong and direct interest in the conservation and availability of landraces on an ongoing basis.

As with the related species of many other crops, the main risks of genetic erosion for wild *Manihot* are the consequence of the expansion of human activity into native habitats, mainly in the form of the expansion of agriculture and urbanization. It appears that for *Manihot*, the former is by far the more pronounced. This relates to the nature of the distribution of the wild species – the largest center of diversity is in central Brazil, in the *cerrado* (savanna) region, where agriculture has expanded rapidly since the 1970s, as technology became available to profitably produce crops, especially soybeans, sugarcane and citrus. For example, of 41 habitats identified and

surveyed in the late 1970s, only one locality remained for wild *Manihot* twenty-five years later (Nassar, 2006). Habitat change is also leading to loss of wild *Manihot* in Mexico.

3 Cassava-related networks

The formation of collaborative mechanisms that bring together a number of institutions working towards a common goal, and with similar or complementary objectives, has proven to be a cost-effective means of enhancing the efficiency of research and development. Best and Henry (1994) noted four levels of cassava and cassava-related networks, summarized and updated in Table 4.

Table 4. Cassava and related networks.

Global	Cassava R&D workers Cassava Biotechnology Network Cassava Genetic Resources Network International Society for Tropical Root Crops (ISTRC) Global Cassava Partnership MOLCAS
Regional	Asian Cassava Research Network Panamerican Cassava Breeders' Network Latin American Integrated Projects Network Collaborators in Root and Tuber Improvement and Systems (CORTIS) African Francophone Cassava Network (CORAF) African Branch of the ISTRC CLAYUCA
Subregional	Eastern and Southern African Root Crop Research Network Southern Cone Cassava Development Network
National	Country Root and Tuber Crop Societies

Source: Modified from Best and Henry, 1994.

International networks for cassava generally appear to be much appreciated by the participants, in view of experiences in participation and feedback. One of the key elements of success is clearly adequate and stable funding. Although, ideally, networks are funded by the participants who benefit, most cassava-related networks have required continuing external funding due to the generally low level of resources available to cassava R&D institutions. In fact, few of the crop networks have realized stable funding, and therefore their effectiveness has been variable over time. Some of the longer-term networks have been able to get support from a series of different donors in order to remain viable. Others have evolved into new networks as priorities and funding sources changed. For example, the Cassava Biotechnology Network evolved into the broader-based Global Cassava Partnership in 2004, in response to participant and donor interest in better integrating biotechnology with a broader range of production, processing and utilization activities.

Some of these networks help facilitate the conservation of cassava genetic resources; nearly all of them benefit from it. The only network specific to genetic resources is the Cassava Genetic Resources Network, described earlier (Section 1.3), and no longer active as an organized entity. Nonetheless, many of the networks rely on and link to genetic resources activities. One of the key activities of CLAYUCA, for example, is to promote and facilitate the

interchange of germplasm among participating countries. The Asian Research Network has, since its beginning as the Asian Cassava Breeders' Network, had a strong emphasis on germplasm, through facilitating exchange of selected materials among participating countries, and through duplication of national genebank accessions at CIAT. Perhaps the future of networking for cassava genetic resources conservation could usefully be left to these more user-focused groups.

4 Cassava in the regional crop strategies

The Trust commissioned the regional PGRFA networks around the world to develop regional strategies for conservation and use of PGRFA. Each of the following regional reports[4] listed cassava as a high-priority crop for conservation, though based on a somewhat different approach to defining priorities: the Americas; Eastern Africa; West and Central Africa; SADC region of Africa; the Pacific; and South, Southeast and East Asia (reports available at http://www.croptrust.org/main/regional.php?itemid=83).

4.1 Americas

During several working sessions, the network coordinators and other stakeholders defined several levels of criteria to prioritize crops and collections: criteria based on the principles of theTrust; criteria used to define important crops; criteria for assessment of the importance of the collections; and criteria for assessment of the quality of collections. From these criteria they developed a weighted scoring system, described in the report as "far from a solid scientific process, but nonetheless provides a useful entry point for prioritization and a reference milestone for future discussions." In this assessment, the ten top priority genera/species, in order of weighted scores, are: *Phaseolus*, *Capsicum*, *Zea*, *Manihot esculenta*, *Arachis*, *Glycine*, *Lycopersicon*, *Theobroma*, *Musa* and *Citrus*.

Table 5. Important collections in the Americas.

Country	Network	Institution
Bolivia	TROPIGEN/REDARFIT	El Vallecito
Several	CAPGERNet	Several
Brazil	TROPIGEN	EMBRAPA
Brazil	TROPIGEN	IAC
Brazil	TROPIGEN	IPAGRO
Colombia	REDARFIT/TROPIGEN	CORPOICA-CIAT
Peru	REDARFIT/TROPIGEN	INIEA
Paraguay	REGENSUR	DIA
Venezuela	REDARFIT/TROPIGEN	INIA
Venezuela	TROPIGEN	UCV-FAGRO

4.2 Eastern Africa

The EAPGREN Regional Steering Committee ranked crops based on: threats of genetic erosion; center of diversity; uniqueness; food security; status of PGR collections – health; status of characterization; conservation facilities – long term; safety duplication; and regeneration needs. Cassava ranked 7th, just behind banana (#5) and sweet potato (#6), and just ahead of rice (#8) and yam (#9). It is listed as important in Burundi, Kenya, Madagascar, Rwanda, Sudan and Uganda. It is noteworthy that several of the vegetatively propagated crops are grouped together among the top

[4] At this writing, the West Africa Report is brief and does not list priority crops. However, the overwhelming importance of cassava in this region makes it evident that the crop should be near the top in terms of priority for conservation of genetic resources.

priority crops. In this region, the collection of most importance was noted as that of NARO, Uganda (300 accessions; 50% of accessions with passport data).

4.3 SADC Region

The Southern African Development Community (SADC) comprises 14 member countries. Most are important cassava producers. The SADC Plant Genetic Resources network has a broad mandate to maintain genetic resources of the region. The mandate list has over 3,000 species, out of which 27 are included in the Annex 1 of the ITPGRFA. Cassava is among the twelve Annex 1 crops in the SADC region considered important at the regional level. The report lists several countries with cassava collections: Malawi, South Africa, Swaziland, and Zambia.

The SADC report lists a number of priority areas for upgrading and capacity building. Most important among these in the case of cassava are:

1. Support to field genebanks for vegetatively propagated crops such as sweet potato, cassava, banana, potato, yams, and aroids, as well as coconut.
2. Support to *in vitro* base collection of the priority field crops mentioned above.

4.4 South and Southeast Asia

The National PGR Coordinators defined criteria for crops of greatest importance to the agriculture of the SSEEA region, or to a few countries in the region, as follows:

- Center of diversity (primary or secondary)
- Level of sub-regional, regional and global importance as food and nutritional crop (including feeds and fodder for animals)
- Presence of regional and /or international collections
- Usefulness as crops for marginal areas and subsistence agriculture
- Livelihood security for smallholders
- Threat to the genetic diversity *in situ*/on-farm
- Crop with unique advantage to the sub-region or region

Table 6. Examples of priority crops and ranking assigned by individual sub-regional network.

	SANPGR	RECSEA-PGR	EA-PGR
Rice	1	1	2
Wheat	3		1
Maize	2		3
Potato	15		10
Sweet potato	19	3	
Cassava	18	6	

Source: SSEEA Regional Report, Table 5.

The SSEEA report provides an integrated ranking among the sub-regions, where cassava is ranked as 13th in terms of priority crops for support. Countries noted as requiring this support are Indonesia, Malaysia, the Philippines. The information was further discussed to define collections of greatest importance and priority for support based on the following criteria (Section 13 of SSEEA report):

- Collections in the public domain
- Distinct collections (landraces, wild relatives)
- Collections with no safety duplication
- Collections under threat
- Collections with specific traits and from specific ecologies
- Collections that meet all the eligibility criteria

- Collections with sufficient eco-geographical representation/Size of the collections
- Collections from institutions where regional/international collaborations are on-going

Based on these criteria, collections from the following countries were recognized as important: India, Indonesia, Malaysia, the Philippines and Sri Lanka.

4.5 Pacific Islands

Cassava is widely distributed throughout the Pacific Islands, but overall is not a major crop. The entire region plants only some 15,000 hectares (2006 data, FAOSTAT). However, it is listed as a priority 2 crop in the regional report, coming in behind coconut, sweet potato, banana/plantain, aroids, yam and breadfruit, which are ranked as first priority. Cassava is most important in the Cook Islands, Vanuatu and Papua New Guinea. Its importance is related to diversity (some evidence of unique material) and to income generation (an important cash crop in some places, and a developing export market in a few). Germplasm conservation and use activities in the Pacific are coordinated by PAPGREN – the Pacific Agricultural PGR Network. Field cassava collections are noted for: Cook Islands, Federated States of Micronesia, Fiji, New Caledonia, Palau, Papua New Guinea and Vanuatu. In addition, there is a regional collection of 28 accessions maintained *in vitro* at SPC-RGS, Suva, Fiji.

4.6 Summary from the regional reports

The working group for the Americas used a useful approach to regional analysis – a SWOT analysis to help describe the region in terms of PGRFA. The following is adapted from that analysis, applied strictly to cassava, and globally.

STRENGTHS

- Large number of genebanks with ample diversity of landraces.
- Major IARCs to partner with: CIAT (headquarters in Latin America and regional office for Asia in Bangkok) and Bioversity International (various regional offices), IITA in Nigeria.
- Some large well-established PGRFA programs in conservation and sustainable utilization, e.g. Brazil and India.
- Regional networks on each continent that support PGRFA conservation.

WEAKENESSES

- Communication difficulties – issues related to language, infrastructure, documentation systems, geographical access, and logistics.
- Vast geographical areas from the tropic of Cancer to the tropic of Capricorn, and including the range from very wet to very dry, lowland to highland regions, and wide soil variations, among others.
- Limited knowledge and public awareness of genetic diversity issues.
- Differences in policy approaches and the involvement in, or ratification of, international agreements and instruments (ITPGRA, CBD, etc.).
- Political stability and related issues.
- Not all countries are parties to the ITPGRFA and thus may not be eligible for funding without agreeing to a “Solemn Undertaking”.
- Limited human resources.
- Inadequate germplasm enhancement efforts.

OPPORTUNITIES

- Large number of partnerships possible – bilateral and multilateral.
- Well established sub-regional networks, but with varying degree of operation and coordination.
- Creative skills of network members in solving problems.

- Considerable potential for new uses – for direct and indirect use in markets, communities, farmers' groups, etc.
- Commitment to common goals, particularly as they relate to the goals of the GPA and the Global Crop Diversity Trust.

THREATS

- Shortage of financial resources – unsustainability.
- Range in development of technical expertise – variable levels between countries and sub-regions.
- Range of infrastructure development – many of which require substantial input and/or investment.
- Environmental variables, e.g. hurricanes, flooding, extreme heat, low temperatures, extreme wet and dry conditions.
- Rapid genetic erosion, especially in areas of agricultural development and urbanization.
- Safety duplication is not complete.

The regional reports clearly indicate the high importance of cassava among priority crops for support for conservation in the Americas, Asia and Africa. The remainder of this report highlights general and specific goals and strategies to accomplish secure global conservation.

5 Overview of cassava collection and conservation

5.1 Collecting strategies, techniques and priorities

We now know that new variation is continually arising through incorporation (intentional or not) of seed-derived volunteer plants in cassava plantations. Each of these is a new genotype. A variety may actually in some cases be a mixture of similar genotypes rather than a clone. It would be very difficult and expensive to collect and conserve a sample of every existing genetic variant of cassava or of the wild *Manihot* species. What to conserve and how to identify and collect these materials are two of the most fundamental questions in developing a conservation strategy.

The techniques and the process of cassava collecting, like those of most crops, have advanced considerably in the past 25 years. Most early collecting was done simply by visiting villages in known cassava-growing regions and taking a few stakes from each of what appeared to be distinct varieties, based either on morphological differences in appearance or information provided by the grower. Usually, the collector recorded the date, the name of the village, the name of the grower, and the local name of the variety. This is the extent of passport information for the large majority of genebank accessions around the world. A brief history is given here of the early establishment of CIAT's cassava genebank, as a baseline for understanding how many collections began and evolved.

CIAT's first annual report (1969) stated among the goals of its newly established root and tubers program, "to explore and collect cultivars and related wild species of *Manihot* in the countries where variability is present, with emphasis in the primary centers of origin (Northern South America and Middle America), in order to establish a germplasm bank representative of the world's variability." (p. 43). In fact, as noted earlier, it is now widely believed that cassava's greatest diversity is in Brazil. In any case, it was certainly understood at the time that there was major variability in Brazil, but importation of cuttings into Colombia was prohibited due to concern about introducing coffee rust (Nestel and Cock, 1976). Nonetheless, based on this goal, CIAT began the systematic collecting of cassava landraces in Colombia, in May of 1969, in collaboration with the Secretaria de Desarrollo y Fomento del Valle. This organization appointed Mr. Victor Manuel Patiño, director of the Cauca Valley Botanical Garden, to work with CIAT in the collecting. In this initial phase of collecting, a total of 611 accessions from 20 departments were assembled and established on the CIAT farm near Palmira, Valle. The following year, the collection was extended to other countries, and by the end of 1970, the genebank consisted of the following accessions:

Colombia	1,884

Ecuador	123
Puerto Rico	60
Panama	118
Peru	8
Venezuela	330
Mexico	70

It was already recognized at the outset that phytosanitary care would be critical for germplasm management, and CIAT initiated a cooperative project with the plant pathologists of ICA (CIAT, 1969). 1971 brought an outbreak of cassava bacterial blight (not yet identified), and in order to prevent the spread of the disease to commercial and experimental plantings, there was an intense eradication of material from the genebank, with attempts to recover clean planting material through cutting plants back to just the woody stems, where there appeared to be less probability of bacterial inoculant (CIAT, 1971). By 1972, it was possible to eradicate the bacterial blight pathogen from the field collection, but there had already been significant losses from the collection. Most of these losses occurred in the earliest years as the techniques for management of pathogens were being developed. Nonetheless, there were continuing losses over the years, especially among accessions that originated from environments that were very different from those of the CIAT station where the genebank had been established in the field.

CIAT's breeding program relied very heavily on this initial set of introductions, through its first decade. Thereafter, when *in vitro* transfer techniques became available, the CIAT genebank steadily increased its accessions and broadened the genetic base of materials used in breeding. Most notably among the newer introductions was a sizable introduction from Brazil. By the mid-1980s to early 1990s, the collection had broadened to include modest numbers of accessions from Asia and Africa, though the majority of these were bred materials rather than landraces.

CIAT initiated an *in vitro* genebank in 1978, soon after the technology for slow growth *in vitro* culturing of cassava was developed at the University of Saskatoon, in Saskatchewan, Canada by Kartha and Gamborg (1975). Since 1998, CIAT's Genetic Resources Unit has further extended the subculturing to 12 to 20 or more months. Clones subcultured under this system have been found to be genetically stable (D. Debouck, pers. comm.). However, it was not until the early 2000s that the decision was taken to eliminate the field collection, due to a combination of factors. These included cutbacks in research budgets at CIAT, increasing difficulties of keeping pest and disease pressures under control (especially whiteflies and frogskin disease), the increasing security of the *in vitro* techniques and the completion of the *in vitro* collection, and finally, the establishment of a black box duplication arrangement with CIP in Peru. In addition, by the late 1990s, the cassava breeding project had finished with the routine morphoagronomic characterization of the collection. It appeared at that time that there might be less urgency to evaluate the collection for new traits. However, this philosophy changed somewhat as it became apparent that the cassava world was moving toward the development of new markets, and there were new needs to evaluate the germplasm, especially for variations in starch quality.

In 1982 an IBPGR-sponsored working group proposed a collecting format that greatly expanded the information to be recorded in the field, to include a broad array of information from the grower about the traits and performance of individual varieties (Gulick et al., 1983). These forms, or something similar, have been used in many subsequent collecting expeditions. Nonetheless, probably fewer than 1000 accessions worldwide have this broader information. While the expanded passport information would be useful, it does not seem to be justified to re-collect extensively in areas already collected over the past decades, in order to obtain this information. The complexity and cost of integrating recollected materials into existing genebanks would be very high.

Collecting additional cassava landraces is a critical part of a long-term conservation strategy. Priorities should be based primarily on: (1) genetic diversity; (2) gaps, and (3) areas in danger of genetic erosion. Globally, it is estimated that some 5,000 more landrace varieties should be collected in order to fully represent the species genetic diversity (Table 3). Priority countries, based on the above criteria, are: Bolivia, Brazil, Colombia, Haiti, Nicaragua, Peru, Venezuela, Democratic Republic of Congo, Uganda, Tanzania and Mozambique. Collecting in these countries should

make full use of the lessons from past collecting in terms of information to collect, as well as the available databases and technologies such as GIS, on-site *in vitro* preparations, and on-site virus indexing.

There is now good evidence that the incorporation of new genetic variability through volunteer seedlings may be either a conscious or an unconscious practice, depending upon the level of knowledge of farmers and their traditions (McKey, 2008; Reichel-Dolmatoff, 1996). It is thus clear that cassava will continue to evolve in farmers' fields, which would indicate the need to plan for continuing future collections. However, the rate of evolution slows with advancing agricultural production technology. The opportunities for mutant types to be selected and propagated by farmers is minimal in situations where known, modern varieties are cultivated in monoclonal situations, and the selection and propagation of seedlings (seed-derived plants) does not consciously occur. Nonetheless, evolutionary forces will continue to produce new genetic variability in the species, in some locations, as they have done for thousands of years. Therefore, the secure conservation of currently existing landrace varieties would reduce the need for future collections to very sporadic and narrowly targeted expeditions. Completing collecting goals in the next decade would reduce to very low levels the need for future investments in this area. At the same time, it would not eliminate the need to continue to monitor on-farm change in varieties, and the positive or negative effects of changes in genetic diversity.

