RESEARCH PROJECT

TITLE Development of techniques for conservation and utilization of wild Manihot ssp.

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PROJECT OBJECTIVES

a. To develop methods for breaking dormancy and overcoming seed sterility of wild Manihot species as a means to increase the percentage of germination and shortening the breeding cycle.

b. To develop techniques for embryo rescue and culture in vitro as a means to micropropagate small seed samples, interspecific hybrids and seeds with rudimentary embryos unable to germinate with conventional germinating methods.

c. To develop methods for in vitro storage of wild Manihot germplasm.

d. To study the cross-compatibility relationships (interspecific crosses) between wild Manihot spp. and cassava with the purpose of improving efficiency of wild germplasm utilization.

BACKGROUND AND JUSTIFICATION

There is evidence that wild Manihot species can provide useful genes for the improvement of cultivated cassava, Among wild cassava germplasm there are sources, for example, for low cyanide content in M. pringlei and for resistance to African cassava mosaic virus (M. glaziovii). There are also sources of useful genes for resistance to cassava bacterial blight in M. pseudoglaziovii and M. reptans, as well as for high starch content in M. tristis and M. angustiloba. Genetic resistance to the most common cassava pests has been found among wild Manihot species, such as M. glaziovii and M. dichotoma, e.g. high level of resistance to mealy bug, a very dangerous insect in West Africa; and resistance to stem borer in M. neusane M. pohlii and M. grahami has been recently reported. The incorporation of new genes from wild species to the crop, especially genes for disease resistance may prove to be a successful line of work. That is the case, for example, of gene transfer of resistance to African cassava mosaic virus from M. glaziovii to M. esculenta made successfully by Hahn (1986) at IITA.

Adaptation and tolerance to different abiotic stresses in the wild gene pool has been pointed out by several cassava researchers. For example M. chlorosticta grows in saline soils in North East Brazil, and M. subspicata as well as M. pseudoglaziovii are adapted to drought conditions. This fact would make M. chlorosticta a good candidate for breeding programmes to improve productivity in arid lands and saline soils. . Another stricking example of adaptation are displayed by M. attenuata and M. rubricaulis which may provide useful genes for tolerance to cool temperatures in cassava breeding because they grow at elevations of about 1600-1700 m. Therefore, the availability of a wide spectrum of wild Manihot germplasm should be stressed, to broaden the genetic base of cultivated cassava (see appendix I and II).

The feasibility of gene transfer from wild Manihot species to cultivated germplasm seems to be promising. Hahn (1982) reported successful hybridization between M. esculenta and M. dichotoma. F_1 hybrids from M esculenta and M. pohlii crosses were obtained by Silva in 1984; and recently Nassar (1986) reported the cross-compatibility of cassava cultigens with M. reptans, M. fruticulosa, M oligantha and M. tripartita. Several attempts to hybridize M. esculenta with other five wild Manihot species were unsuccessful. Althought there is no data on cytology and bilateral compatibility of parental genotypes in these reports, they provide a very useful preliminary information on interspecific crosses within the genus Manihot (see appendix III).

The limited literature, on the other hand, of Manihot cytogenetics suggests that the chromosome number is uniform throughout the genus and that M. esculenta is cross-compatible with several different wild species. However, comparatively few wild species have been available in living collections, and further research on interspecific hybridization may show that currently accepted generalization are not well founded.

In fact, wild Manihot germplasm displays unique potentially useful qualities in resistance to pests and diseases and tolerance to abiotic stresses, as well as dwarf forms that have increased production in may others crops. Because of this, wild Manihot species have been recommended by IBPGR and CIAT for breeding and agronomy studies on drought and pest resistance, various growth characteristics, as well as resistance to pest pressures. The attributes of wild species certainly would justify acquisitions and encourage use of this diverse germplasm in many areas of cassava investigation. It has also been recommended to carry out a general evaluation of wild *Manihot* spp. regarding morphology, root formation, fertility and crossability, thus there should be no delay in preserving the present existing genetic diversity of wild spp. The urgent need of preservation is also supported by the fact that considerable genetic erosion currently takes place in nature among wild species of *Manihot*. For example *M. walkerae*, *M. hassleriana*, *M. guaranitica*, *M. subspicata*, *M. angustiloba* and *M. pringlei* have been reported to be extremely endangered species. Therefore, saving these genotypes and maintaining them in a seed genebank and or in an *in vitro* active genebank could be one of the main objectives of this project.

