Chapter 8
Manihot

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8.1 Introduction

Global food demand has been projected to double by the year 2050 and research for increased agricultural production has been suggested as the key to feed the increasing world population. Cereal crops have been the major and preferred food sources across many nations; however, there is tremendous amount of pressure on these crops to meet the increasing demand which is in turn associated with changing climate, poor soil conditions, and increased disease and pest incidences. Root crops such as cassava currently fits as the best alternative to cereals as it has the ability to tolerate longer periods of drought and disease and insect pressure while producing economic yields for the poor farming communities. Owing to these attributes, cassava plays a very important role as a food security crop in Africa. It is believed that bringing marginal lands and poor soils under cassava cultivation and exploring the additional uses of cassava and its wild relatives can help meet global food demand. Undoubtedly, increased cassava production not only for human consumption and industrial use, but also as feed in poultry and livestock industry will ease the global pressure on cereal crops.

Cassava, *Manihot esculenta* Crantz, a starchy root crop, is one of the major staple of about 800 million people worldwide (Lebot 2009). It is cultivated between 30°N and 30°S of the equator, having a mean annual temperature greater than 18°C (Nassar and Ortiz 2007). It is a major source of carbohydrate in sub-Saharan Africa, ranking sixth among major food crops of the world after wheat, rice, maize, potato, and barley. Native to neotropics, it is mainly cultivated in tropical and subtropical regions in Africa, Asia, and Latin America. The world production of cassava root was 238.5 million tons in 2008 (FAO 2008) with majority of it being produced in Africa, with Nigeria being the world's largest producer (49 million tons in 2008). Worldwide cassava production has increased by 15% between 2005 and 2008 (FAO 2009). This increase is due to elevated prices of cereal and other staple food crops, improved cultivars, improved cassava root processing and productions techniques, alternative utilization of cassava as bio-energy (ethanol), and animal feed resource. These positive attributes made the farmers in cassava growing areas turn toward the cultivation of cassava thus increasing the area under cassava cultivation while utilizing the improved cultivars for production with government support (FAO 2008). Cassava ranked first in production in Nigeria followed by yam and sorghum (FAO 2010) (http://ndn.nigeriadailynews.com).

Cassava – *M. esculenta* Crantz – is classified under kingdom Plantae, subkingdom Tracheobionta, superdivision Spermatophyta, division Magnoliophyta, class Magnoliopsida, subclass Rosidae, order Euphorbiales, family Euphorbiaceae, and genus *Manihot*. Synonyms of this species are *Manihot utilissima* Phol., *Manihot aipi* Phol., *Jatropha manihot* L. and related wild species is *Manihot dulcis*. Common names of *M. esculenta* are "cassava," "yuca," "bitter cassava," "tapioca," "tapioca plant," "manioc," "Brazilian arrowroot," and "sweet potato tree." It has many vernacular names – *yucca*, *dang noi*, *man sum palung*, *pearks sakhoo*, *tapioca*, *manioca*, and *huacamote*. Cassava relatives in the Euphorbiaceae family include several commercially important plants, such as rubber

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trees (*Hevea brasiliensis*), castor oil plants (*Ricinus communis*), and ornamental plants (*Euphorbia* spp.).

There are 12 species in genus *Manihot* viz., *M. angustiloba* (Torr.) Müll. Arg. (desertmountain *Manihot*), *M. caerulescens* Pohl (manicoba), *M. catingae* Ule (manicoba brava), *M. davisiae* Croizat (Arizona *Manihot*), *M. dichotoma* Ule (manicoba rubber), *M. esculenta* Crantz (cassava), *M. glaziovii* Müll. Arg. (ceara rubbertree), *M. grahamii* Hook. (Graham's *Manihot*), *M. subspicata* D.J. Rogers & Appan (spiked *Manihot*), *M. tristis* Müll. Arg. (*Manihot*), *M. tristis* Müll. Arg. ssp. *saxicola* (Lanj.) D.J. Rogers & Appan (*Manihot*), and *M. walkerae* Croizat (Walker's *Manihot*) reported in USDA plant data base (http://plants.usda.gov). These wild species provide a rich source of genes for cassava improvement in the breeding programs.

The wild *Manihot* species are gaining importance due to the presence of potential interspecific variations with useful genes for morphological and physiological traits as well as biotic and abiotic stresses (Allem 1984). The useful gene for low cyanide content was observed in *M. pringlei*, resistance to African cassava mosaic virus from *M. glaziovii*, resistance to cassava bacterial blight from *M. pseduglaziovii* and *M. reptans*, high starch content from *M. tristis* and *M. augustiloba*. Genetic resistance to pests was found in *M. glaziovi* and *M. dichotoma* (mealy bug); *M. neusana*, *M. pohlii*, and *M. grahami* (Stem borer). The abiotic stress-tolerant genes were present in *M. chlorostica* for salinity, *M. pseudoglaziovi*, *M. carthaginesis*, and *M. dichotoma* for drought while *M. attenuata* and *M. rubricaulis* for cold temperatures. The outstanding contribution for tolerance to aluminum toxicity, acid soils, and low phosphorous levels is from *M. irwinii*, *M. tripartita*, and *M. orbicularis*. In Africa, using wild crop relatives through classical breeding, a variety of elite genotypes with improved starch, dry matter, resistance to biotic, and abiotic stress resistance/tolerance as well as with reduced cyanogenic level have been already developed (Hahn 1989). About 98 species have been reported for the genus *Manihot* with cassava (*M. esculenta* ssp. *esculenta*) as the only cultivated staple food crop in the tropics (Rogers and Appan 1973).

In addition to human consumption, cassava also has many industrial uses and cultivated cassava is the cheapest known source of starch, producing more than 300 industrial products (Tonukari 2004). Of these, one promising application is fermentation of the starch to produce ethanol used in biofuel (Nguyen and Gheewala 2008). Despite its growing demand and production potential, it has received less attention from the researchers compared to other globally important crops and is still considered as an "orphan crop." It is grown mainly by small-scale farmers in areas that have little or no access to improved varieties, fertilizer and other production inputs and are often cut off from the marketing channels and agro-processing industries. This chapter presents a brief overview of the origin, evolution, and botany of *Manihot* species; collection and conservation strategies followed to protect landraces from extinction; germplasm characterization using morphological, cytological, biochemical, and molecular markers; limitations of present strategies; and future directions.

8.2 Basic Botany of the Genus

8.2.1 Origin, Geographical Location, and Distribution

The evolutionary origin of cassava (*M. esculenta* ssp. *esculenta*) is still under debate even though several researchers put forth different hypothesis on this topic. Most hypotheses were made around three important questions that probably could determine the actual botanical origin of cassava, such as, the wild species from which it descends; the geographical origin (the area where the progenitor existed); and the agricultural origin (the area of original cultivation of wild ancestor) (Allem 2002). The available knowledge show that there is enough evidence already available on botanical origin of this crop and evidence on geographical origin is in conjecture with the areas where cultivation has begun. The traditional hypotheses suggest that cassava is a "compilo-species," i.e., the result of hybridization events among several species including *Manihot aesculifolia*, a species endemic to Central America (Olsen and Schaal 2001; Olsen 2004), which was considered as the probable center of origin for cultivated cassava. On the other hand, it was reported that all the wild taxa of cassava have a broad ecological range from southwestern Amazonia to the Savannas of the Guianas (Allem 2002).

Allem (1994) proposed that the modern cultivated cassava, *M. esculenta* ssp. *esculenta*, originated from two primitive forms having three subspecies. These are *M. esculenta* ssp. *esculenta* (in which all known cultivars and landraces are included), and two wild types such as *M. esculenta* ssp. *peruviana* (occurring in eastern Peru and western Brazil) and *M. esculenta* ssp. *flabellifolia* (with a distribution from Goiás in Brazil to Venezuelan Amazonia). Allem (1999) considered *M. esculenta* ssp. *flabellifolia* as the wild ancestor of cultivated cassava and included it in the primary gene pool GP-1 together with *M. peruviana* (Allem et al. 2001). This close relationship has since been supported by studies of Roa et al. (1997, 2000) using amplified fragment length polymorphism (AFLP) markers to elucidate the genetic relationships. A detailed molecular analysis based on the single-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase (Olsen and Schaal 1999) indicated that cassava was domesticated specifically from populations of *M. esculenta* ssp. *flabellifolia* occurring along the southern rim of the Amazon basin in the Brazilian states of Acre, Rondônia, and Mato Grosso, and likely extending south into similar conditions in Bolivia. The premise of a southern Amazonian domestication has been further supported by subsequent studies, which consistently showed that genetic variation in cassava is almost entirely a subset of the genetic variation occurring in the wild *M. esculenta* populations from this geographical region (Olsen and Schaal 2001; Léotard and McKey 2004; Olsen 2004). Duputie et al. (2007) further demonstrated, based on molecular studies, that domesticated cassava probably has a single progenitor rather than a pool of hybridizing unidentifiable wild species. They also confirmed that crop has undergone hybridization in nature with the only probable wild relative *M. esculenta* ssp. *flabellifolia* at several places in French Guiana with a single domestication center, rather than multiple sites throughout the neotropics, and hybrids (F_1, F_2 and probable backcrosses) are all fertile.

