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In vitro multiple shoot bud induction and regeneration from plumule junction explants of pigeon pea [*Cajanus cajan* (L.) Mill sp.] cultivars

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The response of eleven Indian cultivars of pigeon pea for *in vitro* multiple shoot bud induction and regeneration from plumule junction explants under variable concentration of 6-benzyl amino purine (BAP), kinetin and thiadiazuron (TDZ) was assessed in the present study. The cultivar IPA-3088 showed best response with a maximum of 20 buds per explants in Murashige and Skoog (MS) media supplemented with 0.05 mgL^{-1} TDZ. Among the hormones tested, lower concentration of TDZ gave the best response for these cultivars though higher concentration of BAP was also effective in multiple shoot bud induction and regeneration from plumule junction explants. The elongation of multiple shoot buds was achieved in the same medium and the nature of the regenerants in most of the cases was shoots, though in few cases shoot buds and shoot primoridia were also observed. Rooting of plumule junction derived shootlets was found to be better in the presence of NAA as compared to IAA and IBA for most of the cultivars producing maximum number of primary roots. The best responding cultivar IPA 3088 showed efficient rooting in the presence of 0.2 mgL^{-1} of NAA. The regenerated plantlets were acclimatized in soil with percentage of acclimatization varying from 40-80% for different cultivars.

Key words: Pigeon pea, cultivars, multiple shoot bud induction, organogenesis, acclimatization, elongation, *Cajanus cajan* (L) Mill sp.

INTRODUCTION

Pigeon pea [*Cajanus cajan* (L) Mill sp.] is an important legume crop widely grown in tropical and subtropical regions of the world whose genome has been recently deciphered (Varshney et al., 2012; Singh et al., 2012). It is an important

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Abbreviations: BAP, 6-Benzyl amino purine; **TDZ,** Thiadiazuron; **NAA,** Naphthalene acetic acid; **IAA,** Indole acetic acid; **IBA,** Indole butyric acid; **MS,** Murashige and Skoog medium.

source of protein (with 20 to 22% protein by dry weight) especially in vegetarian diets (Duke, 1981). The lack of genomic resources, and narrow genetic diversity has been a major hurdle for the breeders to develop strategies for its improvement (Saxena et al., 2010a; Bohra et al., 2011) though recently attempts have been for the development of cytoplasmic-nuclear male sterility for potential application in pigeon pea hybrid programme (Saxena et al., 2010b). Genetic transformation of pigeon pea cultivars has also been attempted (Lawrence and Koundal, 2001; Satyavathi et al., 2003; Thu et al., 2003; Prasad et al., 2004), though there is still limitation of highly efficient and reproducible *in vitro* regeneration protocol owing to its recalcitrance in tissue culture conditions. Although pigeon pea is considered to be recalcitrant crop, plant regeneration has been reported but only a few protocols could be successfully utilized for genetic transformations (Geetha et al., 1999). Recent improvements on genetic transformation (Lawrence and Koundal, 2001; Satyavathi et al., 2003; Dayal et al., 2003; Thu et al., 2003; Mohan and Krishnamurthy, 2003; Prasad et al., 2004) have demonstrated that the crop is not as recalcitrant as assumed earlier; however, limited to cultivars and some genotypes.

In vitro regeneration by organogenesis using diverse explants like leaf discs (Kumar et al., 1983; Eapen and George, 1993; George and Eapen, 1994; Eapen et al., 1998; Geetha et al., 1998; Dayal et al., 2003), cotyledons (Kumar et al., 1983; Mehta and Mohan, 1980; Kumar et al., 1984; Sarangi and Gleba, 1991; Naidu et al., 1995; Srinivasan et al., 2004; Chandra et al., 2003), cotyledonary nodes (Geetha et al., 1998; Shiva et al., 1994) and embryonal axes (George and Eapen, 1994; Sarangi and Gleba, 1991; Naidu et al., 1995; Franklin et al., 2000; Mohan and Krishnamurthy, 1998) has been reported. More than 50 diverse genotypes have been attempted for regeneration via organogenesis to develop suitable regeneration protocol amenable to transformation with desired genes (Krishna et al., 2010).

This paper reports assessment of 11 Indian cultivars of pigeon pea for *in vitro* multiple shoots bud induction and regeneration using plumule junction explants in the presence of different hormones.

MATERIALS AND METHODS

Seeds of 11 cultivars of pigeon pea namely IPA-2013, IPA-3088, Pusa-9, IPA-34, IPA-204, IPA-242, T-7, IPA-61, IPA-337, IPA-341 and IPA-98-3 were obtained from the Indian Institute of Pulses Research Kanpur, India. After repeated washes in running tap water, the seeds were surface sterilized with 1% cetrimide solution for 10 min followed by treatment with 70% ethanol for 30 s and 0.2% $HgCl_2$ for 5 min. Finally the seeds were washed 4 to 5 times with sterile double distilled water and germinated on MS medium (Murashige and Skoog, 1962) and cultures were kept under cool white fluorescent light at 25 ± 2°C. After 10 days, plumule junction explants of approximately 0.5 cm size were excised aseptically and were cultured on MS media supplemented with variable concentration of three hormones that is, BAP, kinetin, TDZ for multiple shoot bud induction and regeneration. Data about the number of buds per explants were recorded after 4 weeks of culture with two passage of sub culturing with a mean value of 10 replicates.

