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Production of hybrids between *Cajanus platycarpus* and *Cajanus cajan*

Nalini Mallikarjuna & J.P. Moss
Cellular and Molecular Biology Division, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India

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Summary

Cajanus platycarpus a wild species of pigeonpea incompatible with the cultigen has many desirable characters important for the improvement of cultivated pigeonpea. In the present study, barriers to hybridization were studied and were identified as post-zygotic. Efficient embryo rescue techniques were developed. As a result, hybrids were produced. Morphological and cytological studies as well as esterase isozyme band pattern confirmed the hybrid nature of the plants. The F_1 hybrids were completely pollen sterile. Meiotic studies were carried out to check for the cause of pollen sterility.

Abbreviations: MS – Murashige & Skoog's (1962) medium, BAP – 6-Benzylaminopurine, IBA – Indole-3-butyric Acid, NAA – α Naphthaleneacetic Acid

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is an important crop of Asia and Africa. Pigeonpea is a multipurpose crop, grown as a sole crop or as intercrop in many farming systems across the tropics and subtropics. It is also grown by small farmers on marginal lands where other crops are not suitable. Dehulled seeds of pigeonpea are used as dhal, seed husk are fed to the cattle, dry stems are used for firewood and to make huts and baskets. Root nodules of pigeonpea fix nitrogen thus increasing the soil fertility, and the deep roots of pigeonpea take up phosphorus, which is thus available to other crops.

Pigeonpea is endowed with a rich germplasm base. According to Van Der Maesen (1990) the gene pool of pigeonpea is classified into three groups. The primary gene pool comprising of cultivar collections, the secondary gene pool consisting of closely related wild species and the tertiary gene pool of wild species which are not crossable with pigeonpea. The wild species of the secondary gene pool have been successfully utilized to produce interspecific derivatives (Remanandan, 1990; Saxena et al., 1987).

Cajanus platycarpus has been assigned to the tertiary gene pool of pigeonpea. It has many desirable characters such as extra-early flowering and maturity, photoperiod insensitivity, prolific flowering and pod setting, high harvest index, annuality and rapid seedling growth (Dundas, 1985). This species also has resistance to phytophthora blight, sterility mosaic, and a limited amount of resistance to cyst nematode, pod borer, and podfly (Saxena et al., in preparation) and also salinity tolerance (Subbarao, 1988).

All earlier attempts to obtain hybrids between *C. platycarpus* and *C. cajan* or vice versa have not been successful (Ariyanayagam & Spence, 1979; Kumar, 1985; Dundas, 1985; Pundir & Singh, 1987).

The present paper is the first study which describes barriers to hybridization in the cross between *C. platycarpus* and *C. cajan* and methods to overcome them. As a result hybrids between *C. platycarpus* and *C. cajan* have been successfully produced.

Material and methods

Seeds of *C. platycarpus* accession ICPW 68 and *C. cajan* cv. ICPL 87 were obtained from the Genetic Resources Program and Pigeonpea Breeding unit of ICRISAT, India. Seeds of *C. platycarpus* have a hard seed coat so were scarified. Seeds were sown and when the plants reached the flowering stage, emasculations were followed by pollination before 10.00 am in the morning.

For fluorescence microscopic studies, pollinated pistils were processed according to the technique of Shivanna & Sastri (1981).

Ovules were supported on filter paper bridges and cultured over MS liquid medium with NAA (0.5 mg L^{-1}) and BAP (0.5 mg L^{-1}). After 3–4 weeks of ovule culture, embryos were dissected from the ovules and transferred to growth medium. The growth medium consisted of MS basal medium with 0.1 mg L^{-1} NAA and 1.0 mg L^{-1} BAP. Shoots of 1.0 cm or more in length were transferred to a root induction medium consisting of 1/10 MS basal salts plus 3.0% sucrose, 0.7% agar with 0.2 mg L^{-1} NAA and 0.1 mg L^{-1} IBA. After 12 days on the root induction medium, shoots were transferred to either 1/10 or full strength MS basal medium with 3.0% sucrose and 0.7% agar.

The method used for cytological study was as described by Singh & Moss (1982). To obtain esterase isozyme profile, crude extracts were made from immature leaves of hybrids and parents. Polyacrylamide gel electrophores (PAGE) was carried out on 9.0% gels. The gels were stained according to the metod of Scandalios (1969).

