INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-5, Issue-1, Jan-Mar-2014 Coden : IJABPT Copyrights@ 2014

ISSN : 0976-4550

Received: 03rd Jan-2014 *Revised: 05th Jan-2014* *Accepted: 18th Jan 2014*

Research article

PATTERN OF PLANT REGENERATION FROM SHOOT TIP EXPLANTS OF PIGEONPEA (*CAJANUS CAJAN* L MILLLSP) VAR LRG-41

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ABSTRACT: An efficient direct shoot bud differentiation and multiple shoot induction from shoot tip explants of pigeon pea (*Cajanus cajan* L.) has been achieved. The frequency of shoot bud regeneration was influenced by the type of explants, genotype and concentrations of cytokinin. Explants viz. shoot tip isolated from 10 day old seedlings showed better explants response Explants were cultured on Murashige and skoog (MS) medium augmented with different concentrations of BAP and NAA. Among the various concentrations tested, 2.0mg/l BAP (Benzyl amino purine) and 0.1 mg/l Napthalene 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 and optimal BAP concentration for shoot regeneration was 2mg/l. The elongated shoots were successfully rooted on MS medium containing different concentrations of auxins. Among them indole buteric acid (IBA) at 1.0mg/l induced maximum frequency of rooting. Regenerated plants were successfully established in soil where 91% 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.

Keywords: Organogenesis. Shoot tip. Pigeonpea. Plant regeneration

INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millsp.) or redgram is one of the most popular legume in the world, especially in the Indian subcontinent. Due to its multiple uses, pigeonpea is widely used in intercropping systems in semi-arid regions and ranks fifth in area after soybean, common bean, peanut and chickpea. It is used in diverse ways as a source of food, feed, and fertilizer. It provides the main source of protein for the poorest populations and plays an important role in reducing malnutrition for millions of people around the world. Improvement of pigeonpea cultivars possessing resistance to pest and diseases, tolerance to abiotic stresses and low allergenic proteins in seeds is therefore desirable. Plant regeneraton in pigeonpea has been reported through cotyledons (George and Eapen 1994; Geetha *et al*. 1998), cotyledonary nodes (Mehta and Mohan Ram 1980; Kumar *et al* 1983,1984; Shiva Prakash *et al* 1994; Naidu et al 1995; Geetha *et al* 1998) and Embryonal axes (Franklin *et al*.2000). In these reported regeneraton systems, time required for the formation of shoot buds and their complete differentiation into shoot was long, and the recovery of fully differentiated plants was long thus making such systems inefficient for genetic transformation work. Hence in the present study, the major emphasis was on the establishment of a regeneration protocol that would provide transgenic plants in large numbers for routine work on the genetic enhancement of pigeonpea

MATERIALS AND METHODS

Plant material

Seeds of pigeonpea (*Cajanus cajan* L. Millsp) variety LRG-41 used in the experiment were obtained from plant breeding (pulses) section, Regional Agricultural Research Station, Acharya NG Ranga Agricultural University, Tirupati-517502, A.P. India

Explant preparation and shoot regeneration

The seeds of pigeonpea var .LRG-41 were washed with 0.1% mercuric chloride for 10 minutes. Shoot tip showed highest response when collected from 10 days old seedling, compared 8,10,12 days aged seedlings. Such response of seedling age in terms of explant response is known to be existed and it has been reported by many workers in red gram. (Eapen *et al.*,1998, Chandra *et al.,* 2003 and Vandanakashyap *et al.,* 2011). The present investigation, different combinations of hormones were used *viz.,* cytokinins like BAP, Kinetin alone or along with the combination of auxins *i.e.,* NAA along with different explants. MS basal medium with different hormonal concentrations of BAP, Kinetin and NAA were evaluated for their effect on days taken for shoot bud initiation, Number of explants producing shoot buds, shooting frequency, mean number of shoots / explant and length of the shoots. The data revealed significant differences between the treatments for all the parameters. Among different combinations, MS medium + BAP 2.0 mg l^{-1} + NAA 0.1 mg l^{-1} recorded significantly lesser number of days taken for shoot bud initiation in shoot tip explants (15 days).

