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Production of triploid cassava, *Manihot esculenta* Crantz by hybrid diploid gametes

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ABSTRACT

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A natural hybrid of *Manihot pseudoglaziovii* and cassava was collected, grown, multiplied vegetatively and studied cytogenetically. The meiotic division showed fairly regular chromosome pairing and formation 2n pollen. When selfed, a triploid was selected from among its progeny and reported here for the first time. Evaluation of this triploid for agronomic traits showed a high productivity of tuberated roots and resistance to stem borers under semi-arid conditions. The implication of intraspecific hybridization correlated with 2n gamete formation in cassava plant breeding perspectives is discussed.

INTRODUCTION

Wild *Manihot* species are a useful source of many genes for cassava improvement (Nassar, 1978a, b, c, 1979, 1985, 1986). However, interspecific barriers may impede the efficient utilization of these genes (Nassar *et al*., 1986). Natural hybrids frequently occur in their habitats due to specific niches and conditions that may lead to sporadic breakdown of these barriers (Nassar, 1984). These natural interspecific hybrids can be a good alternative to crosses that are hard to carry out to scale and may fail under normal conditions. The critical point in localizing a natural hybrid is the familiarity of the breeder with the wild species characteristics and identifying the natural hybrids with the help of marker genes (Nassar, 1989).

The success of tetraploid and triploid cultivars of vegetatively reproduced crops has stimulated cassava breeders. Trials of chromosome doubling in cassava were made by Abraham et al, early in the 60.s using colchicine (1964). However, this attempt has not led to the development of tetraploid or triploid cultivars, probably due to instability of the chimeras produced. The use of gametic restitution for production of polyploids was tried successfully in several crop plants, for example *Zea mays* (Roades and Dempsey, 1966), *Solanum* (Mok and Peloquin, 1975), *Medicago sativa* (Vorsa and Bingham, 1979). Using interspecific hybrids in such polyploidization will lead to the maximum of heterosis as to the development of bilateral sexual doubling. Since the occurrence of 2n gametes appears to be a common phenomenon in angiosperms (Harlan and Wet, 1975, de Wet, 1988), it is likely to be detected in cassava in an extensive, detailed examination is made.

MATERIALS AND METHODS

Though our program of cassava germplasm collection and utilization in Brazil, a natural hybrid of *Manihot pseudoglaziovii* and cassava was collected in 1978 from county Remigio, Paraiba State and grown in the living collection at the Biological Experimental station, Universidade de Brasília (Nassar, 1982). This natural hybrid was identified by the marker genes of winged fruit which came from cassava and peltated, pendurated leaf, two characteristics genes of *M. pseudoglaziovii*. In addition to these marker genes, this hybrid was found in region where the habitat of *M. pseudoglaziovii* overlaps cassava plantations. The hybrid was multiplied vegetatively and cloned 20 to individuals maintened in the living collection and used in this experiment. This clone was left for open pollination .entre si.. Seeds collected were planted in 1980 and progeny of 22 plants were produced. Evaluation of this progeny for root production was carried out in the following years.

The natural hybrid as well as its selected progeny were studied meiotically. The anthers were fixed for 24 h in a 3:1 solution of ethanol and acetic acid, transferred to 70% ethanol and stored at 5°C. The anthers were squashed in 1% acetocarmine. Pollen analysis was conducted by collecting pollen from undehisced natural anthers of a flower onto a microscope slide and staining with 1% acetocarmine. Pollen grain diameter was assessed at 450x using an eye piece micrometer. A minimum of 1000 stained grains were considered fertile and partially filled and unstained ones were considered sterile.

The frequency of mature 2n pollen grains was based on counts of 1000 pollen grains stained with

1% acetocarmine. Classification of n vs. 2n pollen was made by visual size discrimination of stained pollen, given that latter should have twice the nuclear volume of the former.

To obtain data on ploid status, mitosis in root tips was examined and chromosome number was counted to confirm the plant.s chromosome constitution.

RESULTS AND DISCUSSION

Screening the hybrid cloned plants for tuber formation showed them to have complete fibrous roots but they extended deep in the ground, almost 5-6 m in length (See photos gallery fig. 74). The presumed progenitor itself, *M. pseudoglaziovii* (Fig. 22 in this photos gallery) grows in one of the driest pockets of Paraiba State, county Remigio from where the wild species had acquired this deep-rooted character as a mechanism for removal of subterranean water. This character may be useful in subsequent improved generations for production of drought-tolerant cassava cultivars.

Two years after planting, the hybrid reached 3-4 m height. Its leaves are peltatum and frequently pendurate. These characters are typical of *M. pseudoglaziovii* and one of the striking features that distinguish these species from the other 98 species recognized by Rogers and Appan (1973). The hybrid carried few fruit: 4-5 fruits compared to 60-70 fruits in the pure *M. pseudoglaziovii* of the same age. The fruit is winged , a marker gene characterizing cassava fruit (Nassar, 1989).