In the last 20 years a lot of work has been done on taxonomy, ecology and germplasm collections of wild *Manihot* species. Rogers and Appan (1973) described 98 species related to cassava (See Appendix I); and recently another new species, *M. neusana*, has been described, which has tolerance to low temperature and resistance to stem borer (Nassar, 1985).

During the last decade, several expeditions to collect wild *Manihot* species throughout the main centers of diversity of this genus have been carried out. Most of these collecting expeditions were done with IBPGR and CIAT support. As a result of this, some living collections of wild *Manihot* species were raised and are actually maintained in Brazil, (CENARGEN and University of Brasilia) Mexico (INIFAP) and Paraguay (MAP-IAN). Although, from the point of view of genetic conservation, these living collections are not representative of the full range of genetic variability within the wild spp. gene pool, they constitute a very promising genetic resources of cassava, which should be preserved for the future. However it should be emphasized that several wild genotypes in such living collections might get lost because of lack of facilities, support and logistic in the National Programs. Taking these facts into consideration it is our interest at CIAT to preserve the wild *Manihot* germplasm for further utilization in breeding programmes.

From the 98 known wild Manihot species, only 31 species have been collected and stored in the seed bank of CIAT. From which only 11 species have 20 or more seeds with an average of two accessions per species. In addition, there is a probability that the current seed collection stored at CIAT may be in most of the cases unviable. Experience at CIAT has demostrated that viability of these seeds are very low, with percentage of germination ranging from 5-15; even low survival rate after germination has been observed. Preliminary studies carried out at CIAT using wild Manihot sexual seed, suggests that the percentage of germination can be increased with the application of in vitro culture techniques followed by micropropagation. CIAT considers that this is an opportune time to plan towards a germplasm conservation and utilization scheme for wild Manihot germplasm.

GENERAL WORK PLAN

SAMPLING

The applicability of in vitro culture methods normally used for the cultigens to the wild species requires urgent attention. In view of quarantine, plants can not be moved easily form country to country. Vegetative material and sexual seed samples of wild Manihot species will be from the living collections and from national seed banks. If sexual seeds are not available, in vitro shoot tip or bud culture, should be developed to transfer material from living collections to CIAT. The increase of number of seeds per accession, number of accessions and number of wild species at CIAT can be done through germplasm exchange with Mexico, Brazil and Paraguay. Collaborative agreement between CIAT and local institutions should be worked out to facilitate this exchange.

GERMINATION

Effect of heat treatment on wild Manihot seed will be studied. Experience at CIAT has demostrated that seeds of wild Manihot species normally have severe dormancy. For this reason, difficulties have been encountered previously for raising plants from seeds. Attemps to break seed dormancy of wild germplasm were only partially successful. One experiment consisted of subjecting the seeds to alternated temperatures 35°C and 25°C for 16 hours and 8 hours, respectively with light provided during the 16 hours phase. Although few seeds germinated within 7 days, most of them did not germinate. Another experiment consisted of treating seeds at 60°C during 14 days, to break dormancy and enhance germination. These experiences will be tested in this project, including the conventional germinating method, which consists of placing sexual seeds in wet filter paper at 35°C (control).

EMBRYO CULTURE

Mature sexual seeds will be used as source embryo culture to overcome germination problems commonly present in wild Manihot species. Results of experiments in this area carried out at CIAT suggests that the percentage of germination of wild *Manihot* seeds can be increased by *in vitro* embryo culture. The use of modified Murashige and Skoog (MS) medium, supplemented with gibberellic acid, thiourea, and sucrose to achieve embryo germination and plant development in wild *Manihot* species has shown promising results. The pH of Murashige and Skoog medium must be 5,5 with sucrose concentration of 4%. Taking into account previous experience at CIAT, all seeds will be disinfected with mercury bichloride, washed with distilled sterilized water, and the embryos dissected form each accessions or species, placed in culture media and incubated at 32°C and 27°C (day and night respectively) with a photoperiod 12 hours and an illumination of 5000 lux. It has also been found that treatment of seeds before embryo culture at 60°C during 20 days can enhance embryo development, resulting in twice as many plants in comparison with untreated seeds.