Elite breeding lines may or may not become successful varieties. Those that do not reach farmers' fields may be lost unless specific steps are taken to preserve them. If they are not successful, there may be little reason to save them in genebanks, unless they are known to have specific important genetic value, and/or are difficult to obtain (e.g. interspecific crosses). For those that are successful, there is a need for systematic preservation so that a permanent, pure representation of the variety exists – the equivalent of breeder's seed in a seed-propagated crop. This conservation may be the responsibility solely of a breeding program, or may be managed jointly by breeding and genetic resources efforts. These stocks can be the basis of a seed program, for clarifying any possible future problems related to varietal contamination and as a source for distribution to other gene banks or breeding programs. The definition of "elite" is the key to a sensible conservation strategy. Only a very limited number of materials can be assigned elite status, or the costs involved in conservation quickly get out of hand. At CIAT, for example, a clone becomes elite only after passing through all preliminary stages of selection, and multi-site selection in advanced yield trials for at least two years. On average, 10 to 15 clones a year enter the elite category. Even this relatively small number can eventually become burdensome for conservation, and this group may be given a lower management intensity (e.g., fewer replicates). Many countries include elite breeding material or experimental lines in their genebanks, but the criteria for inclusion are not well-documented.

Genetic stocks can be temporary or permanent parts of a collection, depending upon objectives. CIAT (1994) reported on incorporating a mapping population into the germplasm collection as a way to ensure that it would be widely available to participants in the Cassava Biotechnology Network. Stocks for a specific, limited study may not need to be preserved at all.

For small collections, all accessions can normally be treated with an equally high priority for conservation. In larger collections, one may gain efficiencies by assigning levels of importance to different groups and managing their conservation distinctly. Local landraces are nearly always the top priority. Their conservation must be secured. This may be by two separate field locations, duplicate *in vitro* collections, or a field and an *in vitro* collection, for example. If a core collection (see later section) has been defined, this may get the highest of all priorities. Breeding lines and introductions, especially if retained in a collection in the country of origin, may be given a lower status for conservation.

5.2 Conservation alternatives for cassava

The gene combinations found in cassava landraces are normally the result of many decades or even centuries of selection by farmers. Since cassava is highly heterozygous, the only means of conserving the specific gene combinations of landrace varieties is through vegetative propagation. For some purposes it is necessary to maintain

these specific gene combinations. If the goal is to select germplasm accessions to recommend directly to farmers, vegetative conservation is a necessity. For species or clones that rarely or never flower, there may be little alternative to vegetative conservation, until such time as flower induction is possible. Currently, every cassava genebank in the world conserves accessions in vegetative form. The international collections maintained as clonal material by CIAT and IITA are registered into the Multilateral System of the International Treaty.

Alternatively, if the interest is conservation of genes rather than genotypes, germplasm may be maintained as true seed. Germplasm maintained in seed form would ultimately be useful principally as a source of genes in a breeding programme and not directly as a source of varieties. The exception could be if there were means of inducing apomixis, which would duplicate the exact genetic structure of the parent clone, and produce the same result as vegetative propagation.

5.2.1 Field

Cassava collections have traditionally been maintained in field plots. Stem pieces are used as the propagules just as in commercial production. Theoretically, such a collection could be maintained for many years without regeneration. In practice, maintenance problems often increase after a year or two, making replanting at more frequent intervals necessary. Common problems include lodging from excessive growth and build-up of pests and diseases. Adaptation problems typically occur when the edapho-climatic characteristics of the genebank location are very different from the collection site, where the variety is presumably adapted well enough to be selected and propagated year after year by the growers.

In general, field-managed material is not available for international shipment, which is a major limitation. Major advantages of field maintenance of collections are their technical simplicity and the availability of planting material for evaluations, breeding nurseries, or other uses.

The following general recommendations apply to field conservation:

(1) The area where materials are maintained should be as free as possible of diseases and insect pests that could cause losses of clonal material or create difficulties in the transfer of clean planting material to other sites.

(2) A minimum of three to five plants is necessary for practical maintenance. If a plantation is also to be used as a source of production of stakes for planting of other trials, more plants may be required.

(3) Cassava can be maintained in field plantings as a perennial plant, but periodic renewal every one or two years is desirable to avoid problems of excessive vegetative growth, cumulative disease and insect problems and to facilitate maintenance generally.

(4) The distance between plots of different clones should be adequate to prevent undue competition among the plots.

(5) Accurate plot and accession labeling are crucial for long-term reliability of information on cassava genebanks. Because plants may remain in the field two or more years, durable labels are important.

In order to combine the benefits of lower space requirements with continual availability of planting material for experimental use, CIAT devised a slow-growth system based on restricting root development in small planting pots (*bonsai* effect). Plants occupy only a small fraction of the space they would occupy if allowed unlimited growth in the field.

Maintaining a cassava germplasm collection in containers has the potential advantages of space savings; better protection against pests, diseases and weather-related damage; and labor savings. Disadvantages can include difficulty in using plants as a source of planting material for field trials (generally small and weak stems), cost of infrastructure, and cost of materials.

5.2.2 *In vitro*

In the mid-1970s, the University of Saskatoon (Canada) (Kartha and Gamborg, 1975) and CIAT developed techniques for routine *in vitro* conservation of rooted plantlets of cassava. These plantlets can be derived in a number of ways, but for phytosanitary reasons the recommended source is small meristem tips. These can easily be surface-sterilized against superficial organisms, and many systemic pathogens do not advance into the new tissue of a rapidly growing meristem. Extra precautions of chemo- or thermotherapy can also lower chances of contamination. Meristem tips are cultured in nutrient media in glass or plastic jars or test tubes, and maintained under controlled light and temperature conditions. Under minimum growth conditions, cultures can be maintained 12–18 months before renewal. Renewal can be by planting stem pieces or meristem tips from the *in vitro* plantlet into new sterile media, without the need for a field propagation phase. Recent experiments with silver nitrate in the media show promise of further extending the regeneration period for *in vitro* plantlets (Mafla, 2008). CIAT's facilities have the capacity to hold more than 6,000 accessions *in vitro* at 20°C (day)/15°C (night) temperatures, 12-hour photoperiod and 500 to 1,000 lux illumination.

CIAT monitored genetic stability of *in vitro* cultures using a combination of morphological and biochemical traits, and DNA markers. All results have so far been negative, indicating a high level of genetic stability after as many as 15 years of *in vitro* conservation and regeneration (CIAT, 1994).

5.2.3 Cryopreservation

Liquid nitrogen storage of vegetative tissue tips should be the most secure and trouble-free system for conservation of clonal cassava germplasm. The major advantage is the virtual freedom from maintenance problems during storage, with the possible exception of low rates of mutation caused by background ionizing radiation. Conservation could theoretically be carried out indefinitely with no need for renewal. Development of successful cryopreservation techniques has been somewhat slow and sporadic, due to limited funding, as well as what appears to be considerable variation among genotypes in the recovery response. Various laboratories developed basic cryopreservation techniques for meristem tips in the 1980s, using chemical dehydration and programmed freezing in liquid nitrogen. With later developments in encapsulation and quick freezing, more than 80% of accessions tested at CIAT (mainly from the core collection), have recovery rates of greater than 30%. The minimum acceptable level for a long-term conservation strategy is still a matter of some debate. CIAT's Genetic Resources Unit believes this level should be at least 50% (D. Debouck, pers. comm.). Preliminary observations have shown no noticeable changes in plant characters after cryopreservation. However, a cryopreservation strategy would need to include periodic monitoring of stability, using precise molecular measures.

Somatic embryos already represent an efficient regeneration system for rapid propagation, and are a target for transformation. They have the potential to serve as the basis for germplasm conservation as well, especially if they can be adapted to and recovered from cryopreservation. Mycock *et al.* (1995) and Stewart *et al.* (2001) successfully cryopreserved somatic embryos, with a 40–60% post-thaw viability. Danso and Ford-Lloyd (2004) introduced new cryoprotection and dehydration techniques and obtained 95% post-thaw viability (albeit, with a limited range of genotypes). Rate of plant recovery from the cryopreserved embryos was comparable to that of non-preserved ones. The optimal protocol involved induction of embryogenic calli on an induction medium (Murashige and Skoog medium supplemented with 2,4-D and sucrose), cryoprotection on 0.3 M sucrose for 21 days, followed by 16 h of dehydration and immersion in liquid nitrogen. Although plants recovered from somatic embryos appeared to be genetically stable, this needs to be further tested and monitored. Current evidence suggests that sucrose cryoprotection followed by air desiccation provides a viable solution for long-term conservation of cassava genetic resources via cryopreservation. Cryopreservation research for cassava should receive continuing and increased support, given its potential to contribute to long-term conservation for additional security.

Cryopreservation is certainly a technology that is amenable to continued improvement, but the rate of progress in the past 20 years has been disappointing. This is in part because of sporadic funding and in part because there just seems to be a set of germplasm (cassava) that is stubbornly recalcitrant. The good news is that there seems to be a

new optimism about overcoming some of the hurdles in dealing with the recalcitrant types. The other silver lining is that this is one more indication of the fantastic genetic variation that exists in the cassava landraces.

There is little doubt that a protocol will eventually be developed whereby it will be possible to recover all, or nearly all, accessions. So there are two components that are relevant to the cassava conservation strategy: first, the continuing research to fine-tune the technology, and secondly, determining how it fits into an overall conservation strategy. With about 75% of accessions estimated to have an acceptable recovery rate of over 30% (Gonzalez-Arnao et al., 2007), it seems we should be getting close to the point of beginning to incorporate cryopreservation into a conservation scheme. However, taking this next step will be rather expensive. It would involve screening the entire collection of landrace varieties for their recoverability under the established protocol. Given that there seems to be optimism about the near-future potential for good progress in improving recoverability of the recalcitrant accessions (Gonzalez-Arnao et al., 2007 and Escobar, April/May CIAT workshop presentation), it is probably wise to still wait some time before the mass screening of the collection. Perhaps once the recovery rate of the core collection reaches about 95%, then it would be an appropriate time to begin the mass screening to determine the recovery rate of each clone. The set of clones with acceptable recovery rates could then become a secure backup to the collection. At this point, this group might replace the current black box *in vitro* collections at CIP (CIAT collection) and at Cotonou (IITA collection). Or they could also easily be moved to another institution as well. It is important that the *in vitro* base collections and the cryo collections not be held in the same location, if they are to play their full role in secure conservation. Those +/- 5% of clones that are still classified as recalcitrant would have to continue in some scheme of secure backup such as an *in vitro* collection, while research on the freeze/recovery protocol continues. A time-frame for all of this is of course difficult to predict, however, it seems that we should be looking at this type of system to be in place within seven to eight years.

5.2.4 Seed

Most seed storage is done by breeders in their work of crossing and genetic improvement. Seed storage as a method for germplasm conservation in cassava has received limited attention. Varieties have been selected and propagated vegetatively to preserve specific gene combinations. After self- or cross-pollination, these genes are re-assorted into new combinations. Seed conservation can be a means of preserving genes, but not the specific combinations of the parent clone(s).

Cassava seeds are apparently orthodox in behavior and therefore can be stored under conventional conditions of low humidity and low temperatures (Ellis *et al*., 1981). IITA (1979) reported storing seeds at 5°C and 60% relative humidity for up to seven years with no loss in germination ability. Seed can also be preserved in liquid nitrogen and recovered with high viability (Mumford and Grout, 1978; Marin *et al*., 1990). *Manihot* wild species have not been systematically tested for seed storage behavior, a critical need as collections proceed, along with regeneration of seed from field-grown plants.

Although the mechanics of seed storage appear to be straightforward, further studies are needed to define appropriate methodologies from the standpoint of germplasm conservation theory. Various approaches are possible, including uncontrolled open pollination, selfing, or pollination among selected accessions. In each case, the numbers of seeds needed for a defined level of probability of conserving an adequate gene pool (preventing genetic drift) need to be defined.

The simplest method would be open pollination. This would perhaps be an appropriate means of conserving a broad pool of genes in the case of some catastrophe that resulted in loss of clonal accessions. From the point of view of preserving the genetic integrity of an accession, the best approach would appear to be selfing, which retains most of the genes of the accession of interest without intermixing the genes of other accessions. In fact, from the plant breeder's point of view, seed from selfed genebank accessions may be considerably more valuable than the accessions themselves as parents. Selfing should eliminate some level of deleterious genes, although the detailed studies have not yet been done to quantify these effects. Because of the partial homozygocity of S_1 plants (50%, on average), there is a higher breeding value, i.e. greater likelihood that expressed traits (the phenotype) can be

transferred to progeny, which of course is to great advantage of the efficiency of a breeding program. Pollination among selected accessions could be very complicated to design appropriately, such that flowering among selected materials is synchronized, and that the particular crosses chosen are those that are most appropriate in terms of genetic resources conservation.

For any pollination system designed for germplasm conservation, there would be a very large advantage to having the ability to induce flowering -- either to produce flowers in clones that normally do not flower, or to regulate the timing of flowering. The limited research in the area of flower induction has had only moderate success.

The long-term advantages of seed conservation warrant further work in all these areas. Initially seed production and storage would be an additional cost, in combination with some form of vegetative conservation such as *in vitro* slow growth or cryopreservation. However, eventually it should be possible to partially replace some of the vegetatively-maintained accessions with seed collections, for a net cost savings.

Conservation of the wild *Manihot* species as seed requires some different approaches. Since all the wild species seem to be seed-propagated in the wild, seed regeneration *ex situ* can be based on natural systems of cross pollination within heterogeneous populations. However, studies on pollination agents and isolation distances are practically non-existent for *Manihot*. Systematic seed propagation and conservation of the *Manihot* wild species will require a number of studies on pollination behavior and seed response to different storage regimes.

5.2.5 Pollen

Cryopreserved pollen should be a good system of preserving populations of genes. Like selfing, this system would allow sampling the genes within each accession, without introducing genes from others. From a breeder's perspective, pollen has the advantage that it can be used almost immediately, as opposed to seed or *in vitro* conservation, both of which require a cycle of regeneration, to the stage of producing flowers.

One of the limitations to research on cassava pollen conservation remains the difficulty of viability testing. Neither staining nor *in vitro* germination is adequately reliable as an indicator. Protocols for efficient, large-scale and rapid viability testing will be a necessary prerequisite to effective pollen conservation. Leyton (1993) resorted to *in vivo* pollination as a means of testing viability in a series of experiments on pollen cryopreservation. He obtained no seeds from any sub-zero pollen treatment (-4°, -12° or -70°C). Orrego and Hershey (1984) were unsuccessful in storing viable pollen after desiccation over silica gel.

5.3 Conservation costs

Design of a conservation strategy needs to consider, first, the best technical approach to safely preserve the genes and genotypes of the collected materials, and secondly, the cost-effectiveness of that approach. Advantages of *in vitro* conservation are the low space requirements and minimal possibility of loss of materials through diseases, pests, weather or soil factors. Disadvantages are the need for relatively sophisticated facilities for culturing sterile plantlets, and for maintaining reliable conservation conditions. Costs of field versus *in vitro* conservation are highly location-specific, depending upon local costs of labor, energy, supplies and infrastructure. Economies of scale are also a factor. For most small national collections, *in vitro* conservation may not be justified, unless the laboratory forms part of a conservation facility serving other crops as well. Cryoconservation, at this stage of its development, is probably only appropriate for more advanced programs that are able to carry out the continuing research necessary for fine-tuning the technology. As procedures become more routine, cryoconservation should be more broadly applicable, especially to medium and large national programs.

Conservation of vegetatively propagated species has always been laborious and costly relative to seed conservation. *Koo et al. (2004)* carried out a comprehensive study comparing costs of maintaining field, *in vitro* and cryoconserved collections at CIAT (Table 7). Total costs per accession for conservation alone (excluding distribution) were comparable for *in vitro* and field (US$10.34 and US$7.18, respectively), while cryoconservation with regeneration is

much more expensive, at US$40.31. The advantages of cryoconservation enter the picture as regeneration time is extended, since maintenance costs alone are very low. Costs, security and convenience will dictate different strategies in different situations.

Table 7. Comparison of key costs (US$ per accession, 2000 basis) for conserving and distributing a cassava accession for one year in CIAT's genebank.

	In vitro		Cryoconservation		
Cost category	Existing accessions	New accessions	Without regeneration	With regeneration	Field genebank
Conservation					
Storage[a]	1.28	1.28	0.86	0.86	7.18
Subculturing[b]	9.06	9.06			
Viability testing				7.96	
Regeneration (cryo.)				31.49	
Disease indexation		57.27			
Conservation total cost	10.34	67.61	0.86	40.31	7.18
Distribution					
Storage	1.28	1.28			
Subculturing	9.06	9.06			
Dissemination	12.54	12.54			
Distribution total cost	22.88	22.88			

Source: Koo et al., 2004.
[a] Storage costs for the field genebank are the same as the cost of field maintenance.
[b] Storage and subculturing costs for in vitro are allocated equally between conservation and distribution.

6 Overview of wild *Manihot* species collection and conservation

Many wild *Manihot* species are notoriously difficult to maintain either as field collections outside their natural habitat or as *in vitro* plantlets. Seed conservation remains the preferred system. Since all the wild species appear to be seed propagated in nature, populations are assumed to be highly heterogeneous (as opposed to cassava, which normally consists of single clones or clonal mixtures in production fields). There has, however, been inadequate attention given to population biology theory in order to collect, and later conserve, wild species populations by methods that optimally conserve an adequate sample of the available genes. It is certainly to be expected that the genetic variation within and among populations of plants will be very high, and sampling for *ex situ* conservation should have a good theoretical and practical knowledge base at hand to select plants and seeds for collection in the wild, and regeneration *ex situ*.

Low seed production of some species often limits the ability of the genebank curator to optimize a sampling system during regeneration. For example, EMBRAPA collected seeds from wild species across the four locations where field genebanks are established in Brazil. Number of seeds collected varied from 11 for *M. irwinii* to 16,503 for *M. peruviana* (Alves, 2008). Genetic drift is likely to be a major issue in wild *Manihot* species genebanks, although no specific studies have been carried out in this area. With species that produce few seeds, continual field maintenance may be the most efficient means of combining conservation and regeneration.

Response to different treatments to improve seed germination varies among species (CIAT, 1993). *M. quinquipartita* responded to heat treatment and pre-germination at alternating temperatures. Several species benefit from embryo rescue, but others do not. Microwave treatment and mechanical scarification were detrimental to most species. CIAT recommended using a sample for germination by direct seeding, and holding some seeds for reserve in case alternative methods are needed.

