Interspecific Hybrid of Cassava and its Cytogenetic Behaviour[1]

H.N. Nassar*, N.M.A. Nassar*,
C. Vieira**, L.S. Saraiva***

ABSTRACT

An interspecific hybrid between cassava, *Manihot esculenta* Crantz and *M. neusena* Nassar and its progeny were examined meiotically and mitotically. The F1 hybrid showed an irregular meiosis due to unpaired chromosomes. The backcrossed progeny varied from partially fertile to sterile. Meiotic restitution was also recorded in all of the hybrids. Diploid pollen formation was also observed. The irregular meiosis of the F1 hybrid led to production of aneuploidy in its backcrossed progeny. The use of F1 hybrid as a maternal plant in backcrosses with cassava improved seed setting in F2 progeny, while the reciprocal backcross produced sterile progeny.

Key words: Wild species, aneuploid, irregular meiosis, meiotic restitution, dyad, tryad.

RESUMEN

Se examinaron las características meióticas y mitóticas de un híbrido interespecífico entre yuca *Manihot esculenta* Crantz y *M. neusena* y de su progenie. El híbrido F1 mostró una meiosis irregular debido a la disparidad de sus cromosomas. La progenie retrocruzada varió desde parcialmente fértil a estéril. También se registró la restitución meiótica en todos los híbridos, y se observó la formación de polen diploide. La meiosis irregular del híbrido F1 conllevó a la producción de aneuploidía en su progenie retrocruzada. El uso del híbrido F1 como planta madre en los retrocruzamientos con yuca mejoró la viabilidad de las semillas en la progenie de F2, mientras que el retrocruzamiento recíproco produjo una progenie estéril.

INTRODUCTION

Wild species of different crops have been used frequently as a source of useful genes through interspecific hybridization (Harlan 1976; Hawkes 1977; Rick 1978). The only published case of interspecific hybridization in cassava has been that of Nichols (1947) who successfully transferred genes for resistance to mosaic from *M. glaziovii* to cassava, saving it from extinction in East Africa. Since 1976, Nassar has systematically evaluated wild manioc species native to South America for different useful characters (Nassar 1978 a,b,c,e; 1979, 1985, 1986). Moreover, he established and maintained a living collection of these species at the Universidade de Brasilia for systematically interspecific hybridization with cassava. One of the most interesting species for breeding is *Manihot neusana* Nassar. It is tolerant to low temperature, resistant to stem borers, highly resistant to bacteria and is an evergreen (Nassar 1985). An interspecific hybrid between this species and cassava was obtained early by the second author (Nassar 1989). This paper describes the cytogenetic behaviour of this hybrid and its backcrossed progeny.

MATERIAL AND METHODS

The wild manioc species *M. neusana* was hybridized with the cassava clone Catelo through controlled hybridization with the help of pollinating insects (Nassar 1989; Nichols 1947). An interspecific hybrid that combined marker genes of both parents was obtained. The marker genes were ribbed fruit, acquired from cassava, and variegated fruit color from *M. neusana* (Fig. 1). This hybrid (HN) was backcrossed with cassava and used as a pollinator in one of the trials and as a fruit carrier in the second trial.

1 Received for publication on November 22, 1993
This work was carried out with the help of a grant from the *Conselho Nacional de Desenvolvimento Cientifico e Tecnologico* (CNPq), Brasilia. Bra. The living collection was initiated by the Canadian International Development Research Center (IDRC), to whom we are very grateful

* Departamento de Agronomía, Universidade de Brasilia, Brasilia
** Departamento de Fitotecnia, Universidade de Vicoa. Vicosa. Bra
*** Departamento de Biologia General. Universidade de Vicoa. Bra

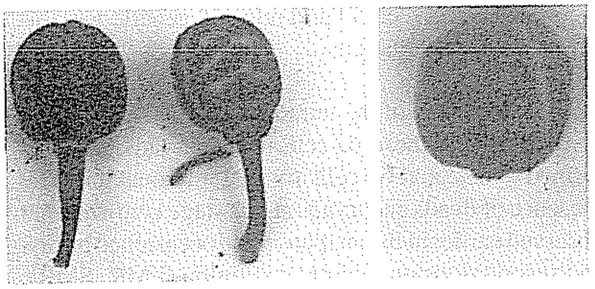

Fig. 1. a) Marker genes, winged fruit of cassava (left) and variegated fruit of *M. neusana* (right); b) fruit of the F1 hybrid (HN).