Different centers of origin have been reported for the genus *Manihot*. Vavilov (1951) assumed that the center of diversity for cassava is in the Brazilian–Bolivian region, which probably is the origin of this crop. However, Harlan (1961) proposed more than one center of diversity for all cultivated species and he also suggested that all centers of diversity may not represent centers of origin of a particular cultivated species. This also applies in case of cassava and its wild progenitors. Therefore, four centers of diversity have been identified for *Manihot* species (Nassar 1978a) such as Mexico, Northeast and Central Brazil, Southwest Brazil and Bolivia following the model described by Dobzhansky (1973) for species formation in Iris, Eucalyptus, Liatris, Penstemon, and Tragopogon. Microcenters of diversity also exist for these species, which probably arose from frequent hybridization between species and heterogenic topography of their habitats (Nassar 2003). About 80 species are considered to have originated from two centers of diversity in Brazil, one being central Brazil and the other as Northeast Brazil (Olsen and Schaal 1999, 2001; Olsen 2004). Another 17 species have been found in Mexico and Central America (Rogers and Appan 1973; Nassar 2001). Sometimes Africa has been also considered as an additional center of diversity for cultivated cassava (Gulick et al. 1983; Pickersgill 1998). Table 8.1 lists the wild species of *Manihot* collected from different localities of northeastern Brazil. It is apparent that

Table 8.1 Wild species of *Manihot* collected from different localities in northeastern Brazil

Species	Locality
M. caerulescens Pohl	Aparipina, PE
M. heptaphylla Ule	Seabra, BA
M. cichotoma Ule	Jequié, BA
M. catingae Ule	Itaberaba, BA
M. brachyandra Pax et Hoffmann	Petrolina, PE
M. maracasensis Ule	Itambé, BA
M. epruinosa Pax et Hoffmann	Bentecoste, Fortaleza, CE
M. glaziovii Mueller	Arcoverde, Ouricure, Serratalada, PE
M. quinquefolia Pohl	Senhor do Bonfim, Juazeiro, BA
M. jacobinensis Mueller	Vitória da Conquista, BA

Source: Nassar (2000)

Table 8.2 Primary and secondary centers of origin for *Manihot*

Center of diversity	Species included
Primary center: Brazil	*M. acuminatissima* Mueller; *M. sparsifolia* Pohl; *M. pruinosa* Pohl; *M. alutacea* Rogers et Appan; *M. divergens* Pohl; *M. cecropiaefolia* Pohl; *M. triphylia* Pohl; *M. pentaphylla* Pohl; *M. anomala* Pohl; *M. procumbens* Mueller; *M. crotalariaformis* Pohl; *M. pusilia* Pohl; *M. togepetiolata* Pohl; *M. tomentosa* Pohl; *M. purpureo-costata* Pohl; *M. attenuata* Mueller; *M. orbicularis* Pohl; *M. tripartita* (Sprengel) Mueller; *M. pilosa* Pohl; *M. sagittato-partita* Pohl; *M. falcata* Rogers et Appan; *M. quinqueloba* Pohl; *M. violacea* Pohl; *M. irwinii* Rogers et Appan; *M. mossamedensis* Taubet; *M. fruiculosa* (Pax) Rogers et Appan; *M. gracilis* Pohl; *M. warmingii* Mueller; *M. replans* Pax; *M. stipularis* Pax ; *M. oligantha* Pax; *M. nana* Mueller; *M. stricta* Baillon; *M. salicifolia* Pohl; *M. weddelliana* Baillon; *M. peltata* Pohl; *M. janiphoides* Mueller; and *M. handroana* N.D. Cruz.
Secondary center: southwestern Mexico	*M. pringlei* Watson; *M. aesculifolia* Pohl; *M. oaxacana* Rogers et Appan; *M. rhomboidea* Mueller; *M. easkerae*; *M. waskearae* Croizat; *M. divisiae* Croizat; *M. michaelis* McVaugh; *M. websterae* Rogers et Appan; *M. aurivulata* Mcvaugh; *M. rubricaulis* I.M. Hohnson; *M. chlorosticta* Standley & Goldman; *M. subspicata* Rogers et Appan; *M. caudata* Greenman; *M. angustiloba* (Torrey) Mueller; *M. tomatophylla* Standley; *M. foetida* Pohl.
Third center: northeastern Brazil	*M. zentneri* Ule; *M. surinamensis* Rogers et Appan; *M. quinquefolia* Pohl; *M. pseudoglaziovii* Pax et Hoffmann; *M. maracasensis* Ule; *M. quinquepartita* Huber; *M. caerulescens* Pohl; *M. marajoara* Chermont de Miranda; *M. tristis* Mueller; *M. glaziovii* Mueller; *M. epruinosa* Paz et Hoffmann; *M. brachyandra* Pax et Hoffmann; *M. dichotoma* Ule; *M. leptophylla* Pax; *M. reniformis* Pohs; and *M. heptaphylla* Ule.
Fourth Center: western Mato Grosso do Sul and Bolivia	*M. guaranitica* Choda et Hassier; *M. pruinosa* Pohl; *M. jacobinsis* Mueller; *M. condesata* Rogers et Appan; *M. xavantinensis* Rogers et Appan; and *M. flemingiana* Rogers et Appan.

western Pernambuco and central Bahia present the greatest variability of *Manihot*. The primary center of diversity is central Brazil, the secondary center is southwestern Mexico, while the third center of diversity is northeastern Brazil, and the fourth center of diversity is western Mato Grosso do Sul and Bolivia (Table 8.2).

Ekanayake et al. (1997) suggested that the major center of origin for cultivated *Manihot* is located between Brazil and Paraguay and the minor center of origin is Central America including Colombia, Venezuela, Guatemala, and southern Mexico since a large number of wild *Manihot* species are present there (Sauer 1952; Rogers 1965). Presently, cultivated *Manihot* is distributed throughout tropical America and in Asia and Africa. It was introduced into Africa and Asia by the Portuguese travelers in the fifteenth century (Jennings and Hershey 1985; Allem 1994). Further studies on *Manihot* species found in the Central American region suggested that they are only distantly related to the cultivated *Manihot* species (Fregene et al. 1994; Schaal et al. 1994; Roa et al. 1997).

A primary gene pool (GP-1) for cassava was established based on the degrees of cross transferability between cassava and its wild relatives (Allem et al. 2001). The GP-1 of a crop is composed of gene reservoirs that cross easily with the domesticated species, while the crosses regularly produce fertile offspring. The GP-1 is further subdivided into cultivated and wild gene pools. The cultivated gene pool encompasses commercial stocks of the crop, as well as landraces. The wild GP-1 of the crop comprises putative ancestors and closely related species that show a fair degree of fertile relationships with the domesticated species. Two South American wild subspecies of cassava (*M. flabellifolia* and *M. peruviana*) were proposed as natural members of the wild GP-1 of the crop. Another Brazilian species (*M. pruinosa*) is morphologically so close to both wild subspecies that it may turn out as another member of the wild GP-1 (Allem et al. 2001).

The debate still continues, however, it has been generally accepted that cultivated cassava originated from a single progenitor, *M. esculenta* ssp. *flabellifolia*, with probable hybridizations among them and the stretch from Brazil to Bolivia is the center of origin for this crop with many microcenters existing in different places.

8.2.2 Morphology and Taxonomy of Manihot

Rogers and Appan (1973) classified *Manihot* species into 19 sections, with trees in the section *Glazioviannae* to subshrubs, nearly acaulescent, in the section *Stipularis*. Other sections, like *Tripatitae* and *Graciles*, are perennial subshrubs with large woody roots (Nassar 1980). The roots of all *Manihot* species are tuberous, fleshy, farinaceous, and can grow up to 90 cm in length and 15 cm in diameter. The roots can weigh up to 40 kg (average is between 4 kg and 7 kg). These roots are rich in starch and contain a venomous volatile chemical compound, hydrocyanic acid (HCN), that can be eliminated by cooking or heat treatment to make them edible (Rickard 1985; Ravindran 1993; Bunyeth and Preston 2006). Contrary to those of the cultivated cassava genotypes, roots of wild species are fibrous and slender; some species frequently exhibit a limited number of tuberous roots. Root surface is smooth or rough, and subepidermis varies from red or yellow to white color. The cortex of tuberous rooted species is white, cream, or yellow colored.

