Predicting *Manihot* species compatibility by molecular analysis

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Abstract

Wild *Manihot* species are sources of useful characters to improve the crop. However, their incompatibility with cassava may impede their utilization for improving the cultigen. This study examined the possibility of detecting their compatibility judging from electrophoresis results. Four *Manihot* species, namely *M. pilosa*, *M. glaziovii*, *M. reptans* and *M. cearulescens*, were used. These species were allowed to hybridize with cassava to give an idea of how much this hybridity coincides with similarity index of electrophoresis analysis. Gene markers of leaf shape, stem surface, disk color, and fruit form were used to detect hybridity. Species *M. pilosa*, *M. glaziovii* had successful hybridization while others failed under natural conditions. This result coincides with the similarity index of electrophoresis.

Key words

Interspecific hybrids, marker genes, wild cassava species, *Manihot*

Introduction

Wild Manihot species are sources of many useful characters for improving cassava (Nassar, 1978a, 1978b, 1978c, 1978d,, 1999). Transferring these genes, however, faces problems of interspecific barriers which impede successful crosses.
To facilitate crosses designated for the transfer of useful genes from the wild to the cultigen, it is necessary to know how much is the distance between a certain wild species and the cultivated crop, and consequently how strong or weak are the barriers.

Grattapaglia and Nassar (1986) analyzed biosystematically the relationship between cassava and its wild relatives on the basis of protein electrophoresis. They constructed a species similarity matrix based on band density and number. Some enquiries were raised as to how much this relationship is reflected in cross trials and fertility. In this study, a trial was designed to hybridize 4 species representative of the wild Manihot groups with the cultigen. They were selected because they represent botanically the extreme ends of distance to cassava (Rogers and Appan, 1973). Our idea was that the more hybrid seed obtained the better the relationship and compatibility are.

Material and Methods

Four wild species in addition to cassava were used in this experiment. These species were M. pilosa, M. glaziovii, M. reptans and M. cearulescens. Seed and or cuttings of these species were planted in September 2000.

The seeds were treated thermally with an alternating temperature, 16/28 C, for periods of 8/16 hours (Nassar & Pio 1982) for one week duration to break dormancy. Mode of planting was in circles, with one plant of the wild species in the center of the circle, surrounded by 8 cassava plants, They were allowed to pollinate by insects. Fruits were collected from the wild species (maternal parent) in the third year. June 2002. Seeds were extracted from the fruits, treated thermally to break dormancy and planted in rows. Raised plants were examined using gene markers to identify interspecific hybrids. These marker genes are dominant gene of prominent nodes on stem (which came from cassava-the paternal parent) against smooth stem, red color of flower disk which are dominant to yellow, setaceous bracteole which is dominant to foliaceous, and winged fruit which is dominant to globose one . The raised plants were also observed for growth habit, height, stem texture and tuber formation.

Results and Discussion

Out of 200 seeds of M. pilosa, only 39 seedlings emerged of which 4 hybrids were identified by dominant markers from cassava: noded stem, setaceous bracteoles, ribbed fruit and tuberous roots (Table 1). Other characters proved to be indirect evidence of hybridization.

The 200 seeds collected from M. glaziovii gave rise to 78 seedlings . Of these, three seedlings showed characteristics of interspecific hybridization . Hybrid plants exhibited dominant phenotypes from cassava, namely ribbed fruit, red color in flower disk, noded stem and tuberous roots (Figs. 1-4) (Table 2).

Table 1. Growth habit and marker genes of Manihot specis

Character	M.pilosa	Cassava	Hybrid
Growth habit	Tall shrub 4m height	small shrub 1.5-2m	medium shrub 3m
Young stem Texture	Hairy	Glabrous	Hairy
Bracteoles	setaceous	setaceous	setaceous
Fruits	Globose without ribs	Ovoid,ribbed	Ovoid ribbed
Flower disc Color	Yellow	Red	Red
Tuber formation	None	Forms tubers	Forms tubers

Table 2. Comparison of morphological characters for M. glaziovii, cassava and their hybrid

Character	M.glaziovii	Cassava	Hybrid
Growth habit	Tree 10 m height	Erect shrub 1.5-2 m	Erect shrub 2.5-3 m
Young stem texture	Glabrous	Glabrous	Glabrous

Bracteoles	Setacious	Setaceous	Setaceous
Fruits	Globose without Ribs	Ovoid ribbed	Ovoid ribbed
Flower disc color	Yellow	Red	Red
Stem nodes	smooth	prominent	prominent
Tuber Formation	None	forms tubers	Forms tubers

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These results show that glabrous stem, setaceous-foliaceous bracteoles, red-creamy color of flower disks, variegated-green color of fruit, and ribbed-nonribbed fruit are simple marker genes that can be used to recognize interspecific hybridization. This is in accordance with what has been found by Nassar in 1989 while working with broadening the genetic base of Cassava by controlled hybridization.

Species of M. reptans and M. cearulescens did not produce any hybrid among the 200 seeds collected from each one of them. In their biosystematic analysis of *Manihot* species using electrophoresis of soluble protein, Grattapaglia and Nassar(1986) elaborated a matrix of similarity index for cassava and wild *Manihot* species examined as follows:

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
0															-	-	51		-	39	37	43
P																	36			-	48	41
Q																					49	56
R																					49	36
S																					50	38
T																						
U																					43	58

This index was based on quantifying density and distance of bands. From this matrix, it is seen that M. *pilosa* and M. *glaziovii* (referred to by letters H and I, while cassava is referred to by the letter A) have the highest similarity index. This similarity index was 68% for M. *pilosa* and 64% for M. *glaziovii*. The similarity index for species M. *reptans* and M. *cearulescens* was far lower. These species are referred to by letters S and U, having a similarity index of 32 and 47, respectively.

Apparently, the weak barriers between cassava and M. *glaziovii* and M. *pilosa* could be broken, while the stronger ones with the other two species could not be easily overcome.

From our observations in this experiment, the pollination by insects played an important role in obtaining successful crosses. This is probably due to the fact that insects as vectors carry an abundant number of cassava pollen grains from one flower to another (Nassar, 2004, 2007). This means a great diversification of gametes which is not possible with manual crosses.

We can conclude that barriers between cassava and other *Manihot* species are weak and recently evolved. A similar deduction was made previously by Nassar et al. (1995, 1997). It seems that they arose not as a primary isolated event, but secondarily after geographic isolation. Nassar (1978c, 1984, 1985, 2007) postulated that cassava itself is an interspecific hybrid that appeared through domestication some 3000 years ago or less.

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Fig.1 Marker gene of fruit shape;winged fruit disk

(left), golobose fruit (right), hybrid fruit (middle)

Fig.2 Red flower and yellow disk

Fig.3 Foliaceous bracteole(right) and setaceous