Hereditas 00: 1–3 (2006)

Chromosome doubling induces apomixis in a cassava × *Manihot anomala* hybrid

NAGIB M. A. NASSAR

Universidade de Brasilia, Brasilia, Brazil

Nassar, N. M. A. 2006. Chromosome doubling induces apomixis in a cassava × *Manihot* hybrid. — *Hereditas 00*: 1–3. Lund, Sweden. eISSN 1601-5223. Received April 22, 2006. Accepted October 5, 2006

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

Nagib. M. A. Nassar, Universidade de Brasilia, Brasilia, Brazil. E-mail: nagnassa@rudah.com.br

Cassava is one of the most important staple crops in the tropics. It is used by more than 800 millions of poor people (Fao 2002). Wild *Manihot* species are still untapped resources for the genetic enhancement of cassava. They possess useful genes that, if incorporated in the cultigen, would enrich the cassava gene pool with useful characters for consumption or adaptation to severe soil and climate conditions. To broaden the genetic base of cultivated cassava with genes from the wild species, systematic interspecific hybridization has been undertaken (Nassar 1980, 1989, 1995).

Interspecific hybridization however, brings practical results only for a few wild species that are genetically close to the cultigen. In other species, which are taxonomically more distant, hybridization faces many problems. One of them is the strong barriers for crossing some wild *Manihot* species and cassava (Nassar et al. 1996). For certain *Manihot* species, when hybridization is carried out successfully, the resulting hybrid with cassava is often sterile, which impedes its utilization for further crosses with cassava cultigens. To overcome this problem, the 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* 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 (Fig. 1).

The hybrid was polyploidized artificially by using colchicine (Fig. 2). The colchicine treatment was carried out by applying a 0.2% colchicine solution to lateral buds three times through a period of 36 h using moist cotton fixed around the bud. The emerging shoots were screened for formation of chimera or total tetraploidy, eliminating all chimeras. 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. Embryo 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 from the original ones. They had broader leaves and slow growth. Not all of the buds 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 larger stomata, 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 (Fig. 4). Cells of 34 bivalents and one quadrivalents were observed. Pollen viability was 81%, viz. a viz. to 94% in the diploid type. The diploid hybrid between *M. anomala* and 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

Fig. 1. Leaves of the hybrid cassava × *M. anomala.*

cases of polyploidizing interspecific hybrids of *Manihot* species, the pairing varied greatly. For example, in the interspecific hybrid of cassava and *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. two seeds per fruit. When planted, only three 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 leave 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.

There was a high frequency (29%) of multiembrionic sacs in the ovules (Fig. 3), viz. a viz. zero frequency of multiembryonic sacs in the same number of diploid

Fig. 2. Leaves of the hybrid polyploid type.

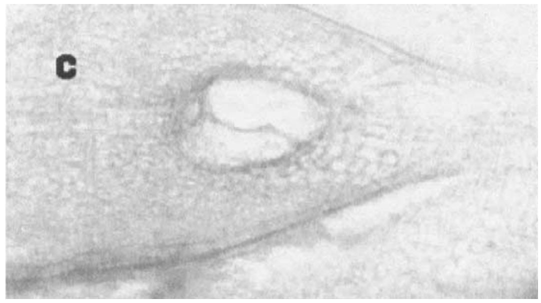

Fig. 3. A multiembrioc sac of the hybrid polyploid type.

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 *Papsalum* may result in apomixis. Carman (1997) states that apomixis may ensue from hybridization of two related species with different reproductive behavior and timing. Asynchronous

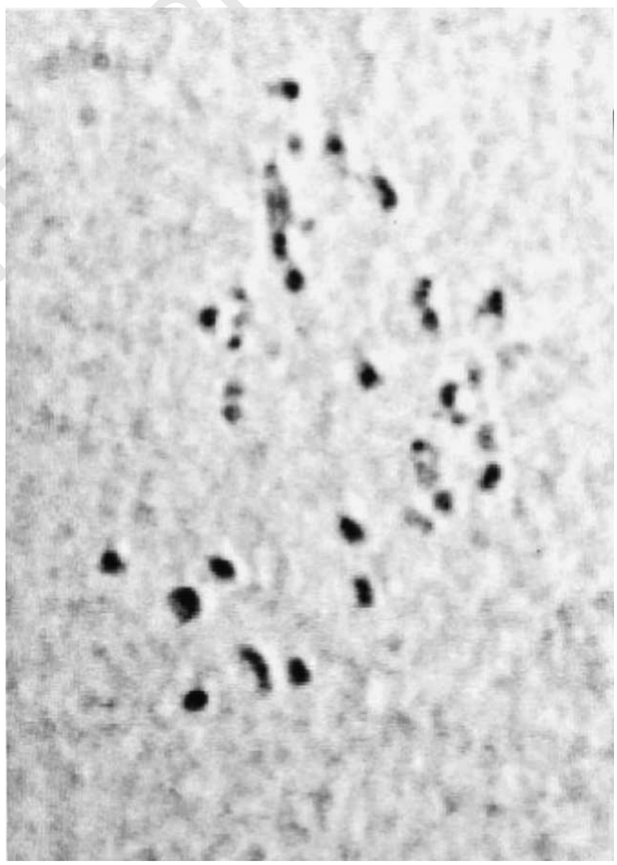

Fig. 4. Metaphase 1 of the hybrid polyploid type.