MICROPROPAGATION

Seedling raised through embryo culture will be micropropagated. Medium 4E (widely used to micropropagate cassava), will be tested. Replicated cultures will be used for *in vitro* storage in a slow growth medium, and another group of plants will be grown in the greenhouse until flowering for cytological and morphological characterizations using IBPGR descriptors.

ELECTROPHORESIS

Electrophoresis pattern relatioships between wild Manihot species will be carried out to recognize evolutionary groups. This work will be performed with the application of $\alpha\beta$ esterase (EST) in polyacrilamide gel electrophoresis. *In vitro* shoots grown from meristem tips will be used for electrophoresis.

WIDE CROSSES

Wild genotypes which successfully reach maturity and flowering stage in the field or greenhouse will be used in wide cross experiments with cassava. Cytological and morphological identification as well as the breeding behavior of parental genotypes and their hybrid progeny will be investigated. The crossability relationships between wild Manihot species and *M. esculenta* will be also investigated using a genetic design. Interspecific hybrids will be studied in the light of their fertility, cytogenetic mature and crossability with the purpose of improving efficiency of wild germplasm utilization and transfer of alien gene variation to cassava germplasm.

FACILITIES

Laboratory, field and greenhouse facilities, as well as, technical assistance and labor will be provided by the Biotechnology Research Unit, Cassava Program and the Genetic Resources Unit of CIAT.

APPENDIX I

WILD MANIHOT SPECIES

(Main source of information: Rogers and Appan-1973) *

Number (Taxon. order)	Abbreviation (proposed)	Species	Country of origen	No. of seeds at CIAT -GR	Remarks
		I. Manihot Sect. Manihot			
1	esc	M. esculenta			2n=4x=36
		II. Manihot Sect. Parvibracteate			
2	pri	M. pringlei	MEX.	5	Low cyanide conten and endagered species.
3	aur	M. auriculata	MEX.		
4	aes	M. aesculifolia	MEX, HON, GUA, NIC. CRI, PAN SAL	118, 12 38	Form starchy storage roots Stored in vitro (IVAG-BRU).
5	rub	M. rubricaulis	MEX	30	
5a	rub	M. rubricaulis subsp. rubricaulis	MEX		
5b	rub	M. rubricaulis subsp. isoloba.	MEX		Tolerant to cool temperatures.
6	oax	M. oaxacana	MEX	13,23	
7	chl	M. chlorosticta	MEX	9, 18, 29 17, 21, 3	Salt tolerant
8	dav	M. davisiae	USA, MEX	1, 2	
9	ang	M angustiloba	USA, MEX	5, 1	Form starchy storage roots. Endangered species
10	rho	M. rhomboidea	MEX, GUA, NIC, SAL, HON.	2	Form starchy storage roots

*Information is also based on several reports from wild Manihot germplasm collecting expeditions made by N. Nassar, C. Allen, J. Salick, F. Cardenas, V.M. Patiño, A. Amaya.

Number (Taxon. order	Abbrevia- tion	Species	Country of origen	No of Seeds at CIAT	Remark
10a	rho	M. rhomboidea subsp. rhomboidea	MEX		
10b	rho	M. rhomboidea subsp. microcarpa	MEX, HON NIC, SAL		
11	sub	M. subspicata	MEX		Form starchy s rage roots and endangered spe cies and droug tolerant.
12	wal	M. walkerae	USA, MEX		2n=4x=36
		III Manihot sect. Foetidae			
13	cau	M. caudata	MEX	11, 12	
14	mic	M. michaelis	MEX	34, 28	
15	tom	M. tomatophyla	MEX		
16	web	M. websterae	MEX	8, 16, 29	
17	foe	M. foetida	MEX		
18	cra	M. crassipetala	MEX	15, 35, 12	
		IV Manihot sect. Heterophylae			
19	zeh	M. zehntneri	BRA		
20	mar	M. marajoara	BRA		
21	sur	M. surinamensis	VEN, GY SUR		
22	tri	M. tristis	VEN, SUR BRA.		High starch co tent

