
Evolution, Domestication, and Agrobiodiversity in the Tropical Crop Cassava

Cassava (*Manihot esculenta*), Euphorbiaceae, is the sixth most important crop globally (Mann, 1997). It is the primary staple crop for more than 500 million people worldwide, serving mostly the poor in tropical developing countries (Best and Henry, 1992). It is the major source of calories in sub-Saharan Africa, where it is grown primarily for its starchy roots, although it can serve as a leaf crop as well (Cock, 1985). Cassava is an inexpensive source of starch and is currently being developed for industrial uses as well as a source of animal feed, primarily in Asia. Nonetheless, most of the world's cassava is consumed by subsistence farmers in Africa and Latin America. In the past cassava was considered an orphan crop. Because the majority of cassava consumers live in poverty with little access to cash, efforts at cassava crop improvement have lacked the economic stimulus that commerce provides. Consequently, much of the basic biology of the crop and its closely related species has gone understudied until recently, in stark contrast to the extensive work on cash crops such as corn, wheat, rice, and soybeans.

Cassava has great potential to increase the food security of people in the developing world. Average yields for cassava in Africa are 8 ton/ha, but potential yields are 80 ton/ha (*FAO News*, November 5, 2002). Unlike most other crops, plants continue to deposit carbohydrates into the storage roots as long as the plant is actively growing. In general, cassava tolerates

moderate levels of drought, and it grows well in the nutrient-poor soils of the tropics. Moreover, the crop is easy to plant and grows especially well in marginal agricultural areas of the world. New plants are started from planting sticks; pieces of stem are stuck into the ground, and 6–9 months later the farmer returns to harvest some or all of the storage roots.

Although cassava has many benefits as a crop of the tropics, it has several limitations that challenge the human populations that subsist on it. Historically, the crop has been cultivated mainly to produce cassava flour and starch. Selection for these traits has resulted in the storage root of most cultivars having high caloric content and low vitamin, protein, and mineral levels. People who subsist solely on cassava are subject to nutritional deficiency diseases such as kwashiorkor and night blindness. Other challenges include root susceptibility to postharvest deterioration. The roots of cassava plants are nonperenniating, and spoilage occurs quickly after harvest through physiological deterioration caused initially by oxidation followed by colonization and growth of various saprophytic microorganisms. In addition, cassava plants are susceptible to various pathogens, including a bacterial blight and the African cassava mosaic virus, a devastating pathogen found in Africa that can reduce yields by as much as 70%. The challenge for crop breeders is to increase nutrition and pathogen resistance while reducing postharvest deterioration. Finally, the value of cassava as a cash crop is low. Many other crops provide inexpensive sources of starch, and the quality of cassava starch is low, making it less desirable as a source of raw material for the food industry. The lack of a cash crop for many farmers of the developing world has significant effects on the well-being of families, who cannot buy such things as medicines and books without cash. Any modification of cassava to enhance its value as a market crop would have a direct and positive effect on the lives of poor families. As we discuss in this chapter, landraces of cassava that sequester carbohydrates other than amylose have great potential as cash crops.

Traditionally, many plant breeders have turned to landraces, wild ancestors, and closely related species as a source of traits for future crop improvement. For example, some of the rice varieties developed during the Green Revolution contained pathogen-resistant genes from rice's wild progenitor, *Oryza rufipogon*. Our own work with cassava has centered on the native and agricultural biodiversity of *Manihot*. We have investigated the wild ancestor of cassava, determined the site of domestication, and studied the relationship of the cassava to its wild relatives, and one of us (L.J.C.B.C.) has discovered and characterized the biodiversity of landraces in the Amazon Basin. These studies identify and describe potential reservoirs of germplasm

for the improvement of cassava. In addition, cassava serves as a model system for understanding evolution in a recently arisen plant genus and for understanding domestication in a perennial, clonally reproducing crop.

