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In vitro regeneration of pigeonpea from leaf with petiole explant

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ABSTRACT

The two genotypes TAT-10 and PKV TARA responded for direct regeneration, 90-100% establishment with good growth of explants of both the genotypes was recorded on all 21 media combinations under study. On shoot bud induction medium, MS + BAP 2.0 mg/l with 0.1 mg/l NAA , significantly higher average number of shoot buds / explant were recorded in both the genotypes. The shoot elongation treatment combination MS + 0.2 mg /l BAP recorded highest per cent conversion of shoot buds to shoot and significantly higher number of shoot elongation / clump of shoots. The rooting medium ½ MS + 1.5 mg/l IAA recorded significantly higher average number of secondary roots / shoot in the genotype TAT-10 and rooting medium ½ MS + 1.0 mg/l IAA recorded significantly higher average number of secondary roots / shoot in the genotype PKV TARA.The rooted plantlets of both the genotypes when hardened in a plastic cups on soilrite initially supplemented with ½ MS liquid medium and covered with polythene for a week showed 75% survival.

Key words : Pigeonpea, *In vitro* regeneration

INTRODUCTION

Pigeonpea is an important grain legume crop of rainfed agriculture in the semi-arid tropics. The conventional breeding methods are the most widely used for crop improvement. But in certain situations, these methods have to be supplemented with modern biotechnological techniques either to increase their efficiency or to be able to achieve the objective, which is not possible through the conventional methods. Transformation is one of the important technology developed which expands the sources of genes for plant improvement to all organisms, far beyond the gene pool accessible via sexual hybridization. Transformation also offers strategies for over expressing or suppressing endogenous genes. Thus, introducing new genes or manipulating endogenous gene expression via transformation generates new phenotypic variation useful for investigating gene function and for crop improvement.

Several attempts and number of reports yet, it has not been possible to achieve direct regeneration from leaf segments of *C. Cajan*, where most of the reports are on callus tissue (Eapen and Georg 1993, Georg and Eapen 1994).

MATERIALS AND METHODS

The experimental material of present investigation comprised of two varieties of pigeonpea *viz.,* TAT- 10 and PKV - TARA. The genetically pure seeds were obtained from Senior Research Scientist, Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The leaf of 5-6 days grown seedling with petiole was used as explants. For preparation of explants mature seeds were used. The seeds were washed with 1% (v/v) lanoline followed by treatment with 0.1 % (w/v) mercuric chloride for 5 minutes,followed by rinsing with sterile distilled water for 5-6 times. The pre-sterilized seed were grown *in vitro* on ½ MS medium with 3% sucrose and 0.8% agar-agar. The 24-36 hrs. germinated seeds were cut to remove seed coat and cotyledons were splits open in a LAF cabinet. The embryo axes were extracted and the shoot apex region and the root pole were removed. The mature embryo axes in which both shoot and root pole were removed (referred to as decapitated mature embryo axes: DCMEA) were used as explants.

RESULTS AND DISCUSSION

At various stages of regeneration different media combination formulated using various concentrations of growth hormones (BAP, NAA, IAA and GA3) with Murashige and Skoog's (MS) basal medium were tried.

Shoot proliferation:

The leaf with petiole explant get increased in size after 7 days of inoculation. Genotype TAT-10 and PKV TARA showed 90.67 to 100 per cent establishment of leaf with petiole explant. The leaf segment and petiolar region enlarged and produced shoot buds at basal region of explants. The observations for initiation of shoot bud induction were recorded daily and data for number of multiple shoots induced was recorded on 30th day, after inoculation of explants. The explant failed to induce shoots on plain MS medium and in both the genotypes hence, all the treatment combinations tried were significantly superior over the control (Plate 1).

Out of different treatments tried for induction of

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Plate 1 : Shoot bud induction from petiolar region of leaf explant

multiple shoots in genotype TAT-10, treatment combination MS + 2.0 mg/l BAP+ 0.1 mg/l NAA induced significantly highest number of multiple shoots *i.e.* 1.40 and the treatment combination MS + 2.0 mg/l BAP showed at par result with 1.13 number of multiple shoots. For genotype PKV TARA, similar treatment combination MS + 2.0 mg/l BAP + 0.1 mg/l NAA induce significantly highest number of multiple shoots *i.e.* 1.47 and at par 1.22 number of multiple shoots on treatment MS + 2.0 mg/l BAP (Table 1).

The various concentrations of NAA (0.1, 0.3,0.5 mg/l) were tried in combination of BAP(2.0,3.0,4.0,5.0 mg/l). At a single concentration of NAA it was observed that increase in concentration of BAP form 2.0 mg/l to 5.0 mg/l had reduced the mean number of shoots produced. This trend was repeatedly observed in all tried concentrations of NAA and also in mean over explant in both the genotypes *i.e.* TAT-10 and PKV TARA.

