



# Introgression of phytophthora blight disease resistance from *Cajanus platycarpus* into short duration pigeonpea [*Cajanus cajan* (L.) Millsp.]

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## Abstract

Phytophthora blight is an important disease of pigeonpea. Resistance to this disease is not present in the germplasm collection. *Cajanus platycarpus* (accession ICPW 61), a wild species of pigeonpea, has many desirable characters including resistance to P<sub>3</sub> (highly virulent) isolate of Phytophthora blight. Unfortunately *C. platycarpus* cannot be crossed with cultivated pigeonpea by conventional techniques. Hybrids were obtained by the use of embryo rescue techniques. F<sub>1</sub> hybrids were 100% pollen sterile, hence were treated with colchicine to double their chromosome number. The resultant plants had double the chromosome number and were tetraploids. F<sub>1</sub> plants were subjected to the pathogen. The results of this screening experiment showed that it was possible to transfer resistance to Phytophthora blight from the wild species *C. platycarpus* to cultivated pigeonpea. The results of F<sub>2</sub> data indicated that the nature of resistance to be monogenic and recessive.

**Key words:** Pigeonpea, disease resistance, microtomy, phytophthora blight, allotetraploid

## Introduction

Amongst the pathogens causing drastic yield reduction in short-duration pigeonpea [*Cajanus cajan* (L.) Millsp.], Phytophthora blight disease caused by *Phytophthora drechsleri* tucker var. *cajani* Pal, Grewal and Sarbhoy, is one of them. There are three isolates of Phytophthora blight (P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub>) affecting pigeonpea. Of these P<sub>1</sub> is the least virulent isolate and P<sub>3</sub> the most virulent one [1]. Virulence of P<sub>2</sub> is in between P<sub>1</sub> and P<sub>3</sub>. Evaluation of over 14000 accessions of cultivated pigeonpea for resistance to Phytophthora blight disease resulted in identification of several lines resistant to P<sub>2</sub> isolate only [2-4]. None of these P<sub>1</sub> resistant lines showed resistance to the more virulent P<sub>3</sub> isolate of Phytophthora blight [3].

Screening for resistance to Phytophthora blight in wild species of pigeonpea resulted in the identification

*Cajanus platycarpus* (Benth) van der Maesen comb. nov., accessions ICPW 61 and ICPW 66 as being resistant to the disease in repeated tests [4]. *Cajanus platycarpus* is a wild species from the tertiary gene pool of pigeonpea [5]. Mallikarjuna and Moss [6] have reported the cross between *C. platycarpus* and cultivated pigeonpea by using embryo rescue techniques [7]. The present investigation reports the successful transfer of disease resistance from the wild species *Cajanus platycarpus* into cultivated pigeonpea.

## Materials and methods

*Cajanus platycarpus* accession ICPW 61, a wild relative of pigeonpea, was grown and maintained in a glasshouse. ICPW 61 has been identified as being highly resistant to Phytophthora blight (PB) disease and especially to P<sub>3</sub> isolate. Pigeonpea cultivar ICPL 85030 is an extra short duration variety and is susceptible to all races of PB. Plants were grown and maintained in the glasshouse. Emasculations, pollinations and growth regulator application was carried out as described by Mallikarjuna and Moss [6]. *Cajanus platycarpus* was used as the female parent in the crossing experiments. Aborting F<sub>1</sub> hybrid seeds were rescued by embryo rescue techniques [7]. The terminal bud of F<sub>1</sub> (2n = 22; diploid) hybrids plants were treated with an aqueous solution of 0.05% colchicine with 10% Tween-20 with the help of a cotton swab to double the chromosome number (Mallikarjuna, unpublished). Axillary buds were removed to facilitate the growth of terminal bud. Tetraploid hybrids (2n = 44; tetraploid) were selfed to obtain F<sub>2</sub> seeds.

The pathogen was isolated from small pieces of 3 mm stem portions having lesions of *Phytophthora* fungi growing on pigeonpea plant. The stem pieces were washed in running tap water and surface sterilized in 2% sodium hypochlorite solution for 1-3 minutes and placed on potato dextrose agar (PDA) slants. On the

basis of growth characteristics, slants with the fungus in pure form were identified and confirmed by microscopic examination. The  $P_3$  isolate was confirmed by virulence test by inoculating 12-15 days old susceptible (ICPL 87119, susceptible to  $P_2$  and  $P_3$ ) and resistant seedlings (ICP 2366, susceptible to  $P_3$  but resistant to  $P_2$ ) with the inoculum. All the susceptible seedlings were killed by  $P_2$  and  $P_3$  isolates but among the resistant seedlings, they were healthy against  $P_2$  isolate but succumbed to  $P_3$  isolate.

$F_2$  seedlings with one trifoliate leaf (15 days) were scored for the disease. The screening procedure was as follows: an inoculation concentration of 1g. of mycelium/100ml of water, was sprayed on the seedlings. The seedlings were incubated at 25-30°C at 95-100% humidity for 36 hours. Plants were sprayed with tap water every 2-3 hours during the day, until 4 days after inoculation. Disease data was taken after 10 days of inoculation. Plants which did not succumb to the disease were scored as resistant and the ones which succumbed to the disease were scored as susceptible. The screening procedure was as described by Gupta *et al.*, [1, 8]. After 30 days of sowing, the seedlings which did not succumb to the disease were again inoculated with *Phytophthora* pathogen and observations were recorded. Seedlings which showed resistance at the seedling stage were found to be resistant at 30 days too. Plants grew normally and set seeds.

