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Overcoming crossing barriers between cassava, *Manihot esculenta* Crantz and a wild relative, *M. pohlii* Warwa

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ABSTRACT

The use of mentor pollen has enabled successful hybridization between cassava, *Manihot esculenta* Crantz, and the wild species *M. pohlii* Warwa. Killed pollen of a cross compatible type produced by freeze-thawing was mixed with incompatible pollen and the mixes were dusted on stigmas. This treatment resulted in production of seed in 4.9% of the total pollinations, compared to 0% in the case of untreated pollinations. The use of a bridge species, *M. neusana* Nassar, through the hybrid *M. pohlii* and *M. neusana* also proved successful in overcoming interspecific barriers between cassava and *M. pohlii*.

INTRODUCTION

Interspecific hybridization has provided means of transferring desirable characters from one species to another. In cassava, *Manihot esculenta* Crantz, many useful traits have been shown to be present in wild *Manihot* species, such as tolerance to stress conditions and adaptation to a wide range of environments (Nassar, 1978, 1985, 1986). *M. pohlii* has a high resistance to stem borers, *Coelosternus* spp, and to bacteria, *Xanthomonas manihotis*. Hybrids of *M. pohlii* and other species with cassava can be obtained, but at a very low frequency due to interspecific crossing barriers (Nassar *et al.*, 1986). However one technique for overcoming such barriers involves the use of pollen mixes which combine the pollen intended for syngamy with "mentor" pollen of the maternal species. This technique has been reported for a number of plant taxa including *Populus* (Stettler, 1968; Knox *et al.*, 1972), *Malus* (Dayton, 1974), *Cosmos* (Howlett *et al.*, 1975), and *Petunia* (Sastri and Shivanna, 1976). The role of the mentor pollen is to facilitate fertilization by foreign pollen. Apparently, the mentor pollen supplies proteinaceous substances which permit the foreign incompatible pollen grains to germinate (Knox *et al.* 1972). A treatment destroys the generative function of the pollen grain without affecting germination and growth of the pollen tube, and consequently does not affect stimulation. The stimulating effect of destroyed pollen is due to protein recognition substances. They are liberated by pollen grains while germinating, and have enzymatic and antigenic properties. These substances are localized in the internal layer of the pollen surface, and are correlated with pollen germination and growth of its tube on the stigma surface. They have been localized by cytochemical means in the cellulose intine (Knox *et al.*, 1972). Many studies using this technique have been successful in overcoming an incompatibility barrier (Brewer and Henstra, 1974, Williams and Church, 1975). Subsequent work indicated the need to refine the preparation of the mentor pollen by using freeze thaw cycles or methanol treatment (Knox *et al.*, 1972). The present communication reports the successful use of

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freezed mentor pollen in hybridizing cassava with *M. pohlii*.

In addition to the mentor effect, other techniques seem to have potential for overcoming interspecific barriers. One of these may be the use of a bridge species. We were inspired by the capacity of a species, *M. neusana* Nassar, to cross easily with all *Manihot* species growing in the vicinity, and have used it as a bridge species. Very little information is available on the bridge technique. Probably the only case is that which came from Dionne (1963) who used *Solanum acaule* Bitter as a bridge between *Solanum tuberosum* L. and *Solanum bulbocostanum* Dunal. We report here the use of *M. neusana* Nassar as a bridge between *M. esculenta* and *M. pohlii*.

MATERIAL AND METHODS

A natural hybrid between *M. pohlii* and *M. neusana* Nassar was used as a bridge species. The hybrid was identified by fruit marker genes, which produce variegation of fruit color in *M. neusana*, and a straight white line in *M. pohlii* fruit (Figure 1). Crosses of this hybrid (named HNP) and cassava (clone EB 05) were carried out from January 1993 to January 1994.

Flowers were taped shut for two days until they had been pollinated manually. Pollination of both the hybrid HNP and cassava was done with pollen mixes of cassava and *M. pohlii*. *M. pohlii* pollen used as mentor pollen was successively frozen for 5 min at -4°C and thawed for 30 min for a period of 105 min. The purpose of this treatment was to kill the mentor pollen and increase the chance of obtaining interspecific hybrid seed. To verify the presence of any autoincompatibility that would interfere in the controlled crosses in *M. pohlii* and cassava, a controlled autopollination was undertaken. Crosses between cassava and *M. pohlii* (POH) were carried out using mentor pollen in one trial and untreated pollen in the second trial. Seeds were collected from both crosses and planted in the next growing season.