Fig. 5. An inflorescence of the hybrid polyploid type.

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.

The name of *Manihot vieira* (Nassar) is given to this new species in honour of deceased professor Clibas Vieira who dedicated a part of his life to study cassava. Description of the species is as follows:

Manihot vieira (Nassar)
Sub-shrub up to 2 meters hight, branched, roots enlarged, young stems glabrous, mature stems woody with large pith, colour 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 non-lobed, median obovate or obovate pandurate, usually 12 to 17 cm long. Inflorescence monoecious, terminal, up to 12 cm long. Bracteoles and bracclets are setaceous color green. Pistillate flowers are restricted to the base of the inflorescence. Staminate buds are ovoid (Fig. 5). Capsules about 2 cm subglobose to slightly rounded having prominent wings (this gene of prominent wings served as a morphological marker and inherited from cassava, Fig. 6).

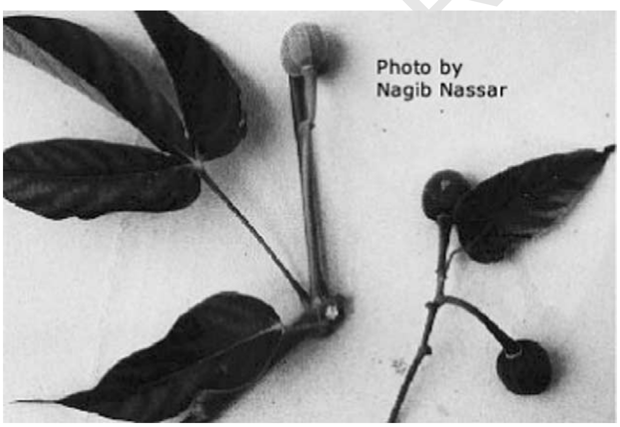

Fig. 6. Ribbed fruit.

UNLINKED REFERENCES

QUARIN et al., 2001, CARMAN, 1977

Acknowledgements – This work was done 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 to Rodomiro Ortiz for edition help and the under graduate students Nayra Nascimento, Pollyana Gomes and Danielle Hashimoto for laboratory assistance.

REFERENCES

Carman, J. G. 1977. A synchronous expression of duplicate genes in angiosperms may cause apomixes, bispory, tetraspory and polyembriony. – Biol. J. Linn. Soc. 61: 51–94.

Carman (1997). **Please provide further details.**

FAO 2002. Annual Production Yearbook. – Food and Agriculture Organization of the United Nations, Rome, Italy.

Grimanelli, D., Leblanc, O., Perotti, E. et al. 2001. Developmental genetics of gametophytic apomixes. – Trends Genet. 17: 597–604.

He, P., Friebe, B. R., Gill, B. G. et al. 2003. Allopolyploidy alter gene expression in the highly stable hexaploid wheat. – Plant Mol. Biol. 2003: 401–414.

Nassar, N. M. A. 1980. Attempts to hybridize wild Manihot species with cassava. – Econ. Bot. 34: 13–15.

Nassar, N. M. A. 1989. Broadening the genetic base of cassava, Manihot esculenta Crantz by interspecific hybridization. – Can. J. Plant Sci. 69: 1071–1073.

Nassar, N. M. A. 1995. Development of cassava interspecific hybrids for savanna (cerrado) conditions. – J. Root Crops 22: 9–17.

Nassar, N. M. A., Carvalho, C. G. and Vieira, C. 1996. Overcoming barriers between cassava Manihot esculenta crantz and wild relative M. pohlii warwa. – Brazilian J. Genetics 19: 617–620.

Nassar, N. M. A., Vieira, C. and Grattapaglia, D. 1998. Molecular and embrionic evidence of apomixis in cassava interspecific hybrids of Manihot spp. – Can. J. Plant. Sci. 78: 348–352.

Quarin (2001). **Please provide further details.**

Quarin, C. L., Espinosa, F., Martinez, E. J. et al. 2001. A rise of ploidy levels effect on meiosis and mode of reproduction in Papsalum notatum. – Sex Plant Reprod. 13: 243–529.