Stem height varies from almost acaulescent in subshrubs to about 20 m in tree species. Shrubs native to the Brazilian savanna frequently have their stem die back to the crown in the dry season. Stem color varies from gray or brown to reddish. The branching pattern is typically dichotomous or trichotomous with branching point exhibiting a terminal inflorescence. In wild species, the young stem frequently has a varying degree of pubescence, a character rarely encountered in the cultigen (Nassar and Grattapaglia 1986).

Leaves are alternate varying from subsessile to long petiolated. They are spirally arranged on the stem with petioles ranging from 5 to 30 cm long, usually longer than the blades. The young leaves vary in color from yellowish green to deep purple. All species produce palmately lobed leaves. The inflorescence of *Manihot* species is terminal and monoecious except in few rare species that are native to Central Brazil with dioecious inflorescence and grouped in racemes or panicles (Watson and Dallwitz 1992). Flowers have a single perianth composed of five petals, with length ranging from 0.5 to 2.0 cm. Buds of staminate flowers are ovoid or spheric, and those of pistillates are conic. All *Manihot* species outcross and pollination is by insects. Fruits are capsules with three locules and can be elliptic, conical, smooth, or with small wings (Watson and Dallwitz 1992). Seeds have caruncles varying in size, playing a major role in water exchange, enhancing germination in dry areas. The cotyledons are flat and large.

8.2.3 Cytology, Karyotype, and Genome Size

All *Manihot* species have a chromosome number of $2n = 36$ and behave like diploids in meiosis. Thirty-nine cultivars of cultivated *Manihot* species and eight related wild species were analyzed for number, morphology and size of chromosomes, prophase condensation pattern and the structure of the interphase nucleus. All investigated accessions showed a similar karyotype with $2n = 36$, small metacentric to submetacentric chromosomes (Bai 1987; Reginaldo and Marcelo 2002).

The current review on chromosome number of *Manihot* species revealed the segmental allopolploid origin of cassava cultivars as suggested by Jennings (1963) and Magoon et al. (1969). Magoon et al. (1969) studied the pacyhytene chromosomes of cassava cultivars and found completely paired pachytene bivalents varying in length from 19.3 to 40.0 μm. These authors also observed the haploid chromosomal complement inter alia has three functional nucleolar chromosomes and six chromosomal types represented in duplicate during pachytene. This study supported the putative tetraploid nature of current cultivar and suggested its diploid ancestor with similar karyotype (Magoon et al. 1969). Umanah and Hartman (1973) analyzed the karyograms of *M. esculenta* and *M. glaziovii* and concluded that the tetraploid level of both species with similar satellite chromosome pairs was observed in each karyotype.

Studies conducted with isozymes and codominant markers also support the hypothesis and show a disomic inheritance at 12 loci (Umanah and Hartman 1973; Jennings and Iglesias 2002). Similar pachytene studies have been carried out in *M. glaziovii* and a comparison with the karyotype of cassava showed many common features, including the same number and a similar morphology of chromosomes (Krishnan

et al. 1970). Further studies on the genetics of *Manihot* species have been very limited and breeders have concentrated on obtaining the basic information required for effective genetic improvement of the crop. A more complete genomic characterization of the species and its relatives is needed to be focused soon. However, some authors have described it as a segmental allotetraploid with basic chromosome number of $x = 9$. Studies of Jos and Nair (1979) on the meiotic behavior of several cassava genotypes revealed regular 18 bivalent formation of the chromosomes typical of its diploid ($2n = 2x = 36$) status. Cassava breeders also observed a similarity in breeding behavior of allotetraploids to that of diploid (Wricke and Weber 1986).

Although most cultivated *Manihot* genotypes studied are diploid, spontaneous polyploids, such as triploids ($3n$) and tetraploids ($4n$) have been reported in some genotypes (Hahn et al. 1980, 1999). Triploid and tetraploid plants differ from diploid plants in vigor and leaf shape and size. Triploid plants usually grow and yield better than tetraploid and diploid plants. The nucleic acid content of diploid cultivated *Manihot* is 0.83 pg per nucleus equivalent to 800 Mbp in the haploid genome (Awoleye et al. 1994). The heterozygosity levels of different *Manihot* accessions have been further confirmed from diversity studies using isozyme (Lefevre and Charrier 1993a, b), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), AFLP, and simple sequence repeat (SSR) markers (Sánchez et al. 1999; Fregene et al. 2001, 2003).

8.2.4 Agricultural Status

Historically a number of wild *Manihot* species were agronomically evaluated and had genes transferred to cassava, particularly for resistance to diseases such as mosaic virus and bacterial blight and to pests such as green spider mites and mealy bugs, but these hybrids rarely reached commercialization. They have also been used to increase protein content and decrease cyanide content. These species, for example, include *M. catinga* and *M. dichotoma* for resistance to mosaic virus, *Manihot pseudoglaziovii* for drought tolerance, *M. tristis* ssp. *saxicola* for high protein content, *M. tripartite* for compact roots, white flesh and high protein content, *M. stipularis* for drought resistance, adapted to low temperature and dwarfiness, *M. caerulescens* for drought resistance, tolerance to toxic soils and adaptation to low temperature. An exception is the species *M. glaziovii*, which hybridize naturally with *M. esculenta* and have been successfully crossed with it in Africa that provided high-yielding, locally adapted commercial varieties with genes for resistance to cassava mosaic virus, bacterial blight, as well as low cyanide content (Hahn et al. 1980).

The wild *Manihot* species are not cultivated for human consumption and are mostly grown in forests. The consumption of cultivated cassava in Brazil's semi-arid northeast region is among the world's highest, with a per capita consumption at just under 100 kg per year (http://www.fao.org/docrep/007/y2413e/y2413e0c.htm). Because cassava cultivation is growing steadily as an important staple food crop, indigenous species are more likely to experience the thinning out or eventual extinction of their populations. There are, therefore, recommendations to conserve these wild forms for their potential contribution toward improvement of cultivated cassava. Rogers and Appan (1973) described 98 species related to cassava; another new species, *M. neusana*, has also been described (Nassar 1985); and few more are being described. Each and every species is considered to possess traits of potential interest to plant breeders along with high interspecific variation for morphological and physiological traits that could be exploited for sustainable production of cassava.

8.3 Conservation Initiatives

8.3.1 Genetic Erosion

Although cassava is more important in the food and industrial economies of Africa and Asia than in the Americas, there is potential for competition between the cultivated and wild *Manihot* species for arable land. The largest producing countries (e.g., Brazil, Colombia and Paraguay) have the most potential for cassava cultivation to displace wild species (http://www.fao.org/docrep/007/y2413e/y2413e0c.htm) as presented in Table 8.3. The Brazilian Amazon, home to an estimated seven forest species of *Manihot*, has lost 15% of its

Table 8.3 Species of *Manihot* of particular concern for humankind

1[a]	2[b]	3	4	5
M. aesculifolia	*M. anomala*	*M. caerulescens*	*M. flabellifolia*	*M. brachyloba*
M. angustiloba	*M. baccata*	*M. diamantinensis*	*M. peruviana*	*M. pilosa*
M. auriculata	*M. brachyloba*	*M. dichotoma*	*M. pruinosa*	*M. triphylla*
M. caudata	*M. compositifolia*	*M. glaziovii*	–	–
M. chlorosticta	*M. flabellifolia*	*M. jacobinensis*	–	–
M. crassisepala	*M. flemingiana*	*M. janiphoides*	–	–
M. davisiae	*M. hassleriana*	*M. maracasensis*	–	–
M. foetida	*M. mossamedensis*	–	–	–
M. michaelis	*M. peruviana*	–	–	–
M. oaxacana	*M. pilosa*	–	–	–
M. pringlei	*M. pruinosa*	–	–	–
M. rhomboidea	*M. quinquepartita*	–	–	–
M. rubricaulis	*M. sagittato-partita*	–	–	–
M. subspicata	*M. tripartita*	–	–	–
M. tomatophylla	*M. triphylla*	–	–	–
M. walkerae	*M. violacea*	–	–	–
M. websterae	–	–	–	–

Source: http://www.fao.org/docrep/007/y2413e/y2413e0c.htm

[a]1 = Mexican species threatened because of development; 2 = Brazilian species threatened because of development and cassava cultivation; 3 = Species of maniçobas economically valuable to dwellers of Brazil's NE semi-arid region; 4 = Species involved in the ancestry of cassava and constituting the wild primary genepool of the crop; 5 = The putative closest wild relatives of cassava and assumed to participate in the secondary genepool of the crop

[b]Includes species of column 3

original vegetation (Walker and Holmes 1996). The importance of cassava in slash-and-burn agriculture in traditional Amazonian communities is well established (Salick et al. 1997; Emperaire et al. 1998). Thus, the threat of cassava cultivation per se to wild *Manihot* populations is not negligible.