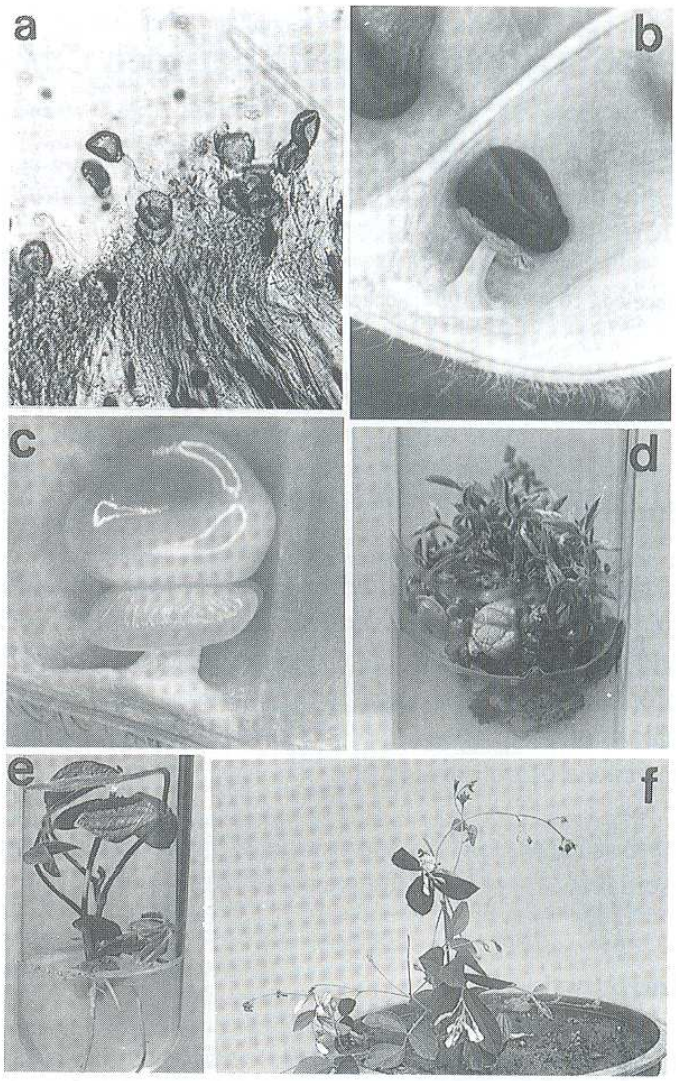

Fig. 1. a: Pigeonpea pollen germinating on *C. platycarpus* stigma; b: aborted black immature seed; c: immature seed at the time of ovule culture; d: multiple shoots from immature embryo; e: *in vitro* rooting; f: F_1 hybrid.

Results

Pollen grains germinated within a few minutes of deposition on the stigma (Fig. 1a) and were observed in the ovary by 24 hours after pollination in successful crosses of *C. platycarpus* × *C. cajan*. By three days after pollination ovaries enlarged, and pod development was observed on the 6th day. The majority of the pods remained on the plant until 20–25 days after pollination, but by 30–35 days most of the pods had abscissed. Selfed pods of *C. platycarpus* pods very rarely abscissed, and mature seeds were obtained by 35–40 days after pollination. Immature seeds which were black and dried, and less than 2.0 mm in size, were observed in all the crossed pods which did remain on the plant even up to 35–40 days after pollination (Fig. 1b).

In the reciprocal crosses using *C. cajan* as the female parent and *C. platycarpus* as the pollen donor, fluorescence microscopy failed to show pollen tubes reaching the ovary. But in one per cent of the pollinations, pod initials were observed by the 6th day after pollination (Table 1). These pods had a stunted appearance, and were devoid of developing seeds.

Hybrid embryos from the cross *C. platycarpus* × *C. cajan* were at late heart to early cotyledonary stage and less than 0.25 mm in size by 20 days after pollination. The embryos aborted at this stage as no later stages were seen when dissected and cultured on the growth medium. Therefore, immature ovules were harvested at

Table 1. Interspecific hybridization between *Cajanus platycarpus* and *C. cajan*

Cross	No. of pollinations	No. of pods (%)	No. of seeds	No. of embryos cultured	No. of embryos growing
C. platycarpus × *C. cajan*	1164	81 (7)	156	74	17
C. cajan × *C. platycarpus*	1295	17 (1)	0	0	0

20 days after pollination (Fig. 1c), and cultured on the ovule culture medium. Morphologically no change was observed except for the colour change from green to pale brown. After 3–4 weeks of ovule culture, embryos were dissected and cultured on the growth medium.

