Chapter 9
Genetic Resources and Conservation

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Taxonomy, Origin and Distribution

Cassava belongs to the botanical species *Manihot esculenta* Crantz of the family Euphorbiaceae, subfamily Crotonoideae, and tribe Manihotae. The genus *Manihot* contains about 100 species of herbs, shrubs and trees among which the production of latex and cyanogenic glucosides is common (Rogers and Fleming, 1973; Bailey, 1976). These species are grouped into 19 taxonomic sections and cassava is classified under the section *Manihot* (Rogers and Appan, 1973). All *Manihot* species originated in the tropical Americas.

Vavilov (1951) placed the origin of cassava in Brazil. In addition, other lowland areas of tropical Americas have been considered as places where cassava could have originated (Smith, 1968). Rogers (1963) identified two geographic centres of speciation of cassava: (i) the drier areas of western and southern Mexico and portions of Guatemala; and (ii) the dry northeastern portions of Brazil. Nassar (1978a,b) identified four areas of diversity of the wild species: (i) central Brazil; (ii) northeastern Brazil; (iii) southwestern Mexico; and (iv) western Mato Grosso (Brazil) and Bolivia (chapter 1).

The cassava crop may have been cultivated in Colombia and Venezuela from 3000 to 7000 years ago (Rouse and Cruxent, 1963, cited in Hershey, 1985). Ugent *et al.* (1986) cited evidence for domestication on the Peruvian coast before 4000 BC. Sauer (1952, quoted by Smith, 1968) proposed the heart of domestication as northwestern South America. Vavilov (1939) suggested early cultivation of cassava in the equatorial region of South America. It is believed that cassava was carried by the Arawak tribes of Central Brazil to the Caribbean Islands and Central America in the 11th century (Brucher, 1989), by the Portuguese to the west coast of Africa, via the Gulf of Benin and the Congo River at the end of the 16th century (Jones, 1959), and to the east coast via the islands of Reunion, Madagascar, and Zanzibar at the end of the 18th century (Barnes, 1975; Jennings, 1976). The crop arrived in India about 1800. The Spaniards took it into the Pacific, but it was not widely used as a food crop there until the 1960s (Jennings, 1976). Cassava is now widely cultivated in the tropics. The tuberous roots provide a major food source for more than 500 million people in Africa, Latin America and Asia. Its leaves are also used for human consumption in parts of Africa and Asia.

The Mesoamerican region extending from the northwestern coast of Mexico and covering parts of Guatemala, El Salvador and Nicaragua is also a potential area for early domestication (Rogers, 1965), where the wild species *Manihot aesculifolia*, *Manihot pringlei* and *Manihot isoloba* may have contributed to the cultigen through extensive hybridization, although this theory has been questioned (Brucher, 1989). Rogers and Fleming (1973) believed that cassava is a complex species with multiple sites of initial

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cultivation. Allem (1994) believed that *M. esculenta* was derived from two wild ancestor species, *Manihot flabellifolia* Pohl and *Manihot peruviana* Mueller. He placed the two wild species in the primary genepool of the cultivated cassava, while *Manihot glaziovii*, *Manihot dichotoma*, *Manihot pringlei*, *Manihot aesculifolia*, *Manihot pilosa*, *Manihot triphylla* and *Manihot pruinosa* he placed in the secondary genepool.

Cassava is monoecious and predominantly outcrossing, which leads to a very high degree of heterozygosity in plants and among populations produced from true seeds. Cassava varieties are heterozygous individuals which are propagated vegetatively to maintain the desired genotypes. Though cultivated germplasm has erratic flowering habits, it produces seed readily in many environments. Its seeds disperse naturally, as the fruit capsules dehisce at maturity. Many cassava plants growing from naturally dispersed seeds occur in farmers' fields in Africa. Through outcrossing among heterogeneous plant populations and subsequent selection by nature and by farmers, specific recombinants or new variants are created under the African agroecosystem. Specialized cultivars, such as those with a high level of resistance to cassava mosaic disease, could have been selected in this way.

Some wild *Manihot* species, such as *Manihot glaziovii* and *Manihot tristis* were introduced to many parts of Africa and Asia, at least since the early 20th century. They have now become naturalized in some parts of Africa and Asia (Rogers and Appan, 1973). These wild species were introduced to Africa and Asia initially for use as a source of rubber and later as shade trees in cocoa plantations and home compounds, and in Africa as a tree for fencing (Allem and Hahn, 1991). Introgression from *Manihot glaziovii* into cassava seems to be occurring under natural conditions in Africa. This phenomenon could have generated additional new variability in cassava in Africa, outside the place where it was first domesticated. Indeed, it has been widely recognized that an important secondary centre of diversity of cassava has become established in Africa (Gulick *et al.*, 1983).

