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Genotype specificity for *in vitro* regenerability of pigeonpea genotypes

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ABSTRACT

***In vitro* response at various stages of regeneration was observed to be genotype specific and it varies according to concentrations of growth hormones.Among the two genotypes under study TAT-10 recorded early response for shoot bud initiation and recorded higher average number of shoots / explant followed by better elongation and rooting as compared to genotype PKV TARA in both the explants over all the treatments understudy. Rooting and hardening were genotype independent.**

Key words : Pigeonpea, genotypes, *In vitro* regeneration

INTRODUCTION

Red gram or pigeonpea [*Cajanus cajan* (L) Millsp.] is an important grain legume of the semi-arid tropics and forms a significant component of the diet of vegetarians. The morphogenetic response of pigeonpea is known to be a genotypes specific phenomenon (Mohan and Krishnamurthy, 1998). Successful transformation of pigeonpea for these reasons will be greatly aided by genotype / variety specific determination of critical parameters on improving *in vitro* regeneration.

Vichita Yadav and Laxmi Chand (1998) used in genotype Bahar and UPAS-120 , Singh *et al*.(2002) reported genotype T7, Bahar and UPAS-120 of pigeonpea for *in vitro* proliferation and regeneration (Naidu *et al.,* 1995).

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 explant used for *in vitro* regeneration was decapitated mature embryonic axes and leaf with petiole.Different combination of MS basal medium with varied concentrations of auxin (NAA,IAA) and cytokinin (BAP) and plain MS basal medium as control were tried.

Statistical analysis:

Five culture bottles each with three explants were used per replication for each treatment. Every treatment was replicated trice. Thus observations were recorded for 45 explants.

The data of present investigation was analysed and ANOVA was carried out by using Factorial Completely Randomized Design. The mean and standard error, critical difference were calculated as per procedure given by Panse and Sukhatme (1958). F test was used to test the significance.

RESULTS AND DISCUSSION

The results of the present experiment as well as relevant discussions have been presented under following heads :

Establishment of explants and shoot induction:

Different combination of MS basal medium with varied concentrations of auxin (NAA) and cytokinin (BAP) and plain MS basal medium as control were tried for establishment of the explants, in both the genotypes under study namely TAT-10 and PKV-TARA. The DCMEA explants get increased in size and showed swelling at base and the leaf with petiole explant get increased in size after 7 days of inoculation. The genotype TAT 10 and PKV TARA showed per cent establishment of DCMEA explant ranged between 90.67 to 100 per cent.

The genotype TAT-10 had recorded minimum 9 and 15 days, respectively where as the genotype PKV-TARA had recorded minimum 15 and 22 days, respectively for explants DCMEA and leaf with petiole for initiation of shoot bud after inoculation. Thus from table.1, it is concluded that among the two genotypes under study TAT-10 showed early response for both the explants as compared to genotype PKV-TARA over all the treatments under study.

These results showed the critical role of genotype in initiation of shoot bud induction. The variation in response of genotype could be attributed to the early physiological duration of genotype TAT-10 (120 days- early) as compared to mid-late genotype PKV-TARA (180 days-

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Table 1 : Days required to initiate the shoot buds in culture