The growth of hybrid embryos was very slow. Direct germination of embryos was never observed. Initial response was enlargement of the embryo accompanied by multiple shoot bud formation (Fig. 1d), followed by a period of 3–4 months for the shoot buds to grow 1.0 cm long. Elongated shoots were transferred to root induction medium. In the first set, 12 shoots from 12 different hybrid embryos were transferred to root induction medium, of which 7 shoots rooted (Fig. 1e), but only 4 with a robust root system were successfully transferred to soil (Fig. 1f). All the 4 hybrid plants survived. The growth of the hybrids was initially slow when kept in the growth chamber for acclimatization, but when transferred to the glasshouse, the growth of the hybrid plants was vigorous.

Meiotic analyses of the hybrids showed much variation in chromosome configurations with mean of 6 univalents (rang 5.2–6.7) and 8 bivalents (7.4–8.4) per cell; trivalents were rarely observed (Table 2). Mature pollen grains were stained in acetocarmine and 99.5% of the grains did not stain and had negligible cell contents.

The morphology of the leaf from the hybrid plant was intermediate between the two parents, whereas the growth habit of the hybrid, and the flowering axes resembled the male parent, *C. cajan* (Fig. 1f).

The hybrids had an esterase pattern distinct from each parent though hybrids had bands common with both the parents (Fig. 2).

Table 2. Chromosome association per cell[a] in hybrids (*C. platycarpus* × *C. cajan*)

No.	Univalents	Bivalents	Trivalents
1	5.2	8.4	0.1
2	6.0	8.0	0.0
3	6.0	7.4	0.3
4	6.7	7.5	0.0

[a] Mean of 25 cells.

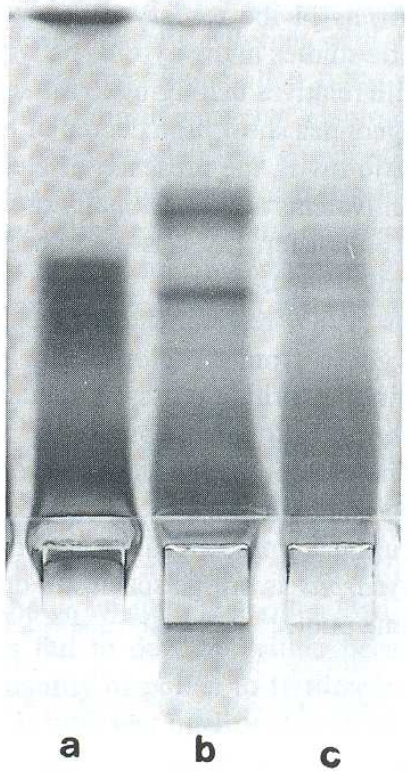

Fig. 2. Esterase isozyme pattern. a: *Cajanus cajan*; b: The hybrid; c: *C. platycarpus*.

Discussion

All earlier attempts to cross *C. platycarpus* with *C. cajan* were unsuccessful due to lack of information on barriers to hybridization. From the present investigations it was clear that barriers to hybridization were post-zygotic. In the reciprocal cross, using *C. cajan* as the female parent the barriers to hybridization were pre-zygotic. This is exemplified by the fact that pollen grains germinated and pollen tubes grew in the style in the former cross whereas pollen did not germinate in the latter (reciprocal) cross. The low percentages of pod formation observed in the cross *C. cajan* × *C. platycarpus* may have been due to the parthenocarpic development of the pod, probably as the result of stimuli received from the cross pollination.

To obtain hybrid plants, an initial 20–25 days of in-ovule embryo culture on liquid MS medium was essential. Immature embryos were dissected and cultured to obtain hybrid plants in the present study. A similar procedure has been followed to obtain interspecific hybrids in groundnut (Nalini & Sastri, 1985).

The high degree of pollen sterility observed in the hybrids between *C. platycarpus* × *C. cajan* as well as the presence of a large number of laggards shows the extent of nonhomology between the two parental genomes. This observation justifies the placement of *C. platycarpus* in the tertiary gene pool of pigeonpea. Meiotic studies in the hybrids between pigeonpea and the wild relatives belonging to the secondary gene pool of pigeonpea show high degree of homology and recombination with the presence of eleven bivalents, although univalents are occasionally observed. A high degree of pollen fertility was also observed (Kumar, 1985; Reddy, 1983).

Dundas (1985) recommended that the transfer of desirable genes from *C. platycarpus* may be possible by finding bridge-cross combinations between *C. platycarpus* and other compatible wild *Cajanus* species. The present investigations have demonstrated a direct method to obtain hybrids between *C. platycarpus* and cultivated pigeonpea, as well as possible use of *C. platycarpus* as bridge species to other species in tertiary gene pool.

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