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Cytogenetic behaviour of the interspecific hybrid of *Manihot neusana* Nassar and cassava, *M. esculenta* Crantz, and its backcross progeny

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Nassar, H. N., Nassar, N. M. A., Vieira, C. and Saraiva L. S. 1995. **Cytogenetic behaviour of the interspecific hybrid of *Manihot neusana* Nassar and cassava, *M. esculenta* Crantz and its backcross progeny**. Can. J. Plant Sci. 75: 675-678. An interspecific hybrid between cassava, *Manihot esculenta* Crantz, and *M. neusana* Nassar and backcrossed progenies was examined meiotically and mitotically. The F1 hybrid showed irregular meiosis due to unpaired chromosomes. The backcrossed progeny ranged in fertility from 17.7 to 35.8%. Meiotic restitution was also observed in all of the hybrids. Diploid pollen formation also occurred and ranged from 0.6 to 1.6%. Irregular meiosis among the F1 hybrid produced aneuploidy in backcrossed progeny. When the F1 hybrid was used as the maternal plant in backcrosses with cassava, this improved seed setting in the BC1 progenies, whereas the reciprocal backcross produced sterile progenies.

Key words: Wild species, meiotic restitution, aneuploidy, diploid gamete.

Nassar, H. N., Nassar, N. M. A., Vieira, C. et Saraiva L. S. 1995. **Comportament cytogénétique de l'hybride interspécifique de *Manihot neusana* Nassar et du manioc cultivé *M. esculenta* Crantz ainsi que de sés descendances de rétrocroisement**. Can. J. Plant Sci 75: 675-678. Un hyrid interspécifique entre le manioc cultivé Manihot esculenta Crantz et M. Neusana Nassar ainsi que ses descendances de rétrocroisement ont été examinés au plan de la méïose te de la mitose. L'hygride F1 manifestait une méïose irrégulière due à la présence de chromosomes non appariés. La fertileté dês descendances de rétrocroisement allait de 17,7 à 35,8%. On observait en outre des phénomènes de restitution méïotique chez toutes les descendances hybrides. La formaton de pollen diploïde (0,6 à 1,6%) était également observée. La méïose anormale observée à la F1 provoquait l'apparition d'aneuplïdie dans les descendances de rétrocroisement. Lorsque la F1 était utlisée comme plante maternalle en rétrocroisement avec le manioc cultivé, on constatait une amélioration de la nouaison dans la BC1 tandis que le rétrocroisement réciproque produisait des descendances stériles.

Mots clés: Espèce sauvage, restitution méïotique, aneuplïdie, gamète diploïde.

Wild species of different crops have frequently been used as sources of useful genes transferred through interspecific hybridization (Harlan 1976; Hawkes 1977; Rick 1978). The only reported case of interspecific hybridization involving cassava [(*Manihot esculenta* (Crantz)] was that of Nichols (1947) who sucessfully transferred a gene for resistance to mosaic virus from *Manihot glaziovii* to cassava. Wild *Manihot* species native to South America have been systematically evaluated for useful charactestics (Nassar 1978a, b, c, d, 1979, 1985, 1986). One fo the most useful species for plant breeding is *Manihot neusana* Nassar. It is tolerant to low temperature, resistant to stem borers, highly resistant to bacterial diseases and has an evergreen habit (Nassa 1985). An interspecific hybrid between this species and cassava was obtained earlier (Nassar 1989). This paper describes the cytogenetic behaviour of this hybrid and its backcrossed progeny.

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; ribbed fruit was acquired from cassava and the variegated fruit color was acquired from M. neusana. This hybrid (called HN) was backcrossed to cassava using it as the male in one of the trials, and as the female in the second trial. BC1 Seed was obtained from both backcrosses. A single plant from each backcross was obtained and identified as HO1 when HN was used as the maternal parent and HO4 whem HN was used as the paternal parent. The three hybrids plants, HN, HO1 and HO4 as well as their parents, *M. neusana* and the cassava clone

Catelo, were studied cytogenetically during meiosis and mitosis. For the meiotic studies, inflorescences were fixed in a 3:1 mixture of absolute alcohol and glacial acetic acid and kept at 5°C for 24 h. The anthers were smeared in acetocarmim stain. Chromosome configurations at metaphase I, chromosome distribution at anaphase I, and tetrad formation were studied. Pollen viability was determined using acetocarmine and iodine strains (Nassar 1978d). For mitotic studies, root tips were left in a 2.0% colchicine solution for 2h, then fixed in acetic alcohol for 24h. Before smearing in acetocarmine root tips were treated in 1 N HCL for 10 min.

RESULTS AND DISCUSSION

Meiotic Behaviour of the F1 Hybrids (HN)

A sample of 100 PMCs was studied at metaphase I of the interspecific hybrid M. neusana with cassava. Thirty PMCs in metaphase II and 1000 tetrads of the same material were also investigated. The study of metaphase I revealed several different chromosome configurations. Forty-eight PMCs exhibited bivalents only; 34 PMCs had 17 bivalents and 2 univalents; 12 PMCs exhibited 16 bivalents and4 univalents. Four PMCs had 15 bivalentes and 6 univalents; and in the case of two cells, one had 14 bivalents and 8 univalents. The mean number of bivalents in all cell of the metaphase I was 16.13 per cell. The hight frequency of univalents is attributed to a lack of synapsis between chromosomes os the two species or their failure to remain associated.