The clearing of land to cultivate cassava in the Brazilian semi-arid region has been most prevalent in areas inhabited by seven wild *Manihot* species known as maniçobas. These species live in the thorny bushy vegetation called carrascos and in the harsh conditions of the innermost area called sertão. They are: *M. caerulescens*, *M. diamantinensis*, *M. dichotoma*, *M. glaziovii*, *M. jacobinensis*, *M. janiphoides*, and *M. maracasensis*. The clearing and burning of the vegetation in drought-plagued areas of the Caatinga has the potential to hit hard local populations of *Manihot* because they are usual components of the vegetation. About 100 municipalities of the area have annual rainfall rates below 500 mm, and seldom between 0 and 250 mm (http://www.fao.org/docrep/007/y2413e/y2413e0c.htm). The urgent need of collection and preservation of wild germplasm is also supported by the fact that considerable genetic erosion is currently taking place among wild *Manihot* species in nature. Nassar and Cardenas (1985) described that *M. walkerae*, *M. guaranitica*, *M. subspicate*, *M. angustiloba*, *M. longipetiolata*, and *M. pringlei* are extremely endangered species.

Socio-economic data as well as farmers' estimation of genetic erosion were the potential indicators of genetic erosion or diversity (Guarino 1995; Brush 1999) in cassava. The study area, the Ucayali region of the Peruvian Amazon, was surveyed through interviews with 285 cassava farmers in 50 communities, while diversity was assessed based on agro-morphological characterization of 295 cultivated *Manihot* accessions (Willemen et al. 2007). The study revealed also that farmers are a good direct source of information on the diversity present at community level, which can contribute to the development of methodologies to assess diversity more rapidly.

8.3.2 Germplasm Conservation

The international 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) along with national institutes made several collection efforts to conserve landraces, improved cultivars, and wild relatives of cassava. About 20,000 accessions of cassava and its wild relatives are presently conserved as ex situ germplasm collection in CIAT, IITA, and national institutes of more than 45 different countries worldwide (Bonierbale et al. 1997). All the accessions in the two CGIAR institutes are held "in trust" under the auspices of Food and Agricultural Organization (FAO) of the United Nations for public access and are freely distributed to all the users through Standard Material Transfer Agreement (SMTA). However, these collections may include duplicates and the total number of unique accessions is likely to be much smaller. South America has the largest collection, followed by West and Central Africa. Such collections are the reservoir for genetic improvement in cassava worldwide (Kawano 2003), although only a sample of these accessions have been thoroughly assessed by the breeders for their useful variation. Hence, a core subset of 630 accessions from 23 countries was established by CIAT (Bonierbale et al. 1997) to provide an entry point and access to the entire collection. A total of 295 cultivated and wild *Manihot* accessions are maintained in Peru by Instituto Nacional de Investigaciony Extension Agraria (INIEA) (Fukuda and Guevara 1998). More than 1,635 accessions (785 exotic and 850 indigenous) have been collected and characterized at Central Tuber Crops Research Institute (CTCRI), India (Pillai et al. 2002). Plant quarantine regulations for cassava made it clear in the 1980s that only in vitro plants would be accepted for distribution worldwide, and technical guidelines for the safe movement of *Manihot* germplasm were formulated (Frison and Feliu 1991).

Various conservation methods are followed such as field gene bank, seed storage, in vitro storage, and cryopreservation of shoot tips and pollen. On farm conservation methods are yet to be established but would be of great advantage in complementing conventional ex situ conservation. Even DNA bank can be one of the options for cassava germplasm conservation.

8.3.3 Conservation Methods

There are two basic conservation methods for plant genetic resources, ex situ and in situ, and the method adopted depends mainly on cost as well as the targeted gene pool of a species. Cassava is an outcrossing species and produces botanical seeds, however, it is mainly vegetatively propagated using stem cuttings or by shoot tips in in vitro cultures to maintain genotypes. The wild relatives are also predominantly outcrossing, and are propagated mainly by botanical seeds, and in some cases, through stem cuttings (Iglesias 1994; Ng and Ng 1997).

Field genebanks are another conservation method wherein cassava germplasm are maintained in the field, as living collection, which are relatively easier to establish and cheaper to maintain with plant materials readily available for evaluation/characterization and cross-pollination. However, germplasm conserved in field are under constant pressure of prevailing diseases and pests, which could lead to a loss of germplasm accessions or even genetic drift (Ng and Ng 2002). Similarly, seed banks are also maintained for cassava seeds, which are orthodox and stored best in cool and dry conditions. The in vitro propagation and techniques are well established in cassava and are routinely used in many genebanks, particularly in CIAT, IITA and in national programs in Brazil, Argentina, Paraguay, and Cuba (Bonierbale et al. 1997; Ng et al. 1999). Shoot tip cultures of cassava clonal accessions are usually conserved under reduced growth or slow growth culture media or reduced incubation conditions (temperature and light intensity). Cultures are checked regularly that undergoes virus indexing for certification by Plant Quarantine Services for multiplication and distribution.

Cryopreservation has been recently gaining importance as it enables long-term conservation of germplasm. Methods to cryopreserve cassava germplasm were developed by Escobar et al. (1997) using classical protocols (chemical dehydration and programmed freezing). New protocols such as encapsulation, dehydration, and quick-freezing have also been developed and validated in cassava. More than 82% of the accessions tested have recovery rates of more than 30%, the minimum required for cryopreservation. Protocols are now being tested for wild relatives of cassava, species of which sometimes behave very poorly in vitro or even in the field, making their conservation troublesome. Plants have been recovered for *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *Peruviana*, and *M. carthaginensis*.

The droplet freezing method that uses dimethyl sulphoxide (DMSO) as the cryoprotectant, and is termed droplet-vitrification when applied in combination with PVS2 vitrification solutions (Panis et al. 2005; Sakai and Engelmann 2007), has also been used in cassava. In this case, shoot-tips require pregrowth treatments before proceeding to the cryoprotectant stage. In the case of droplet-freezing, 10% (v/v) DMSO is dispensed as 2.5–20 µL droplets of cryoprotectant onto sterile aluminum foil strips of 2–3 × 0.5–1.0 × 0.003 to 0.005 cm^3 dimensions with a loading of 5–10 droplets/strip. The shoot-tip meristems are added to each droplet using liquid nitrogen (LN)-tolerant sterile forceps and the foils are directly exposed to liquid phase LN and transferred to cryovials at two foils per vial and stored in the liquid phase of LN. For rewarming, the foils are removed from the vials and placed directly into liquid medium at ambient room temperatures (ca. 25°C). The shoots dislodge on rewarming and are plated onto recovery medium. The cost estimates show that USD5 per year per accession is required for maintaining field collections, whereas the in vitro collection costs USD4.20 per year per accession and cryopreservation maintenance costs are USD1 per accession per year.

The in situ or on-farm conservation of cassava is still not fully operational as it requires broad knowledge on social, biological, and environmental factors. Generally in situ conservation involves maintenance of genetic materials, mainly wild relatives in their natural ecosystems. There is lack of proper documentation of information on cassava and its wild relatives to develop effective in situ conservation strategy. However, the demand for wild *Manihot* species on the part of cassava breeders had a positive effect on their conservation and study. The plants became better known and this in turn raised interest in further collection and conservation efforts. In addition, the wild *Manihot* germplasm assembled in research institutions in Africa and Asia from the early 1930s through the late 1950s was multiplied for generations and made available to the community for other characterization studies. Ex situ conservation will still remain the most effective and reliable way of conserving cassava genetic resources.

Genetic resources of cassava comprise local or introduced landraces, improved cultivars, and related wild species. The CIAT cassava germplasm collection consists of 6,000 accessions with landraces from Latin America and Asia, elite clones selected by CIAT, and the International Institute of Tropical Agriculture (IITA) in Nigeria conserves about 3,000 accessions including several wild *Manihot* species. These are mainly conserved in the form of slow-growth in vitro plantlets and also as living collection in the field. Efforts are underway in IITA to develop a DNA bank for cultivated cassava accessions, which will further be extended to other *Manihot* species.