For induction of shoot bud in pigeonpea MS basal medium as employed in present study was most commonly used by various workers. George and Eapen (1984) reported induction of multiple shoots in pigeonpea on 1.0 mg/l BAP enriched medium. Prakash *et al.* (1994) reported MS + 2.0 mg/l BAP, Geetha *et al.* (1998) reported MS + 2.0 mg/l BAP or KIN, for shoot bud induction. Mohan and Krishnamurthy (1998) reported 22.2 µM BAP, 2.3 µM KIN and 271 µM adenine sulphate for induction of large number of shoot buds from explants of genotypes T-15-15 and GAUT 32-90 cultured on six different basal media.

Shoot elongation:

Among both the genotypes, the treatment combination (MS +0.2 mg/l BAP) was found to show highest number of shoot elongation per clump of shoot buds. In both the genotypes the MS medium used as control failed to induce elongation of shoots.

The two treatment combination 0.1 mg/l and 0.2 mg/l BAP used for induction of multiple shoots has shown elongation in same media and hence subsequently tried for elongation. MS + 0.2 mg/l BAP was found to be the best combination for elongation with 0.44 and 0.38 number of shoot elongated in genotype TAT-10 and PKV TARA (Table 2 and Plate 2). Increasing the concentration of NAA (0.1 to 0.3 mg/l) increased induction of callus which interfered with the shoot bud elongation and inhibit the elongation activity. Data not shown.

Lower concentration of BAP was used for elongation which showed good separation and well elongation as compared to other treatments. Same results were reported by Thu *et al.* (2003). Jain and Chaturvedi, 2004, Misra 2002 reported MS + BA 0.2 µM for elongation. MS + 1.0 mg/l BAP medium were used for elongation of shoot buds from leaf explants (Balarama and Padmaja, 2003). In both the genotypes the explants under study failed to elongate on MS basal medium, where as Frankline *et al.* (2004) reported elongation on MS basal

Table 1 : Proliferation of explants of genotypesTAT-10 and PKV TARA on different shoot bud induction media combination

Treatments	Avg. no. of multiple shoots		
	TATA-10	PKV TARA	Callus
MS + 0.1mg/l BAP	0.51	0.53	+
MS + 0.2mg/l BAP	0.38	0.33	+
MS + 0.5mg/l BAP	0.82	0.96	+
MS + 1mg/l BAP	1.02	1.00	-
MS + 2mg/l BAP	1.13	1.22	-
MS + 3mg/l BAP	1.04	1.22	-
MS + 4mg/l BAP	0.67	0.69	-
MS + 5mg/l BAP	0.53	0.49	-
MS + 0.1mg/l NAA + 2mg/l BAP	1.40	1.47	+
MS + 0.1mg/l NAA + 3mg/l BAP	0.80	0.87	+
MS + 0.1mg/l NAA + 4mg/l BAP	0.51	0.51	+
MS + 0.1mg/l NAA + 5mg/l BAP	0.16	0.20	+
MS + 0.3mg/l NAA + 2mg/l BAP	1.02	1.09	++
MS + 0.3mg/l NAA + 3mg/l BAP	0.60	0.53	++
MS + 0.3mg/l NAA + 4mg/l BAP	0.40	0.33	++
MS + 0.3mg/l NAA + 5mg/l BAP	0.29	0.27	++
MS + 0.5mg/l NAA + 2mg/l BAP	0.82	0.78	+++
MS + 0.5mg/l NAA + 3mg/l BAP	0.53	0.47	+++
MS + 0.5mg/l NAA + 4mg/l BAP	0.33	0.33	+++
MS + 0.5mg/l NAA + 5mg/l BAP	0.18	0.16	+++
MS plain	0.00	-	-

Note: Observations recorded on 30th day of inoculation
+: Low callus (0.5 mm),
++ : medium callus(1-1.5 mm),
+++: large callus (2 mm and above)

Table 2 : Shoot elongation in genotypes TAT-10 and PKV TARA on shoot elongation media

Treatments	No. of shoots elongation	
	TAT-10	PKV TARA
MS+0.1mg/l NAA+0.1mg/l BAP+0.5mg/l GA3	0.20	0.16
MS+0.1mg/l NAA+0.1mg/l BAP+1.0mg/l GA3	0.24	0.20
MS+0.1mg/l NAA+0.1mg/l BAP+1.5mg/l GA3	0.22	0.18
M+0.1mg/l BAP	0.36	0.31
MS+0.2mg/l BAP	0.44	0.38
MS Control	0.00	0.00

