Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Compatibility of interspecific *Manihot* crosses presaged by protein electrophoresis

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Genet. Mol. Res. 9 (1): 107-112 (2010)
Received September 24, 2009
Accepted October 19, 2009
Published January 19, 2010

ABSTRACT. Cross incompatibility of wild *Manihot* species with cassava (*M. esculenta*) can impede their utilization for improving this cultigen. We tested whether compatibility could be determined based on electrophoresis results. *Manihot pilosa*, *M. glaziovii*, *M. reptans*, and *M. cearulescens* were tested. These species were allowed to hybridize with cassava to determine whether hybridization coincides with the similarity index based on electrophoresis analysis. Gene markers of leaf shape, stem surface, disk color, and fruit shape were used to confirm hybridization. *Manihot pilosa* and *M. glaziovii* successfully hybridized with cassava, while the others failed to do so under natural conditions. This result coincided with the similarity index from electrophoresis.

Key words: Interspecific crosses; Marker genes; *Manihot*; Wild cassava species

INTRODUCTION

Wild *Manihot* species are sources of many useful characters for improving cassava (Nassar, 1978a,b,c,d; 2000, 2006a; Nassar and Sousa, 2007). Transfer of these genes, however, faces the problem of interspecific barriers, which impede successful crosses.

To facilitate crosses aimed at transferring useful genes from the wild plants 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 et al. (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 trial crosses and in fertility. In this paper, a trial was designed to hybridize 4 species representative of wild *Manihot* groups with the cultigen. They were selected because they represent botanically the extreme ends of distance to cassava (Rogers and Appan, 1973; Nassar, 2003a,b). Our idea is that the more hybrid seed obtained the greater the relationship and compatibility are.

MATERIAL AND METHODS

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

The seeds were treated thermally by alternating temperatures of 16º and 28ºC for periods of 8/16 h (Nassar, 1983; Nassar, 2006b; Nassar and Hashimoto, 2006) for one week to break dormancy. Mode of planting was in circles where one plant of the wild species was in the center of a circle of 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 fruits, treated thermally to break dormancy and planted in rows. Raised plants were observed using gene markers to identify interspecific hybrids. These marker genes are dominant genes of prominent nodes on stem (which came from cassava, the paternal parent) versus smooth stem, red color of flower disk, which is dominant to yellow, setaceous bracteole, which is dominant to foliaceous, and winged fruit, which is dominant to globose one. The plants raised were also examined for growth habit, height, stem texture, and tuber formation.

RESULTS AND DISCUSSION

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.

Table 1. Growth habit and marker genes of *Manihot* species.

Character	*M. pilosa*	Cassava	Hybrid
Growth habit	Tall shrub 4 m in height	Small shrub 1.5-2 m	Medium shrub 3 m
Young stem texture	Hairy	Glabrous	Hairy
Bracteoles	Setaceous	Setaceous	Setaceous
Fruits	Globose without ribs	Ovoid, ribbed	Ovoid, ribbed
Flower disk color	Yellow	Red	Red
Tuber formation	None	Forms tubers	Forms tubers

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 of cassava, namely ribbed fruit, red color of flower disk, noded stem, and tuberous roots (Figures 1-4; Table 2).

Table 2. Comparison of morphological characters of *Manihot glaziovii*, cassava and their hybrid.

Character	*M. glaziovii*	Cassava	Hybrid
Growth habit	Tree 10 m in height	Erect shrub 1.5-2 m	Erect shrub 2.5-3 m
Young stem texture	Glabrous	Glabrous	Glabrous
Bracteoles	Setaceous	Setaceous	Setaceous
Fruits	Globose without ribs	Ovoid, ribbed	Ovoid, ribbed
Flower disk color	Yellow	Red	Red
Steam nodes	Smooth	Prominent	Prominent
Tuber formation	None	Forms tubers	Forms tubers

Figure 1. Marker gene of fruit shape; winged fruit.

Figure 2. Red flower disk (left), globose fruit (right) and hybrid fruit (middle), and yellow disk.

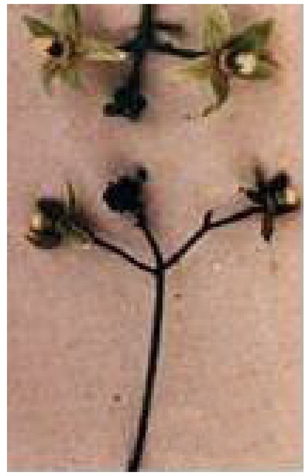

Figure 3. Foliaceous (above) and setaceous bracteole (below).

Figure 4. Smooth stem (right), noded stem (left) and hybrid form (middle).

These results show that glabrous stem, setaceous-folicaceous 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 was 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 within 200 seeds collected from each of them. In their biosystematic analysis of *Manihot* species using electrophoresis of soluble protein, Grattapaglia et al. (1986) elaborated a matrix of similarity index for cassava and other wild *Manihot* species examined as shown in Table 3.