The native range of *Manihot* species is from southern Arizona (*Manihot davisiae* and *Manihot angustiloba*) to Argentina (*Manihot grahami* and *Manihot anisophylla*). Only one wild species, *Manihot brachyloba*, is native to the West Indies. The species of *Manihot* are all rather sporadic in their distribution. However, there are two major concentrations of species, one in Mexico, the other in Brazil (Rogers, 1963). The species found in the two areas are remarkably disjunct; with the exception of cultivated species, none of the North American species is found in South America. The preponderant number of species are South American; the greatest number are found in eastern central Brazil (Rogers and Appan, 1973). Most *Manihot* species are found in relatively dry areas, and only a few are found typically in rainforest regions. The North American species are mostly found on limestone-derived soils. All of the species in the genus are sensitive to frost. There are only two species, *M. grahami* and *M. anisophylla* whose native distributions are in regions with occasional frost.

Germplasm Conservation

The importance of plant genetic resources to global food security as well as to the security of the livelihood of millions of rural families was underlined at the first United Nations Conference on the Human Environment in Stockholm, 1972. A decade before that conference, widespread loss of diversity at different levels of biological organization was recognized (TAC/FAO, 1972; Frankel, 1973). The Stockholm conference called for concerted efforts to conserve and utilize naturally occurring genetic variability in all plants, whilst considering the interests of both present and future generations.

Several international research institutes of the Consultative Group for International Agricultural Research (CGIAR), such as Centro Internacional de Agricultura Tropical (CIAT) and International Institute of Tropical Agriculture (IITA), were already established at that time. They had started assembling genetic resources of their mandated crops, consisting of local or introduced landraces, improved cultivars and related wild species for use in their breeding programmes. This initial effort of collecting plant genetic resources was formalized when many CGIAR centres established *ex situ* conservation programmes to collect and conserve the genetic resources of their own mandated crops, as an integral part of their activities. CIAT and IITA share the CGIAR mandate for cassava. They

collaborated with many national programmes with active programmes in cassava breeding or genetic resources conservation in collection, utilization and conservation of the genetic resources of this crop. The 1992 United Nations Conference on Environment and Development was held in Rio de Janeiro, where the Convention on Biological Diversity (CBD) was signed by over 150 heads of state. This marked a historic commitment by the nations of the world to conserve biodiversity and to ensure that biological resources are used sustainably and that the benefits of such use are shared equitably.

Germplasm collections

Recent reports indicated that some 20,000 accessions of cassava germplasm and its wild relatives are being preserved *ex situ* in CIAT, IITA, and national programmes in more than 45 countries throughout the world (Bonierbale *et al.*, 1997; IITA, 1997, 1998). Table 9.1 lists the number of accessions held in the two CGIAR centres and national programmes. The figures may include duplicates, especially among those that are held by CIAT and national programmes in the Americas, and by IITA and national programmes in West and Central Africa. The collection at CIAT includes germplasm from Argentina (72), Brazil (1334), Colombia (2001), Costa Rica (148), Cuba (77), Ecuador (117), Guatemala (91), Indonesia (51), Malaysia (67), Mexico (102), Nigeria (19), Panama (43), Paraguay (231), Peru (405), Puerto Rico (15), Thailand (31), USA (10), Venezuela (249), and five other countries (Bonierbale *et al.*, 1997). The IITA collection includes materials from Cameroon (247), Togo, (289), Ghana (81), Nigeria (363), Republic of Benin (378), Congo (64), Kenya (12), East Africa (29), Brazil (49), and five other countries in Africa. The total number of unique accessions is likely to be much smaller than 20,000. South America has the largest collection, followed by West and Central Africa. The collection held at CIAT represents the largest diversity of cassava from the Americas and Asia, and from West and Central Africa at IITA. The IITA-coordinated research networks, East Africa Root Crops Research Network (EARRNET) based in Uganda, and the Southern Africa Root Crops Research Network (SARRNET) based in Malawi, initiated activities in network member countries to collect and evaluate the local cassava germplasm. A total of 1245 accessions were assembled and maintained in EARRNET member countries, including Burundi, Kenya, Madagascar, Rwanda and Uganda (IITA, 1997). Similarly, a total of 797 accessions of local germplasm were collected and maintained in SARRNET member countries, including Angola, Botswana, Malawi, Mozambique, Namibia, Tanzania, Swaziland, South Africa, Zambia and Zimbabwe (IITA, 1998). Cassava germplasm accessions preserved at CIAT and IITA have been designated as 'in trust' collections under the auspices of the Food and Agriculture Organization of the United Nations (FAO) for public access. They are freely available to researchers worldwide.