Treatments	Initiation of shoot bud				
	TAT 10		PKV TARA		callus
	DCMEA	Leaf with petiole	DCMEA	Leaf with petiole	
MS + 0.1mg/l BAP	9th day	15th day	15th day	21st day	+
MS + 0.2mg/l BAP	9th day	15th day	15th day	21st day	+
MS + 0.5mg/l BAP	10th day	16th day	16th day	22rd day	+
MS + 1mg/l BAP	10th day	16th day	18th day	24th day	-
MS + 2mg/l BAP	10th day	16th day	17th day	23rd day	-
MS + 3mg/l BAP	11th day	17th day	18th day	24th day	-
MS + 4mg/l BAP	11th day	18th day	18th day	24th day	-
MS + 5mg/l BAP	12th day	18th day	18th day	24th day	-
MS + 0.1mg/lNAA + 2mg/l BAP	10th day	16th day	17th day	23rd day	+
MS + 0.1mg/l NAA + 3mg/l BAP	10th day	16th day	17th day	23rd day	+
MS + 0.1mg/l NAA + 4mg/l BAP	10th day	16th day	18th day	24th day	+
MS + 0.1mg/l NAA + 5mg/l BAP	12th day	18th day	18th day	24th day	+
MS + 0.3mg/l NAA + 2mg/l BAP	10th day	16th day	17th day	23rd day	++
MS + 0.3mg/l NAA + 3mg/l BAP	10th day	16th day	18th day	24th day	++
MS + 0.3mg/l NAA + 4mg/l BAP	11th day	17th day	18th day	24th day	++
MS + 0.3mg/l NAA + 5mg/l BAP	12th day	18th day	18th day	24th day	++
MS + 0.5mg/l NAA + 2mg/l BAP	11th day	17th day	17th day	23rd day	+++
MS + 0.5mg/l NAA + 3mg/l BAP	12th day	18th day	17th day	23rd day	+++
MS + 0.5mg/l NAA + 4mg/l BAP	12th day	18th day	17th day	23rd day	+++
MS + 0.5mg/l NAA + 5mg/l BAP	12th day	18th day	17th day	23rd day	+++
MS plain	9th day	-	15th day	-	

+: Low callus (0.5 mm),
++ : medium callus(1-1.5 mm),
+++: large callus (2 mm and above)

late).

Over two explants the genotype TAT-10 recorded 11.46 mean number of average shoots and genotype PKV TARA recorded 8.21 mean number of average shoots on treatment MS+0.1 mg/l NAA+2.0 mg/l BAP(Table 2). Similarly, at par treatment combination MS+2.0 mg/l BAP recorded 11.06 mean numbers of average shoots in genotype TAT-10 and 7.97 mean number of average shoots in genotype PKV TARA (Plate1 and 2).

Thus the comparison between the genotype TAT-10 and PKV TARA revealed that the treatment means over

Plate 1 : Shoot bud induction from TAT-10

Plate 2 : Shoot bud induction from PKV TARA

Table 2 : 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	1.43	1.46	+
MS + 0.2mg/l BAP	2.02	1.82	+
MS + 0.5mg/l BAP	2.59	2.75	+
MS + 1mg/l BAP	4.09	3.78	-
MS + 2mg/l BAP	11.06	7.97	-
MS + 3mg/l BAP	6.37	6.02	-
MS + 4mg/l BAP	3.50	3.49	-
MS + 5mg/l BAP	1.01	0.85	-
MS + 0.1mg/l NAA + 2mg/l BAP	11.46	8.21	+
MS + 0.1mg/l NAA + 3mg/l BAP	8.03	6.20	+
MS + 0.1mg/l NAA + 4mg/l BAP	1.83	1.51	+
MS + 0.1mg/l NAA + 5mg/l BAP	0.70	0.61	+
MS + 0.3mg/l NAA + 2mg/l BAP	1.73	1.43	++
MS + 0.3mg/l NAA + 3mg/l BAP	0.99	0.78	++
MS + 0.3mg/l NAA + 4mg/l BAP	0.55	0.34	++
MS + 0.3mg/l NAA + 5mg/l BAP	0.60	0.35	++
MS + 0.5mg/l NAA + 2mg/l BAP	0.69	0.69	+++
MS + 0.5mg/l NAA + 3mg/l BAP	0.61	0.37	+++
MS + 0.5mg/l NAA + 4mg/l BAP	0.33	0.29	+++
MS + 0.5mg/l NAA + 5mg/l BAP	0.17	0.15	+++
MS plain	0.75	0.65	-

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)

both the explants under study of the two best treatments varied from genotype to genotype.