Work on *in vitro* culture shows that species vary widely in their media requirements for optimum conservation and regeneration (CIAT, 1984). Research at CIAT (CIAT, 1993) on methods to improve vegetative establishment of wild species compared leaf buds, shoots from rooted stakes, air layering, shoots from source plant, kinetin treatment and Hormonagro® treatment. Air layering was the most broadly successful method across species, but still resulted in a low rate of multiplication.

Several species have been recovered successfully from cryopreservation, including *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *peruviana* and *M. carthaginensis*. If most or all of the wild *Manihot* species have orthodox seeds (which is the indication so far from preliminary experience), there may be little justification to develop alternatives to seed preservation in low temperature/low humidity conditions. Field growth is useful as a means of having material for study, and for regeneration, but in the longer term, just as for cassava, will probably not be a recommendable system for genetic resources conservation.

7 Overview of current cassava genebanks

This overview of *ex situ* collections is based mainly on a survey supported by the Trust during the first half of 2008. The survey instrument is included in Appendix III. Additional sources were used to fill gaps, especially for countries that had not yet responded to the survey as of this writing. The main sources were two relatively recent published reviews, with global coverage, of the materials existing in *ex situ* collections. The report of the first meeting of the International Network for Cassava Genetic Resources held at CIAT in Colombia, in August 1992, covered genebanks globally, by region. Hillocks et al. (2002) provided a more recent summary. However, both these sources provide only rudimentary genebank information -- mainly total number of accessions by country.

The surveys were sent to the curators of 50 genebanks throughout the cassava-producing world. As of August 2008, 34 surveys were returned, and these data are summarized in various sections of this report, a good rate. Several other curators committed to returning the surveys at a later date. A consolidated summary of key information from these various sources is given in Table 3, and Appendix V summarizes additional information from the Latin American, African and Asian collections based on survey returns.

7.1 Genebank holdings of landrace varieties and collection needs

Genebank curators often choose to conserve some combination of local landraces, introduced landraces, and breeding or other experimental materials. The approaches vary widely in terms of emphasis on these different categories. Local landraces are the nucleus of most collections, especially in the Americas. Breeder or experimental material generally involves rearranging the genes found in landraces, but does not introduce new genes. The one notable, but relatively rare, exception would be mutation-based breeding, which produces heritable new traits not necessarily found in any landrace variety. Therefore, from the perspective of cataloguing genetic diversity, this study considers a local landrace (collected *within* the country) to be the unit of interest.

Most genebanks are centralized within a country, with possible duplications at other sites. Brazil has instituted a regional system as a rational way to deal with very broad genetic diversity in a large country. For field-grown collections, this has the major advantage that most accessions are maintained in an environment similar to that of their site of collection (Fukuda, April/May CIAT workshop).

Generally genebanks aim to maintain all collected material (except possibly material positively identified as internal copies or duplicates), but there is rarely, if ever, a precise count of the number of distinct landraces in a country. The goal of collecting and conservation should be to sample all the locally frequent genes available in landraces, but the sampling strategy to achieve that is normally not based on good genetic information. The molecular characterization of landraces is only beginning, so it is not yet a rational guide for collecting, but may be in the future. The concern is

that collecting in areas not yet covered should probably not await the level of scientific information that would be ideal; there is too much risk of continuing losses of material while waiting for research to provide this information.

It must be understood that much of the data in Table 3 are highly speculative - current "best guesses" that need to be researched further and continually updated with greater precision. Information in the Table is referred to by its column number in the following discussion.

In order to place cassava conservation in the perspective of importance of the crop in each country, Column 2 shows area planted for each country, according to 2006 FAO data. Columns 3 and 4 show the number of accessions based on previous reports. Column 5 reports only those materials indicated as local landraces in the current survey information. Column 6 is an estimate of the number of landraces from each country that are held by either CIAT or IITA.

A global conservation strategy must rely on reasonable estimates of the number of materials that should be preserved from each country. Columns 8 and 9 of Table 3 estimate the number of unique landraces (excluding duplicates) held in *ex situ* genebanks, and the total number of distinct genotypes that exist *in situ* in each country. Although in theory it would be possible to arrive at nearly exact numbers by meticulous collecting and extensive molecular analysis, the costs of doing so could probably not be justified, relative to other research priorities. These estimates place total *ex situ* landrace accessions at 10,068, and total *in situ* landraces at 26,986.

There are two principal issues that confound the interpretation of most published, summarized registers of cassava genebanks, and probably for the genebanks of other species as well: in general, it is not possible to distinguish what proportion of the accessions are landraces, as opposed to bred varieties, or some other form or origin of material. And often it is also not possible to distinguish local landraces from landraces introduced from another country. These factors tend to inflate the level of genetic diversity held in genebanks. For the most part, it is the local landraces that represent the breadth of the genetic diversity available within a species (although, of course, there may be much broader variability available in the wild species). The ideal information, in order to best assess genetic variability, would be a comprehensive list of the number of accessions of local landraces held in *ex situ* collections, compared to the total number of landraces that exist in a country (collected or uncollected). The germplasm survey reported here attempted to correct this deficiency. Table 3 attempts to estimate this information by combining results from the current survey data, from previous genebank analysis, and from personal contacts and experience of the author. This information is combined to make estimates about the density of landrace accessions in each country (number of hectares per landrace accession); total number of landraces in each country; proportion of these that are maintained *ex situ*, and conversely, proportion that remain to be collected; and number of accessions from each country that are conserved in one of the CGIAR centers (CIAT or IITA). From these data, we make estimates of the total number of accessions that are not in CGIAR centers, and what would be involved in obtaining secure, complete duplication of all landrace accessions in the IARCs.

In some cases, there is good agreement in numbers across different reports, but in many cases there are wide variations in terms of number of accessions being reported for a given genebank.

One way to compare across countries and regions is to look at landrace density, e.g. the number of hectares, on average, occupied by a landrace in a given country or region (total hectares planted divided by number of landrace varieties). By these estimates, as one would expect, landrace density is much higher in the Americas (center of origin) than either Africa or Asia. *In situ* densities (hectares per landrace) are estimated as follows: Americas – 176; Africa – 1,619; and Asia/Oceania – 1,245. On a global basis, the estimate is 690 hectares per landrace variety.

These numbers are very rough estimates. Also, these averages are made up of some extremely variable within-region estimates. For example, Thailand is estimated at 97,346 hectares per landrace. This is in fact a situation where it is known that there are few landraces and a very large area of production. Countries like Mexico, Puerto

Rico, Surinam and Vanuatu appear to have rather broad genetic diversity, but very few hectares planted, so that, for these four countries, the estimated landrace density varies from 2 to 8 hectares per variety.

The estimate of 26,986 total landraces in the world (Column 9) may at first seem daunting in terms of the resources required for conservation. But there is little justification for attempting to conserve every genetic variant that exists – our interest is instead primarily in conserving the totality of genetic variation, which will most likely be contained in a subset of the total number of genotypes. Column 13 suggests numbers that might represent the minimum number of landrace varieties that would need to be collected in order to conserve nearly all the genes in each country's total of landrace varieties. On a global basis, this is just over half the total estimated number of landrace accessions – 14,791 out of a total of 26,986. This in fact is not an unreasonably large number of accessions to consider conserving *ex situ*, given that CIAT alone has nearly half that number in its *in vitro* gene bank.

Column 14 indicates the importance of introducing national program accessions that are not yet represented in the international centers, for safety duplication (see also later sections). These low, medium and high priorities are based on number of *in situ* or *ex situ* materials not yet at CIAT or IITA, and the relative importance of that country's cassava genetic diversity. Critical countries for further representation in the international centers are: Brazil, Peru, Republic of Congo, Côte d'Ivoire, D.R. Congo, Malawi, Mozambique, Rwanda, Uganda and Tanzania, along with several other countries of medium urgency.

Priority for further collecting (Column 15) is based on likely genetic diversity that is not yet represented in any *ex situ* collections. Highest urgency is for Bolivia, Brazil, Haiti, Angola, D.R. Congo, Ghana, Madagascar, and Mozambique.

Collecting of cassava has already advanced very well on a global basis. This report estimates that about 14,791 distinct landraces should be conserved in genebanks in order to adequately represent global genetic diversity of cassava. Currently there are probably about 10,000, or two-thirds of the goal (see Table 3). However, it is probably fair to conclude that most of the "easy" collecting has been done. The remaining priority areas tend to be somewhat difficult to access because of lack of infrastructure, or are in countries with very low levels of available funding and personnel for collecting and conservation activities.

One of the lessons from the genebank surveys is that truly accurate information is difficult to obtain in a survey format. In order to obtain accurate information, each genebank should be visited, the curator extensively interviewed, and historical records studied. Updating and correcting the information in Table 3 is a daunting task, but with a combination of local and international expertise committed to obtaining these data, it should be possible to arrive at reasonably accurate estimates in the next two to three years. Having these data will contribute considerably to a rational conservation strategy.

7.2 Collaboration arrangements in conservation

It is common for cassava genebanks to be managed by scientists who may not have a background in genetic resources management. These managers are often breeders or agronomists, who were the main founders of early genebanks as a practical way of providing for their own needs for broad genetic diversity. While the largest banks, especially those of the international centers and a few national programs, are now managed by germplasm specialists, most are not. Plant breeders have a good sense of the economically significant genetic diversity in a collection and its long-term importance, but may be less interested in providing the necessary management input to conservation of materials with less immediate use in genetic improvement. In the 1980s, as the technology for *in vitro* conservation was developed for broad use, there was a trend for separation of management of cassava genebanks -- field genebanks continued to be managed by plant breeders or agronomists, and *in vitro* collections by physiologists or botanists, but not necessarily genetic resources specialists. Not only are the *in vitro* managers often not genetic resources specialists, but also they frequently are not at all familiar with the field-grown plants or the general needs of germplasm users, such as entomologists, pathologists or breeders.

The lesson in this overview is that the effective conservation of cassava genetic resources is necessarily a collaborative venture. The good news is that there is considerable expertise around the world in this area, and communications technology is making it ever more readily available to whoever needs it. The less-than-good news is that consistent, adequate, long-term funding to support cassava genetic resources conservation is often not available, and the integrity of genebanks suffers.

Collaboration of several types can enter the equation to improve conservation – training, infrastructure support, holding of duplicate genebanks, phytosanitary status testing/monitoring, GIS analysis in support of collecting, and others. The mechanisms to foster collaboration reside mainly in networks – formal or informal, and range from intra-institutional to global. The formal networks were described in Section 3, and generally genebank managers will be aware of the networks relevant to their own situation. What is often missing in genebank management is creating and taking advantage of the less formal networks. The good management of *Manihot* genebanks is in the interest of a wide range of people, including future generations of farmers, agronomists and breeders, and all these people should be part of formal or informal networks to support genebank management. Many times this support can come from people within the same institution where the genebank resides, or in related institutions.

Below are some hypothetical examples at different levels of collaboration that may fit the needs of specific genebanks. The way this collaboration is arranged will depend on each institution's structure and *modus operandi*. For example, in some situations, a contractual arrangement may work, such as a genebank curator contracting the agronomy department in the same institution to maintain a field collection, or a pathologist to develop an indexing protocol for a virus. Other situations may call for a joint grant application. A soil scientist could request permission to evaluate the collection at the expense of his or her own project, for tolerance to a soil stress condition, under guidelines agreed with the curator, and later load the data into the genebank database. In general the genebank curator will be responsible for seeking out and creating these collaborative relationships, but should also welcome proposals from others.

Intra-institutional or local inter-institutional

- Field genebank establishment and agronomic management by agronomist
- Pest and disease evaluations by specialists
- Virus indexing protocols by a pathologist
- *In vitro* conservation by a private firm with appropriate facilities and expertise

National

- Molecular characterization in a university laboratory
- Starch quality analyses by a private company lab

International

- Duplicate accessions held by an international center
- Virus indexing in a third-party lab prior to import or export of materials
- Molecular characterization in a university laboratory
- Taxonomic studies in an university

7.3 The most important collections

Criteria to determine the most important collections need to be identified for defining programs that merit support for cassava conservation. The April/May CIAT workshop participants discussed this topic and arrived at the following recommendations:

Criteria to define the most important cassava collections

- Recognized as a collection from a country or region with highly significant genetic variability for cassava
- Inclusion of a major part of the country's total genetic diversity in the genebank

- Greater than X number of accessions
- Duplication of collection at an IARC, or intent to do so
- Signatory to the International Treaty
- Demonstrated long-term institutional support
- Ability to maintain entire collection "safely" *in vitro* for long-term conservation
- Facilities for phytosanitary testing of *in vitro* collection
- Ability to distribute to requesting entities within country
- Ability to distribute to requesting entities outside country

This set of criteria does not name specific genebanks, but establishes criteria that can be used to evaluate the characteristics of the world's cassava genebanks by donors. The Working Group was reluctant to indicate a specific number of accessions required to be considered "important." Rather, it was felt that this number would need to be considered in the context of the balance of other criteria. For example, a small collection from a country with broad or unique genetic diversity should be classified as "important" for long-term conservation. In such case, however, it may be more practical to emphasize support to a centralized institution that maintains a duplicate of such materials.

It is also recognized that the list of "important" collections will be dynamic. Countries whose current collections may not be considered important may have a large genetic variability of uncollected landrace varieties. There needs to be support for collecting these varieties and establishing them in *ex situ* collections. At that point, they may be considered important, and worthy of international support for long-term secure conservation. For example, even though Guatemala is a small producer of cassava, and has only a modest genebank (see Table 3), recent diversity studies have shown that the Guatemalan germplasm is apparently genetically distinct from other groups in either Africa or Latin America (Hurtado and Fregene, 2008). By this definition, this collection would be considered as important for the global conservation effort.

It should be pointed out that currently very few national collections, and perhaps none of them, meet all the criteria in this list. This became apparent after the workshop, as more of the genebank surveys were returned and analyzed. For example, among the genebanks that returned the surveys, only a few of the smaller ones maintain their full collection *in vitro*. Many genebanks have some capacity for *in vitro* conservation, but it would need to be upgraded in order to securely conserve the entire genebank. Alternatively, donors could consider support to genebanks whose accessions are conserved *in vitro* in a duplicate collection, for example in one of the international centers. There are also few countries that have the ability, at a high confidence level, to index accessions for virus infection, a prerequisite for international exchange. Few are equipped for, or interested in, international exchange, other than to send to or receive from the international centers.

The implications of these conclusions concerning important collections are included in the final recommendations for cassava conservation (Section 13).

8 Overview of current wild *Manihot* species genebanks

The wild *Manihot* species have long been a frustrating challenge to collectors, genebank curators and breeders. Funding for their collecting has been sporadic and inadequate. Their conservation in the field, *in vitro* or as seeds all present difficulties and a number of species-specific research approaches. Breeders have long viewed some of their potential traits with great interest, but have been generally reluctant to commit to the time and difficulty of recovering those traits from the poor agronomic genetic background that result from crossing with *M. esculenta*. In addition, the threats to their existence in natural populations continue, and in many instances is increasing. Despite proposals since more than 20 years ago to *create in situ* reserves for wild *Manihot* species, this has not been realized.

On the positive side, technological progress in all these areas is creating renewed interest in the *Manihot* species. The habitats of many of these species are known. This potentially allows the use of GIS to target specific sites for

more efficient collecting. Progress has been made in conservation in all forms, including field, seed and *in vitro*. Developments in marker assisted selection are enabling much greater rates of progress in recovering target genes from wide crosses. Brazil's EMBRAPA has continued making periodic wild *Manihot* collections, and is giving renewed efforts to their safe conservation (Alves, 2008).

During the founding meeting of the *Manihot* Genetic Resources Network in 1992, participants updated and revised collecting priorities. The group recommended that collecting be prioritized to solve bottlenecks affecting existing breeding programs. For wild *Manihot* there are still too many unknowns to define a detailed strategy, so Allem (1994) proposed using crossability with *M. esculenta* as an initial guideline. He described Gene Pool 1 (GP1) as the species known to cross readily with cassava and yield fertile offspring. In this GP1 he included only *M. flabellifolia* and *M. peruviana*, now believed to be the direct ancestors of cassava. Taxa crossing with difficulty with cassava but giving some positive results make up GP2. This pool includes *M. glaziovii*, *M. dichotoma*, *M. pringlei*, *M. aesculifolia* and *M. pilosa*. In practical terms there should probably be a combined weight given to environmental threats to populations in the wild, along with expected potential use in cassava genetic improvement.

Table 8 is a complete list of the *Manihot* species, as per Rogers and Appan (with notes on more recent taxonomic updates). The list notes where species are maintained in the principal *ex situ* collections, and species of concern for potential loss in their natural habitats. There are several genebanks around the world that maintain a few species for experimental purposes, but only EMBRAPA, Universidade de Brasilia (Nagib Nassar) and CIAT have a serious program for long-term conservation of wild *Manihot*. IITA maintains eight species. The shaded bars in the Table indicate species of concern, which have no apparent representation in *ex situ* collections.[5] This includes ten of the South American species. However, many of those species that are conserved *ex situ* are seriously under-represented in terms of genetic diversity of the wild populations.

In view of this small number of genebanks, determining the wild *Manihot* species collections of importance is rather straightforward: they are all critical to the safe long-term conservation of the genus. Due to its mandata, the Trust is contemplating support only of *ex situ* collections, but *in situ* conservation should be supported by other means.

[5] As of this writing, there is no information available on the *ex situ* genebank of Mexico. Therefore none of the Mesoamerican species are highlighted as species of concern, but it is nearly certain that many of them will later be shown to be at risk.

Table 8. Wild *Manihot* species in genebanks and of concern for genetic erosion. (Shaded rows indicate "Species of concern" that are not represented in any *ex situ* genebank).