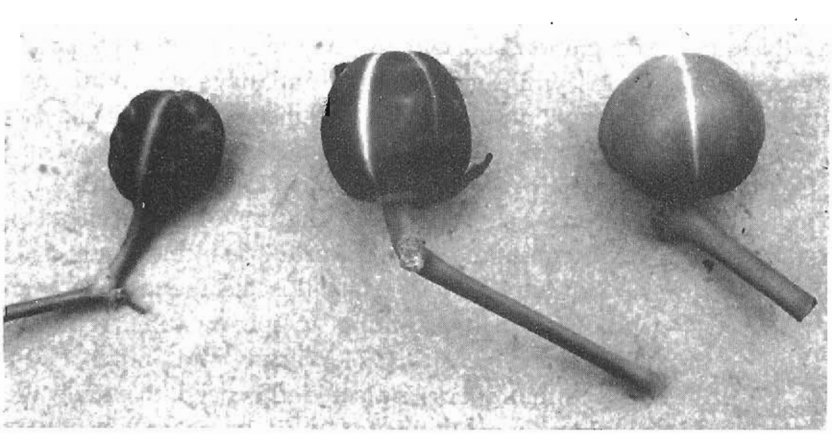

Figure 1 - Fruit of *Manihot neusana-M.pohlii* hybrid (middle) marked by variegation of *M. neusana* and straight white line of *M. pohlii*.

RESULTS AND DISCUSSION

The pollination of *M. pohlii* by untreated cassava pollen did not produce any fruit set (Table I), while crosses with mixed pollen of cassava and mentor resulted in the production of 21 seeds, 4.9% of the possible maximum (assuming that every fruit has three ovules). Using cassava as a maternal parent, pollination by untreated pollen of *M. pohlii* did not result in any seed. This clearly demonstrates the effect of mentor pollen in the crosses of cassava with *M. pohlii*. It likewise means that the freeze-thawing treatment administered to *M. pohlii* pollen, although killing it, did not affect its stimulatory function, so that all of the seed produced by the mentor effect had embryos and endosperm.

Table I - Crosses attempted between *Manihot pohlii* and *M. esculenta* (cassava).

Treatment 1 - without mentor 2 - with mentor	Flowers pollinated	Fruit No.	Seed No.
1o. Cassava x *M. pohlii*	145	0	0
2o. *M. pohlii* x Cassava	142	10	21

This result indicates that only the stigmatic barrier functions in preventing crossing in these species. Postfertilization mechanisms fail to prevent crossing. Only one plant germinated and could be raised from these seeds to severe dormancy. This plant bores fruits carrying the marker genes of both *M. pohlii* and cassava; a straight white line from *M. pohlii*, and winged fruit from cassava (Figure 2). The mentor effect has also been successfully used in *Populus* and *Cosmos*. In these genera, interspecific incompatibilities have been overcome by using compatible but dead pollen (Knox *et al.*, 1972). These studies have suggested that this phenomenon is due to proteinaceous recognition factors released from the wall of the killed compatible pollen, masking the rejection reaction of the recipient stigma. Our report represents the first case of obtaining hybrid seed of *M. pohlii* and cassava and its further reproduction. In spite of the useful characters of *M. pohlii*, no successful breeding program has been carried out, due to a lock of hybrids between this species and cassava. Our study is the first to successfully cross these two species.

