

# Induction of a productive aneuploid in cassava, *Manihot esculenta* Crantz

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## ABSTRACT

Progenies of two interspecific hybrids of cassava (*Manihot esculenta* Crantz) with wild *Manihot* species were studied meiotically as well as mitotically. An aneuploid ( $2n + 2$ ) was isolated among progeny of the interspecific hybrid of cassava with *M. pseudoglaziovii*, and two  $2n + 2$  aneuploids were isolated from progeny of the interspecific hybrid of cassava with *M. neusana*. One of these aneuploids derived from progeny of the interspecific hybrid cassava  $\times$  *M. pseudoglaziovii* had a very large starchy root, while the other two aneuploids had fibrous roots. It is presumed that root formation in cassava is controlled by additive polygenes that are distributed on more than one chromosome.

## INTRODUCTION

The first case of an artificially induced aneuploid in plants was reported in *Datura stramonium* by Blackeslee and Avery (1938). Later, a few plant species were reported to have had aneuploidy induced artificially (Khush, 1973). In cultivated crops of economic value, it seems on first analysis that aneuploid constitution will not improve production. In cassava, *Manihot esculenta* Crantz, this type of chromosome constitution is unknown.

## MATERIAL AND METHODS

Wild *Manihot* species have been systematically evaluated and hybridized with cassava to transfer useful genes (Nassar, 1978a, 1980, 1986). Two inter-

specific hybrids between cassava and wild *Manihot* species have been obtained and/or maintained in our program. One of the two interspecific hybrids was between cassava and *M. pseudoglaziovii* Pax (Nassar, 1982); the second hybrid was between cassava and *M. neusana* Nassar (Nassar, 1989). These hybrids were cloned by vegetative reproduction and seed produced by open pollination. Ten seeds were collected from each hybrid for germination studies. Four seeds from progenies of cassava with *M. pseudoglaziovii* germinated, of which one plant was selected for vigor. Only two seeds of progenies of the hybrid of cassava with *M. neusana* germinated, of which one plant was selected for vigor.

These three plants were cloned by vegetative propagation of cuttings. When the plants flowered, the three clones and their parents were studied meiotically for chromosome association in metaphase I, segregation in anaphase I and pollen viability. Mitotic analysis was also performed on root tips taken from cuttings. After nine months of growth, these clones were examined for root productivity. For the study of meiosis, buds were fixed in a 3:1 mixture of ethyl alcohol and glacial acetic acid for 24 h, transferred to 70% alcohol, and the smear

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stained with 1% acetic carmin. For mitotic counting of chromosomes, root tips were treated with 0.2% colchicine for two hours, washed in distilled water, transferred to the above mentioned fixative fluid for 24 h, treated with 1 N HCl for 10 min, and smears stained with 1% acetic carmin.

## RESULTS AND DISCUSSION

Meiotic examination in metaphase I of the clone, progeny of the interspecific hybrid of cassava with *M. pseudoglaziovii* (HPS), showed a mean of 16.19 bivalents, 1.54 trivalents, and 1.00 univalent for the 15 sporocytes examined (Figure 1). These results suggested an aneuploid having a  $2n + 2$  chromosome constitution. The parental hybrid showed  $2n = 36$ , with a mean association of 17.42 bivalents and 1.58 univalents. Estimation of pollen viability revealed a viability percentage of 54% in 2917 aneuploid grains and 40% of 1350 in the parental hybrid.

The hybrid of cassava and *M. neusana* (HO1) had a mean of 1.86 trivalents, 16.13 bivalents, and 0.13 univalents among 30 PMCs examined. The chromosome constitution was  $2n = 38$ . This was confirmed by chromosome counting at mitosis of root tip cells. The second clone of this cross (HO4) showed a mean chromosome association of 1.63 trivalents, 12.41 bivalents, and 8.84 univalents in metaphase I. This clone had  $2n = 38$  also. The parent had a mean of 17.42 bivalents and 1.58 univalents, which means  $2n = 36$ . Pollen viability was found to be 35.8% in HO1, 17.7% in HO4, and 36.8% in their parent.

The progeny of the interspecific hybrid of cassava with *M. pseudoglaziovii* had a very large starched

root, with a mean of 6.1 kg per plant at 10 months for the 15 plants evaluated, compared to 3.7 kg for the cultivated cassava clones. The other two aneuploids had fibrous roots.

