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High frequency shoot regeneration in nodal and shoot tip explants of pygmy ground cherry (*Physalis minima* L.) via. Two different modes of organogenesis

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Abstract
Physalis minima L. a pubescent annual herb possess both horticultural as well as medicinal properties. The present study describes a reproducible protocol for high frequency *in vitro* shoot production through nodal explants and shoots tip explants when cultured on Murashige and Skoog (MS) medium supplemented with different Cytokinins. Optimum response was achieved through Shoot tip explants when cultured on 10.0 µM BA + 0.5 µM NAA which in turn resulted in the production of a maximum number of 77.4±0.50 adventitious shoots with a mean shoot length of 1.42±0.03 cm. Gibberellic Acid (GA) resulted in further proliferation and elongation when added to the optimized cytokinin concentration. Rooting was observed on half-strength MS medium fortified with different auxins either singly or in combination. Regenerated plantlets were successfully acclimatized, hardened and then transferred to green house with 90% survival rate.

Keywords: adventitious shoots, axillary shoots, 6-benzyladenine (BA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), kinetin (KN), nodal explants, plant growth regulators (PGRs), shoot tip explants, thiadiazuron (TDZ)

Introduction
Physalis minima L. belonging to the family Solanaceae, is a native throughout south-east Asia and into northern Australia. The plant has anti-inflammatory, analgesic and antipyretic activities. The fruits are considered to be tonic, diuretic and purgative (Parmar and Kaushal, 1982; Chopra *et al*., 1986) [31, 10]. Two active anti-cancer compounds have been isolated from its methanol extract of stem and leaf and were identified as physalin B and physalin F (Lee and Houghton, 2005) [22]. Decoction of roots and leaves is also used for diabetes. Extracts from leaves showed anti-microbial activity against (Nayeemulla *et al*., 2006) [30]. Increasing human and livestock populations have already affected the status of wild plants; particularly those used in herbal medicine (Alagumanian *et al*., 2004) [3]. Forest cleaning activities are also leading to depletion of valuable plant resources. Thus the conservation of these valuable genotypes is imperative (Karuppusamy *et al*., 2006) [20]. India's share in global export of horticultural commodities is negligible due to low productivity, lack of infrastructural facilities and inadequate post-harvest management. Currently modern applications of plant biotechnology viz. micropropagation offer a wide range of possibilities for crop and horticultural species to increase productivity and diversification via. Sustainable agriculture. There are a few reports available for *in vitro* plant regeneration of *Physalis minima* using various explants like nodal segments and shoot tip (Afroz *et al*., 2009) [2], leaves and nodes (Sheeba *et al*., 2010) [35], leaf, node and root explant derived calli (Arvind *et al*., 2011) [5]. Thus considering the medicinal and food value of this plant in the present investigation a reproducible protocol has been developed for high frequency *in vitro* shoot production through nodal and shoot tip explants through two different modes of organogenesis simultaneously viz. axillary and adventitious.

Materials and Methods
Collection of explant
The explants were collected from the *in vivo* grown plants of *P. minima* in the botanical garden of the Department of Botany, AMU, Aligarh. The visibly healthy plant amongst all was chosen as explants source. The excised nodal explants were kept in a beaker under running tap water for half an hour for preliminary washing followed by immersing in 1% (w/v) solution of bavistin, a fungicide, for 15 min to remove adherent particles from the surface and thereafter washed thoroughly with 5% (v/v) teepol solution (a mild detergent) for 15 min by continuous stirring. Then the explants were washed with water thoroughly to remove all the detergent present. Thereafter, under Laminar air flow cabinet explants were surface sterilized by treating with 0.1% $HgCl_2$ followed by through rinsing for 2-3 times in DDW so as to completely remove the traces of $HgCl_2$. The surface sterilized explants containing a single node were inoculated vertically on the culture medium.

