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Role of PGR on in vitro shoot propagation in *Cyamopsis tetragonoloba* L. (Taub.): a drought tolerant grain legume

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Abstract An efficient in vitro mass propagation protocol for *Cyamopsis tetragonoloba* L. (Taub.), a drought tolerant multipurpose grain legume, has been developed. A comparative range of cytokinins have been investigated for multiple shoot induction and plant regeneration with cotyledonary node, node and shoot tip explants. Four weeks exposure with thidiazuron (TDZ) containing medium, prior to transfer to BA supplemented medium was sufficient to induce maximum number of shoots. In descending order regarding shoot formation, the four different cytokinins at the optimum concentration could be ranked as TDZ, BA, Kn and 2iP. Optimum multiple shoot induction occurred in MS medium containing 5.0 μM (TDZ) or 10.0 μM (BA) where an average of 14.6 ± 0.67 or 7.8 ± 0.73 shoots, respectively, were produced in cotyledonary node explant after 8 weeks of incubation. Elongated shoots were rooted on half strength MS medium containing 5.0 μM (IBA) with about 45 % efficiency, the plantlets thus obtained were transferred to thermocol cups containing sand:soil (3:1) mixture and placed in a hardening room. Survival rate of plantlets in pots was more than 80 % and all the regenerated plants appeared morphologically normal.

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Abbreviations

BA	6-Benzyladenine Kn Kinetin
PGRs	Plant growth regulators
2iP	2-Isopentenyl adenine
TDZ	Thidiazuron
IBA	Indole-3-butyric acid
MS	Murashige and Skoog (1962) medium
NAA	α-Naphthalene acetic acid

1 Introduction

Legumes are a large, diverse family ranging from herbaceous annuals to woody perennials, because of their capacity to fix nitrogen, have been also domesticated for the production of food, feed, forage, fiber, industrial and medicinal compounds. Cluster bean (*Cyamopsis tetragonoloba*) is a branched, summer, annual legume, native to tropical Africa and Asia. The young pods are eaten as a vegetable and seeds are used as cattle feed in India and Pakistan. Recently, this legume gained the status of an industrial crop due to the presence of 40 % guar gum (galactomannans) in its seeds which is extensively used for food, mining and explosives, cosmetic, pharmaceutical and oil industries (Whitler and Hymowitz 1979).

Being an economically important crop, biotechnological applications could be utilized for its improvement. Modern biotechnology, including tissue culture, genetic engineering and genetic transformation offers strategies for over expressing or suppressing endogenous genes, introducing new genes or manipulating endogenous gene expression via transformation generates new phenotypic variation

useful for investigating gene function and also for crop improvement. A reproducible shoot regeneration protocol is a prerequisite for an efficient application in crop improvement and genetic manipulation strategies. Few reports are available on shoot organogenesis in *C. tetragonoloba* via callus culture (Prem et al. 2005) and direct differentiation from cotyledonary nodes (Prem et al. 2003) using cytokinins and also TDZ (Ahmad and Anis 2007). Ramulu and Rao (1991), Bansal et al. (1994) and Joersbo et al. (1999) reported *Agrobacterium*-mediated genetic transformation in *C. tetragonoloba*. As far as we know, BA and Kn were the only cytokinins applied for axillary shoot formation (Prem et al. 2003). The activity of other cytokinins has so far not been investigated which influence or increase the rate of shoot multiplication. Different cytokinins generally show different activities in affecting axillary shoot formation in vitro. Besides the N^6-substituted adenine derivatives, other compounds also have cytokinin activity, e.g. the thidiazolylurea derivatives, thidiazuron (TDZ) has been reported to possess cytokinin-like activity and is similar to that of highly active *N*-phenyl-*N*,-4-pyridyl urea derivatives (Mok et al. 1982). TDZ could also substitute for BA in tissue culture studies of several leguminous species (Malik and Saxena 1992; Sanago et al. 1996; Victor et al. 1999). In our on going efforts, we have carried out an extensive work on various factors that can influence synchronous regeneration of multiple shoots from various explants. The aim of the study was to evaluate the response of different types of explants (cotyledonary node, nodal and shoot tip explant) and the comparative performance of cytokinins on the induction, multiplication and finally rooting in the microshoots followed by their successful establishment in green house.

