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Research Article

IN VITRO PROLIFERATION OF SHOOT REGENERATION FROM SHOOT TIP OF *CAJANUS CAJAN* L. (VAR. LRG-41)

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Abstract

An efficient multiple shoot induction from embryo explants of pigeon pea (*Cajanus Cajan* L) has been achieved. The frequency of shoot regeneration was influenced by the type of explants, genotype and concentrations of cytokine. Explants shoot tip were cultured on Murashine and Skoog (MS) medium augmented with different concentrations of Benzyl amino purine (BAP). Among the various concentrations tested, 2.0 mg/l BAP and 0.1 mg/l naphthalene acetic acid (NAA) was found to be the best for maximum shoot proliferation. Percentage as well as the number of shoots per explants showing higher on MS media supplement with BAP. The optimal BAP concentration for shoot regeneration was 2.0 mg/l. Elongation of multiple shoots was obtained in MS medium with the concentration 0.4 mg/l gibberillic acid (GA_3). The elongated shoots were successfully rooted on MS medium containing different concentrations of auxins. Among them indole butyric acid (IBA) at 1.0 mg/l induced maximum frequency of rooting. Regenerated plants were successfully established in soil rite 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, red gram, embryo, multiple shoots

Introduction

The importance of grain legume is multipurpose and their seeds are mostly used to supply vegetative protein for humans. Red gram or pigeon pea high among the grain legumes of India, consumed by large population of the

country. Genetic improvement through molecular techniques has been considered for wide range of grain legumes. The availability of a genetic transformation system would facilitate the agronomic traits affecting production efficiency as well as the nutritional quality of red gram. This paper describes the regeneration protocol for genetic improvement for red gram.

Materials and Methods

Seeds of *Cajanus Cajan* L., (var. LRG-41) are procured from Regional Agricultural Research Station, Acharya N G Ranga Agricultural University, Tirupati were used in this studies. Seeds were surface sterilized with 0.1% mercuric chloride solution for 10 min and then rinsed 5 times with sterile distilled water .The seeds were inoculated in MS medium and kept in dark for 2 days and then transfer to photoperiod at 25 ± 2^0c with a light intensity of 60µE M^2S^1.After 12days shoot tip are cut into 5mm and they are inoculated in regeneration medium (Prakash et *al., 1983*) (1mg/l BAP and 0.1mg/l NAA). After one week shoot buds appear (Venkatachalam *et al.,* 1998*)* and later they are converting into shoots. These shoots are sub culture into shoot elongation medium (0.4mg/l GA3). Then they are transfer into rooting medium 1mg/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

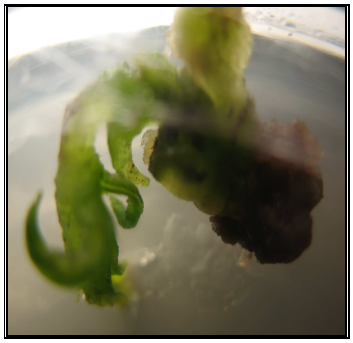
The shoot tip was placed on MS medium with 3% (w/v) sucrose supplemented with BAP (1-5mg/l) for direct shoot bud regeneration (Kumar et *al., 1984*). All media were adjusted to P^H 5.8 prior to the addition of 0.8% (w/v) agar and autoclaved at 121^0C and 15lb for 15 min. The culture were maintained at 25 ± 2^0C in the culture room with 16 hours photoperiod with 60µ E m^2s^2 light intensity provided by cool white fluorescent tubes. The regenerated explants (Sharma *et al*., 2003) were taken and sub-culture in the regeneration media (Mohan *et al.,* 1998) for every 15 days. After regenerated up to 2 cm meter long(Lakshmisita *et al*., 1999), then they were placed in the shoot elongation media (SEM) supplement with MS salts(George *et al*., 1998) and hormone GA3 (0.4 mg/l) concentration. Then shoots were sub cultured for every 15 days .The sub-cultured shoots were then transferred to the rooting medium supplemented with 1mg/l IBA. For every 15 days, the sub-culture repeats (Frankalin *et al.,* 1998) .After getting the roots, then they were transferred to the soil rite for hardening .Later they transfer to green house.

Results and Discussion

The shoot regeneration was observed from embryo after 3-4 weeks in the regeneration medium (MS) of various concentrations (0-2). From the table-1 it is evident that with the BAP at concentrations 1mg/l and 0.1mg/l in combination with NAA showed maximum number of shoots per explants. Along with the shoot buds, some buds were found to be yellowish in colour. Green and healthy shoots (Lawrence et *al*., 2001) were measured to be 2-3 cm in length were excised and sub-culture into the shoot elongation medium (GA_3 0.4 mg/l). For every 15 days the sub-culture must be repeated. When shoots grows to their maximum lengths (Jaiwal *et al.*, 2003), then they were sub-cultured into the rooting medium. From the table 2, it is clear that IBA at concentration 1mg/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 soil rite, vermiculate and peat (Jacobs *et al*, 2003)

Our study proved that efficient regeneration could be done with shoot tip as explants in red gram (var.LRG-41). However, proper medium conditions should be employed for better shoot and root growth after regeneration. Further studies are in progress in transgenic for the exploitation of the results obtained in this experiment.

a) Shoot tip with multiple shoots



b) shoot lets in elongation medium.

Shoot let in rooting medium

Table-1: **Effect of BAP and NAA against embryo explants for shoot regeneration.**

BAP	NAA	No.of explants keptfor 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 rootsper 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

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