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Variability of chloroplast DNA and nuclear ribosomal DNA in cassava (*Manihot esculenta* Crantz) and its wild relatives

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Abstract Chloroplast DNA (cp) and nuclear ribosomal DNA (rDNA) variation was investigated in 45 accessions of cultivated and wild *Manihot* species. Ten independent mutations, 8 point mutations and 2 length mutations were identified, using eight restriction enzymes and 12 heterologous cpDNA probes from mungbean. Restriction fragment length polymorphism analysis defined nine distinct chloroplast types, three of which were found among the cultivated accessions and six among the wild species. Cladistic analysis of the cpDNA data using parsimony yielded a hypothetical phylogeny of lineages among the cpDNAs of cassava and its wild relatives that is congruent with morphological evolutionary differentiation in the genus. The results of our survey of cpDNA, together with rDNA restriction site change at the intergenic spacer region and rDNA repeat unit length variation (using rDNA cloned fragments from taro as probe), suggest that cassava might have arisen from the domestication of wild tuberous accessions of some *Manihot* species, followed by intensive selection. *M. esculenta* subspp *flabellifolia* is probably a wild progenitor. Introgressive hybridization with wild forms and pressures to adapt to the widely varying climates and topography in which cassava is found might have enhanced the crop's present day variability.

Key words Cassava · Wild *Manihot* species
Chloroplast DNA · Nuclear ribosomal DNA · Phylogeny

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Introduction

Cassava (*Manihot esculenta* Crantz) is the fourth most important source of calories in the tropics. It is the staple food of over 200 million people in Africa (IITA 1990) and also occupies a prominent position in the agroecosystems of Latin America and Asia. Africa accounts for roughly half of the world production, Asia for 30% and Latin America for 20% (FAO 1991). Cassava, a monoecious perennial shrub, is well adapted to drought, poor soils and high temperatures. It is the major cultivated species of the genus *Manihot*, and it is important mainly because of its starchy, thick, tuberous roots, which are a cheap source of calories.

All species of the genus *Manihot* are native to the New World tropics and occur naturally only in the Western Hemisphere between the southwestern USA (33°N) and Argentina (33°S). Cultivated and wild accessions were introduced to the Old World tropics in the 16th century (Jones 1959; Rogers 1963). The genus *Manihot* consists of two sections: the arboreae, which contains the tree species and is considered more primitive, and the fructicosae, which contains shrubs adapted to the savanna, grassland or desert (Jennings 1976). Although four centers of diversity have been described for the wild species, most of their diversity is concentrated in two areas, one in northeastern Brazil and the other in western and southern Mexico (Nassar 1978).

In a morphological description of 98 *Manihot* species from the southern USA to Argentina, Rogers and Appan (1973) hypothesized that cassava was domesticated through natural hybridization between two closely related species. The likely wild species include *M. aesculifolia* (H.B.K.) Pohl, *M. pringlei* Watson, *M. isoloba* (Standley) Rogers and Appan and *M. rubricaulis* I.M. Johnston. More recent botanical collections in South America indicate that cassava is conspecific to some of its closely related wild relatives, particularly *M. trisits* Muell-Arg, a conclusion

drawn from the morphology and growth habit of the specimens (Allem 1984). Cassava is usually outcrossing mediated by protogyny; pollination is aided by a wide variety of insects. This produces a heterozygous gene pool with great phenotypic diversity, thereby leading to considerable inflation in taxonomic classifications based on morphology (Allem and Hahn 1988).

All *Manihot* species examined to date have a chromosome number of 2n=36. This high diploid chromosome number reflects the evolutionary polyploid nature of the genus. On the basis of observed numbers of satellite chromosomes and the karyology of chromosomes at the pachytene stage of meiosis, it has been postulated that *Manihot* species originated through segmental allotetraploidy and allotetraploidy from two closely related taxa (Magoon et al. 1969b; Umannah and Hartman 1973). Cytogenetic studies of spontaneous sexual tetraploids and triploids obtained from interspecific hybrids of cassava, *M. glaziovii* Muell-Arg and *M. epruinosa* Pax & K. Hoffman, revealed a mixture of autosyndetic and allosyndetic pairing at M-I (Bai 1985; Hahn et al. 1990). This suggests that cassava and its close wild relatives have common chromosome segments. Cytogenetic data on the meiotic behavior of chromosomes in several *Manihot* species and in interspecific hybrids at prophase I and M-I suggest that the genus is fully diploidized (Bai et al. 1992; Allem 1984).