8.4 Role of Wild *Manihot* Species on Elucidation of Origin and Evolution of the Cultivated *Manihot* Species

One of the questions that remained unsolved until recent times is about the evolutionary and geographical origin of cultivated cassava, and the actual wild progenitors of this crop. In general, *Manihot* species possesses morphological traits that could be reliable for species delimitation but most species show tremendous intraspecific morphological variability. These led to conclusions that hybridization is extensive among co-occurring (sympatric) species in nature (Rogers and Appan 1973). On the basis of morphological traits, the Central American species, *M. aesculifolia* (Kunth, Pohl) and *M. carthaginensis*, was considered as the closest wild relatives and putative ancestors of the crop, although populations were observed in South America that have closer physical appearance to cassava. Tracing cassava's origin through ethno-botanical studies has complicated it further, owing to the crops long-term geographically widespread use (Sauer 1993). Studies using DNA markers such as RFLPs (Fregene et al. 1994), AFLPs (Roa et al. 1997) and DNA sequences (Schaal et al. 1997) indicated that South American and Central American species form two distinct evolutionary lineages, and cultivated cassava is genetically more closer to South American lineage. During the same time, Allem (1994) indicated that the naturally occurring *Manihot* populations (*M. esculenta* ssp. *flabellifolia*) in South America are morphologically more similar to cassava, and could be the wild progenitor of cassava. However, work with AFLP markers and microsatellite analysis showed that *M. aesculifolia*, *M. carthaginensis*, and *M. brachyloba* are the most distant relatives of the crop, whereas the

wild forms *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana* appear to be the closest (Roa et al. 2000). Later, Olsen and Schaal (2001) confirmed, based on DNA sequences and SSR markers that cultivated cassava has a single wild progenitor, *M. esculenta* ssp. *flabellifolia* and the geographical origin of the crop lies along the southern border of the Amazonian basin. The findings of this study also indicated that hybridization between other wild species, such as *M. pruinosa*, might be occurring in nature but it is unlikely that this hybridization would have played any role in the origin of this crop. There could be other progenitors involved in the evolution of cassava; however, strong evidences are lacking. The polymerase chain reaction (PCR)-based markers, such as SSRs and EST-SSRs, also indicated a strong grouping of varieties related to the region of cultivation in Brazil (Carvalho and Schaal 2001). The occurrence of pubescent cassava cultivars in Peru that might have descended from *M. peruviana* indicate that cassava might have been domesticated in eastern Peru (Hahn 1993). It can be summarized that the regions where cassava and wild relatives co-occur, there is possibility of hybridization and introgression; however, it is well proved now that *M. esculenta* ssp. *flabellifolia* is the single progenitor of cassava and its geographical origin lies in southern America.

8.5 Role of Wild *Manihot* Species in the Development of Cytogenetic Stocks and Their Utility for Cassava

8.5.1 Addition and Substitution Lines

Cultivated cassava is a diploid with $2n = 36$ chromosomes based on regular meiosis (Allem 1984). However, meiotic irregularities such as laggards, delayed separation of bivalents, non-orientation, and non-congression of bivalents, monads, dyads, and polyads were also reported (Bai 1987). Interspecific hybrids of cassava with its wild relatives show fair regular meiosis and the backcrossed generations exhibit high fertility (Nassar 2000). Similarly, polyploids were found to be produced in *Manihot* by unreduced gamete fertilization (Nassar 1992) playing a vital role in plant evolution as a means of preserving favorable hybrid combinations during sexual reproduction. The formation of unreduced microspores, which are gametes with somatic chromosome number, appears to be a common phenomenon in angiosperms (de Wet 1980).

Unreduced microspores are very important in plant breeding as they may lead to the development of highly productive triploids and tetraploids by sexual reproduction resulting in preservation of their heterozygosity (Mendiburu and Peloquin 1977). Unreduced microspores occur in interspecific hybrids of cassava involving wild species as one of the parents, as a consequence of meiotic irregularity, and now there is a general agreement that dyads form due to spindle abnormalities, which may be visible at meiotic metaphases, I and II (Nassar 1992; Nassar and Freitas 1997). The causes of formation of unreduced microspores in cassava are variable. It was reported that unreduced microspore formation is due to simple recessive genes (Mok and Peloquin 1975) and also due to the occurrence of non-functional spindles, due to which all the resulting metaphase chromosomes remain in the center instead of separating to the poles (Vorsa and Bingham 1979). Triploidy has been used successfully in cassava improvement especially for the production of high starch varieties in India. In Brazil, the most drought-tolerant cultivar is a natural triploid (Manebeba Branca). Spontaneous tetraploids, triploids and $2n$ pollen (unreduced gametes) were also obtained in IITA (Hahn et al. 1990) from diploid interspecific crosses and from open-pollinated interspecific hybrids, involving female diploid plants of *M. esculenta* crossed with male parents of *M. pruinosa* or *M. glaziovii*. These two related species are highly apomictic but can also hybridize naturally with cassava. Spontaneous sexual and asexual polyploids occur in polyploid breeding of cassava. Sexual polyploidization has advantages over asexual polyploidization in terms of variability of gametes, heterosis, vigor, plant architecture, and productivity of the crop (Hahn et al. 1990). Many polyploid cassava clones have been generated at IITA utilizing bilateral and unilateral polyploidization. Triploids obtained from the cross between normal diploid and colchicine-induced autotetraploids outyielded the autotetraploid parents (Jos et al. 1987). A natural hybrid of *M. pseudoglaziovii* and cassava was collected and multiplied vegetatively and studied cytogenetically (Nassar 1991). A triploid was selected from the selfed progeny of this hybrid and its evaluation showed a high

productivity of tuber roots and resistance to stem borer pest. All these results indicate that triploids are more promising than tetraploids in cassava improvement.

Breeding for characters governed by recessive genes is difficult in cassava owing to its allotetraploid nature; therefore, production of haploids utilizing microspore cultures or dihaploids from $4x \times 4x$ matings in *Manihot* could be useful for cassava breeding in various ways. Reducing ploidy levels from tetraploidy to diploidy level could be useful and be efficiently applied for cassava improvement because genetic manipulation is much easier at diploid level than at tetraploid level (Hahn et al. 1990). Thus wild relatives have traditionally been used as sources of useful traits and their use will continue to introgress new genes into cassava.

8.5.2 Aneuploids

Availability of aneuploids greatly facilitates the construction of cytogenetic maps in which it is possible to locate the marker loci not only on the chromosomes but also on specific regions of the chromosomes. The meiotic division in interspecific hybrids may lead to a higher frequency of aneuploid gametes, making it possible to select polyploids from their progeny (Nassar et al. 1995). Progenies of two interspecific hybrids of cassava with wild *Manihot* species were studied meiotically as well as mitotically (Nassar et al. 1996). A tetrasomic aneuploid from the progeny of the above-mentioned hybrids had a very large starchy root. These results indicated that root formation in *Manihot* species is controlled by additive polygenes that are distributed on more than one chromosome (Nassar et al. 1996). Apomixis in *Manihot* is frequently associated with aneuploidy but it does occur in some diploid types. It is due to the formation of aposporic sacs, which can easily be detected by clearing tissue preparations (Young et al. 1979; Nassar and Santos 2002).

8.5.3 Interspecific Hybridization

Most species of the *Manihot* genus can be easily crossed with cultivars of *M. esculenta* and desirable alleles can be transferred for cassava improvement (Hahn et al. 1980). However, only limited number of possible interspecific crosses has, so far, been attempted. Crosses were made between *M. esculenta* and *M. glaziovii* for resistance to cassava mosaic disease (CMD), hybrids from *M. esculenta* and *M. melanobasis* crosses had superior seed set and root yield, and crosses between cassava and *M. saxicola* resulted in hybrids with better yield and higher protein content. Successful crosses were also obtained between cassava and *M. dichotama* and *M. catingae*. Four interspecific hybrids were obtained between cassava and *Manihot* species for polyploidization. These are *M. neusana* × *M. esculenta*, *M. glaziovii* × *M. esculenta*, *M. aesculifolia* × *M. esculenta* and *M. pohlii* × *M. esculenta* (Nassar 2000).

Interspecific hybrids of several wild *Manihot* species with cassava through controlled crosses by insect vectors have been reported (Nassar 1978b, c, d, 1989, 1994). These were identified by the dominant morphological markers from cassava such as noded stem, setaceous bracteole, ribbed fruit, and tuberculated root. Wild *Manihot* species were hybridized with cultigens in order to incorporate their desirable genes for drought tolerance, high protein content, apomixis, high yield and pest and disease resistance into the cultivars (Nassar 1997, 1999), and also for nutritional quality and yield of roots (Nassar and Dorea 1982).