			Genebanks				Species of concern[1]					
	Species	Approximate geographical range[a]	CIAT	EMBRAPA	U. Brasilia*	IITA	Mexican species[2]	Brazilian species[3]	Brazil maniçobas[4]	Primary gene pool[5]	Secondary gene pool[6]	High genetic erosion[7]
1	*M. angustiloba* (Torrey) Muell.-Arg. emend Rogers & Appan	southwest USA, Mexico					x					
2	*M. davisae* Croizat	southwest USA, Mexico					x					
3	*M. walkerae* Croizat	southwest USA, Mexico					x					
4	*M. aesculifolia* (HBK) Pohl	Mexico	x		x		x					
5	*M. auriculata* McVaugh	Mexico					x					
6	*M. caudata* Greenman	Mexico					x					
7	*M. chlorosticta* Standley & Goldman	Mexico	x				x					
8	*M. crassisepala* Pax & K. Hoffmann	Mexico					x					
9	*M. foetida* (HBK) Pohl	Mexico					x					
10	*M. michaelis* McVaugh	Mexico					x					
11	*M. oaxacana* Rogers & Appan	Mexico					x					
12	*M. pringlei* Watson	Mexico					x					
13	*M. rhomboidea* Muell.-Arg.	Mexico					x					
14	*M. rubricaulis* I.M. Johnston	Mexico	x				x					
15	*M. subspicata* Rogers & Appan	Mexico					x					
16	*M. tomatophylla* Standley	Mexico					x					
17	*M. websterae* Rogers & Appan	Mexico					x					
18	*Manihotoides pauciflora* (T.S. Brandegee) Rogers & Appan	Mexico										
19	*M. carthaginensis* (Jacquin) Muell.-Arg.	Colombia, Venezuela, West Indies	x									
20	*M. tristis* Muell.-Arg.	Venezuela, northern Brazil	x									
21	*M. surinamensis* Rogers & Appan	Venezuela, Guayana, Suriname										
22	*M. filamentosa* Pittier	Venezuela	x									

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23	*M. maguireiana* Rogers & Appan	Venezuela										
24	*M. brachyloba* Muell.-Arg.	Central America, West Indies, northern & central South America	x					x			x	
25	*M. marajoara* Chermonte de Miranda apud Huber	northern Brazil										
26	*M. caerulescens* Pohl	northern, northeastern, and central Brazil	x	x	x	x			x			
27	*M. glaziovii* Muell.-Arg.	northeastern Brazil; introduced throughout tropical America, Africa, India, Pacific Islands	x	x	x	x			x			
28	*M. brachyandra* Pax & K. Hoffmann	northeastern Brazil			x							
29	*M. catingae* Ule	northeastern Brazil			x							
30	*M. dichotoma* Ule	northeastern Brazil	x	x	x				x			
31	*M. epruinosa* Pax & K. Hoffmann	northeastern Brazil	x		x	x						
32	*M. heptaphylla* Ule	northeastern Brazil			x							
33	*M. maracasensis* Ule	northeastern Brazil							x			
34	*M. pseudoglaziovii* Pax & K. Hoffmann	northeastern Brazil	x		x							
35	*M. quinquefolia* Pohl	northeastern Brazil										
36	*M. reniformis* Pohl	northeastern Brazil										
37	*M. zehntneri* Ule	northeastern Brazil										
38	*M. acuminatissima* Muell.-Arg.	eastern Brazil										
39	*M. handroana* N.D. Cruz	eastern Brazil										
40	*M. janiphoides* Muell.-Arg.	eastern Brazil	x						x			
41	*M. pilosa* Pohl	eastern Brazil	x		x			x			x	

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42	*M. pohlii* Wawra	eastern Brazil			x							
43	*M. sagittato-partita* Pohl	eastern Brazil						x				
44	*M. warmingii* Muell.-Arg.	eastern Brazil										
45	*M. tripartita* (Sprengel) Muell.-Arg.	central and eastern Brazil			x			x				
46	*M. quinquepartita* Huber ex Rogers & Appan	northern and central Brazil	x					x				
47	*M. alutacea* Rogers & Appan	central Brazil	x									
48	*M. attenuata* Muell.-Arg.	central Brazil										x
49	*M. cecropiaefolia* Pohl	central Brazil	x	x								
50	*M. crotalariaeformis* Pohl	central Brazil										
51	*M. divergens* Pohl	central Brazil										x
52	*M. falcata* Rogers & Appan	central Brazil										
53	*M. flemingiana* Rogers & Appan	central Brazil						x				
54	*M. fruticulosa* (Pax) Rogers & Appan	central Brazil	x									
55	*M. irwinii* Rogers & Appan	central Brazil	x	x								
56	*M. jacobinensis* Muell.-Arg.	central Brazil	x	x					x			
57	*M. longepetiolata* Pohl	central Brazil	x									
58	*M. mossamedensis* Taubert	central Brazil				x		x				
59	*M. nana* Muell.-Arg.	central Brazil										
60	*M. oligantha* Pax	central Brazil										x
61	*M. orbicularis* Pohl	central Brazil	x									
62	*M. paviaefolia* Pohl	central Brazil										
63	*M. peltata* Pohl	central Brazil	x									
64	*M. pruinosa* Pohl	central Brazil						x		x		

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65	*M. purpureo-costata* Pohl	central Brazil	x									
66	*M. pusilla* Pohl	central Brazil										
67	*M. quinqueloba* Pohl	central Brazil										
68	*M. reptans* Pax	central Brazil			x							
69	*M. salicifolia* Pohl	central Brazil										
70	*M. sparsifolia* Pohl	central Brazil	x									x
71	*M. stipularis* Pax	central Brazil										
72	*M. tomentosa* Pohl	central Brazil		x								
73	*M. triphylla* Pohl	central Brazil	x					x			x	
74	*M. weddelliana* Baillon	central Brazil										
75	*M. violacea* Pohl	central Brazil	x			x		x				x
76	*M. xavatinensis* Rogers & Appan	central Brazil										
77	*M. esculenta* subsp. *flabellifolia* (Pohl) Ciferri	western and central Brazil	x	x		x		x		x		
78	*M. stricta* Baillon	Peru, western and central Brazil										
79	*M. leptophylla* Pax	Ecuador, Peru, western and central Brazil				x						
80	*M. grahami* Hooker	southeastern Brazil, northern Argentina, Paraguay, Uruguay										
81	*M. inflata* Muell.-Arg.	southern Brazil										
82	*M. corymbiflora* Pax	southeastern Brazil			x							
83	*M. leptopoda* (Muell.-Arg.) Rogers & Appan	southeastern Brazil										
84	*M. jolyana* N.D. Cruz	southeastern Brazil										

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85	*M. condensata* Rogers & Appan	Bolivia										
86	*M. guaranitica* Chodat & Hassler	Bolivia	x									
87	*M. anomala* Pohl	central Brazil, Paraguay	x	x	x			x				
88	*M. gracilis* Pohl	central Brazil, Paraguay			x							
89	*M. pentaphylla* Pohl	central Brazil, Paraguay	x									
90	*M. hassleriana* Chodat	Paraguay						x				
91	*M. mirabilis* Pax	Paraguay										
92	*M. variifolia* Pax	Paraguay										
93	*M. populifolia* Pax	Paraguay										
94	*M. procumbens* Muell.-Arg.	southern Brazil, Paraguay										
95	*M. affinis* Pax	southern Brazil										
96	*M. tenella* Muell.-Arg.	southern Brazil										
97	*M. hunzikeriana* Martinez-Corvetto	southern Brazil, Argentina										
98	*M. anisophylla* (Grisebach) Muell.-Arg.	Argentina										
	Species that exist in at least one location:		42									
	[a]*Source: Halsey, M.E., K.M. Olsen, N.J. Taylor and P. Chavarriaga-Aguirre. 2008. Reproductive biology of cassava (Manihot esculenta Crantz) and isolation of experimental field trials. Crop Sci. 48:49-58.*											
	Species added after Rogers and Appan											
99	*M. baccata*							x				

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	M. compositifolia							x				
100	*M. peruvianua*		x	x		x		x		x		
	M. diamantinensis								x			
101	*M. hastatiloba*		x									
102	*M.neusana* Nassar (CJPS,1985)				x							
103	*M.swaminii* Nassar (CJPS, 2008)				x							
	Cultivated varieties with "wild" traits											
	"maniçoba"			x								
	"pornúncia"			x								
	"sete anos"			x								
	Synthetic species											
	M. rogersii Nassar				x							
	M. vieirii Nassar				x							
	Interspecific hybrid											
	M. oligantha X cassava				x							
	M. pilosa X cassava				x							
	M. pohlii X cassava				x							
	M. glaziovii X cassava				x							
	M. pseudoglaziovii X cassava				x							
	M. neusana X cassava				x							
	M. dichotoma X cassava				x							

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		CIAT	EMBRAPA	U. Brasilia*	IITA	Mexican species[2]	Brazilian species[3]	Brazil maniçobas[4]	Primary gene pool[5]	Secondary gene pool[6]	High genetic erosion[7]
M. caerulescens X cassava				x							
M. aesculifolia X cassava				x							
M. anomala X cassava				x							
M. reptans X cassava				x							
Polyploidized interspecific hybrid											
M. oligantha X cassava				x							
M. aesculifolia X cassava				x							
M. cearulescens X cassava				x							
M. anomala X cassava				x							
M. glaziovii X cassava				x							
Apomictic clone 307 and its polyploid type				x							

* Each species is represented in the field by about 30 plants (Nagib Nassar, pers. comm.)

[1] Source: Howeler et al., 2001

[2] Mexican species threatened because of development

[3] Brazilian species threatened because of development and cassava cultivation

[4] Species of maniçobas economically valuable to dwellers of Brazil's NE semi-arid region

[5] Species involved in the ancestry of cassava and constituting th wild primary gene pool of the crop

[6] The putative closest relatives of cassava and assumed to participate n the secondary gene pool of the crop

[7] According to Nassar, 1979 (cited in IPGRI, 1994)

9 Improving the efficiency of conservation

Various management strategies are available to make germplasm conservation more efficient. Two of these emerged with some frequency in the genebank surveys – the definition of core collections, and the identification of duplicates.

9.1 Core collections

The core collection concept grew out of the need to streamline and prioritize conservation and evaluation, particularly in large genebanks. Originally conceived by Frankel (1984), a core collection would represent "with a minimum of repetitiveness, the genetic diversity of a crop species and its wild relatives." These collections are normally 5-10% of the total. CIAT defined a core collection of 630 accessions based on geographic origin, morphological diversity, diversity of esterase isozyme banding patterns, common landraces, and elite breeding lines (Hershey *et al*., 1994). CENARGEN, in coordination with CNPMF in Brazil, defined a core collection of Brazilian accessions.

Defining a core collection has several implications for management of the whole collection. Conservation strategies may be tailored to give a higher priority for the core. The core may be duplicated in several institutions, or it may be held in various forms (e.g. field and *in vitro*), while the remainder is kept only *in vitro* or in seed form. Use of the core as a conservation strategy should be temporary, at best. For example, based on recommendations of the *Manihot* Genetic Resources Network (IPGRI, 1994), CIAT sent the core collection to Brazil and Thailand for duplication. Longer-term plans call for Africa to also receive this subcollection, when introduction of vegetative material is accepted and managed more routinely.

A core collection permits a better understanding of genetic diversity in the whole collection, through more efficient use of resources for evaluation. Evaluation of the core for a given trait should indicate total diversity, and may direct the scientist to specific geographical areas or groups of germplasm with special promise for further study. It is a common experience in large collections that evaluation for a single trait can take years and considerable resources. Evaluation of the core as a first step can be far more efficient. CIAT began extensively evaluating the core collection soon after its formation, especially for traits that had previously been considered too costly to evaluate in the entire collection.

As with any sampling procedure, a core collection definition is subject to sampling errors. The probability of a gene occurring in the core collection is significantly different from its frequency in the entire collection only if that gene is related in some way to the criteria for defining the core. One of the main risks is the difficulty of identifying rare genes that may escape inclusion in the core.

Defining a core collection is strategically useful only in large collections. While a number of factors may influence the decision, as a rule, defining a core collection may not be very worthwhile except in cassava collections of about 1,000 or more accessions, and where most of the accessions are landraces.

The surveys identified considerable interest in the development of core collections by the national program genebanks, although only Brazil and India appear to have already established core collections. Some of the interest could be the result of an incomplete understanding of the functions of a core collection. In fact, for practical purposes, there is probably limited value in defining a core collection for all but a very few of the largest collections. This would appear to limit the utility of core collections to CIAT and IITA, plus Brazil, and Peru (Table 3).

There is some question about the need for, and the efficacy of, core collections for purposes of safe conservation, given the development in recent years by both IITA and CIAT of black box duplicates in other institutions. This does not mean that core collections may not have other purposes, especially with regard to determining likely areas to search for particular traits, or for other types of genetic diversity studies. Of course, the ideal would be a global core collection, including material from multiple collections around the world. But centralized and standardized data management in a global register would be needed for that. There is more on this in later sections.

9.2 Duplicate identification

It is common during collection expeditions to sample inadvertently the same genotype more than once. Situations that increase the probability of collecting duplicates are: (1) different local name for the same clone; (2) a clone widely grown across a region; (3) collecting expeditions to the same region at different times; (4) clones sensitive to environmental variations and displaying variable phenotypes across microenvironments; and (5) inexperienced collectors.

Hershey (1994) estimated that CIAT's global collection could be reduced by 20-25% by identifying duplicates. This has to be done with great care, however, and by relying on methods that will identify genetic duplicates with a high degree of confidence. CIAT established a four-step procedure (Hershey *et al*., 1991; CIAT, 1993): (1) identification of candidate duplicate genotypes by comparison of eight key morphological characteristics; (2) side-by-side field comparison of putative duplicates grown together in the same year; (3) re-characterization for morphological traits; and (4) characterization of putative duplicates with molecular markers. The efficacy of the molecular probe M-13 was demonstrated in that 20% of genotypes identified by other criteria as probable duplicates showed distinct fingerprints.

Ocampo *et al*. (1993) analysed 4,304 accessions from CIAT's germplasm collection, with the -esterase isozyme system. From a total of 22 distinct bands, accessions grouped into 2,146 different banding patterns. A further analysis of the Colombian accessions within the collection, using molecular markers, indicated a likelihood of about 10% duplication (Debouck, 2008). If this figure is extrapolated across the CIAT collection, some 500 accessions (10% of about 5,000 landrace accessions in the genebank) could be combined or merged, with an annual savings of some $5,000 per year in conservation costs.

In similar work, Sumarani *et al*. (2004) analyzed 70 sets of tentative duplicates (total of 139 accessions from 786 indigenous accessions in India's national collection). The esterase isozyme system produced a maximum of five bands per accession, and among the multiple sets, a total of 35 bands, proving to be a highly polymorphic system. Altogether, 62 out of 218 accessions (28%) were found to be duplicates. The authors suggest that duplicate identification should proceed in a logical manner from creating tentative groupings among a large number of genotypes, with rapid and inexpensive methods, to isozyme analysis with a reduced number of clones, and finally, confirmation by molecular probes. If facilities for molecular probes are readily available in a potential collaborating institution, isozyme analysis might be eliminated altogether.

Duplicate accessions may either be eliminated, assigned to a lower level of conservation priority, or combined into a single accession. As the number of molecular markers is now in the thousands, the level of confidence in identifying duplicate clones is becoming quite high. It is a question of whether the cost of identifying these duplicates is lower than the cost of maintaining them in the field or in the laboratory. As the cost of processing molecular markers falls dramatically, their application to identifying duplicate accessions becomes very practical.

9.3 Improved slow-growth conditions

The annual cost of sub-culturing *in vitro* plantlets is about seven times that of storage (Koo et al., 2004). The main way to reduce this cost will probably be to further slow the growth rate. This has been one of the main goals of cassava *in vitro* research since its initial development 30 years ago, and constant progress is being made. This is research that can have a high return, and needs to continue in the main institutions that employ *in vitro* storage. The incorporation of silver nitrate into the media is one recent example of new techniques with promise for extending the time until regeneration (Mafla, 2008).

10 Characterization and preliminary evaluation

A germplasm collection is useful as a resource to its users only when accessions are well-described in terms of characteristics of interest. Bioversity International (and its predecessors IBPGR and IPGRI) has developed standardized descriptor lists for many crops, but has not yet done so for cassava. As noted in Appendix I, the April/May CIAT workshop on cassava germplasm conservation was followed by a mini-workshop on cassava descriptors, facilitated by Bioversity. This initiative should lead to a minimum descriptor list for cassava.

There are two generally recognized basic categories of documentation for germplasm collections, apart from passport data: (1) characterization – those characters that are highly heritable, clearly visible and are expressed in all environments; and (2) preliminary evaluation – a limited number of additional traits of lower heritability considered desirable by a consensus of users of the crop. Characterization is important basically as a tool for varietal description, identification of duplicates in a collection, monitoring genotypic stability of clones stored *in vitro* or in other non-conventional forms, and varietal fingerprinting. Preliminary evaluation is often the starting point for breeders to identify an accession's potential value in a breeding program. A breeder's general objective is typically to identify clones that can be used directly as recommended varieties, or as parents in a breeding program. Many other crucial decisions hinge on this general objective, related to target production areas and their physical and biological characteristics, management practices to be employed, and processing and marketing characteristics.

Most germplasm curators will see characterization, as a means of describing and cataloguing an accession, as clearly part of their mandate, but often draw a line at evaluation, considering that the preserve of breeders. There is room for attitudinal change here, on both sides, and greater collaboration.

Preliminary evaluation consists of six broad categories: (1) general adaptation, (2) resistance, (3) plant architecture, (4) yield, (5) root quality, and (6) other locally important traits.[6] The procedures for evaluation of germplasm accessions may be very similar, or identical to evaluation of breeding lines. Much of the detail on evaluation and selection given in later chapters can be applied also to germplasm collections. There is, however, an important procedural difference: all germplasm accessions should be equally and fully evaluated. On the other hand, breeding lines may be pre-selected on the basis of a few key criteria, and only those passing this first step receive further evaluation. If large numbers of germplasm accessions need to be evaluated, some compromises may be made with regard to level of precision. With up to a few hundred accessions, multi-location evaluation in replicated trials may be possible. If accessions number in the thousands, the breeder or germplasm curator may only be able to manage unreplicated single row trials.

Many characters may appropriately be evaluated within a field-planted genebank itself. Stresses that impose risks to the collection, and may result in accession losses if uncontrolled, should be evaluated in separate, specially designed trials. Serious pests and diseases or major soil problems are examples. The field collection often is not an appropriate place to evaluate yield or quality because of inappropriate plot design or the need to leave plants in the ground well beyond the normal harvest period.

