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

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*In honor of deceased Professor Vieira who dedicated a part of his life to cassava breeding

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ABSTRACT. A new species was synthesized artificially by chromosome doubling in a hybrid. The ensuing polyploid type exhibits an apomictic nature and maintains its morphological characteristics in the progeny. It showed a frequency of multiembryonic sacs of 29% in the ovules examined, whereas 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 million 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 into the cultigen would enrich its gene pool with useful characters related to its consumption or adaptation to severe conditions of soil and climate. Systematic interspecific hybridization was undertaken to broaden its genetic base with genes of the wild species (Nassar, 1980, 1989, 1995). Interspecific hybridization, however, produces a practical result only for a few species, which are genetically near to the cultigen. In other species that are taxonomically distant, crossing faces many problems. One of them is the strong barriers between hybridized species and cassava (Nassar et al., 1996). In certain species, even when hybridization is carried out successfully, it results in high sterility of the hybrid which impedes its utilization for further backcrosses. To overcome this problem, the chromosome number of the interspecific hybrids was systematically duplicated. The present study reports the chromosome duplication of the interspecific hybrid between cassava and *Manihot anomala*.

MATERIAL AND METHODS

An interspecific hybrid between cassava and *Manihot anomala* (Figure 1) was obtained in 1989 (Nassar, 1989). This hybrid was propagated vegetatively and grown at the Biological Experimental Station of the Universidade de Brasília, and further screening revealed its host plant resistance to borers and to bacterial blight as well as its exuberant vegetative growth.

Figure 1. Leaves of the hybrid cassava x *Manihot anomala*.

This hybrid was polyploidized artificially by the use of colchicine (Figure 2) applied as a solution of 0.2% to lateral buds 3 times over a period of 36 h using moist cotton fixed around the bud. The emerging shoots were screened for the formation of chimeras or total tetraploids with the chimeras eliminated. To identify sectorial chimeras, 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.

Embryo sacs of 100 ovules were taken from flowers of treated shoots and observed using the clearing method (Nassar et al., 1998).

Figure 2. Leaves of the hybrid polyploid type.

RESULTS AND DISCUSSION

Colchicine treatment of stem lateral buds resulted in shoots that differed morphologically from the original ones. They had broader leaves and slow growth rate. Not all of them were tetraploids. Some were sectorial chimeras whereas others were periclinal. A sectorial chimera was identified by having broad leaves in the lateral part of a shoot while the other part had the original form. The periclinal chimeras had larger stomata, but pollen mother cells with a diploid number of chromosomes. Somatic selection was applied by eliminating chimeral shoots, maintaining only the polyploidized types.

A meiotic study confirmed the tetraploidy of the selected shoot (Figure 2). Cells of 34 bivalents and one quadrivalent were examined (Figure 3). Pollen viability was 81% compared 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 a quadrivalent chromosome configuration. In other cases of polyploidizing interspecific hybrids of *Manihot* species, the pairing was extremely different. For example, in 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 phylogenic 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 seeds per fruit. When planted, only 3 seedlings were raised and grown to the stage of mature plants. The cause for non-germinating seeds 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 (Figure 4).

There was a high frequency (29%) of multiembryonic sacs in the ovules (Figure 4), compared to 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

Figure 3. Metaphase 1 of the hybrid polyploid type.

Figure 4. An inflorescence of the hybrid polyploid type.

to have increased the expression profiles of certain genes, especially 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 et al. (2001) stated also that doubling of a sexual *Papsalum* may result in apomixis. Carman (1997) states that apomixis may ensue from the 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 polyploid 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, and apomixis then improves and perpetuates genotypes that are well adapted to a certain environment.

DESCRIPTION OF THE SPECIES *MANIHOT VIEIRI* NASSAR

Sub-shrub of up to two meters high, branched, roots are enlarged, young stems glabrous, mature stems woody with large pith, color greenish brown. Leaves alternate, stipules caduceus. 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 nonelobed. Median obovate or obovate pandurate usually 12 to 17 cm long. Inflorescence monoecious, terminal, up to 12 cm long (Figure 5). 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 Figure 6).

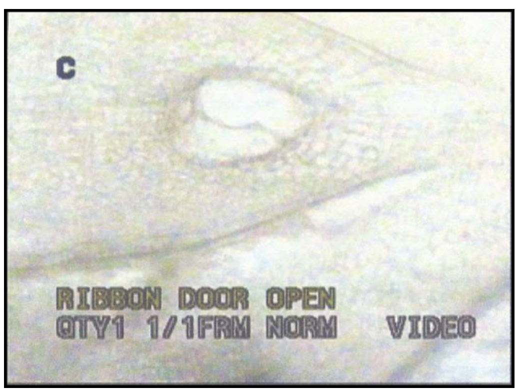

Figure 5. A multiemrionic sac of the hybrid polyploid type.

Figure 6. Ribbed fruit.

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