

Review

Apomixis in cassava: advances and challenges

D.Y.H. Freitas¹ and N.M.A. Nassar²

¹Programa de Pós-Graduação em Botânica, Universidade de Brasília, Brasília, DF, Brasil

²Departamento de Genética e Morfologia, Universidade de Brasília, Brasília, DF, Brasil

Corresponding author: N.M.A. Nassar
E-mail: nagnassa@rudah.com.br

Genet. Mol. Res. 12 (2): 988-994 (2013)
Received December 13, 2012
Accepted February 8, 2013
Published April 2, 2013
DOI <http://dx.doi.org/10.4238/2013.April.2.14>

ABSTRACT. Cassava is the most important staple crop in the Tropics and Subtropics. Apomixis may revolutionize its production due to various attributes. These potential advantages include production by true seed, maintaining cultivar superiority over generations without segregation, and avoiding contamination by bacteria and viruses. Historically, apomixis was initially observed by International Institute of Tropical Agriculture researchers, in the 1980s, in homogenous progeny of hybrid crosses. Later, from 1980 through 2010, apomixis was extensively studied by Universidade de Brasília, in order to determine contributing mechanisms and occurrence. Apomixis genes occur naturally at low frequencies in cultivated cassava and can be transferred by crosses with wild species. Apparently, apomixis in cassava is controlled by more than one recessive gene, which act in an additive form. Aneuploidy is associated with apomixis in cassava and can provide the double dosages necessary for recessive gene action. By using molecular techniques, genetic homogeneous progeny has been demonstrated, while embryonic exams have shown nucellar multiembryos. Polyploidy was found to increase apomixis percentage. From an evolutionary viewpoint, polyploidy has contributed to production of new species, when combined with apomixis. Recently,

somatic embryos have been detected in the integument, revealing a rare model of apomixis that has only been documented in cassava.

Key words: Adventitious embryony; Aneuploidy; Apospory; Sterility; Hybridization; Polyploidy

SOME CONSIDERATIONS ON APOMIXIS IN CASSAVA

Apomixis means seed formation without fertilization. It has been investigated in various crops, other than cassava (*Manihot esculenta* Crantz), since the 1940s by many authors (Gustafsson, 1946; Brown and Emery, 1958; Nygren, 1967; Nogler, 1984; Hanna and Bashaw, 1987; Asker and Jerling, 1992; Savidan, 1992; Grimanelli et al., 2001; Koltunow and Grossniklaus, 2003; Ozias-Akins, 2006; Yudakova, 2009; Tucker et al., 2012; Hojsgaard et al., 2013). It has been studied in 400 species within 300 genera and 40 plant families of Angiosperms (Carman, 1997; Singh et al., 2011). Apomixis occurs only in a few species of agricultural importance, such as forages - *Panicum*, *Brachiaria*, *Cenchrus*, *Trip-sacum*, *Hieracium*, and *Paspalum* - citrus, apple, mango, and orchids (Singh et al., 2011).

The first report of apomixis in cassava came from the International Institute of Tropical Agriculture (IITA, 1984), where morphological similarities were observed in the progenies of crosses of the wild species with the cultivated plant instead of the expected highly segregated progeny. The first experimental documentation on apomixis in cassava came from observations of offspring morphological uniformity, and abundant fruit formation in individuals supposed to be sterile (Nassar, 1995). The characters observed by this author included root color and surface, stem color, scars, and storey length and flower and fruit color. The author analyzed 4 cassava clones (namely UnB 011, UnB 040, UnB 060, and Branca Santa Catarina) and a hybrid between cassava and *M. dichotoma* Ule (UnB 031) for apomixis, based on morphological offspring homogeneity. One of them, UnB 011, reached 77% offspring homogeneity. Pollen viability and chromosome number indicated an association between aneuploidy and higher apomictic rate. Wild *Manihot* species, considered the source of many useful characters (Nassar, 1978), seemed to have also conferred apomixis genes to cultivated cassava through natural hybridization. Therefore, apomixis is frequently reported to occur in indigenous cultivars, which cross frequently with wild species in natural habitats (Nassar, 1978, 1992).

APOMIXIS GENE FREQUENCY IN CASSAVA

In the early 1990s, 2 putative facultative apomictic clones, UnB 011 and UnB 031, were investigated using RAPD (Grattapaglia et al., 1995). A greater extent of DNA polymorphism was observed between maternal parent and offsprings of UnB 011. Several markers were found in the maternal parent and absent in the progeny and vice versa, clearly rejecting the hypothesis of an apomictic origin for all offsprings. This result differs from the conclusion of a previous morphological study (Nassar, 1995), which indicated that this clone has 77% apomixis rate. Clearly, the rate of apomixis based on morphological markers was overestimated. It seems that incomplete penetrance of the morphological gene markers or modifications due to environmental effects masked the correct pheno-

type, especially in UnB 011.