There appear to be no interspecific barriers to hybridization in *Manihot* (Bryne 1984). A number of experimental crosses suggest that the boundaries in this genus are ambigiuous (Nichols 1947; Bolhuis 1953; Jennings 1957, 1963; Abraham 1957; Magoon et al. 1969a; Nassar 1979; IITA 1988). The most successful crosses were those in which cassava was used as the female parent. This instability of species boundaries has led some authors to conclude that divergence of the different species in the genus *Manihot* is recent and still evolving rapidly (Rogers and Appan 1973). While wild *Manihot* species are sources of abiotic and biotic resistance, they have not been used much for cassava improvement (Allem and Hahn 1988). Relative to normal cassava, spontaneous triploids derived from interspecific crosses involving *M. glaziovii* and cassava have shown a near doubling of their crop yield and a more vigorous canopy formation (Hahn et al. 1990).

Chloroplast DNA (cpDNA) is gaining importance as an aid to elucidating the origin and evolution of plant species (Palmer 1987; Harris and Ingram 1990). Restriction fragment length polymorphism (RFLP) analysis of cpDNA has yielded insights into allopolyploid speciation (Erickson et al. 1983; Soltis and Soltis 1989). It has also provided operational taxonomic units (OTUs) for eliminating the confusion that exists over the taxonomy of vegetatively propagated crops, which arises from the plasticity of morphological characters in these species (Hosaka and Hanneman 1988; Terauchi et al. 1992).

Eukaryote ribosomal RNA (rDNA) genes plus their spacer regions are organized as multigene families of moderately to highly repeated sequences, some or all of which may comprise the nucleolar organizer region (NOR) of chromosomes (Saghai-Maroof et al. 1984; Appels and Honeycutt 1986). Amongst higher plants these genes differ in terms of the number and length of tandem repeats; length variability is often localized to the intergenic spacer region and is species-specific in sequence (Appels and Dvorak 1982). The pattern of length variation generally shows a high level of rDNA family heterogeneity between species and a high level of homogeneity within species (Sano and Sano 1990). This kind of variation has been exploited as a source of useful molecular markers for elucidating phylogenetic relationships among related species (Doyle and Beachy 1985b; Systma and Schaal 1985). In hybrids, rDNA variation in restriction sites, repeat length and copy number has been used to identify putative parents in both plant and animal species (Appels and Honeycutt 1986). In this paper we report our assessment of the intra-and interspecific cpDNA and rDNA variability of cassava cultivars adapted to three major growing regions and of wild *Manihot* species. From these results we derive tentative insights into the origins of cassava and the evolution of gene pools in the cassava cultigen.

Materials and methods

Plant materials

Forty five accessions of *Manihot* germ plasm maintained in the CIAT and IITA collections were studied: 29 accessions of 16 wild species, identified according to the classification of Rogers and Appan (1973); 21 highly successful cassava cultivars and landraces from three major cassava growing regions in Latin America, Asia and Africa; and 1 local African arborescent (tree-type) cassava (Table 1).

DNA extraction

Total DNA was extracted from young fresh leaf tissue according to Dellaporta et al. (1983), with some modifications that made it possible to extract good quality DNA without the normal polysaccharide contaminants. In all cases, tissue was collected from a single plant per accession. Between 500 and 1000 µg of total DNA was obtained per gram of fresh leaf tissue.

Restriction digestion and Southern blots

About 2 µg of total DNA was routinely digested overnight with five 6-base-cutters (*Bam*HI, *Pst*I, *Eco*RI, *Hin*dIII and *Eco*RV) and the four 4-base-cutters (*Hae*III, *Msp*I, *Alu*I and *Sau*3A). The digested DNA was electrophoresed in TBE buffer at 22 V for 14 h in 0.8% agarose gels for digests with 6-base-cutters and in 1.5% gels for those with 4-base-cutters. Separated fragments were transferred to Hybond N^+ membranes (Amersham) by Southern (1975) blotting. Irradiation by UV light for 12 s was used for cross-linking transferred DNA to the membrane.

Chloroplast DNA probes

Twelve cloned fragments of cpDNA from mungbean (Palmer and Zamir 1982) were used as heterologous probes for detecting variation in the *Manihot* chloroplast genome (Fig. 1). The mungbean probes included 10 cloned fragments from the *Pst*I mungbean chloroplast library, corresponding to 80% of the entire genome, and 2 *Sal*I cloned fragments from the same library (Fig. 1).