2 Materials and methods

Certified guar seeds (*C. tetragonoloba* cv. Neelam-51) procured from the local market, Aligarh were used in all the experiments. These were first washed under running tap water for half an hour to remove adherent particles, immersed in 5 % labolene detergent (Qualigens fine chemicals, Mumbai) for 10 min followed by washing with tap water and ethanol for 1 min. Thereafter, the seeds were treated in 0.1 % (w/v) solution of mercuric chloride (Qualigens Fine Chemicals, Mumbai) for 5 min and washed 5–6 times with sterile deionised water to remove all traces of disinfectant. Ten seeds were placed in a jam bottle culture vessel containing 50 ml of basic culture medium which contained the salts and vitamins of MS (Murashige and Skoog 1962) medium, 3 % (w/v) sucrose and a solidifying agent, 0.9 % (w/v) bacteriological grade agar agar (Qualigens Fine Chemicals, Mumbai) or 0.25 % Phytagel (Sigma-Aldrich, India). Cotyledonary node, nodal and shoot tips derived from 5, 10 and 15 days old aseptic seedlings were used as explant.

Thidiazuron (TDZ) and three other cytokinins viz. BA, Kn and 2iP were included in basic culture medium at equimolar concentrations (0.1, 1.0, 2.5, 5.0, 10.0, 20.0 μM). The pH of the medium was adjusted to 5.8 with KOH before dissolving agar. The molten medium was dispensed in glass culture tubes (Borosil, India) 25 × 150 mm (20 ml/tube) before sterilization. All media were sterilized by autoclaving at 0.122 MPa (121 °C) for 15 min and used within 48 h of preparation. Single explants were inoculated into each tube and maintained in a culture room at a temperature of 26 ± 1 °C with a photon fluence of 50 μmol m^{-1} s^{-1} provided by white fluorescent tubes (2 tubes × 40 W; by Philips, India Ltd.) with a photoperiod of 16 h.

After 3 weeks of incubation, the developed shoot cultures were subcultured onto the same shoot induction medium, but in case of TDZ-exposed explants, shoot bud clusters were transferred to PGR-free MS medium for further growth and development.

The elongated (>4 cm) microshoots were transferred to half strength MS medium with different concentrations of IBA and NAA (1, 2, 5 and 10 μM) for rooting. Rooted plantlets were washed thoroughly with distilled water to remove intact medium and transferred to thermocol cups (6 cm diameter) filled with sand: soil (3:1) mixture and placed in the culture room at 26 ± 1 °C under 16-h photoperiod (1 × 40 W, fluorescent tube, Philips India) and covered with transparent polyethylene bags to ensure high humidity. The plantlets were irrigated regularly with half strength MS medium lacking organic constituents. After 2 weeks, the poly bags were removed in order to acclimatize the plantlet and then under semi sterile conditions, regenerated plantlets were transferred to earthen pots (8 cm diameter) containing garden soil in green house and finally in the net house.

All the experiments were repeated thrice with 20 explants for each treatment. The regeneration frequency, number of shoots and shoot length were recorded after 4 and 8 weeks. Data obtained were analyzed statistically using SPSS Version 10 (SPSS Inc., Chicago, USA) significant differences between means were assessed by least significant differences (LSD) at $P < 0.05$ using Duncan's Multiple Range Test. Results of the experiment were expressed in terms of mean value ± standard error.