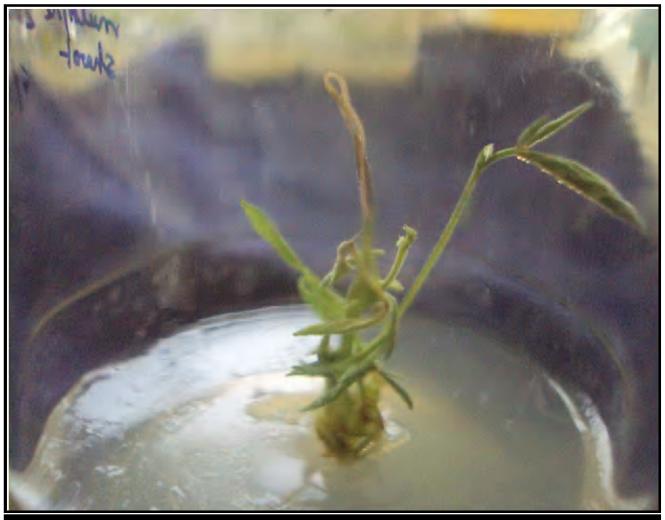

Plate 2 : Elogation of shoots

medium. This variation could be attributed to the genotype, explant difference and also pre culture conditions of proliferation.

Root induction:

The shoots which were elongated 3-4 cm length were excised from the shoot clumps and transferred to medium augmented with full and half strength MS basal medium and different concentration of IAA.

Leaf with petiole explant showed significantly higher secondary roots in ½ MS + 1.2 mg/l IAA with 6.82 average numbers of roots and at par result were found in 1.0 mg/l and 0.5 mg/l IAA with 6.77 and 6.42 average numbers of roots (Table 3 and Plate 3).

In leaf with petiole explant significantly higher secondary roots over control were obtained in ½ MS + 1.0 mg/l IAA (7.60 secondary roots) and 7.24 average secondary roots were obtained in at par treatment ½ MS + 1.5 mg/l IAA were at par with best treatment.

Different scientist used different auxins for rooting

Table 3 : Rooting response in genotype TAT-10 and PKV TARA

Treatments	Average number of secondary roots	
	TAT-10	PKV TARA
MS+IAA		
0.1mg/l	2.02	1.95
0.2mg/l	2.57	1.93
0.5mg/l	3.00	2.04
1.0mg/l	3.33	3.29
1.5mg/l	4.13	6.75
½ MS+IAA		
0.1mg/l	4.71	4.71
0.2mg/l	5.66	5.66
0.5mg/l	6.42	6.80
1.0mg/l	6.77	7.60
1.5mg/l	6.82	7.24
MS plain	1.71	1.71
½ MS plain	2.62	2.68
Mean	4.14	4.36

Plate 3 : Rooting of shooted plant

purpose. In present study used auxin were IAA was used with full and half MS as a basal medium. By increasing the concentration there was increased in average number of secondary roots per explants. Similar observation recorded by scientist Balarama and Padmaja (2003).

Misra (2002) used 0.24 mg/l IAA for rooting. Pulse dipping to shooted plant into the 2.0 mg/l IAA for 2 min. followed by cultured on MS medium was reported by Villiers *et al.* (2008). MS + 1.0 mg/l IAA + 0.1 mg/l KIN showed good results for rooting by Balarama and Padmaja (2003). The frequency of rooting was highest in 0.3 mg/l IBA as reported by Majumdar and Banerjee in 2004.

George and Eapen (1994) had observed 90 % rooting

on NAA medium where as Geetha *et al.* (1998) and Prakash *et al.* (1994) found IBA as best auxin for rooting. Half-strength MS medium as basal medium for rooting was reported by Eapen and george (1993), George and Eapen (1984), Naidu *et al.* (1995) and Frankliune *et al.* (2000); Prakash *et al.* (1994), Geetha *et al.* (1998), (Mehta and Ram, 1980) reported B5 as basal medium.

Hardening

Plants with well developed roots were transferred to pots containing autoclaved soilrite. Soilrite initially watered with liquid ½ MS medium, showed 75 % survival of plantlets in both the genotype. Another treatment in which soilrite was supplemented with water showed low percentage of survival upto 40 % in TAT-10 and 35 % in PKV TARA (Plate 4).

Plate 4 : Hardening

The pots were covered with poly bags to maintain the moisture. After 1 week the covers were removed. Almost the 90-100 % survival percentage was reported by George and Eapen (1994), and Prakash *et al.* (1994), Dayal *et al.* (2003) and Geetha *et al.* (1998), which is higher than reported in present study.

Naidu *et al.* (1995), Yadav and Chand (2001), Misra (2002), Mohan and Krishnamurthy (2003) reported 60-80% survival of *in vitro* regenerated plants on various medium like soil, sand, vermiculate etc.

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