Systematics of *Manihot*: Cassava's Close Relatives

Manihot (Euphorbiaceae) is a genus of an estimated 98 neotropical species (Rogers and Appan, 1973). Plants range in habit from herbs to small trees. There are two centers of diversity for the genus: one in Brazil, with some 80 species, and another in Mexico, with approximately 17 species. Four major centers of species diversity are found in Brazil. The region of greatest diversity is in the Central Plateau, with 58 of 80 species. Eight species are found each in the northeast and southeast of Brazil, with six species localized in the Amazon region (for distribution maps, see www.cenargen.embrapa.br). The taxonomy of *Manihot* is enigmatic. Early work by Rogers (1965) and Rogers and Appan (1973) noted the overlap in morphology between species, the phenotypic plasticity of characters, the lack of chromosome variation, and the limited number of taxonomically informative traits. Rogers and Appan and, more recently, Allem (Allem, 1987, 1994, 1999) have struggled with a morphologically based taxonomy. The paucity of reliable taxonomic traits has made studies on the origin of cassava difficult because based on morphology alone, several species of *Manihot* from Mexico, Central America, and South America are potential ancestors, with no single species being morphologically so similar to cassava to be unambiguously assigned the wild ancestor.

The lack of clear morphological affinities of cassava with any single wild *Manihot* species led to hypotheses that cassava may be a hybrid derivative, a hybrid between two to several species. This "compilospecies" origin is most closely associated with the species complex in Mexico, but a similar hybrid origin has also been suggested among South American species (Rogers, 1963, 1965; Rogers and Appan, 1973; Ugent et al., 1986; Sauer, 1993). As an alternative to a multiple-species origin, Costa Allem in the early 1990s suggested that wild *Manihot* populations occurring in Brazil were so similar to domesticated cassava that they were part of the same species (Allem, 1987, 1994). These wild populations, *M. esculenta* ssp. *flabellifolia*, differ from domesticated cassava almost entirely in traits that appear to be associated with domestication such as shortened internodes, thickened stems, swollen leaf scars, a more erect stature, and increased size of storage roots.

To address the issue of cassava's origins, we initially used a traditional molecular phylogenetic approach to understanding species relationships (Hillis et al., 1996). We obtained collections of cassava species from Mesoamerica and from South America, including species that had been previously identified as potential progenitors of cassava such as *M. aesculifolia* from Mexico, *M. cartaginensis* from Colombia, and a suite of species that Allem considered the secondary gene pool for cassava, *M. pilosa*, *M. triphylla*, and *M. esculenta* ssp. *flabellifolia*. We used several DNA sequences to reconstruct the phylogeny of this group of species, including chloroplast DNA, and nuclear regions such as the internal transcribed spacer region of ribosomal DNA, linamerase, and aspartate transaminase. Typical results are shown in figure 12.1. First and most notable was the overall sequence similarity between species within the genus. *Manihot* is thought to be a recently arisen genus, based on the morphological similarity of species and the lack of chromosome differentiation. These molecular results indicating low sequence divergence (1–2% in some cases) are consistent with a hypothesis of recent origin. In many cases the relationship between species could not be resolved, as indicated by polytomies on the tree. However, in all cases the phylogenies consisted of two clades, supported by bootstrap

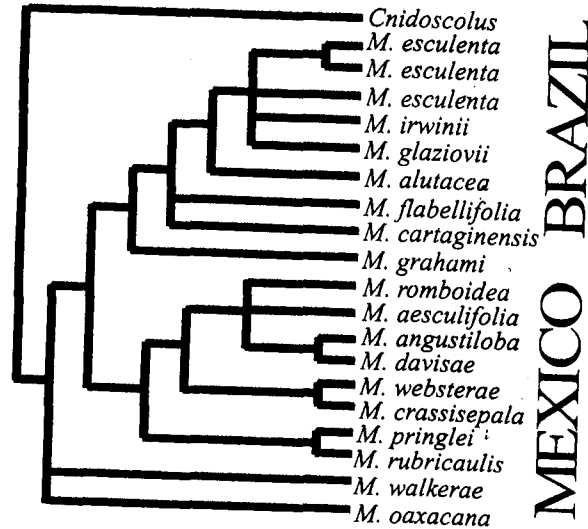


FIGURE 12.1 Phylogeny reconstruction in *Manihot*: maximum parsimony tree using internal transcribed spacer. Only the node separating the Mexican and Brazilian species is well supported (bootstrap >90%).

values greater than 90%; one clade represents the South American species, and the other represents species from Mesoamerica. These results are similar to Bertram's (1993) findings from chloroplast DNA restriction site data that also indicated South American and Mesoamerican lineages within the genus. Finally, in every case, cassava is nested within the South American clade.