Ex situ conservation methods

There are two basic approaches to the conservation of plant genetic resources, *ex situ* and *in situ*, and they are complementary. There are various methods for the conservation of genetic resources. The best way is to adopt a combination of cost-effective and practical methods, to conserve the targeted genepool of a species. The cassava crop is an outcrossing species and produces botanical seed in many environments, but is mainly propagated vegetatively using stem cuttings with two to six nodes, or by shoot tips in *in vitro* cultures to maintain genotypes. Its wild relatives are also predominantly outcrossing and are propagated mainly by botanical seed, and in some species, by stem cuttings. Many wild species could also be propagated in *in vitro* cultures (Iwanaga and Iglesias, 1994; Ng and Ng, 1997). Various *ex situ* techniques and options are available for the conservation of cassava genetic resources. These are the field genebank, seed storage, *in vitro* reduced growth storage and cryopreservation of shoot tips and pollen. DNA storage could also eventually become one of the options for cassava germplasm conservation. However, strategies and procedures for the utilization of the stored DNA have yet to be devised. Recent advances in cassava biotechnology which facilitate the selective transfer of genes, stored DNA from outside the genepool of cassava is likely to become more relevant in breeding (Withers, 1994).

Table 9.1. Cassava germplasm collection in some national and international research centres (maintained as field genebanks).

Region/country	No. accessions	Institute/programme
South America		
Colombia	4695	CIAT
Brazil	4132	CNPMF/CENARGEN
Paraguay	360	IAN
Ecuador	101	INIAP
Argentina	177	INTA
Bolivia	18	IIA
Central America		
Costa Rica	154	CATIE
Mexico	225	INIFAP
Panama	50	IIA
Nicaragua	37	UNA
Caribbean		
Cuba	495	INIVIT
Dominican Rep.	46	–
Eastern and Southern Africa		
Angola	13	
Botswana	11	
Tanzania	254	RTCP
Malawi	170	RTCP
Uganda	413	RTCP
Kenya	250	RTCP
Mozambique	81	INIA
Zambia	96	
Rwanda	280	
Zimbabwe	6	
South Africa	100	
West and Central Africa		
Benin	340	SRCV
Cameroon	250	
Côte d'Ivoire	300	
Gabon	42	
Ghana	2000	PGRC/CRI
Burkina Faso	14	
Nigeria	435	NRCRI
	2861	IITA
Guinea, Conakry	168	
Senegal	57	ISRA/CDH
Sierra Leone	134	IAR
Togo	734	
D.R. Congo	250	
Asia – Oceania		
China	86	SCATC/UCRI/GAAS
India	1507	CTCRI
Indonesia	251	CRIFC/MARIF
Israel	5	Israel Genebank for Agric. Crop
Malaysia	92	MARDI
Myanmar	21	ARI
Pakistan	3	Plant Introduction Center
Philippines	384	PRCRTC/IPB
Sri Lanka	112	CARI/PGRC
Thailand	250	RFCRC
Vietnam	36	Hung Loc. Agric. Centre

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Field genebanks

Cassava germplasm can be maintained in the field, as living collections which are relatively easy to establish and maintain with little need of sophisticated equipment. This method has an advantage over other conservation methods in that it provides plant materials readily for evaluation/characterization and for cross-pollination. A major disadvantage is the requirement for large fields to maintain germplasm collections. Germplasm materials in a field genebank are under pressure of constant exposure to diseases and pests, which could lead to a loss of genetic materials or genetic drift. Proper maintenance of a field genebank in a cost-effective way is important to ensure the survival of germplasm in a sustainable manner.

The planting distance of cassava for food production is usually a minimum of 1 m × 1 m between plants. Cassava stakes (stems with two to six nodes cut from mature stems) are used as propagules, and planted directly in the ground or in soil ridges/mounds, with at least one node buried under the surface of the soil. To achieve greater root yields, cassava is more frequently planted on soil ridges/mounds. For the purpose of maintaining a germplasm collection, it is less important to produce high root yields or improved root quality, than to maintain the clonal materials in perpetuity. A common practice used by IITA for cassava field genebank maintenance is to plant cassava stakes on flat ground in rows. Each accession is planted on a 2.5-m row plot, at a distance of 25 cm between hills within the row, and 50 cm between rows, with a total of 11 plants. The close spacing between plants suppresses weed growth and minimizes the area of land required. Nine months after planting, the plants are pruned; after 18–24 months the materials are planted in a new field. Where the activities for germplasm characterization/preliminary evaluation at IITA are combined with the maintenance of germplasm, cassava stakes are planted on ridges at a normal planting distance of 1 m × 1 m within and between rows, with 10 plants per accession. The materials are planted in new land every year. IITA maintains a collection of 24 wild *Manihot* species and CIAT has 26 species. Both the landraces and wild species are important sources of resistance to pests and diseases as well as quality characters. Work at IITA and CIAT has confirmed previous reports on useful traits in several wild *Manihot* species, such as high protein content, insect resistance and high levels of carotene (Asiedu *et al.*, 1992). IITA has used several wild *Manihot* species in interspecific hybridization with cassava to transfer their desirable genes into cassava (Hahn *et al.*, 1980, 1990).

