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Full Length Research Paper

In vitro proliferation of shoot regeneration from embryo of *Cajanus cajan* L (var.LGG-29)

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An efficient and direct shoot bud differentiation and multiple shoot induction from embryo explants of pigeon pea (*Cajanus cajan* L.) has been achieved. The frequency of shoot bud regeneration was influenced by the type of explant, genotype and concentrations of cytokinin. Explant embryo were cultured on Murashine and Skoog (MS) medium augmented with different concentrations of Benzyl amino purine (BAP). Among the various concentrations tested, 1.0 mg/l BAP and 0.1 mg/l naphthalene acetic acid (NAA) were found to be the best for maximum shoot bud differentiation. Percentage, as well as the number of shoots per explant showing differentiation of shoot buds was higher on MS media supplement with BAP. The optimal BAP concentration for shoot regeneration was 1.0 mg/l. Elongation of multiple shoots was obtained in MS medium with the concentration 0.4 mg/l gibberillic acid (GA3). The elongated shoots were successfully rooted on MS medium containing different concentrations of auxins. Among them indole buteric acid (IBA) at 1.0 mg/l induced maximum frequency of rooting followed by NAA and indole acetic acid (IAA). Regenerated plants were successfully established in soil where 90 to 95% of them have been developed into morphologically normal and fertile plants. This method can thus be advantageously applied in the production of transgenic pigeon pea plants.

Key words: Tissue culture, redgram, embryo, multiple shoots.

INTRODUCTION

The importance of grain legume is multipurpose and their seeds are mostly used to supply vegetative proteins for humans. Red gram or pigeon pea was high among the grain legumes of India, consumed by large population of the country. Genetic improvement through molecular techniques (Lawrence et al., 2001) has been considered for a wide range of grain legumes. The availability of a genetic transformation system would facilitate the agronomic traits (Singh et al., 2003) affecting production efficiency as well as the nutritional quality of redgram. This paper describes the regeneration protocol for genetic improvement for redgram (Venkatachalam et al., 1999).

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MATERIALS AND METHODS

Seeds of *Cajanus cajan* L. (var.LGG-29) are procured from Directorate of oil seed research, Hyderabad were used in this study. Seeds were sterilized on surface with 0.1% mercuric chloride solution for 10 min and then rinsed 5 times with sterile distilled water. The seeds were soaked for overnight and the embryo was excised and inoculated in dark for 2 days and then transfer to photoperiod at 25±2°C with a light intensity of 60 μEm^2s^1. After 5 days embryos are cut into 5 mm (Geetha et al., 1998) and they are inoculated in regeneration medium (1 mg/l BAP and 0.1 mg/l NAA). After one week shoot buds appear and later they are convert into shoots. These shoots are sub cultured in shoot elongation medium

Table 1. Effect of BAP and NAA against embryo explant for shoot regeneration.

BAP	NAA	No. of explants kept for regeneration	No. of responding explants	No. of shoots per explants
0	0	8	NR	NR
0.5	0	8	NR	NR
1	0	8	5	NR
1.5	0	8	4	3
2	0	8	2	1
0	0.1	8	NR	NR
0.5	0.1	8	NR	NR
1	0.1	8	5	7
1.5	0.1	8	2	1
2	0.1	8	3	4

NR: Not responded.

Table 2. Effect of IBA against root induction.

Growth regulator	Rooting frequency	Days of rooting	Mean no. of roots per shoot	Mean length of roots per shoot
0	ND	ND	ND	ND
0.5	ND	ND	ND	ND
1	70	21	5.5±0.03	3.7±0.1
1.5	60	17	3.2±0.03	2.3±0.2
2	40	14	3.5±0.03	1.6±0.3

ND: Not detected.

(0.4 mg/l GA3). Then they are transferred into rooting medium 1 mg/l IBA. The adventitious root appeared within 2 weeks and developed further in 4 weeks. Then plants are ready for transplantation to pots.

Culture media and conditions

The embryo was placed on MS medium with 3% (w/v) sucrose supplemented with BAP (1 to 5 mg/l) for direct shoot bud regeneration. Similar conditions of the media have been reported by (Prakash et al., 1994; Kumar et al., 1983). All media were adjusted to pH 5.8 prior to the addition of 0.8% (w/v) agar and autoclaved at 121°C and 15l b for 15 min. The culture was maintained at 25±2°C in the culture room with 16 h photoperiod with 60 μEm^2s^2 light intensity provided by cool white fluorescent tubes.

The regenerated explant were taken and sub-cultured in the regeneration media for every 15 days. After regeneration upto 2 cm long, they were placed in the shoot elongation media (SEM) supplement with MS salts and hormone GA3 (0.4 mg/l) concentration (Mohan et al., 1998). Then shoots were sub cultured for every 15 days. The sub-cultured shoots were then transferred to the rooting medium supplemented with 1 mg/l IBA (Daal et al., 2003). For every 15 days, the sub-culture repeated. After getting the roots, they were transferred to the soilrite for hardening (George et al., 1998). Later plants were transferred to the green house.