Seeds were obtained from both crosses, but only one plant could be raised from each; HO1 was the result of the interspecific hybrid (HN) as maternal plant (seed carrier), and HO4 resulted from crosses where the interspecific hybrid (HN) was used as pollinator. The three hybrid plants (HN, H1 and H4) were studied cytogenetically for both meiotic and mitotic behavior. For the study of meiosis, inflorescenses were fixed in a mixture of three parts absolute alcohol and one part glacial acetic acid and kept in the refrigerator for 24 hours. The anthers were smeared with acetic carmine. Chromosome configurations in the metaphase, chromosome distribution in anaphase I and tetrad formation were also studied. Pollen viability had been determined by using acetocarmine and iodine stain (Nassar 1978d). For mitotic study, root tips were left in 0.2% colchicine for 2 h, then fixed in acetic alcohol for 24 hours. Before smearing with acetocarmine, they were treated with HCL 1N for 10 minutes.

RESULTS AND DISCUSSION

Meiotic behaviour of the El hybrids (HN)

One hundred pollen mother cells (PMCs) were studied in metaphase I of the interspecific hybrid *M. neusana* with cassava; 30 PMCs in metaphase II and 1000 tetrads of the same material were also investigated. The study of metaphase I showed different chromosome configurations, as seen in Table 1. The average of bivalent frequency in all cells of metaphase I was 16.13 per cell. The high frequency of univalents was attributed to lack of synapses between chromosomes, or failure of the two species to remain associated.

Table 1. Frequency of chromosome configurations of metaphase I in interspecific *Manihot* hybrids and their parents.

	PMCs (no.)	Trivalents	Mean Bibalents	Univalents
N neusana	20	-	18.00	-
Cassava	20	-	18.00	-
HN	100	-	17.00	1.58
HO1	30	1.86	16.13	0.13
HO4	100	1.63	12.41	8.84

Virtually the only other report on this subject is that of Magoon *et al.* (1970) where chromosome pairing in the interspecific hybrid *M. glaziovii* (rubber tree) and cassava was studied, and a regular synapsis that led them to conclude that there is a strong relationship between this species and cassava. Nassar, Silva and Vieira (1985) suggested that the material used by Magoon was not a pure *M. glaziovii*, but rather a natural interspecific hybrid between this species and cassava. If this is true, the supposed interspecific hybrid would be a backcrossed progeny. The study of anaphase I showed that out of 40 PMCs studied, 38 cells exhibited laggards, which were attributed to the occurrence of univalents resulting from non-homologous chromosomes (Fig. 2).

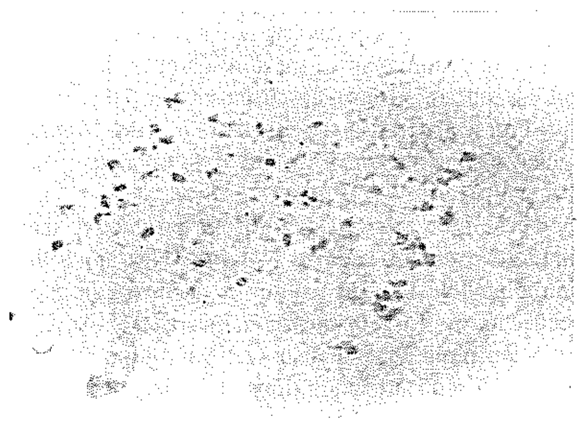

Fig. 2. Breakdown of anaphase I in F1 hybrid (HN).

Anaphase II showed meiotic restitution. Of 33 PMCs studied in this phase, five cells exhibited a second meiotic restitution (SMR), forming 36 chromosomes on each pole. Apparently this phenomenon is a consequence of meiotic disturbance in the hybrid. An example of this disturbance was the breakdown of anaphase I. Probably this was due to disharmony between the two different genomes. Nassar (1992) previously documented this phenomenon in the interspecific hybrids of cassava with *M. pseudoglaziovii*. The presence of such restitution was confirmed in the following tetrad stage, where the formation of both dyads and terads was observed (Fig. 3).

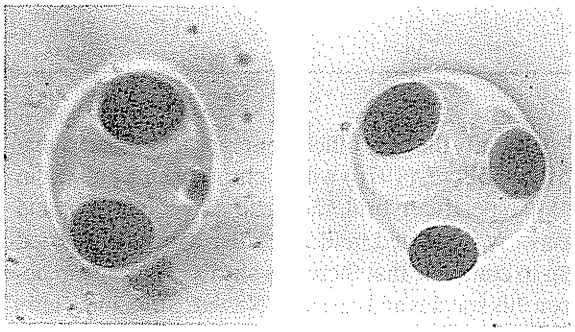

Fig. 3. Dyad and tryad in F1 hybrid (HN).