8.6 Role of Wild *Manihot* Species in Classical and Molecular Genetic Studies of Cassava

8.6.1 Classical Genetic Studies

Not much research has been carried out on cassava genetics and the earliest work in the field of genetics was done in Indonesia, Zaire, East Africa, Madagascar, India, and Brazil. During the 1970s, with the establishment of CIAT, Columbia, and IITA, Nigeria research was focused more on cassava classification and variability. These two international centers collaborated with national programs such as EMBRAPA, Brazil, CTCRI, etc. to increase yield per unit area and also root quality. The breeding strategies varied from one country to another and depended mainly on the

final use of the crop, whether for human consumption or industrial use or for subsistence. In Africa, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the major constraints. Bacterial blight caused by *Xanthomonas axonopodis* pv. *Manihotis* is prevalent in Asia, Africa, and Latin America, and is also a major constraint. There were other difficulties with cassava being allogamous and highly heterozygous in nature because of which its sexual progenies are highly heterogeneous with wide morphological variation among them, something which is not preferred for commercial cultivation. The breeding strategy for Africa is higher yield coupled with resistance to diseases and pests, and IITA continued its research on crossing selected wild species, particularly *M. glaziovii*, with cassava with the aim of producing improved genotypes, especially with resistance to African mosaic virus (Hahn et al. 1980). In South America, breeders had access to a much broader range of genetic variability within cassava, and did not resort to use of wild species for obtaining novel traits. However, the African institutes crossed cassava and the Brazilian species *M. dichotoma*, *M. catingae*, and *M. glaziovii*, besides the Surinamese species *M. saxicola* and *M. melanobasis* (Nichols 1947; Jennings 1959) for targeted traits.

Achievements in cassava breeding using its wild relatives in Africa includes the development of a range of elite cassava genotypes, such as TMS 30572 and TMS 4(2)1425, that combined high stable yields, agronomic traits, and consumer quality with acceptable levels of resistance to CMD and cassava bacterial blight (CBB). These genotypes are widely cultivated (Hahn et al. 1989) and adopted (Otim-Nape et al. 1994) in Africa. The introduction of Latin American germplasm into the breeding programs in Africa has resulted in significant broadening of the genetic base of cassava in Africa. Classical breeding has contributed to improve dry matter and starch content as well as reducing the cyanogenic potential in cassava. Subsequently, a farmer participatory plant breeding (PPB) scheme was employed to evaluate recombinant progenies and select several varieties adapted to the semi-arid region of northeastern Brazil (Fukuda and Saad 2001).

Cassava mosaic disease (CMD) in Africa, a disease that is not found in Latin America, limited immediate use of the African germplasm but required introgression of CMD resistance into the Latin American germplasm. Resistance to CMD was introduced by backcrosses and thousands of recombinant seeds were produced and distributed to participating countries in West and Central Africa. The improved cassava germplasm has extended considerably the range of cultivation of cassava beyond its traditional area in the humid and subhumid tropics into the semi-arid zone of West and Central Africa by more than 100,000 ha between 1989 and 1999 (IITA 2000b, c, d).

Conventional breeding programs in Africa, Asia, and South America have been successful in developing and releasing varieties with enhanced harvest index and resistance to prevailing diseases. However, the rate of improvement in cassava yields has been much lower than its potential except for some Asian countries (Kawano 1978). The classical breeding in cassava is also complicated due to strong heterozygosity and inbreeding depression coupled with lengthy breeding cycles and the need for making large number of crossings to obtain the first generation progenies, which are then screened for desired traits. Selected individuals are then propagated vegetatively to generate sufficient number of clones for further screening and multilocation trials (Ceballos et al. 2004). There is a general acceptance that better progress can be made in cassava improvement through incorporation of modern biotechnological tools and molecular marker-assisted selection in breeding programs.

8.6.2 Molecular Marker Studies

In recent times, genetic or DNA markers have become one of the fundamental tools for understanding the inheritance and diversity present in any crop. The availability of markers has made it possible to construct genetic/linkage maps, cloning of genes, and quantitative trait loci (QTL) analysis along with whole genome sequencing. The earliest markers used in cassava were morphological and Graner (1942) described leaf shape and root color as two morphological markers to study their inheritance. Later many more morphological markers were described based on stem, leaves, and root characteristics (Harshey and Ocampo 1989). The second generation markers were biochemical ones such as isozymes, which provided a useful tool for genetic fingerprinting and to study genetic diversity in cassava (Ramirez et al. 1987; Lefevre and Charrier 1993a). Isozymes have also been used to determine genetic

relationships among African cassava germplasm (Lefevre and Charrier 1993b; Wanyera et al. 1994) and also to fingerprint the CIAT germplasm (Ocampo et al. 1992). However, with the advent of DNA/molecular markers, they gained importance rapidly in the studies involving inheritance, genomics, and genetic diversity. The most prominent molecular markers systems included minisatellites (Jeffreys et al. 1985), RFLPs (Botstein et al. 1980), RAPDs (Williams et al. 1990), and more recent ones are microsatellites/SSRs (Litt and Lutty 1989), AFLPs (Vos et al. 1995), DNA sequencing of internal transcribed spacer (ITS) of ribosomal DNA, DNA chips or oligonucleotide arrays, and single nucleotide polymorphisms (SNPs); the list is ever-growing. Currently, the cassava research community has developed several hundreds of molecular markers of which SSRs account for the largest proportion (Fregene et al. 1997; Mba et al. 2001; Okogbenin et al. 2006; Raji et al. 2009). Recently, following development of ESTs in various labs around the world (Anderson et al. 2004; Lokko et al. 2007; Sakurai et al. 2007), SNP markers are becoming the markers of choice owing to their suitability to the latest high-throughput genotyping platforms (Gedil and Sartie 2010).

8.6.2.1 Genetic Diversity Studies

The germplasm collection of cassava consisting of cultivated and wild accessions is a very important resource for the future improvement of the crop, and understanding the genetic relationships among accessions using molecular markers has made it possible to select diverse parents in the breeding programs. The genetic relationships among cassava accessions and wild *Manihot* species was studied using DNA markers (Jeffreys et al. 1985; Ocampo et al. 1995) and possible duplicate accessions were identified. The molecular analysis of *M. aesculifolia* contradicted its origin in Meso-America as described based on morphological characters and suggested a possible domestication of this species from Brazil along with some other close wild relatives such as *M. trsitis* and *M. esculenta* ssp. *flabellifolia*. Another study using AFLPs demonstrated that *M. esculenta* ssp. *flabellifolia*, *M. trsitis* and *M. peruviana*, all originating from Brazil, are more closely related to cassava than its Mexican close relative, *M. aesculifolia* (Roa et al. 1997; Carvalho and Schaal 2001).

Molecular markers have made immense contribution to cassava breeding and genetics, in assessment of genetic diversity, taxonomical studies, understanding the phylogenetic relationships in the genus, confirmation of ploidy, and development of genetic maps (Fregene et al. 1997, 2001; Lokko et al. 2004).

8.6.2.2 Construction of Genetic Maps

As most of the economic traits studied in cassava are polygenic, efforts were made to identify important QTLs through molecular mapping studies (Hahn et al. 1989; Rajendran 1989; Amma et al. 1995; Bryne et al. 1997; CIAT 2003).

First Generation Maps

Angel et al. (1993) initiated work on a detailed genetic map of cassava for tagging agronomically important traits and to clone cassava genes using a range of cassava accessions and a wild *Manihot* species. It was concluded that a combined use of RFLP and RAPD markers would lead to the construction of a detailed map of cassava. Gomez et al. (1996) used 328 RAPD markers for linkage analysis in cassava. Following this, the first cassava linkage map was developed by Fregene et al. (1997). This map was based on an F_1 population of two geographically divergent parents. The female parent TMS I30572, with resistance to CMD, was derived through introgression from *M. glazovii* (a wild relative of cassava), while the South American male parent CM 2177–2 (ICA-Cebucan) was susceptible.