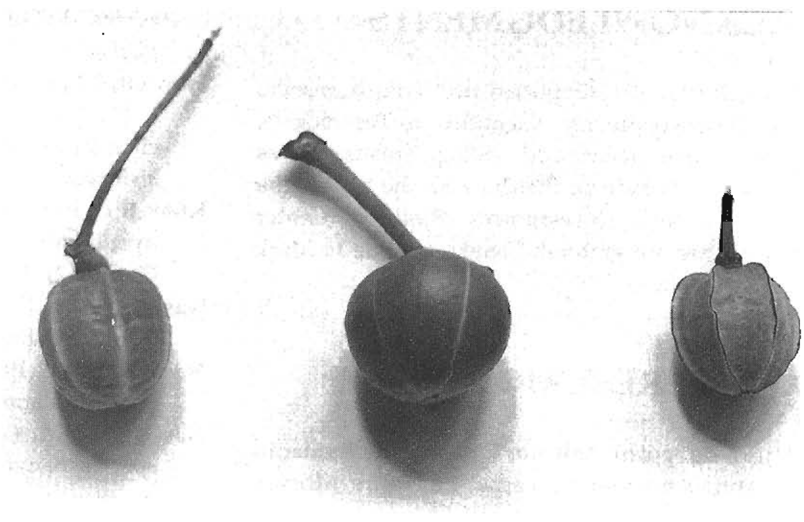

Figure 2 - Fruit of *Manihot pohlii*-cassava hybrid (right) marked by straight white line of *M. pohlii* and winged fruit of cassava.

The use of *M. neusana* as a bridge species through the hybrid *M. neusana*-*M. pohlii* has improved seed set. When used as a maternal parent pollinated by cassava it gave seed in 3.5% of cases while the reciprocal crosses had greatly improved seed set, yielding 25.9% (Table II). The combined treatment of both species (bridge and mentor) produced 3.5% seed production. The success of *M. neusana* as a bridge species between *M. pohlii* and cassava may occur because the two genomes of *M. neusana* carry different genetic mechanisms of cross incompatibility. This hypothesis is confirmed by our observations on crossing behavior of this species in the living collection of wild *Manihot* species. *M. neusana* has hybridized naturally with several *Manihot* species grown in the vicinity, e.g. *M. pseudoglaziovii* Pax et Hoffm., *M. glaziovii* Mull. Arg., *M. tripartita* Mull. Arg., *M. caerulescens* Pohl, *M. salicifolia* Pohl, *M. pohlii* and *M. esculenta* itself (Nassar, 1989). Its hybrids with the above mentioned species were easily identified by the marker dominant gene of variegated fruit which came from *M. neusana* (Figure 3). *M. neusana* is a newly emerging species, described and identified recently (Nassar, 1985).

Table II - Crosses attempted between cassava and *Manihot neusana* hybrids; fruit and seed produced following different treatments.

Treatment	Flower pollinated	Fruit No.	Seed No.
HNP x cassava	161	14	17
Cassava x HNP	90	14	70
HNP x cassava + mentor	208	19	22

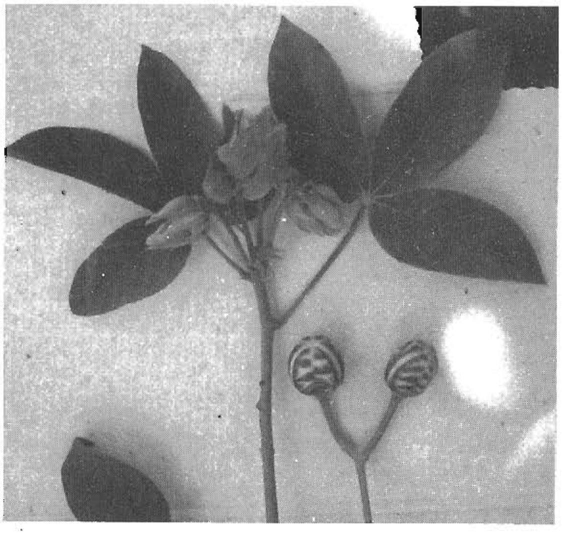

Figure 3 - *Manihot neusana* fruit showing marker gene of variegation.

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RESUMO

O uso de pólen "Mentor" permitiu hibridação interspecífica entre a mandioca e a espécie silvestre, *Manihot pohlii* Warwa. Para aplicar esta técnica, o pólen do tipo compatível foi destruído pelo congelamento, em seguida misturado com o pólen incompatível, e a mistura foi usada na polinização. Além do efeito "Mentor", a manipulação de espécies *M. neusana* Nassar como ponte através do híbrido *M. pohlii-M. neusana* mostrou também sucesso na hibridação interespecífica.

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