Another result noted in our molecular sequencing studies was the almost identical sequence similarity between cassava and *M. esculenta* ssp. *flabellifolia*. Although species relationships could not be statistically resolved within the South American clade, even with multiple loci, in every case cassava showed greater sequence similarity to *M. esculenta* ssp. *flabellifolia* than to other *Manihot* species. The close genetic similarity of this subspecies to cassava has been noted in a number of amplified fragment length polymorphisms (AFLPs) and other DNA marker studies (Fregene et al., 1994; Roa et al., 1997). These molecular data and Allem's morphological data (Allem, 1994), which first indicated *M. esculenta* ssp. *flabellifolia* as a potential ancestor, make a strong case for examining the relationship between populations of this native taxon and domesticated cassava by high-resolution population genetic analyses.

Cassava's Wild Ancestor

M. esculenta ssp. *flabellifolia* occurs in the transition zone between the southern Amazon forest and the drier Cerrado region of Brazil and Peru. Populations are found in gallery forests, the mesic forest patches often associated with river drainages. *M. esculenta* ssp. *flabellifolia* is a clambering understory vine-like shrub. A total of 27 populations of *M. esculenta* ssp. *flabellifolia* from its entire known range in Brazil were collected for genetic analysis (Olsen, 2000). In addition, several populations of *M. pruinosa* were also collected to test the traditional hybridization hypotheses. *M. pruinosa* is a member of the secondary gene pool of cassava and is the closest relative of cassava that overlaps in distribution, within the eastern part of *M. esculenta* ssp. *flabellifolia*'s geographic range (Allem, 1999). Domesticated cassava was represented by a collection of 20 landraces of cassava, the "world core collection" maintained by the Centro Internacional de Agricultura Tropical in Cali, Colombia.

Populations were scored for two distinct types of genetic markers (Olsen and Schaal, 1999, 2001). We used different markers because both the precision and type of evolutionary inferences that can be drawn vary depending on marker choice. First, DNA sequence variation of the gene glyceraldehyde

3-phosphate dehydrogenase (*G3pdh*) was used for a historical, phylogeographic analysis. An analysis of sequence variation can provide information on the mutational relationships between variants (haplotypes), which are related by a haplotype network. In turn, a haplotype network can be used to infer historical, evolutionary processes and provide information on the geographic sorting of lineages. Second, we used a suite of five microsatellite loci, with a total of 73 alleles, to analyze genetic variation and population differentiation. Microsatellites provide high levels of variability, and alleles are codominant, which allows detailed population genetic analysis. The comparative analysis with these two markers sought to document that *M. esculenta* ssp. *flabellifolia* was indeed the wild progenitor of cassava and to determine the geographic site of domestication.

Figure 12.2 shows the results of the phylogeographic study of the *G3pdh* locus. Variants of *G3pdh* (haplotypes) are shown on the haplotype tree. The tree orders haplotypes based on their mutational relationships, with each line connecting haplotypes representing a single nucleotide substitution. Haplotype trees can be constructed either by hand or by a computer program, using parsimony to order the haplotypes. Several conclusions are apparent from figure 12.2. First, cassava contains much less haplotype diversity than does the wild taxon *M. esculenta* ssp. *flabellifolia*. Only 6 of the 23 haplotype variants of *M. esculenta* are found in domesticated cassava. The haplotypes of cassava are a subset of those found in *M. esculenta* ssp. *flabellifolia*, with the exception of a single haplotype not detected in the wild populations. These data are consistent with a hypothesis of cassava being derived from *M. esculenta* ssp. *flabellifolia*. Second, there appears to be no evidence of hybridization with *M. pruinosa*. The haplotypes of *M. pruinosa*, often shared with *flabellifolia*, are not observed in cassava. The absence of *M. pruinosa* haplotypes in domesticated cassava weakens the case for hybridization being a dominant process in cassava domestication. Finally, the geographic location of domestication can be inferred by the distribution of alleles contained both in cassava and in wild *M. esculenta* ssp. *flabellifolia* populations. Populations of *flabellifolia* that contain alleles also found in cassava are geographically limited to the southern and western parts of *flabellifolia*'s range, the transition zone between the humid Amazon forest and the dry Cerrado (figure 12.3).