Since the mid 1990s, the accession information and some evaluations from CIAT's germplasm collection have been available on-line at www.singer.cgiar.org. This website is managed by the System-wide Information Network for Genetic Resources, the germplasm information exchange network of the System-wide Genetic Resources Programme of the CGIAR. While this is a reasonable first step to search for traits of interest, it is best done with additional consultation with breeders and germplasm curators who are familiar with the details of the evaluations and the germplasm itself. Clearly there is great value in the germplasm information database. At the same time it will be most useful to a breeding program if the evaluations are understood in the context of a complete picture that includes agro-climatic conditions, and the complete range of traits that are of importance to the breeder. To that end, a

[6] For example, consumer preferences for eating quality, etc., or color traits such as yellow roots.

cassava common registry has been recently established, with passport and preliminary characterization data provided by CIAT and IITA at this stage (see http://www.cassavaregistry.com).

There is an urgent need to digitize data currently held only as hand-written files. Many genebanks are still managed without benefit of computerized information. Few of those that do maintain digitized files make this information broadly available online.

11 Distribution

Many genebanks and breeding programs obtain new genetic diversity through introduction from outside sources. The principles and methods associated with germplasm exchange are fundamental to the functioning of most genebanks. The large majority of cassava genebanks distribute materials only within the county, and only a few engage in regular international exchange. This discussion focuses on international cassava germplasm movement.

11.1 Benefits and risks

The potential benefits of germplasm introduction are essentially a function of the genetic variability available in local germplasm, and more specifically, of the strengths and weaknesses of that germplasm. Typically, the range of variability in local germplasm depends on the region, with the highest variability usually in the crop's centre of origin, the Americas. Even in areas of high variability, there can be large advantages to germplasm introductions or to capitalize upon advances made in breeding programs elsewhere to introduce specific characters.

Two types of risks accompany germplasm introductions: phytosanitary and genetic. The phytosanitary risks – introducing new biotypes or species of pests or pathogens – are of paramount importance. Minimizing these risks must take very high priority in any germplasm exchange. The genetic risks are the risks of knowingly or unknowingly introducing undesirable alleles along with the known desirable ones. Undesirable alleles may be those that confer susceptibility or non-tolerance to a particular environmental factor; alleles for poor quality; or in general, any that are considered less desirable than those controlling the same traits in local germplasm. These genetic risks are minimized by an appropriately designed evaluation and selection program.

11.2 Forms of exchange

11.2.1 Vegetative

Generally, the international exchange of genebank accessions between institutions is through *in vitro* culture. The principal advantage of *in vitro* introduction is phytosanitary. Insects, mites, bacteria and fungi are easily eliminated, and cultures can be indexed for several viruses to provide a high level of assurance of pest and pathogen-free material. From a standpoint of international quarantine, *in vitro* introductions are widely accepted within and among Asian and Latin American countries. Regulations on exchange within Africa, and between Africa and other continents, are more variable and generally more restrictive. No method is free of risk, but the technology for detecting pathogens is well-advanced.

In vitro introductions first need to be propagated and grown in specialized conditions, resulting in some delay until agronomically useful evaluations can be made. Under ideal conditions, and using rapid propagation techniques, agronomic trials can be established within one and a half years after *in vitro* introductions. Normally, however, three or more years are required to obtain sufficient planting material (including one cycle of field propagation to obtain lignified stakes). Many scientists receiving *in vitro* cultures have initially been too optimistic about the time required for regeneration and evaluation.

In the case that international exchange is for the purpose of introducing new variability to genebanks that serve the needs of breeders, the amount of genetic variability that can be managed is a major limitation for vegetative exchange. For large numbers of clones, expense of preparation and difficulty of management by the recipient, may be prohibitive. This generally means that only a limited number of clones are sent in any given shipment, usually on the order of ten or less, but up to a few hundred in special cases.

11.2.2 Seeds

The two outstanding advantages of seed introductions are ease of handling broad genetic variability and the relatively high tolerance of seeds to storage and shipping. The fundamental property of seed introductions is that any plant derived from a botanical seed of cassava is a new, distinct genotype – necessarily different from the clone from which it was derived. Seed introductions into a genebank will not duplicate the accessions of the donor bank. One constraint for some programs to utilize seed introductions is the need for specialized training for management of seed and seed-derived plants. Some programs combine both seed and vegetative introductions, taking advantage of the positive features of each.

11.3 Quarantine considerations

The exchange of cassava stem cuttings through unofficial means (farmers, tourists, entrepreneurs) is probably the major means of disseminating pathogens and pests across international boundaries. The bacterial blight pathogen can survive in the xylem vessels of infested stems for months. Cassava viruses and mycoplasmas are efficiently harbored in stem cuttings from infected plants and readily transferred to new plants via infested cuttings. The cassava green mites, mealybugs and scale insects can survive for months, feeding on the lateral buds of stem cuttings. Introductions of green mites, mealybugs and the bacterial blight pathogen into Africa are important examples of the risks of inappropriate and unmonitored germplasm movement.

Some pathogens can be disseminated through botanical seeds. These fit into two broad groups: (1) those that infest the seed; and (2) those that infect it. Infestation may follow fruit dehiscence. If the seeds fall to the ground, the probability of infestation is higher than when the seeds are collected prior to dehiscence and stored under controlled conditions. Pathogens of cassava that can most effectively infest the seeds and survive on them are those producing abundant mucilaginous propagules, such as *Colletotrichum*, *Phoma* and *Diplodia* spp., and *Xanthomonas campestris* pv. *manihotis*. Infestation of storage containers is also a risk. Disinfested seed should be repacked in clean containers.

Pathogens that infect seeds include *X. campestris* pv. *manihotis*, *Diplodia manihotis, Fusarium* spp. and *Cladosporium* spp. However, the limited research in this area does not preclude the possibility of other fungal and bacterial pathogens.

Determination of the potential for seed transmission of all cassava viruses is essential for the safe interchange of botanical seeds, but information is far from adequate. The main virus concerns, namely, cassava mosaic virus, cassava common mosaic virus and frogskin virus, are apparently not transmitted via cassava seeds. Two more recently discovered nepoviruses, the cassava green mottle virus (apparently a minor virus limited to some South Pacific islands), and the cassava American latent virus, found in Brazil and Guyana, raise some concern about seed transmission in view of the type of virus.

A few mycoplasma-like organisms (MLOs) affect cassava, causing antholysis (leaf distortion) and witches' broom diseases. These MLOs are not seed-transmitted.

There are few insects that attack cassava seeds so the risk of disseminating arthropod pests is relatively low. Seeds may however be superficially infested, especially with mites. A seed insecticide/miticide treatment is recommended

as a precaution, especially for any seed to be shipped internationally. However, some quarantine agencies, including that of Brazil, have expressed concern about exposing quarantine personnel to pesticides as they examine seeds.

FAO and IPGRI jointly published technical guidelines that include general and technical recommendations for cassava exchange (Frison and Feliu, 1991).[7] These phytosanitary measures, independent of others legally established by quarantine regulations of importing countries, would reduce the risk of disseminating pathogens and pests through propagative material of cassava. Their effectiveness depends on the strict application of such measures by both the sender and the recipient. Technical recommendations are provided for: (1) seeds; (2) pathogen-tested *in vitro* cultures; (3) cuttings from pathogen-tested *in vitro* cultures; and (4) untested vegetative material. By the end of 2008, CIAT intends to have the entire in-trust collection of cassava landraces disease tested and ready for international shipment (Cuervo, 2008)

The importing country should be especially cautious in the introduction of cassava propagating material from countries or areas where exotic diseases exist. For example, because of cassava mosaic disease, vegetative material should not be imported from Africa or India, except after very thorough virus indexing both at the source and in a third party institution in a non-cassava growing country. Indexing can be done in the mother clone (a field-grown plant for example), in *in vitro* tissue in the country of origin, and/or in *in vitro* material by the third party. A third party should be an independent entity that has a high level of trust by both the country of origin and the recipient country.

Detection methods can be based on the observation of symptoms in the mother plants, symptoms in grafts or indicator plants, or on the detection of virus particles and viral products. The reliability of detection methods based on plant symptoms can be increased by growing plants under optimal conditions for symptom expression. For example, the symptoms of cassava mosaic disease are poorly expressed at temperatures above 28°C. In this case plants may be grown in a cooler environment to enhance symptom development.

The bioassay of mechanically transmissible cassava viruses to indicator hosts is a sensitive indexing method if a very susceptible host is available, virus concentration in the test plant is high and environmental conditions are optimal for symptom expression. The Nigerian isolate of cassava mosaic disease produces a severe, systemic infection in inoculated *Nicotiana benthamiana* plants. The Kenyan isolate of cassava brown streak virus can be bioassayed on *N. debneyi*.

Grafting is a method for indexing viruses and virus-like agents that are not mechanically transmissible. Graft indexing is very sensitive if a highly susceptible indicator clone is used in the graft. The native Colombian clone *Secundina* is highly susceptible to frogskin disease (the same causal agent as for the Caribbean mosaic). When a *Secundina* scion is grafted onto an infected rootstock, leaves express moderate to severe mosaic symptoms. Although a graft-indexing programme requires minimal facilities and training, the procedure is labor intensive and indexing results are not available for several weeks. Another major constraint can be the difficulty of maintaining virus-free stocks of the indicator clone.

Sensitive serological tests are available for viruses that have been isolated, purified and an antiserum produced. ELISA is a highly sensitive, efficient and rapid method for detecting CMD and cassava common mosaic virus (CCMV). The immunoabsorbent electron microscopy (ISEM) test can also be used for detecting CMD and CCMV. ELISA is suited to a large-scale virus-indexing programme, where hundreds of plants can be tested in a day with results available within 36 hours. The preparation of test material and examination of grids is simple and rapid. Although ISEM is not as sensitive as ELISA, it has the advantage of providing results within several hours.

Nucleic acid or spot hybridization and isolation of viral-specific double-stranded RNAs (dsRNAs) can detect some cassava viruses. Spot hybridization has been adapted for detecting CMD. The procedure is based on the use of a

[7] There are plans to update the guidelines in the near future (D. Debouck, pers. comm.).

radioactively labelled DNA molecule that is complementary to the viral genome, to probe spots of leaf sap for the presence of viruses. The test is highly sensitive and suited for processing large numbers of samples.

Isolation of dsRNAs is especially suited to detecting uncharacterized viruses for which an antiserum or nucleic acid probe is not available. The extraction and analysis of dsRNAs are somewhat laborious, making the test more appropriate for indexing a limited number of mother plants rather than as a general screening method.

One of the principal concerns of shipments from Latin America to either Asia or Africa has been frogskin disease. Until recently, detection was done mainly by grafting onto a sensitive scion. A new test for the virus (rt-PCR) has been standardized and the results are in the process of being published, as the first step in gaining international acceptance for certification of frogskin-free material. The method cuts the diagnostic time from more than 20 weeks (for a grafting-based test) to just a few days (Cuervo; Debouck, workshop presentations).

11.4 Procedures for distribution

11.4.1 Sources

There are few genebanks with the capacity to act as sources of cassava germplasm on a regular basis and provide the essential phytosanitary safeguards. These functions have been assumed mainly by the international centers – CIAT for Asia and Latin America, and IITA for Africa. Both centers use the latest indexing and preparation techniques to give the highest possible assurance that material being distributed is pathogen-free.

The Field Crops Research Institute of the Thailand Department of Agriculture, in collaboration with the CIAT Asia Cassava Program, has distributed germplasm from various sources (mainly Thailand and CIAT breeding program) throughout Asia. Several quality *in vitro* laboratories are situated in the region, and the generally low level of problems of quarantine significance simplifies distribution. Within the context of a major international project for cassava breeding in the 1990s, CNPMF and CENARGEN of Brazil developed a protocol for distribution of cassava seed to Africa. A few other countries may respond to germplasm requests, but normally are not prepared to do so on a regular basis. The international centers can often act as intermediaries to facilitate germplasm exchange between two countries that may not have complete capacity for pathogen indexing.

11.4.2 Legal aspects

The international agricultural research system (including formal or informal collaboration among national programs, universities, the private sector and international centres) has depended on the free exchange of materials and information for continued success. The results of plant breeding research, both from private and public sectors, are increasingly protected with various forms of intellectual property protection, including patents, material transfer agreements, plant breeders' rights and trade secrets. Since implementation of the International Treaty on Plant Genetic Resources for Food and Agriculture (www.planttreaty.org), the principal means of formalizing exchange of cassava germplasm has been the standard material transfer agreement (SMTA), required for material in the Multilateral System for access and benefit-sharing (see ftp://ftp.fao.org/ag/cgrfa/gb1/SMTAe.pdf). Patents and trade secrets associated with genetically modified plants or tissues are coming more into play, but to a far lesser degree than for crops important in temperate agriculture.

Africa, in particular, has less capacity to replicate research results patented elsewhere, for the benefit of poor farmers (Devries and Toenniessen, 2001). While there are many publicly funded partners who would be willing in theory to share their most important discoveries freely, they are often unable to do so because of agreements made with private donors who want to protect their market advantages.

Table 3 (Column 16) indicates the ITPGRFA status of the world's major cassava-producing countries. Only twenty-five (about one-third) of these countries have ratified the Treaty, so there is clearly a constraint to the free exchange of cassava germplasm under the Treaty's terms.

It is important to note that wild cassava is explicitly excluded from the Annex 1 list of crops included in the Treaty's Multilateral System. Such material in the international collections maintained by CIAT and IITA is included in the MLS via Article 15, but the same cannot be said of wild *Manihot* in national programme genebanks, unless the country explicitly includes it.

12 Documentation of germplasm management

Generally, an institution assumes responsibility for germplasm management as a permanent, ongoing activity. An effective information management system becomes a critical part of the process. Survey returns indicated that most national programs have only rudimentary information management systems in place for genebank management. Most, however, also indicated an intention to computerize information in the next three years. This is an area where genebanks could greatly benefit by availability of standardized procedures and protocols, while at the same time having the flexibility to utilize locally familiar systems.

Accuracy in information management is critical. For example, the cumulative effects of even a low error rate in the identification of accessions will have devastating effects on the validity of information in the long term. Historically, there is often considerable instability in the personnel responsible for conservation of collections, and this can contribute to some lack of consistency in information management. Information for many small collections is not well organized, and the evaluation data are of dubious quality.

As electronic information management becomes more widely available, including positive ID systems like bar codes or RFID, genebank information management should improve. A database integrating information across all components of germplasm management (Perry, 1994) would provide a means to:

- assess the current status of conservation and characterization of the genetic resources in all participating collections;
- provide an indication of gaps that may exist in geographical representation or phenotypic/genotypic variability inherent in the collection;
- provide an indication of duplication (including intentional security duplication) of material between collections;
- assess the regeneration requirements at international level.

Some of the above objectives are currently filled by the Cassava Common Registry (accessible freely at http://www.cassavaregistry.com), with data inputs provided so far by CIAT and IITA. There one can consult the cassava databases of both institutions and introduce cassava germplasm requests to any of them.

13 Rationalizing a conservation strategy – *Manihot esculenta*

13.1 Elements of a conservation strategy

A conservation strategy for cassava should be comprehensive, secure, efficient and cost-effective. It needs to be agreed upon by all partner institutions that maintain or utilize genebanks. The current collections held by national programs, state or regional research and extension programs, universities, international centers, or other entities, vary considerably in the types of materials that are maintained and in the protocols for conservation and distribution. To some degree, each is free to determine independently what is in their best interest in this regard. However, those that have signed the International Treaty are committed to its standards and protocols. The international centers – CIAT and IITA – have broad international obligations to conserve cassava in a manner which meets long-term needs

of the global community. The range of holdings reflects these needs and interests. They include local landraces, introduced landraces, bred varieties (released materials), experimental breeding materials, mapping populations, and others.

A global conservation strategy should focus on landraces. These represent the range of diversity that has evolved over time in farmers' fields, under a combination of natural and human selections. All current genotypes (excepting those few that are the result of mutation or transformation events) are the result of recombinations of existing or extinct landrace varieties. The large majority of future genotypes will, as well, consist of genes that derive from existing or extinct landrace varieties. This is the set of genotypes that is of highest priority for secure conservation in perpetuity. Table 3 of this report gives some preliminary estimates of number of landrace varieties in most cassava-producing countries, but ultimately this number will be determined by extensive field collecting. Perhaps a more important question for conservation, however, is, how many genotypes are needed to represent the diversity of all landrace varieties of a country or region? This is a question that can only be answered with an extensive base of information from evaluations and molecular studies on diversity. For the present, we can only provide an educated guess. For the long-term, this will be a critical basis for determining the resources required for conservation. The numbers presented in Table 3 need to be broadly reviewed and evaluated by experts familiar with the diversity of each country. Although a draft of this report was widely distributed, feedback on this particular element of conservation was minimal. It appears to be an aspect of cassava conservation that has not received much thought or analysis up to now. The Table 3 estimate of almost 15,000 accessions needing to be conserved on a global basis, in order to fully represent cassava genetic diversity, should be considered a tentative number, but at the same time provides a starting point in planning for resource requirements in a global conservation strategy.

In the long term, a critical question to be answered is the desirability of conservation of genes versus conservation of genotypes. The question is fundamental to a conservation strategy both in terms of genetics and of financial resources. The conservation of genes can be done in either vegetative or seed form, whereas the conservation of specific genotypes (where perpetual regeneration is practiced) is only possible in vegetative form. The implications of this choice need to be a basic part of the discussions among cassava germplasm curators and users in the coming years (and probably of other vegetatively-propagated crop species as well). Insofar as breeders make direct recommendations of landraces to farmers, it is essential that these landraces be conserved vegetatively. But this is a strategy likely to be of diminishing importance, as continual breeding improvements are made and landraces play a continually lesser role for direct use as new varieties. However, their role as sources of genes will continue as long as cassava breeding continues. The genes of value can be derived from either seed-derived material, or from the original landraces maintained continually in vegetative form. Based on current information, seed conservation should be possible at a far lower cost than vegetative conservation. While it appears that a cassava conservation strategy should evolve in the long term toward seed conservation, both for reasons of genetic utility and of efficient resources management, in the short and medium term, we should continue a strategy based on vegetative conservation. Understanding that the longer term aim is seed conservation is an important factor in the design of a vegetative conservation strategy. This report has focused on a vegetative strategy, since it is the one of immediate concern, and needs to be established as a precursor to a seed conservation strategy.

