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UNREDUCED MICROSPORES IN CASSAVA, *MANIHOT ESCULENTA* CRANTZ CLONES

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ABSTRACT

Nine indigenous Costa Rican cultivars of cassava were studied cytogenetically to detect unreduced microspores. Out of these nine clones, four showed about 1% triads among the tetrads 1–1.3%. One of them also showed 3.7% diads. The meiotic study revealed presence of sectorial chimera in one of these clones. This is the first report of sectorial chimera in cassava. This chimera formed two parallel sectors in the inflorescence, where sector had regular chromosome pairing, and the other sector showed predominant chromosome asynapsis.

Key words: Microsporogenesis, ploidy levels, chimera cassava.

The formation of unreduced microspores (gametes with somatic chromosome number) appears to be a common phenomenon in angiosperms [1, 2]. They, most likely, have a major role in the evolution and origin of polyploids. From a plant breeding viewpoint, these gametes may lead to the development of highly productive tetraploids and triploids by sexual means, and also for preserving their heterozygosity [3]. Probably, the most convincing cytological evidence of their occurrence in higher plants was came from Prakken and Swaminathan [4], who observed that diads (with two unreduced microspores) and tetrads (with four reduced microspores) occur together in the same plants of several *Solanum* species. This was confirmed later by several workers in different crop plants [5–8]. Nassar and associates [9, 10] reported it for the first time in cassava interspecific hybrids as a consequence of meiotic irregularity. Now, there is agreement that diads form due to spindle abnormalities which may be visible at meiotic metaphases I and II [9].

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MATERIALS AND METHODS

Nine cassava clones grown in the germplasm bank of the Centro Agronomico Tropical de Investigacion y Enseñanza, CATIE were used in this study. Floral buds were collected and fixed for 24 h in 3 : 1 ethanol–acetic acid, mixture, transferred to 70% ethanol and stored at 5°C. The anthers were squashed in a drop of 1% acetocarmine. Metaphase I was used for chromosome counting and study of chromosome associations. The tetrad stage was observed to determine the frequency of diads and triads. About 300 tetrads were counted in each clone. Photomicrographs were taken from temporary preparations using the Zeiss standard research microscope.

RESULTS AND DISCUSSION

Out of the nine cassava clones studied, eight showed regular metaphase with complete pairing and formation of 18 bivalents (Table 1, Fig. 1). The ninth clone (No. 6477), popularly known as Chioriqui showed a sectorial chimera in the inflorescence. One sector of

Table 1. Chromosome associations and frequency of diads and triads in eight clones of cassava

Cultivar	Chromosome association	Diads		Triads	
		frequency	2%	frequency	2%
9959 (Mangi)	18 II	—	—	4	1.33
6417 (White)	18 II	—	—	—	—
10861 (Num 4-RB)	18 II	—	—	—	—
6429 (Negra Muchera)	18 II	—	—	4	—
11965 (Sin Nombre)	18 II	11	3.7	3	1
6379 (Amarilla-1)	18 II	—	—	3	1
6473 (Vagana 4208)	18 II	—	—	—	—
6477 (Chiriqui):					
Chimera a	18 II				
Chimera b	11 II + 14 I				

inflorescence developed flowers with normal metaphase I and complete pairing of chromosomes. No laggards at anaphase I and no micronuclei at the tetrad stage were observed. The other sector of the inflorescence had flowers with extreme abnormal metaphases I in all the flowers investigated. This was accompanied by the presence of empty anthers. The abnormality at metaphase I was due to lack of chromosome pairing or asynapsis. In the 20 metaphases examined the chromosome associations ranged from 11 to 12 bivalents instead of 18 normal bivalents (Fig. 2). The remaining chromosomes formed

Fig. 1. Regular metaphase I with 18 bivalents.

