

Prospect of polyploidizing cassava, *Manihot esculenta* Crantz, by unreduced microspores

N. M. A. NASSAR AND M. FREITAS

Departamento de Agronomia, Universidade de Brasília, CX. 04477, Brasília, 70919-000
Brasil

Abstract

Eleven interspecific hybrids of cassava with the wild relatives *Manihot glaziovii*, *M. pseudoglaziovii*, *M. dichotoma* and *M. anomala* were examined to detect unreduced microspores. The frequency of dyads and triads were counted, as well as pollen viability. Of these hybrids, the second generation progeny of cassava & times; *M. glaziovii* showed a high frequency of unreduced microspores (3.7%) permitting their selection for further manipulation of this character. An association of vegetatively-reproduced genotypes and the occurrence of unreduced microspores has also been observed.

Key words: *Manihot esculenta* – interspecific hybrids – meiotic irregularity – mutation

Cassava is a major source of energy in humid tropics and subtropics. It ranks as the fifth largest starchy crop and supplies more than 500 million people (FAO 1994). One of the most promising approaches to improving it is to produce triploid types which may offer resistance to drought and insects, combined with high productivity (Hahn *et al.* 1990, Nassar 1992). The triploid tree produced by Nassar (1992) showed a doubling of root production under semi arid conditions.

Polyploid types were obtained by the first author and were found to be produced by unreduced gamete fertilization (Nassar 1992). It is believed that if parents with a high frequency of unreduced gametes could be selected, this would enhance the possibility of obtaining polyploidy types in their progeny.

The causes of unreduced microspores in plant are variable, ranging from simple recessive genes (Mok and Peloquin 1975) to disturbed spindle function in interspecific hybrids. Nassar *et al.* (1995) suggested that the disturbance of meiotic division in

interspecific hybrids may lead to a higher frequency of unreduced gametes, consequently making it possible to select polyploids among their progeny.

Brazilian *Manihot* species are believed to be progenitors of cassava, and Brazil contains its centers of diversity (Nassar 1978a). Interspecific hybridizations have been carried out systematically by the first author since 1980 and a large number of interspecific produced (Nassar 1989). Some of these, which represent different interspecific hybrids and cover a vast array of variation, are used in our present investigation.

Eleven interspecific hybrids and/or their progenies were used in this investigation. These hybrids are: F₁ *M. glaziovii* x cassava (three genotypes), F₂ *M. epruinosa* x cassava, F₂ *M. anomala* x cassava (three genotypes), F₃ *M. pseudoglaziovii* x cassava (two genotypes), F₄ *M. pseudoglaziovii* x cassava. These hybrids and progenies were chosen because they provide large genetic variation.

Chromosome associations in meiotic metaphase I and the occurrence of dyads, triads, and also pollen viability were studied. For the meiotic study, inflorescences were fixed in a 3:1 mixture of absolute alcohol and glacial acetic acid and kept at 5°C for 24 hours. The anthers were smeared in an acetocarmine stain. Chromosome configurations at metaphase I, together with microspore formation were studied.

For the pollen viability study, one to three flowers per plant were selected, their pollen being crushed in acetocarmine and scanned. Pollen counts and percentage of stained normal pollen were calculated.

Chromosome associations and their frequency in meiotic metaphase I of pollen mother cells (PMCs) of all interspecific hybrids have shown regular pairing of 18 bivalents (Fig. 1a) except the F₂ progeny of *M. glaziovii* x cassava (first genotype), which revealed the presence of two univalents in 5% of the cells examined. This was the same genotype that showed a high frequency (3.7%) of dyad formation (Fig. 1b), and low pollen viability (42%). The study of PMCs in the tetrad phase revealed the formation of abnormal tetrads having 1-3 micronuclei in all hybrids used in the experiment, except F₁ *M. glaziovii* x cassava (2nd genotype). The abnormal tetrads have ranged from 0.8% in F₁ *M. glaziovii* x cassava (1st genotype) to 4.38% in F₃ *M. pseudoglaziovii* x cassava (Table 1). Pollen

viability has ranged from 23.5% in F₁ *M. dichotoma* x cassava (2nd genotype) to 94% in the F₁ hybrid *M. epruinosa* x cassava (Table 1).