In various crops, interspecific hybridization has led to the disturbance of meiotic division, with consequent meiotic restitution, e.g., in *Trifolium pratense* by Parrott and Smith (n.d.), and in *Medicago* spp. by Vorsa and Bingham (1979). In manioc species, Hahn *et al.* (1990) reported 2n pollen formation in wild species, in addition to certain clones of cassava itself. The detection of this phenomenon enabled these researchers to isolate triploid and tetraploid types from progeny that came from crosses of cassava with certain wild *Manihot* species, namely *M. glaziovii* and *M. epruinosa*.

These types proved much more productive than commercial clones used in Nigeria. Nassar (1992) manipulated the meiotic restitution occurring in interspecific hybrids of *M. pseudoglaziovii* with cassava to produce triploid types that showed very good productivity under semi-arid conditions. The discovery of the frequent occurrence of this phenomenon in interspecific hybrids of cassava offers an effective tool for the production of polyploid types by sexual means, instead of the traditional method of colchicine applied to vegetative parts, which normally induces unstable, chimeral types (Abraham *et al.* 1964). An additional advantage is that production of triploid types may lead to production of trissomics among their progeny. If genes which control productivity in cassava are polygenes with additive model action, as it is the case for many crops, certain trissomics of this crop may be more productive than their diploid ancestors. In general, the production of polyploidy via sexual means is advantageous from both genetic and evolution viewpoints, as it offers a vigorous heterotic effect and releases genetic variability useful for adaptations.

For the study of the tetrad stage, 1065 PMC were investigated. Of these, 62 cells formed tryads, while 47 formed dyads, and 62 cells formed micronuclei. The presence of dyads and tryads at this stage confirms that observed earlier in the anaphase: the occurrence of first and second division meiotic restitution (FMR, SMR). Both these types are capable of producing 2n gametes. However, the FMR is more valuable since it preserves the major part of its heterosis and epistatic interaction (Mendiburu and Peloquin 1977; Parrot and Smith n.d.; Vorsa and Bingham 1979). For the viability study (Table 3), 1235 pollen grain were analyzed, of which 36.68% were found viable.

Table 2. Diploid pollen in interspecific *Manihot* hybrids and their parents.

	Pollen grains examined (no.)	Diploid pollen (no.)	Diploid pollen (%)
M. neusana	818	3	0.36
Cassava	1 162	3	0.26
HN	1 235	20	1.62
HO1	1 128	8	0.71
HO	1 007	6	0.60

This low viability was attributed to irregularity of chromosome pairing. Of these 1235 pollen grains, 20 were found to have a diploid size pattern (Table 2).

Cytogenetic behaviour of the backcrossed generation (HOI)

Table 1 shows frequency of chromosome configurations at metaphase I. 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 aneuploidy in this hybrid (Fig. 4). This was confirmed by mitotic counting, which sowed 2n=38, i.e., 2n+2 (Fig. 5).

In anaphase I, the presence of laggards with different frequencies was recorded. The study of 900 tetrads showed 644 normal ones, 218 micronuclei, 12 dyads and 26 tryads. Analysis of pollen viability revealed that only 35.8% were viable (Table 3.).

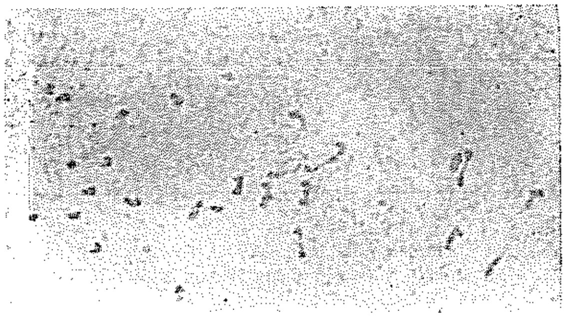

Fig. 4. Chromosome configuration in metaphase I of hybrid HO1 shows presence of two trivalents.