Number (Taxon. order	Abbrevia-tion	Species	Country of origen	No. of seeds at CIAT	Remarks
22a	tri	M. tristis subsp. tristis	VEN		
22b	tri	M. tristis subsp. saxicola	SUR, BRA		
22c	tri	M. tristis subsp. surumuensis	BRA		
23	jan	M. janiphoides	BRA		
24	gra	M. grahami	BRA, ARG		Resistent to st borer
25	nif	M. inflata	BRA		
26	pil	M. pilosa	BRA		
27	cor	M. corymbiflora	BRA		
28	lep	M. leptopoda	BRA		
29	qui	M. quinquefolia	BRA		
30	con	M. condensata	BOL		
31	jol	M. jolyana	BRA		
32	lan	M. landroana	BRA		
33	poh	M. pohlii	BRA		Resistent to st borer and compa ble with esc
		V. Manihot sect. Anisophyllae			
34	ani	M. anisophylla	ARG		
35	gua	M. guaranitica	BOL, PAR, ARG.		Endangered spe-cies.

Number (Taxon. order	Abbrevia-tion	Species	Country of origen	No of seeds at CIAT	Remarks
35a	gua	M. guaranitica subsp. guaranitica	BOL, PAR ARG, BRA		
35b	gua	M. guaranitica subsp. boliviana	BOL		
VI Manihot Sect. Carthaginensis					
36	car	M. carthaginensis	COL, VEN CARIBE	58	Form starchy storage root
37	fil	M. filamentosa	VEN		
VII Manihot Sect. Quinquelobae					
38	acu	M. acuminatissima	BRA		
39	sag	M. sagittato-partita	BRA		
40	xav	M. xavantinensis	BRA		
41	spa	M. sparsifolia	BRA		
42	fal	M. falcata	BRA	1	
43	pru	M. pruinosa	BRA		
44	qun	M. quinqueloba	BRA		
45	alu	M. alutacea	BRA	2	
46	vio	M. violacea	BRA		
46a	vio	M. violacea subsp. violacea	BRA		
46b	vio	M. violacea subsp. recurvata	BRA		
47	jac	M. jacobinensis	BRA		

Number (Taxon. order	Abbrebia-tion	Species	Country of origen	No. of seeds at CIAT	Remarks
48	div	*M. divergens*	BRA		
49	irw	*M. irwinii*	BRA		
50	cec	*M. cecropiaeofolia*	BRA		Stored *in vitr* (IVAG-BRU).
51	mos	*M. mossamedensis*	BRA		
VIII *Manihot* Sect. *Graciles*					
52	fle	*M. flemingiana*	BRA		
53	lun	*M. hunzikeriana*	BRA,ARG		
54	las	*M. hassleriana*	PAR		Endangered spe cies
55	trp	*M. triphylla*	BRA		
56	fru	*M. fruticulosa*	BRA	2	Compatible wit *esc*
57	pen	*M. pentaphylla*	BRA,PAR	2	
57a	pen	*M. pentaphylla* subsp. *pentaphylla*	BRA		
57b	pen	*M. pentaphylla* subsp. *rigidula*	BRA		
57c	pen	*M. pentaphylla* subsp. *tenuifolia*	BRA		
57d	pen	*M. pentaphylla* subsp. *graminifolia*	PAR		
58	ten	*M. tenella*	BRA,PAR		
59	grc	*M. gracilis*	BRA,PAR	3	