Culture Media and Conditions
The MS (Murashige and Skoog, 1962) [27] medium was used throughout the experiments. All media were gelled using 0.8% (w/v) agar (Qualigens, India) supplemented with 3% (w/v) sucrose (Qualigens, India). The pH of the

medium was adjusted to 5.8 using 1 N NaOH or HCl before autoclaving at 121ºC with a pressure of 104 K Pa for 20 min. The cultures were maintained in a culture room under controlled environmental conditions of 25±2ºC with 16/8 h photoperiod with a photosynthetic photon flux density (PPED) of 50µM $m^{-2}s^{-1}$provided by cool fluorescent tubes (Philips India) and relative humidity 55 ± 5 %.

Axillary and adventitious shoot induction and proliferation

Both Nodal and shoot tip explants were cultured on MS medium fortified with PGRs viz. BA (1.0, 2.5, 5.0, 10.0 and 12.5 µM), TDZ (0.5, 1.0, 2.5, 5.0 and 10.0 µM) and KN (0.5, 1.0, 2.5, and 5.0, µM) either singly or in combination with 1-Napthalene acetic acid NAA (0.5, 1.0 µM) for axillary as well as adventitious shoot induction. For further proliferation and elongation cultures were transferred on optimum cytokinin concentration i.e., BA (10.0 µM) and TDZ (5.0 µM) in combination with Gibberellic Acid GA (0.1, 0.5, and 1.0 µM).

Rooting and plant establishment

For root induction elongated microshoots were excised and transferred to root induction medium consisting of single as well as combined concentrations of IAA (0.5, 1.0µM), IBA (0.5,1.0 µM) and NAA (0.5,1.0µM) in half strength MS basal medium. Data based on the observations was collected periodically after every three to five days. After 4 weeks the rooted microshoots were carefully taken out of culture tubes and roots were washed gently to remove agar adhered to them. Thereafter, these rooted microshoots were planted in thermo Cole cups containing soilrite and kept in culture room conditions for proper hardening. For first three days these plantlets were watered with MS basal medium which was gradually replaced by water after short interval of three day. After one and a half month hardened plantlets were transferred to earthen pots containing garden soil and farmyard manure in 1:1 ratio and kept in greenhouse.

Statistical analysis

For each experiment ten replicates were raised and each experiment was repeated thrice. The data was analyzed statistically through one-way ANOVA using SPPS ver. 10 (SPPS inc., Chicago, USA). The significance of difference among means was carried out by Duncan's multiple range test at p=0.05 and the results are expressed as mean ± SE of three repeated experiments.

Results and Discussion

Explant type and application of cytokinins

Two types of explants viz. nodal and shoot tips were taken to optimize the regeneration protocol. Both the explants showed a nullified effect when treated on MS medium devoid of growth regulators. In contrary to this, addition of different cytokinins proved effective. Different cytokinins were tested either singly or in combination with auxins and other growth regulators. The response initiated in the form of axillary bud break in both the explants but the time period of initiation varied accordingly with the type of PGR used. Thereafter the axillary bud transformed into a flattened sheath like structure where multiplication of meristemoids started and ultimately a large number of axillary shoots resulted from the flattened structure. Gradually a nodule formation started at the base of both the explants. The elongated axillary shoots were transferred on rooting media and the nodule was cut and transferred on single as well as combination media. This nodule resulted in the formation of a large number of adventitious shoots. Cytokinins are most critical for shoot induction and elongation in several systems including medicinal plants such as *Clitoria ternatea* (Rout, 2005) [32] and *Justicia gendarussa* (Thomas and Yoichro, 2010) [39]. Additionally, BA is well known for promotion of in vitro shoot formation.

Shoot proliferation

Response occurred in the form of axillary as well as adventitious bud break thus depicting two different pathways of differentiation on same explant source. Axillary bud break occurred earlier whereas adventitious shoot formation was a bit delayed in both the types of explants.