Rooting of shoots and acclimatization

The regenerated shoots were transferred to different combinations of IBA, NAA alone and BAP and Kinetin in combination with IBA were used with full strength MS medium for root induction. Mean number of days taken for root initiation was significantly lower when MS medium was supplemented with IBA 1.0 mg l^{-1} compared to all other combinations. *In vitro* rooted plants established with 91 % success in when soilrite mixture was used and days taken for acclimatization is 12.8 days followed by Soil : Sand : Soilrite (1:2:1).

Culture media and conditions

MS basal medium containing 3% sucrose was used for all in vitro cultures. The pH of the medium was adjusted to 5.8 prior to adding 0.8% agar; media were autoclaved at 121^{0}C for 15 min. Cultures were maintained at 26 ±1^{0}C under continuous light provided by white cool fluorescent tubes of 60 µE m^{-2} s^{-1} light intensity. The growth regulators BAP, kinetin, NAA and IBA were filter-sterilized prior to addition to culture media. The explants were cultured on sterile Petri dishes containing SIM (Shoot induction medium), explants bearing adventitious shoot buds were subsequently transferred to culture tubes for shoot elongation and rooting of shoots. Data on the frequency of shoot bud regeneration from each explant was recorded. All experiments were repeated three times and the data were analyzed by calculating mean and standard error.

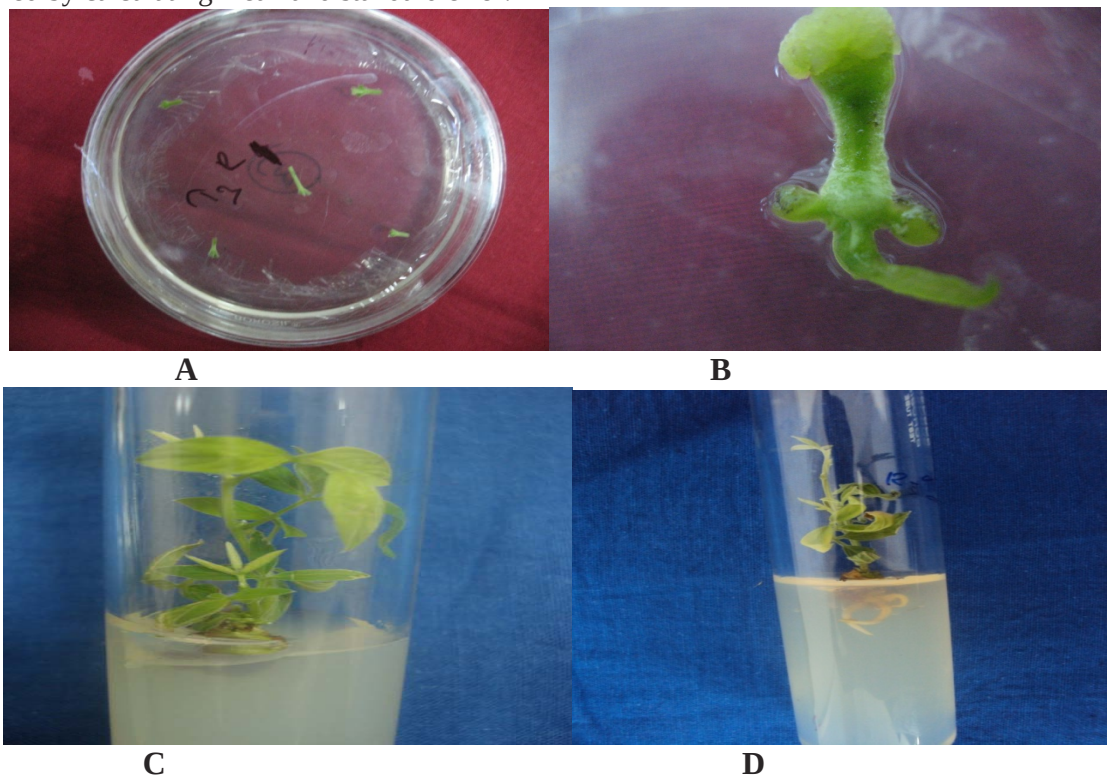

Fig 1. Regeneration of multiple shoots from different explants (A) Shoot tip explants inoculated on MS medium supplemented with BAP and NAA (B) shoot buds originating from explants (C) Multiple shoot production (D) Root induction

Statistical analysis

The data were analysed using analysis of variance for a completely randomized design and the treatment means were compared.