Number (Taxon. order	Abbrevia-tion	Species	Country of origen	No of seeds at CIAT	Remarks
59a	grc	M. gracilis subsp gracilis	BRA,PAR		
60	pav	M. paviaefolia	BRA	5	
61	mag	M. maguireiana	VEN		
		IX Manihot sect. Sinuate			
62	anm	M. anomala	BRA,PER, BOL,ARG.	2, 2, 91, 3, 3, 3, 48	Stored in vitro (IVAG-BRU).
62a	anm	M. anomala subsp. anomala	BRA		
62b	anm	M. anomala subsp. pubescens	BRA		
62c	anm	M. anomala subsp. cujabensis	BRA		
62d	anm	M. anomala subsp. glabrata	PAR		Form starchy storage roots Stored in vitr
62e	anm	M. anomala subsp. pavoniana	PER,BOL ARG.		(IVAG-BRU).
63	war	M. warmingii	BRA		
		X Manihot sect. Variifoliae			
64	mir	M. mirabilis	PAR		
65	var	M. variifolia	PAR		

Number (Taxon. order)	Abbrevia-tion	Species	Country of origen	No of seeds at CIAT	Remarks
		XI Manihot sect. Glaziovianna e			
66	gla	M. glaziovii	BRA	3, 3	2n=4x=36 Resistant to mealy bug and C. African vi Compatible wi esc.
67	pse	M. pseudoglaziovii	BRA		Resistant to c cassava bacte-rial blight an drought
68	epr	M. epruinosa	BRA		
69	bra	M. brachyandra	BRA		
70	maa	M. maracasensis	BRA		
71	cat	M. catingae	BRA		
72	dic	M. dichotoma	BRA		2n=4x=3 Resistant to mealy bug and compatible wi esc
		XII Manihot sect. Peruvianae			
73	bra	M. brachyloba	CRI,RDOM COL,VEN, SUR,PER,.		Form starchy storage roots
74	lep	M. leptophylla	ECU,PER, BRA		Form starchy storage roots
75	quq	M. quinquepartita	BRA		
76	per	M. peruviana	PER		

Number (Taxon. order)	Abbreviation	Species	Country of origen	No. of seeds at CIAT	Remarks
		XIII Manihot sect. Crotalariaeformes			
77	pro	M. procumbens	BRA		
78	aff	M. affinis	BRA		
79	rep	M. reptans	BRA	2	Resistent to bac rial blight and patible with esc
80	cro	M. crotalariaeformis	BRA		
		XIV Manihot sect. Stipulares			
81	sti	M. stipularis	BRA		
82	pus	M. pusilla	BRA		
83	oli	M. oligantha	BRA	2	Compatible with esc
84	lon	M. longepetiolata	BRA	2	
85	nan	M. nana	BRA		
		XV Manihot sect. Grandibracteate			
86	tom	M. tomentosa	BRA		
86a	tom	M. tomentosa subsp. tomentosa	BRA		
86b	tom	M. tomentosa subsp. araliaefolia	BRA		
		XVI Manihot sect. Brevipetiolatae			
87	str	M. stricta	BRA,PER		

Number (Taxon. order	Abbrevia-tion	Species	Country of origen	No. of seeds at CIAT	Remarks
88	pur	*M. purpureo-costata*	BRA	2	
89	sal	*M. salicifolia*	BRA	2	
90	att	*M. attenuata*	BRA	2	Tolerant to coo temperatures
91	wed	*M. weddelliana*	BRA		
92	orb	*M. orbicularis*	BRA		
		XVII *Manihot* sect. *Peltatae*			
93	pel	*M. peltata*	BRA	2	
94	pop	*M. populifolia*	PAR		
95	ren	*M. reniformis*	BRA		
		XVIII *Manihot* sect. *Tripartitae*			
96	tri	*M. tripartita*	BRA,PAR		Stored *in vitro* (IVAG-BRU).Compa tible with *esc*
96a	tri	*M. tripartita* subsp. *tripartita*	BRA		
96b	tri	*M. tripartita* subsp. *humilis*	BRA		
96c	tri	*M. tripartita* subsp. *vestita*	BRA		
96d	tri	*M. tripartita* subsp. *laciniosa*	BRA		

Number (Taxon. order)	Abbreviation	Species	Country of origen	No. of seeds at CIAT	Remarks
		XIX *Manihot* sect. *Caerulescentes*			
97	cae	*M. caerulescens*	BRA,PAR.	5,2,24, 19	Stored *in vitr* (IVAG-BRU).
97a	cae	*M. caerulescens* subsp. *caerulescens*	BRA		
97b	cae	*M. caerulescens* subsp. *paraensis*	BRA		
97c	cae	*M. caerulescens* subsp. *macrantha*	PAR		
98	hep	*M. heptaphylla*	BRA		
99	neu	*M. neusana*	BRA		Resistent to st borer, tolerant to low temperat re.

