

## Biosystematic of wild cassava species (*Manihot* spp) based on soluble seed protein patterns

By

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Grattapaglia *et al.* (1986) conducted a biosystematic analysis of wild *Manihot* species based on soluble seed protein patterns. Nineteen species were analyzed electrophoretically (Table III) and a species similarity matrix was constructed based on differences in band density and number (Table IV). Several species were found to be very similar, e.g., *M. fruticulosa* and *M. pentaplylla*, and *M. pilosa* and *M. corymbiflora*; these results correlate well with the taximetric analysis made by Rogers and Appan (1973) and show that *M. pilosa* and *M. corymbiflora* are the two species most similar to cassava. Profile analysis confirmed the introgression between *M. cearulescens* and cassava. Electrophoresis was carried out according to Laemilli (1970) using 0.1% SDS and 5.5% acrylamide gel in Tris-HCL (pH=6.8), with gels being fixed for 12 h in 5% trichloroacetic acid followed by staining with 0.65% Coomassie brilliant blue. Four replicate gels were made for each species. The approximate molecular mass (AMM) of each band was determined according to Webber and Osborn (1969). The protein profiles varied in band intensity, and fifteen bands were selected as reference bands.

Table 2 shows the 15 selected reference bands (based on AMM) analyzed in each of the four replicates, with the banding classified as absent (a) or very intense (e). The total number of bands was calculated for each species in order to quantitatively compare the protein patterns between species. The variability of wild *Manihot* species in morphology, growth habit, and geographic distribution was reflected in the electrophoretic profiles as differences in the number and intensity of visible bands (Table VI).

The two cassava varieties, *M. esculenta* Crantz (var. EAB) and *M. esculenta* Crantz (var. RB), shared a similarity index (SI) of 78% and were similar to some species from the Glaziovinae (54-66% SI with *M. esculenta*), especially *M. glaziovii* Muell (66% SI with *M. esculenta*) and *M. pseudoglaziovii* Pax & Hoff (64% SI with *M. esculenta*) which themselves share an SI of 74%. The Heterophyllae contained species which were most similar to the cultigen, where *M. pilosa* (67-68% SI with *M. esculenta*) and *M. corymbiflora* (64-68% SI with *M. esculenta*) also shared morphological similarities with cassava, indicating that they are probably part of the complex from which the cultigen originated (Nassar, 1978b). A high SI was also found between the two species of *Gracilis* (SI=78%). High similarity between species in the various sections reflects their recent speciation and is in accordance with the taxonomic classification; genetically speaking they are probably part of the same gene pool.

**Table 1** - Wild *Manihot* species and their identification number in the germplasm bank at the Universidade de Brasília

Species	Section	Habitat	Nº	Nº de Coleta de herbário
<i>M. esculenta</i> Crantz (Var. EAB)	I (A) Manihot	Brasília(DF)	001	01
<i>M. esculenta</i> Crantz (Var. RB)	(B) Manihot	Brasília(DF)	002	01/a
<i>M. zehntneri</i> Ule	(C) Heterophyllae	Goiânia(GO)	173	02
<i>M. grahami</i> Hooker	II (D) Heterophyllae	Maringá(PR)	375	03
<i>M. pilosa</i> Pohl	(E) Heterophyllae	São Miguel de Antes(MG)	601	04
<i>M. corymbiflora</i> Pax	(F) Heterophyllae	São Miguel de Antes(MG)	605	05
<i>M. pohlii</i> Wawra	(G) Heterophyllae	Lençóis(BA)	139	06
<i>M. glaziovii</i> Muell	III (H) Glaziovinae	Pentecoste(CE)	221	08
<i>M. pseudoglaziovii</i> Pax & Hoff	(I) Glaziovinae	Remigio(PB)	545	09
<i>M. epruinosa</i> Pax & Hoff	(J) Glaziovinae	Serra Talhada(PE)	554	10
<i>M. brachyandra</i>	(K) Glaziovinae	Currais Novos(RN)	524	11
<i>M. reptans</i> Pax	IV (L) Clotalariaeformes	Corumbá(GO)	602	13
<i>M. alutacea</i> Rogers & Appan	V (M) Quinquelobae	Goiás Velho(GO)	115	07
<i>M. fruticulosa</i> Rogers & Appan	VI (N) Graciles	Alexânia(GO)	162	10938
<i>M. pentaplylla</i> Pohl	(O) Graciles	Goiás Velho(GO)	103	11755

<i>M. stipularis</i> Pax	VII	(P) Stipulares	Alexânia(GO)	184	14
<i>M. salicifolia</i> Pohl	VIII	(Q) Bravipetiolatae	Xavantina(MT)	195	-
<i>M. cearulescens</i> subsp Cearulescentes	IX	(R) Cearulescentes	Picos(PI)	258	15
<i>M. cearulescens</i> (não classificada)		(S) Cearulescentes	Morro do Chapéu(BA)	567	16
<i>M. cearulescens</i> (não classificada)		(T) Cearulescentes	Jequié(BA)	269	17
<i>M. leptophylla</i> Pax	X	(U) Peruvinae	Barra do Corda(MA)	517	12
<i>M. neusana</i> Nassar	-	(V) -	Maringá(PR)	360	18