Table 1 Source, cpDNA type and rDNA spacer length variant (slv) of germ plasm studied

	Species	Accession number	Source/ locality	CpDNA type	rDNA spacer length variant (size in kb)
1	*M. aesculifolia*	002/4	CIAT	IV	slv 2 (11.35)
2		aes/C	CIAT	IV	slv 2 (11.35)
3	*M. brachyloba*	blo 001/1	CIAT	VI	slv 9 (15.0)
4	*M. caerulescen*	cae 002/1	CIAT	VI	slv 3 (11.5)
5	*M. chlorosticta*	chl 001	CIAT	VI	slv 6 (13.0)
6	*M. carthaginensis*	cth 190	CIAT	VII	slv 1 (11.2)
7	*M. crassisepala*	cra 011	CIAT	VI	slv 3 (11.5)
8	*M. epruinosa*	epr 002/1	CIAT	VIII	slv 2 (11.35)
9	*M. esculenta* sub spp *flabellifolia*	fla 072/2	CIAT	I	slv 2 (11.35)
10		fla 055/2	CIAT	I	slv 2 (11.35)
11		fla 075/9	CIAT	II	slv 2 (11.35)
12		fla 057/1	CIAT	I	slv 2 (11.35)
13	*M. glaziovii*	gla 002	CIAT	VI	slv 4 (11.65)
14	*M. grahami*	grh 007	CIAT	IX	slv 8 (14.0)
15	*M. typhylla*	tph 002	CIAT	VI	slv 9 (15.0)
16	*M. irwinii*	irw 006	CIAT	II	slv 3 (11.5)
17	*M. michaelis*	mic 006	CIAT	VI	slv3 (11.5)
18	*M. rubricaulis*	rub 004/2	CIAT	V	slv 7 (11.35)
19		rub 016	CIAT	V	slv 7 (13.5)
20	*M. tristis*	tst 011/1	CIAT	I	slv 8 (14.0)
21		tst 008/4	CIAT	I	slv 8 (14.0)
22		tst 014/4	CIAT	I	slv 8 (14.0)
23		tst 002/1	CIAT	I	slv 8 (14.0)
24		tst 003/2	CIAT	I	slv 8 (14.0)
25		tst 038/2	CIAT	I	slv 8 (14.0)
26		tst 051/1	CIAT	I	slv 8 (14.0)
27	*M. violacea*	vio 002	CIAT	VI	slv 9 (15.0)
28	*Modakeke* (Tree cassava)	mod 003	IITA/Nigeria	VI	slv 7 (11.35)
29	*M. esculenta*	MArg 11/1	CIAT/Argentina	II	slv 5 (12.85)
30		MBra 335	CIAT/Brazil	I	slv 3 (11.5)
31		Mbra 110	CIAT/Brazil	I	slv 2 (11.35)
32		MBol 3	CIAT/Bolivia	I	slv 9 (15.0)
33		MCol 1505	CIAT/Colombia	II	slv 2 (11.5)
34		MCol 22	CIAT/Colombia	I	slv 2 (11.35)
35		MCub 21	CIAT/Cuba	I	slv 2 (11.2)
36		MChn 21	CIAT/China	I	slv 2 (11.35)
37		MEcu 82	CIAT/Ecuador	I	slv 5 (12.85)
38		MMex 1	CIAT/Mexico	II	slv 2 (11.35)
39		MMal 37	CIAT/Malaysia	I	slv 6 (13.0)
40		Mind 1	CIAT/Indonesia	I	slv 3 (11.5)
41		MThai 1	CIAT/Thailand	II	slv 6 (13.0)
42		MThai 8	CIAT/Thailand	I	slv 6 (13.0)
43		TMS 30001	IITA	II	slv 2 (11.35)
44		TMS 30572	IITA	III	slv 2 (11.35)
45		MVen 25	CIAT/Venezuela	I	slv 2 (11.35)

Nuclear rDNA probes

Four heterologous nuclear rDNA probes were used; PGMr-1, a recombinant plasmid containing 18S, 5.8S and 26S rRNA genes from soybean, *Glycine max* (Jackson and Lark 1982), and clones pCE34.1, pCE34.2 and pCE34.12, which span the entire rRNA gene region of taro, *Colocasia esculenta* (Matthews 1990) (Fig. 2).