Cytological analysis of PMCs revealed a regular occurrence of 18 bivalents at metaphase I. No multivalents were observed in 50 metaphase I cells studied. However, disturbed anaphase I was noted in six of the 30 anaphase I cells studied. Dyads and triads were noted totaling 14, compared to 86 normal tetrads. Pollen viability as measured by stainability with acetocarmine was as low as 41%. Out of these viable pollen, 21% were small.
The high percentage of sterility in spite of regular pairing, may attributed to the maintenance of the hybrid through vegetative reproduction by farmers in Paraiba State. This mode of reproduction may have led to accumulation of sterility mutations which have not been eliminated by natural selection through the sexual cycle (Nassar, 1978c).

TABLE 1

Averaged productivity per plant of the triploid (selection 121) and other cassava clones under Savanna and semi-arid conditions

Clone	No. plants	Productivity (kg/plant)	Region, location of trial and harvest age
This triploid	30	2.13	Central Brazil, conducted at the Experimental station, Univ. of Brasília. Harvest age 12 months
Sonora	30	1.67	
This triploid	29	5,9	Under semi arid conditions. Trial conducted by the CPATS, Petrolina, north-eastern Brazil. Harvest age 18 months
Nagib 01/84	27	1,8	
Nagib 02/84	29	3,7	
Nagib 03/84	28	1,1	
Nagib 04/84	22	4,1	
Cigana	28	2,9	

TABLE 2

Chromosome associations at Metaphase I in the triploid and its parent

Type	No. PCMs examinated	Average chromosome association		
		I	II	III
Natural hybrid	50	-	18	-
Triploid	30	10.2	9.2	3.6

Out of the 20 plants cloned from the hybrid, 82 fruits were collected. They gave 48 seeds with an average of 0.59 seed per fruit. The average number of fruits are per plant is 4.1 compared to 60-70 fruits in the wild species plant. This low fertility is expected in view of the high sterility of pollen grains. By planting these seeds, 25 seedlings were obtained but all of them were chlorotic or deformed and died with the exception of four plants that survived. These were identified in our living collection by numbers 120, 121, 122 and 123. Out of these four plants, three gave fibrous roots, but the fourth (no. 121) was very vigorous, resistant to stem borers and produced large tuberated roots by the end of the year. When multiplied vegetatively and compared with other cassava clones it showed superiority in tuber formation by the age of 12 months. This superiority reached approximately 28% more tubers than the .Sonora. clone, one of the best commercial cultivars. Its production was 2.13 kg/plant compared 1.67 for .Sonora. (Table 1). An evaluation of this selection under arid conditions was carried out during the years 1985-1989 by the national Center for Tropical Semi-arid Region and confirmed its superiority (Table 1). The meiotic study of this plant showed formation of trivalents, bivalents and univalents in metaphase I with frequencies of 10.2 III, 9.2 II and 3.6 I in a sum of 30 metaphases (Table 2). The mitotic division counting proved a triploid chromosome number of 2n=54. Apparently this triploid resulted from the fertilization of 2n gamete with n gamete in the progenitor hybrid. Viability of pollen of this triploid is as low as 19%. Seeds rarely formed. However, five seeds were collected and have been reproduced in our program.

The 2n pollen formation which has led to production of this triploid is reported here in cassava for the first time (See fig. 54 in geneconserve photos gallery). The cytological mechanism of its formation is probably inhibition of spindle at metaphase I and metaphase II judging from the triad formation (Fig. 5a). In the literature, several mechanisms were reported. Clement and Stanford (1961) and Tyagi (1988) attributed it to abnormal cytokinesis, while Mendiburu and Peloquin (1977) proposed parallel spindle as an additional mechanism. The 2n pollen mutations were described in several plant species: *Zea mays* (Roades and Dempsey, 1966), *Solanum* (Hogland, 1970), *Medicago sativa* (Vorsa and Bingham, 1979) and *Lolium* (Sala et al.,1989). The vigour of our selected triploid as seen in its productivity both under Central Brazil conditions and in the semi-arid tropic adds an evidence of usefulness of this 2n gamete phenomenon as a powerful mechanism for transferring variability and fitness to polyploid offspring. This vigorous sexually reproduced triploid is important in establishing a founder population of new chromosome race. Its progeny may rehybridize with new polyploids and diverse genotypes, producing additive heterotic potentialities. Since trivalent occurrence in this triploid is predominant, demonstrating gene exchange between wild and cultivated genomes, it is likely that this will generate more variability in the progeny.

The wild parent and its interspecific hybrid progeny are highly tolerant to drought as seen from their deep roots (Fig. 74 in this photos gallery). They normally survive the frequent years of drought in their natural habitat. This triploid is a potential progenitor of cultivars adapted to these conditions.

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