The MS media used in all experiments contained 3% sucrose gelled with 0.8% agar-agar (Hi-media Mumbai) and was sterilized by autoclaving after adjusting the pH to 5.8. Each sterilized culture tube (150 × 25 mm, Borosil) containing 20 ml of medium was inoculated with plumule junction explant and plugged with non-absorbent cotton (wrapped in one layer of cheese cloth), incubated under light-dark (16 to 8 h) at 25 ± 2°C with cool fluorescent light illumination. The explants with or without shoot initials were sub cultured repeatedly after 15 days. Numbers of shoot buds were counted after 30 days of inoculation.

For each experimental set up 10 explants were used with each concentration and experiment was repeated twice. After each successive subculture within 15 days, the well developed shoots were rooted on MS media with different concentration of NAA, IAA and IBA.

Rooted plants were removed from the medium, agar sticking to their roots was washed with tap water and transplanted into plastic cups or small pots, filled with autoclaved mixture of soil and sand (3:1) for hardening (Dayal et al., 2003). Plants were kept in high (90% or more) humidity and, initially low light intensities. The data were statistically analysed by standard ANOVA tool and treatment means were compared.

RESULTS AND DISCUSSION

The different explants namely leaf, shoot tips, nodes and embryonal axis along with plumule junction were subjected to *in-vitro* regeneration studies but based on regenerative as well as acclimatization percentage, plumule junction explants was further studied for multiple shoot bud induction and regeneration. The multiple shoot bud induction from plumule junction explants were analysed for 11 cultivars of pigeon pea by subjecting to MS media supplemented with variable concentration of BAP ranging from 0.5 to 4.0 mgL^{-1}. Substantial differences in the response of cultivars were observed with different concentration of BAP in terms of number of buds formed per explants. The response of multiple shoot bud induction among all 11 pigeon pea cultivars under different concentration of BAP is presented in Table 1. The overall response of cultivars for shoot bud induction can be summarized as IPA-3088> T-7> IPA-2013> IPA-61> IPA-337> IPA-242> IPA-34> IPA204= IPA-341> IPA-98-3> Pusa-9.

The cultivar IPA-3088 with a maximum of 7 buds per explants showed best response with 2.5 mgL^{-1} to BAP. Shoot bud induction from cotyledonary callus of pigeon pea on Blaydes medium with 2.25 mgL^{-1} BAP has been reported (Kumar et al., 1983). Similarly, 65% shoot buds from cotyledonary node explants of cultivar T-15-15 and SPMA-4 has been reported (Shiva et al., 1994; Mohan and Krishnamurthy, 1998). However, the maximum number of shoot buds regenerated per explants is not clear. A maximum of about 13-15 shoots from cotyedonary nodes were reported in MS media supplemented with 2 mgL^{-1} of BAP (Geetha et al., 1998). Overall the higher concentration of BAP comparatively showed better response for shoot bud induction among these cultivars. The present work enumerates the comparative account of shoot bud

Table 1. Effect of BAP on multiple shoot bud induction using plumule junction explants (number of shoots / explant) for eleven cultivars of pigeon pea after 4 weeks of culture with an average of 10 replicates and means with different letters differ significantly at p=0.05.

Cultivars	Concentration of BAP (mgL^{-1})							
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
	Number of shoots (Mean±S.D.)							
IPA-2013	2.5±0.6^{a}	2.4±0.4^{a}	2.3±0.4^{a}	3.9±0.9^{b}	4.3±0.6^{b}	3.8±1.9^{b}	4.8±0.5^{ab}	4.1±0.5^{b}
IPA-3088	4.9±0.8^{b}	2.7±1.1^{b}	2.8±1.1^{b}	4.4±2.2^{b}	5.4±1.8^{b}	4.4±1.2^{b}	4.1±1.3^{b}	4.1±2.5^{b}
Pusa-9	1.7±0.4^{a}	3.0±0.0^{b}	3.5±0.5^{ab}	2.5±0.5^{b}	2.6±1.2^{b}	1.3±0.4^{a}	1.7±0.4^{a}	1.4±0.4^{a}
IPA-34	3.1±0.3^{b}	1.9±0.3^{a}	3.7±0.4^{b}	3.0±0.0^{b}	4.1±0.7^{b}	3.3±0.4^{b}	4.3±1.1^{ab}	4.2±0.4^{b}
IPA-204	2.0±0.0^{a}	2.0±0.0^{a}	2.5±0.6^{a}	2.6±0.4^{a}	3.8±0.4^{b}	4.2±0.6^{ab}	2.5±0.5^{a}	2.0±0.0^{a}
IPA-242	2.3±0.4^{a}	2.3±0.4^{a}	2.3±0.4^{a}	2.4±0.4^{a}	2.4±0.4^{a}	4.4±0.4^{a}	3.2±0.4^{a}	3.0±0.0^{a}
T-7	2.2±0.4^{a}	2.8±0.4^{a}	3.2±0.8^{a}	2.0±0.0^{a}	3.3±1.1^{b}	3.0±0.8^{a}	5.1±0.7^{ab}	3.2±0.4^{a}
IPA-61	3.1±0.9^{a}	3.2±0.9^{b}	3.0±0.0^{a}	3.6±0.4^{a}	2.3±0.4^{a}	3.0±0.6^{a}	3.5±0.8^{b}	4.6±0.4^{ab}
IPA-337	4.5±0.5^{a}	3.1±0.3^{a}	3.3±0.45^{a}	3.3±0.6^{a}	2.6±0.4^{a}	2.2±0.6^{a}	2.0±0.0^{a}	2.0±0.0^{a}
IPA-341	2.0±0.0^{a}	2.0±0.0^{a}	3.0±0.0^{a}	3.0±0.0^{a}	3.0±0.0^{a}	4.2±0.7^{ab}	3.6±0.4^{b}	2.0±0.0^{a}
IPA-98-3	1.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	3.8±0.4^{a}	2.0±0.0^{a}	2.5±0.5^{a}	2.0±0.0^{a}	2.0±0.0^{a}