13.2 Conservation scenarios

The elements of a strategy can be brought together in multiple ways. Some of these options are presented here as scenarios, that present choices based on activities, partnerships and funding. There is no single best choice. In part, the pathways chosen will depend on level of external funding available, and the level of commitment by participating institutions. Optimizing the organization of shared services can create substantial cost benefits and efficiencies, but at the same time requires a large investment in institutional planning and interaction. Networks abound that may play a role in conservation planning and activities, but there seems to be a balance between investments in assuring that networks function, and in investments that are directly targeted to operational matters. There is little point in funding planning meetings while the lack of funding for basic conservation functions like field genebank maintenance results

in germplasm losses. One of the more ambitious networks of the past 25 years, the International Network for Cassava Genetic Resources, was conceived well in every aspect except for the unexpected sharp decline of national and international funding that would be available. The entry of private funding into cassava research and development has been very slow, but that now appears to be changing. The starch and animal feed industries especially are dynamic and enthusiastic about the potential of cassava in these markets. Both have shown willingness to invest in genetic resources in a narrow way – where the benefits have a clear possibility of helping their industry – but these initial experiences can and should lead to productive partnerships between private and public sector institutions for cassava conservation.

Networks will be critical in establishing standards and agreements on conservation strategies and methods. These need not be formal networks, but rather groups that have the ability to meet, or communicate electronically, to develop agreements on conservation standards, on information management standards, on germplasm exchange procedures and standards, and on the means to achieve long-term support for conservation. South-south partnerships should be a significant part of the future of *Manihot* genebanks. The regions of less variation (e.g. Asia) should see a role in supporting conservation in the Americas for their own future benefit.

Table 9 summarizes some of the short- and medium-term research needs in cassava and *Manihot* conservation, who can do the research, and key elements of funding to achieve success. The most notable point is that there is a wide range of needs, and these will need to be tackled by an equally wide range of institutions doing diverse research.

Table 9 indicates a series of alternative scenarios in the broad scheme of a global conservation strategy for cassava, involving national programs and international centers, and a range of conservation methods. The following highlights some of the main points to consider:

Table 9. Alternative scenarios for conservation, based on an individual landrace variety.

Alternative conservation sites and methods										
National program		International center				*Black box*		Analysis of the global system		
Field	In vitro	Field	In vitro	Cryo	Seed	In vitro	Cryo	Security	Cost	Efficiency[a]
Current situation:[b]								Low	na	na
7,000	2,000	2,000	7,500	670	0	6,000	0	Med	Med	Med
Alternative future scenarios										
x		x						Low	Low	Low
x		x	x					Med	Med	Med
x	x		x			x		High	Med	Med
x	x		x	x			x	High	High	Med
	x		x					High	Med	Med
x			x			x		High	Low	High
x				x			x	High	Low	High
x				x	x		x	High	Med	High

[a] Cost-effectiveness of obtaining the designated level of *ex situ* security.
b Uncollected landrace varieties (existing in situ only) are estimated at about 17,000.

Security of conservation is a major consideration in a global strategy. This is basically a product of the level of security that any particular method provides, and the number of sites where each accession is replicated. Security involves inherent characteristics, management factors, and the unknown or unpredictable externalities. For example, recovery from cryopreservation is known to be successful only for a certain percentage of genotypes. This is an inherent trait of each clone (for a given protocol). Management has a critical influence on all forms of conservation. Externalities could include serious threats like war or long-term electrical outages. Therefore, any single site for conservation is at some level of risk, and the most cost-effective means of obtaining a high level of security is to

conserve accessions in different sites and in different forms. There is no magic formula to determine the right balance between level of security and cost. It seems clear that more than one site is essential, but it is also evident that resources are not unlimited, and there can be little justification for more than two or three sites for secure conservation of a given clone. Based on the Table 3 estimates, nearly two-thirds of landrace varieties exist only *in situ*. We did not have enough information to estimate number of varieties that are held in only one *ex situ* genebank.

In the long-term, a highly secure system for vegetative conservation could include *in vitro* conservation in one site and cryopreservation at two sites.

- There is a large number of uncollected landrace varieties in farmers' fields, which currently fall outside the realm of management by *ex situ* genebanks.
- Under the current situation, clones that are duplicated between national programs and IARCs are relatively secure, but there are probably a few thousand accessions in national programs that do not exist in the IARCs. These are in various states of security.
- There are multiple scenarios by which a high level of security of conservation can be achieved. Between national programs and IARCs, there should be at least three replications of each landrace accession, in order to achieve optimum conservation security.
- Greater than three replications *ex situ* is probably not cost efficient in terms of security, but it may be cost efficient for other reasons, such as specific research objectives of a genebank.
- The ideal combination of high security, low cost and high efficiency can be achieved with careful coordination between national genebanks and international centers.
- The international centers should provide a secure duplicate for national program genebanks, such that national programs can reduce their investment in duplication, while at the same time having access to field collections for efficient evaluation and breeding.
- Any national program is of course free to duplicate their collection as often as they wish, for whatever reason, in their own country.
- While cryoconservation can provide a high level of security at a low cost, there will probably always be an advantage in having *in vitro* slow growth or field collections at the national program level, as a means of easier facility of field regeneration for evaluation purposes.

13.3 A consensus strategy: collaborative centralization

In terms of a global strategy, there appears to be a relatively logical way forward that meets the cassava genetic resources conservation needs expressed by the scientists providing input to this report. The components of this strategy are:

- Collecting in priority areas is carried out to fill gaps, with the aid of genetic diversity studies and geographical information systems (GIS).
- National program genebanks and the CG centers (CIAT and IITA) develop a common cassava registry at a global level, based on passport, morphological and molecular information.
- The CG centers continue their long-term commitment to the secure conservation of landraces. In the short- to medium-term, they should continue with their continental mandates – Asia and the Americas for CIAT and Africa for IITA – due to the virus problems exclusive to each continent, and the risks associated with moving vegetative material.
- In the long-term, the CG centers should consider a unified conservation strategy, where each holds a global collection, and each serves as a backup to the other.
- The CG centers will duplicate landraces that are currently held only by national programs. Currently the centers appear to hold about 50-60% of the total *ex situ* accessions.
- The CG centers formalize a long-term commitment to replacing any materials lost from national program collections, at the request of the national program.

- The CG centers maintain at least two forms of each accession. Currently this may be an *in vitro* active genebank plus a black box duplicate kept in another center. In the future, cryopreserved accessions will be either the main or the backup genebank.
- The CG centers commit to meeting the demands and phytosanitary requirements for international exchange of cassava landrace varieties under terms of the International Treaty. Along with this, it is urgent to develop protocols for the safe movement of vegetative germplasm between the Americas and Africa.
- The national program genebanks commit to long-term conservation of their national genetic resources, at a level of security that assures low risk to the collection, but without the need to invest in expensive infrastructure, or in a duplicate collection within the country.
- Many national programs will find that field collections are the most cost effective and practical means of conservation. This system normally allows a combination of moderately secure year-to-year conservation if proper precautions are followed, and has the additional advantage that planting material is readily available for evaluations.
- A structure is developed for periodic interaction among stakeholders. Most notably this will be between the CG centers and the national programs. Each will have a formal responsibility to periodically inform the other of the status of each collection.

This strategy could be described as one of collaborative centralization. There are compelling reasons to rethink a decentralized strategy where each national program has the ability to conserve its germplasm in a highly secure system, which normally involves a field collection backed up by an *in vitro* collection. There have been some significant changes in the world of cassava genetic resources that impact the structure of an optimum conservation strategy. First, the status of the collections maintained by the CGIAR has been clarified. These collections are now part of the Multilateral System of the International treaty under its Article 15. Secondly, international exchange has become much safer and more acceptable with advances in virus indexing.

This environment allows us to think in new ways about the optimum conservation system for cassava. Conservation *in vitro* (slow growth or cryopreserved) is highly non site-specific and therefore large efficiencies can be gained by centralization. This centralization in the international centers now becomes politically viable, because ownership is clarified, and international exchange is clarified and more secure from a quarantine perspective. We now have an opportunity to develop a strategy that is biologically and economically rational, creates a structure of interdependence and collaboration among genebanks, and at the same time conforms to the new policy environment.

13.4 Implementation and funding

The first step in implementation of the collaborative centralization strategy will be to formalize agreement at the international level, among the CG centers and at least the major national cassava genebanks. This formal discussion and agreement is important, since this recommendation represents a major shift in strategy, not just strengthening of a current strategy. The consensus among the participants in the cassava conservation workshop (CIAT, 30 April – 2 May 2008), and among the respondents to the genebank survey serves as an important step toward formal agreement. It is this formal international agreement that will provide the basis and the impetus to initiate a broad range of conservation-related activities. This one step is the most fundamental action required, since it is a prerequisite to setting in motion most of the subsequent activities in the global strategy.

The greatest needs in cassava genetic resources conservation are often not those of greatest technical difficulty. Rather, they tend to be issues of inadequate funding for fairly simple and routine tasks. Substantial progress has been made in some of the more complex issues of cassava germplasm management, such as virus identification and indexing, fine-tuning of *in vitro* media for slower growth, cryopreservation pre- and post-treatments, and molecular fingerprinting. It is true, of course, that there are still many challenging scientific questions that will require significant investment.

At the same time, many programs struggle with the basic ability to maintain a field collection, manage the information systematically and accurately, and evaluate it in appropriate environments for the benefit of growers and consumers. The most urgent challenges in terms of securing the long-term preservation of cassava germplasm appear to be in providing support to the programs most at risk of permanently losing accessions. Providing support to programs that meet the criteria for standard conservation protocols and for international exchange attacks the other side of the same problem. This dual thrust of reducing the risks of loss of genetic diversity within at-risk genebanks, along with supporting the ability to duplicate these materials in an international center for secure back-up, will go a long way toward preventing serious erosion of cassava genetic resources.

It must be emphasized that the conservation strategy proposed here by the stakeholders of cassava germplasm represents a major shift in the operational structure of the global conservation system. The emphasis placed on centralized secure conservation, and the role of the international centers, collaboratively with the national programs, means that there will need to be high level discussions to put into place the agreements that will allow these new efficiencies to take place. This strategy does not simply involve a series of projects to strengthen existing components of a conservation strategy, but changes the strategy itself. Table 10 lists a set of key short- and medium-term research areas for cassava conservation and related strategies – activities that can only proceed after agreements are reached at the research director level (national and international institutions) to implement the new strategy.

Table 10. Key short and medium-term (up to 10 years) research needs in cassava conservation and related activities

Research area	Priority	Lead institutions	Collaborators	Funding needs
Comprehensive register of global genebank holdings	High	IARCs	Cassava genebanks	Consultant(s) to visit or correspond with major genebanks
Duplication of natl. progr. collections in IARCs	High	IARCs	Cassava genebanks	Preparation and shipping; expanded *in vitro* storage
Identify priority collection areas (see also Section 5.1)	High	National PGR programs	Cassava genebanks; IARCs	Molecular studies of landraces; GIS studies
Collection in priority regions	High	National PGR programs	Cassava genebanks	Expeditions; post-collection management
Establish mechanisms for genebank communication and coordination	High	PGR Networks	IARCs and National PGR programs	Periodic meetings and consultations; published proceedings
Evaluation for traits of importance, especially for future novel uses	High	National breeding programs	IARCs and National PGR and programs	Support for field and lab studies
In-country safety backup of natl. progr. collections	Med	National PGR programs	Cassava genebanks	Field or *in vitro* facilities and personnel
Coordinated documentation	Med	IARCs; Cassava genebanks	National PGR programs	Workshops; consultations
Cryopreservation	Med	IARCs	National PGR programs	Pre/post freezing research
In vitro very slow growth	Med	IARCs	Cassava genebanks; universities	Media and environment studies
Flower induction for seed genebank	Med	IARCs	Universities; cassava genebanks	Physiology and hormone studies

14 Rationalizing a conservation strategy – *Manihot* wild species

Apart from the fact that cassava and the wild species are part of the same genus, there is not much resemblance between the two groups in terms of conservation strategies. First, cassava is included in Annex I of the International Treaty, while the wild *Manihot* species are not (though the wild accessions maintained by the CG Centres come under the Multilateral System through Article 15). This has implications for the support that may be available for the wild species management, and for international exchange. It is important that this be a topic of continuing conversation in the Treaty deliberations in the future.

The wild species are seed-propagated in nature and cassava is universally vegetatively propagated in production systems (excepting the occasional volunteer seedlings that are found and tended by farmers). While cassava collections are held in some 75 genebanks around the world, there are only a handful of wild *Manihot* collections. Brazil (EMBRAPA and Universidade de Brasilia) and CIAT hold the only major collections. While *in vitro* culture is routine for cassava, most of the wild species have not been tested for their suitability to this conservation system. Where species have been tested, there are frequently species-specific requirements for optimum *in vitro* growth. Many of the conservation techniques and procedures are still experimental in the case of the wild species, so standardizing recommendations for a coordinated global system is somewhat more difficult.

Given the difficulties and costs of *Manihot* wild species conservation, it is expected that only a few institutions will take on these challenges in a comprehensive manner, for the long-term future. Table 11 lists the critical research areas for these species. It should be noted that even though there are only a few wild species genebanks, there is a broad array of research needs, in which many institutions can participate. Given the importance of the wild species to the long-term goals of cassava improvement, all the tools need to be made available for their secure conservation, taxonomic classification, phylogeny, evaluation and use in breeding.

Table 11. Key short and medium-term (up to 10 years) research needs in wild *Manihot* conservation and related activities.

Research area	Priority	Lead institutions	Collaborators	Funding needs
Taxonomy	High	National PGR programs	Universities; botanical gardens; *Manihot* genebanks	Post-graduate research and national program scientists
Threats to habitat	High	National PGR programs	Universities; national environmental agencies; *Manihot* genebanks	Support to ongoing studies, esp. Brazil and Mexico, to include *Manihot;* policy advocacy
Collection in priority regions	High	National PGR programs	*Manihot* genebanks	Support to national PGR programs for direct costs
Crossability studies	High	National PGR programs	IARCs	Support for field and lab studies
Evaluation for traits of importance	High	National PGR programs	IARCs	Support for field and lab studies
Phylogeny studies	Med	National PGR programs	Universities; botanical gardens; *Manihot* genebanks	Post-graduate research and national program scientists
Population genetics for conservation	Med	National PGR programs	Universities; *Manihot* genebanks	Theoretical studies; field test
Seed physiology	Med	*Manihot* genebanks	Universities	Basic standard lab studies
In vitro techniques	Med	*Manihot* genebanks	Universities; IARCs	Media and light/temperature studies

Table 11. Key short and medium-term (up to 10 years) research needs in wild *Manihot* conservation and related activities.

Research area	Priority	Lead institutions	Collaborators	Funding needs
Cryopreservation	Low	*Manihot* genebanks	Universities; IARCs	Pre/post freezing research
Field propagation techniques	Med	*Manihot* genebanks	Universities; IARCs	Lab/field studies under semi-controlled conditions

Appendix I. Workshop program

Manihot Genetic Resources: Strategies for Long-Term Conservation

30 April - 2 May 2008

Calima Room

CIAT Headquarters, Cali, Colombia

		Presenters and Moderators
Wednesday 30 April 2008		
Workshop Opening		
8:00 - 8:15	**Registration**	
8:15 - 8:30	**Welcome and Workshop Opening**	**Joe Tohme**
8:30 - 9:00	**Workshop goals and organization; program overview; introduction of participants**	**Clair Hershey**
9:00 - 9:20	**Goals and strategies of GCDT for CGR conservation; specific goals of study**	**Luigi Guarino**
9:20 - 9:50	**Keynote**	**Anthony Bellotti**
SESSION I: Understanding *Manihot G*enetic Diversity		
Session Chair:	**Alfredo Alves**	
9:50 - 10:20	**Studies on Manihot evolution; mutants and novel traits recently discovered**	**Luiz Carvalho**
10:20 - 10:40	**Coffee**	
10:40 - 11:10	**Quantifying genetic diversity of landrace varieties: experiences and conclusions from Latin America and Africa, and implications for conservation strategies**	**Paula Ximena Hurtado and Martin Fregene**
11:10 - 11:40	**Conclusions from molecular fingerprinting of CIAT core collection and local germplasm**	**Peaingpen Sarawat**
11:40 - 12:10	**Functional classification of cassava genes and implications for the germplasm base of cassava**	**German Plata and Joe Tohme**
12:10 - 13:10	**Lunch - CIAT restaurant**	

13:10 - 13:40	**Geographical Information Systems support to cassava and wild Manihot collection and in situ conservation**	**Luigi Guarino and Andrew Jarvis**
13:40 - 14:10	**Cassava pre-breeding at CIAT and implications for germplasm conservation, evaluation and exchange**	**Hernan Ceballos**
14:10 - 14:30	**Coffee**	
TOUR		
14:30 - 16:30	**Tour of CIAT Genetic Resources Unit**	**Daniel Debouck, Graciela Mafla, Maritza Cuervo, Roosevelt Escobar, Cesar Ocampo, Ericson Aranzales, Angela Hernandez**
16:30 - 17:30	**Wrap of GRU tour: Criteria and studies for decision-making in cassava conservation**	**Daniel Debouck**

Thursday 1 May 2008

SESSION II: Status and Needs of Existing Genebanks

Session Chair:	**Daniel Debouck**	
8:00 - 8:30	**Status and needs of cassava germplasm conservation in Brazil**	**Wania Fukuda**
8:30 - 9:00	**Wild Manihot collection and conservation in Brazil**	**Alfredo Alves**
9:00 - 9:30	**Status and needs of cassava germplasm conservation in Meso-America and the Caribbean**	**Sergio Rodriguez**
9:30 - 10:00	**Status and needs of cassava germplasm conservation in Venezuela, Colombia, Ecuador and the Guyanas**	**Antonio Lopez**
10:00 - 10:20	**Coffee**	
10:20 - 10:40	**Status and needs of cassava germplasm conservation in Peru, Bolivia, Paraguay and Argentina**	**Llerme Rios Lobos**
10:40 - 11:10	**Status and needs of cassava germplasm conservation in Africa**	**Paul Ilona**
11:10 - 11:40	**Overview of IITA cassava genebank**	**Dominique Dumet**

11:40 - 12:10	**Status and needs of cassava germplasm conservation in Asia**	**Peaigpen Sarawat**
12:10 - 13:10	**Lunch – CIAT restaurant**	
1:10 - 2:30	**Group discussion: Genebank surveys -- status and follow-up**	**Moderator: Clair Hershey**