However, apomixis did occur in the clones, but at lower rates. The observation of multiple seedlings per seed (Nassar et al, 1998a; Nassar, 1995, 2002) supports the hypothesis that apomictic seedlings originate through nucellar polyembryony. Nassar (1995) reported that apomixis genes occur in common cassava gene pool naturally at a low frequency and played an important role in evolving cassava species.

Molecular markers have also been used to determine apomictic frequencies in cassava hybrids. Nassar and Collevatti (2005a, 2008) and Nassar et al. (1998b, 2006, 2009) analyzed a large number of samples of parents, F_1 and F_2 hybrids of *M. glaziovii* x cassava, namely 307. According to their results, heterozygosity was low for all loci; F_2 samples of mother plant were all sibs with loci different from each other and from the maternal plant. For 307/2 F_2 , 2 of 4 were similar to the parental. For 307/5 F_2 , 1 of 6 was identical to the parental.

TYPES OF APOMIXIS IN CASSAVA AND ITS TRANSFER FROM THE WILD

Following the same line of molecular detection of apomixis in cassava, Nassar et al. (1998b) analyzed the F_2 progeny of UnB 031 and UnB 200, a hybrid between cultivated cassava and *M. glaziovii* Müll. Arg. They combined RAPD markers with embryological studies and found a more precise estimation of 2% apomixis; they also detected apomixis in a second genotype within the F_1 progeny besides evidence of apomixis occurrence as apospory in cassava. The fact that apomixis was detected in an F_1 interspecific hybrid hinted at the possibility of directly transferring genes through apomixis from a wild relative to cultivated cassava, a hypothesis predicted earlier by these authors.

Based on RAPD analysis, progenies of clones UnB 031 and UnB 200 clearly indicated that the type of apomixis detected in this study was facultative and occurred at a very low frequency in cassava.

In an embryological analysis in the same paper, both clones showed aposporic sacs inside the sexual embryo sacs. Apparently, one of them was derived from somatic cells in the ovule, while the second embryo sac was derived from a normal megaspore mother cell. The normal sacs showed 1 egg, 2 polar nuclei, and 3 antipodals. Synergids were occasionally seen. The egg was often inconspicuous. The antipodals were distinguished by a swollen, tear-drop shape, dense cytoplasm, chalazal position, and absence of a wall separating them from the cavity of the sac. The aposporous sacs lacked antipodals and had only one nucleus per sac. Sometimes, there was a single polar nucleus and an egg. These results strongly suggested that the mechanism responsible for apomixis in cassava is apospory.

In a study of a polyploidized cassava, Nassar et al. (2011) reported adventitious embryos (Figure 1A), along with 2 embryo sacs within an ovule (Figure 1B). This was the first documentation of this phenomenon to occur in a cultivated plant particularly in an interspecific hybrid. Apparently, in addition to apospory, somatic embryony also occurs in cassava apomictic hybrids, showing some plasticity in development involving different types of apomixis. It was also found that multiembryo formation can occur independently from pollination and fertilization. This latter observation is one of the most striking features of apomixis in cassava.

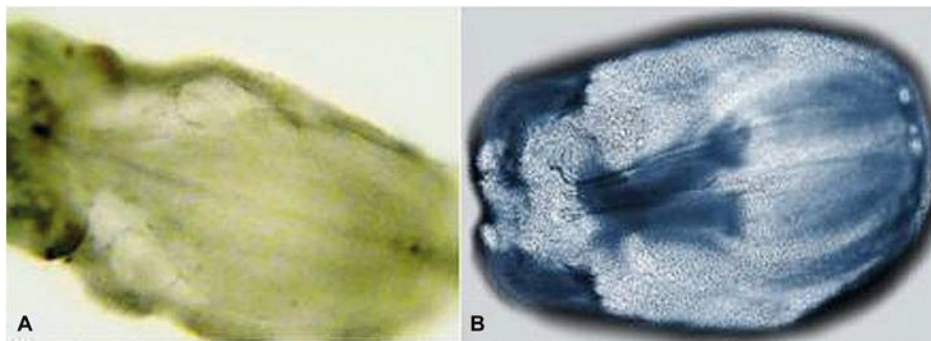


Figure 1. A. Adventitious embryo sacs between the inner and the outer integuments. B. Two embryo sacs in an ovule.