RESULTS AND DISCUSSION

Shoot tips collected from 10 days old seedlings were cultured on MS medium + BAP 2.0 mg l^{-1}+NAA 0.1 mg l^{-1} recorded significantly lesser number of days taken for shoot bud initiation (15.0 days) the highest shoot length was recorded when MS medium was fortified with BAP 2.0 mg l^{-1} + NAA 0.1 mg l^{-1} (3.8 cm) shown in Table 1. Singh *et al,* (2004) reported that shoot apices from 16 days old seedlings of pigeonpea induced multiple shoots with low concentration of TDZ 0.05 to 1.0 µM. For root induction Different combinations of IBA, NAA alone and BAP and Kinetin in combination with IBA were used with full strength MS medium.

Table 1: Effect of MS medium with different hormonal treatments on shoot induction and elongation in Shoot tip explants of Redgram var. LRG-41

S. No	Concentrations (mg l^{-1})	Days taken for shoot bud initiation	No. of explants producing shoot buds	Shooting frequency (%)	Length of shoots (cm)
1	MS+BAP 0.5	NR	0	0	0
2	MS+BAP 1.0	21.5	0.6	8.91(17.36)	1.8
3	MS+BAP 2.0	20.0	1.0	17.80(24.95)	1.9
4	MS+BAP 3.0	22.5	1.7	27.85(31.82)	2.5
5	MS+BAP 4.0	18.2	2.0	28.56(32.270	2.4
6	MS+BAP 5.0	21.5	0.6	8.91(17.360	2.8
7	MS+Kinetin 0.5	NR	0	0	0
8	MS+Kinetin 1.0	NR	0	0	0
9	MS+Kinetin 2.0	NR	0	0	0
10	MS+Kinetin 3.0	20.0	1.7	27.85(31.63)	2.8
11	MS+Kinetin 4.0	21.5	2.7	38.85(38.53)	2.6
12	MS+Kinetin 5.0	23.5	2.0	28.56(32.27)	2.2
13	MS+BAP0.2+NAA0.1	16.2	1.7	27.85(31.82)	2.0
14	MS+BAP0.4+NAA0.1	17.0	2.0	28.56(32.27)	2.2
15	MS+BAP0.6+NAA0.1	15.6	2.7	38.85(38.53)	1.8
16	MS+BAP0.8+NAA0.1	17.4	2.0	28.56(32.27)	2.6
17	MS+BAP1.0+NAA0.1	16.5	2.3	35.71(36.690	2.4
18	MS+BAP1.2+NAA0.1	15.0	2.7	38.85(38.53)	3.0
19	MS+BAP1.4+NAA0.1	17.8	2.3	35.71(36.60)	2.1
20	MS+BAP1.6+NAA0.1	18.2	3.0	42.83(40.86)	3.0
21	MS+BAP1.8+NAA0.1	19.1	3.3	47.61(43.620	2.1
22	MS+BAP2.0+NAA0.1	18.8	4.3	61.90(51.88)	3.8
23	MS+BAP2.2+NAA0.1	15.2	3.6	52.37(46.32)	2.0
24	MS+BAP2.4+NAA0.1	16.5	2.3	35.71(36.69)	1.6
25	MS+BAP3.0+NAA0.1	21.2	3.3	47.61(43.68)	1.8
26	MS+NAA0.5+Kinetin0.5	18.6	2.3	35.71(36.69)	3.2
27	MS+NAA1.0+Kinetin1.0	19.6	2.7	38.85(38.53)	3.0
28	MS+NAA1.5+Kinetin1.5	22.0	3.0	42.83(40.86)	2.9
29	MS+NAA2.0+Kinetin2.0	24.2	3.6	52.37(46.32)	2.4
30	MS+NAA2.5+Kinetin2.5	20.0	2.3	35.71(36.69)	2.2
31	MS+NAA3.0+Kinetin3.0	21.5	2.0	28.56(32.27)	1.9
	(±)S.Em	0.816	0.68	0.89	1.274
	C.D at 5%	1.63	0.98	1.98	3.618

Note: Figures in parantheses represent arc sine transformed values No multiple shoots observed