Footnote for Table 9.1.
Acronyms: ARI, Agricultural Research Institute, Yezin, Myanmar; CARI/PGRC, Central Agricultural Research Institute, Gannoruwa, Peradeniya, Sri Lanka/Plant Genetic Resources Centre, Peradeniya, Sri Lanka; CATIE, Centro Agronomico Tropical de Investigacion y Ensenanza, Turrialba, Costa Rica; CENARGEN, Centro Nacional de Recursos Geneticos e Biotecnologia (of EMBRAPA), Brasilia, Brazil; CNPMF, Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical, Brazil; CRIFC/MARIF, Central Research for Food Crops, Indonesia/Malang Research Institute for Food Crop, Indonesia; CTCRI, Central Tuber Crops Research Institute, India; IAN, Instituto Agronomico Nacional, Paraguay; IAR, Institute of Agronomic Research, Sierra Leone; IIA, Instituto de Investigacion Agricola, Bolivia; IIA, Institute de Investigaciones Agropecuarias, Panama; INIA, Instituto Nacional de Investigacao Agronomica, Mozambique; INIAP, Instituto Nacional de Investigacion Agropecuarias, Ecuador; INIFAP, Instituto Nacional de Investigaciones Forestales y Agropecuarias, Mexico; INIVIT, Instituto Nacional de Investigacion de Viandas Tropicales, Cuba; INTA, Instituto Nacional de Tecnologia Agropecuaria, Argentina; ISRA, Institut Senegalais de Recherches Agricole/Centro pour le Developement de l'horticulture, Senegal; MARDI, Malaysian Agricultural Research and Development Institute, Malaysia; NRCRI, National Root Crop Research Institute, Nigeria; PGRC/CRI, Plant Genetic Resources Centre/Crop Research Institute, Ghana; PRCRTC/IPB, Philippine Root Crop Research and Training Centre/Institute of Plant Breeding, Philippines; RFCRC, Rayong Crop Research Centre, Thailand; RTCP, Root and Tuber Crop Research Programme; SCATC/UCRI/GAAS, South China Academy of Tropical Crops/ Upland Crop Research Institute/Guang Dong Academy of Agricultural Science, China; UNA, Universidad Nacional Agraria, Facultad de Agronomia, Nicaragua.

Seed genebanks

It was reported that cassava seeds could tolerate desiccation to 3.2% moisture content (MC), and there was no loss in seed viability following 4 months hermetic storage at −20°C with 6% MC (Ellis *et al.*, 1981), and after 14 years hermetic storage at −20°C with 6% MC (unpublished results, Seed Science Laboratory, Department of Agriculture, the University of Reading, cited by Hong *et al.*, 1996). Cassava seeds lost their viability after 2 years of storage under ambient conditions in Ibadan, Nigeria. Thus, cassava seeds are orthodox: they store best in cool and dry conditions.

At IITA, cassava seeds are harvested in bulk from all plants of each individual clonal accession and kept separately as a population of the clonal accession. Freshly harvested seeds are dried to 5–7% seed MC, sealed in aluminium foil envelopes, and stored at −20°C for long-term conservation. Seeds for use in breeding programmes and for distribution are stored in paper bags in a cold store maintained at 5°C and *c.* 30% relative humidity.

In vitro genebanks

In vitro propagation and conservation techniques for cassava have been well developed and have been applied routinely in many genebanks, particularly in CIAT and IITA, and in national programmes in Brazil, Argentina, Paraguay and Cuba (Bonierbale *et al.*, 1997; Ng and Ng, 1997; Ng *et al.*, 1999). Table 9.2 summarizes information on the cassava germplasm collections that are maintained in an *in vitro* genebank, either by reduced growth storage or under normal culture conditions. Shoot-tip cultures of cassava clonal accessions are usually conserved under reduced-growth or slow-growth culture media,

Table 9.2. Cassava germplasm collections maintained *in vitro* in some national and international research institutes.

Country/institution	No. accessions (Reference)	Culture condition	Storage duration (months)
Argentina	120 (Bonierbale *et al.*, 1997)	–	–
Brazil	1307 (De Goes *et al.*, 1999)	–	3–14
Caribbean	28 (Bateson, 1999)	18–22°C	–
CIAT	5714 (Guevara and Mafla, 1999)	23–24°C, 1000 lux Low sucrose medium	10–18
Cuba	– (Morales *et al.*, 1999)	20–22°C, 500 lux Normal culture medium	–
Ghana	9 (Acheampong, 1999)	28°C	–
IITA	727 (Ng and Ng, 1997)	18–22°C	8–12
India	30 (Bonierbale *et al.*, 1997)	–	–
Paraguay	101 (Bonierbale *et al.*, 1997)	–	–
Philippines	13 (Zamora and Paet, 1999)	8 h light, normal culture medium	2
South Pacific	19 (Taylor, 1999)	20°C	9
Sri Lanka	56 (Bonierbale *et al.*, 1997)	–	–
Vietnam	10 (Bonierbale *et al.*, 1997)	–	–