Axillary shoot proliferation

The effect on multiple shoot induction of three cytokinins (BA, TDZ and KN) were studied on both the explants. On BA supplemented MS medium response initiated in the form of axillary bud break after three days of inoculation. In nodal explants, among all the concentrations of BA tested, MS +10.0 µM BA resulted in an optimum production of 33.4 ± 0.67 mean no. of shoots/explant with a mean shoot length of 3.98±0.03 cm, whereas in shoot tip explants a maximum production of 33.8±0.58 mean no. of shoots/explant with a mean shoot length of 3.64±0.08 cm was obtained on the same concentration (Table 1). The lower concentrations of BA were not much effective as well as the higher concentrations responded less effectively. On TDZ supplemented MS medium response initiated after one week of inoculation and was a bit early in shoot tip explants rather than nodal explants. Among all the concentrations of TDZ tested, MS + 5.0 µM TDZ proved to be optimum for both the explants with a maximum production of 28.6 ± 0.87 mean no. of shoots/explant with a mean shoot length of 3.56 ± 0.08 cm on nodal explants and a maximum production of 36.8±1.15 mean no. of shoots/explant with a mean shoot length of 3.30±0.06 cm on shoot tip explants (Table 1). The lower concentrations of TDZ were less effective and the higher concentrations were found to be inhibitory. On KN supplemented MS medium 5.0 µM KN responded with a maximum production of 5.4 ± 0.24 mean no. of shoots/explant with a mean shoot length of

1.20 ± 0.12 cm for nodal explants and a maximum production of 6.60 ± 0.67 mean no. of shoots/explant with a mean shoot length of 1.02 ± 0.07cm for shoot tip explants (Table 1). Lower concentrations of KN proved inhibitory as well as on higher concentrations of KN response was very less effective. Comparatively shoot tip explants showed a much better response than nodal explants in terms of shoot number on 5.0 µM TDZ supplemented MS medium but in contradiction to this maximum shoot length was achieved on nodal explants augmented with MS +10.0 µM BA. BA showed optimum response on nodal explants whereas TDZ showed a much better response than BA on shoot tip explants.

Adventitious shoot proliferation

Besides axillary shoot proliferation adventitious shoot proliferation was also obtained on both the explants. Adventitious regeneration in plants occurs after cells from organized tissues dedifferentiate and then reorganize into meristemoids forming shoots either directly or indirectly via., an intervening callus phase. In this study direct shoot formation has been achieved adventitiously in addition to axillary shoot formation. Surface of explant around axillary meristem differentiated into adventitious shoots forming a circular cluster of shoots around the nodal region. Regeneration capacity on the nodal surface might be due to the presence of inherent meristematic cells at the axils which influence the adjacent non-meristematic cells in certain way to dedifferentiate and induce adventitious shoot formation. Effect of BA and TDZ also differed for frequency of shoot production and elongation. However, in indirect mode of organogenesis that resulted in adventitious shoot proliferation, among all the concentrations of BA tested, an optimum effect was obtained on MS +10.0 µM BA with the production of 49.60±0.87 mean no. of shoots/explant and a mean shoot length of 2.58±0.07 cm on nodal explants and a maximum production of 50.8±0.80 mean no. of shoots/explant with a shoot length of 2.30±0.04 cm on shoot tip explants (Table 2). Among all the concentrations of TDZ, MS + 5.0 µM TDZ proved to be optimum for both the explants with a maximum production of 41.40±0.74 mean no. of shoots/explant with a mean shoot length of 2.28±0.06 cm on nodal explants and a maximum production of 58.8±1.11 mean no. of shoots/explant with a mean shoot length of 2.06±0.50 cm on shoot tip explants (Table 2). Response of shoot tip explant was better than nodal segment on every treatment tested. Better regeneration potential of shoot tip could be attributed to the higher content of endogenous auxin present on comparison to nodal explant because of the intact shoot apex. Thus endogenous auxin incorpates with exogenously supplied cytokinin to promote shoot multiplication. In contradiction with the present results nodal explant was found to be more suitable than shoot tip in *Physalis minima* (Afroz *et al,* 2009) [2]. It is not unusual for explants to respond to TDZ to induce more adventitious shoots compared to BA (Huetteman and Preece 1993; Lu, 1993) [17, 24]. However, it was clearly observed that after several subculture passage TDZ induced cultures exhibited vitrification of shoots. While cultures initiated on BA were still normal. Vitrification is a result of exposure to high cytokinin for longer duration. Instances of vitrification on TDZ containing medium have been reported in *Arnedia exchroma* (Malik *et al.* 2010) [26], *Arachis stenosperma* (Vijayalaxmi and Giri. 2003) [41] and *Cineraria maritime* (Banerjee *et al.,* 2004) [6].