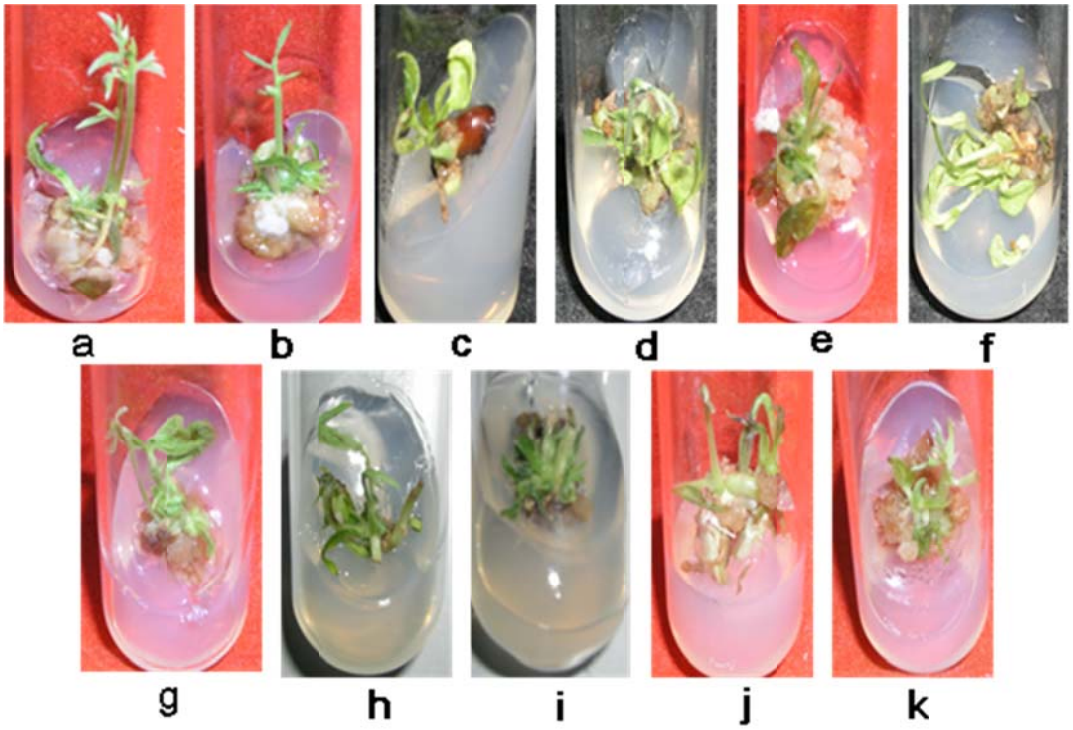

Figure 1. Multiple shoot induction from plumule junction explants of different cultivars of pigeon pea showing best response with variable concentration of BAP. **(a)** IPA2013 (3.5 mgL^{-1}); **(b)** IPA-3088 (2.5 mgL^{-1}); **(c)** Pusa-9 (1.5 mgL^{-1}); **(d)** IPA-34 (3.5 mgL^{-1}); **(e)** IPA-204 (3.0 mgL^{-1}); **(f)** IPA-242 (3.0 mgL^{-1}); **(g)** T-7 (3.5 mgL^{-1}); **(h)** IPA-61(4.0 mgL^{-1}); (i) IPA-337(0.5 mgL^{-1}); **(j)** IPA-341(3.0 mgL^{-1}); **(k)** IPA-98-3(2.0 mgL^{-1}).

induction in the case of 11 cultivars. Moreover, in this study, best responsive concentrations of BAP has been worked out and presented in Figure 1.

The cultivar IPA 3088 showed best response among all the 11 cultivars with a maximum of 10 buds per explants while only 2 buds were observed in case of IPA-61 when

Table 2. Effect of Kinetin on multiple shoot bud induction using plumule junction explants (number of shoots / explant) for eleven cultivars of pigeon pea after 4 weeks of culture with an average of 10 replicates and means with different letters differ significantly at p=0.05.