Hybridization

Two nonradioactive methods of random chemical labelling and enzymatic detection were used: the enhanced chemiluminescence (ECL) system of Amersham PLC and the Digoxigenin-dUTP labelling and detection system of Boehringer Mannheim Gmbh. Labelled probes were used repeatedly, and membranes were hybridized several times after stripping off previous hybridized probes in 0.5% SDS boiled for 5 min. Hybridization was done as described in the manufacturer's manual.

Results

Chloroplast DNA variation

Twelve heterologous cpDNA probes were hybridized sequentially to DNA of the 45 accessions digested with three 4-base cutters and five 6-base cutters. Of the 48 successful hybridizations, 16 gave rise to polymorphic patterns. Autoradiograms of the different enzyme digests hybridized to identical probes were cross-checked, and only independent mutations are presented (Fig. 3a–d). These RFLPs appear to have arisen from 8 restriction site changes and 2 length mutations. Expected additivity for mutated samples was demonstrated for 6 point mutations (Fig. 3a, c, d and

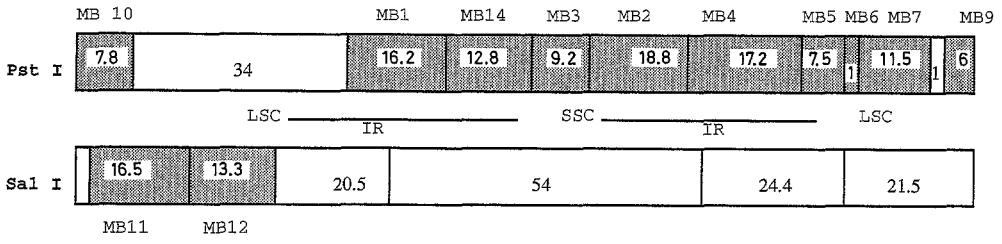

Fig. 1 Restriction fragment map (*Pst*I and *Sal*I) of mungbean (Palmer and Zamir 1982). *Shaded areas* were used in hybridization

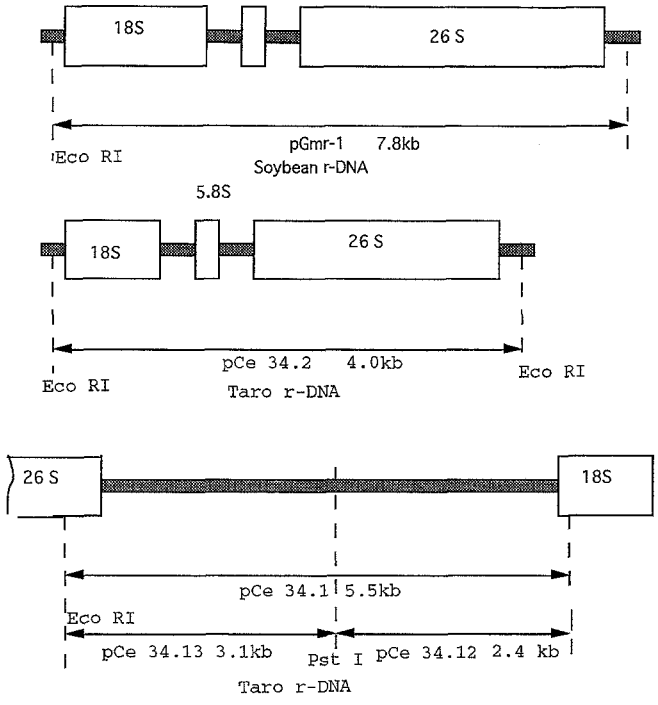

Fig. 2 Structure of ribosomal DNA fragments used as probes

Table 2). Changes were assumed to have occurred at the other 2 restriction sites for which additivity was not observed, since similar size alterations were not observed in other enzyme digests hybridized to the same probe. A missing mutated fragment might have overlapped with fragments of similar size, occurred beyond the extent of the probe or been too small to be detected. Two length mutations were observed with at least three of the restriction endonucleases employed and were judged to be the result of small insertions (Fig. 3a, b and Table 2). Restriction site mutations and length mutations were found with probes belonging to the large single copy region, small single copy region and inverted repeat of the mungbean chloroplast genome.

Ribosomal DNA variation

Four heterologous rDNA probes were hybridized one by one to DNA digests of *Sau*3A1, *Eco*RI, *Hin*dIII, *Msp*I and *Eco*RV. Hybridization with enzymes such as *Eco*RI, *Hin*dIII and *Eco*RV, which cut once per rDNA repeat unit, yielded a single band varying in length between 11.20 and 15 kb with pCE34.1 and pGMr-1 for most *Manihot* accessions; *M. glaziovii* and a cassava accession from Brazil displayed 2 repeat unit length variants each after considerable exposure of the filters (Fig. 4a). Nine spacer length variants (slvs) were found in the wild *Manihot* accessions and 8 among the cultivated species (Table 1).