In a heterozygous species, the segregating F_1 population is obtained by crossing a wild relative with a cassava cultivar (Pillay and Kenny 1996; Fregene et al. 1997). In a cross with three isoenzyme loci segregating as single-dose restriction fragments (SDFs, for more details read Wu et al. 1992) in the gametes of the female parent plus a total of 150 RFLPs, 30 RAPDs, and five microsatellites, 20 linkage groups were defined spanning 950 cM map length with an average marker density of one per 6 cM. In another study with 120 RFLPs, 50 RAPDs, four microsatellites and one isoenzyme single dose marker in the gametes of the male parent, 24 linkage groups were defined with a total length of 1,220 cM and

average marker density of one marker every 8 cM. Intervals were observed to be larger in the male-derived map than in the female-derived map and a paired *t*-test showed significantly ($P = 0.01$) greater distances in the male-derived map, suggesting a reduced recombination rate in gametes of the female parent. The mean interval length between adjacent allelic bridges (markers common to both parents) in the female-derived map was 38% less than in the male derived map. The male- and female-derived maps together covered 300 markers and 80% of the cassava genome requiring more number of markers to complete the genetic map.

Second-Generation Maps

Most of the markers present in first-generation map are based on RFLPs or RAPDs, which are not the best for large-scale, high-throughput marker-assisted breeding of plant populations. Therefore, attempts were made to develop second-generation map using PCR-based, highly polymorphic markers such as SSRs and sequence-tagged sites (STSs), which can be easily integrated into the existing genetic maps or can be used to develop new linkage maps. Mba et al. (2001) developed and characterized 172 SSR markers to saturate the existing linkage map of TMS I30572 × CM 2177–2 based on 150 progenies. Currently, the total number of RAPD, RFLP, and SSR markers on the cassava genetic map is 830 and for the first time 18 analogous linkage groups representing 18 chromosomes of cassava were identified (Fregene et al. 2001). With over 1,000 markers available, further efforts have been made to generate linkage maps in cassava from diverse genetic backgrounds and saturate the map (Akano et al. 2002; Lokko et al. 2003, 2004).

8.7 Role of Wild *Manihot* Species in Cassava Improvement Through Traditional and Advanced Tools

According to Allem (1999), wild genetic resources of *Manihot* have five important utilities (1) agricultural value (agrobiodiversity – direct or indirect economic application either as food suppliers or as income generators); (2) scientific value (enlargement of knowledge); (3) social value (recreation); (4) cultural value (broader spectrum of interest to the community); and (5) ecological value (keystone species or as participants in nature's food chains).

8.7.1 Traditional and Molecular Breeding Efforts

The importance of cultivated *Manihot* as a global food source demands the production of improved germplasm (Scott et al. 2000). Clonal selection was the predominant method in conventional breeding for cassava improvement at the national centers in Africa and Brazil. The only exception to this was the production of the CMD-resistant clones by hybridizing *M. glaziovii* with cassava (Storey and Nichols 1938). However, traditional breeding systems for cassava are hindered by its highly heterozygous nature, asynchronous flowering, and inbreeding depression. In addition, traditional breeding techniques for cultivated *Manihot* are cumbersome, requiring screening of approximately 100,000 seedlings after the first sexual crossing and at least 10 years for the improved product to reach the farmer. The CIAT, Columbia established a cassava breeding program in early 1970s with the aim of improving yield potential and resistance/tolerance to pests and diseases. As a part of this program development, the following processes were accomplished: germplasm collection and evaluation, generation of advanced breeding materials, and varietal selection and dissemination (Kawano 2003).

The development of transgenic technologies in cassava could circumvent many of the problems inherent in traditional improvement programs. Cassava cultigens are deficient in many desirable agronomic characters such as pest and disease resistance, drought tolerance, and low protein content (Nassar 2000). Developing interspecific hybrids and employing genetic transformation systems could help facilitating introgression of heterologous genes that could potentially improve the genetic diversity and revolutionize the improvement of cassava breeding program. The capacity to integrate transgenes into cassava is now established and being utilized to generate plants expressing traits of agronomic interest (Nigel et al. 2005). In 2000, CIAT initiated a program to introgress

genes for several traits such as root yield, quality, and dry matter content from wild cassava relatives into its germplasm collection (Ojulong et al. 2008).

Apomixis is the alternative means of reproduction in cassava, which helps in avoiding the systemic pathogens, excluding the genetic segregation in the progeny, and facilitating rapid speciation in this genus. Apomixis is present at very low level in cassava (1–2%). Facultative apomixis was discovered in the wild relatives of cassava (Nassar et al. 1998; Nassar 2001) and interspecific hybridization was carried out to transfer the useful genes to the cultigens (Nassar and Collevatti 2005).

Polyploidization of interspecific hybrids was attempted by colchicine treatment, which resulted in the production of tissues having different ploidy levels (Nassar 1991). In addition, a somatic polyploidization technique has also been proposed for cassava breeding in which in vitro plantlets were treated with colchicine and oryzalin (Awoleye et al. 1994). Thus interspecific hybridization, apomixis, and polyploidy contributed to the evolution of the *Manihot* in which interspecific hybridization and polyploidy produced the genetic variability necessary for speciation, while apomixis was responsible for perpetuating new hybrid types adapted to various environments.

8.7.2 Genetic Transformation

Efforts to develop a genetic transformation system for cassava were initiated in early 1990s, but remained elusive until 1996 because of its recalcitrance to in vitro manipulation. Initial efforts reported a system of somatic embryogenesis in cassava (Stamp and Henshaw 1982). For this purpose, embryogenic cultures are induced to develop from immature leaf explants after 3 weeks culture on medium supplemented with growth hormones (Stamp 1987) and also by particle bombardment or *Agrobacterium tumefaciens*-mediated transformation (Raemakers et al. 1996, 1997). However, only chimeric tissues were recovered because of the multicellular nature of the morphogenic events and highly organized nature of the embryonic structures (Schöpke et al. 1993).

By manipulating the embryonic culture systems of cassava, the genetic transformation capability has been improved. Four different techniques have been reported for recovering transgenic cassava plants with integration of transgenes for beneficial agronomic traits (Li et al. 1996; Schöpke et al. 1996; Taylor et al. 2004). All the four systems are reliant on the production of embryogenic tissues from in vitro mother plants (leaf explants). These four transformation systems are: production of somatic embryos from friable embryogenic callus, cotyledon fragments, embryogenic cultures, and immature leaf explants (Taylor et al. 2004).

Thus, cassava improvement continues to tap genetic variation from wild *Manihot* through conventional breeding and advanced techniques such as transgenics. However, novel sources of variation are required to genetically advance this important food crop of Africa and other areas in the tropics of the developing world.

8.8 Genomics Resources in *Manihot* Species

To augment the cost-effectiveness of achieving the required goals, a number of genomic resources and molecular tools have been developed during the recent years to advance breeding of cassava cultivars.

The incentive for the genome sequence of cassava began in 2003 but the whole genome project gathered momentum in early 2009 utilizing 454 sequencing technology. More than 61 million sequencing reads were generated and assembled into a draft genome that contains an estimated 95% of cassava genes. It is one of the first large genome projects to primarily use 454 Life Sciences' long-read sequencing platform, which enabled both improved quality of the draft, and its rapid generation. The annotated draft genome sequence is available at the US Department of Energy-Joint Genomic Institute (DOE-JGI) Phytozome Web site (http://www.phytozome.net/cassava). The other genomic resources of cassava include expressed sequence tag (EST) databases, which provide resources for producing Euphorbiaceae-specific DNA microarrays and cross-species comparisons. A full-length cDNA library of cassava plants under normal, heat, drought, aluminum, and post-harvest physiological deterioration conditions was built (Cortés et al. 2002). As of August 2010, there are 83,537 nucleotide and EST sequences at public genomic databases such

as GenBank, which is a very small number compared to the number of sequences from maize (4,459,127), rice (4,430,606), soybean (2,097,140), potato (385,881), or sugarcane (295,578).

In order to characterize the library and find the number and putative functions of the transcripts that were captured, nearly 20,000 clones were sequenced from both ends (Sakurai et al. 2007). These studies were able to determine the information about 5′ UTR's of 1,949 sequences and 3′ UTR's of 2,241 sequences as well as the complete coding sequence of 732 genes, all of which is an essential resource for gene discovery, characterization, and cloning, and to assist in the annotation of the cassava genome. The ESTs were assembled into 6,355 contigs and 9,026 singletons that were further grouped into 10,577 scaffolds. About 4,621 new cassava sequences and 1,521 sequences were found with no significant similarity to plant protein databases. Transcripts of 7,796 distinct genes were captured and functional classification was assigned to 78% of them, while more than half of the enzymes annotated to metabolic pathways in *Arabidopsis* (Sakurai et al. 2007).

To increase the tools for understanding and manipulating drought tolerance in cassava, another group of scientists generated ESTs from normalized cDNA libraries prepared from dehydration-stressed and control well-watered tissues yielding a total of 18,166 ESTs with an average read length of 586 nucleotides (Lokko et al. 2007; Raji et al. 2009). Analyses of these ESTs resulted in the identification of 8,577 unique gene clusters, which can be utilized for the development of microarrays and gene-derived molecular markers to further dissect the molecular basis of drought tolerance in cassava.