Cultivars	Concentration of kinetin (mgL^{-1})							
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
	Number of shoots (Mean±S.D.)							
IPA-2013	3.0±0.0^{a}	3.7±0.4^{a}	3.6±0.4^{a}	2.0±0.0^{a}	3.0±0.0^{a}	2.0±0.0^{a}	4.9±0.5^{a}	2.0±0.0^{a}
IPA-3088	4.2±1.2^{b}	4.1±1.3^{b}	5.4±0.8^{b}	3.6±0.4^{a}	7.5±2.1^{ab}	2.7±0.9^{a}	3.3±0.9^{a}	2.9±1.1^{a}
Pusa-9	3.5±0.5^{b}	2.0±0.0^{a}	3.5±0.5^{b}	2.0±0.0^{a}	4.1±0.7^{ab}	2.0±0.0^{a}	2.0±0.0^{a}	2.7±0.7^{b}
IPA-34	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.3±0.4^{b}	2.2±0.4^{b}	2.0±0.0^{b}	2.0±0.0^{b}
IPA-204	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	1.8±0.4^{a}	2.0±0.0^{a}	2.6±0.4^{b}	3.6±0.4^{ab}	2.0±0.0^{a}
IPA-242	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.8±0.8^{b}	2.0±0.0^{b}	2.0±0.0^{b}
T-7	1.0±0.0	2.0±0.0	1.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	1.0±0.0	2.0±0.0
IPA-61	1.5±0.5^{b}	1.0±0.0	2.0±0.0	1.0±0.0	1.4±0.4^{a}	1.0±0.0	1.0±0.0	1.0±0.0
IPA-337	2.0±0.0^{a}	3.5±1.3^{a}	2.0±0.0^{a}	1.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	1.0±0.0^{a}	2.0±0.0^{a}
IPA-341	2.0±0.0	2.0±0.0	1.0±0.0	2.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	2.0±0.0
IPA-98-3	1.0±0.0	2.0±0.0^{b}	2.0±0.0	1.0±0.0	2.0±0.0	1.0±0.0	1.6±0.4^{b}	1.0±0.0

subjected to MS media supplemented with variable concentration of kinetin in the range of 0.5 to 4.0 mgL^{-1}. The overall response of cultivars in terms of number of shoot buds formed per explants can be summarized as IPA-3088> IPA-2013> Pusa-9> IPA-204> IPA-337> IPA-242> IPA-334> IPA-61> T-7> IPA-341> IPA-98-3. In most of the cultivars, concentration range of 2.5 to 3.5 mgL^{-1} of kinetin was found to be most effective for shoot bud induction as shown in Table 2. In case of cultivars IPA-337 and IPA-61 lower concentration of kinetin that is, 1.0 and 1.5 mgL^{-1} was found to be effective for shoot bud induction. Regeneration using distal cotyledonary segment *via* organogenesis for diverse cultivar BDN-1, BDN-2, Gaut-82-90, ICP 7182, ICPL 87, ICPL 87119, T-15-15, TV-1 has been reported in the presence of kinetin, BAP and /or adenine sulphate (AdS) (Mohan and Krishnamurthy, 1998). The cultivar T-15-15 showed a maximum of 33 shoots in the presence of BAP + KIN + AdS. The shoot bud induction for all the eleven cultivars subjected to best responsive cocentration of kinetin is shown in Figure 2.

The same cultivar IPA-3088 responded best with 20 shoot buds per explants when cultured under TDZ at 0.05 mgL^{-1} which is quite efficient than regeneration *via* organogenesis using cotyledonary node reported for Pusa 33, ICP 8863 and ICPH cultivars with 13, 6.2 and 4.7 shoots respectively (Singh et al., 2002). The cultivar IPA-98-3 resulted in the formation of only 3 buds per explants subjected to variable concentration of TDZ. The overall response of cultivars subjected to different concentration of TDZ in terms of number of shoot buds formed per explants can be summarized as IPA 3088> IPA-61> IPA-204> T-7> IPA-34> IPA-2013> Pusa-9> IPA-341> IPA-337> IPA-242> IPA-98-3. Multiple shoot bud induction from plumule junction was comparatively better when subjected to lower concentration of TDZ, though in case of IPA-61 higher concentration that is, 0.35 mgL^{-1} was found to be better resulting in the formation of 9 buds per explants. The overall response of different concentration of TDZ for multiple shoot bud induction in all the 11 cultivars is provided in Table 3.

The shoot bud induction for all the 11 cultivars subjected to best responsive concentration of TDZ is shown in Figure 3. The percentage of explants response for multiple shooting, nature of regenerants and length of regenerated shootlets for best responsive cultivar IPA-3088 in the presence of BAP, kinetin and TDZ are presented in Table 4.

In most of the cultivars, the nature of regenerants were shoots (size more than 1 cm) though in few cases shoot buds (size less than 1 cm) were also observed. The overall response of three hormones influencing multiple shoot bud induction among all 11 cultivars clearly indicates TDZ to be better than BAP and kinetin as shown in Table-5. Further it has been observed that among the concentrations of BAP, kinetin and TDZ tried for multiple shoot bud induction, higher concentration of BAP and kinetin and lower concentration of TDZ was comparatively better for multiple shoot bud induction irrespective of cultivars.

