

## S U M M A R Y

Pigeonpea (Cajanus cajan) is the most important high protein grain legume of semiarid tropics. However, this crop severely suffers from wilt disease caused by a fungus Fusarium udum. Most of the high yielding cultivars are susceptible to Fusarium wilt which can reduce the yield by nearly 99 % if the wilt occurs at or prior to early pod-setting (Nene et al 1979). Thus wilt control is important for boosting pigeonpea production. Development of resistance through biotechnological methods can substantially benefit crop improvement.

With <sup>this</sup> background in view, studies have been conducted on the following aspects;

- (i) identification of the optimal culture conditions for plantlet regeneration from various explants of C. cajan,
- (ii) to attempt in vitro embryo rescue and ovule culture techniques for obtaining intergeneric and inter-varietal viable hybrid embryos and plants,
- (iii) varietal screening of C. cajan for wilt disease, using culture filtrate of F. udum at the whole plant and leaf discs level and
- (iv) to obtain a resistant line by applying selection pressure.

Various seedling explants viz., epicotyl, distal halves of cotyledon and leaf (7 days old seedling) of C. cajan (var. Bandapalera) were cultured on MMS medium supplemented with various hormones. The best morphogenetic response was registered, when these explants were transferred from MMS medium supplemented with BAP (5 mg/l), Kn (0.5 mg/l) and AdS (50 mg/l), after clearing off the primary callus, to the medium containing low levels of auxin (0.1 mg/l IBA) and gibberelin (0.05 mg/l GA<sub>3</sub>) with epicotyl explants giving rise to direct shoots and the cotyledonary explants forming somatic embryos. In case of leaf explants, shoot differentiation was completely inhibited by even low concentration of auxin (1.0 mg/l IBA), while incorporation of TIBA (0.5 mg/l) along with Kn (0.02 mg/l) was found beneficial for shoot bud induction.

Regenerated shoots were grown to plantlets on half strength MMS media with IBA (0.1 mg/l) and GA<sub>3</sub> (0.05 mg/l).

Somatic embryogenesis was achieved using distal halves of cotyledons in a number of genotypes. Formation of somatic embryos was markedly influenced by the concentrations of specific cytokinins, mineral nutrient formulation and soaking period of seeds.

The distal halves of the cotyledons excised from seeds soaked in sterile distilled water for 16 h, expanded rapidly, turned green and formed embryos on a high cytokinin medium (5 mg/l BAP with 0.5 mg/l Kn and 50 mg/l AdS). Reduction of cytokinins favoured maturation of the embryos. While complete withdrawal of the cytokinins with the introduction of small amounts of IBA (0.1 mg/l) and  $GA_3$  (0.05 mg/l) proved effective in germination of the embryos and development into complete plantlets.

In another set of experiments, auxin (NAA) was used in presence of cytokinins (BAP, Kn and AdS) for improvement of embryo induction from cotyledons. Maximum response, in terms of per cent responsive cotyledons as well as frequency of embryo induction was registered with cytokinins alone (5 mg/l BAP, 0.5 mg/l Kn and 50 mg/l AdS). Incorporation of NAA (0.5 to 20 mg/l) alongwith BAP in the nutrient medium suppressed the embrogenic response.

The influence of size and age of cotyledons after removing embryonic axis on the induction of somatic embryogenesis is recorded. Increase in the response in terms of per cent responsive cotyledons, embryo inductive cotyledons and frequency of embryo induction was registered with increasing age and size of the cotyledons in case of pigeonpea var. T-15-15. The cotyledon measuring 7 mm x 9 mm in size and 35 days in age after anthesis were highly

responsive, forming either callus or embryos. The frequency of embryo induction was also very high in these cotyledons.

The two amino acids viz. glutamine and asparagine at 200 mg/l as well as ABA at 0.025 or 0.25 mg/l concentration alone or in combinations, reduced embryo maturation and germination. On the other hand, when two ethylene antagonists, namely silver nitrate and salicylic acid, were tried to increase embryo maturation and germination, silver nitrate (20 mg/l) enhanced somatic embryos maturation and germination, but salicylic acid (200 mg/l) had little effect.

Attempts to transfer wilt resistance, by making crosses with Atylosia lineata using pigeonpea varieties T-15-15 and CAUT-82-90 as female parents failed. In A. lineata x T-15-15 cross, two pods were obtained but seed setting did not occur in the pods. In the present investigation different levels of success in the inter-cultivar crosses of C. cajan, ranging from about 3 to 5.2 % depending on the parental genotypes were obtained.

The selfed immature embryos of various genotypes of C. cajan germinated and complete plantlets were recorded with high frequency on MMS medium containing IAA (1 mg/l), Kn (0.2 mg/l) and CM (10% v/v). The frequency of embryo germination varied significantly among genotypes. The

hybrid plantlets obtained in the intercultivar crosses of C. cajan are transferred to pots for further development of F<sub>1</sub> plants.

Four to twenty days old ovules after anthesis of C. cajan (var. T-15-15) were excised from the pods and inoculated on different media for further development and germination. Very young (2 days old) as well as later stages of ovules (more than 12 days old) failed to develop further and germinate irrespective of the sucrose and hormone combinations used in vitro. However, 4, 6, and 8 days old ovules, germinated and developed into either single or multiple shoots.

In present investigation the pathogenicity of the fungus was studied using fungal mycelium as well as CF. The relative susceptibility of different varieties was also evaluated. It was found that 15% (v/v) CF of F. udum was capable to mimic the action of fungal hyphae and spores in bringing about all the wilt symptoms much earlier than the fungus.

The liquid culture and leaf disc techniques were standardized for screening pigeonpea varieties against wilt disease using CF of F. udum. The results of the present investigation have clearly indicated that they could be

very efficient techniques as compared to field screening of different pigeonpea varieties for wilt caused by F. udum.

Then CF as selection agent was incorporated into the culture medium used to obtain somatic embryogenic response from the cotyledonary explants.

LD-50 dose of CF (20% v/v) was employed as selection pressure for in vitro culture of cotyledons, at all the stages of embryo formation till the development of complete plantlets in five varieties (var. T-15-15, GAUT-82-90, Bandapalera, NP (WR)15 and BDN2) of pigeonpea. The data obtained on the basis of per cent responsive cotyledons and frequency of embryo induction showed that, var. NP (WR)15 was having high regeneration capability as compared to other varieties, Based on these results NP (WR)15, BDN2. and Bandapalera varieties were selected as disease resistant, while T-15-15 and GAUT-82-90 varieties were considered as disease susceptible.