Apomixis in different ploidy levels of cassava

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Two polyploid hybrids between cassava (Manihot esculenta) cultivar 307-2 and its wild relatives M. glaziovii and M. anomala, were studied to examine the relationship between ploidy level and the production of seeds without fertilization. A clearing method was applied to assess ovule sizes as an indication of multiembryony. The diploid cultivar 307-2 had regular 18 bivalents at meiotic metaphase I while the polyploid types showed chromosome configurations varying from 3 to 4 quadrivalents and 28 to 30 bivalents. A total of 14% of studied ovules of the polyploid hybrid involving M. glaziovii were multiebryonic, while the percentage of multiembryony was as low as 2% in the polyploid hybrid M. anomal x M. esculenta. Diploid hybrid types did not show any multiembryony. Adventitious embryos were found and documented for the first time in polyploid hybrids M. esculenta × M. glaziovii. The association of multiple embryo formation with ovary size and pollination showed that apomictic embryos form independently from fertilization. Simple iodized carmine stain for measuring pollen viability proved as efficient as the sophisticated Alexander method.

In apomixis seeds are produced without fertilization so that the embryo develops from an unreduced somatic cell. It fixes heterozygosity and may perpetuate superior types (KOLTUNOW et al. 1995).

Polyploidy is often associated with apomixis (ASKER and JERLING 1992; NASSAR 2006). Some wild Manihot species such as M. glaziovii and M. neuana reproduce through apomixis (NASSAR 2001; NASSAR et al. 2007). We have tried to transfer this character further and to overcome any expected hybrid sterility through polyploidization. NASSAR (2006) and NASSAR et al. (2007, 2009), working with polyploidized between species hybrids of Manihot, recommend this as a possible solution to problems in cassava breeding.

Here we study the relationship between ploidy level and apomixis and whether pollination is necessary for triggering apomictic embryogenesis as seen in by polyploidy formation.

MATERIAL AND METHODS

We use the diploid cultivar 307-2, selected for productivity from the progeny of the hybrid M. esculenta × M. glaziovii. This hybrid does not express apomixis while the M. glaziovii parent is apomictic. We attempt to see if any of the second generation offspring may segregate for apomixis, as seen through multiembryo formation (OZIAS-AKINS 2006). In addition to this variety, we used two between species hybrids: M. esculenta × M. glaziovii and M. esculenta × M. anomal a. These hybrids were polyploidized artificially so that colchicine was applied to lateral buds. The resulting polyploid shoots were multiplied vegetatively.

Multiembryo formation in ovules, the nucellus and teguments was taken as evidence for apomixis (NASSAR et al. 2007, 2008). To study possible role of pollination and the stage of flower development in forming polylembryonic ovules, flowers of different sizes with ovaries measuring 3, 4, 5, 6, 7 and 8 mm in diameter were studied. The flowers were protected with PVC plastic.

To study embryos the flowers were fixed for 24 h in Carnoy’s fluid (alcohol and glacial acetic acid 3:1). They were thereafter examined using the clearing technique (HERR 1982; NASSAR et al. 1998). Images were captured using software HonestechTVR 2.5. This technique allows the visualization of the embryo sac in toto and any abnormalities in embry development.

To study eventual meiotic behaviour, 15 metaphase cells from every plant were analyzed using the smear method. Images were digitized as mentioned above.

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<th>Table 1. Apomixis in different Manihot types.</th>
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<td>Cultivar Treatment</td>
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FAA = flower before anthesis; FP = flower protected; FPA = flower after anthesis.
To assess pollen viability, two methods were applied: iodized carmine (NASSAR et al. 2000 and Alexander's stain (ALEXANDER 1969)).

RESULTS AND DISCUSSION

Table 1 and Fig. 1 and 2 give the results in a concise form. The diploid cultivar 307-2 has a regular set of 18 bivalents at meiotic metaphase I, while the polyploid types studied here showed chromosome configurations involving 3 through 4 quadrivalents and 28 through 30 bivalents (Fig. 1).

The clarified ovules of cultivar 307-2 showed embryo development between the inner integument and the outer one, (Fig. 1-A). Diploid hybrid types do not show any multiembryony. Multiembryos were found in ovules measuring 3, 4 and 5 mm. Figure 2 shows that ovules measuring 8 mm across had both adventitious and multiembryos present.