Multiple shoot buds obtained from plumule junction explants were subjected to rooting on full strength MS basal medium supplemented with three different hormones *viz*. NAA, IAA and IBA at three different concentrations namely 0.1, 0.2 and 0.3 mgL^{-1}. NAA was found to better than other two hormones with a maximum numbers of primary roots observed by subjecting the plumule junction derived shootlets to rooting under 0.2 mgL^{-1} of NAA. The rooting response of four selected cultivars IPA-3088, IPA-Pusa-9, IPA-34 and IPA-242 is shown in Figure 4. The percentage of rooting varied from 50 to 100%. The overall response to rooting of all the 11 cultivars at three

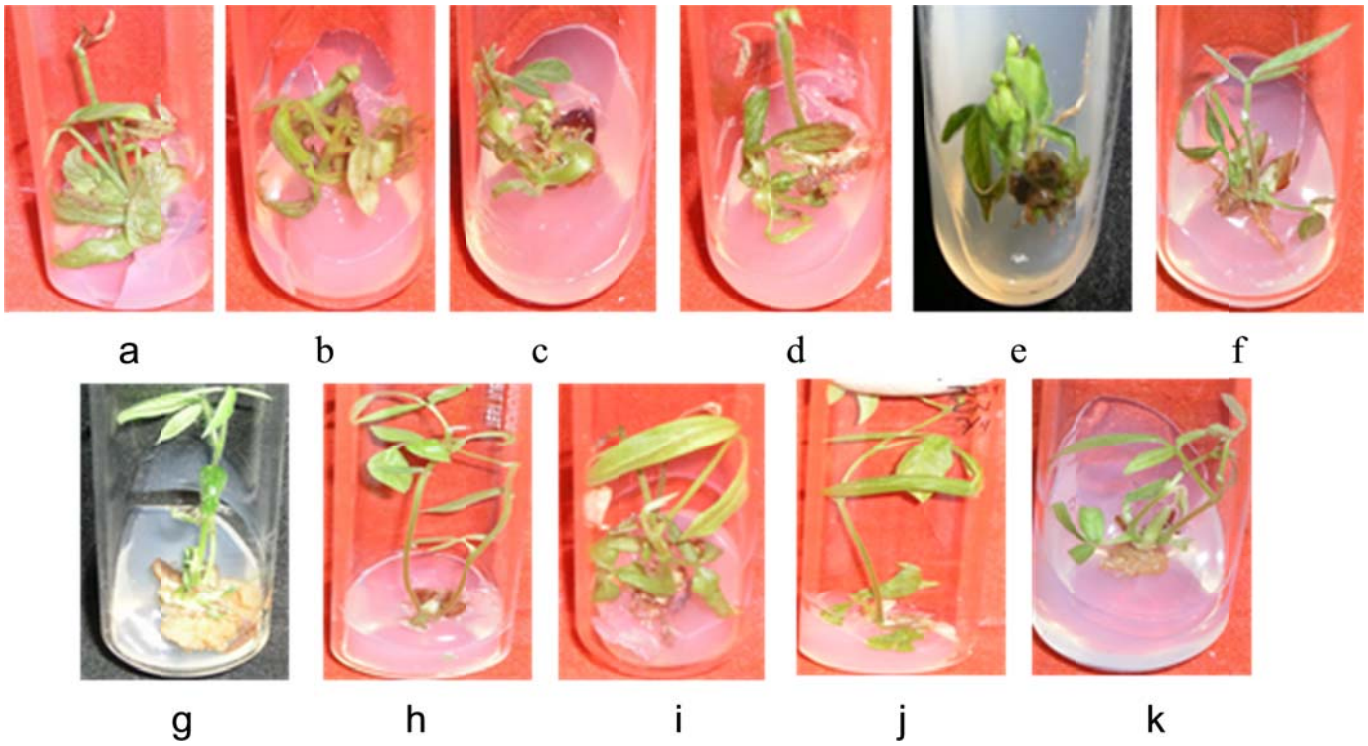

Figure 2. Multiple shoot induction from plumule junction explants of different cultivars of pigeon pea showing best response with variable concentration of kinetin **(a)** IPA2013 (3.5 mgL^{-1}); **(b)** IPA-3088 (2.5 mgL^{-1}); **(c)** Pusa-9 (2.5 mgL^{-1}); **(d)** IPA-34 (2.5 mgL^{-1}); **(e)** IPA-204 (3.5 mgL^{-1}); **(f)** IPA-242 (3.0 mgL^{-1}); **(g)** T-7 (1.0 mgL^{-1}); **(h)** IPA-61(1.5 mgL^{-1}); **(i)** IPA-337(1.0 mgL^{-1}); **(j)** IPA-341(1.0 mgL^{-1}); **(k)** IPA-98-3 (1.0 mgL^{-1}).

Table 3. Effect of TDZ on multiple shoot bud induction using plumule junction explants (number of shoots / explant) for eleven cultivars of pigeon pea after 4 weeks of culture with an average of 10 replicates and means with different letters differ significantly at p=0.05.