One of the criticisms of evolutionary studies based on single gene sequences is that the sequence may reveal only a gene tree. That is, the relationships between haplotypes of a gene are reflected, not necessarily the relationships between populations. The organismal tree, the phylogeny of the species or

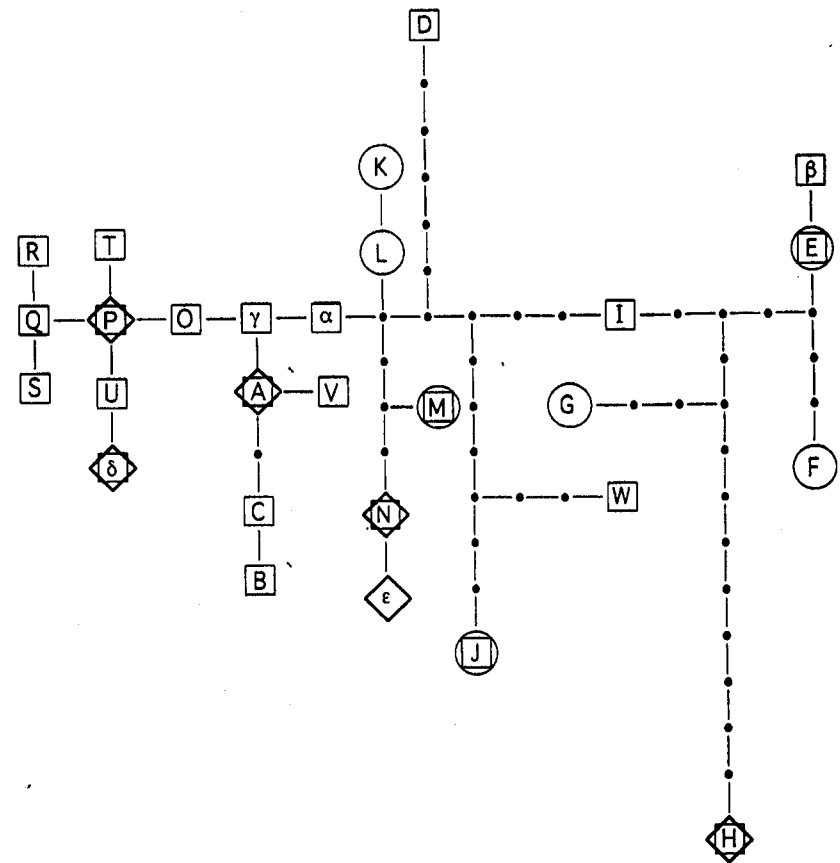


FIGURE 12.2 Haplotype network for *G3pdh*. Letters represent observed haplotypes. Lines represent single mutations, and dots are haplotypes not detected in the sample. Squares represent haplotypes of *M. esculenta* ssp. *flabellifolia*; diamonds represent haplotypes found in domesticated cassava. Circles are haplotypes found in *M. pruinosa*.

populations, may be different from the relationships between alleles at a locus. In phylogenetic studies multiple genes, often from different genomes such as the nuclear, chloroplast, or mitochondrion, are used to address this concern. Population-level studies often use high-resolution markers to assess relationships. Microsatellites and some other markers, such as AFLPs, represent a broader segment of the genome and thus provide good distance measures (Gepts, 1993). (Distance measures have their own assumptions and limitations, particularly for inferring historical relationships.) A disadvantage of microsatellites is the difficulty of developing a set of markers for

a specific plant species; we were fortunate to have a suite of microsatellites already developed for cassava (Chavarriga-Aguirre et al., 1998).