3 Results and discussion

The nodal explants placed on MS basal medium lacking PGRs (served as control) did not show any morphogenetic

response and failed to produce shoots even after 8 weeks of incubation. The comparative effect of the four cytokinins (BA, Kn, 2iP, TDZ) on multiple shoot induction was studied on cotyledonary node, node and shoot tip explants. In case of N^6-substituted adenine derivatives BA, Kn, and 2iP, shoot buds were observed within 2 weeks of incubation but it got delayed by 3–4 weeks in explants placed on the medium containing TDZ. Analysis of variance (ANOVA) revealed a significant effect ($P < 0.05$) of explants, time of explant collection, cytokinins concentration on number of shoot and shoot length per explant. A comparison of the different explants showed that the maximum average number of multiple shoot per explant was observed on cotyledonary nodes followed by nodes and shoot tip explants (Tables 1, 2). Time of explant collection also influences the organogenic capacity of explant. Explant collected from 10-day-old aseptic seedling exhibited a greater capacity for multiple shoot induction compared to 5 and 15 days old seedling (Table 2).

Amongst the various cytokinins tested, the highest multiple shoot formation per cotyledonary node explant was obtained in cultures containing MS medium supplemented with 5.0 μM TDZ or 10.0 μM BA (Fig. 1a, b). A significant decrease in the number of shoots/explants occurred at lower (0.1, 1.0 μM) or higher (20.0 μM) BA levels. An increase TDZ or BA concentration beyond optimum (5.0 and 10.0 μM, respectively) did not affect the number of shoots/explant significantly, but the shoot appeared to be developmentally suppressed and did not grow further. Although a decrease in number of shoots/explant was also observed with 20.0 μM Kn, but regeneration frequency continued to increase up to 85 % (Table 3).

The cytokinin BA has been commonly used for the induction of lateral shoot branching in various legumes; however, comprehensive studies comprising BA with other cytokinin for shoot production in legume are lacking (Parrott et al. 1992). A comparison of the relative

Table 1 Effect of different cytokinins and explant on number of shoot formation in *Cyamopsis tetragonoloba* L. after 8 weeks of incubation

Treatment	Conc. (μM)	Mean number of shoots/explant		
		Cotyledonary node	Node	Shoot tip
BA	10	7.8 ± 0.73^{b}	6.6 ± 0.92^{b}	2.4 ± 0.74^{a}
Kn	10	2.8 ± 0.73^{c}	2.8 ± 0.58^{c}	1.6 ± 0.40^{ab}
2iP	10	2.4 ± 0.60^{c}	1.6 ± 0.40^{c}	1.2 ± 0.20^{b}
TDZ	5	14.6 ± 0.67^{a}	8.6 ± 0.50^{a}	2.8 ± 0.37^{a}

Values represent mean ± standard error of 20 replicates per treatment in three repeated experiments. Means followed by the same letter are not significantly different by the DMRT at 0.05 % probability level

Table 2 Effect of different explant and days of collections of explant on in vitro shoot regeneration in *Cyamopsis tetragonoloba* L. after 8 weeks of incubation

Explant	Mean no. of shoots/explant (time of explant collection)		
	5 days	10 days	15 days
Cotyledonary node	7.8 ± 0.58^{a} (66.6 ± 4.40^{a})	14.6 ± 0.67^{a} (97.0 ± 3.00^{a})	6.8 ± 0.58^{a} (61.6 ± 4.40^{a})
Node	0.00 ± 0.00^{b} (0.00 ± 0.00^{b})	8.6 ± 0.50^{b} (91.0 ± 4.93^{ab})	5.8 ± 0.37^{a} (53.3 ± 3.33^{a})
Shoot tip	0.00 ± 0.00^{b} (0.00 ± 0.00^{b})	(2.8 ± 0.37^{c}) (77.3 ± 3.92^{b})	1.4 ± 0.54^{b} (40.0 ± 2.88^{b})

Values represent mean ± standard error of 20 replicates per treatment in three repeated experiments. Means followed by the same letter are not significantly different by the DMRT at 0.05 % probability level