APPENDIX II
WILD MANIHOT SPECIES
(In alfabetical order)

	Species	Number (taxon-order)*	Abbreviation (proposed)
1	*M. acuminatissima*	38	acu
2	*M. aesculifolia*	4	aes
3	*M. affinis*	78	aff
4	*M. alutacea*	45	alu
5	*M. angustiloba*	9	ang
6	*M. anisophylla*	34	ani
7	*M. anomala*	62	anm
7	*M. anomala* subps. *anomala*	62a	anm
7	*M. anomala* subsp. *pubescens*	62b	anm
7	*M. anomala* subsp. *cujabensis*	62c	anm
7	*M. anomala* subsp. *glabrata*	62d	anm
7	*M. anomala* subsp. *pavoniana*	62e	anm
8	*M. attenuata*	90	att
9	*M. auriculata*	3	aur
10	*M. brachyandra*	69	bra
11	*M. brachyloba*	73	brc
12	*M. caerulescens*	97	cae
12	*M. caerulescens* subsp. *caerulescens*	97a	cae
12	*M. caerulescens* subsp. *paraensis*	97b	cae

* according to Rogers and Appan (1973)

	Species	Number (taxon-order)*	Abbreviation (proposed)
12	M. caerulescens subsp. macrantha	97c	cae
13	M. carthaginensis	36	car
14	M. catingae	71	cat
15	M. caudata	13	cau
16	M. cecropiaeofolia	50	cec
17	M. chlorosticta	7	chl
18	M. condensata	30	con
19	M. corymbiflora	27	cor
20	M. crassipetala	18	cra
21	M. crotalariaeformis	80	cro
22	M. davisiae	8	dav
23	M. dichotoma	72	dic
24	M. divergens	48	div
25	M. epruinosa	68	epr
26	M. esculenta	1	esc
27	M. falcata	42	fal
28	M. filamentosa	37	fil
29	M. flemingiana	52	fle
30	M. foetida	17	foe
31	M. fruticulosa	56	fru
32	M. glaziovii	66	gla
33	M. gracilis	59	gra
33	M. gracilis subsp. gracilis	59a	gra

	Species	Number (taxon-order)	Abbreviation (proposed)
34	*M. grahami*	34	grh
35	*M. guaranitica*	35	gua
35	*M. guaranitica* subsp. *guaranitica*	35a	gua
35	*M. guaranitica* subsp. *boliviana*	35b	gua
36	*M. handroana*	32	han
37	*M. hassleriana*	54	has
38	*M. heptaphylla*	98	hep
39	*M. hunzikeriana*	53	hun
40	*M. irwinii*	49	irw
41	*M. jacobinensis*	47	jac
42	*M. janiphoides*	23	jan
43	*M. jolyana*	31	jol
44	*M. leptophylla*	74	lep
45	*M. leptopoda*	28	let
46	*M. longepetiolata*	84	lon
47	*M. maguireiana*	61	mag
48	*M. maracasensis*	70	mar
49	*M. marajoara*	20	maj
50	*M. michaelis*	14	mic
51	*M. mirabilis*	64	mir
52	*M. mossamedensis*	51	mos
53	*M. nana*	85	nan
54	*M. neusana*	99	neu