Cytogenetic behaviour of the backcrossed generation (HO4)

One hundred PMCs at metaphase I were studied Again, bivalents, trivalents and univalents with averages of 12.4, 1.6 and 8.8, respectively, were observed. The total chromosome count for the different configurations was 38. This showed a constitution of 2n + 1 + 1, which was confirmed by root tip mitotic counting. In

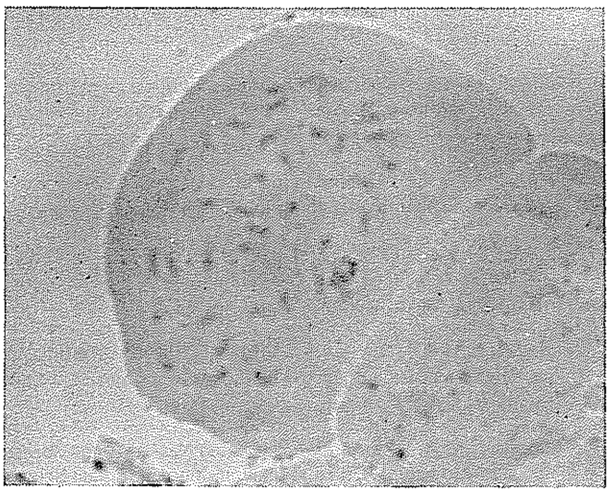

Fig. 5. Mitotic metaphase in root tip of hybrid HO1, with 38 chromosomes (2n+1+1).

anaphase I, of 32 PMCs studied, 31 proved to have laggards. In anaphase II, 35 PMCs were examined; of these, seven cells appeared as restitution nuclei, later confirmed in the tetrad stage

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

	Pollen analyzed (no.)	Viable pollen (no.)	Viable pollen (%)	Non-viable pollen
M. neusana	1 001	818	81 72	183
HN	1 235	1 162	94 09	73
HO1	1 830	655	35 80	1 175
H04	1 542	273	17 70	1 269

In the tetrad stage, 1196 sporocytes were observed. Of these, 326 were normal, 826 contained micronuclei, 25 were tryads, and 19 were dyads. The study of pollen viability showed a very low viable grain percentage of 17.7% (Table 3).

Cytogenetic behaviour of the parents

The cassava clone EB01 showed a regular meiotic division in all of the 20 PMCs studied, with 18 bivalents formed. A total of 950 tetrads were examined, of which 942 were normal, five contained micronuclei and three were tryads (Table 4).

The study of *M. neusana* used as a parent in this experiment showed it to have a regular meiosis with 18 bivalent formations. Of 1011 tetrads studied, 1003 were normal, while six had micronuclei, and two were dyads. The pollen viability was 81.72% (Table 3). Mitotic counting of root tips showed 2n=36. This was the first report of a chromosome number for this new species, recently described by Nassar (1985).

Evolutionary and plant breeding significance

The fertility of the hybrid H01 shows the possibility of further manipulation of this hybrid through backcrosses to transfer useful genes of *M. neusana* to cassava. The backcrossed generations produced were aneuploid 2n + 1 + 1 in both cases studied (H01 and H04). In the case of hybrid H04, the plant was completely sterile, having a chromosomal constitution of 2n + 1 + 1. Obviously, this hybrid resulted from fertilization of a pollen gamete n + 1 + 1 of the parent hybrid (HN), with a cassava ovule of "n". On the other hand, when the interspecific hybrid HN was used as maternal plant, a fertile progeny was obtained. When it was used

Table 4. Analysis of tetrads of interspecific *Manihot* hybrids and their parents.

	Tetrads (no.)			PMCs with		Tryads		Dyads	
	Total	Normal	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)
M neusana	1 011	1 003	99 22	6	0 59	2	0 19	-	-
Cassava	950	942	99 15	5	0 53	3	0 31	-	-
HN	1 065	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

as a pollen parent in the backcross with cassava, it resulted in the production of a sterile progeny (H04). Probably this was due to the elimination of fertile embryo genotypes in the progeny because of incompatibility or disharmony between them and the endosperm. The partial fertility of the backcrossed generation (H01) shows that the species *M. neusana* may be classified within the secondary gene pool of cassava according to the concept of Harlan and de Wet (1971). Other *Manihot* species that may fit in this category are: *M. melanobasis* (Jennings 1959), *M glaziovii* (Magoon 1970), *M. reptans*, *M. zenhtneri*, *M. anomala*, *M. oligantha*, *M. pohlii* (Nassar *et al.* 1985), and *M. dichotoma*, *M. epruinosa*, *M. leptophylla* (Hahn *et al.* 1990).