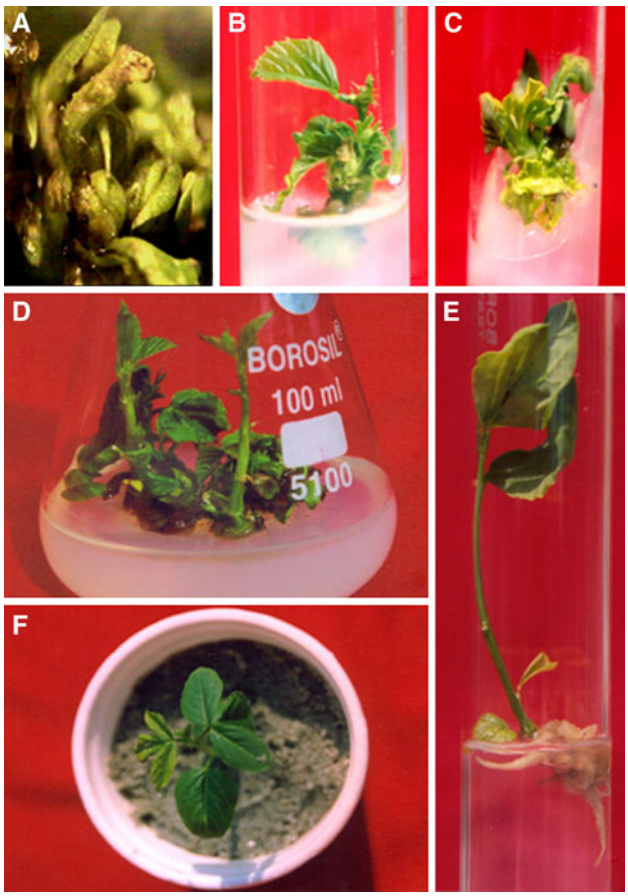

Fig. 1 Shoot bud differentiated in *Cyamopsis tetragonoloba* L. **a** Multiple shoot buds on MS medium with 5 μM TDZ in CN explant after 4 weeks. **b** Shoot buds differentiated in CN explant on MS medium with 10 μM BA, after 4 weeks. **c** Rosette of shoot buds in CN explant on MS medium with continuous exposure of TDZ after 6 weeks. **d** Shoot elongation on MS basal medium after 10 weeks of incubation. **e** Rooting of in vitro regenerated shoots on MS medium with 5.0 μM IBA. **f** An example of an acclimatized guar plant in the net house

Table 3 Effects of different PGRs and explants on in vitro multiple shoot induction in *Cyamopsis tetragonoloba* after 10 weeks of incubation