These aneuploids apparently resulted from disturbance of meiotic division of their interspecific hybrid parents. This disturbance was interpreted by Nassar *et al.* (1995) as an inhibition of spindle function and chromosome asynapsis. Both have led to laggard formation and unbalanced gametes. Thus, the origin of these aneuploids can be attributed to the fertilization of an  $n + 1$  egg by an  $n + 1$  male gamete, based on the fact that gametes with this structure are more viable than gametes with  $n + 2$ . The other possibility is fertilization of an  $n + 2$  egg with an external  $n$  male gamete because the plants were open pollinated. It was not possible to determine whether the male gamete came from selfing or from another pollinator plant. In various crops, interspecific hybridization has led to the disturbance of meiotic division. Similar findings were reported in *Trifolium pratense* by Parrott and Smith (1984), and in *Medicago spp.* by Vorsa and Bingham (1979). No aneuploids however were obtained in these crops.

The two additional chromosomes in clone HPS resulted in the production of a large tuber root that is superior to normal root production in cultivated cassava clones. In all likelihood this size increase is due to additive polygenes. These polygenes may be distributed on more than one chromosome.

Nassar (1978b) postulated the origin of cassava as a product of hybridization between two wild species, both having fibrous non-starchy roots. Their hybrid, cassava, apparently acquired a starchy root as a result of an accumulation of additive polygenes derived from ancestral wild parents.

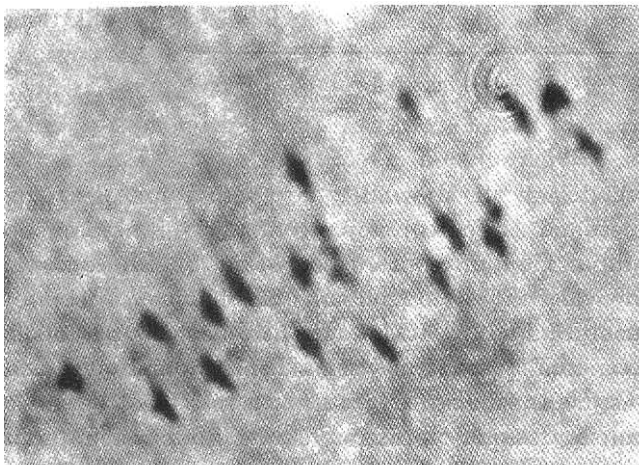


Figure 1 - Metaphase I of double trisomic (progeny of hybrid of *M. pseudoglaziovii* X cassava) showing  $1\text{ III} + 17\text{ II} + 1\text{ I}$ .

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## RESUMO

Progenie de dois híbridos interespecíficos da mandioca com espécies do gênero *Manihot* foram estudadas meioticamente e mitoticamente. Um aneuploide muito produtivo ( $2n + 2$ ) foi isolado da progênie de um híbrido da

mandioca com *Manihot pseudoglaziovii* enquanto aneuplóides com raízes fibrosas foram isoladas da progênie da mandioca com *M. neusana*. Supõe-se que o caráter de formação de raízes seja controlado através do modelo aditivo.

## REFERENCES

- Blackeslee, A.F. and Avery, A.C.** (1938). Fifteen year breeding records of  $2n + 1$  types of *Datura stramonium*. *Cop. in Res., Carnegie Inst. Wash. Pub.* 501: 315-361.
- Khush, G.S.** (1973). *Cytogenetics of aneuploids*. Academic Press. N.Y.
- Nassar, N.M.A.** (1978a). Wild *Manihot* species of central Brazil for cassava breeding. *Can. J. Plant. Sci.* 58: 257-261.
- Nassar, N.M.A.** (1978b). Conservation of the genetic resources of cassava (*Manihot esculenta* Crantz): Determination of wild species localities with emphasis on probable origin. *Econ. Bot.* 32: 311-332.
- Nassar, N.M.A.** (1980). Attempts to hybridize wild *Manihot* species with cassava. *Econ. Bot.* 34: 13-15.
- Nassar, N.M.A.** (1982). Collecting wild cassava in Brazil. *Indian J. Genet.* 42: 405-411.
- Nassar, N.M.A.** (1986). Genetic variation of wild *Manihot* species native to Brazil and its potential for cassava improvement. *Field Crop. Res.* 13: 177-184.
- Nassar, N.M.A.** (1989). Broadening the genetic base of cassava, *Manihot esculenta* Crantz by interspecific hybridization. *Can. J. Plant Sci.* 69: 1071-1073.
- Nassar, H.N.M.A., Nassar, N.M.A., Vieira, C. and Saraiva, I.S.** (1995). Cytogenetic behaviour of interspecific hybrid of *Manihot neusana* Nassar and cassava, *M. esculenta* Crantz, and its backcross progeny. *Can. J. Plant Sci.* 75: 675-678.
- Parrott, W.A. and Smith, R.R.** (1984). Production of  $2n$  pollen in red clover. *Crop Sci.* 24: 467-472.
- Vorsa, N. and Bingham, E.T.** (1979). Cytology of pollen formation in diploid alfalfa, *Medicago sativa*. *Can. J. Genet. Cytol.* 21: 526-530.

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