For microtomy, flower buds were fixed at 10, 18, 25 and 30 days after pollination in FAA (formaldehyde 10 ml + glacial acetic acid 5 ml + alcohol (95%) 50 ml + water 35 ml). The technique of fixing, and staining the specimens was as mentioned in historesin embedding kit by Reichert-Jung. Microtome sections were cut at 4-6  $\mu$  thick. They were stained in methylene blue-azur I and basic fuchsin.

## Results and discussion

Anatomical study of the  $F_1$  hybrid embryo at 10 days after pollination (DAP) showed a growing embryo at early cotyledonary stage of development with a few cells floating in the largely coenocytic endosperm. At 18 DAP, revealed that the outer integument had intensified sclereid formation on the dorsal region of the ovule which resulted in the collapse of the ovule wall and crushing of the embryo cavity (Fig. 1, I). Endosperm was seen as a papery layer. By 30 DAP, the embryo sac was crushed with no traces of the hybrid embryo.

One of the reasons for embryo abortion in the  $F_1$  (*C. platycarpus*  $\times$  *C. cajan*) could be abnormal/incomplete endosperm development. The message for such incomplete development could be from the mother plant itself. Since *C. platycarpus* is not closely related

to cultivated pigeonpea, and meiotic analysis of  $F_1$  hybrids have shown that large number of univalents (5-6 univalents) [6], it might be nature's way to purge out unwanted/ abnormal seeds.

A total of 118  $F_1$  ( $2n = 22$ ) hybrid plants were treated with colchicine from which 12 tetraploid  $F_1$  ( $2n = 44$ ) hybrid plants were recovered. Tetraploid  $F_1$ s, although were feeble in their growth habit to begin with, were later robust in growth and flowered profusely. Seeds were buiked from all 12  $F_1$  hybrids and only mature seeds (Fig. 1, F) were selected for generation of  $F_2$  progeny.

Fifty four  $F_2$  plants were randomly selected and screened for *Phytophthora* blight pathogen  $P_3$  isolate. Screening the parents to the  $P_3$  isolate of PB showed the resistant nature of *C. platycarpus* accession and susceptibility of *C. cajan* cultivars. Amongst the 54 plants screened for *Phytophthora* blight disease, 14 plants showed resistant reaction similar to *C. platycarpus*. The rest of the 40 plants succumbed to the disease pressure (Fig. 1 H). Chi square test showed that the segregation followed the 1 resistant: 3 susceptible ratio ( $P > 0.05$ ). Plants showing resistant reaction were grown in the glasshouse. After 30 days of growth seedlings were again subjected to the pathogen. None of the 14 resistant selections showed any disease symptoms (Fig. 1 G).

According to Gupta *et al.*, [1], the resistant reaction of  $P_3$  isolate of *Phytophthora* blight in pigeonpea germplasm was completely dominant over susceptibility and was monogenic. It was designated as  $Pd_1$  by Sharma *et al.*, [9]. They also stated that since not all screened plants were fully resistant, there might be minor genes involved in controlling resistance. In the case of the wild species *C. platycarpus*, the gene conferring resistance to  $P_3$  isolate of PB was not dominant. Only 14 out of 54 plants showed a resistant reaction. Based on the  $F_2$  data and Chi square test it can be said that the gene operating is probably recessive.  $F_1$  hybrids could not be scored at the seedling stage as the hybrids were obtained by embryo rescue techniques and later rooting hybrid shoots in vitro [6].

The resistant plants identified at seedling stage were found to be resistant to the disease at all stages of their life cycle. There is a possibility that the resistant gene transferred from the wild species *C. platycarpus* may be different from the gene present in pigeonpea germplasm.

This is the first report of gene transfer from *C. platycarpus* into cultivated pigeonpea for *Phytophthora* blight resistance. These results open up possibilities of gene transfer for biotic and abiotic constraints from

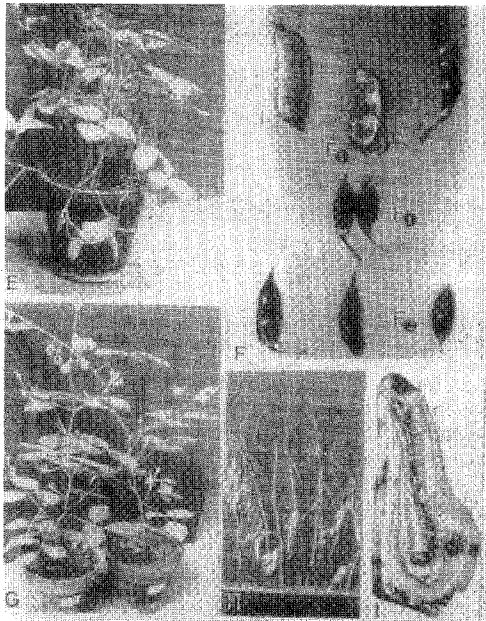


Fig. 1. Gene transfer in the cross *Cajanus platycarpus* × *C. cajan*

other incompatible wild species of pigeonpea. The results also show that although *C. platycarpus* is placed in the tertiary gene pool, it is not very divergent to access genes for pigeonpea improvement.

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