Effect of Combination media on shoot proliferation

Cytokinins in combination with auxins were also evaluated for their effect on *in vitro* shoot regenerations from both nodal and shoot tip explants. Synergistic influence of auxin-cytokinin combination has previously been stated in many solanaceae members (Gleddie *et al.,* 1983; Sharma and Rajam, 1995; Seetharam *et al.,*2003) [16, 34, 33]. Lower amount of auxin with high concentration of cytokinin was known to have promotive effect on *in vitro* regeneration of *Crataegus sinaica* (Maharik *et al.,* 2009) [25] and *Datura insignis* (Figueiredo and Esquibel, 1991) [14]. Of all the cytokinins and auxins, BA and NAA have been used most commonly for shoot induction (Tripepi, 1997; Arockiasamy *et al.,* 2002; Nasiruddin *et al.,* 2003) [40, 4, 29]. However, observations revealed that the frequency of shoot multiplication was rather lower on optimum BA + NAA combination i.e, 10.0 µM BA + 0.5 µM NAA which resulted in the production of 72.2±0.96 mean no. shoots/explants with a mean shoot length of 1.62±0.08 cm) in comparison to optimum TDZ + NAA combination i.e, 5.0 µM BA + 0.5 µM NAA which resulted in the production of 80.0±0.89 mean no. shoots/explants with a mean shoot length of 1.14±0.04 cm (Table2). Cytokinins commonly stimulate shoot proliferation and inhibit shoot elongation, particularly BA (Brassard *et al.,* 1996) [8]. Thus proper elongation of shoots was not achieved on combination medium. Observations also revealed that phenomenon of vitrification that was observed by cultures maintained on TDZ containing medium was eliminated in case of cultures induced on TDZ + NAA combination. This might be due to the presence of auxin which nullified the adverse effect of high cytokinin concentration in cultures. Instances of vitrification on TDZ containing medium have been reported in *Arnedia exchroma* (Malik *et al.* 2010) [26], *Arachis stenosperma* (Vijayalaxmi and Giri, 2003) [41] and *Cineraria maritime* (Banerjee *et al.,* 2004) [6].

Effect of GA on shoot elongation and proliferation

Established cultures were further transferred to proliferation medium to stimulate proper shoot elongation. Observation revealed increase in shoot length under influence of GA on proliferation medium. GA is reported to be conducive for promotion of growth, biomass production and xylem fibre length (Ericksson *et al.,* 2000) [13]. Striking difference was noticed in percentage elongation on BA 10.0 µM + NAA 0.5 µ + GA 0.5 µM containing MS medium and TDZ 5.0 µM + NAA 0.5 µ + GA 0.5 µM supplemented medium (Graph 1). Effect of GA was limited by the presence of TDZ thus restricting frequency of elongation. TDZ is reported to suppress shoot

elongation in many studies (Chand *et al*., 1999; Geneve, 2005) [9, 15]. However presence of BA did not seem to hinder shoot elongation capacity of GA. Similar finding on positive effect of GA on increased shoot length were reported by (Detrez *et al.,* 1994, Abadi and Hamidoghli, 2009) [11, 1].

Rooting of Microshoots

Elongated micro shoots were excised and transferred for *in vitro* root induction on auxin containing media. Variation in root length and type were revealed depending on type and concentration of auxin used (Table 4). While IBA induced short and fibrous roots, IAA increased mean root length leading to long and thin root formation. Combination of IAA and IBA yielded long fibrous root development, thus proving that IAA played the central role in proper elongation also. Indirect rooting with intervening callus formation at the base of microshoots occurred on NAA containing nutrient medium. Incidences of callus formation during rooting on NAA were also reported in *Solanum tuberosum* (Khadiga *et al*., 2009) [21]. Contrary to present observations NAA was found to be efficient for rhizogenesis in several members of Solanaceae (Husain *et al*., 1999; Bodhipadma and Leung, 2003) [19, 7].