or reduced incubation conditions (temperature and light intensity). Cassava clones in the *in vitro* genebank at CIAT are conserved under the following conditions: (i) constant temperature of 23–25°C for 24 h through the day and night, with 12 h light illumination of 1000–1500 lux; (ii) a slightly modified Murashige–Skoog (MS) culture medium (Murashige and Skoog, 1962). Three to five tubes per accession are maintained (Iwanaga and Iglesias, 1994). At IITA, the cultures are maintained under lower incubation temperatures, and lower light intensity than at CIAT, with five to ten tubes per accession (Ng and Ng, 1997; Ng *et al.*, 1999). Shoot-tip and nodal cultures have also been used at IITA to transfer cassava germplasm collections from collaborating National Agricultural Research Systems in Cameroon, Ghana and the Republic of Benin.

The source of materials for *in vitro* propagation are *apical* buds or nodes collected at their active growth stage, from cassava plants growing in a field genebank or screen house. Buds collected are surface disinfected with 70% ethanol, followed by 7% sodium hypochlorite solution with Tween 20 for 20 min. They are then rinsed with three changes of sterile distilled water. Meristems with one to two leaf primordia are excised from the buds and inoculated in MS basal medium supplemented with 3% sugar, 80 mg l^{-1} adenine sulphate, 0.15 mg l^{-1} benzyl amino purine (BAP), 0.2 mg l^{-1} naphthalene acetic acids (NAA), 0.04 mg l^{-1} gibberellic acid (GA_3) and 0.6% agar. For nodal cutting culture, nodes from young green shoots are surface disinfected with 70% ethanol for 5 min, followed by 10% sodium hypochlorite solution with Tween 20 for 20 min and then by 5% sodium hypochlorite solution for 10 min. The nodes are rinsed three times with sterile distilled water, then placed on MS medium supplemented with 3% sugar, 0.01 mg l^{-1} NAA, 0.05 mg l^{-1} BAP and 0.7% agar. Ten tubes (16 × 125 mm) per accession are cultured. Cultures are incubated in a culture room maintained at 28–30°C with 12 h light illumination of 1000–1500 lux intensity, for 3–4 weeks. Regenerated plantlets are then micropropagated for initial increase and transferred to stores at 18–24°C, and low light intensity. Cultures are checked regularly. Those that are contaminated with fungi or bacteria are discarded and those that show deterioration are subcultured. Cultures can be kept for 8–12 months before subcultures are made. Theoretically, this cycle can be repeated indefinitely and plantlets can also be transplanted to the isolation room for virus and other disease indexing. Through disease or virus indexing, healthy germplasm accessions certified by Plant Quarantine Services are then multiplied for use in exchange with research partners.

Cryopreservation

Cryopreservation enables long-term conservation of germplasm. Recent developments in cryopreservation, especially those reducing cryodamage, offer an improvement of survival after freezing and the list of successfully cryopreserved species is increasing.

Early research by Bajaj (1983) showed that cassava shoot tips frozen directly in liquid nitrogen (LN) with cryoprotectants resumed growth and formed plantlets after thawing. A survival percentage of 26 was obtained after 3 years storage in LN. Other successful approaches involving controlled cooling regimes have been described for shoot tips (Escobar *et al.*, 1993), seeds and zygotic embryos (Marin *et al.*, 1990) and somatic embryos (Sudarmonowati and Henshaw, 1990). Pollen/anthers are also a good source of plant material for long-term conservation. Of the different explants used, the shoot tip is the most suitable plant material for use in the cryopreservation of cassava clonal germplasm. It is most amenable to tissue culture and can be available at any time of the year in any quantity.

The classical cryopreservation protocols have normally been by slow cooling with the use of cryoprotectants and these require sophisticated and expensive programmable freezing equipment. The new techniques recently developed offer an opportunity to use fast freezing, and direct immersion in LN. Pregrowth of shoot tips in proliferation medium, followed by treatment with cryoprotectants and dehydration before direct immersion of shoot tips in LN resulted in a plant recovery rate as high as that with programmable freezing (Escobar *et al.*, 1995). Shoot tips were also successfully cryopreserved by encapsulation–dehydration followed by direct immersion in LN (Escobar *et al.*, 1998). The vitrification technique has been employed to simplify

handling of explants and has secured a high level of recovery. Shoot tips were cryopreserved successfully using this technique on cassava germplasm from Thailand (Charoensub *et al.*, 2000) and from Africa (Ng and Ng, 2000). Some important factors influencing the recovery include pre-culture, treatment with vitrification solution, thawing, size of shoot tips and genotype. The procedure involves pre-culture of shoot tips in a high sucrose proliferation medium, treatment with vitrification solution, followed by direct plunging into LN. Frozen plant materials after retrieval from LN are thawed either by fast or slow thawing, washed with an appropriate solution and transferred to culture medium for re-growth. Up to 60% recovery was recorded in cassava using this procedure (Ng and Ng, unpublished).