A single monomorphic band measuring 4.0 kb was obtained from hybridization with pCE34.2 using the same restriction enzymes mentioned above (data not shown). Clone pCE34.2 is a complete rDNA repeat unit without the intergenic spacer (IGS) region, whereas pCE34.1 and PGMr-1 are rDNA repeat units with the IGS regions. The length variation in *Manihot* rDNA repeat units appeared to be localized at the 5′ end of the IGS region and is judged to have arisen from differences in the number of 150-bp subrepeats contained in the IGS regions. Restriction profiles from the hybridization of pCE34.12 to DNA digests with restriction enzymes that possess sites in the IGS region, such as *Sau*3AI, resulted in ladders of dimers, trimers and higher order multimers and was used to assess restriction site polymorphism in the IGS region of *Manihot* species (Fig. 4b). Hybridization of the same *Sau*3AI DNA digests with pCE34.13 yielded several monomorphic bands for all *Manihot* accessions (data not shown). Both pCE34.12 and pCE34.13 are subclones from pCE34.1. The hybridization of probe pCE34.12 to *Msp*I digests yielded minor restriction site polymorphism in 3 *Manihot* species: *M. caerulescens* (Pohl) emend Rogers and Appan, *M. rubricaulis* and *M. michaelis* McVaugh (data not shown).

Discussion

Chloroplast genome types in cassava and its wild relatives

Nine cpDNA types (plastome types) were defined by 10 independent mutations in 45 cDNAs of cassava and its wild

Fig. 3 **a** Southern hybridization of mungbean chloroplast probe MB12 to *Hae*III digest of cpDNA of cassava and its wild relatives. **b** Southern hybridization of mungbean chloroplast probe MB3 ro *Sau*3A digests of cpDNA of cassava and its wild relatives. **c** Southern hybridization of mungbean chloroplast probe MB7 to *Hin*dIII digests of cpDNA of cassava and its wild relatives. **d** Southern hybridization of mungbean chloroplast probe MB9 to *Bam*HI digests of cpDNA of cassava and its wild relatives. **e** Southern hybridization of mungbean chloroplast probe MB9 to *Pst*I digests of cpDNA of cassava and its wild relatives. **f** Southern hybridization of mungbean chloroplast probe MB1 to *Hin*dIII digests of cpDNA of cassava and its wild relatives

a
M. aes
M. bra
M. cae
M. chl
M. cth
M. cra
M. epr
M. esc fla
M. gla
M. tph
M. grh
M. irvinii
M. mic
M. rub
M. tristis
M. vio
modakeke
M. esc
11.5
8.8
4.6
2.5
b
M. aes
M. rub
M. esc fla
M. esc
M. tristis
5.1
2.7
c
M. tristis
TMS 30572
M. esc
M. esc fla
5.6
5.2
d
M. tristis
M. esc
TMS 30572
M. esc
M. esc fla
M. aes
M. rub
2.5kb
e
M. cth
12.2kb
12.0kb
f
M. rub
M. aes
M. esc fla
M. esc
M. tristis
11.4kb
6.3kb
5.0kb
1.0kb
0.9kb

Table 2 Mutations in cpDNA of cassava and its wild relatives (*LSC* Large single-copy region; *IR* inverted repeat; *SSC* small single-copy region)

Mutation	Enzyme	Probe	Region	Lost fragment (kb)	Gained fragment (kb)	Mutated samples
1	*Hae*III	MB12	LSC	8.8	4.6 + 4.2	1, 2
2	*Hin*dIII	MB1	IR	1.0, 5.0, 6.3	11.4, 0.9	1, 2
3	*Hae*III	MB12	LSC	8.8	2.5 (+5.3)[b]	10–12, 16, 20–26, 29–45
4	*Hae*III	MB12	LSC	6.9 + 4.6	11.5	14
5	*Bam*HI	MB6	LSC	11.5	9.0 + 2.5	44
6	*Bam*HI	MB12	LSC	3.2	2.2 + 1.0	6,8
7	*Pst*I	MB4	IR	4.0	3.2 + 0.8	11, 29, 33, 38, 41, 44
8	*Sau*3A1	MB3	SSC	5.1	2.7 (+2.4)	18, 19
9	–	MB9	LSC	–	0.2 insertion	6
10	–	MB7	LSC	–	0.4 insertion	44