Cultivars	Concentration of TDZ (mgL^{-1})							
	0.05	0.1	0.15	0.20	0.25	0.30	0.35	0.40
	Number of shoots (Mean±S.D.)							
IPA-2013	5.0±0.0^{a}	4.1±0.5^{a}	3.2±0.4^{a}	2.0±0.0	2.0±0.0	2.0±0.0	1.0±0.0	1.0±0.0
IPA-3088	12.6±3.7^{ab}	10.2±1.1^{b}	6.2±1.7^{a}	7.9±3.0^{b}	4.2±1.2^{a}	10.3±3.1^{b}	1.7±1.1^{a}	2.2±1.1^{a}
Pusa-9	3.8±0.6^{b}	4.9±0.7^{ab}	2.9±0.8^{a}	4.1±0.5^{b}	2.5±0.5^{a}	3.8±0.6^{b}	2.4±0.4^{a}	3.6±0.4^{a}
IPA-34	1.4±0.4^{a}	3.5±0.5^{a}	5.2±0.7^{a}	3.5±0.5^{a}	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	1.6±0.5^{a}
IPA-204	2.3±0.4^{a}	4.5±0.6^{a}	4.4±0.4^{a}	7.7±1.5^{a}	3.5±0.5^{a}	3.5±0.5^{a}	3.0±0.0^{a}	3.0±0.0^{a}
IPA-242	1.3±0.4^{a}	2.0±0.0^{a}	1.3±0.4^{a}	2.5±0.5^{b}	3.3±0.4^{ab}	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}
T-7	2.7±0.4^{a}	3.2±0.4^{a}	3.4±0.4^{a}	5.6±0.4^{a}	3.2±0.4^{a}	2.8±0.4^{a}	2.6±0.4^{a}	2.5±0.5^{a}
IPA-61	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	4.3±0.4^{a}	7.8±0.7^{a}	3.0±0.0^{a}
IPA-337	3.3±0.4^{b}	2.0±0.0^{a}	2.8±0.4^{a}	2.0±0.0^{a}	4.3±0.6^{ab}	2.0±0.0^{a}	2.0±0.0^{a}	3.1±1.2^{b}
IPA-341	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	4.4±0.4^{a}	1.6±0.4^{a}	2.0±0.0^{a}	2.0±0.0^{a}
IPA-98-3	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0	3.0±0.0

different concentrations of NAA is represented in Table 6.

Among all the cultivars, IPA-242 showed better response to rooting, though the shootlets derived from best responsive cultivar for multiple shoot bud induction that is, IPA-3088 also showed good response in MS media supplanted with 0.2 mg/L of NAA. Similarly the shootlets derived from plumule junction explants of different cultivars were also subjected to rooting with

