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Evidence of apomixis in cassava (*Manihot esculenta* Crantz)

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ABSTRACT

Apomixis maintains heterosis and avoids transmission of systemic pathogens which accompany vegetative propagation of cassava. An embryonic study of cleared ovules of two cassava clones *in toto* showed them to be of aposporic nature. Cytogenetic analysis of the two clones revealed an aneuploid structure (2n + 1) in apomictic individuals, whereas it was 2n in the sexually reproduced plants.

INTRODUCTION

Cassava *Manihot esculenta* (yuca, mandioca or manioc) is the most important staple crop in the tropics and subtropics, and serves as a food for more than 600 million people (FAO, 1994). Cassava is propagated vegetatively through stem cuttings. Vegetative propagation perpetuates superior genetic combinations, but also favors the accumulation of viral and bacterial diseases that reduce productivity and may lead to the extinction of superior genotypes. If true seeds were used to propagate crops, systemic pathogen contamination could be avoided. However, breakdown of genetic superiority due to genetic segregation in the progeny has always excluded this approach.

Heterozygosity, responsible for vigor, could be efficiently fixed by apomixis in cassava. This phenomenon is defined as a process by which certain plants produce seeds without fertilization. This means bypassing female meiosis and syngamy to produce embryos genetically identical to the maternal parent. Superior cassava genotypes could be maintained through successive generations by apomictic reprodution. Apomixis in cassava was discovered by Nassar (1980) in a study on interspecific hybridization. Later it was shown genetically (Nassar, 1994) and confirmed by RAPD marker analysis in a single clone (Grattapaglia *et al.*, 1996). The present work presents further evidence of apomixis through embryonic and cytogenetic studies.

MATERIAL AND METHODS

Plant material

Two putative facultatively apomictic cassava clones identified by numbers 31 and 200 were used in this study. Descriptions of these clones, according to Nassar and Grattapaglia (1986), are as follows:

Clone 31: Surface of root - rough; root flesh color - cream; root shape - obovate; color of stem - silver; nature of scars on stem - moderately raised; leaf lobe shape - obovate; sinuosity of lobes - linear; length of median lobe - more than 5 cm; petiole color - green; color of young foliage - reddish blue; color of flower corolla - white; fruit color - green with red wings.

Clone 200: Surface of root - smooth; root flesh color - white; external root color - brown; root shape - cylindrical; color of stem - brown; nature of scars on stem - moderately raised; leaf lobe shape - obovate; sinuosity of lobes - linear; length of median lobe - less than 5 cm; petiole color - green; color of young foliage - reddish blue; color of flower corolla - white; fruit color - green.

Clone 31 was selected based on vigor in an F_2 population resulting from a cross between an interespecific hybrid (*M. dichotoma x M. esculenta)* and cultivated clone Branca Santa Catarina. Clone 200 is an F_1 from a cross between cultivated cassava and *M. glaziovii*. Progenies from both clones were obtained and studied anatomically and cytogenetically.

Embryo sac analysis

The morphostructural development of embryo sacs was studied histologically. Unpollinated pistillate buds at presumed one day pre-anthesis and pollinated pistils at post-anthesis were sampled and immediately fixed in Farmer's fixative (1:3 glacial acetic acid: 95% ethanol) in the field between 7:30 and 12:00 a.m. Fixed pistils were dissected under a dissection microscope (magnification 40X, transmitted light). Dissected nucelli and ovules were dehydrated in ethanol series and cleared overnight in the benzyl-benzoate-four-and-a half (BB-41/2) fluid (lactic acid:chloral hydrate:phenol:clove oil:xylene:benzyl benzoate = 2:2:2:2:1:1, w/w) as described by Herr (1982), and treated in modified Herr's fluid as reported by Ogburia and Adachi (1994). Transparent ovules were then observed using an Olympus BX5O microscope equipped with Nomarski's differential interference contrast (DIC) optics and a 100-W high pressure mercury light bulb with appropriate filters for optimal viewing and photography. Both megasporangium and megagametophyte components were photographed and printed by a Sony color video printer.