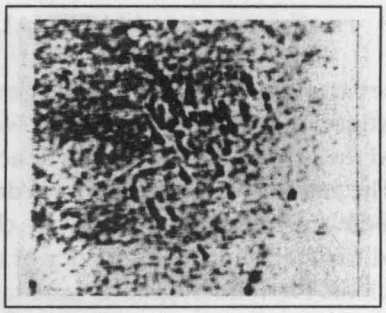

Fig. 2. Irregular metaphase I with 14 bivalents and 8 univalents.

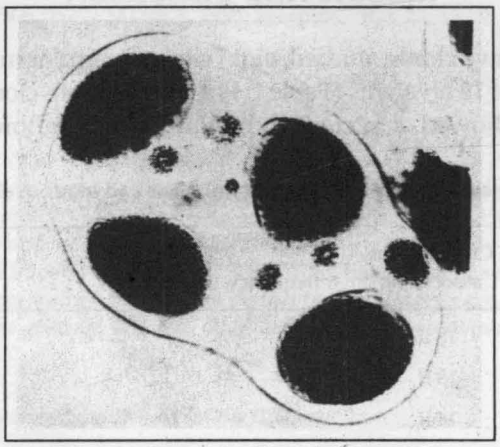

Fig. 3. A tetrad with multiple nuclei.

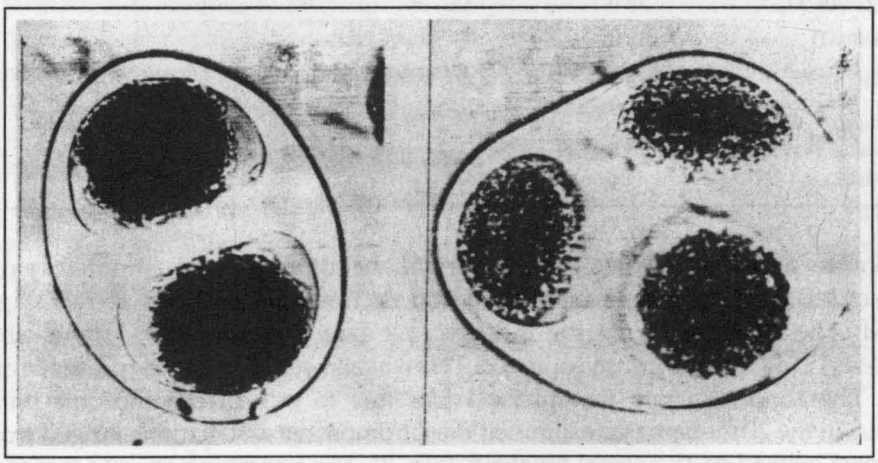

Fig. 4. A diad and a triad.

only univalents. This resulted in the formation of micronuclei at tetrad stage. There were 1–15 micronuclei per tetrad (Table 2, Fig. 3). Apparently, this chimeral sector formed due to a gene mutation in the second layer (LII) of the apex of the growing shoot. Since only a lateral sector exhibits this abnormality, the chimera must be sectorial rather than mericlinal or periclinal.

This is the first report of a mutation which affects chromosome pairing in cassava. Since cassava reproduces vegetatively, it is likely that this mutation has been preserved for a long time in this indigenous Costa Rican clone. To trace unreduced microspore formation, diads and triads were checked among 300 tetrads of each clone. The most interesting case was found in clone No. 11965 which formed 11 diads per 300 sporocytes studied (Table 1). indicating first meiotic restitution. No other clone showed diad formation. As for triads formation, four clones, namely, 9959 (Mangi), 6429, Vegna Mochera, and 6399 formed triads in the range of 1 to 1.3%. The clone 11965, the only diad producer among the clones studied, also produced triads with a frequency of 1%.

