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Production of hybrids between *Cajanus acutifolius* and *C. cajan*

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Summary

There are many wild species of pigeonpea which are endemic to Australia. These wild species are cross incompatible with cultivated species of Indian origin. *Cajanus acutifolius* is one such species which does not easily cross with cultivated pigeonpea. Interspecific pollinations lead to hybrid seeds which were semi-shrivelled. Very few seeds germinated to give rise to F1 plants. Backcrossing the hybrid plants to C. cajan, the male parent, gave rise to aborting seeds which did not germinate in vivo hence BC1 plants are obtained after saving the aborting embryos *in vitro*. BC_1 plants showed normal meiotic pairing, but had low pollen fertility. The reasons for embryo abortion and low pollen fertility in spite of normal meiosis could be due to the effect of wild species cytoplasm.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp., is an important legume crop grown on approximately 4 million hectares, mainly as an intercrop in the drought prone area of Asia, Latin America, Caribbean and Southern and Eastern Africa. Its seeds are rich in dietary protein and serve as good supplementary food for those who take cereals as the main diet. Besides enriching the soil, it provides thepotential of providing 3 t ha^{-1} grain, but the realized yields are low (0.7 t ha^{-1}) and unstable.

Helicoverpa armigera is the most important insect, causing huge economic losses (Reed & Lateef, 1990) every year in the semi arid tropics and tons of insecticides are applied to protect the crop (Shanower et al., 1999). In certain years the intensity of insect damage is so high that even 5–6 insecticide sprays fail to control crop damage and there are also reports of insects developing resistance to the commonly used insecticides (Armes et al., 1993). Therefore, genetic resistance against this pest is best approach for protecting both the crop as well as the environment. In pigeonpea germplasm, durable resistance to *H. armigera* is not available. Hence a search for the resistance in the wild relatives of pigeonpea is a logical breeding approach.

Cajanus acutifolius (F. Muell) van der Maesen (= *Atylosia acutifolia*), a native of northern Australia, is a wild relative of pigeonpea. It is an erect perennial shrub with short silvery hairs on the leaves, giving attractive silvery appearance to the plant. Studies at ICRISAT have shown that *A. acutifolius* has resistance to *H. armigera* (Mallikarjuna, personal observation), and its transfer to cultivated types will significantly enhance the productivity and stability of the crop. Earlier efforts to cross *C. acutifolius* with *C. cajan* through conventional hybridization techniques did not succeed. With information on methods to overcome crossability barriers (Mallikarjuna & Moss, 1996; Mallikarjuna, 1998), these two species have been successfully crossed, and this paper describes the results of the research.

Materials and methods

Cajanus acutifolius (ICPW 2) and *C. cajan* (ICP 1140) plants were grown and maintained in the glasshouse. Crosses were made using *C. acutifolius* as the female parent. Pollinations were carried out soon after emasculations and in the morning before 10 am. From 281 pollinations, only 5 pods were obtained. Pods