It was concluded that the cassava hybrid with *M. neusana* sowed irregular meiotic behavior in the lack of complete chromosome pairing, formation of univalents in metaphase I, chromosome retardation in anaphase I, micronuclei in the tetrad, meiotic restitution and reduced pollen viability. When backcrossed to cassava, this interspecific hybrid produced two aneuploids 2n + 1 + 1. These showed irregular meiosis, partial chromosome pairing and the presence of meiotic restitution. The two backcrossed F2 hybrids differed as regards pollen viability and fertility, which was attributed to variable genetic constitutions. The meiotic restitution continued to occur in F2 hybrids, which sowed that it must be correlate with interspecific meiotic irregularity.

LITERATURE CITED

ABRAHAM, A ; PANICHER, P K ; MATHEW, P M 1964 Polyploids in relation to breeding in tuber crops Journal of the Indian Botanical Society 43:278-283

HAHN, S K ; BAI, K.V ; ASIEDU R 1990 Tetraploids, triploids and 2n pollen from diploid interspecific crosses with cassava Theoretical and Applied Genetics (Germany) 79:433-439

HARLAN, J R 1976. Genetic resources in wild relatives of crops Crop Science (EE UU) 16:329-333

HARLAN, J R ; DE WET, J W J 1971 Towards a rational classification of cultivated plants Taxon 20:509-517

HAWKES, J G 1977 Importance of wild germplasm in plant breeding Euphytica 26:615-621

JENNINGS, D L 1959 *Manihot melanobasis* Muell -Arg.: A useful parent for cassava breeding Euphytica 8:157-162

MAGOON, M L ; KRISHNAN R ; VIJYABAI, K 1970 Cytogenetics of F1 hybrids between cassava and *M Glaziovii* (Ceara rubber) and its backcrosses Genetics 41:425-436

MENDIBURU, A O ; PELOQUIN, S J 1977 Bilateral sexual polyploidization in potatoes Euphytica 26:573-583

NASSAR, N M A 1978a Conservation of the genetic resources of cassava: Determination of wild species localities with emphasis on probable origin Economic Botany (EE UU) 32:311-320

NASSAR, N M A 1978b Wild *Manihot* species of Central Brazil for cassava breeding Canadian Journal of Plant Science 58:257-261

NASSAR, N M A 1978c Some further *Manihot* species of potential value for cassava breeding Canadian Journal of Plant Science 58:915-916

NASSAR, N M A 1878d Genetic resources of cassava: Chromosome number of *Manihot* species Indian Journal of Genetics and Plant Breeding 38:135-137

NASSAR, N M.A 1978e Microcenters of cassava diversity in Central Brazil Turrialba (C R) 28:345-347

NASSAR, N.M.A 1979 Three wild *Manihot* species of Central Brazil with tolerance to stress conditions Canadian Journal of Plant Science 59:553-555

NASSAR, N M A ; SILVA, J R ; VIEIRA, C 1985 Hibridacoes interespecificas no genero Manihot Adans Ciencia e Cultura 38:1050-1055

NASSAR, N M A 1985. *Manihot neusana* Nassar: A new species native to Parana, Brazil Canadian Journal of Plant Science 65:1097-1100

NASSAR, N M A 1986 Genetic variation of a wild *Manihot* species native to Brazil and its potential for cassava improvement Field Crop Research 13:177-184

NASSAR, N.M A 1989 Broadening the genetic base of cassava *Manihot esculenta* Crantz by interspecific hybridization Canadian Journal of Plant Science 69:1071-1073

NASSAR, N.M.A.; CARVALHO, C.G. 1991. Insetos polinizadores e seus comportamento nas especies silvestres da mandioca, *Manihot* spp. Ciencia e Cultura 42:703-706.

NASSAR, N.M.A. 1992. Production of triploid cassava: *Manihot esculenta* Crantz by diploid hybrid gamete. Field Crop Research 30:173-182.

NICHOLS, R.F.W. 1947. Breeding cassava for virus resistance. East African Agricultural Journal 12:184-194.

PARROTT, W.A.; SMITH, R.R. n.d. Production of 2n pollen in red clover. Crop Science 24:469-472.

RICK, C.M. 1978. Potential improvement of tomatoes by controlled introgression of genes from wild species. In Broadening the genetic base of crops. A.C. Zeven, M.A. Harten (Eds.). Wageningen. 347 p.

VORSA, N.; BINGHAM, E.T. 1979. Cytology of pollen formation in dipoid alfalfa, *Medicago sativa*. Canadian Journal of Genetics and Cytology 21:525-530.