NR: No Response

Mean number of days taken for root initiation was significantly lower when MS medium was supplemented with IBA 1.0 mg l^{-1} (17.9 days) compared to all other combinations followed by Kinetin 1.5 mg l^{-1} +IBA 0.6 mg l^{-1} (18.0 days) and BAP 0.8 mg l^{-1} + IBA 0.8 mg l^{-1} (18.2 days). Similarly MS medium with IBA 1.0 mg l^{-1} resulted in the highest mean number of shoots (3.5 Shoots /5 Shoots) producing roots with the highest frequency of 70.6 % compared to all other treatments. The hormonal combination recorded high mean number of roots (8.6) and root length (5.3 cm) is shown in Table 2. Sharma *et al.* (2006) reported that elongated shoots of pigeonpea rooted on MS medium containing 25 µM IAA. However Yadav and Padmaja (2003) reported that low concentrations of growth regulators at 1.0 mg l^{-1} of IAA/NAA + 0.1 mg l^{-1} of Kinetin triggered root induction. *In vitro* rooted plants established with 91 % success in when soilrite mixture was used and days taken for acclimatization is 12.8 followed by Soil : Sand : Soilrite (1:2:1) with 76 % and 14.3 days for acclimatization shown in Table 3. Then the plants were subsequently transferred to earthen pots. The present investigation, the multiplication of shoot buds from explant suggest the shoot bud induction was De novo. This was also confirmed by histological observations. The regeneration system described above may be useful for introducing new genes in to the pigeonpea genome by microprojectile-bombardment-mediated or *Agrobacterium*-mediated genetic transformation.

Table 2: Effect of different MS media fortified with different hormonal treatments on Rooting from shootlets in var. LRG-41

S. No	Concentration (mg l^{-1})	Days taken for root initiation	No. of shoots producing roots	Frequency of rooting (%)	Mean no of roots /shoot	Mean length of roots (cm)
1	MS+IBA 0.2	NR	0	0	0	0
2	MS+IBA 0.4	22.2	0.6	13.3(21.38)	3.3	2.4
3	MS+IBA 0.6	20.8	1.3	26.6(31.04)	3.8	2.56
4	MS+IBA 0.8	18.6	1.0	20.0(26.56)	2.6	2.63
5	MS+IBA 1.0	17.9	3.53	70.6(57.11)	8.6	5.3
6	MS+NAA 1.0	NR	0	0	0	0
7	MS+NAA 1.5	20.2	0.6	13.3(21.38)	3.3	2.9
8	MS+NAA 2.0	24.2	0.3	6.6(14.88)	3.3	2.2
9	MS+BAP0.2+IBA0.2	28.4	1.6	33.3(35.24)	4.3	2.8
10	MS+BAP0.4+IBA0.4	19.8	2.52	50.2(45.11)	6.9	3.2
11	MS+BAP0.6+IBA0.6	21.2	2.64	53.3(46.89)	7.4	3.4
12	MS+BAP0.8+IBA0.8	18.2	3.08	61.6(51.17)	6.3	4.0
13	MS+Kinetin0.5+IBA0.2	23.6	3.16	63.3(52.71)	7.9	3.0
14	MS+Kinetin1.0+IBA0.4	18.9	2.52	50.2(45.11)	6.9	3.8
15	MS+Kinetin1.5+IBA0.6	18.0	3.25	65.0(53.72)	7.0	2.8
16	MS+Kinetin2.0+IBA0.8	0.594	2.64	53.3(46.89)	7.3	4.3
	(±)S.Em	1.728	0.463	0.539	0.54	0.574
	C.D at 5%	0.594	1.341	1.559	1.563	1.664

Note : Figures in parentheses represent arc sine transformed values Observations were taken from five shootlets
NR: No Response

Table 3: Effect of different soil mineral mixtures on acclimatization and survival of regenerated plantlets in Redgram var. LRG-41.

S. No	Soil mineral mixture	No. of days taken for acclimatization	Survival percentage
1	Soilrite	12.8	91(71.57)
2	Sand	16.6	45(42.13)
3	Soil	15.3	62(51.94)
4	Soil:sand:soilrite	14.3	76(60.67)
	± S.Em	0.38	0.43
	C.D at 5%	1.3	1.51

Note: Figures in parentheses represent arc sine transformed values

ACKNOWLEDGEMENTS

The Authors are grateful to Acharya N G Ranga Agricultural university for providing research facilities at Institute of Frontier Technology.

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