SESSION III: Elements of a Long-Term Conservation Strategy (Part I) (Group discussions)

Session Chair: **Llerme Rios**

14:30 - 15:00	**Characterization, evaluation and information management**	**Moderator: Antonio Lopez**
15:00 - 15:20	**Coffee**	
15:20 - 16:10	**Regeneration, conservation and safety duplication needs**	**Moderator: Sergio Rodriguez**
16:10 - 16:40	**Networks and international cooperative programs**	**Moderator: Dominique Dumet**
16:40 - 17:00	**Capacity-building needs**	**Moderator: Alfredo Alves**
19:00	**Depart to Cali**	
20:00	**Dinner in Cali**	

SESSION III: Elements of a Long-Term Conservation Strategy (Part II) (Group discussions)

Session Chair: **Wania Fukuda**

Friday 2 May 2008

8:00 - 8:20	**Bioversity International: goals and strategies for crop genetic resources in the Americas**	**Xavier Scheldeman**
8:20 - 8:50	**International exchange: genebank needs and obligations**	**Moderator: Paul Ilona**
8:50 - 9:20	**Conservation and germplasm users: developing a collaborative relationship**	**Moderator: Sergio Rodriguez**
9:20 - 9:50	**Genebank services and service providers: a look at future scenarios**	**Moderator: Antonio Lopez**
9:50 - 10:10	**Coffee**	
10:10 - 10:40	**Criteria for the most important collections for GCDT support: group recommendations**	**Moderator: Luiz Carvalho**

10:40 - 11:10	**Conservation strategies for smaller collections**	**Moderator: Peaingpen Sarawat**
11:10 - 11:40	**Conservation in perpetuity: overview of what is required**	**Moderator: Daniel Debouck**
11:40 - 12:30	**Wrap-up and conclusions**	**Clair Hershey and Daniel Debouck**
12:30 - 13:30	**Lunch**	
Cassava Descriptors - a Mini-Workshop of Bioversity International		
13:30 - 14:30	**A strategy for developing cassava descriptors**	**Xavier Scheldeman and Clara Ines Quintero Gonzales**

Appendix II. Workshop list of presenters and participants

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*** Presenters (25)**

Appendix III. Cassava and wild *Manihot* survey form

Feb. 2008
A Survey to Build a Global Conservation Strategy for Cassava and Wild *Manihot* Species

Background
The Global Crop Diversity Trust is supporting efforts to develop strategies for the efficient and effective conservation of crop diversity on both a regional and global crop basis. This questionnaire has been developed in order to seek the advice and input of representatives of the world's major cassava and wild *Manihot* collections in the development of the conservation strategy. In particular the questionnaire seeks to assess the status of cassava conservation throughout the world and to identify major needs. It is intended that the Global Crop Diversity Trust (Trust) will base its support for the conservation of cassava genetic resources on this strategy, once developed and adopted. We kindly request you to review questionnaire in advance, improve it and use it as a reference for your presentation or the discussion. We are keen to ensure your active participation in the development of the global cassava conservation strategy.

This survey is divided into two sections.

A. **Cultivated cassava collections**
B. **Wild *Manihot* species collections**

Please fill out all sections that are relevant to your situation. If you manage only cultivated species, there is no need to complete Section B, or if you manage only wild species, there is no need to complete Section A.

SECTION A. CULTIVATED CASSAVA COLLECTIONS

1. <u>Institutional information</u>

1.1. Name and address of organization holding/maintaining the collection	
Name:	
Address:	
City:	
Postal Code:	
Country:	
Web site:	
Curator in charge of the collection:	
Name:	
Address:	
City:	
Telephone:	
Fax:	
Email:	
Name of respondent to this questionnaire if different than above	
Contact details:	
Date of response:	

1.2. Is the organization holding the cassava collection:

- ☐ An independent organization
- ☐ Part of a larger organization
- ☐ A government organization
- ☐ Other (specify): _____

In the case of (B) please provide the name and address of the larger organization: _____
_____
_____

1.3. Who is financing of the conservation of the cassava collection?

- ☐ Government _____%
- ☐ Private sector _____%
- ☐ International or regional funding _____%
- ☐ Other (specify): _____ _____%

1.4. Who is the legal owner of the collection?

- ☐ Institution in charge
- ☐ Other (specify): _____

1.5. How much time is devoted to the management of the cassava collection?

_____ Full time equivalent (fte) per year (1 fte means that a person is working for 100% on the cassava collection)

2. <u>Details on the cassava collection</u>

2.1. Year of formal establishment of the collection:_____

2.2. What is the main objective of the conservation of the collection (in terms of use and of conservation): _____

2.3. Present size of the cassava collection:

Type of cassava germplasm	Total number of accessions	% available for distribution
Farmers' varieties		
Breeders' varieties		
Experimental materials		
Others		
Total		

2.4. Origin of the collection. Please state the percentage of accessions included in the collection of:

- Local origin previously collected in own country: _____%
- Introduced from abroad from the centre of diversity: _____%
- Introduced from abroad, outside the centre of diversity: ____ %
- Other origin _____%

3. <u>Management of the cassava collection</u>

3.1 Acquisition

3.1.1. Was the collection increased during the last 10 years with new accessions (after the international agreement on genetic resources movement)?

☐ yes ☐ no

- If yes, how many new accessions were included of the following:
  - Landrace varieties: _____
  - Modern varieties: _____
  - Breeding material: _____
  - Other: _____

3.1.2. How was the acquisition of the newly obtained germplasm conducted?

☐ Collecting in own country
☐ Collecting in other countries
☐ Introduction from other collections, institutes or private organizations
☐ Other sources – please specify: _____

3.1.3. Are there important gaps in the collection?

☐ yes ☐ no

- If yes, what are the main gaps: _____

_____

3.1.4. Do you plan to fill in these gaps in the next 5 years? ☐ yes ☐ partly ☐ no

- If yes, how
  - ☐ Collection
  - ☐ Introduction
  - ☐ Other
- If no, what are the main reasons:_____

3.2 Storage and maintenance (seed, *in vitro*, field)

3.2.1. Please indicate how germplasm is maintained for long- and medium-term storage (check all boxes that apply and indicate percent of total)

Type of germplasm	Botanical seeds	Field	*In vitro*	Greenhouse/ screenhouse	*Cryo-con-servation*
Farmers' varieties	☐ %____	☐ %____	☐ %____	☐ %____	☐ %____
Breeders' varieties	☐ %____	☐ %____	☐ %____	☐ %____	☐ %____
Experimental materials	☐ %____	☐ %____	☐ %____	☐ %____	☐ %____
Others	☐ %____	☐ %____	☐ %____	☐ %____	☐ %____

3.2.2. What are the storage facilities and conditions of the cassava genebank?

	Type of facility	Temperature (ºC)	RH %	Packing material
Botanical seeds				
Field				
In vitro				
Greenhouse/screenhouse				
Cryo-conservation				

3.2.3. What is the field (F) or greenhouse/screenhouse (G) maintenance protocol for the cassava genebank?

	Number of plants per accession*	Distance between rows	Distance between plants
Farmers' varieties	F:____ G:____	F:____ G:____	F:____ G:____
Breeders' varieties	F:____ G:____	F:____ G:____	F:____ G:____
Experimental materials	F:____ G:____	F:____ G:____	F:____ G:____
Others	F:____ G:____	F:____ G:____	F:____ G:____

* In case of 1, it would be considered as 1 plant/pot within a greenhouse/screenhouse.

3.2.4. Do you apply tests to control the quality of stored germplasm?

☐ yes ☐ partly ☐ no

If yes or partly, which tests are conducted?
☐ Germination test of sexual seed
☐ Control of the vitality and health of stem cuttings
☐ Control of true-to-type-ness of *in vitro* plantlets
☐ Other _____

3.3 Regeneration

3.3.1. Method of regeneration: Please indicate how the cassava germplasm is regenerated.

- As population (sexual seed): ☐ yes ☐ no
- Vegetative by means of stem cuttings or other: ☐ yes ☐ no
- In vitro: ☐ yes ☐ no

Note: More than one option for the same type of material is possible

3.3.2. On how many plants (pl) is the regeneration (population) normally based?

☐ < 10 pl ☐ 10- 20 pl ☐ 21 – 30pl ☐ > 30 pl

3.3.3. How many cuttings (cu) are planted for the next vegetative regeneration?

☐ < 15 cu ☐ 15 –30 cu ☐ 31 to 45 cu ☐ > 45 cu

3.3.4. How many plantlets (pl) are maintained for *in vitro* regeneration?

☐ < 10 pl ☐ 11 –30 pl ☐ >30 pl

3.3.5. Annual capacity of regeneration/multiplication (number of accessions)

Type of germplasm	As population (sexual seed)	Vegetative by means of cuttings	*In vitro*
Farmers' varieties			
Breeders' varieties			
Experimental materials			
Others			

Note: More than one option for the same type of material is possible

3.3.6. Percentage of the collection that needs to be urgently regenerated:

- Primitive forms _____%
- Modern varieties _____%

- Others & research material, etc_____%

3.4 Identification (classification) and characterization

3.4.1. Which type of material of the cassava collection is characterized?

Type of germplasm	*Morphological characterization*	Molecular characterization
Farmers' varieties	☐ yes ☐ no	☐ yes ☐ no
Breeders' varieties	☐ yes ☐ no	☐ yes ☐ no
Experimental materials	☐ yes ☐ no	☐ yes ☐ no
Others	☐ yes ☐ no	☐ yes ☐ no

3.4.2. Which type of descriptor list is used for characterization?

☐ Standard list of IPGRI
☐ Your own independently developed list
☐ List developed by another organization (specify): ……

3.5 Documentation and access to information about the collection

3.5.1. Do you use a database information system for the management of the cassava collection?

☐ yes ☐ partly ☐ no

If yes, what software is used for the documentation? _____

3.5.2. Which kind of data of the collection has been computerized? Please check the appropriate answer.

Type of germplasm	Passport data	Characterization/ evaluation data	Management data*
Farmers' varieties	☐ yes ☐ partly ☐ no	☐ yes ☐ partly ☐ no	☐ yes ☐ partly ☐ no
Breeders' varieties	☐ yes ☐ partly ☐ no	☐ yes ☐ partly ☐ no	☐ yes ☐ partly ☐ no
Experimental materials	☐ yes ☐ partly ☐ no	☐ yes ☐ partly ☐ no	☐ yes ☐ partly ☐ no
Others	☐ yes ☐ partly ☐ no	☐ yes ☐ partly ☐ no	☐ yes ☐ partly ☐ no

* data related to storage, germination, distribution, etc.

3.5.3. In case the cassava collection is not computerized, are there plans to do so in the future?

☐ No plans
☐ Computerization planned within 3 years

3.5.4. Is information of the cassava collection accessible through the Internet?

☐ yes ☐ partly ☐ no URL: _____

3.5.5. Are data of the cassava collection included in other databases? If yes or partly, specify the database

- National ☐ yes ☐ partly ☐ no _____
- Regional ☐ yes ☐ partly ☐ no _____
- International ☐ yes ☐ partly ☐ no _____

3.6 Health of germplasm

3.6.1. Is the cassava collection affected by diseases that can restrict the distribution of the germplasm?

☐ **yes** ☐ **slightly, or only few accessions** ☐ **no**

If yes or slightly, which types of diseases are causing this restriction?

☐ Seed-borne diseases in sexual seed
☐ Infection in maintained plants
☐ Virus or viroid-infected in vitro plantlets

3.6.2. If in vitro samples are distributed within the country are they virus indexed?

☐ **yes** ☐ **some** ☐ **no**

3.6.3. If in vitro samples are distributed outside the country are they virus indexed?

☐ **yes** ☐ **some** ☐ **no**

3.6.4. Is knowledge available at your institution and are there facilities for eradication of these diseases?

☐ **yes** ☐ **limited** ☐ **no**

3.6.5. Do you need assistance to improve the health status of the cassava collection?

☐ **yes** ☐ **limited** ☐ **no**

If yes, what type of assistance will be required?

1) _____
2) _____
3) _____

3.7 Distribution

3.7.1. Do you distribute material outside your institute, within the country? ☐ yes ☐ no

(If **no**, please go to Section 3.7.3)

3.7.2. How many accessions have you distributed within the country in the past 5 years to the following users?

Sent to:	Wild species	Landrace varieties	Experimental materials	Other
Breeders, other researchers				
Farmers				
Genebanks				
Extensionists				
Others (specify)				

3.7.3. Do you distribute material outside the country? ☐ yes ☐ no

(If **no**, please go to Section 3.8)

3.7.4. How many accessions have you distributed outside the country in the past 5 years to the following users?

Sent to:	Wild species	Landrace varieties	Experimental materials	Other
Breeders, other				

researchers				
Farmers				
Genebanks				
Extensionists				
Others (specify) _____				

3.7.5. Do you set specific conditions for distribution? ☐ yes ☐ no
If yes, please specify: _____

3.7.6. Is the germplasm sufficiently available for distribution?

- Sexual seed: ☐ yes ☐ partly ☐ no
- Cuttings: ☐ yes ☐ partly ☐ no
- In vitro plantlets: ☐ yes ☐ partly ☐ no

3.7.7. Compared to 5 years ago, are you now distributing <u>less</u>, the <u>same</u> amount, or <u>more</u> germplasm? ☐ less ☐ same ☐ more

3.7.8. Do you expect to distribute <u>less</u>, the <u>same</u> amount, or <u>more</u> germplasm 5 years from now?
☐ **less** ☐ **same** ☐ **more**

3.7.9. Do you keep records of the distribution? ☐ yes ☐ No

3.7.10 What information is kept in these records?

_____

_____

_____

3.7.11 Do you request and get any feedback from the recipients? ☐ yes ☐ No

3.7.12. What use is made of the information? _____

_____

3.7.13. How are the services of the collection publicized to users and how effective are these methods in terms of increased use of the collection?

	High impact	Medium impact	Low impact	Don't know
Scientific publications				
Institution reports				
Extension leaflets				
Oral presentations				
Visits to collection				
Other (specify) _____				

3.7.14. Have any requests for material been refused? If yes, specify: _____
_____

3.7.15. How do the users of the germplasm influence the management of the collection? Indicate *yes* or *no* in table below.

	Through feedback on material?	Through formal consultations	Through participating n the governing body of the genebank	Other (specify) _____ _____
Breeders, other researchers				
Farmers				
Genebanks				
Extensionists				
Others (specify) _____				

3.8 Safety duplication

3.8.1. Are the accessions of the cassava collection safety-duplicated in another genebank?

☐ yes _____% duplicated ☐ no ☐ uncertain

If yes, please specify where the germplasm is safety-duplicated _____

Storage conditions _____

3.8.2. Is there any germplasm of other cassava collections safety-duplicated at your facilities?

☐ yes ☐ no

If yes, can you specify the name of the holder of the cassava collection safety-duplicated at your genebank including the number of accessions duplicated?

Collection holder: _____ Accessions duplicated (no.): _____

3.9 General management

3.9.1. How many staff are working on the collection (full-time staff equivalents)?

Mark the appropriate boxes with an X.

	<1	1	2	3-5	>5
In the field or greenhouse/screenhouse					
scientists					
technicians					
field workers					
students					
In the lab					
scientists					
technicians					
students					

3.9.2. Have you established a quality management system or written procedures and protocols for: (check each that applies)

☐ Acquisition (including collecting, introduction and exchange)
☐ Regeneration

- ☐ Characterization
- ☐ Storage and maintenance
- ☐ Documentation
- ☐ Health of germplasm
- ☐ Distribution
- ☐ Safety duplication

3.9.3. In case you have procedures and protocols, are you able to provide the Trust with this information or include a copy of it? ☐ yes ☐ no

3.9.4.Does the existing capacity in numbers and skills meet the needs of the collection in the long term (e.g. greater than 10 years)? ☐ yes ☐ no

If no, please describe what is needed. _____

_____

4. Utilization of the cassava collection

4.1. For what purposes is the cassava collection used? Check all that apply.

- ☐ Research (e.g. taxonomic, biosystematic, inheritance, evolutionary studies)
- ☐ Characterization
- ☐ Evaluation for important productivity & quality traits
- ☐ Plant breeding
- ☐ Biotechnology, e.g. gene isolation, molecular studies, functional genomics, etc
- ☐ Distribution to farmers
- ☐ Return of germplasm to country of origin

4.2. Do you have a systematic evaluation program to evaluate the collection for traits?

☐ yes ☐ planned ☐ no

If yes, can you list the most important traits the cassava collection is evaluated for?

_____

_____

_____

5. Networks of cassava genetic resources

5.1. Do you collaborate in (a) network(s) as a cassava collection holder?

☐ yes ☐ no

	National level	Regional level	Global level	None
Exchange of germplasm				
Exchange of information				
Training				
Other (specify): _____				

5.2. What are the major objectives of the network(s) in which you participate?

☐ Joint conservation of cassava germplasm
☐ Evaluation or characterization of cassava germplasm
☐ Establishment of central cassava database
☐ Rationalization of the collections
☐ Safety duplication of cassava germplasm
Remark: more than one option is possible

5.3. Do you consider a worldwide network for cassava genetic resources important and would you consider participating in such network?
☐ yes ☐ no

5.4. What will be your major interest for participation in a cassava genetic resources network?

6. <u>Policies with regard to access of the cassava collection</u>

6.1. What is your policy regarding distribution of cassava germplasm?
☐ Distribution to any *bona fide* users, without further conditions
☐ Distribution to any *bona fide* users after signing of a MTA (Material Transfer Agreement)
☐ Distribution only to users in own country
☐ Distribution only to users in certain countries after signing of a MTA
☐ Distribution only on a mutually agreed exchange basis
☐ Other flows of distribution, please specify: _____

6.2. Cost for distribution of cassava germplasm:
☐ No cost, free distribution
☐ No cost, but only on the basis of reciprocal exchange of material
☐ Request to contribute for processing and shipping, specify amount: _____
☐ Request to pay for each requested accession, specify amount: _____
☐ Other conditions requested, please specify: _____

6.3. Please attach examples of your organization's long-term commitment to long term conservation of cassava collection, for example:
☐ Legal status
☐ Institutional constitution
☐ Mandates
☐ Published strategic plans
☐ National conservation strategy
☐ Action plans
☐ Other: _____

7. <u>Future developments regarding the cassava collection</u>

7.1. Will the cassava collection be extended with new material or rationalized in the next five years?
☐ The collection will keep approximately the same size
☐ The collection will be expanded to a limited extent (5-10 %)
☐ The collection will be substantially increased (> 20%)
☐ The collection will be reduced due to duplication with other collections and internal rationalization
☐ The collection will be reduced as a result of lack of funding or facilities

7.2. Are there any constraints for maintenance of the cassava collection? ☐ yes ☐ no
If yes, what type of constraints do you face?
☐ Insufficiently trained staff

- ☐ Regeneration capacity to maintain the collection limited
- ☐ Facilities for optimal maintenance of the collection not satisfactory
- ☐ Others (please specify): _____

7.3. Will some of the above constraints result in a loss of cassava germplasm?

☐ yes ☐ only incidentally ☐ no

If yes, what is the most important constraint, which may contribute to genetic erosion within the collection? _____

8. <u>Core collection</u>

8.1. Have you identified a core collection within the total collection? ☐ yes ☐ no

8.2. If no, is there any plan to do so in the future? ☐ yes ☐ no

8.3. If yes, how many accessions are included in the core?

Number ____ Percent of total _____

8.4. What criteria were used to define the core collection?

- ☐ Geographic or agro-ecological origin
- ☐ Morphological characterization
- ☐ Molecular characterization
- ☐ Agronomic and market traits
- ☐ Others (please specify): _____

9. <u>Further remarks</u>

If you are responsible for a collection of wild *Manihot* species, please complete Section II. Otherwise, please see directions at the end of the document for returning the completed survey.