[a] Lost and gained fragments are all with respect to plastome type VI
[b] Parenthesis are given for fragments not seen but assumed to exist since other enzymes with the same probe failed to detect length mutations

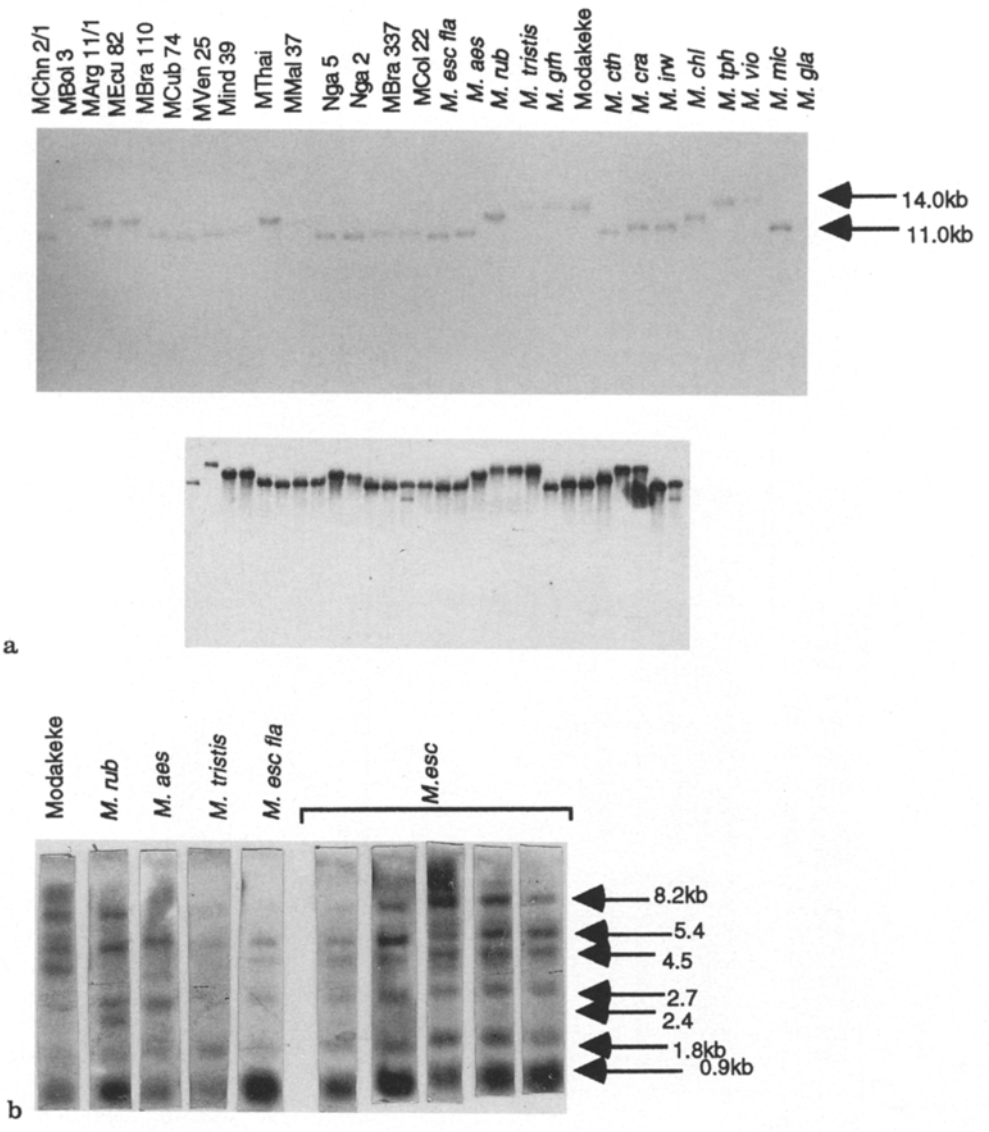

Fig. 4 **a** Southern hybridization of taro rDNA probe PCE34.2 to *Hin*dIII digests of DNA of cassava and its wild relatives. The *lower figure* is an autoradiogram of the same hybridization after a longer exposure. **b** Southern hybridization of taro rDNA probe PCE34.2 to *Sau*3A digests of DNA from some cassava cultivars closely related wild relatives

