IN VITRO PLANT REGENERATION FROM SHOOT TIP EXPLANTS OF PIGEONPEA (*CAJANUS CAJAN*.L)

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ABSTRACT

In vitro plant regeneration of shoot buds from shoot tip explants of the pigeon pea, 6-8 days-old isolated shoot tips from seedling was studied under defined cultural conditions. Shoot tip explants were cultured on MS medium supplemented with various concentration of auxin and cytokinin. Among various concentrations evaluated, IAA (1.5 mg/L) + TDZ (2.0 mg/L) resulted in callus initiation with in a week, which extended over the surface of the explants followed by induction of shoot buds with in 4 weeks of inoculation. Elongated shoots were rooted (100%) on MS medium fortified with 0.5mg/L of IBA. These present study deals with the development of a protocol for callus mediated regeneration from shoot tip explants in *Cajanus cajan* L. cv ICPL 87119(Asha).

Key words : Pigeon pea, *Cajanus cajan* callus, multiple shoots regeneration.

Abbreviation: TDZ (1-Phenyl -3 - (1, 2, 3-Thiadiazo l-5-yl) Urea, IAA-Indole -3- acetic acid , IBA-Indole-3-butyric acid

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.,) Mill sp.) is a major pulse crop of India. The country accounts for 85% of world pigeon pea production and more than 5.0 million ha area is under pigeonpea . The yield potential of pigeonpea and its wider adaptation however is constrained by various biotic and abiotic stresses of which wilt disease (*Fusarium udum*) and pod borer (*Helicoverpa armigera*) are most important. An efforts to develop pod borer resistant pigeonpea varieties through conventional breeding has not been so far successful due to non-availability of reliable resistant donors in the germplasm. Hence, development of pod borer resistant transgenic lines is considered as a potential strategy for pigeonpea improvement; however, a robust regeneration protocol combined with transformation system is a pre-requisite for development of insert resistant transgenic pigeonpea varieties .

In vitro plant regeneration in pigeonpea has been reported from cotyledons, leaf discs, embryo and stems through callus induction (Mehta and Mohanram 1980) (Kumar *et al.* 1983) and Kumari *et al.* 2001). Similarly, direct organogenesis from different explants circumventing the need for callusing has also been reported in pigeonpea by different workers (Eapen and George 1993,George and Eapen1994) (Prakash *et al.* 1994, Geeta *et al.* 1998). Further somatic embryogenesis has also been reported in Pigeon pea (Sreenivasu *et al.* 1998). In recent year some regeneration protocols have been successfully coupled to *Agrobacterium tumefaciens* mediated transformation system (Lawrence and Kondal 2000, Devi 2004). However most of the protocols developed in the past suffer from low reproducibility low regeneration frequency and genotype dependent responses. The present paper described high frequency regeneration of pigeonpea shoots from apical shoot meristem through direct organogenesis. This protocol is simple reproducible and genotype independent.

MATERIALS AND METHOD

Seeds of Pigeonpea cultivar ICPL87119 (Asha) were obtained from the ICRISAT Hyderabad, India, Seeds were surface sterilized in 70% (v/v) ethanol and seeds were surface sterilized with 0.1%(w/v) mercuric chloride ($HgCl_2$) for 5 minutes and then rinsed thoroughly with sterile double distilled water 3-4 time. The seeds were germinated on MS (Murashige.and Skoog 1962) basal medium with 3% (w/v) sucrose 4 weeks old seedlings served as the source of shoot explants.

Shoot tips measuring 4-6mm in size were excised aseptically and inoculated on to MS (Murashige and Skoog 1962) medium supplemented with different concentrations of TDZ (0.5-5.0mg/L) and IAA (0.5-3.0mg/ L).One explant per culture tube was inoculated. Growth regulators were added prior to autoclaving, pH of the medium was adjusted to 5.8 with NaOH or HCl prior to the addition of agar 0.8% (w/v). After melting the agar the medium (15-20ml (test tube) was dispensed into culture tubes (25X150mm) the tubes were plugged with cotton. The medium was sterilized by autoclaving at 121°C at 15lbs pressure for 20minutes. The cultures were incubated at 16/8-h. light and dark photo period, provided by cool white fluorescent lamps of 2000lux and temperature of 26-2°C. Plant growth regulators namely IAA and TDZ were used in different concentrations for

the induction of shoot buds and shots from shoot tip explants.

RESULTS AND DISCUSSION

Plant regeneration through callus was reported earlier in several legumes (Sal and Bajaj 1979), made an attempt to induced organogenesis from callus culture of *Cicer auritinum Vig*na *radiate Vigna mungo* and *Pisum sativum* using different explants. Hormones especially cytokinins are known to influence shoot bud differentiation *in vitro* (Allik and Saxena 1992, Sanago *et al.* 1996). Among the cytokinins TDZ has been commonly used for the induction of shoot tip explants were inoculated on MS (Murashige.and Skoog 1962) nutrient medium containing different concentration of TDZ (0.5-5.0 mg/L) and IAA (0.5-3.0 mg/L) The effect of various concentration of hormones is presented in the (Table-1} A combination of TDZ (0.5mg/L) + IAA (0.5mg/L) resulted in the induction of callus. High frequency callus induction was noticed when TDZ (1.0mg/L) + IAA (1.0mg/L) was used Increase in the concentration of hormones lead to a decrease in the frequency of shoot buds formation. Further increase in the concentration resulted in callus formation only no shoot buds differentiation when shoot tip explants cultured on MS (Murashige and Skoog 1962) medium supplemented with 2.0mg/L TDZ + 1.5mg/L IAA resulted in callus, which extended all over the surface of the explant with in a week followed by shoots formation with in 3 weeks in culture (Fig-1A). These shoots were sub-cultured on to a medium containing TDZ (2.0mg/L)+ IAA (1.5mg/L) which resulted in proliferation and

Figure 1: A. Initiation of multiple shoots B. Shoot elongation C. Rooting of *in vitro* regenerated shoot. D. Hardening of regenerated plantlet

Table 1: Effect of TDZ and IAA on regeneration from shoot tip explants of Pigeonpea (*Cajanus cajan*.L)

Growth regulators Concentration(mg/L)		Frequency of explants responding	% of response	Growth response
TDZ	IAA			
0.5	0.5	19/25	76	Profuse callus formation
1.0	1.0	18/25	72	Profuse callus formation
2.0	1.5	14/25	56	Callus with shoots
3.0	2.0	15/25	60	Callus with shoots
4.0	2.5	14/25	56	Callus no shoot buds
5.0	3.0	10/25	40	Callus no shoot buds

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