

The nature of apomixis in cassava (*Manihot esculentum*, Crantz)

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Apomixis in cassava is formed by aposporic embryo sacs which arise from cells in nucellus or from cells in the sexual embryo sac. There is an association between sterility and apomixis. It seems that sterility caused by defected meioses triggers a specific gene for exhibiting apomixis. This apomictic mechanism is favoured by natural selection since it offers an escape from lethality and provides a perpetuation of the current genotype.

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Cassava is an important staple crop and food for more than 800 million people in the tropics and subtropics (FAO 1999). Apomixis, which means production of asexual embryos and seed without fertilization, offers many advantages for cassava development. It makes it possible to avoid contamination by bacterial germs and virus which prevail in case of using stalk in reproduction. Moreover, it fixes heterosis since the plants are perpetuated through identical offspring for successive generations. We have previously investigated the occurrence of apomixis in cassava and the development of apomictic clones (Nassar 1994, 1995; Nassar et al. 1998a, b). We have now proceeded towards transferring the genes from wild to cultivated plants (Nassar et al. 2000). However, the nature and action still needs to be clarified.

MATERIALS AND METHODS

The facultative apomictic cassava clone UnB 200, obtained earlier through selection within the progeny of a cassava hybrid with *Manihot glaziovii*, was left for open pollination. From its progeny 25 plants were selected. These were studied cytogenetically and anatomically. For cytogenetic studies, mitosis and meioses were examined.

For the studies of mitosis, cuttings of the clones were rooted in the tubes and root tips treated with 8-hydroxyquinoline (0.2 mM) for approximately 24 h. The root tips were fixed in 1:3 mixture of acetic acid and absolute alcohol for 24 h, hydrolyzed in 5 N HCl for 10 min, washed in distilled water and smeared in 1% acetocarmine.

For meiotic studies, flower buds were fixed in acetic alcohol for 24 h, and then hydrolyzed in 5 N HCl for 5 min. The anthers were isolated and squashed in 1% acetocarmine. Pollen grains from all 25 clones were investigated for viability using a mixture of acetocarmine and iodine.

The embryo sac analysis were carried out on both pollinated and unpollinated pistils. Unpollinated pistillate buds were collected 1 day before anthesis. The pollinated ones were collected 2 days after anthesis. Approximately 200 pistils from each clone were collected for embryo sac analysis. They were fixed in 1:3 acetic alcohol in the field between 7:30 and 12:00. Fixed pistils were dissected under a dissection microscope (magnification x 40, transmitted light). Dissected nucellus and ovules were dehydrated in ethanol series and cleared overnight in benzyl-benzoate-4½ (BB-4½) fluid (lactic acid-chloral hydrate:phenol:clove oil:xylene:benzyl benzoate = 2:2:2:2:1:1, w/twt, devised by Young et al (1970) and treated in a modified Herr's fluid as previously reported by Ogburia and Adachi (1994). Transparent ovules were then observed and photographed microscopically at 400 x magnification using Normarski's differential interference contrast.

RESULTS AND DISCUSSIONS

The anatomical studies of ovules showed that the embryo was formed by apospory from a somatic cell in the nucellus. The megasporogenesis in ovules with aposporous development proceeds normally up to a certain moment when nucellar cells enlarge and the nuclei divides to form aposporous embryo sacs (see Fig.47 and Fig 58). These aposporous embryo sacs appear to develop faster than sexual embryo sacs, probably they are not delayed by meiotic division. This is in accordance with Asker (1979) and Nogler (1984). In some cases, development of apospory embryo sacs from cells within the sexual one was noted (see Fig 46 in photos gallery). Both the aposporous and sexual embryo grew in parallel and finally coexisted.

Fig 1a-c. a. Three apospory embryo sacs growing in the nucellus tissue. b. Developed apospory embryo sac within the sexual one. c. Two developed apospory sacs in the nucellus tissue.

This observation confirms results from a previous study (Nassar 1995), where two seedlings grew side by side; one of which was apomictic and one of sexual origin. Nogler (1984) reported that in *Potentilla*, aposporous and sexual processes coexisted in one individual ovule producing several embryos. This study documents the survival of two aposporous embryo sacs beside a sexual one, all of them in a developed stage in the ovule. (see Fig.47 in photos gallery)

The cytogenetical study showed that out of the 25 individuals examined, 13 plants were

sterile; the percentage of pollen viability ranging from 4 to 15% (Table 1). Two plants had 2n+1 while the rest were 2n. The other 12 plants were highly fertile with pollen viability ranging from 92 to 97%. Their chromosome number was 2n.

The embryonic study revealed that all of the sterile plants were partially apomictic while the fertile plants were sexuals. Sterility apparently leads to apomixis. Sterility is caused by consistent defects of meiosis due to lack of pairing. All of these sterile plants showed asynapsis in meiotic metaphase. Formation of univalents ranged from 4 to 6 per cell. The irregular chromosome segregation in these sporocytes lead to genetically unbalanced and aborted gametes. It seems that that sterility triggers certain genes of apomixis to act. Apomixis will function and be established in such genotypes since it is favoured by natural selection as it offers an escape from lethality, providing a perpetuation of the current genotype.

Table 1. Chromosome constitution in relation to pollen viability and apospory in apomictic progeny.

Clone	Pollen viability %	Apospory %	Chromosome number
1	9.2	1.2	2n
2	6.8	1.5	2n+1
3	4.9	1.7	2n
4	2.0	1.6	2n+1
5	4.7	1.1	2n
6	6.1	1.2	2n
7	15.6	1.3	2n
8	4.1	1.4	2n
9	8.3	1.7	2n
10	5.6	1.3	2n
11	4.1	1.8	2n
12	4.6	1.4	2n
13	4.7	1.3	2n

It can be concluded that: 1) The nature of apomixis in cassava is different from other types found in other crops since it is present at very low levels, 1 – 2%. 2) It depends on meiotic irregularity which often causes sterility in plants. 3) This sterility triggers a certain gene in cassava which activates a number of somatic cells in the nucellus or in the sexual embryo sac to form aposporic embryo sacs. 4) The natural selection favours this apomictic genetic structure since it is an escape from extinction and a mode of perpetuation for the current genotype.

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