PLANT REGENERATION FROM CALLUS OF IMMATURE COTYLEDONS OF PIGEONPEA

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Pigeonpea (Cajanus cajan Millsp. L) is one of the major grain legume in India occupying an important place in Inidan agriculture. Pigeonpea alone shares around 21 % of the total area under pulse production in inida. More than 90 percent of the world's area and production of pigeonpea is in India. The major objective in pigeonpea breeding is to increase grain yield and provide resistance against insect pest remains the major problems and cause of loss of this important legume. One of the major insect pest is pod borer (Helicoverpa armigera) a lepidopteron insect, which cause extensive damage to the crop (Lateef & Reed, 1983) due to which the production goes down by more than 20 %. recent advances in gentic engineering have demonstrated the possibility of incorporating foreign genes for desired traits while preserving the existing characteristics of improved genotypes (johansen, 1940).

Attempts to obtain pest-resistant genotype of pigeon pea by conventional breeding method have limited success due to its narrow genetic variation, and sexual incompatibility with wild relative (Nen et.al., 1990). Genetic engineering approaches to introduce Basillus thuringiensis(Bt) genes coding for Insecticidal Crystal Proteins into pigeonpea may prove useful in obtaining pest-resistant genotypes (Kumar et.al., 1996). Hence, introduction ofsuitable Bt gene in pigeonpea is expected to give protection against pod borer, the major insect pest of the crop.

For any transformation system a regeneration protocol is a pre-requisite. An efficient plant regeneration system from the callus of immature cotyledons of pigeonpea, var. UPAS 120 and Tripura local has been established. Surface sterilized immature cotyledons of both the genotype were in Murashige and Skoog's (MS) medium along with various hormonal combinations. Among the combination tried. BAP (6-benzyl-aminopurine) 2 mg/L along with NAA (1-naphthalene acetic acid)).2 mg'L proved to be the best for both the genotypes. The culture were incubated at 25± 20 C under white fluorescent light at a 16 hr. photoperiod. Susan et.al., (1993) regenerated plants from leaf disc of pigeon pea. Sreenivasu et.al. (1998) reported plant regeneration via somatic embryogenesis in pigeonpea from cotyledons and leaf explants using thidiazuron. In this experiment minimum of 50 explants were used and all the experiments were repeated three times. Callusing starts after about 5-6 days of inoculation. The percentage of callusing was more than 90 %. Cotyledons of both the genotypes responded to this cytokinin and auxin combination. After 20 days of culture initiation the calli were transferred to the same medium but hormonal concentrations reduced to half and the medium gelled with 0.8 % agarose. After 2 sub-culture tye regenerated calli were transferred to hormone free MS medium. Afte the plants attained a height of 3-4 cms. It was then transferred to ½ MS medium supplemented with 0.2 mg/L indole butyric acid (IBA) for rooting. The regenerated plants were transferred to pots for acclimatization. The highest percentage of calli showing embryogenesis was using 2 mg/L BAP and 0.2 mb/L NAA. For plant regeneration it was essential to have both BAP and NAA. The survival rate of regenerated plants was more more than 50 %. This protocol may be ideal for use in genetic transformation of this recalcitrant grain legume to recover pod-borer resistant pigeonpea plants.

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Table 1. Effect of BAP and NAA on induction of callus from immature cotyledon explant of 2 genotypes of pigeonpea (Cajanus cajan L)

Genotype	Callusing percentage (%)			
	BAP(1mg/L) + NAA (0.1mg)/L	BAP (2 mg.L)+ NAA (0.1mg /L)	BAP (2mg/L)+ NAA (0.2mg/L)	BAP (3mg/L)+ NAA (0.2mg/L)
UPAS 120	65.0	74.4	87.4	51.2
Tripura local	57.1	71.2	94.0	59 0