Few studies have been reported on the cytogenetics of interspecific hybrids of *Manihot* species and their chromosome behaviour in relation to metaphase pairing. Magoon et al. (1970) studied chromosome pairing in the interspecific hybrid of *Manihot glaziovii* (rubber tree) and cassava and found regular synapsis. This led them to conclude that there is a strong relationship between this species and cassava. Nassar et al. (1985) suggested that the material used by Magoon was not pure *M. glaziovii*, but rather a natural interspecific hybrid between this species and cassava. If this is true, the reported interspecific hybrid would be a backcrossed progeny. The study of anaphase I showed that of 40 PMCs studied, 38 cells exhibited laggards. This was attributes to the occurrence of univalents that resulted from non-homologous chromosomes and difficulty in chiasmata terminalization.

The study of anaphase II made the observation of meiotic restitution possible. Of the 33 PMCs studied, 5 cells exhibited second meitic restitution (SMR), 36 chromosomes forming each pole. This phenomenon is apparently caused by meiotic disturbance in the hybrid. An example of this disturbance was the breakdown of anaphase I. This is probaly due to the disharmony between the two different genomes. Nassar (1992) documented this phenomenon in the interspecific hybrids of cassava with *M. pseudoglaziovii* the presence of such restitution was confirmed in the tetrad stage where the formation of both dyads and tetrad stage where the formation of both dyads and terads was observed. In various crops, interspecific hybridization has lead to the disturbance of meiotic division, with consequent meiotic restitution, e.g. in *Trifolium pratense* (Parrott and Smith 1988) ande in *Medicago* spp. (Vorsa and Bingham 1979). This is an important cytogenetic phenomenon in crop breeding as it leads to 20 gamete formation and consequently sexual polyploidization (Mock and Peloquim 1975; Mendiburu and Peloquim 1976; Mendiburu and Peloquim 1977). In the case of *Manihot* species, Hahn et al. (1990) reported 20 pollen formation in wild species in addition to certain clones of cassava itself. The detection of this phenomenon enable Hahn et al. (1991) to isolate triploid and tetraploid types from progeny derived from crosses of cassava with *Manihot glaziovii* and *M. epruinosa*. These types proved much more productive than commercial clones used in Nigeria. Nassar (1992) manipulated this phenomenon in interspecific hybrids of *Manihot pseudoglaziovii* with cassava to produce triploid types with very hight productivity under semi-arid conditions. The occurrence of this phenomenon with high frequency in interspecific hybrids of cassava offers an effective tool for the traditional method of treating vegetative parts with colchicine may induce unstable chimeral types (Abraham et al. 1964). An additional advantage is that production of triploid types may lead to production of trisomics among their progeny. If genes controlling productivity in cassava are polygenes with additive gene action, as is the case of man crops, certain trisomics of this crop may be more productive than their diploid ancenstors. Generally, obtaining polyploidy by sexual means is advantageous from the standpoint of both genetics and evolution since the heterotic effect may increase vigour and genetic variability, a useful feature for further adaptation.

For the study of the tetrad stage (Table 2), 1065 PMCs were examined. Of these, 62 cells formed tryads while 47 formed dyads. Two hundred and sixty-two cells formed micronuclei.

Table 1 - Chromosome configurations at metaphase I in interspecific *Manihot* hybrids and their parents.

		Mean		
	Nº PMCs	*Trivalents*	*Bivalents*	*Univalents*
M. Neusana	20	-	18.00	-
cassava	20	-	18.00	-
HN	100	-	16.13	1.58
HO1	30	1.86	16.13	0.13
HO4	100	1.63	12.41	8.84

HN, F_1 interspecific hybrid; HO1 and HO4, F_2 progenies

Table 2 - Analysis of tretads of the interspecific *Manihot* hybrids and their parents.

	Nº Tetrads			*PCMS with Micronuclei*		*Tryads*		*Dyads*	
	Total	*Normal*	*%*	*Nº*	*%*	*Nº*	*%*	*Nº*	*%*
M. Neusana	1011	1003	99.22	6	0.59	2	0.19	-	-
cassava	950	942	99.15	6	0.53	2	0.31	-	-
HN	1065	694	65.15	262	24.60	62	5.82	47	4.41
HO1	900	644	71.55	218	24.22	26	2.88	12	1.33

HN, F_1 interspecific hybrid; HO1, F_2 progeny

Table 3 - Viability of pollen of the interspecific *Manihot* hybrids and their parents.

	Nº Pollen analyzes	*Viable Pollen*		*Inviable Pollen*
	Nº	*Nº*	*%*	*Nº*
M. Neusana	1001	818	81.72	183
cassava	1235	1162	94.09	73
HN	1235	453	36.68	782
HO1	1830	655	35.80	1175
HO4	1542	273	17.70	1269

HN, F_1 interspecific hybrid; HO1, HO4; F_2 progeny

Table 4 - Diploid pollen in interspecific *Manihot* hybrids and their parents.