In situ and on-farm conservation

In situ conservation of genetic resources is to maintain genetic material, usually the wild relatives of crop plants or trees, in their natural ecosystems. On-farm conservation is to conserve local landraces/cultivars in farmers' fields. *In situ* or on-farm conservation of genetic resources maintains the evolutionary processes of the targeted plant species. The process can make a direct contribution to the wellbeing of farmers and communities by ensuring that adapted plant types remain directly available to them for their own continuing use.

Maintenance of landraces or traditional crop varieties on-farm and in home gardens or wild relatives of crop plants *in situ* has gained recognition and been widely promoted as an effective way of conserving traditional crop varieties and wild relatives of crop species. For thousands of years, farmers selected and managed their plant genetic resources through gathering and domestication, continuous selection and cultivation of cultivars most suitable to the surrounding environments in which the farmers work and live. Farmers are creators and conservators of crop genetic resources, while at the same time they abandon what they do not need or cannot maintain because of social factors or biological constraints. It is impossible at this stage to rely solely on farmers to save all traditional cassava varieties they themselves have created or selected, unless there are some incentives to do so and support from institutions and governments. Development of on-farm or *in situ* conservation strategies require very broad knowledge, ranging from social, biological and environmental factors. Such information required for the development of effective *in situ* conservation strategies for cassava species and their wild relatives is not well documented or understood. It requires a multidisciplinary team approach to gather data and analyse the information for the design of an appropriate *in situ* conservation strategy for cassava.

Before a well-designed strategy for *in situ* or on-farm conservation of cassava and their wild relatives is in place, scientific communities, social workers and policy makers should encourage/support farmers or volunteers and institutions to conserve as many of their traditional varieties on-farm as possible. *Ex situ* conservation of genetic resources for the foreseeable future still remains the most effective and reliable way of conserving cassava genetic resources.

Conclusions

With the increasing importance of cassava around the world for human consumption, animal feed and industrial uses, there will be an increasing need for a wide range of genetic diversity to develop cultivars having specific characteristics and for adaptation to different ecologies. It is important that the existing cassava genetic resources held in various institutes be well maintained, and safely duplicated using a combination of available conservation methods.

There are some gaps in the existing *ex situ* collection, with respect to the representation of genetic diversity from many geographical areas (Bonierbale *et al.*, 1997). Few collections of wild relatives of cassava have been assembled and conserved in *ex situ* genebanks. Because of the destruction of natural habitats where wild relatives of cassava are growing and also abandonment of old traditional cassava cultivars by farmers, there is some urgent need to collect the cassava diversity not represented in the existing collections for *ex situ* conservation. Concurrently, germplasm characterization using agrobotanical descriptors and molecular markers should

be intensified and accelerated. Information obtained from the characterization will assist in the selection of core collection(s) and the elimination of duplicates, thus increasing the efficiency of germplasm management and use. Recent progress in cryopreservation research in cassava is very encouraging, especially the vitrification and fast freezing protocols. This technique when fully developed offers a relatively simple and inexpensive method for long-term conservation of cassava germplasm. This technique will be affordable for cassava conservation in national programmes. Research in this area should focus on the optimization of the protocol to achieve high recovery rate and application to a wide range of cassava germplasm.

Investigation of *in situ* or on-farm conservation of cassava genetic resources by national programmes and international research institutes should be encouraged. Appropriate strategies for *in situ* or on-farm conservation should be developed to complement *ex situ* conservation. Ideal locations for *in situ* or on-farm conservation should be identified. National/regional/international policies governing the use of the areas for the conservation of cassava genetic resources and their access should be well articulated to ensure that the conserved genetic resources would be accessible to researchers worldwide.

References

Acheampong, E. (1999) *In vitro* genebank management of clonally propagated crops under minimal conditions. In: Engelmann, F. (ed.) *Management of Field and* in vitro *Germplasm Collections*. IPGRI, Rome, Italy, pp. 73–75.

Allem, A.C. (1994) The origin of *Manihot esculenta* Crantz (Euphorbiaceae). *Genetic Resources & Crop Evolution* 41(3), 133–150.

Allem, A.C. and Hahn, S.K. (1991) Cassava germplasm strategies for Africa. In: Ng, N.Q., Perrino, P., Attere, F. and Zedan, H. (eds) *Crop Genetic Resources of Africa*, Vol. II. Ibadan, Nigeria, pp. 127–149.

Asiedu, R., Hahn, S.K., Bai, K.V. and Dixon, A.G.O. (1992) Introgression of genes from wild relatives into cassava. In: Akoroda, M.O. and Arene, O.B. (eds) *Proceedings of the Fourth Triennial Symposium of the International Society of Tropical Root Crops – Africa Branch*. ISTRC-AB/IDRC/CTA/IITA, Nigeria, pp. 89–91.