Table 2. Frequency of diads and five kinds of tetrads in the sectorial chimera of clone 6477 (No. of tetrads studied 300)

Parameter	Diad	Normal tetrads	Tetrads with different Nos. of micronuclei			
			1	2	3	4
Frequency	1	18	26	41	26	188
Percent	0.3	6	8.7	13.9	8.9	62.6

As can be seen from Table 1, there is variation for 2n gamete production among the cassava clones. Several workers have reported that in species with a tendency to form unreduced microspores, the frequency of such gametes may vary from one line to another [11–13]. In the meantime, stable high 2n gamete production has been observed in 2n gamete producer lines [13]. Thus, the variation in the frequency of 2n gametes may be attributed mainly to their genotypic differences [13]. It seems that unreduced microspore formation is gene controlled and not due to the disturbance of chromosome asynapsis. There is strong evidence for this in our example of sectorial chimera in clone 6477 which asynapsis and yet showed very low frequency of unreduced gametes despite predominance of chromosome asynapsis. It is, however, agreed that this is due to the occurrence of nonfunctional spindle, resulting in all the metaphase chromosomes remaining in the center instead of separating to the two poles [14–17].

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REFERENCES

1. J. R. Harlan and J. M. de Wet. 1975. On 0 wings and a prayer; the origin of polyploids. Bot. Rev., **41**: 361–391.

2. J. M. J. de Wet. 1980. Origin of polyploids. *In*: Polyploidy: Biological Relevance (ed. W.H. Lewis). Plenum Press, New York, U.S.A.

3. A. O. Mendiburu and S. J. Peloquin. 1977. The significance of 2n gametes in potato breeding. Theor. Appl. Genet., **54**: 53–61.

4. R. Prakken and M. S. Swaminathan. 1952. Cytological behaviour of some interspecific hybrids in the genus *Solanum* sect. *tuberosum*. Genetica, **26**: 77–101.

5. M. M. Roades and E. Dempsey. 1966. Induction of chromosome doubling at meiosis by elongated gene in maize. Genetics, **54**: 505–522.

6. M. Hogland. 1970. Meiosis in *Solanum phureja*. Hereditas, **66**: 183–188.

7. B. R. Tyagi. 1988. The mechanism of 2n pollen formation in diploids of *Costus speciosus* J. E. Smith and role of sexual polyploidization in the origin of intraspecific chromosomal races. Cytologia, **53**: 763–770.

8. C. A. Sala, E. L. Camadro, M. T. Saleberry and A. C. Mendiburu. 1989. Cytological mechanism of 2n pollen formation and unilateral sexual polyploidization. Euphytica, **43**: 1–6.

9. H. N. M. A. Nassar, N. M. A. Nassar, C. Vieira and L. S. Saraiva. 1983. Interspecific hybrids of cassava with *Manihot neusana* Nassar and their cytogenetic behaviour. Cienc. Cult. (in press).

10. N. M. A. Nassar. 1992. Production of triploid cassava, *Manihot esculenta* Crantz, by hybrid diploid gametes. Field Crops Res., **30**: 173–182.

11. D. W. S. Mok and S. J. Peloquin. 1975. The inheritance of three mechanisms of diplandroid (2n pollen) formation in diploid potatoes. Heredity, **35**: 295–302.

12. J. R. Ballington and G. J. Galleta. 1976. Potential fertility levels in four diploid *Vaccinium* species. J. Amer. Soc. Hort. Sci., **101**: 507–509.

13. R. Veilleux and F. I. Lauer. 1981. Variation for 2n pollen production in clones of *Solanum phureja*. Theor. Appl. Genet., **59**: 95–100.

14. M. S. Ramanna. 1974. The origin of unreduced microspores due to aberrant cytokinesis in the meiocytes of potato and its genetic significance. Euphytica, **23**: 20–30.

15. E. H. Standfor, W. M. Clement Jr. and E. T. Bingham. 1972. Cytology and evolution of the *Medicago sativa falcata* complex. *In*: Alfalfa Science and Technology (ed. C. H. Hanson). Amer. Soc. Agron. Madison, Agronomy No. 15: 87–101.

16. R. Veilleux. 1985. Diploid and polyploid gametes in crop plants: mechanisms of formation and utilization in plant breeding. Pl. Breed. Rev., **3**: 41–46.

17. N. Vorsa and E. T. Bingham. 1979. Cytology of 2n pollen formation in diploid alfalfa, *Medicago sativa*. Can. J. Genet. Cytol., **21**: 525–530.