Fig. 1. (A) Tegmental embryos of flower before anthesis. Mag. ×50. (B) Ovule with two embryo sacs. Mag. ×50. (C) Normal ovule. (D) Metaphase I of polyploid hybrid M. esculenta × M. glaziovii. Mag. ×400. (E, F) Pollen viability assessed using carmin stain and the Alexander method, respectively. Mag. ×100.
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Fig. 2. Effect of ovule size on multiembryo formation in different Manihot types: cultivar 307-2, hybrid *M. esculenta* × *M. glaziovii* and *M. esculenta* × *M. anomala*. FAA = flower before anthesis; FP = flower protected; FPA = flower after anthesis.

(Fig. 2). The association of multiple embryo formation with ovary size and pollination showed that apomictic embryos form independently from fertilization.

Adventitious embryos are here found and documented for the first time in polyploid hybrids *M. esculenta* × *M. glaziovii*. Accordingly, in addition to apomictic apospory reported by Nassar et al. (2000) cassava may have adventitious embryos. It shows that cassava has a plasticity of development involving different types of apomixis.

Multiembryos were found to form before anthesis. Their formation does not depend on fertilization. This is seen in cultivar 307-2 where the highest percentage of apomixis; 15% was found in protected flowers. In flowers examined before anthesis we observed 14% multiembryos. The lower rate of apomixis detected was in the post-anthesis flowers 8.7%. The higher level of apomixis in protected flowers may be due to that unprotected ones were frequently attacked by insects and aborted.

A total of 14% of studied ovules of the polyploid hybrid involving *M. glaziovii* were multiebryonic, while the percentage of multiembryony was as low as 2% in the polyploid hybrid *M. anomala* × *M. esculenta*.

The polyploidized *M. esculenta* × *M. glaziovii* had 25% multiple embryos in the examined 300 ovules while the diploid type showed 0.0%. Apparently apomixis is controlled by a model of additive gene action.

In contrast to studies suggesting monogenic inheritance (Leblanc et al. 1995), this finding may indicate polygenic interaction and additive action of genes for apomixis in *Manihot*. In some species of *Hieracium*, apomixis is reported to be controlled by dominant one dominant allele (Bucknell et al. 2000), while in *Paspalum* more than one pair of genes is required for apomixis to manifest itself (Askar and Jerling 1992). Clearly, the kind of apomixis in *Manihot* is different from that in *Hieracium*.

Polyploid plants of the hybrid *M. anomala* × *M. esculenta* showed a high proportion of hermaphrodite flowers (80%). Pollen of the these flowers showed viability equal with the staminate flowers. It is likely that hybridization followed by polyploidization seems to act in certain genotypes to canalize expression flowering genes in this way (Soltis and Soltis 2009).

The diploid cultivar 307-2 had a regular set of 18 bivalents at meiotic metaphase I, with high pollen viability. The polyploid types studied here showed chromosome configurations involving 3 through 4 quadrivalents and 28 through 30 bivalents (Fig. 1) but they had a high proportion of viable pollen as well.

Our data indicate that the genetic nature of apomixis in *Manihot* could be recessive since it was absent in the F1 and showed a segregation in F2. It can also be a polygenic trait since it was manifested in polyploid type while it was absent in the diploid one. This is in agreement with the findings reported earlier (Nassar et al. 2007, 2009, 2010).

Measuring pollen viability by both iodized carmine and the Alexander method showed the same result of 71–73% viability without any significant difference. Accordingly, we recommended the use of carmin in such experiments because it is very much simpler to prepare and apply in comparison with the sophisticated Alexander method.

In conclusion we may state that there is no association between polyploidy and apomixis in hybrids of cassava with *M. glaziovii* or with *M. anomala*. It is apparent that the genetic behaviour of apomixis in *Manihot* does not follow the model found in some genera like *Paspalum* (Askar and Jerling 1992). Apomixis in *Manihot* is different in nature from other types since it combines both adventitious embryos and apospory. The formation of multieembryos in apomictic cassava ovule does not depend on pollination and fertilization of ovule. The use of simple iodinated carmine stain was as effecient as the sophisticated Alexander stain.

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REFERENCES