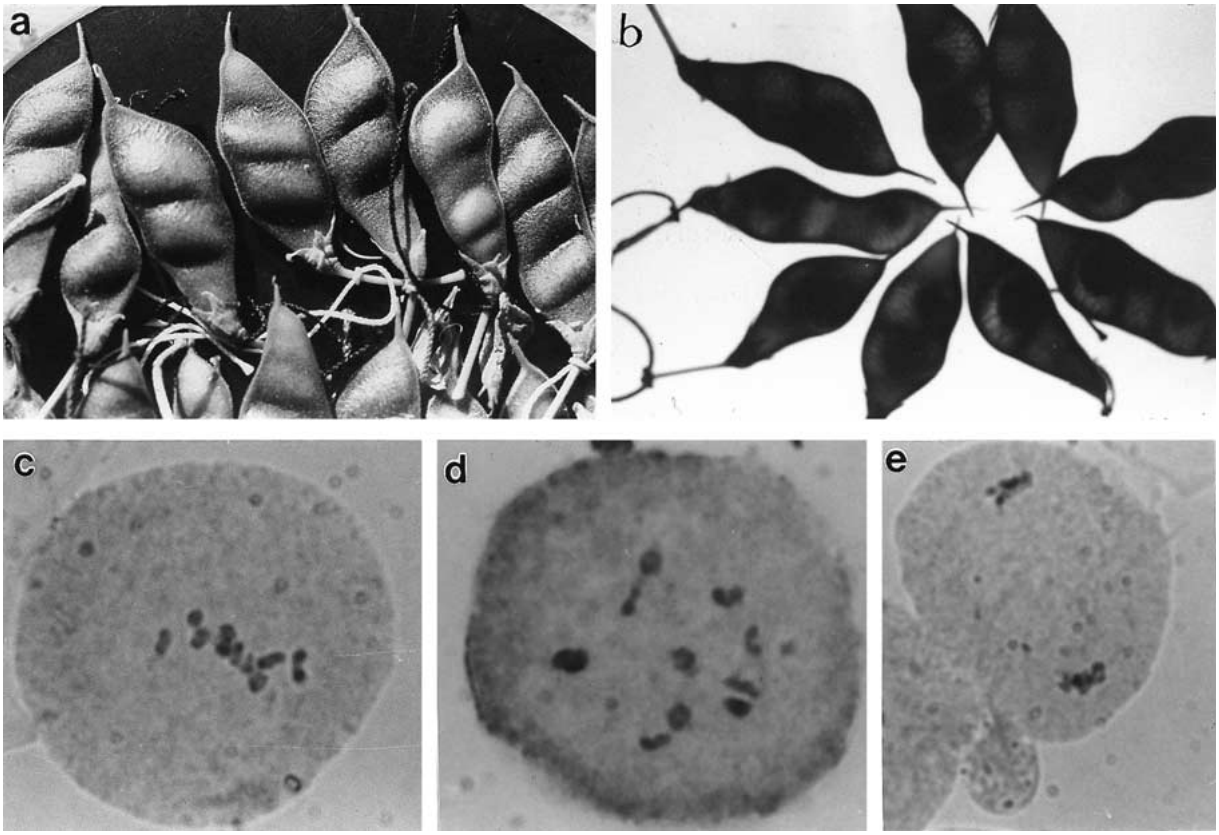

Figure 1. Interspecific hybridization between *C. acutifolius* and *C. cajan*. a. Pod formation in the cross (*Cajanus acutifolius* × *C. cajan*) × *C. cajan*. Morphologically the pods resemble those of *C. acutifolius*; b. Immature seeds seen in BC_1F_1 hybrid pods (*C. acutifolius* × *C. cajan*) × *C.cajan*; c. Normal meiosis with 11 bivalents; d. The ten bivalents comprises of 6 ring and 5 rod bivalents; e. Normal disjunction of chromosomes at anaphase I.

were harvested 20–25 days after pollination. From these pods four semi-shriveled seeds, 6 mm in size, were obtained. Hybrid seeds were germinated in the glasshouse of which two seeds germinated. Hybrid plants grew normally and flowered profusely. Young buds were used for cytological studies. Application of gibberellic acid (50 mg/l) was mandatory to obtain BC_1F_1 seeds. Out of 305 pollinations made, 51 pods were obtained. Externally the pods appeared normal but the seeds were shrunken and had embryos at different stages of abortion. Seeds failed to germinate *in vivo*. Seeds with embryos more than 3 mm in size were selected for embryo culture. Pigeonpea embryos less than 3 mm in size are difficult to germinate *in vitro* (Mallikarjuna, 1998). The embryo culture method and medium was basically as described by Mallikarjuna, (1998). Culture medium comprised of MS (Murashige & Skoog's medium) basal medium with naphthyl acetic-acid (NAA; 0.1 mg/l) and benzylamino purine (BAP; 1.0 mg/l). Hybrid embryos formed multiple shoots, and these were rooted on MS medium with NAA (2 mg/l) and IBA (1 mg/l). Rooted shoots were acclimatized in a growth chamber with 75–80% relative humidity and 25 ± 2°C with a photoperiod of 16 hr light and 8 hr dark.

