Fertility and Chimera Induction in cassava, *Manihot esculenta* Crantz interspecific hybrids

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ABSTRACT

Four interspecific hybrids between cassava and wild *Manihot* species were polyploidized by the application of colchicine to cutting buds. Totally tetraploid types of 2n=72 as well as sectorial and periclinal chimeras were produced. Morphological, cytogenetial and anatomical exames were efficient in identifying different types of chimers. Somatic selection applied to lateral buds of sectorial chimeras enabled obtaining totally tetraploids.

The fertility of sterile interspecific hybrids was restored by chromosome duplication and reached 93% of viable pollen in tetraploid types compared to 13% in the diploid forms. New Manihot species have evolved due to this induced fertility.

Periclinal chimeras showed high vigorisity compared to both tetraploid and diploid types. The fertile interspecific hybrids will be crossed with apomictic clones to produce triploid apomictic ones.

Key words - Polyploidy, periclinal and sectorial chimera, pollen viability, hybrid fertility

Cassava is an important staple crop and a food for more than 800 million people. Its wild species have proved potential resources for many useful genes (Nassar,1978,1986 ,1999). For the use of these species in the plant breeding program, they were hybridized with the cultivate and interspecific hybrids were obtained (Nassar,1980,1989). Hybrids were propagated vegetatively maintained in the living collection at the Universidade de Brasilia. The hybridity nature was confirmed by the use of morphological gene markers but the hybrids showed high sterility which impeded further backcrosses in the plant breeding program. This sterility is due to lack of pairing of the parental chromosomes. To overcome this problem, it was planned polyploidizing these hybrids artificially by the use of colchicine.

MATERIAL AND METHODS

Four interspecific hybrids between cassava and relative wild *Manihot* species , obtained earlier by this author, were used initially for polyploidization. These hybrids are: *M.neusana* X cassava; *M.glaziovii* X cassava; *M.aesculifolia* X cassava and *M.pohlii* X cassava. Twenty lateral buds of cuttings of each hybrid were moisted by 0.2%colchicine aqueous solution for a period of 24 hours. Sproutng stems were examined for leaf shape. When flowered, viability of pollen was estimated and PMC'ss were studied too for chromosome number. To study PMCs meiotic division, buds were fixed in absolute alcohol-glacial acetic acid , smeared and stained by acetocarmin. Pollen viability was estimated by acetocarmine-iodine mixture. Five hundreds each exame were counted. Stomata, guard cells and leaf form were examined in both sides of the emerging stem after colchicine treatment.

RESULTS AND DISCUSSION

The colchicine treatment resulted in production of both tetraploid stems and chimeral ones. Chimera means the growth of different genotypes side by side in the same stem. It is due to the stratified arrangement of the cells in the meristem treated by colchicine, and the derivation of nature tissue from these layers (Boertjs and van Harten, 1978). The derivative cells of the outermost layer of the tunica forms the epidermis of the plant. The second tunica layer LII forms the subepidermal tissues such as gametes and other part of the cortex. Derivatives of the corpus (LIII and inner tissue) form the central tissues such as the pith and vascular tissue. The chimeras were distinguished to sectorial and periclinal. Out of the 20 buds treated each plant it was obtained the following types:

Hybrid	total tetraploid	Chimera
Cassava X *M. neusana*	1	3
Cassava X *M. glaziovii*	5	-
Cassava X *M. pohlii*	2	2
Cassava X *M aesculifolia*	-	3

Identification of chimera

It was possible identifying chimera by the use of both of pollen grain viability, leaf shape and stem anatomy. In case of sectorial chimera, a lateral section of stem showed the broad and short polyploidy shape of the leaf while the other side showed the narrow and longer diploid form. In case of periclinal chimera, the pollen viability estimation, leaf shape and stomata size were used as a criterion, pollen viability are formed by LII layer while leaf form and stomata are formed by LI. In periclinal chimera, leaf form and stomata size showed typical size of tetraploid type while and pollen viability expressed the low viability of diploid ones (see in the photo gallery Fig. shows the leaf shape of tetraploid leave against diploid one).

Moreover, comparing stem section of diploiod and chimera one shows different thickness of different tissues of the same age (see photo gallery, photo no.).

In the case of the interspecific hybrid cassava X *M.neusana* and cassava X *M.pseudoglaziovii* all the chimeras were sectorial while in the case of cassava X M.*aesculifolia*, it was obtained 2 sectorial chimeras and a periclinal one.

In sectorial chimeras, Chromosome counting in PMC's of 2n=36 and pollen grains viability in one side showing the low characteristic level of a diploid while on the other side, chromosome counting in PMC's of 2n=72 and high pollen viability were characteristic of a tetraploid, in addition their size was notably larger. Table 2 shows viability of pollen grains in the both diploid and tetraploid types of the intespecific hybrids. In Periclinal chimera where LII is different in constitution from LI, the pollen grains being produced by LII have expressed the ploidy level of this layer.

Instability of chimeras

A little informations are available about production of chimera in root crops, far less on cassava. However, there is a report on producing totally tetraploidy in cassava by the treatment of colchicine.. Sine production of chimera is eminent in case of the application of colchicine to somatic tissues, it is not clear from the referred report if the the resulting chimeras were overlooked or simply ignored.

In all the identified sectorial chimeras, the stem exhibited diploid condition after about six months growth restoring the normal characteristics of leaf shape. In place of the broader leaf it becomes narrow, and the pollen viability when measured showed tetraploid sterility. It seems that the rythm of tetraploid tissue growth is slower than diploid ones and it is overgrown by diploid ones. However it was possible to apply somatic selection to promote tetraploid type growth. This was done by cutting the apical buds of the sectorial stem, selecting the growing lateral buds in the tetraploid side and removing buds on the diploid one.

Table 2. Pollen viability in diploid and tetraploid types

Interspecific hybrid	viability %	
	diploid type	tetraploid type
cassava X *M. neusana*	18	92
cassava X *M. glaziovii*	11	90
cassava X *M. pohlii*	13	93
cassava X *M. aesculifolia*	15	91

One of the most striking features in this interspecific hybrids polyploidization is the restoration of their fertility by polyploidization. Yet a very little portion of inviable pollen was recorded due to formation of a very low frequency of multivalents of about 3 % in the polyploidized complement. This quadrivalent does exist in the cassava itself. Restoring fertility of the interspecific hybrid assures efficiency of artificial polyploidization in manipulation of wild species for the cassava improvement. Botanically it means the evolving of new species since these tax , because of its high fertility, will be autoreproduced maintaining its new characteristics and creating a new closed gene pool of every interspecific hybrid (Nassar,2002).

By this technique, breeder can incorporate desirable genes in further crosses. The strategy of developing such polyploidiuzed interspecific hybrids is to backcross them with cassava followed by selection for desirable traits in the progeny. This technique may succeed due to the preferencial autosyndetic pairing between chromosomes of cassava resulting in the elimination of majority of wild species chromosomes during meiotic segregation and maintaing few chromosomes of the wild parent which may carry useful genes. By selecting these genotypes, useful genotypes can be obtained. Since vegetative propagation is the predominant mode in reproducing this crop, selected genotypes can be perpetuated. By selfing the fertile hybrid, a progress can be achieved too, since we may obtain useful combinations between the wild and the cultivate, and propagate it vegetatively.

One of the interesting approaches in utilizing the induced polyploid types is to cross them facultative apomictic clones obtained earlier by this author (Nassar, 1995). This may lead to production of apomictic triploid clones that combine both of heteroses and polyploidy vigor.

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