The genotype specificity to regeneration has been reported in a number of plants. The success in obtaining regeneration in leguminous species, once regarded as recalcitrant group (Bhojwani *et al.,* 1977), has been mainly due to shift in emphasis from media selection to genotype selection (Bhojwani and Mukhopadhyay, 1986).

Mohan and Krishnamurthy (1998) reported genotypic differences in genotype T-15-15 and GAUT 32-90 in their regeneration ability. Archana Tiwari *et al.*(1999) observed best response of Bahar genotype of pigeonpea followed by T21 and Paras for shoot induction. Yadav and Chand (2001) reported better multiple shoot induction in variety UPAS- 120 using DCMEA explants as compared to variety Bahar. Singh *et al.*(2002) reported that T7 gave best *in vitro* response followed by Bahar and UPAS-120.

The genotype specificity for induction of shoot had also been reported by Naidu *et al.*, 1995, Tyagi *et al.,* 2001 and Ramana *et al.,* 2003 in various genotypes of pigeonpea. Dayal *et al*. (2003) gave the effect of explants donor genotype on shoot bud regeneration in pigeonpea. All these results justify the variation in ability of genotype TAT-10 and PKV TARA for shoot bud induction.

In addition to number of repots on genotype specificity for regeneration, Kumar *et al.* (2003) had reported MS + 2.0 mg/l BA as best media to induced shoot buds and claimed that the protocol developed was genotype independent for cotyledon node explants. The same shoot induction media had responded differently in the genotype studied during present investigation.

Shoot elongation:

Among the two genotypes TAT-10 and PKV TARA the treatment combination (MS +0.2 mg/l BAP) was found to show highest number of shoot elongation per clump of shoot buds.

For comparison of response of genotypes the treatment mean over two explants are presented here. The treatment mean of (2.76 in TAT-10 and 1.29 in PKV TARA) was significantly superior over control and all other treatment combinations.

In both the genotypes the MS medium used as control failed to induce elongation of shoots induced from DCMEA as well as leaf with petiole explants.

Table 3 revealed that on the treatment combination MS+0.2mg/l BAP , DCMEA explants induced highest conversion of shoot buds to shoots in TAT-10 (66.87%) followed by genotype PKV TARA (49.97%). On the

Table 3 : Conversion of shoot buds in to shoot in genotype TAT 10 and PKV TARA with explants DCMEA and leaf with petiole

	DCMEA		Leaf with petiole	
	TAT-10	PKV TARA	TAT-10	PKV TARA
MS+0.1mg/l NAA+0.1mg/l BAP+0.5mg/l GA3	25.42	14.18	18.75	12.28
MS+0.1mg/l NAA+0.1mg/l BAP+1.0mg/l GA3	49.53	24.07	24.44	18.75
MS+0.1mg/l NAA+0.1mg/l BAP+1.5mg/l GA3	24.52	15.33	22.22	17.78
M+0.1mg/l BAP	43.47	32.60	35.56	31.11
MS+0.2mg/l BAP	66.67	49.97	41.67	37.78
MS control	0.00	0.00	0.00	0.00

same MS+0.2mg/l BAP treatment combination leaf with petiole explants induced highest conversion of shoot buds to shoots in TAT-10 (41.67%) followed by genotype PKV TARA (37.78%).

The numerical comparison between two genotypes for number of shoots elongated per clumps and percentage of conversion of shoot buds to shoots in each treatment combination revealed that the genotype TAT-10 had responded better as compared to genotype PKV TARA for elongation of shoots in the media.

The results of present investigation are supported by study conducted by Naidu *et al.* (1995) in pigeonpea. He reported that growth and elongation of shoot buds into plants was genotype dependent and it was restricted to two genotypes out of seven that had showed successful establishment.

The genotypic differences were attributed to the genetics of genotype and their endogenous levels of hormones.

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