Cytokinin	Conc. (μM)	Cotyledonary node		Node		Shoot tip	
		Mean no. of shoots	Mean shoot length (cm)	Mean no. of shoots	Mean shoot length (cm)	Mean no. of shoots	Mean shoot length (cm)
BA	0.0	0.0 ± 0.00^{l} (0.0 ± 0.00^{n})	0.00 ± 0.00^{f}	0.0 ± 0.00^{g} (0.00 ± 0.00^{m})	0.00 ± 0.00^{g}	0.0 ± 0.00^{g} (00.0 ± 0.00^{l})	0.00 ± 00.00^{d}
	0.1	3.6 ± 0.50^{efgh} (31.3 ± 2.72^{klm})	1.58 ± 0.31^{e}	1.4 ± 0.50^{defg} (32.3 ± 2.66^{kl})	1.70 ± 0.34^{f}	1.0 ± 0.31^{de} (23.0 ± 2.51^{k})	1.70 ± 0.37^{cd}
	1.0	4.4 ± 0.81^{ef} (42.6 ± 3.71^{ijk})	2.08 ± 0.47^{de}	2.8 ± 0.66^{cdef} (43.0 ± 2.30^{ij})	2.18 ± 0.36^{ef}	1.4 ± 0.50^{bcdef} (36.0 ± 2.08^{ij})	1.94 ± 0.58^{c}
	2.5	4.6 ± 0.50^{de} (43.3 ± 4.40^{ijk})	2.86 ± 0.35^{abcde}	3.2 ± 0.86^{cde} (44.3 ± 1.76^{hij})	2.29 ± 0.38^{ef}	2.0 ± 0.44^{abcd} (37.3 ± 3.71^{hij})	2.14 ± 0.50^{bc}
	5.0	6.2 ± 0.73^{cd} (51.6 ± 4.40^{ghij})	2.64 ± 0.55^{bcde}	3.4 ± 0.50^{cd} (52.6 ± 4.05)	2.78 ± 0.51^{bcdef}	2.4 ± 0.50^{abc} (48.0 ± 3.0^{efg})	2.86 ± 0.58^{bc}
	10.0	7.8 ± 0.73^{c} (73.6 ± 4.09^{bcd})	2.86 ± 0.17	6.6 ± 0.92^{b} (78.3 ± 2.18^{bc})	2.88 ± 0.59^{bcdef}	2.4 ± 0.74^{abc} (64.0 ± 3.48^{bc})	3.20 ± 0.40^{abc}
	20.0	6.6 ± 0.50^{c} (55.6 ± 3.48^{fghi})	2.80 ± 0.38^{abcde}	3.4 ± 0.50^{cd} (55.6 ± 2.33^{efg})	2.74 ± 0.20^{cdef}	1.6 ± 0.40^{abcde} (46.0 ± 3.05^{fgh})	2.68 ± 0.47^{abc}
Kn	0.1	1.8 ± 0.20^{fghijk} (27.6 ± 4.33^{m})	2.90 ± 0.53^{abcde}	$1.2 \pm 0 \pm .20^{efg}$ (28.3 ± 3.71^{l})	3.06 ± 0.38^{bcdef}	0.4 ± 0.24^{efg} (27.6 ± 2.60^{jk})	3.00 ± 1063^{abc}
	1.0	2.6 ± 0.50^{fghijk} (48.0 ± 3.05^{hij})	3.26 ± 0.56^{abcde}	1.0 ± 0.44^{fg} ($53.0 \pm 0.40f^{gh}$)	3.18 ± 0.44^{abcdef}	0.6 ± 0.24^{efg} (43.6 ± 2.96^{ghi})	2.98 ± 0.59^{abc}
	2.5	2.6 ± 0.50^{efghij} (51.3 ± 4.93^{ghij})	3.08 ± 0.64^{bcde}	1.6 ± 0.60^{defg} (49.3 ± 4.05^{ghij})	3.14 ± 0.33^{abcdef}	1.0 ± 0.31^{defg} (47.3 ± 3.71^{efg})	3.26 ± 0.67^{abc}
	5.0	2.8 ± 0.58^{efghij} (56.3 ± 4.48^{fgh})	3.24 ± 0.58^{abcde}	1.8 ± 0.80^{defg} (59.6 ± 3.28^{def})	3.24 ± 0.55^{abcdef}	1.2 ± 0.58^{cdefg} (51.6 ± 2.02^{efg})	3.14 ± 0.62^{abc}
	10.0	2.8 ± 0.73^{efghij} (76.0 ± 3.48^{bc})	3.66 ± 0.79^{abcd}	2.8 ± 0.58^{cdef} (80.6 ± 3.17^{bc})	4.06 ± 0.42^{abc}	1.6 ± 0.40^{abcde} (68.6 ± 2.84^{ab})	3.60 ± 0.55^{abc}
