

The synthesis of a new cassava-derived species *Manihot vieiri* Nassar*

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* In honour of deceased Professor Clibas Vieira, who dedicated a part of his life to cassava breeding

Abstract

A new species was synthesized artificially by chromosome doubling of an interspecific hybrid. The ensuing polyploid type exhibits an apomictic nature and maintains its morphological characteristics in the progeny. It showed a frequency of 29% of multiembryonic sacs of its examined ovules whereas the multiembryonic sacs were absent in the diploid type

Key words Apomixis, interspecific crosses, polyploidy

Introduction

Cassava is the most important staple crop in the tropics, where more than 800 millions of poor people eat this crop (FAO, 2002). Wild *Manihot* species are still untapped resources for the genetic enhancement of cassava. They possess useful genes that if incorporated to the cultigen would enrich its gene pool by useful characters of consumption or adaptation to severe conditions of soil and climate. To broaden its genetic base by genes of the wild species, systematic interspecific hybridization was undertaken (Nassar 1980, 1989, 1995). Interspecific hybridization however, brings a practical result only for a few species, which are genetically near to the cultigen. In other species, which are distantly taxonomically, crossing faces many problems. One of them is the strong barriers between hybridized species and cassava (Nassar et al. 1996). In certain species, even the hybridization carried successfully, it results in a high sterility of the hybrid that impedes its utilization for further backcrosses. To overcome this problem chromosome number of the interspecific hybrids was systematically duplicated. This research reports the chromosome duplication of the interspecific hybrid between cassava and *M. anomala*.

Material and Methods

An interspecific hybrid between cassava and *Manihot anomala* (see photos gallery, fig. no. 85) was obtained in 1989 (Nassar, 1989). This hybrid was propagated vegetatively and grown at the Biological Experimental Station of the Universidade de Brasilia and further screening revealed its host plant resistance to borers and to bacteria blight as well as its exuberant vegetative growth

This hybrid was polyploidized artificially by the use of colchicines (see photos gallery, fig. 86). To apply colchicine, a solution of 0.2% concentration was applied to lateral buds 3 times through a period of 36 hours using moist cotton fixed around the bud. The emerging shoots were screened for formation of chimera or total tetraploid, having the chimeras eliminated. To identify sectorial chimera, leaf shape and form on both sides of the shoot were compared as well as stomata. For identifying periclinal chimera, buds were observed for meiotic counting in addition to observing leaf shape and form of the developed shoot. Embryos sacs of 100 ovules were taken from flowers of treated shoots and observed using the clearing method (Nassar et al. 1998).

Results and discussion

The colchicine treatment to stem lateral buds resulted in shoots that differed morphologically of the original ones. It have broader leaves and slow rhythm of growth. Not all of them were tetraploids. Some were sectorial chimeras whereas others were periclinal. A sectorial chimera was identified by having broad leaves in a lateral part of a shoot while the other part has the original form. The periclinal chimeras had their stomata of larger size, but pollen mother cells (PMC) have the diploid number of chromosomes. Somatic selection has been applied by eliminating chimeral shoots, maintaining only the polyploidized types.

The meiotic study confirmed the tetraploidy of the selected shoot. Cells of 34 bivalents and one quadrivalents were observed (see photos gallery, photo no. 84). Pollen viability was 81%, viz. a viz. to 94% in the diploid type. The diploid hybrid of *M. anomala* with cassava is one of the few that shows complete chromosome pairing. Hence, its tetraploid type tends to have less fertility due to quadrivalent chromosome configuration. In other cases of polyploidizing interspecific hybrids of *Manihot* species, the pairing was extremely different. For example the interspecific hybrid of cassava with *M. glaziovii*, the viability of the polyploidized type was 92% compared to 13% in the diploid type. Apparently there is a greater phylogenetic affinity between *M. esculenta* and *M. anomala* than that between *M. glaziovii* and *M. esculenta*.

Eight fruits were collected from the polyploidized plant. They had 16 seeds; i.e., 2 seed per fruit. When planted, only 3 seedlings were raised and grown to the stage of mature plants. The cause for

non-germinating seed was the severe dormancy of the wild *Manihot* species. The three plants and their mother plant were identical in leaf shape, form and texture, color of the stem and leaves, apical vegetative growth, inflorescence color and shape, fruit color and shape, bract and bracteoles size (see photos gallery fig. no 87).

There was a high frequency (29%) of multiembryonic sacs in the ovules (see photos gallery Fig. no. 88), viz. a viz. zero frequency of multiembryonic sacs in the same number of diploid type ovules. Apparently the apomictic nature was further induced by polyploidy. This polyploid level seems to have increased the expression profiles in certain genes specially those involved in reproduction, thus producing apomixis. In support of this theory, certain plants such as *Arabidopsis* and wheat have the expression of a number of genes determined or activated upon polyploidization (Grimanelli et al. 2001; He et al. 2003). Quarin (2001) stated also that doubling of a sexual *Paspalum* may result in apomixis. Carman (1997) states that apomixis may ensue from hybridization of two related species with different reproductive behavior and timing. Asynchronous mis-expression of parent genes in the hybrid could then lead to all components of apomixis. The combination of hybridity and polyploidy nature in this hybrid between cassava and *M. anomala* supports the idea that both of the two phenomena must have played an important role in the evolution of apomixis in *Manihot* species. Hybridity and polyploidy confer variability to a certain population, then apomixis fixes and perpetuates genotypes that are well adapted to a certain environment.

Description of the species *M.vieiri* Nassar:

Sub-shrub up to two meters high, branched, roots are enlarged, young stems glabrous, mature stems woody with large pith, colour greenish brown. Leaves alternate, stipules caduceous. Petioles ca 15 cm long, glabrous, yellowish green. Petiole attachment to lamina basal, nonepeltate, lamina 3 to 5 lobed. Leaves associated with the inflorescence are nonlobed. Median obovate or obovate pandurate usually 12 to 17 cm long. Inflorescence monoecious, terminal, up to 12 cm long (see photos gallery, Fig. 87). Bracteoles and bractlets are setaceous color green. Pistillate flowers are restricted to the base of the inflorescence. Staminate buds are ovoid. Capsules about 2 cm subglobose to slightly rounded having prominent wings (This gene of prominent wings served as a morphological marker that came from cassava (see photos gallery fig. 32).

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Acknowledgement

This work is being carried out with the help of the Brazilian National Council for Scientific Development –CNPq. The above mentioned living collection has been established at the Universidade de Brasilia with the help of the International Development Research Center- IDRC. Thanks are due to Rodomiro Ortiz for edition help and the under graduate students Nayra Bonfim, Pollyana Gomes, Adalgisa Shaib and Danielle Hashimoto for laboratory assistance.