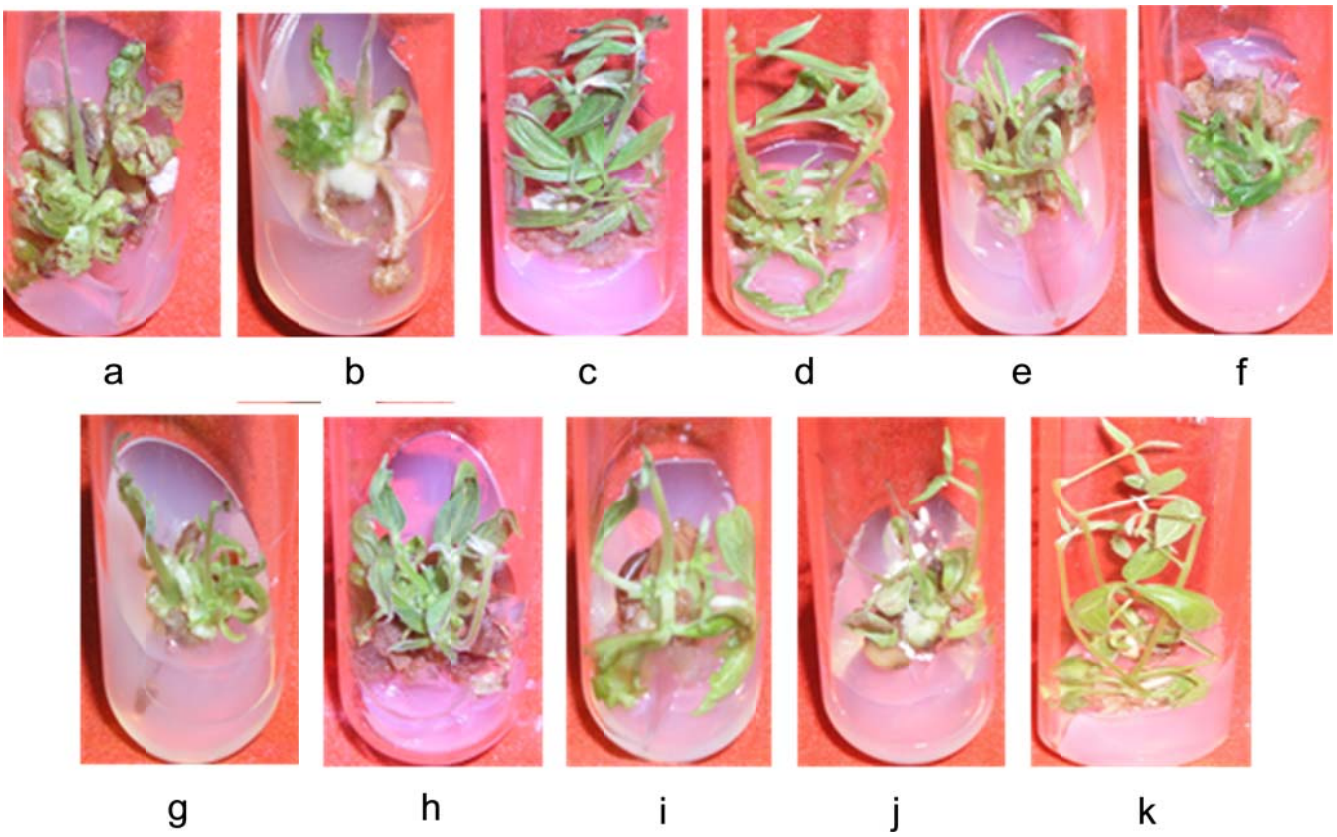

Figure 3. Multiple shoot induction from plumule junction explants of different cultivars of pigeon pea showing best response with variable concentration of TDZ (in mgL^{-1}). **(a)** IPA2013 (0.05 mgL^{-1}); **(b)** IPA-3088 (0.05 mgL^{-1}); **(c)** Pusa-9 (0.1 mgL^{-1}); **(d)** IPA-34(0.15 mgL^{-1}); **(e)** IPA-204 (0.20 mgL^{-1}); **(f)** IPA-242 (0.25 mgL^{-1}); **(g)** T-7 (0.20 mgL^{-1}); **(h)** IPA-61(0.35 mgL^{-1}); **(i)** IPA-337(0.25 mgL^{-1}); **(j)** IPA-341(0.25 mgL^{-1}); **(k)** IPA-98-3 (0.05 mgL^{-1}).

Table 4. Effect of different concentration of BAP, Kinetin and TDZ on frequency of multiple shooting from plumule junction explants indicating height and nature of regenerants for cultivar IPA-3088. Date recorded from 10 replicates of explants for each concentration after 4 weeks of culture [S= Shoot (Size more than 1 cm), SB= Shoot buds (size up to 1cm) and SP= shoot primordia].

Hormone concentration (mgL^{-1})	Multiple shooting (%)	Length of regenerants in cm (Mean ± S.D.)	Nature of regenerants and their occurrence (%)
BAP			
0.5	100	1.0±0.3	12.24%SP+57.14%SB+30.61%S
1.0	50	1.8±1.5	29.62%SP+ 100%S
1.5	40	4.8±2.0	100%S
2.0	100	2.1±1.0	22.72%SB+ 77.27%S
2.5	90	3.8±1.7	9.25%SB+ 90.74%S
3.0	100	5.6±1.2	100%S
3.5	90	2.3±0.7	14.63%SB+ 85.36%S
4.0	60	2.9±0.9	24.39%SB+ 75.61%S
Kinetin			
0.5	90	11.4±2.3	100%S
1.0	90	6.7±0.9	100%S
1.5	100	4.3±0.9	100%S
2.0	100	6.2±0.8	100%S
2.5	100	4.6±0.9	100%S
3.0	50	4.7±1.4	100%S

Table 4. Contd

3.5	80	7.4±0.9	100%S
4.0	60	4.7±1.5	100%S
TDZ			
0.05	100	3.3±0.2	100%S
0.10	100	1.6±0.4	0.98%SP+ 99%S
0.15	100	1.3±0.6	3.23%SP+ 25.81%SB+ 70.97%S
0.20	100	2.7±1.1	2.53%SP+ 97.47%S
0.25	100	1.0±0.3	4.08%SP+ 63.27%SB+ 32.65%S
0.30	100	1.5±0.6	1.94%SP+ 98.06%S
0.35	70	0.7±0.3	23.53%SP+ 70.59%SB+ 5.8%S
0.40	100	1.3±0.8	18.18%SP+ 81.82%S

Table 5. Comparative response of plumule junction explant of different cultivars showing multiple shooting (Mean ± SD in %). Average was taken from multiple shooting responses under different concentration of each growth regulator for each cultivar.

Name of cultivars	% Multiple shooting (Mean±SD)		
	BAP	Kinetin	TDZ
IPA-2013	74±30	63±48	38±48
IPA-3088	79±23	84±18	90±18
Pusa-9	36±38	44±47	83±24
IPA-34	88±33	4.0±9.0	38±48
IPA-204	44±39	20±36	90±26
IPA-242	59±32	6.0±17	19±35
T-7	63±38	0	83±19
IPA-61	83±24	0	38±48
IPA-337	60±42	8.0±20	41±44
IPA-341	63±48	0	13±33
IPA-98-3	19±35	0	100±0.0