	20.0	2.4 ± 0.50^{ghijk} (84.3 ± 3.48^{ab})	2.60 ± 0.26^{bcde}	1.4 ± 0.50^{defg} (86.6 ± 4.40^{b})	3.78 ± 0.76^{abcde}	0.8 ± 0.20^{defg} (75.6 ± 3.48^{a})	3.46 ± 0.82^{abc}
2iP	0.1	1.4 ± 0.40^{ijkl} (29.3 ± 2.33^{lm})	3.00 ± 0.37^{abcde}	1.2 ± 20.20^{efg} (33.6 ± 2.33^{kl})	2.70 ± 0.25^{cdef}	0.2 ± 0.20^{gf} (24.0 ± 2.33^{k})	2.26 ± 0.45^{bc}
	1.0	2.4 ± 0.50^{ghijk} (41.0 ± 4.58^{jkl})	3.66 ± 0.81^{abcd}	1.4 ± 0.24^{defg} (41.3 ± 2.02^{ijk})	3.26 ± 0.449^{abcdef}	0.8 ± 0.20^{defg} (34.0 ± 3.48^{ij})	2.90 ± 0.82^{abc}
	2.5	2.4 ± 0.60^{ghijk} (48.3 ± 4.40^{hij})	3.92 ± 0.67^{abc}	1.6 ± 0.40^{defg} (43.6 ± 2.96^{hij})	3.92 ± 0.68^{abcd}	1.2 ± 0.20^{cdefg} (48.0 ± 3.46^{efg})	3.60 ± 0.91^{abc}
	5.0	2.0 ± 0.54^{hijk} (61.6 ± 4.91^{defg})	4.26 ± 0.71^{ab}	1.6 ± 0.50^{defg} (49.6 ± 3.84^{ghij})	4.06 ± 0.64^{abc}	1.4 ± 0.24^{bcdef} (53.6 ± 2.96^{defg})	3.86 ± 0.69^{abc}
	10.0	1.0 ± 0.44^{jkl} (64.6 ± 3.75^{cdef})	4.44 ± 0.75^{a}	1.8 ± 0.58^{defg} (60.3 ± 3.46^{def})	4.76 ± 0.74^{a}	1.6 ± 0.40^{abcde} (53.0 ± 3.21^{defg})	4.60 ± 0.79^{a}
	20.0	0.8 ± 0.48^{kl} (59.3 ± 4.48^{fgh})	1.28 ± 0.41^{e}	1.2 ± 0.20^{efg} (64.0 ± 3.60^{de})	3.56 ± 0.86^{abcde}	0.8 ± 0.20^{efg} (56.3 ± 4.09^{cdef})	3.46 ± 0.89^{abc}
TDZ	0.1	2.4 ± 0.74^{ghijk} (60.6 ± 4.37^{efgh})	2.52 ± 0.26^{bcde}	1.0 ± 0.31^{fg} (65.3 ± 2.90^{d})	1.90 ± 0.43^{f}	1.0 ± 31^{defg} (56.6 ± 3.33^{cde})	1.78 ± 0.41^{cd}
	1.0	3.0 ± 0.54^{efghi} (72.3 ± 2.60^{bcde})	2.94 ± 0.23^{abcde}	1.8 ± 0.66^{defg} (76.0 ± 3.21^{c})	2.60 ± 0.30^{cdef}	1.2 ± 0.37^{cdef} (65.0 ± 2.8^{bc})	2.50 ± 0.43^{abc}
	2.5	4.0 ± 0.89^{efg} (84.6 ± 4.48^{ab})	3.70 ± 0.48^{abcd}	4.0 ± 0.83^{c} (87.6 ± 2.33^{b})	3.04 ± 0.23^{bcdef}	1.4 ± 0.50^{bcdef} (75.3 ± 3.71^{a})	2.76 ± 0.52^{abc}
	5.0	14.6 ± 0.67^{a} (91.0 ± 4.93^{a})	4.06 ± 0.43^{ab}	8.6 ± 0.50^{a} (97.0 ± 3.00^{a})	4.42 ± 0.28^{ab}	2.8 ± 0.37^{a} (77.3 ± 3.92^{a})	4.20 ± 0.71^{ab}
	10.0	9.6 ± 0.50^{b} (83.0 ± 2.51^{ab})	2.20 ± 0.47^{cde}	4.0 ± 0.89^{c} (86.3 ± 2.72^{b})	3.22 ± 0.21^{abcdef}	2.6 ± 0.67^{ab} (71.0 ± 3.46^{ab})	3.08 ± 0.54^{abc}
	20.0	6.2 ± 0.66^{cd} (78.0 ± 4.16^{b})	2.00 ± 0.43^{de}	2.8 ± 0.86^{cdef} (79.3 ± 2.33^{bc})	$2.32 \pm 0.44d^{ef}$	1.4 ± 0.40^{bcdef} (62.6 ± 3.71^{bcd})	2.04 ± 0.70^{c}