Table 3. Matrix of similarity between the *Manihot* species studied.

Section species	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			-	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 the letters H and I, and cassava referred to by the letter A) have the highest similarity indices, i.e., 68% for *M. pilosa* and 64% for *M. glaziovii*. The similarity indices for species *M. reptans* and *M. cearulescens* are far lower. These species are referred to by the 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 (Nassar 2006c,d).

From our observations in this experiment, insect pollination played an important role in obtaining successful crosses. This is probably due to the fact that insects as vectors carry abundant amounts of cassava pollen grains from one flower to another (Nassar, 2004, 2007a). This means a great diversification of gametes, which is available when manual crosses are applied.

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

ACKNOWLEDGMENTS

Research supported by Conselho Nacional de Desenvolvimento Cientifico - CNPq. The above mentioned living collection was established with the support of the Canadian International Development Research Center, Ottawa, to which we are grateful.

REFERENCES

Nassar NMA (1978a). Conservation of the genetic resources of cassava (*Manihot esculenta*). Determination of wild species localities with emphasis on probable origin. *Econ. Bot.* 32: 311-320.

Nassar NMA (1978b). Genetic resources of cassava - Chromosome behavior in some wild *Manihot* species. *Indian J. Genet. Plant Breed.* 38: 135-137.

Nassar NMA (1978c). Hydrocyanic acid content in some wild *Manihot* (cassava) species. *Can. J. Plant Sci.* 58: 577-578.

Nassar NMA (1978d). Microcenters of wild cassava, *Manihot* ssp diversity in central Brazil. *Turrialba* 28: 345-347.

Nassar NMA (1983). A quebra da dormência da semente das espécies selvagens da mandioca, *Manihot* spp. *Cienc. Cult.* 35: 630-632.

Nassar NMA (1984). Natural hybrids between *Manihot reptans* Pax and *Manihot alutacea* Rogers and Appan. *Can. J. Plant Sci.* 64: 423-425.

Nassar NMA (1985). *Manihot neusana* Nassar: A new species native to Paraná, Brazil. *Can. J. Plant Sci.* 65: 1097-1100.

Nassar NMA (1989). Broadening the genetic base of cassava, *Manihot esculenta* Crantz, by interspecific hybridization. *Can. J. Plant Sci.* 69: 1071-1073.

Nassar NMA (1997). Development of cassava interspecific hybrids for savanna conditions. *J. Root Crops* 22: 9-17.

Gratapaglia DE, Nassar NMA and Dianese JC (1986). Biossistemática de espécies brasileiras do gênero *Manihot* baseada em padrões de proteína da semente. *Cienc. Cult.* 19: 294-300.

Nassar NMA (2000). Cassava, *Manihot esculenta* Crantz, genetic resources: their collection, evaluation, and manipulation. *Adv. Agron.* 69: 179-230.

Nassar NMA (2003a). Cassava, *Manihot esculenta* Crantz genetic resources VI. Anatomy of a diversity center. *Genet. Mol. Res.* 2: 214-222.

Nassar NMA (2003b). Gene flow between cassava, *Manihot esculenta* Crantz, and wild relatives. *Genet. Mol. Res.* 2: 334-347.

Nassar NMA (2004). Cassava: some ecological and physiological aspects related to plant breeding. *Gene Conserv.* 3: 229-245.

Nassar NMA (2006a). Letter to the Editor. *Genet. Mol. Res.* 5: 419-420.

Nassar NMA (2006b). Cassava genetic resources: extinct everywhere in Brazil. *Genet. Res. Crop. Evol.* 53: 975-983.

Nassar NMA (2006c). The synthesis of a new cassava-derived species, *Manihot vieiri* Nassar. *Genet. Mol. Res.* 5: 536-541.

Nassar NMA (2006d). Cassava in South America, Brazil's contribution and the lesson to be learned from India. *Genet. Mol. Res.* 5: 688-695.

Nassar NMA (2007a). Wild cassava, *Manihot* spp. to improve the crop. *Gene Conserv.* 26: 387-414.

Nassar NMA (2007b). Cassava genetic resources and their utilization for breeding of the crop. *Genet. Mol. Res.* 6: 1151-1168.

Nassar NMA and Hashimoto DYC (2006). Wild cassava, *Manihot* spp.: a survey of useful species with reference to economic value. *Gene Conserv.* 5: 284-295.

Nassar NMA and Sousa MV (2007). Amino acid profile in cassava and its interspecific hybrid. *Genet. Mol. Res.* 6: 292-297.

Rogers DJ and Appan SG (1973). *Manihot* and Manihotoids (Euphorbiaceae) (Flora Neotropica Monograph, 13). Hafner Press, New York.

Nassar HN, Nassar NMA, Vieira C and Saraiva LS (1995). Cytogenetic behavior of the interspecific hybrid of *Manihot neusana* Nassar and Cassava, *M. esculenta* Crantz, and its backcross progeny. *Can. J. Plant Sci.* 75: 675-678.