APOMIXIS TRANSFER FROM *MANIHOT NEUSANA* NASSAR

The finding of apomixis transfer from *M. glaziovii* in the 1990s was followed by a report of its transfer from *M. neusana* (Nassar et al., 2000; Nassar, 2003a). First and second generations and parental plants of cassava x *M. neusana* hybrids were analyzed embryologically and cytogenetically.

M. neusana was found to have a 1.5% apomixis rate; while this feature was found to be absent in cassava and the F₁ generation. In Nassar et al. (2000), normal embryo sacs were found to contain 8 nuclei (Figure 2A). Two embryo sac components were found for the first time, as they grew side by side in an F₂ individual (Figure 2B).

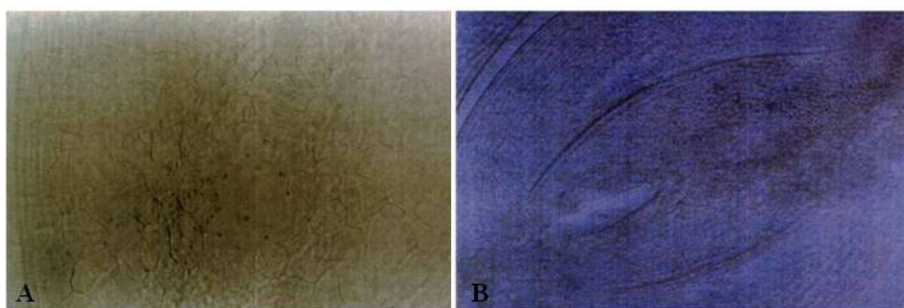


Figure 2. A. Sexual embryo sac with 8 nuclei. B. Aposporic and sexual embryo sacs.

While the hybrid F₁ did not reveal apomixis in the embryonic analysis, it was found in the F₂ plant. This could mean that apomixis is controlled by more than one recessive gene, which acts in an additive mode, probably carried on the same chromosome (Asker, 1979). In this case, additional chromosomes in the aneuploid F₂ plant (2n = 38) have allowed for homozygosity and dosages necessary for the action of these genes. The connection between apomixis and aneuploidy was confirmed in more recent research (Nassar, 2002, 2003b; Nassar et al., 2000, 2009).

An interesting phenomenon noted on apomixis transference from the wild to the crop is sterility, which is frequently associated with apomixis in cassava hybrid progenies (Nassar, 2001; Nassar and Collevatti, 2005b). This was observed in several plants and studied in detail in the cultivar UnB 200, which is a hybrid between cassava and *M. glaziovii*. Twenty-five plants were obtained from this hybrid by open pollination. These plants were studied cytogenetically and anatomically. Embryo sacs were also analyzed by clearing *in toto* (Nassar et al., 2000). Anatomical study of these progenies showed aposporic embryo sacs in all of them (Figure 3). In some cases, development of aposporic embryo sacs from cells within the sexual embryo sac was also noted. Both the aposporous and the sexual embryo grew in parallel and finally coexisted.



Figure 3. Aposporic embryo sacs. **A.** Three aposporic embryo sacs in nucellar tissue. **B.** Developed aposporic embryo sac within the sexual embryo sac. **C.** Two developed aposporic sacs.

Apparently, apomixis in cassava is strongly associated with meiotic irregularity and sterility. This sterility probably activates certain genes in somatic cells in the nucellus or in the sexual embryo sac to form aposporic embryo sacs. Apparently natural selection has favored this structure as an escape from extinction and a mode of perpetuating selected genotypes.

INCREASE IN APOMICTIC FREQUENCY BY BREEDING

As concluded previously, apomixis rates in cassava cultivars range from 1-2%. However, recurrent selection has proven to increase its rate to up to 11% judging from polyembryo frequencies (Nassar et al., 2000). This result supports the hypothesis that apomixis in cassava is controlled by multiple factors; accumulation of such factors in a certain genotype might result in a higher level of apomixis. Breeding aimed at accumulating apomixis additive genes in superior clones could be a relevant challenge for cassava breeders in the future.

POLYPLOIDY, HYBRIDIZATION AND APOMIXIS IN CASSAVA

Nassar (2002, 2006) and Nassar et al. (2008a, 2010, 2011) explained the role of polyploidization and interspecific hybridizations in the speciation of this genus. Sterile hybrids adapted to certain environment may be perpetuated by apomixis, giving rise to new species. Apomixis enables hybrids to maintain their heterogenic structure under unfavorable conditions, protecting them against future recombination.