Values represent mean ± standard error of 20 replicates per treatment in three repeated experiments. Means followed by the same letter are not significantly different by the DMRT at 0.05 % probability level

Values given in parenthesis are mean percentage response per explant

effectiveness of different cytokinins for multiple shoot formation revealed the order of effectiveness TDZ > BA > Kn > 2iP.

Kn and 2iP tested in the range of 0.1–20.0 μM were either ineffective or less effective in the induction of additional shoots beyond the normal two cotyledonary axillaries; similar observations were also made by Malik and Saxena (1992) in *Phaseolus vulgaris*.

Cytokinin concentration with time of exposure had a marked effect on shoot regeneration frequency from different explant of *Cyamopsis* (Table 3). In general, the number of shoot buds per explant increased up to an optimum concentration depending on the cytokinin and type of explant (Table 3). Significantly more regenerants were formed on the cotyledonary explant exposed to TDZ than BA-treated explant. The optimal concentration of TDZ supplementation in media was 5.0 μM, wherein maximum (14.6 ± 0.67) shoot buds/explant were produced within 8 weeks of culture. Shoot buds obtained from different explants on TDZ containing media did not elongate and resulted in rosette (Fig. 1c) of shoots when continued to culture on to the same shoot induction medium. The shoot clusters were than transferred onto MS basal medium lacking PGRs for shoot/stem elongation (Fig. 1d), because shoot elongation has repeatedly been found as a major obstacle in obtaining normal plantlets from TDZ-exposed explant (Sanago et al. 1996; Victor et al. 1999). However, continuous presence of BA is necessary for the entire 10 weeks period for the induction of shoot buds and their further growth and development. If multiple shoots exposed to BA (2–3 weeks) were transferred to MS basal medium, differentiated buds did not develop further and no new buds were initiated, which is in consonance with the procedure mentioned in *P. vulgaris* (Malik and Saxena 1992).

Morphologically normal looking shoots were detached from the shoot clumps and transferred to half strength MS basal medium containing different concentrations of IBA and NAA for in vitro rooting. No root formation was observed in medium lacking auxins. Presence of auxins in medium exhibited rhizogenesis. However, the maximum frequency (55 %) of root formation (Fig. 1e) and the number (3.0 ± 0.55) of roots with root length (2.5 ± 0.47a) were achieved on half strength MS medium supplemented with 5.0 M IBA (Table 4). As MS medium fortified with IBA supported good rooting in comparison to NAA, therefore, IBA was found to be a superior auxin. Our observations are consistent with the earlier finding in which IBA was successfully employed for rooting in *Capsium annuum* (Ahmad et al. 2006) and *Sansevieria cylindrica* (Shahzad et al. 2009).

Acclimatization is the final step in a successful micropropagation system. During this stage, plants have to adapt to the new environment of greenhouse or field. The plantlets usually need some weeks of acclimation in shade with the gradual lowering of air humidity (Pospíšilová et al. 1998). The regenerated plantlets with well-developed shoot and roots were then hardened (as described in materials and methods) prior to transfer to the green house and then in net house. 90 plantlets were hardened and transferred to the net house of the Botanical garden, out of which about 75 plants (Fig. 1f) survived (with 80 % survival rate) and grew to maturity. They showed vigorous growth and normal phenotype.

Table 4 Effect of NAA and IBA on rooting in in vitro raised microshoots of *Cyamopsis tertragonoloba* cultured on half strength MS medium after 4 weeks of incubation

NAA	IBA	% response	Mean no. of roots/shoot	Mean root length (cm)
0.0		0	0.0 ± 0.00^{c}	0.0 ± 0.00^{e}
1.0		35	1.4 ± 0.24^{b}	0.7 ± 0.15^{e}
2.0		42	1.6 ± 0.24^{b}	1.1 ± 0.32^{bcd}
5.0		45	2.2 ± 0.37^{ab}	1.4 ± 0.37^{bcd}
10.0		40	1.4 ± 0.51^{b}	0.9 ± 0.30^{de}
	1.0	48	1.2 ± 0.20^{b}	1.4 ± 0.40^{bcd}
	2.0	50	1.8 ± 0.37^{b}	1.9 ± 0.24^{abc}
	5.0	55	3.0 ± 0.55^{a}	2.5 ± 0.47^{a}
	10.0	45	1.8 ± 0.37^{b}	2.1 ± 0.33^{ab}