Table 1: Frequency of unreduced microspores and pollen viability in different hybrids.

Manihot hybrids	Microspores examined	Frequency and percentage of different sporads								Pollen examined	Pollen viability	
		Diad		Triad		Tetrad		Abnormal tetrad			n	%
		n	%	n	%	n	%	n	%			
<i>M. glaziovii</i> x cassava	1217	10	0.82	8	0.66	1158	95.15	41	3.37	2979	1197	40.18
F1 <i>M. glaziovii</i> x cassava x cassava (1st genotype)	1168	43	3.70	8	0.70	1107	94.80	10	0.80	1713	723	42.21
F1 <i>M. glaziovii</i> x cassava x cassava (2nd genotype)	1044	0	0.00	4	0.40	1040	99.60	0	0.00	1463	1123	76.76
<i>M. epriminoso</i> x cassava	1252	0	0.00	0	0.00	1252	100.00	0	0.00	603	567	94.03
F2 <i>M. anomala</i> x cassava (1st genotype)	1374	1	0.07	0	0.00	1326	96.51	47	3.42	836	183	21.89
F2 <i>M. anomala</i> x cassava (2nd genotype)	1145	0	0.00	0	0.00	1117	96.56	28	2.44	1297	441	34.00
F2 <i>M. anomala</i> x cassava (3rd genotype)	1136	1	0.09	0	0.00	1106	97.36	29	2.55	801	452	56.43
F3 <i>M. pseudoglaziovii</i> x cassava	1416	0	0.00	0	0.00	1134	95.62	62	4.38	1427	873	61.18
F4 <i>M. pseudoglaziovii</i> x cassava	1210	0	0.00	0	0.00	1207	98.80	3	0.20	1130	1034	91.50
F1 <i>M. dichotoma</i> x cassava (1st genotype)	1138	1	0.09	0	0.00	1125	98.86	12	1.05	1704	862	50.59
F1 <i>M. dicotoma</i> x cassava (2nd genotype)	491	2	0.40	3	0.60	476	97.0	10	2.04	1273	299	23.49

Fig. 1: Pollen mother cells in Manihot: a. meiotic metaphase I with 18 bivalents; b. dyad; c. meiotic restitution showing gametic number 36.

The high frequency of unreduced microspores in this F₂ progeny of *M. glaziovii* x cassava will facilitate selection of this genotype and its progeny as a possible progenitor of polyploids in the future. Apparently, the dyad formation is due to meiotic restitution (Fig. 1c). The low pollen viability may be due to univalent formation and the consequent irregular chromosome distribution leading to unbalanced gametes.

Cassava is a natural allopolyploid, judging by its chromosome number and the complete pairing of its meiotic metaphase chromosomes (Nassar 1978b, Vásquez and Nassar 1994). If unreduced gametes were responsive for its natural polyploidization in the past, it is possible that one can detect them among wild relatives and their hybrids with cassava. The presence of unreduced microspore in the hybrid progeny, as mentioned before, confirms this hypothesis.

Vásquez and Nassar (1994) have reported a high frequency of unreduced microspores among cassava clones. It is believed, therefore, that this character is heritable and genetically controlled. It was probably acquired by cassava from its wild ancestors (Nassar *et al.* 1995), representing an evolutionary remnant in cassava. The significance of this phenomenon is that it provides direct evidence on polyploidization from lower ploidy

levels through the mechanism of unreduced gametes and not through other types of somatic doubling (Harlan and de Wet 1975, de Wet 1980). According to the genes of wild ancestors have probably had the opportunity to combine and produce larger rooted cassava (Nassar 1992, Nassar *et al.* 1996). It seems that this character is correlated with meiotic irregularities provided there is some univalent formation in the genotype producing unreduced microspores.

It is worth mentioning that this unreduced-microspore-producing genotype is a progeny of a natural hybrid of *M. glaziovii* with cassava. This natural hybrid has been maintained by farmers for hundreds of years through vegetative reproduction. The unreduced microspore gene has probably arisen through recurrent mutation and been maintained through vegetative reproduction. In other germplasm that reproduces sexually, it would be eliminated by natural selection due to the abortion of gametes which carry it.

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