Cytogenetic analysis

Cytological studies were carried out on the maternal plant and five progeny individuals to examine their meiotic behavior. Flower buds were fixed in acetic alcohol 3:1 for 24 h, preserved in 70% ethanol, smeared and stained with l% acetocarmin. Pollen viability was estimated by staining with 1% aceto carmin.

RESULTS AND DISCUSSION

Two hundred sixty-one ovules of the two clones were histologically analyzed. We found aposporic sacs inside the sexual embryo sacs in both clones (Figure l). The presence of two embryo sacs in an ovule is an indicator of aposporic nature of apomixis. It is supposed that one of the embryo sacs is derived from somatic cells at its location in the ovule, while the second embryo sac is derived from a normal megaspore mother cell. At a certain stage, before the complete maturation of the sexual embryo sac, it may abort and be replaced by the developping aposporous sac, otherwise, it continues to develop, giving rise to two embryo sacs and two embryos in the ovule. This abnormality was found in 2.4 % and 1.5% of the ovules of clones 31 and 200, respectively. Similar results using the same histological clarification technique were reported in *Cenchrus ciliaris* (Young *et al.*, 1979).

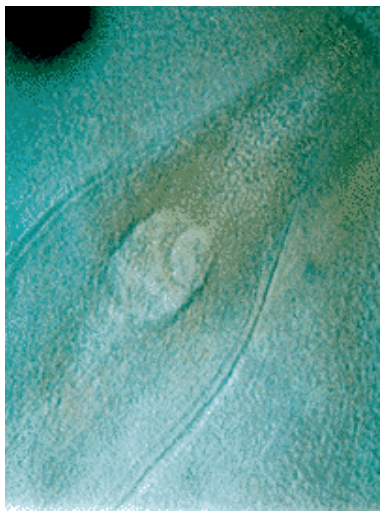

Figure 1 - Differential interphase contrast (DIC) micrograph of a cleared ovule showing two embryo sacs: aposporic and sexual (one sexual embryo is normal). Identification of the aposporous and sexual embryos can be made on the basis of their location in the ovule. The sexual member is located opposite to the micropylar region.

It was possible to see details of cassava embryo sac in the cleared pistils with a differential interphase contrast (DIC) microscope. 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. 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 suggest that the mechanism responsible for apomixis in cassava is apospory (development of aposporic embryo sacs). Apospory is the most common mechanism responsible for apomixis in the angiosperms. In this type of apomixis the apospory embryo sac originates from one or more somatic cells of the ovule, which becomes enlarged and vacuolated (Asker, 1980).

Apomixis by apospory in the angiosperms explains the multiple plants per seed found in clone 31 by Nassar (1994). The presence of apomixis in clones 31 and 200 shows the potential of the utilization of wild species as a source of genes for apomixis in cassava, since clone 31 represents the F_2 generation of a cross between cassava and *M. dichotoma,* and clone 200 is an F_1 of cassava and *M. glaziovii*. Sources of genes controlling apomixis in wild species, relative to corn, sugar beet, wheat, and several forage grasses, have already been identified (Asker, 1979).