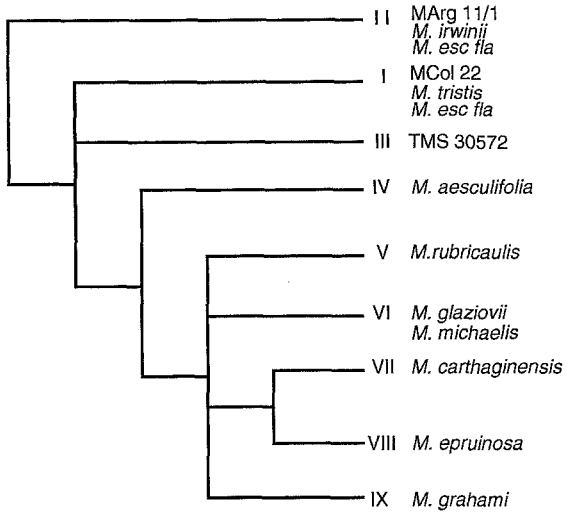

Fig. 5 Phylogenetic tree of cassava and its wild relatives derived from cladistic assessment of cpDNA using the branch and bound search option of PAUP (Swofford 1991). Accessions displayed are representative of cp plastome types

relatives (Table 1). We define a plastome type as a cpDNA differing from the others by at least 1 independent mutation. Plastome type I was shared by all accessions of *M. tristis*, by 3 accessions of *M. esculenta* sub spp *flabellifolia* (fla 072/2, fla 055/2 and fla 057/1) and by the cultivated accessions MBra 335, MBra 110, MBol 3, Chn 2/1, MPar1, MThai 8, MEcu 82, MCol 22 and MVen 25. The fourth accession of *M. esculenta* subspp *flabellifolia*, 075/9, *M. irwinii* Rogers and Appan and the cultivated accessions Arg 11/1, MThai 1, TMS 30001 and MCol 1505 possessed plastome type II. The cultivar 'TMS 30572' displayed plastome type III, which was not found in any other accession. Six plastome types were detected in the other wild species. All accessions of *M. aesculifolia* displayed plastome type IV, and all of those of *M. rubricaulis* showed type V. *M. glaziovii, M. crassisepala, M. tryphylla* Pohl emend Rogers and Appan, *M. brachyloba* Muell-Arg, M. *caerulescens, M. chlorosticta* Ule, *M. michaelis, M. violacea* Pohl and *M. pseudo glaziovii* Pax&K. Hoffman (a local tree cassava) had plastome type VI. *M. carthaginensis* Rogers and Appan displayed plastome types VII, *M. epruinosa*, type VIII; and *M. grahami* Hooker, type IX. No mutations were detected in *Manihot* species from the section arboreae, which is thought to contain more primitive species than the section fructicosae.

Since the chloroplast genome in angiosperms is most often inherited maternally (Palmer et al. 1988), these results suggest that some cassava accessions are of maternal origin in their wild relatives, such as *M. tristis*, *M.esculenta* subspp *flabellifolia* and *M. irwinii*. Alternatively, the cassava genotypes examined might have introgressed with these species, which have identical plastome types, after domestication from unknown ancestry, or the observed change in restriction site might be a convergent event in evolutionary pathways to present forms.

Phylogenetic relationships among the nine chloroplast types in cassava and its wild relatives

Raw cpDNA RFLP data were organized into cpDNA types, and a data matrix was generated. Cladistic analysis of the data matrix was done by the computer program Phylogenetic Analysis Using Parsimony (PAUP 3.0) (Swofford 1990), using the branch and bound search option. A single most parsimonious unrooted tree was obtained with a homoplasy of 0 and a retention index of 1 (Fig. 5). Two primary clusters, which correspond to the primary and secondary gene pools in the cultigen *M. esculenta*, were inferred from clusterings on the tree. The phylogenetic tree topology shows an overall similarity to dendograms of relationships in the genus *Manihot* that are based on morphology (Rogers and Appan 1973). In particular, members of the *Manihot* section Arborea, which contains the tree species and is considered to be more primitive, occupy the least derived phylogenetic position in the network. *M. aesculifolia*, an erect shrub, occupies an intermediate position in the transition from the primitive to more recent plastome types.