In our microsatellite study, the analyses were consistent with the results of the phylogeographic study. First, cassava appeared to contain a subset of the variation contained in wild *M. esculenta* ssp. *flabellifolia* populations. Only 15 of the observed 73 alleles found in *M. esculenta* ssp. *flabellifolia* were detected in cassava, again suggesting that cassava is a derivative of *M. esculenta* ssp. *flabellifolia*. As an aside, if *flabellifolia* were feral cassava populations, as has been suggested by some, these populations should contain less, not more variation than domesticated cassava. Second, the microsatellite data and the phylogeographic analysis are concordant: Cassava contains microsatellite alleles associated with populations from the same region of Brazil as in the phylogeographic study (figure 12.3). Thus we have concluded from these two independent data sets that cassava was domesticated from *M. esculenta* ssp.

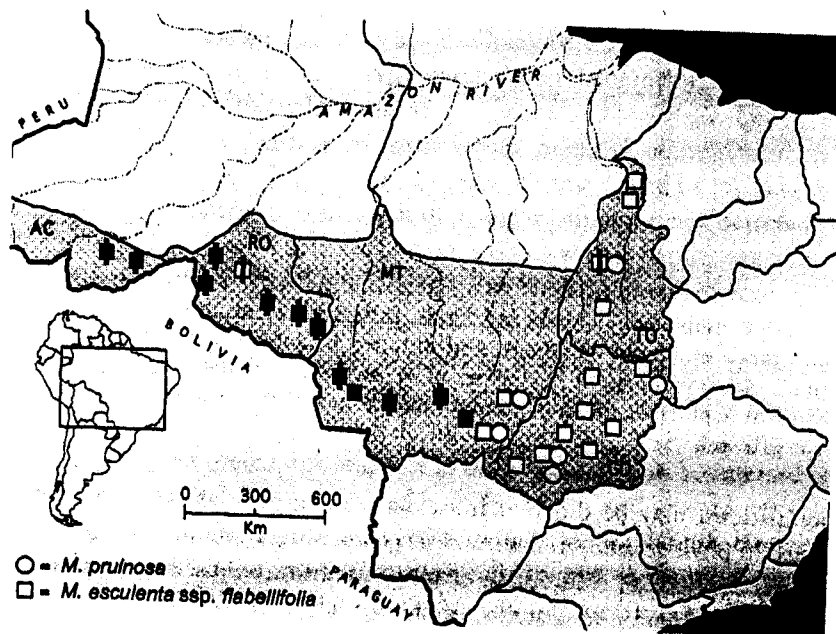


FIGURE 12.3 Wild *Manihot* populations and their genetic relationship with cultivated cassava. Black shading indicates populations containing *G3pdh* haplotypes detected in cassava samples. Vertical bars indicate populations clustered with cassava accessions in a distance analysis of microsatellite allele frequencies. AC = Acre, GO = Goiás, MT = Mato Grosso, RT = Rondônia, TO = Tocantins.

flabellifolia in the southern region of the transition zone between the lower Amazon forest and the Cerrado. Interestingly, this region is thought to be the location of an agricultural complex that includes domestication of the peanut, chili pepper, and jack bean (Pearsall, 1992; Piperno and Pearsall, 1998). Archaeological sites from this region suggest very early agricultural settlements, at about 4800 years BP (Miller, 1992). Finally, although neither the *G3pdh* data nor the microsatellite data indicate that substantial interspecific hybridization has taken place during the origin of cassava, the study cannot totally exclude hybridization, either before or, more importantly, after domestication. There are numerous reports of natural hybridization among *Manihot* species, based primarily on morphological evidence. Morphology of *Manihot* species is notoriously plastic, making such studies difficult to evaluate. Additional genetic studies would be extremely useful for understanding the role of hybridization in the evolution of the genus.