	Species	Number (taxon-order)	Abbreviation (proposed)
55	*M. niflata*	25	nif
56	*M. oaxacana*	6	oax
57	*M. oligantha*	83	oli
58	*M. orbicularis*	92	orb
59	*M. paviaefolia*	60	pav
60	*M. peltata*	93	pel
61	*M. pentaphylla*	57	pen
61	*M. pentaphylla* subsp. *pentaphylla*	57a	pen
61	*M. pentaphylla* subsp. *rigidula*	57b	pen
61	*M. pentaphylla* subsp. *tenuifolia*	57c	pen
61	*M. pentaphylla* subsp. *graminifolia*	57d	pen
62	*M. peruviana*	76	per
63	*M. pilosa*	26	pil
64	*M. pohlii*	33	poh
65	*M. populifolia*	94	pop
66	*M. pringlei*	2	pri
67	*M. procumbens*	77	pro
68	*M. pruinosa*	43	pru
69	*M. pseudoglaziovii*	67	pse
70	*M. purpureo-costata*	88	pur
71	*M. pusilla*	82	pus

	Species	Number (taxon-order)	Abbreviation (proposed)
72	M. quinquefolia	29	qui
73	M. quinqueloba	44	qun
74	M. quinquepartita	75	quq
75	M. reniformis	95	ren
76	M. reptans	79	rep
77	M. rhomboidea	10	rho
77	M. rhomboidea subsp. rhomboidea	10a	rho
77	M. rhomboidea subsp. microcarpa	10b	rho
78	M. rubricaulis	5	rub
78	M. rubricaulis subsp. rubricaulis	5a	rub
78	M. rubricaulis subsp. isoloba	5b	rub
79	M. sagittato-partita	39	sag
80	M. salicifolia	89	sal
81	M. sparsifolia	41	spa
82	M. stipularis	81	sti
83	M. stricta	87	str
84	M. subspicata	11	sub
85	M. surinamensis	21	sur
86	M. tenella	58	ten
87	M. tomatophylla	15	tom

	Species	Number (taxon-order)*	Abbreviation (proposed)
88	M. tomentosa	86	toe
88	M. tomentosa subsp. tomentosa	86a	toe
88	M. tomentosa subsp. araliaefolia	86b	toe
89	M. tripartita	96	tri
89	M. tripartita subsp. tripartita	96a	tri
89	M. tripartita subsp. humilis	96b	tri
89	M. tripartita subsp. vestita	96c	tri
89	M. tripartita subsp. laciniosa	96d	tri
90	M. triphylla	55	trp
91	M. tristis	22	trs
91	M. tristis subsp. tristis	22a	trs
91	M. tristis subsp. saxicola	22b	trs
91	M. tristis subsp. surumuensis	22c	trs
92	M. variifolia	65	var
93	M. violacea	46	vio
93	M. violacea subsp. violacea	46a	vio

	Species	Number (taxon-order)*	Abbreviation (proposed)
93	*M. violacea* subsp. *recurvata*	46b	vio
94	*M. walkerae*	12	wal
95	*M. warmingii*	63	war
96	*M. websterae*	16	web
97	*M. weddelliana*	91	wed
98	*M. xavantinensis*	40	xav
99	*M. zehntneri*	19	zeh

APPENDIX III

Crossability of wild *Manihot* species with *M. esculenta*

No.	CROSS	CROSSABILITY *	Source of Information (Reports)
		Compatible	Hahn (1986)
1	*M. esculenta* X *M. glaziovii*	Incompatible	Silva, J.R. (1984)
2	*M. esculenta* X *M. caerulescens*	Incompatible	Silva, J.R. (1984)
3	*M. esculenta* X *M. pseudoglaziovii*	Incompatible	Silva, J.R. (1984)
4	*M. esculenta* X *M. pohlii*	Compatible	Silva, J.R. (1984)
5	*M. esculenta* X *M. dichotoma*	Compatible	Hahn,(1982)
6	*M. esculenta* X *M. leptophylla*	Incompatible	Nassar, (1986)
7	*M. esculenta* X *M. reptans*	Compatible	Nassar, (1986)
8	*M. esculenta* X *M. fruticulosa*	Compatible	Nassar, (1986)
9	*M. esculenta* X *M. neussana*	Incompatible	Nassar, (1986)
10	*M. esculenta* X *M. oligantha*	Compatible	Nassar, (1986)
11	*M. esculenta* X *M. tripartita*	Compatible	Nassar, (1986)

* Reports on crossability experiments do not specify whether the crosscompatibility is unilateral or bilateral.