**Table 2** - Classification of bands in wild *Manihot* species according to approximate molecular weight (AMW)

AMW (Kda)	Section																					
	I		II					III					IV	V	VI	VII	VIII	IX			X	-
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
81-75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75-66	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	3	2	2
66-62	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	-	1	-	-	1	-	1
62-50	3	4	4	3	3	4	4	3	4	4	3	3	3	3	3	1	5	1	1	4	3	3
50-37,5	5	5	6	6	6	6	6	5	6	6	6	4	5	3	4	4	5	4	4	5	3	4
37,5-33	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
33-30	1	1	2	2	1	1	-	2	2	-	2	3	1	2	2	3	1	1	1	2	1	2
30-27	2	2	2	2	2	2	2	1	1	1	1	2	3	1	1	3	1	1	1	1	1	1
27-25	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
25-24	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
24-21	1	1	-	-	1	-	-	1	1	-	1	1	1	1	1	1	1	-	-	-	-	1
21-20	-	-	2	2	-	-	1	1	-	-	-	1	1	-	1	-	-	-	-	1	-	-
20-18	1	1	1	2	-	-	1	-	-	1	-	-	1	-	-	-	1	-	-	1	-	-
18-13	3	1	2	3	3	3	3	3	2	3	3	3	3	3	2	2	2	2	3	2	3	3
Nº bands	21	20	24	24	20	20	21	19	20	19	20	21	20	17	18	18	20	11	12	22	15	20
Nº reference	15	15	15	14	15	15	14	15	15	15	15	15	15	14	14	14	14	15	15	15	15	14
Nº total bands	36	35	39	38	35	35	35	34	35	34	36	36	35	31	32	32	34	26	27	37	30	34

\*See Table III for species identification.

**Table 3** - Matrix of similarity between studied *Manihot* species

Section species	Section																					
	I		II					III					IV	V	VI	VII	VIII	IX			X	-
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
A	-	78	54	45	67	64	58	66	64	58	58	58	50	45	43	43	54	30	32	54	47	50
B		-	49	38	68	68	68	61	60	56	54	50	52	42	41	44	52	28	31	53	43	50
C			-	62	51	65	48	51	49	51	54	54	59	31	30	32	45	33	33	50	44	40
D				-	47	53	65	40	45	40	50	54	47	30	29	30	39	32	33	50	39	59
E					-	75	61	70	75	63	74	66	62	46	44	45	60	36	39	66	53	62
F						-	58	67	71	67	70	70	71	42	40	41	58	36	38	58	56	56
G							-	51	54	51	65	65	52	38	39	38	50	34	36	50	41	78
H								-	74	71	60	55	61	49	50	48	56	35	37	64	54	52
I									-	59	64	70	45	45	43	43	62	41	45	70	69	60
J										-	74	55	52	43	41	42	52	45	44	36	49	48
K											-	71	59	41	39	38	52	32	34	37	47	54
L												-	59	38	39	46	56	33	36	38	51	59
M													-	40	35	50	50	32	35	37	47	50
N														-	78	55	50	-	88	38	43	42
O															-	-	51	-	-	39	37	43
P																	36					
Q																						49 56
R																						49 36
S																						50 38
T																						
U																						43 58

\*See Table III for species identification.

**Table 4** - Distribution of reference bands according to density in studied wild *Manihot* species profiles

Reference bands	Section																			
	I	II	III	IV	V	VI	VII	VIII	IX	X	-									

Nº	AMW	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	81	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2	75	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
3	66	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
4	62	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
5	50	C	D	C	C	D	C	C	B	C	C	C	C	B	C	B	B	C	B	B	C	C	C
6	37,5	C	D	C	C	C	C	C	C	C	C	C	C	B	C	B	B	C	B	B	C	C	C
7	33	E	E	B	B	D	B	C	D	D	C	C	B	B	D	D	D	D	B	B	D	D	D
8	30	C	B	B	C	C	C	C	C	C	E	D	C	B	C	C	B	C	C	C	C	C	C
9	27	C	C	C	C	C	C	C	B	B	D	D	C	B	B	B	A	C	C	C	C	C	C
10	25	B	B	B	B	B	B	C	B	B	B	B	C	B	B	C	B	C	D	D	B	B	C
11	24	B	B	B	C	B	B	B	B	D	B	B	D	B	B	B	B	C	C	C	D	D	C
12	21	D	D	B	B	C	B	C	C	C	C	C	C	B	E	E	E	C	B	B	B	B	C
13	20	D	D	B	A	C	C	A	C	C	C	C	C	B	E	E	E	B	B	C	B	B	A
14	18	D	D	D	C	C	D	C	D	D	D	C	C	C	B	B	B	D	B	B	D	D	B
15	13	D	D	D	C	C	C	C	D	C	D	C	C	C	A	A	C	A	C	C	C	C	C

\*See Table III for species identification. A=Absent band; B=little visible band; C=visible band; D=dense band; E=very dense band. AMW=Approximate molecular weight.