Morphology and Domestication

Determining the wild ancestor of cassava allows us to examine changes associated with domestication. Morphological distinctions between *M. esculenta* ssp. *flabellifolia* and modern cassava cultivars identify traits that have been altered during domestication. *M. esculenta* ssp. *flabellifolia* is a clambering vinelike shrub with a rudimentary storage root. The species is highly plastic; if the surrounding forest is removed, regrowth forms an erect shrub with a hard storage root that contains small amounts of starch and high fiber content. This erect habit is much more similar to that of domesticated cassava than the vinelike form. A second change associated with domestication is the development of a fleshy storage root high in starch, certainly the most striking difference between the ancestor and crop. A third major change is related to vegetative propagation of the crop. *M. esculenta* ssp. *flabellifolia* flowers freely, whereas in modern cassava cultivars flowering may be limited, often to small number of flowers and partial fruit set. This reduction in flowering of the modern varieties could result from the long history of vegetative propagation of the crop; many plants show a trade-off between sexual and vegetative reproduction. This later change in particular has implications for modern cassava germplasm collections around the world. For instance, most of the germplasm collections of cassava are based on local cultivars, and few accessions are derived from crosses of conventional breeding programs, in part because of flowering limitations that in turn

restrict the ability to cross specific cultivars. Thus finding and identifying new landraces of cassava that have both good flowering ability and useful and variable traits are critical for enhancing cassava as a staple crop.

Agrobiodiversity in the Amazon

Because cassava was domesticated in the lower Amazon region, one expects that this would be a geographic area where humans have had a long traditional association with cassava. Many visitors to the region have noted the diverse uses of cassava among villages. Cassava meal is used in sauces, flour is baked into flatbreads, the leaves are ground and cooked, and several fermented drinks are produced from the storage root. This diversity of uses is quite different from the use of cassava cultivated in much of the rest of the world, where it is grown as a source of starch for flour. Moreover, in the Amazon cassava is grown mostly in small, intercropped fields or in backyard gardens that often contain several distinct landraces of cassava. Again this is in contrast to the monoculture of cassava observed for improved varieties grown in many parts of the world. Given that both the uses of cassava and the mode of cultivation are more diverse in the Amazon region (Carvalho and Schaal, 2001), there may be underlying variability in key agronomic characters for cassava that could be useful in addressing the challenges cassava faces as a crop. The discovery of the site of domestication allows agricultural biologists to focus their areas of study and collection.

One of us (L.J.C.B.C.) has made several field trips to the Brazilian Amazon to learn of new uses and varieties of cassava and to collect diverse storage root variants. Smallholder farmers, isolated rural communities, local markets, and regions with different systems of cassava cultivation were visited in the states of Mato Grosso, Rondonia, Amazon, Para, Marajo Island, and Amapa. The landraces in this region showed an astounding diversity in unusual storage root traits related to root shape, color, and structure as well as carbohydrate content and type (figure 12.4). A field test for starch based on iodine allows one to identify starch in a cross-section of the storage root and to identify the type of starch and the pattern of starch distribution. Cross-sections of various landraces of cassava clearly show diversity in both the presence or absence of starch and the pattern of starch distribution (figure 12.4). Biochemical studies of the carbohydrates of these landraces revealed a new type of cassava, sugary cassava, which contained large amounts of free sugar (primarily glucose). These landraces also contained amylose-free starch and glycogen-like starch (phytoglycogen) (Carvalho et al., 2004). Many of these landraces

