



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 P₃ (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. F₁ 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. F₁ 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 F₂ 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 (P₁, P₂ and P₃) affecting pigeonpea. Of these P₁ is the least virulent isolate and P₃ the most virulent one [1]. Virulence of P₂ is in between P₁ and P₃. Evaluation of over 14000 accessions of cultivated pigeonpea for resistance to Phytophthora blight disease resulted in identification of several lines resistant to P₂ isolate only [2-4]. None of these P₁ resistant lines showed resistance to the more virulent P₃ 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 P₃ 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 F₁ hybrid seeds were rescued by embryo rescue techniques [7]. The terminal bud of F₁ (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 F₂ 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 μ 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 bulked 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

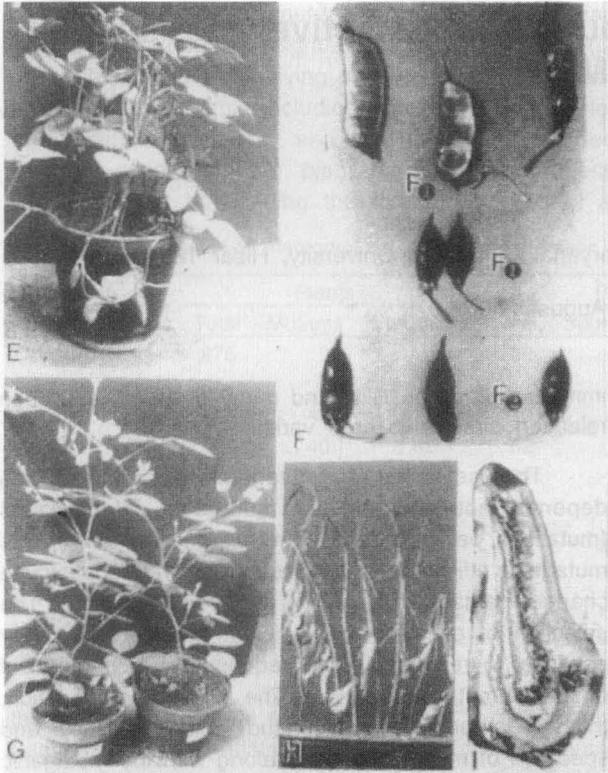


Fig. 1. Gene transfer in the cross *Cajanus platycarpus* × *C. cajan*

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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