Values represent mean ± standard error of 20 replicates per treatment in three repeated experiments. Means followed by the same letter are not significantly different by the DMRT at 0.05 % probability level

In conclusion, we have established a promising and reproducible protocol for an efficient in vitro shoot multiplication from different explants of *C. tetragonoloba* using TDZ. It employs that a large number of shoot meristems differentiated simultaneously, all the differentiated shoot meristems developed into shoots and many healthy plants can be grown ex vitro under field condition. Therefore, the described regeneration system may be adapted for regeneration investigations and for in vitro manipulations of Guar.

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References

Ahmad N, Anis M (2007) Rapid plant regeneration protocol for cluster bean (*Cyamopsis tetragonoloba* L. Taub.). J Hortic Sci Biotech 82:585–589

Ahmad N, Siddique I, Anis M (2006) Improved plant regeneration in *Capsicum annuum* L. from nodal segments. Biol Plant 50:701–704

Bansal YK, Chibbar T, Bansal S, Singh M (1994) Plant regeneration from hypocotyls and cotyledon explant of cluster bean (*Cyamopsis tetragonoloba* L.). J Physiol Res 7:47–60

Joersbo M, Brunstedt J, Marcussen J, Okkels FT (1999) Transformation of endospermous legume guar (*Cyamopsis tetragonoloba*) and analysis of transgene transformation. Mol Breed 5:521–529

Malik KA, Saxena PK (1992) Regeneration in *Phaseolus vulgaris* L: high frequency induction of direct shoot formation in intact seedlings by N^6-benzylaminopurine and thidiazuron. Planta 186:384–389

Mok MC, Mok DWS, Armstrong DJ, Shudo K, Isogai Y, Okamoto T (1982) Cytokinin activity of N-phenyl-1,2,3-thidiazol-5-ylurea (Thidiazuron). Phytochemistry 21:1509–1511

Murashige T, Skoog S (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Parrott WA, Baily MA, Durham RE, Mathew HV (1992) Tissue culture and regeneration in legumes. In: Moss DP (ed) Biotechnology and crop improvement in Asia. ICRISAT, Patancheru, pp 115–148

Pospíšilová J, Wilhelmová N, Synková H, Čatský J, Krebs D, Tichá I, Hanáčková B, Snopek J (1998) Acclimation of tobacco plantlets to ex vitro conditions as affected by application of abscisic acid. J Exp Bot 49:863–869

Prem D, Singh S, Gupta PP, Singh J, Yadav G (2003) High frequency multiple shoot regeneration from cotyledonary nodes of Guar (*Cyamopsis tetragonoloba* L. Taub). In Vitro Cell Dev Biol 39:384–387

Prem D, Singh S, Gupta PP, Singh J, Kadyan SPS (2005) Callus induction and de novo regeneration from callus in guar [*Cyamopsis tetragonoloba* L. Taub.]. Plant Cell Tissue Organ Cult 80:209–214

Ramulu CA, Rao D (1991) Tissue culture studies of differentiation in a grain Legume *Cyamopsis tetragonoloba* (L.) Taub. J Physiol Res 4:183–185

Sanago MHM, Shattuck VI, Strommer J (1996) Rapid plant regeneration of pea using thidiazuron. Plant Cell Tissue Organ Cult 45:165–168

Shahzad A, Ahmad N, Rather MA, Husain MK, Anis M (2009) Improved shoot regeneration system through leaf derived callus and nodule culture of *Sansevieria cylindrica*. Biol Plant 53:745–749

Victor JMR, Murch SJ, Krishna Raj S, Saxena PK (1999) Somatic embryogenesis and organogenesis in peanut: the role of thidiazuron and N^6 benzylaminopurine in the induction of plant morphogenesis. Plant Growth Regul 28:9–15

Whitler RL, Hymowitz T (1979) Guar: agronomy production, industrial use and nutrition. Purdue University Press, West Lafayette