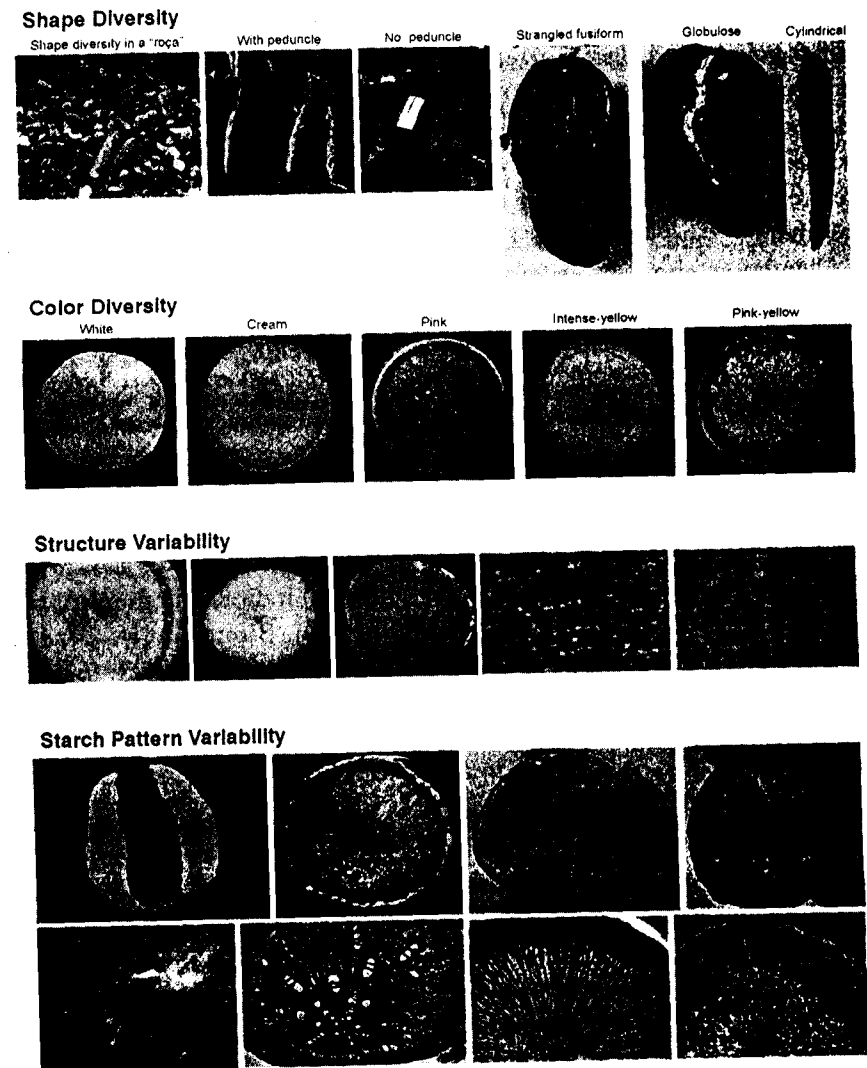


FIGURE 12.4 Shape, color, structure, and starch pattern variation between landraces of cassava. Cassava varies both in overall root color and shape and in the deposition of starch. Colors range from white to pink and intense yellow. Cassava roots also vary in the pattern of secondary xylem and parenchyma cells in the root. The dark regions in the lower 8 photographs are cells stained with iodine to detect starch. The presence of starch and the pattern of deposition vary between landraces. (Full-color version of this figure follows page 230.)

with unique carbohydrates have been domesticated for specific local uses. For example, the landrace that accumulates phytyglycogen is used as a food for very young children. Phytyglycogen is a highly branched molecule with short linear polymerized glucose, which makes it soluble in cold water and much easier to digest than common starch, amylopectin. The sugary cassava, with high amounts of free sugar, is used to prepare a glucose syrup for local desert dishes and a sweet smoked cassava cake. The sugary cassava is also used to produce a fermented alcohol drink used during the community's religious ceremonies.

Landraces also vary in pigments found in the storage root. Color variants of cassava often are observed in several germplasm collections around the world (India; Moorthy et al., 1990; Brazil: Ortega-Flores, 1991; Guimaraes and Barros, 1971; Marinho et al., 1996; and Colombia: Iglesias et al., 1997). The variants found in the Amazon are unusual in their diversity of colors, their carotenoid content, and their tissue-specific patterns of pigment distribution across the root. Figure 12.4 shows the range of color variants from standard white cassava to intense yellow, cream, and pink cassava. These color variants are closely associated with the type of carotenoid present. Biochemical analysis (figure 12.5a) indicates accumulation of a number of different carotenoid forms, including β -carotene, lycopene, and lutein in amount higher than previously reported. This diversity in the