For cytological analysis of meiocytes, immature buds were fixed in Carnoy's II solution (aceticacid 1: chloroform 3: and ethanol 6) for 24 hr at 4 °C and transferred to Carnoy's I solution (acetic acid 1: ethanol 3). Meiocytes were squashed and stained in 2% acetocarmine, and well spread meiotic preparations were photographed. Anthers were harvested on the day of anthesis and squashed in 2% acetocarmine. Pollen fertility counts were made on brightly stained pollen grains.

Genomic DNA was extracted from immature leaves of plants grown in a glasshouse. Fresh immature leaves were harvested, lyophilized in liquid nitrogen and stored at –70 °C and DNA was extracted whenever necessary by the CTAB method (Saghai-

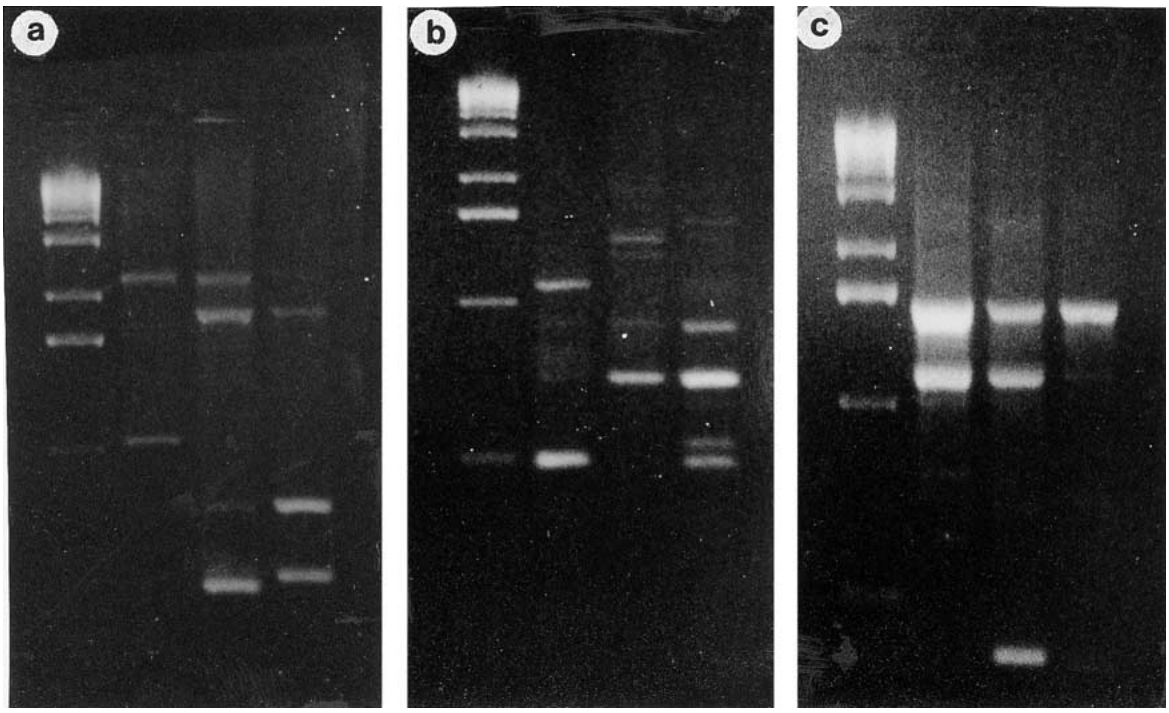

Figure 2. RAPD analysis with primers OPH-11, OPH-12, OPH-5. a. Primer OPH-11. Lane 1 = 1000 bp DNA ladder, lane 2 = cv ICP 1140, lane 3 = Hybrid, lane 4 = *Cajanus acutifolius*; b. Primer OPH-12. Lane 1 = 1000 bp DNA ladder, lane 2 = cv ICP 1140, lane 3 = Hybrid, lane 4 = *Cajanus acutifolius*; c. Primer OPH-5. Lane 1 = 1000 bp DNA ladder, lane 2 = cv ICP 1140, lane 3 = Hybrid, lane 4 = *Cajanus acutifolius.*

maroof et al., 1984). RAPD-PCR was performed according to the protocols of Williams et al. (1990). Twenty random 10-mer primers (Operon Technologies) were used to amplify DNA in Perkin GeneAmp 9600 thermal cycler and PCR products were electrophoresed on 1.6% Agarose gels, stained in ethidium bromide and photographed under UV illumination.