Ribosomal DNA variation and the origin of cassava

Length heterogeneity of rDNA repeats is often caused by the repetition of perfectly or imperfectly repeated elements measuring 0.1–0.35 kb in the IGS region (Appels and Honeycutt 1986; Yakura et al. 1984). The spacer length variants within *Manihot* species varied more in length than those within *M. esculenta*. Similar patterns have been found in wheat (Appels and Dvorak 1982), barley (Saghai-Maroof et al. 1984) and rice (Sano and Sano 1990). The almost equal number of length variants found in the wild species and cassava may have resulted from the larger size of the *M. esculenta* genotypes examined. Spacer length variants within cassava can be divided into four broad classes (Fig. 3f). The first contains slvs measuring 11.5 kb and 11.5 kb+(150 bp)n, where n ranges from 1–12. This class contains a majority of the cultivated accessions, mainly landraces from Latin America and elite clones widely grown in Africa that were derived from recurrent selection of a cross betwen Latin American and African cassava germ plasm introgressed with the wild *Manihot* species, *M. glaziovii*. The second class, measuring 12 kb, contains MMal 37 and MThai 1, both landraces from Asia. The third class, of a similar size (12.15 kb), has 2 landraces, MEcu 82 from Ecuador and MArg 11/1 from Argentina. The fourth class contains only 1 cassava accession, MBol 3, a landrace from Bolivia. *M. aesculifolia, M. esculenta* subspp *flabellifolia, M. irwinii, M. cartaginensis* and *M.glaziovii* also possess slvs similar to those of the first class in cultivated accessions. Associations among rDNA alleles and environmental factors might be responsible for the observed distribution of slvs in cassava cultivars. The effects of natural selection on rDNA alleles under conditions of domestication have been to fix wild-type rDNA alleles that appear to enhance survival in the area

while eliminating those that have adverse effects on survival (Saghai-Maroof et al. 1984). The more than 6000 landraces of cassava are known to be adapted to diverse climates and topographies (Rogers and Appan 1973).

*Sau*3AI restriction sites in the IGS region appear to be well conserved in the genus *Manihot*. Cassava accessions from widely differing regions and showing considerable polymorpism in rDNA repeat length displayed no differences in *Sau*3A restriction site profile (Fig. 3f). A survey of *Sau*3A restriction site polymorphism in cassava, its close wild relatives and the presumed progenitors reveals putatively that *M. esculenta*, as a species grouping that includes the wild subspecies *M. esculenta* sub spp *flabellifolia*, shows evidence of divergence from other *Manihot* species. Similar observations of restriction site conservation in the IGS region between species have been made in the genus *Dioscorea* and used to determine origin of a cultigen in the genus (Terauchi et al. 1992). Cassava apparently arose from the domestication of some wild tuberous *Manihot* species followed by intensive selection. Major morphlogical differences between cassava cultivars and a likely wild progenitor, *M. esculenta* sub spp *flabellifolia*, a lack of woody stem, nonprofuse flowering and swollen leaf scars are traits usually associated with selection (under domestication) for large roots, erect growth, and an ability to establish easily from stem cuttings (Jennings 1976). We will continue examination of rDNA repeat unit length variation and restriction site polymorphism of the rDNA IGS region in a larger number of cassava cultivars and populations of close wild relatives (such as *M. esculenta* subspp *flabellifolia*, *M. irwinii*, *M. tristis*) to further elucidate our hypothesis of evolution to present forms in the primary gene pools of the cassava cultigen.

Polyploidization in Manihot species

M. glaziovii and a cassava accession from Brazil each displayed 2 repeat unit length variants each after considerable exposure of the filters (Fig. 3e). These are likely due to the presence of two satellited chromosomes that contain nucleolar organizer regions in which the rRNA genes are located (Flavell 1982). This supports the observation from cytogenetic studies that there are two distinct NORs per haploid genome of *Manihot* species. The abundance of these secondary rDNA repeat units in both a wild and cultivated accession suggests that the enormous variability in cassava may have been enhanced by introgressive hybridization with wild species. Even though more than 1 rDNA length variant was observed within an individual accession, this study is unable to shed more light on the postulated allotetraploid origin of *Manihot* species. Ribosomal DNA repeat length heterogeneity within an individual plant, or the lack of it, might not be correlated with the allotetraploid origin of plant species. The fixed hybridity of rDNA repeat variants has not always been proven in well characterized allotetraploids due to the lack of general rules governing the incorporation, elimination or homogenization of variant rDNA repeat types (Rogers and Bendich 1987; Doyle and Beachy 1985b).

Despite the limitations in some instances of sampling only 1 individual per cultivar and per species, our results still demonstrate that cpDNA and rDNA RFLP analysis is a powerful tool for elucidating the origin and evolution of gene pools in cassava and phylogenetic relationships in the genus *Manihot*.

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