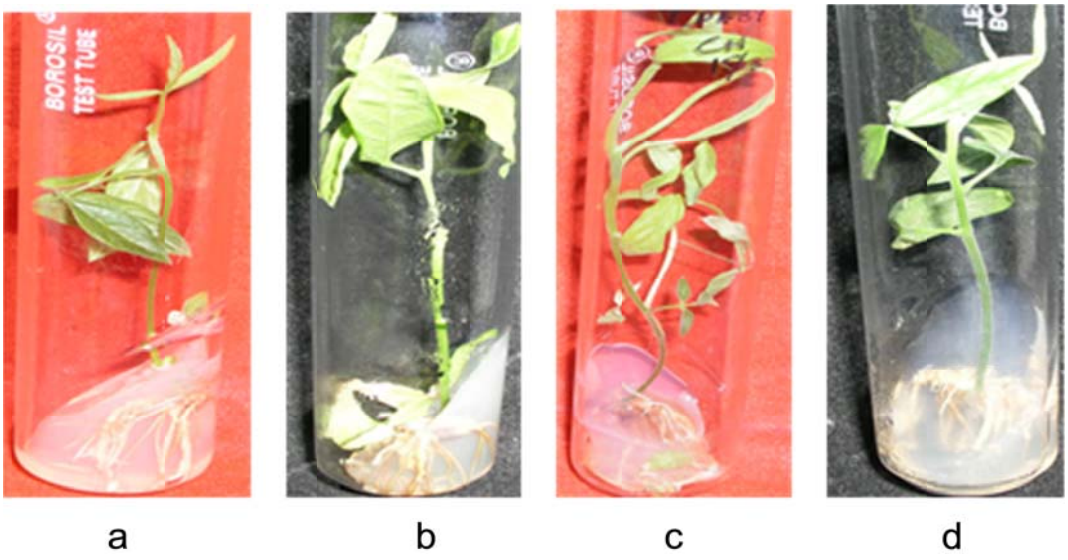

Figure 4. Rooting response for multiple shoot buds from plumule junction explants of different cultivars of pigeon pea on MS media supplemented with different concentration of NAA. **(a)** IPA-3088 (0.2mgL^{-1}); (**b)** Pusa-9 (0.2 mgL^{-1}); (c) IPA-34 (0.1 mgL^{-1}); **(d)** IPA-242 (0.2 mgL^{-1}).

Table 6. Rooting responses of *in vitro* regenerated shoots from plumule junction explants under different concentrations of NAA. Date recorded after 4 weeks of culture with 10 replicates for each treatment and experiment was repeated twice.

Cultivars	NAA (0.1 mgL^{-1})		NAA (0.2 mgL^{-1})		NAA (0.3 mgL^{-1})	
	% of rooting	Number of primary roots Mean±S.D.	% of rooting	Number of primary roots Mean±S.D	% of rooting	Number of primary roots Mean±S.D
IPA-2013	50	0.5±0.5	100	5.0±0.0	NR	NR
IPA-3088	70	0.7±0.5	100	11.6±0.9	80	3.2±1.6
Pusa-9	90	3.6±1.2	100	11.3±0.9	NR	NR
IPA-34	100	10.5±0.5	100	6.6±2.0	NR	NR
IPA-204	NR	NR	100	3.6±0.5	NR	NR
IPA-242	100	1.0±0.0	100	12.2±0.9	50	0.5±0.5
T-7	NR	NR	100	1.0±0.0	NR	NR
IPA-61	100	5.0±0.4	NR	NR	NR	NR
IPA-337	NR	NR	NR	NR	NR	NR
IPA-341	100	5.1±0.5	100	2.7±0.5	NR	NR
IPA-98-3	NR	NR	100	4.0±0.6	NR	NR

Figure 5. Acclimatized plants of cultivar IPA-3088.

three different concentrations (0.1, 0.2 and 0.3 mgL^{-1}) of IAA and IBA. The rooting response was better at 0.1 mgL^{-1} concentration of IAA with overall 70 to 100% rooting in few of the cultivars. The cultivar IPA-204 showed best response for rooting with 0.1 mgL^{-1} IAA. The rooting response was very poor in the presence of IBA and only two cultivars IPA-204 and IPA-61 showed rooting response when subjected to 0.1 mg/l IBA with 90 and 50% of rooting respectively. The percentage acclimatization of multiple shoot buds derived from plumule junction explants with proper rooting in soil ranged from 40 to 85% with cultivar IPA-3088 showing maximum percentage of acclimatization (Figure 5).

In most of the earlier reports two different media with different hormones were used for shoot bud induction and elongation. Shoot bud induction was attempted on MS media supplemented with different concentration of BAP (Geetha et al., 1998; Shiva Prakesh et al., 1994) or TDZ (Eapen et al., 1998) while for elongation, different media containing IAA (Shiva Prakesh et al., 1994) or Gibberelic acid (Mohan and Krishnamurthy, 1998) or in combination of both (Eapen et al., 1998) or lower concentration of BAP and NAA (Geetha et al., 1998) has been used. In the present study same media was used for induction and elongation similar to what has been reported by Singh et al. (2002). In this study, the direct organogenesis protocol involving induction and elongation of shoot buds in same media supplemented with BAP, Kinetin and TDZ might be preferred as the plantlets developed directly without an intervening callus phase, which minimizes the chance of somaclonal variations in the regenerants. Further, our work has shown the comparative account of multiple shoot regeneration from eleven cultivars using one type of explant in order to identify the most responsive cultivar for further transformation use. The results clearly indicates superiority of IPA-3088 which is a candidate for choice for further investigation instead of trying with poorly responding cultivars which may end up with recalcitrance for plant regeneration.

Conclusion

The *in vitro* multiple shoot bud induction and regeneration among 11 Indian cultivars of pigeon pea using plumule junction explants under the influence of three different

hormones has been studied. The genotype dependent variation was observed. The cultivar IPA-3088 is found to be highly efficient for multiple shoot but induction and *in vitro* regeneration among these cultivars at lower concentration of TDZ results in a maximum of 20 shoots buds per explants. This cultivar should be a suitable candidate for developing genetic transformation protocols with desired agronomic traits using either *Agrobacterium* or microprojectile based methods of transformation.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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