SECTION B. WILD *MANIHOT* COLLECTIONS

1. Institutional information

1.1. Name and address of organization holding/maintaining the collection	
Name:	
Address:	
City:	
Postal Code:	
Country:	
Web site:	
Curator in charge of the collection:	
Name:	
Address:	
City:	
Telephone:	
Fax:	
Email:	
Name of respondent to this questionnaire if different then above	
Contact details:	
Date of response:	

1.2. Is the organization holding the *Manihot* collection:

- ☐ An independent organization
- ☐ Part of a larger organization
- ☐ A government organization
- ☐ Other (specify): _____

In the case of (B) please provide the name and address of the larger organization: _____
_____
_____

1.3. Who is financing of the conservation of the *Manihot* collection?

- ☐ Government _____%
- ☐ Private sector _____%
- ☐ International or regional funding _____%
- ☐ Other (specify): _____ _____%

1.4. Who is the legal owner of the collection?

- ☐ Institution in charge
- ☐ Other (specify): _____

1.5. How much time is devoted to the management of the *Manihot* collection?

_____ Full time equivalent (fte) per year (1 fte means that a person is working for 100% on the cassava collection)

2. Details on the *Manihot* collection

2.1. Year of formal establishment of the collection: _____

2.2. What is the main objective of the conservation of the collection (in terms of use and of conservation): _____

Note: Please attach or insert a list of the number of accessions of each species, along with their origin.

2.3. Present size of the *Manihot* collection:

Number of species: _____
Total number of accessions: _____
% available for distribution: _____

2.4. Origin of the collection. Please state the percentage of accessions included in the collection of:

Local origin previously collected in own country: ____ %
Introduced from abroad from the centre of diversity: ____ %
Introduced from abroad, outside the centre of diversity: ____ %
Other origin: ____ %

3. Management of the *Manihot* collection

3.1 Acquisition

3.1.1. Was the collection increased during the last 10 years with new accessions (after the international agreement on genetic resources movement)? ☐ yes ☐ no

If yes, which species were included, and how many new accessions of each (insert or attach separate list if desired):

3.1.2. How was the acquisition of the newly obtained germplasm conducted?

☐ Collecting in own country
☐ Collecting in other countries
☐ Introduction from other collections, institutes or private organizations
☐ Other sources – please specify: _____

3.1.3. Are there important gaps in the collection? ☐ yes ☐ no

If yes, what are the main gaps: _____

3.1.4. Do you plan to fill in these gaps in the next 5 years? ☐ yes ☐ partly ☐ no

If yes, how
☐ Collection
☐ Introduction
☐ Other
If no, what are the main reasons:_____

3.2 Storage and maintenance

3.2.1. Please indicate how germplasm is maintained for long- and medium-term storage

(check all boxes that apply and indicate percent of total)

Botanical seeds: ☐ %____

Field: ☐ %____

In vitro: ☐ %____

Greenhouse/screenhouse: ☐ *%____*

Cryo-conservation: ☐ *%____*

3.2.2. What are the storage facilities and conditions of the *Manihot* species genebank?

	Type of facility	Part of collection represented (%)	Temp. (ºC)	RH (%)	Packing material
Botanical seeds					
Field					
In vitro					
Greenhouse/screenhouse					
Cryo-conservation					

3.2.3. What is the field (F)or greenhouse/screenhouse (G) maintenance protocol for the *Manihot* genebank?

Number of plants per accession*: F:____ G:____

Distance between rows: F:____ G:____

Distance between plants: F:____ G:____

* In case of 1, it would be considered as 1 plant/pot within a greenhouse/screenhouse.

3.2.4. Do you apply tests to control the quality of stored germplasm? ☐ yes ☐ partly ☐ **no**

If yes or partly, which tests are conducted?

☐ Germination test of sexual seed

☐ Control of the vitality and health of stem cuttings

☐ Control of true-to-type-ness of *in vitro* plantlets

☐ Other _____

3.3 <u>Regeneration</u>

3.3.1. Method of regeneration: Please indicate how the *Manihot* germplasm is regenerated.

As population (sexual seed) ☐ yes ☐ no

Vegetative by means of cuttings or other ☐ yes ☐ no

In vitro ☐ yes ☐ no

Note: More than one option for the same type of material is possible

3.3.2. On how many plants (pl) is the regeneration (population) normally based?

☐ < 10 pl ☐ 10- 20 pl ☐ 21 – 30pl ☐ > 30 pl

3.3.3. How many cuttings (cu) are planted for the next vegetative regeneration?

☐ < 15 cu ☐ 15 –30 cu ☐ 31 to 45 cu ☐ > 45 cu

3.3.4. How many plantlets (pl) are maintained for *in vitro* regeneration?

☐ < 10 pl ☐ 11 –30 pl ☐ >30 pl

3.3.5. Annual capacity of regeneration/multiplication (number of accessions)

As population (sexual seed) _____ (no. of seeds)

Vegetative by means of cuttings _____ (no. of cuttings)

In vitro _____ (no. of in vitro plantlets)

Note: More than one option for the same type of material is possible

3.3.6. Percentage of the collection that needs to be urgently regenerated: _____ %

3.4 <u>Identification (classification) and characterization</u>

3.4.1. Is the collection of wild *Manihot* species taxonomically classified?

☐ yes ☐ no ☐ partial (percent classified _____%)

3.4.2. Do you have assistance of a taxonomist for the classification of the *Manihot* germplasm?

☐ yes ☐ some ☐ no

3.4.3. Which type of characterization is done?

Morphological characterization	☐ yes	☐ no
Molecular characterization	☐ yes	☐ no

3.4.4. If morphological characterization is done, which type of descriptor list is used for characterization?

☐ Standard list of IPGRI
☐ Your own independently developed list
☐ List developed by another organization (specify): ……

<u>3.5 Documentation and access to information about the collection</u>

3.5.1. Do you use a database information system for the management of the *Manihot* species collection?

☐ yes ☐ partly ☐ no

If yes, what software is used for the documentation? _____

3.5.2. Which kind of data of the collection has been computerized? Please check the appropriate answer.

Passport data	☐ yes	☐ partly	☐ no
Characterization/ evaluation data	☐ yes	☐ partly	☐ no

Management data* ☐ yes ☐ partly ☐ no

* data related to storage, germination, distribution, etc.

3.5.3. In case the *Manihot* collection is not computerized, are there plans to do so in the future?

☐ No plans
☐ Computerization planned within 3 years

3.5.4. Is information of the *Manihot* collection accessible through the Internet?

☐ yes ☐ partly ☐ no URL: _____

3.5.5. Are data of the cassava collection included in other databases? If yes or partly, specify the database

National ☐ yes ☐ partly ☐ no _____
Regional ☐ yes ☐ partly ☐ no _____
International ☐ yes ☐ partly ☐ no _____

3.6 Health of germplasm

3.6.1. Is the cassava collection affected by diseases that can restrict the distribution of the germplasm?

☐ **yes** ☐ **slightly, or only few accessions** ☐ **no**

If yes or slightly, which types of diseases are causing this restriction?

☐ Seed-borne diseases in sexual seed
☐ Infection in maintained plants
☐ Virus or viroid-infected in vitro plantlets

3.6.2. If in vitro samples are distributed within the country are they virus indexed?

☐ **yes** ☐ **some** ☐ **no**

3.6.3. If in vitro samples are distributed outside the country are they virus indexed?

☐ **yes** ☐ **some** ☐ **no**

3.6.4. Is knowledge available at your institution and are there facilities for eradication of these diseases?

☐ **yes** ☐ **limited** ☐ **no**

3.6.5. Do you need assistance to improve the health status of the cassava collection?

☐ **yes** ☐ **limited** ☐ **no**

If yes, what type of assistance will be required?

1) _____
2) _____
3) _____

3.7 Distribution

3.7.1. Do you distribute material outside your institute, within the country?

☐ yes ☐ no

If **no**, go to Section 3.7.3

3.7.2. How many accessions have you distributed within the country in the past 3 years to the following users?

Sent to:	Wild species	Landrace varieties	Experimental materials	Other
Breeders, other researchers				
Farmers				
Genebanks				
Extensionists				
Others (specify)				

3.7.3. Do you distribute material outside the country?

☐ yes ☐ no

If **no**, go to Section 3.8

3.7.4. How many accessions have you distributed outside the country in the past 3 years to the following users?

Sent to:	Wild species	Landrace varieties	Experimental materials	Other
Breeders, other researchers				
Farmers				
Genebanks				
Extensionists				
Others (specify) _____				

3.7.5. Do you set specific conditions for distribution? ☐ yes ☐ no

If yes, please specify: _____

3.7.6. Is the germplasm sufficiently available for distribution?

Sexual seed:	☐ yes	☐ partly	☐ no
Cuttings:	☐ yes	☐ partly	☐ no
In vitro plantlets:	☐ yes	☐ partly	☐ no

3.7.7. Compared to 5 years ago, are you now distributing <u>less</u>, the <u>same</u> amount, or <u>more</u> germplasm? ☐ less ☐ same ☐ more

3.7.8. Do you expect to distribute <u>less</u>, the <u>same</u> amount, or <u>more</u> germplasm 5 years from now?

☐ **less** ☐ **same** ☐ **more**

3.7.9. Do you keep records of the distribution? ☐ yes ☐ No

3.7.10. What information is kept in these records?

_____

_____

3.7.11. Do you request and get any feedback from the recipients? ☐ yes ☐ No

3.7.12. What use is made of the information? _____
_____

3.7.13. How are the services of the collection publicized to users and how effective are these methods in terms of increased use of the collection?

	High impact	Medium impact	Low impact	Don't know
Scientific publications				
Institution reports				
Extension leaflets				
Oral presentations				
Visits to collection				
Other (specify) _____				

3.7.14. Have any requests for material been refused? If yes, specify: _____
_____

3.7.15. How do the users of the germplasm influence the management of the collection? Indicate *yes* or *no* in table below.

	Through feedback on material?	Through formal consultations	Through participating in the governance of the genebank	Other (specify) _____ _____
Breeders, other researchers				
Farmers				
Genebanks				
Extensionists				
Others (specify) _____				

3.8 Safety duplication

3.8.1. Are the accessions of the *Manihot* collection safety-duplicated in another genebank?

☐ yes _____% duplicated ☐ no ☐ uncertain

If yes, please specify where the germplasm is safety-duplicated _____

Storage conditions _____

3.8.2. Is there any germplasm of other Manihot collections safety-duplicated at your facilities?

☐ yes ☐ no

If yes, can you specify the name of the holder of the cassava collection safety-duplicated at your genebank including the number of accessions duplicated?

Collection holder: _____ Accessions duplicated (no.): _____

3.9 General management

3.9.1. How many staff are working on the collection (full-time staff equivalents)?

Mark the appropriate boxes with an X.

	<1	1	2	3-5	>5
In the field or greenhouse/screenhouse					
scientists					
technicians					
field workers					
students					
In the lab					
scientists					
technicians					
students					

3.9.2. Have you established a quality management system or written procedures and protocols for (check each that applies):

- ☐ Acquisition (including collecting, introduction and exchange)
- ☐ Regeneration
- ☐ Characterization
- ☐ Storage and maintenance
- ☐ Documentation
- ☐ Health of germplasm
- ☐ Distribution
- ☐ Safety duplication

3.9.3. In case you have procedures and protocols, are you able to provide the Trust with this information or include a copy of it? ☐ yes ☐ no

3.9.4. Does the existing capacity in numbers and skills meet the needs of the collection in the long term (e.g. greater than 10 years)? ☐ yes ☐ no

If no, please describe what is needed. _____

_____

4. Utilization of the* Manihot *collection germplasm

4.1. For what purposes is the *Manihot* collection used? Check all that apply.

- ☐ Research (e.g. taxonomic, biosystematic, inheritance, evolutionary studies)
- ☐ Characterization
- ☐ Evaluation for important productivity & quality traits
- ☐ Plant breeding
- ☐ Biotechnology, e.g. gene isolation, molecular studies, functional genomics, etc
- ☐ Distribution to farmers
- ☐ Return of germplasm to country of origin

4.2. Do you have a systematic evaluation program to evaluate the collection for traits?

☐ yes ☐ planned ☐ no

If yes, can you list the most important traits the cassava collection is evaluated for?

_____

_____

_____

5. Networks of* Manihot *genetic resources

5.1. Do you collaborate in (a) network(s) as a cassava collection holder?

☐ yes ☐ no

	National level	Regional level	Global level	None
Exchange of germplasm				
Exchange of information				
Training				
Other (specify): _____				

5.2. What are the major objectives of the network(s) in which you participate?

☐ Joint conservation of cassava germplasm
☐ Evaluation or characterization of cassava germplasm
☐ Establishment of central cassava database
☐ Rationalization of the collections
☐ Safety duplication of cassava germplasm
Note: more than one option is possible

5.3. Do you consider a worldwide network for *Manihot* genetic resources important and would you consider participating in such network?

☐ yes ☐ no

5.4. What will be your major interest for participation in a *Manihot* genetic resources network?

_____

6. Policies with regard to access of the *Manihot* collection

6.1. What is your policy regarding distribution of *Manihot* germplasm?

☐ Distribution to any *bona fide* users, without further conditions
☐ Distribution to any *bona fide* users after signing of a MTA (Material Transfer Agreement)
☐ Distribution only to users in own country
☐ Distribution only to users in certain countries after signing of a MTA
☐ Distribution only on a mutually agreed exchange basis
☐ Other flows of distribution, please specify: _____

6.2. Cost for distribution of *Manihot* germplasm:

☐ No cost, free distribution
☐ No cost, but only on the basis of reciprocal exchange of material
☐ Request to contribute for processing and shipping, specify amount: _____
☐ Request to pay for each requested accession, specify amount: _____
☐ Other conditions requested, please specify: _____

6.3. Please insert or attach examples of your organization's long-term commitment to long term conservation of *Manihot* collection, for example:

- ☐ Legal status
- ☐ Institutional constitution
- ☐ Mandates
- ☐ Published strategic plans
- ☐ National conservation strategy
- ☐ Action plans
- ☐ Other: _____

7. Future developments regarding the* Manihot *collection

7.1. Will the *Manihot* collection be extended with new material or rationalized in the next five years?

- ☐ The collection will remain approximately the same size
- ☐ The collection will be expanded to a limited extent (5-10 %)
- ☐ The collection will be substantially increased (> 20%)
- ☐ The collection will be reduced due to duplication with other collections and internal rationalization
- ☐ The collection will be reduced as a result of lack of funding or facilities

7.2. Are there any constraints for maintenance of the *Manihot* collection? ☐ yes ☐ no

If yes, what type of constraints do you face?

- ☐ Insufficiently trained staff
- ☐ Regeneration capacity to maintain the collection limited
- ☐ Facilities for optimal maintenance of the collection not satisfactory
- ☐ Others (please specify): _____

7.3. Will some of the above constraints result in a loss of germplasm?

☐ yes ☐ only incidentally ☐ no

If yes, what is the most important constraint, which may contribute to genetic erosion within the collection? _____

8. <u>Further remarks</u>

Please send the completed questionnaire as an e-mail attachment to:

Clair Hershey
chh23@cornell.edu

Appendix IV. Register of cassava and *Manihot* species survey respondents

Cultivated cassava

1	Bolivia	Instituto de Investigaciones agrícolas "El Vallecito" Universidad Gabriel René Moreno	Ma. Lizzie Cuellar Gutierrez (in vitro collection)	mlizzie@hotmail.com	
1	Bolivia	Instituto de Investigaciones agrícolas "El Vallecito" Universidad Gabriel René Moreno	Mateo Vargas Banco (field collection)	mateovar@yahoo.com	
2	Brazil	IAC	Teresa Losada Valle	teresalv@iac.sp.gov.br	
3	Brazil	CNPMF (Embrapa Mandioca e Fruticultura Tropical)	Wania Maria Gonçalves Fukuda	Wfukuda@cnpmf.embrapa.br	
4	Brazil	Genetics Department – "Luiz de Queiroz College of Agriculture / University of São Paulo	Elizabeth Ann Veasey	eaveasey@esalq.usp.br	
5	Chad	Institut Tchadien de Recherche Agronomique pour le Developpement	MBAILAO Kemdingao	mbailaok@yahoo.fr	
6	China	Liu Guo-dao, Hainan	Li Kai-mian	likaimian@sohu.com	
7	CIAT	CIAT, Cali, Colombia	Daniel G. Debouck	d.debouck@cgiar.org	
7	CIAT	CIAT, Cali, Colombia	Graciela Mafla	g.mafla@cgiar.org	
8	Costa Rica	Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)	Carlos Alberto Cordero Vargas	cordero@catie.ac.cr	
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