Bailey, H. (1976) *Hortus Third. A Concise Dictionary of Plants Cultivated in the United States and Canada*. McMillan, New York.

Bajaj, Y.P.S. (1983) Cassava plant from meristem cultures freeze-preserved for three years. *Field Crops Research* 7, 161–167.

Barnes, H. (1975) The diffusion of the manioc plant from South America to Africa: an essay in ethnobotanical culture history. Dissertation, Colombia University.

Bateson, J.M. (1999) *In vitro* genebank management at CARDI tissue laboratory. In: Engelmann, F. (ed.) *Management of Field and* in vitro *Germplasm Collections*. IPGRI, Rome, Italy, pp. 90–92.

Bonierbale, M., Guevara, C., Dixon, A.G.O., Ng, N.Q., Asiedu, R. and Ng, S.Y.C. (1997) Chapter 1. Cassava. In: Fuccillo, D., Sears, L. and Stapleton, P. (eds) *Biodiversity in Trust*. Cambridge University Press, Cambridge, pp. 1–20.

Brucher, H. (1989) *Useful Plants of Neotropical Origin and their Wild Relatives*. Springer-Verlag, Berlin.

Charoensub, R., Phansiri, S., Sakai, A. and Yongmanitchai, W. (2000) Cryopreservation of *in vitro* grown shoot-tips of cassava cooled to –196°C by vitrification. In: Engelmann, F. and Takagi, H. (eds) *Cryopreservation of Tropical Plant Germplasm – Current Research Progress and Applications*. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan and International Plant Genetic Resources Institute, Rome, Italy, pp. 401–403.

De Goes, M., Mendes, R.A., Labuto, L.B.D. and Cardoso, L.D. (1999) Present status of germplasm conservation *in vitro* at CENARGEN/EMBRAPA, Brazil. In: Engelmann, F. (ed.) *Management of Field and* in vitro *Germplasm Collections*. IPGRI, Rome, Italy, pp. 86–87.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1981) The influence of desiccation on cassava seed germination and longevity. *Annals of Botany* 47, 173–175.

Escobar, R.H., Roca, W.M. and Mafla, G. (1993) Cryopreservation of cassava shoot tips. In: Roca, W.M. and Thro, A.M. (eds) *Proceedings of the 1st International Scientific Meeting of the Cassava Biotechnology Network*. Colombia, pp. 116–119.

Escobar, R.H., Mafla, G. and Roca W.M. (1995) Cryopreservation for long-term conservation of cassava genetic resources. In: *Proceedings of the 2nd Scientific Meeting of the Cassava Biotechnology Network*, Vol. 1, Colombia, pp. 190–193.

Escobar, R.H., Palacio, J.D., Rangel, M.P. and Roca, W.M. (1998) Cassava cryopreservation II. In: *Abstract of JIRCAS/IPGRI Joint International Workshop on Cryopreservation of Tropical Plant Germplasm – Current Research Progress and*

Applications, 20–23 October 1998, Tsukuba, Japan, p. 25.

Frankel, O.H. (1973) *Survey of Crop Genetic Resources in Their Centers of Diversity*. First Report. FAO/IBP, Rome, Italy.

Guevara, C.L. and Mafla, G. (1999) *Manihot* collections held at CIAT. In: Engelmann, F. (ed.) *Management of Field and* in vitro *Germplasm Collections*. IPGRI, Rome, Italy, pp. 109–112.

Gulick, P., Hershey, C. and Esquinas-Alcazar, J. (1983) *Genetic Resources of Cassava and Wild Relatives*. International Board for Plant Genetic Resources, Rome, Italy.

Hahn, S.K., Terry, E. and Leuschner, R. (1980) Cassava breeding for resistance to cassava mosaic disease. *Euphytica* 29, 673–683.

Hahn, S.K., Bai, K.V. and Asiedu, R. (1990) Tetraploids, triploids and 2*n* pollen from diploid interspecific crosses with cassava. *Theoretical and Applied Genetics* 79, 433–439.

Hershey, C. (1985) Cassava germplasm resources. Presentation at a Workshop on Cassava Breeding: a Multidisciplinary Review, 4–7 March 1985, Philippines.

Hong, T.D., Linington, S. and Ellis, R.H. (1996) *Seed Storage Behaviour: a Compendium*. Handbooks for Genebanks: No.4. International Plant Genetic Resources Institute, Rome, p. 287

IITA (1997) *Project 16. Conservation and Genetic Enhancement of Plant Biodiversity Annual Report 1996*. IITA, Ibadan, Nigeria, p. 2.

IITA (1998) *Project 16. Conservation and Genetic Enhancement of Plant Biodiversity Annual Report 1997*. IITA, Ibadan, Nigeria, p. 4.