Results and discussion

The number of pods formed when *C. acutifolius* was crossed with *C. cajan* was very low (2%). Morphologically the pods resembled those of *A. acutifolius* with silvery hair on the surface. The seeds were semi-shrunken. Only two seeds germinated into plants under in vivo germination conditions. The plants initially grew slowly, but later normal growth was observed. Morphologically the hybrid plants had intermediate growth habit being semi-erect, whereas *C. acutifolius*, the female parent had most of its branches drooping to the ground. The pollen parent had upright growth habit. The leaves of the hybrid plants resembled *C. acutifolius* by the presence of short silvery hairs.

Hybrid plants were backcrossed to ICP 1140. None of the pollinations resulted in pod formation. Pods were obtained only after the application of gibberellic acid to pollinated pistils. Seventeen percent of the pollinations formed pods (Figure 1a). Hybrid seeds did not reach maturity (Figure 1b). Embryos inside aborted at different stages of growth. To obtain BC_1 plants, embryos were dissected out of aborting seeds and cultured. Not all embryos grew in culture. Only embryos, which were 3 mm or more in size and at cotyledonary stage of development, grew in culture and hybrids plants were obtained.

Cytological investigation of the F_1 meiocytes revealed that 96% of them had normal chromosome segregation at metaphase with 11 bivalents (Figure 1c). The number of ring and rod bivalents varied. On an average six ring and five rod bivalents were observed (Figure 1d). The segregation of chrosomes in anaphase was devoid of abnormality with equal number of chromosomes at each pole (Figure 1e). Pollen fertility in F_1 plants ranged between 12–16%. Cytological investigations of BC_1 plants showed normal disjunction of 11 pairs of chromosomes, with 11 chromosomes at each

pole at anaphase II. Pollen fertility ranged between 18–22%.

RAPD analysis of the parents and the hybrid showed that with primer OPH-05 one of the band was common across both the parents as well as the hybrid, but one band of the hybrid was in common with the band of cv ICP 1140 which was absent in the female parent *C. acutifolius*. With primer OPH-11 only one band of the hybrid was in common with a band of *C. acutifolius* and none with cv ICP 1140. With primer OPH-12 the banding pattern of the hybrid was similar to *C. acutifolius* except for one band in common with cv ICP 1140.

The present studies suggest that *C. acutifolius and C. cajan* differ morphologically as well as for crossability. But cytological investigations show that the genomes of the two are may be closely related. Hence The following conclusion can be drawn that the genomes of *C. acutifolius* and *C. cajan* although closely related, the cytoplasm of *C. acutifolius* might have undergone some significant changes, differing with the cytoplasm of *C. cajan* ICP 1140, a species native to India, leading to post meiotic changes, thus bringing about diversification of the cytoplasms, which had an effect on crossability between the two species.

ICRISAT gene bank holds 4 accessions of *C. acutifolius*. It will be interesting to study if all the accessions of *C. acutifolius* show cross incompatibility with *C. cajan*. Saxena et al. (1990) reported variation for crossability between different *C. scaraboides* accessions and *C. cajan*. Ariyanayagam et al. (1995) reported significant differences with respect to cross compatibility between different accessions of *C. sereceus* and *C. cajan*, and in the production of male sterile F_1 plants. Sterility observed in the cross *C. sereceus* $\times$ *C. cajan* has been effectively utilized in developing cytoplasmic-genic male sterile system to develop commercial hybrids in pigeonpea (Saxena & Vijaykumar, 1999). Similar male-sterile system can be developed using *C. acutifolius*, not only to diversify the cytoplasmic base of the male-sterile system, but also to produce insect resistant/tolerant pigeonpea hybrids.

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