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The transference of apomixis genes from *Manihot neusana* Nassar to cassava, *M. esculenta* Crantz

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Manihot neusana Nassar exhibits useful characters such as resistance to bacteriose and stem borers, in addition to partial apomixis. Hybridization of this species with cassava was carried out. F1 and F2 plants were obtained. An embrylogic study of a F2 plant showed it to have aposporyous embryo sacs in addition to the sexual ones. It was aneuploid with 2n = 38.

Apomixis means seed formation without fertilization. In cassava, it is an alternative to reproduction by cuttings which normally is practiced by farmers. The latter type of propagation leads to accumulation of viral and bacterial diseases that reduce productivity and may cause extinction of superior genotypes. Thus, by the use of apomictic plant in propagation, systemic pathogens could be avoided, and the genetic segregation in the progeny is excluded. An additional and important advantage of the induction of apomixis in the cultivated cassava is that it will assure preservation of superior clones in place of their extinction. Plant-produced stems through apomixis from a contaminated clone will be free from viral and bacterial germs and can begin a new cycle of clone life in place of its extinction. If apomixis was found or had been introduced into the excellent Brazilian clones like Guaxupe and Vassourinha, they would not have been extinct, and had been preserved for a long time. The use of apomixis in preserving superior genotypes and filtering the bacterial contamination beneficiates also intemational cassava programs who export routinely their germplasm. It is sufficient in this case, for the destination country to produce only one plant and further reproduce it vegetatively to maintain the original superior genotype. In the earlier work of the first author, partial apomixis was discovered in the wild cassavas *M. dichotoma* and *M. glaziovii* (NASSAR. l994, l995; NASSAR et al. 1998b). It was noted by him earlier in *M. neusana*. This species is characterized by extreme resistance to bacteriose and stem borers (NASSAR. 1985). In earlier work of the first author interspecific hybridization was carried out to transfer these useful genes to the cultigen (NASSAR 1989). This paper presents results of studies of apomixis in its hybrids with cassava.

MATERIAL AND METHODS

The wild *Manihot* species *M. neusana* Nassar was hybridized with cassava, Catelo clone, employing pollinator insects (NASSAR 1989). An interspecific hybrid that combined marker genes of both parents was obtained (see photo gallery Fig. 29); ribbed fruit was acquired from cassava and the variegated fruit color was acquired from *M. neusana* (see photo gallery Fig. 18). This hybrid (called HN) was selfed and seeds were obtained. One plant raised and called here (S1) has been examined. These two plants as well as their parents *M. neusana* and cassava clone Catelo were studied embryonically and cytogenetically.

Embryo sac analysis

The morphological development of embryo sacs was studied. Unpollinated pistillate buds collected, about 1 day before anthesis, and poillinated pistils, collected 2 d after anthesis, were fixed in Farmer's fixative (1:3 glacial acetic acid:95 % ethanol) in the field between 07:30 h and 12:00 h. 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 the benzyl-benzoate-four-and-a-p half (BB-41/2) fluid (lactic acid:chloral hydrate: phenol:clove oil:xylene: benzyl benzoate = 2:2:2:2:1:1 , w /twt devised by HERR (1971), YOUNG et al. (1979) 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.

Cytogenetic analysis

For mitotic study root tips were collected from germinating cuttings. They were pretreated by 8-hydroxy quinoline (0.002 M) for approximately 24 hours at 8ºC, and fixed in ethanol-acetic 3:1, for 24 hrs, then washed in distilled water, hydrolyzed in 5 N HCL for 20 min. at room temperature, and squashed in aceto carmin 2 %.

For meiotic study, flower buds were fixed in ethanol-acetic for 24 hrs, then hydrolyzed in 5 N HCL for 5 min. The anthers were iso1ated and squashed in 2 % acetocarmin. pollen viabi1ity was percent in 200 ovu1es investigated. The ovules exam-estimated by the use of carmin 1 % iodine mixture.

RESULTS AND DISCUSSION

Two hundred ovules each of *M. neusana*, cassava, the F1 plant and its progeny

were examined for apomixis. In both *M. neusana* and the second generation hybrid it aposporyous embryo sacs were detected. Their percentage in *M. neusana* was 1.50 percent in 200 ovules investigated. The ovules examined of cassava and the first generation hybrid showed total absence of apomixis. By using clearing method and the differential interphase contrast (DIC) microscope it was possible to document photographically the cassava embryo sac structure (Fig.55 in the photo gallery). Normal sacs showed one egg, two polar nuclei, and three antipodals. Synergids were occasionally seen. The egg was often inconspicuous. Antipodals were distinguished by a swollen. tear drop shape, dense cytoplasm, chalazal position, and absence of a wall separating them from the sac cavity. In the (S1) plant it was possible to document photographically for the first time the presence of two sacs growing side by side. One of these sacs apparently is the aposporious one while the second is the sexual embryo sac (Fig. 56 in the photo gallery). The aposporous sacs lacked antipodes and had only one nucleus per sac. The development of two sacs jointly is a characteristic pattern of aposporic apomixis in cassava, and explains why we find in some cases 2 seedlings growing jointly from a cassava seed. The two joint embryo sacs are of the same size and phase of development. It seems that a certain external factor has triggered its growing in the same moment as the sexual embryo sac formation.

Meiotic metafase was studied in cassava, *M. neusana*, the hybrid (HN), and the F2. Regular formation of 18 bivalents was found both in *M. neusana* and cassava. The pollen viability was 94 and 96 percent respectively. In the hybrid F1, bivalents and univalents were observed and in medium 17 bivalents and 2 univalents were noted. This lack of complete synapsis was accompanied by low pollen viability which reached 26 percent. It gave a very low number of fruits; 6 fruits compared to 70 fruits in case of normal cassava. The meiotic metaphase study of the F2 plant showed chromosome association of trivalents, bivalents and univalents with a medium of 1.8, 16 and 0.2 respectively .The pollen viability was very low, 8 percent. The mitotic chromosome number was 2n = 38.

While the hybrid (F1 did not acquire apomixis as seen from the embryonic analysis, the F2 plant did show it. This could mean that apomixis is controlled by more than one recessive gene, which works out in an additive mode, probably carried in the same chromosome. (BROWN 1958; ASKER 1979). In our case, the presence of 2 additional chromosomes in the aneuploid plant with 2n = 38 could offer homozygozity and dosages necessary for the action of these genes. The connection between apomixis and aneuploidy has been observed by the first author (NASSAR 1994, 1995; NASSAR et al. 1998a). This report further confirms this fact. The F2 plant carried abundant fruits, about 70 fruits compared to 6 found in the F1 plant. Thus abundant fruitfulness accompanied by high sterility in plants of hybrid origin is one of the strongest indicators of apomixis in plants.

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