The polyploidized hybrids of cassava with *M. anomala* Pohl, *M. glaziovii* and *M. oligantha* Pax provided data in support of this hypothesis. They were studied extensively by cytogenetical, embryonic, and morphological means.

These polyploidized plants in all of the hybrids showed 72 chromosome counts and multiembryos, which were observed microscopically (Figure 4), increasing to about 29% in the hybrids of *M. oligantha* compared to 1% in maternal plants.

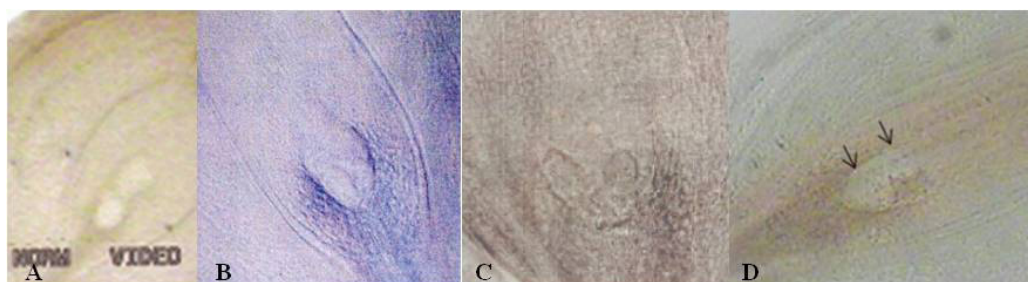


Figure 4. A.-C. Apomictic embryo sacs in *Manihot glaziovii* x cassava polyploid hybrids. D. Apomictic sac in an *M. oligantha* x cassava polyploidy hybrid.

In his survey on polyploidy and apomixis, Carman (1997) reported that they maintain a tight relationship, and emphasized their simultaneous occurrence in Angiosperms. More recently, Nassar et al. (2008b) detected apomictic nature through embryo sac examination of a natural hybrid between *M. tripartita* Müll. Arg. and *M. caerulescens* Pohl. It bred truly over several generations, demonstrating that this is a new evolved species. This observation of a combination of hybridity and polyploidy supports the idea that both phenomena must have played an important role in the evolution of apomixis in *Manihot* species. Hybridity leads to new types that can be adaptive for certain environments while polyploidy increases additive genes responsible for apomixis perpetuating these genotypes.

ACKNOWLEDGMENTS

Research supported by the Foundation for Qualifying Graduate Personnel (CAPES) and the National Council for Scientific Development (CNPq). The above mentioned Living Collection of *Manihot* was established at the Universidade de Brasília in the 1970s, with support from the International Development Research Centre (IDRC), Canada, to whom we are grateful.

REFERENCES

- Asker S (1979). Progress in apomixis research. *Hereditas* 91: 231-240.
 Asker S and Jerling E (1992). Apomixis in Plants. CRC Press, Boca Raton, London.
 Brown WV and Emery WPH (1958). Apomixis in the Gramineae: Panicoideae. *Am. J. Bot.* 45: 253-263.
 Carman JG (1997). Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispority, tetraspority, and polyembryony. *Biol. J. Linn. Soc.* 61: 94.
 Grattapaglia D, Costa e Silva C and Nassar NM (1995). Strict maternal inheritance of RAPD fingerprints confirms