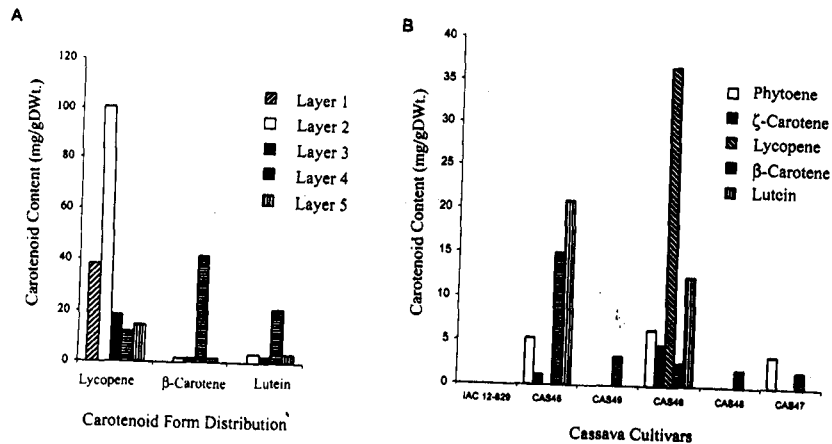


FIGURE 12.5 Diversity in carotenoid forms and content in pigmented cassava in the Amazon. (a) Different carotenoid forms and content in the storage root of local landraces. (b) Distribution pattern of carotenoid content across storage root in local landraces.

amount and type of carotenoids underscores the high potential of cassava for development of an improved staple food combining macronutrients (starch) with micronutrients (β -carotene, provitamin A). Importantly, the accumulation of β -carotene in the intense yellow cassava is accompanied by a higher protein content in the storage root. The intense yellow cassava has 40% more aqueous extractable protein content than does standard white cassava (figure 12.4), representing an opportunity for cassava to also supply protein as a staple food crop. The color diversity has been used locally in the Amazon in several food preparations. For example, the cassava juice called *tucupi*, made from the intense yellow cassava rich in β -carotene, is used in a soup. The fresh yellow roots are boiled and served in many dishes. Colored cassava varieties also are pickled as a way to preserve the roots.

How do these carbohydrate and pigment biochemical variants arise? Analysis of enzymes in the starch synthesis pathway and protein blot analysis indicate that in several of the novel carbohydrate varieties, specific enzymes of the starch biosynthetic pathway are no longer active, and their corresponding proteins are no longer present in a crude protein extract. Thus it appears that mutations that have reduced or eliminated key enzyme functions have in turn altered the flow of carbohydrates through the starch metabolic pathway. The absence of enzyme activity results in the accumulation of novel carbohydrates. Gene expression analysis shows that the sugary cassava that accumulates phytyglycogen no longer expresses the gene coding for starch branching enzyme I (Carvalho et al., 2004). In the case of pigmented cassava, we speculate that the accumulation of novel pigments is also the result of either natural mutations of key enzymes in the pigment synthesis pathway or mutations in the sequestering protein of the chloroplast in a particular cassava variety. These alternative explanations are being explored. It is quite likely that once these mutations initially occurred within a landrace, they are selected by the native peoples to enhance the concentration of either type of novel compound.

Conclusions

The results of what were initially solely academic studies have proven useful in an applied sense. First, the identification of *M. esculenta* ssp. *flabellifolia* as the wild progenitor of cassava allows one to target both the species and specific populations of *Manihot* that potentially have the most important germplasm for cassava improvement. These species and populations may contain

useful genes for important traits of agronomic interest. Second, populations of the wild ancestor as well as primitive landraces and modern mass-selected landraces could improve our understanding of the morphological and genetic processes associated with domestication. Third, focusing landrace collection on the Amazon region has identified several extremely important landraces that can be used to address some of the challenges that confront human populations subsisting on cassava. In addition, these landraces also offer opportunities for better understanding the biological processes that lead to useful biochemical variants. Because the carbohydrate diversity of cassava landraces is the result of specific enzymes losing function, could gene knockouts for other metabolic pathways lead to other useful biochemical variants? The high-sugar cassava and new carbohydrate variants could serve as a cash crop for poor farmers. The β -carotene variant could be very useful for preventing night blindness caused by vitamin A deficiency, a scourge for many poor populations in the tropical developing world.

Many more landraces and the biodiversity of native populations remain to be characterized. Unfortunately, both native populations of *M. esculenta* ssp. *flabellifolia* and traditional landraces are threatened as more land is cleared for modern agriculture. Conserving the agrobiodiversity of cassava should be of high priority, given the value and potential benefit that can be derived from cassava's germplasm resources.

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