The meiotic study revealed the euploid structure of clone 31. Chromosome number was 37 in the maternal plant. Some progeny showed embryonic aposporic apomixis (Figure 2), while other progeny plants showed a 2n number = 36. Chromosome association in the aneuploid ranged from 17 bivalents and three univalents to 17 bivalents and two univalents in the diploid constitution. Pollen viability was 17.83% for the maternal parent plants, and 17.30% for progeny number 4. It was 97.5, 93.6, 92.5, and 96.7% for progeny numbers 1, 2, 3 and 5, respectively. Several authors, such as Gustafsson (1946), Warmke (1954), Brown and Emery (1958), have correlated apomixis with polyploidy, referring to euploidy. The latter authors, in their study on the subfamily Ponicoideae, Gramineae, found apomixis restricted to euploid especies. Association between apomixis and aneuploidy may be explained by the hypothesis that any apomictic cycle must involve at least two genetic divergences from the normal sexual cycle: one which provides a substitute for meiosis, and one which provides a substitute for fertilization. According to this hypothesis, the prevalence of apomixis among aneuploids would be a consequence of the fact that they are partially or completely sterile and do not need a second mutation for apomixis development. The presence of aneuploidy in cassava apomictic clones also demonstrates that while apomixis can be induced naturally at the diploid levels by favorable gene combinations, there is a stronger action of these genes on the aneuploid form where duplication of certain chromosomes exists. The interspecific hybridization origin of Brazilian indigenous cassava clones as explained and documented by Nassar (1992) and Nassar *et al.* (1995) may be reponsible for this apomictic nature since it leads to partial or complete sterility, as well as formation of aneuploid forms (Nassar *et al*. 1995) in the progeny of interspecific hybrids of cassava with *M. neusana* Nassar.

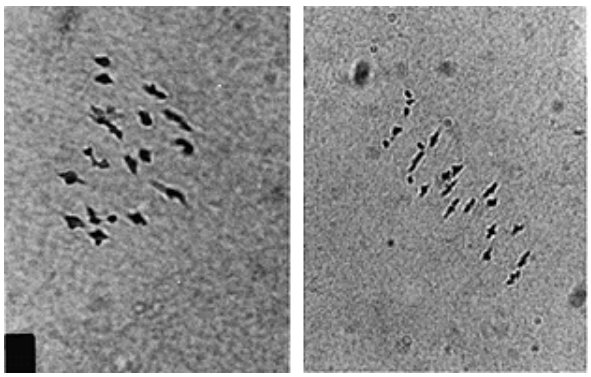

Figure 2 - Metaphase I in pollen mother cells of apomictic (left) and sexual (right) individuals.

An International Institute of Tropical Agriculture report suggested apomixis in wild *Manihot* species and hybrids (IITA, 1988). Genes controlling different levels of apomixis have been found in wild relatives of cultivated crops, for example *Panicum* (Riedy *et al.*, 1992). Several F_2 genotypes which result from interspecific hybridization of cassava with wild relatives have shown strong evidence of apomixis, and abundant fruitfulness, accompanied by sterility. It seems, therefore, that wild *Manihot* species are capable of conferring genes for apomixis to cassava. Clone 31, found in this study to bear apomictic seedlings, is an F_2 genotype derived from an interspecific hybrid of *Manihot dichotoma* x *M. esculenta.*

In conclusion, this report further validates the occurrence of apomixis in cassava by presenting a combination of embryonic and cytogenetic evidence in a large set of progeny individuals from two putative, apomictic clones. By analyzing a larger set of progeny we reinforced the earlier report of apomixis in clone 31 in five fundamental ways: 1) we estimated facultative apomixis to be about 2%; 2) we detected apomixis in a second genotype, clone 200, derived from a different interspecific cross; 3) apomictic behavior was demonstrated in an F_1 individual; 4) parallel embryonic evidence corroborated the evidence for apomixis in the two genotypes investigated. Furthermore, the fact that clone 200 is an F_1 interespecific hybrid hints to the possibility of directly transferring genes for apomixis from a wild relative to cultivated cassava and 5) a cytogenetical study showed association of aneuploidy with apomixis behavior in cassava clones. This is probably due to the additive gene action of this character.

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RESUMO

A Apomixia mantém a heterose e evita a transmissão de patógenos sistêmicos que acompanham a propagação vegetativa da mandioca. Um estudo embriônico de ovos clareados de dois clones de mandioca *in toto* mostrou que eles são de natureza apospórica. A análise citogenética dos 2 clones revelou uma estrutura aneuploide (2n + 1) em indivíduos apomíticos, ao passo que era 2n em plantas reproduzidas sexualmente.

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