Another genomic resource for cassava includes bacterial artificial chromosome (BAC) library from a wild relative of cassava that was constructed with the objective of map-based cloning of disease and pest resistance and root quality genes (Fregene et al. 2001). This library has 5*x* coverage of the genome and a 95% probability of finding any desired clone. This BAC library is publicly available through CIAT.

The other area where progress has been made is to target the resistance gene analogs (RGAs). The rapid accumulation of genome sequence data has paved a way to develop arrays of functional genomic tools to understand the complex pathways of host–pathogen interactions. A study is underway where several pairs of degenerate primers matching the conserved domains of *R*-genes to amplify putative RGAs in cassava and its wild relatives such as *M. glaziovii* and *M. eprunosa* along with castor bean (*R. communis*) has been identified (M Gedil et al. personal communication). In addition, a cassava DArT (diversity array technology) chip with 735 polymorphic markers has been used to fingerprint a diverse range of cassava populations including genotypes from Africa, Latin America, Asia, and breeder lines maintained at IITA along with few wild relatives (IITA unpublished data).

8.9 Gene Flow Between *Manihot* Species

The major constraint in *Manihot* is interbreeding between the species that can cause a "swamping" of the rarer species' gene pool, creating hybrids that drive the originally purebred native stock to complete extinction. It has been confirmed that cassava varieties can cross-pollinate naturally with wild relatives, as a result of which, in South America, transgenes could move easily from a genetically modified variety to other species. The cultivated *Manihot* is developed from wild *Manihot* through controlled selection and hybridization; therefore it is expected that there is highly successful cross-breeding between cassava and its wild relatives, which might pose issues related to gene flow in near future. The gene flow has been closely observed in Africa between cassava (*M. esculenta* ssp. *esculenta*) and *M. glaziovii* in Nigeria and Cote d'Ivoire (Beeching et al. 1993). Therefore, cross breeding between a transgenic cassava resistant to stem borer and the wild relative *M. glaziovii* could take place in regions such as Northeast Brazil where cassava is widely cultivated.

The wild relative of cassava, *M. esculenta* ssp. *flabellifolia* is another potential source to carry genetically modified traits. It was revealed in Central America that only five seeds (very few) formed from two crosses made between cassava and *M. aesculifolia*, a distant relative of cassava (http://books.google.com). Therefore, it is not accountable information on gene flow. The gene flow was well explained by Chavarriaga-Aguirre et al. (1999) between Mexican and Guatemalan cassava accessions using molecular markers. Based on this preliminary data, the authors concluded that there is no concrete information

about gene flow between the cultivated cassava and wild *Manihot* in the actively growing regions of cassava. Further, the biosafety issues of transgenic cassava and potential weed problems still need to be studied in-depth. It was reported by Allem (1984) that the new genes for resistance to African mosaic virus have been incorporated from *M. glaziovii* to *M. esculenta*.

8.10 Recommendations for Future Actions

To boost the role of cultivated *Manihot* as a food security and biofuel crop, there is need for increased research to improve and stabilize yields by developing genetic resistance to major pests and diseases (IITA 2000b, c, d). Part of this could be achieved through increased efforts toward collection and conservation of wild species that are reservoirs of desirable genes for resistance and tolerance to prevailing insects and diseases and also newly emerging diseases and pests owing to changing environment. The 10–12 wild *Manihot* species native to Brazil's southern (subtropical) and southeastern (Cerrado or Savanna) regions are under tremendous threat of erosion due to excessive deforestation over the last five decades for the cultivation of cash crops (http://www.fao.org/docrep/007/y2413e/y2413e0c.htm). Therefore, it is recommended that wild *Manihot* species needs protection from extinction through on-farm conservation in their natural habitat, mainly in the Caatinga region of Northeast Brazil and the Cerrado of central-west Brazil.

Furthermore, wild *Manihot* species should be collected and conserved ex situ, especially the six species most closely related to *M. esculenta*, for possible future interspecific breeding to transfer desirable traits to the cassava. The urgent need for collection and preservation of germplasm was also supported by the reports of genetic erosion by Nassar and Cardenas (1985) for extremely endangered species such as *M. walkerae, M. guaranitica, M. subspicate, M. angustiloba, M. longipetiolata*, and *M. pringlei*.

The conservation efforts should also target the group of species known as "the cassava species complex," which is composed of the wild progenitor of the crop and four other closely related species from the Brazilian tropics. Study of this complex, from the perspectives of taxonomy, biosystematics, and cladistics, will shed new light on the origin, phylogeny, and evolutionary patterns of cultivated *Manihot*. It was only through recent systematic studies of the biodiversity that the long-searched origin of cassava seems close to completion (Allem 1994, 1999; Olsen and Schaal 1999). Similarly, pest and disease resistance genes can be easily introgressed from these valuable wild species into cultivated genotypes by traditional and molecular breeding approaches (Charoenrath et al. 2006). High starch content in *Manihot* species makes it one of the best alternative candidates for ethanol production (Ziskaa et al. 2009). The technology for converting *Manihot* into biofuel ethanol source is currently being perfected and will be very soon applicable anywhere in the world (FAO 2008).

The second conservation effort should select the so-called species, maniçobas, from Northeast Brazil's semi-arid Caatinga region. Three of them in particular merit attention: *M. caerulescens* ("maniçoba do piauí," a source of cheap latex), *M. dichotoma* ("maniçoba de jequié," a minor supplier of latex and domestic utensils such as wooden spoons), and *M. glaziovii* ("maniçoba do ceará," a source of resistance to diseases and pests). The latter is a most versatile species. It provides commercially useful latex and wood, and has been studied for potential use as feed for goats and cattle in Brazil's semi-arid northeast (Ravindran 1993). Most importantly, *M. glaziovii* supplied African cassava breeders with genes for resistance to the African cassava mosaic virus and the cassava bacterial blight (Hahn et al. 1980). A more far-reaching implication is the possibility that commercial cassava stocks worldwide share much of their genome with that of the wild progenitor. Wild materials related to the ancestry of the crop, and making up the wild primary gene pool, should be in storage to enable comparative tests.

Breeding efforts to increase the yield potential and disease/pest resistance of cassava have in some cases reduced the number of commercially grown varieties and thus eliminated the use of certain farmers' landraces. However, extensive collections of *M. esculenta* varieties in the centers of origin in Latin America, and their conservation in field-grown germplasm banks, in vitro culture, cryopreservation, have helped to prevent the loss of biodiversity in *M. esculenta*.

Cassava production has had a minimal effect on the biodiversity of *Manihot* in the center of origin of the species, i.e., in Mexico and Brazil, with a possible exception of the semi-arid northeast of Brazil where intensive monocropping of cassava may threaten the survival of seven *Manihot* species native to that area. Narrowing of the genetic base of commercial cassava varieties by the use of a small number of widely-adapted, high-yielding varieties should be avoided, by the continued release of new varieties with a broad genetic background. This will reduce the risk of widespread crop failure, for example, in case of adverse climatic conditions or appearance of new diseases or pests.

Global organizations such as FAO, CIAT, and IITA actively advocating for cassava crop improvement and effective utilization of biological control agents for mealy bug and green mite in Africa are two examples of such global efforts. Global collaborative and conservation efforts will help preserve the natural diversity of cassava; enhance the development of high-yielding, disease and pest tolerant varieties; improve crop management; and post-harvest practices while avoiding duplicated efforts. To bring the fruits of these global collaborations to farmers, multiplication and distribution of the improved cassava vegetative stocks should be taken up by local, national, and international government agencies.

Cultivation of transgenic crops is rapidly spreading across the world. Accessibility of novel transgenic technology at a nominal licensing fee from commercial and academic institutions around the world to researchers would help to trap the resources from wild *Manihot* to enhance the current crop improvement efforts. Increased investment and international collaboration in global *Manihot* research will definitely help meet the global food and biofuel demand at a rapid pace.

The completion of whole genome sequencing in cassava (*M. esculenta* ssp. *esculenta*) has been a major and most significant achievement for the entire cassava research community. This will not only accelerate the development of large number of new and efficient molecular markers for the characterization of other *Manihot* species but will also enhance the use of next-generation technologies to identify genes involved in many important traits. To meet the demand for rapidly growing population and to mitigate the impact of climate change that has resulted in global food crisis, use of wild relatives and integration of newer and innovative technologies in breeding programs will accelerate the process of improving multiple traits in a more precise way and in turn improve the productivity of this major staple crop.

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