Iwanaga, M. and Iglesias, C. (1994) Cassava genetic resources management at CIAT. In: *Report of the First Meeting of the International Network for Cassava Genetic Resources. International Crop Network Series No. 10*. IPGRI, Rome, Italy, pp. 77–86.

Jennings, D.L. (1976) Cassava, *Manihot esculenta* (Euphorbiaceae). In: Simmonds, N. (ed.) *Evolution of Crop Plants*. Longman, London, pp. 81–84.

Jones, W.O. (1959) *Manioc in Africa*. Stanford University Press, Stanford, Connecticut.

Marin, M.L., Mafla, G., Roca, W.M. and Withers, L.A. (1990) Cryopreservation of cassava zygotic embryos and whole seeds in liquid nitrogen. *Cryo-Letters* 1, 251–264.

Morales, S.R., Garcia, M.G., Torres, J.L., Vega, V.M. and Martin, J.C.V. (1999) *In vitro* germplasm storage of tropical root and tuber crops, and banana and plantain in the Cuban Republic. In: Engelmann, F. (ed.) *Management of Field and* in vitro *Germplasm Collections*. IPGRI, Rome, Italy, pp. 84–85.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plantarum* 15, 473–497.

Nassar, N. (1978a) Conservation of the genetic resources of cassava (*Manihot esculenta*) – determination of wild species localities with emphasis on probable origin. *Economic Botany* 32(3), 311–320.

Nassar, N. (1978b) Microcenters of wild cassava, *Manihot* spp. diversity in central Brazil. *Turrialba* 28(4), 345–347.

Ng, S.Y.C and Ng, N.Q. (1997) Cassava *in vitro* germplasm management at the International Institute of Tropical Agriculture. *African Journal of Root Crops* 2(1&2), 232–233.

Ng, S.Y.C. and Ng, N.Q. (2000) Cryopreservation of cassava and yam by fast freezing. In: Engelmann, F. and Takagi, H. (eds) *Cryopreservation of Tropical Plant Germplasm – Current Research Progress and Applications*. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan and International Plant Genetic Resources Institute, Rome, Italy, pp. 418–420.

Ng, S.Y.C., Mantell, S.H. and Ng, N.Q. (1999) Biotechnology in germplasm management of cassava and yams. In: Benson, E.E. (ed.) *Plant Conservation Biotechnology*. Taylor and Francis, London, pp. 179–202.

Rogers, D. (1963) Studies of *Manihot esculenta* Crantz and related species. Bulletin Torrey Botany Club 90(1), 43–54.

Rogers, D.J. (1965) Some botanical and ethnological considerations of *Manihot esculenta*. *Economic Botany* 19(4), 369–377.

Rogers, D.J. and Appan, S.G. (1973) *Flora Neotropica. Monograph No. 13 Manihot Manihotoides (Euphorbiaceae)*. Hafner Press, New York.

Rogers, D.J. and Fleming, H.S. (1973) A monograph of *Manihot esculenta* with an explanation of the taximetrics methods used. *Economic Botany* 27, 1–113.

Smith, C.E. (1968) The New World centers of origin of cultivated plants and the archeological evidence. *Economic Botany* 22(3), 253–266.

Sudarmonowati, E. and Henshaw, G.G. (1990) Cryopreservation of cassava somatic embryos and embryogenic tissue. In: *Book of Abstract of International Congress for Plant Tissue and Cell Culture*. Amsterdam, The Netherlands, p. 140.

TAC/FAO (1972) The collection, evaluation and conservation of plant genetic resources. *Report of the TAC ad hoc Working Group*. Beltsville, TAC Secretariat/FAO, Rome, Italy.

Taylor, M. (1999) *In vitro* conservation of root and tuber crops in the South Pacific. In: Engelmann, F. (ed.) *Management of Field and* in vitro *Germplasm Collections*. IPGRI, Rome, Italy, pp. 93–95.

Ugent, D., Pozorski, S. and Pozorki, T. (1986) Archeological manioc (*Manihot*) from coastal Peru. *Economic Botany* 40, 78–102.

Vavilov, N.I. (1939) The important agricultural crops of pre-Columbian America and their mutual relationship. *Izd. Gos. Georgr. O-va* [Public. Nat. Dept. Geogr. USSR], 71(10), 1–25.

Vavilov, N.I. (1951) The origin, variation, immunity and breeding of cultivated plants. Translated by Starr, C.K. *Chronica Botanica* 13, 1–366.

Withers, L.A. (1994) Methods and strategies for cassava germplasm. In: *Report of the First Meeting of the International Network for Cassava Genetic Resources. International Crop Network Series No. 10*. IPGRI, Rome, Italy, pp. 135–140.

Zamora, A.B. and Paet, C.N. (1999) *In vitro* gene-banking activities at the Institute of Plant Breeding, College of Agriculture, University of the Philippines at Los Banos. In: Engelmann, F. (ed.) *Management of Field and* in vitro *Germplasm Collections*. IPGRI, Rome, Italy, pp. 76–83.