RESULTS AND DISCUSSION

The shoot regeneration was observed from embryo after 3 to 4 weeks in the regeneration medium (MS) of various concentrations (0 to 2) (Frankalin et al., 1998). From the Table 1, it is evident that with the BAP at concentrations 1 and 0.1 mg/l in combination with NAA showed maximum number of shoots per explant. Along with the shoot buds, some buds were found to be greenish in colour. Green and healthy shoots were measured to be 2 to 3 cm in length were excised and sub-cultured into the shoot elongation medium (GA3, 0.4 mg/l). For every 15 days the sub-culture must be repeated. When shoots grow to their maximum lengths, then they were sub-cultured into the rooting medium. From the Table 2, it is clear that IBA at concentration 1 mg/l showed the maximum root length in 21 days compared to other concentrations of NAA and IAA. After reaching maximum root length it has been acclimatized in sand clay and vermiculate in the ratio of 1:1:1. It has also been acclimatized in soilrite, vermiculate and peat. Similar *in vitro* regeneration and transformation of pigeon pea were reported by Thu et al. (2003).

Conclusions

Our study proved that efficient regeneration could be done with embryo as an explant in redgram (var.LRG-41). However, proper medium conditions should be employed for better shoot and root growth after regeneration. Further studies are in progress in

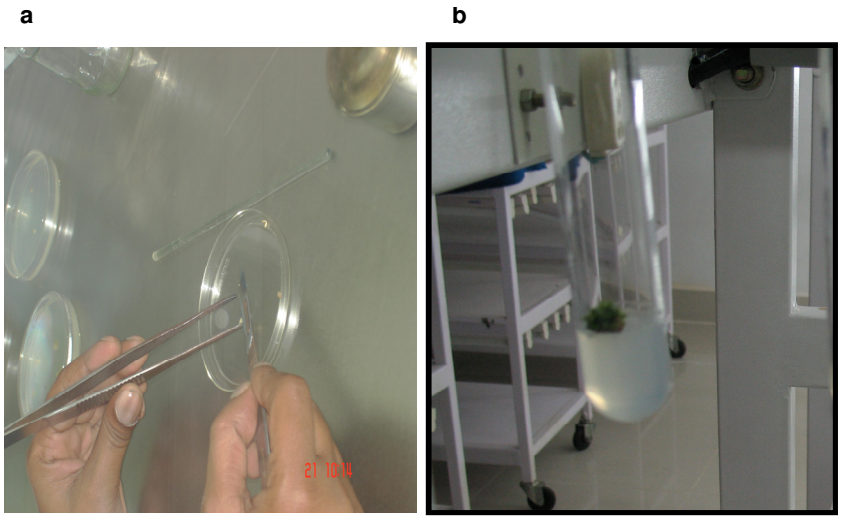

Figure 1. (a) Excised embryo of redgram (var.LGG- 29), (b) Multiple shoots regeneration from embryo.

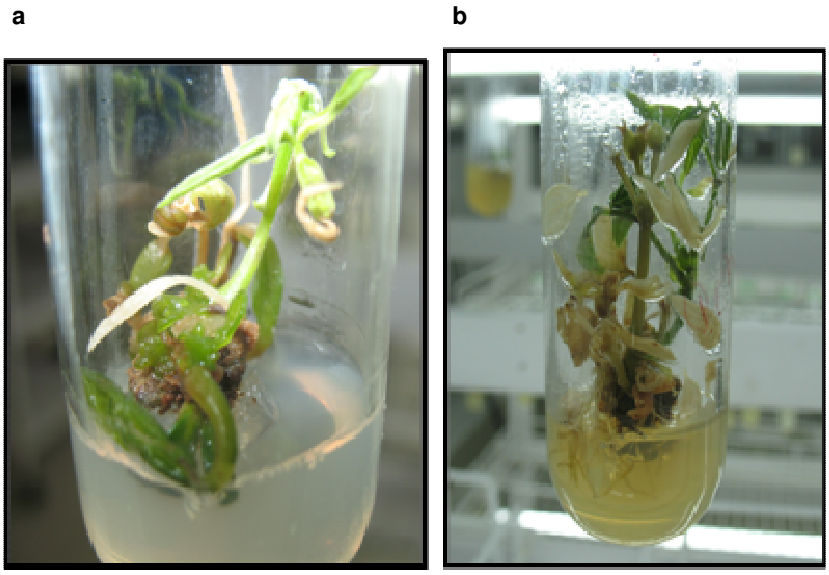

Figure 2. (a) Shoots sub-cultured from embryo (b) Rooted shoot let from embryo.

transgenics for the exploitation of the results obtained from this experiment (Figures 1 and 2).

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