- apomixis in cassava (*Manihot esculenta* Crantz). *Can. J. Plant Sci.* 76: 379-382.
- Grimanelli D, Leblanc O, Perotti E and Grossniklaus U (2001). Developmental genetics of gametophytic apomixis. *Trends Genet.* 17: 597-604.
- Gustafsson A (1946). Apomixis in the higher plants. I. The mechanism of apomixis. *Lunds Univ. Arsskr.* 42: 1-66.
- Hanna WW and Bashaw EC (1987). Apomixis: its identification and use in plant breeding. *Crop Sci.* 27: 1136-1139.
- Hojsgaard DH, Martinez EJ and Quarin CL (2013). Competition between meiotic and apomictic pathways during ovule and seed development results in clonality. *New Phytol.* 197: 336-347.
- IITA (International Institute of Tropical Agriculture) (1984). Annual Report. IITA, Nigeria.
- Koltunow AM and Grossniklaus U (2003). Apomixis: a developmental perspective. *Annu. Rev. Plant Biol.* 54: 547-574.
- Nassar NM (1978). Some further species of *Manihot* with potential value to cassava breeding. *Can. J. Plant Sci.* 58: 915-916.
- Nassar NM (1992). Cassava in South America: a plant breeder's viewpoint. *Braz. J. Ass. Adv. Sci.* 44: 25-28.
- Nassar NM (1995). Development and selection for apomixis in cassava *Manihot esculenta* Crantz. *Can. J. Plant Sci.* 74: 857-858.
- Nassar NM (2001). The nature of apomixis in cassava (*Manihot esculentum*, Crantz). *Hereditas* 134: 185-187.
- Nassar NM (2002). Apomixis and cassava. *Genet. Mol. Res.* 1: 147-152.
- Nassar NM (2003a). Gene flow between cassava, *Manihot esculenta* Crantz, and wild relatives. *Genet. Mol. Res.* 2: 334-347.
- Nassar NM (2003b). Is apomixis in cassava (*Manihot esculenta*, Crantz) associated with aneuploidy? *Gene Conserve* 2: 106-110.
- Nassar NM (2006). Chromosome doubling induces apomixis in a cassava x *Manihot anomala* hybrid. *Hereditas* 143: 246-248.
- Nassar NM and Collevatti RG (2005a). Breeding cassava for apomixis. *Genet. Mol. Res.* 4: 710-715.
- Nassar NM and Collevatti RG (2005b). Microsatellite markers confirm high apomixis level in cassava bred clones. *Hereditas* 142: 33-37.
- Nassar NM and Collevatti R (2008). Embryonic, meiotic and molecular analysis of apomictic cassava (*Manihot esculenta* Crantz). *Gene Conserve* 7: 497-519.
- Nassar NM, Vieira MA, Vieira C and Grattapaglia D (1998a). Evidence of apomixis in cassava, *Manihot esculenta* Crantz. *Genet. Mol. Biol.* 21: 527-530.
- Nassar NM, Vieira MAR, Vieira C and Grattapaglia D (1998b). Molecular and embryonic evidence of apomixis in cassava interspecific hybrids (*Manihot* spp.). *Can. J. Plant Sci.* 78: 349-352.
- Nassar NM, Dos Santos E and David SRO (2000). The transference of apomixis genes from *Manihot neusana* Nassar to cassava, *M. esculenta* Crantz. *Hereditas* 132: 167-170.
- Nassar NM, Kalkmann DC and Collevatti R (2006). A further study of microsatellite on apomixis in cassava. *Gene Conserve* 5: 320-326.
- Nassar NM, Kalkmann D, Hashimoto D, Chaib A, et al. (2008a). A clue to the role of apomixis in *Manihot* speciation. *Gene Conserve* 7: 608-619.
- Nassar NM, Hashimoto D and Castilho AP (2008b). Apomixis induces new species of *Manihot*. *Gene Conserve* 7: 636-642.
- Nassar NM, Gomes PT, Chaib AM, Bomfim NN, et al. (2009). Cytogenetic and molecular analysis of an apomictic cassava hybrid and its progeny. *Genet. Mol. Res.* 8: 1323-1330.
- Nassar NM, Graciano-Ribeiro D, Gomes PF and Hashimoto DY (2010). Alterations of reproduction system in a polyploidized cassava interspecific hybrid. *Hereditas* 147: 58-61.
- Nassar NM, Chaib A and Elsayed AY (2011). Apomixis in different ploidy levels of cassava. *Hereditas* 148: 125-128.
- Nogler GA (1984). Gametophytic Apomixis. In: *Embryology of Angiosperms* (Johri BM, ed.). Springer Verlag, Berlin, 475-518.
- Nygren A (1967). Apomixis in the Angiosperms. In: *Encyclopedia of Plant Physiology* (Ruhland W, ed.). Springer-Verlag, Berlin, 551-596.
- Ozias-Akins P (2006). Apomixis: developmental characteristics and genetics. *Crit. Rev. Plant Sci.* 25: 199-214.
- Savidan Y (1992). Progress in Research on Apomixis and its Transfer to Major Grain Crops. In: *Reproductive Biology and Plant Breeding* (Dattée Y, Dumas C and Gallais A, eds.). Springer-Verlag, Berlin, 269-279.
- Singh M, Goel S, Meeley RB, Dantec C, et al. (2011). Production of viable gametes without meiosis in maize deficient for an ARGONAUTE protein. *Plant Cell* 23: 443-458.
- Tucker MR, Okada T, Johnson SD, Takaiwa F, et al. (2012). Sporophytic ovule tissues modulate the initiation and progression of apomixis in *Hieracium*. *J. Exp. Bot.* 63: 3229-3241.
- Yudakova OI (2009). Abnormalities of female gametophyte development in apomictic bluegrass forms. *Russ. J. Dev. Biol.* 40: 150-156.