		Diploid pollen	
	Nº pollen examined	*Nº*	*%*
M. neusana	818	3	0.36
cassava	1162	3	0.26
HN	1235	20	1.62
HO1	1128	8	0.71
HO4	1007	6	0.60

HN, F_1 interspecific hybrid; HO1, HO4, F_2 progeny

The presence of dyads and tryads at this stage confirms earlier observations of the occurrence of FMR and SMR during anaphase. Both types are capable of producing 2n gametes. However, FMR is more valuable since it preserves most heterosis and epistatic interactions (Mendiburu and Peloquin 1976, 1977). For analysis of pollen grain viability (Table 3) and diploid pollen formation (Table 4), 1235 pollen grain were studied. Of these only 453 (36.68%) were viable. Such low viability is attributed to irregularity of chromosome pairing. Of these 1235 pollen grains, only 20 were found to be diploid.

Cytogenetic Behaviour of the Backcrossed Generation (HO1)

The frequency of chromosome configurations at metaphase I is shown in Table 1. Bivalents, trivalents and univalents were present with a mean of 16.1 bivalents, 1.86 trivalents and 0.13 univalents. The presence of trivalents indicated the presence of aneuploidy in this hybrid. This was confirmed by miotic couting which showed 2n = 38 i.e, 2n + 2. Laggards were found with varying frequency in anaphase I. The study of 900 tetrads showed 644 to be normal while 218 had micronuclei. Twelve were dyads and 26 were triads. The study of pollen viability revealed that only 35.8% were viable.

Cytogenetic Behaviour of the Backcrossed Generation (HO4)

One hundred PMCs at metaphase I were studied. Again, bivalents, trivalents and univalents with means of 12.4, 1.6 and 8.8, respectively, were observed (Table 1). The total chromosome count in different configurations was 38. This means it had a 2n + 1 + 1 make-up which was confirmed by root tip mitotic counts. In anaphase I, of 32 PCMs studied, 31 contained laggards. In anaphase II, 35 PMCs were examined, of which 7 appeared as restitution nuclei, wich was later confirmed in the tatrad stage. In the tetrad stage 1196 sporocytes were observed, of wich 326 were normal, 826 contained micronuclei, 25 were tryads, and 19 were dyads. The study of pollen viability showed a low frequency of viable grains (17.7%).

Cytogenetic Behaviour of the Parents

The EBO1 cassava clone showed regular meiotic division in all of the 20 PMCs studied white 18 bivalents formed. A total of 950 tetrads were examined, of which 942 were normal, 5 contained micronuclei and 3 were tryads. The use of *M. neusana* as the parent in this experiment showed it to have regular meiosis with 18 bivalent formation. Out of 1011 tetrads studied, 1003 were normal while 6 had micronuclei, and two were dyads. The pollen viability was 81.72%. The mitotic counting of root tips showed 2n = 36. This is the first report of chromosome number of this new species recently described by Nassar (1985).

Evolutionary and Plant Breeding Significance

The fertility of hybrid HO1 was sufficient to further manipulate it through backcrosses to tranfer useful genes of *M. neusana* to cassava. The backcrossed generation produced were aneuploid (2n + 1 + 1) in the two cases studied (HO1 and HO4). The HO4 hydrid was completely sterile having the chromosomal constitution of 2n + 1 + 1. Obviously this hybrid resulted from fertilization of an n + 1 + 1 pollen gamete of the parent hybrid (HN) whit an n cassava ovule. However, when the first generation HN interspecific hybrid was used as a maternal plant fertile progeny were produced. When it was used as a pollen parent in the backcross whit cassava, sterile progeny (HO4) were produced. This may be due to the elimination of fertile embryo genotypes of the progeny because of incompatility or disharmony between the embryo and the endosperm. The partial fertility of the backcrossed generation (HO1) shows that *M. neusana* may be classified whithin the secondary gene pool of cassava according to Harlan and de Wet (1971). Other *Manihot* species that can be inclued in this category due to their partial crossability with cassava are: *M. melanobasis* (Jennings 1959), *M. glaziovii* (Magoon et. al 1970), *M. reptans*, *M. zenhtneri*, *M. anamola*, *M. oligantha*, *M. pohlii* (Nassar et al. 1985), and *M. dichotoma*, *M. epruinosa*, *M. leptophylla* (Hahn et al. 1990).

The cassava – *M. neusana* hybrid shows irregular meiotic behaviour, demonstrated by the lack of complete chromosome pairing, meiotic restitution and reduced pollen viability. When backcrossed to cassava, this interspecific hybrid produced aneuploids with irregular meiosis and meiotic restitution. The presence of meiotic restitution in both the hybrid and BC1 appear to be correlated with interspecific meiotic irregularity.

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Abbreviations:

FMR - first meiotic restitution
PMC - pollen mother cell
SMR - second meiotic restitution