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Polyploidy, chimera and fertility of interspecific cassava (*Manihot esculenta* Crantz) hybrids

Nagib M. A. Nassar

Departamento de Genetica, Universidade de Brasilia, Brasilia, Brazil

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Abstract

Four interspecific hybrids between cassava and wild Manihot species were polyploidized by colchicine application to buds of cuttings. Totally tetraploid types as well as sectorial and periclinal chimeras were produced. Somatic selection applied to lateral buds of sectorial chimeras induced totally tetraploids. Fertility was restored in the sterile interspecific hybrids by chromosome doubling up to 93% viable pollen production in the tetraploids compared to 13% in diploids, which could lead to the evolution of new Manihot species. Periclinal chimeras showed high vigour compared to both tetraploid and diploid plants.

Key words: Cassava, polyploidy, chimera, pollen viability, hybrid fertility

Introduction

Cassava, (*Manihot esculenta* Crantz) is an important staple food crop for more than 800 million people. Wild species of Manihot are potential resources for many useful genes [1]. For the use of these species in the plant breeding program, their hybridization with the cultivate was carried out, and interspecific hybrids were obtained [2]. This was followed by vegetative propagation and maintainance in the living collection at the University of Brasilia. The hybrids were confirmed by morphological marker traits. However, high sterility of the hybrids impeded further backcrosses. This sterility is due to lack of pairing between chromosomes. Of the parents overcome this problem, these hybrids were polyploidized with colchicines applications.

Material and methods

Four interspecific hybrids between cassava and wild Manihot species, obtained earlier by this author [2], were used for polyploidization. These hybrids are: *M. neusana* × *M. esculenta; M. glaziovii* × *M. esculenta; M. aesculifolia* × *M. esculenta* and *M. pohlii* × *M. esculenta*. Twenty vegetative buds of stem cuttings of each hybrid were soaked in 0.2% colchicine aqueous solution for 24 hours. Sprouting shoots were examined for leaf shape. Pollen viability of the pollen was estimated and pollen mother cells [PMCs] were studied to determine chromosome number. To study PMCs at meiotic division, the buds were fixed in absolute alcohol-glacial acetic acid, smeared and stained with acetocarmin. Pollen viability was estimated by acetocarmine-iodine mixure. Five hundreds pollen grains were examined in each cross. To distinguish different types of chimeras and tetraploid tissue, stomata and guard cells on leaf surface and leaf shape were examined on both sides of the emerging stem after colchicine treatment.

Results and discussion

The colchicine treatment resulted in production of both complete and chimeral tetraploid stems with tissues having different ploidy levels growing side by side on the same stem. This is due to the stratified arrangement of cells in the meristem treated by the colchicines, and derivation of mature tissue from these layers [3]. The derivative cells of the outermost layer of the tunica form epidermis. The second layer LII forms the subepidermal tisuues. LIII form the pith and vascular tissue. The chimeras were distinguished to sectorial and periclinal. The frequency of polylpoids obtained from 20 buds treated is given below:

Table 1. Frequency of polyploids obtained in four interspecific hybrids between Cassava and wild Manihot

Hybrid	Total tetra-ploids	Chimera	Total poly-ploids	Freqe-ncy of poly-ploids%
M. esculenta × *M. neusana*	1	3	4	20
M. esculenta × *M. glaziovii*	5	-	5	25
M. esculenta × *M. pohlii*	2	2	4	20
M. esculenta × *M. aesculifolia*	-	3	-	15

Identification of chimera: It was possible to identify the chimera tissues on the basis of pollen grain viability, leaf shape and stem anatomy. The polyploid section of the stem in sectorial chimeras had the broad and short leaves while the diploid side developed narrow and longer diploid form. In case of periclinal chimera, pollen viability, leaf shape and stomata size were used as a selection criterion. Pollen formed from LII layer

while leaves are differentiated form the LI layer. In periclinal chimeras, leaf is different and stomata enlareged and pollen viability is much higher than diploid plants. All the chimeras in the interspecific hybrid *M. esculenta* × *M. neusana* and *M. esculenta* × *M. glaziovii* were sectorial while two sectorial, one periclinal chimeras were obtained in the cross cassava × *M. aesculifolia*. In sectorial chimeras, pollen grain viability on one side was low as in a diploid while on the other side, it was as high as observed in the tetraploids. The size of pollen grains was notably larger in the latter part. The pollen grain size in the periclinal chimeras reflects the ploidy level of this layer Table 2.

Table 2. Pollen viability in diploid and tetraploid sectors

Interspecific hybrid	Pollen viability %	
	Diploid tissue	Tetraploid tissue
Cassava × *M. neusana*	18	92
Cassava × *M. glaziovii*	11	90
Cassava × *M. pohlii*	13	93
Cassava × *M. aesculifolia*	15	91

Instability of chimeras: A little informations is available about production of chimera in root crops and much less in cassava, but the use of polyploidy in cassava breeding is frequently reported by the Indian breeders.. Probably, the most interesting reports came from the Indian group at Thiruvanthpuram [4]. These articles report production of total tetraploids in cassava but no mention is made about chimera induction. Since appearance of chimera is a frequent phenomenon after colchicines treatment, it is possible that the resulting chimeras were overlooked or simply ignored in the above studies. Due to stratification of the shoot apex, cytochimeras with different ploidy levels appear in each layer of tissue and their derivativesr when the buds are trated by colchicine. A competition between tetraploid and diploid in chimeras was reported earlier [5]. This competition can lead to the loss of desired traits. Only the chimeras in LII (periclinal) layer have a chance of transmitting desirable characteristic to their progeny. In the chimeral sectors observed by us, the stem exhibited diploid phenotype after about six months growth restoring the normal leaf shape which became narrow, and the pollen viability when measured show characteristic of the diploid. It seems that the growth rate of tetraploid tissue is slower than diploid ones and it is often overgrown by diploid tissue. However it is possible to proliferate tetraploid tissue through somatic selection. This was done by cutting the apical buds of the chimeral stem, followed by removal of the lateral shoots growing from the diploid sector and allowing only the tetraploid side branches to grow.

One of the striking features of polyploidization is the restoration of the fertility of the interspecific hybrids. Yet a very small portion of inviable pollen formed in the polyploids due to formation with 3% multivalents of in the polyploidized tissue. Quadrivalent formation occurs even in cassava itself. Fertility restoration in the interspecific hybrids through polyploidization improvess chances of using the wild species for crop improvement. This means the creation of new tetraploid species with high fertility and capable of self reproduction maintaining their unique characteristics with a new closed gene pool in every interspecific hybrid [6,7]. This technique allows to incorporate desirable genes in further crosses. The strategy involves back-crossing the polyploidized interspecific hybrids with cassava followed by selection for the desirable traits in the progeny. Preferencial autosyndetic pairing between chromosomes of cassava may result into elimination of the majority of chromosomes of the wild species during meiotic segregation. Even selfing of a fertile hybrid may produce useful recombinations between the wild Manihot species and cassava. One interesting approach in utilizing the induced polyploid types could be to cross them with the facultative apomictic clones obtained earlier in our studies. This may lead to the production of apomictic triploid clones that combine both heterosis and polyplody.

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