Table 1: Effect of BA, TDZ and KN on nodal and shoot tip explants of *P. minima* via axillary proliferation after 6 weeks of culture

PGR (µM)			Nodal Explant			Shoot Tip Expant		
BA	TDZ	KN	% Response	Mean no. of shoots/explant	Mean shoot length (cm)	% Response	Mean no. of shoots/explant	Mean shoot length (cm)
1.0	-	-	43.2±1.65^{e}	6.2±0.37^{g}	1.86±0.10^{fg}	63.6±1.36^{d}	8.00±0.54^{h}	1.48±0.58^{g}
2.5	-	-	83.2±2.00^{c}	9.2±0.58^{f}	2.64±0.13^{cd}	83.2±1.39^{c}	12.6±0.60^{f}	2.04±0.60^{e}
5.0	-	-	93.2±1.42^{b}	18.2±0.58^{d}	2.80±0.08^{c}	92.6±1.12^{b}	23.8±0.37^{d}	2.62±0.12^{c}
10.0	-	-	99.2±0.37^{a}	33.4±0.67^{a}	3.98±0.03^{a}	97.8±0.58^{a}	33.8±0.58^{b}	3.64±0.08^{a}
12.5	-	-	63.6±2.92^{d}	17.6±0.92^{d}	2.02±0.07^{ef}	91.8±0.73^{b}	22.2±0.66^{d}	1.70±0.07^{f}
-	0.5	-	32.6±1.02^{f}	7.80±0.73^{f}	1.72±0.11^{g}	43.4±1.28^{e}	10.8±0.96^{g}	1.46±0.07^{g}
-	1.0	-	82.8±1.15^{c}	10.8±0.58^{e}	2.12±0.06^{e}	62.2±1.20^{d}	16.0±0.94^{e}	2.00±0.04^{e}
-	2.5	-	82.6±2.20^{c}	20.2±0.86^{c}	2.44±0.08^{d}	82.0±1.51^{c}	25.8±0.66^{c}	2.24±0.08^{d}
-	5.0	-	98.8±0.37^{a}	28.6±0.87^{b}	3.56±0.08^{b}	99.2±0.37^{a}	36.8±1.15^{a}	3.30±0.06^{b}
-	10.0	-	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{h}	0.00±0.00^{j}	0.00±0.00^{j}
-	-	0.5	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{h}	0.00±0.00^{j}	0.00±0.00^{j}
-	-	1.0	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{h}	0.00±0.00^{j}	0.00±0.00^{j}
-	-	2.5	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{h}	0.00±0.00^{j}	0.00±0.00^{j}
-	-	5.0	23.2±1.35^{g}	5.4±0.24^{g}	1.20±0.12^{h}	30.0±0.83^{f}	6.60±0.67^{h}	1.02±0.07^{h}
-	-	10.0	9.40±0.67^{h}	1.80±0.37^{h}	1.04±0.08^{h}	7.60±0.81^{g}	3.00±0.44^{i}	0.66±0.07^{i}

Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments. Mean value followed by the same letter are not significantly different according to Duncan's Test at 5% probability.

Table 2: Effect of BA, TDZ and KN on nodal and shoot tip explants of *P. minima* via. adventitious proliferation after 6 weeks of culture

PGR (µM)			Nodal Explant			Shoot TIP Expant		
BA	TDZ	KN	% Response	Mean no. of shoots/explant	Mean shoot length (cm)	% Response	Mean no. of shoots/explant	Mean shoot length (cm)
1.0	-	-	50.20±1.31^{f}	14.00±0.44^{f}	1.28±0.04^{e}	67.0±1.04^{e}	17.8±0.37^{h}	1.18±0.37^{e}
2.5	-	-	87.80±0.58^{c}	19.40±0.50^{e}	1.56±0.05^{d}	85.6±0.87^{d}	25.6±0.60^{f}	1.40±0.06^{d}
5.0	-	-	93.40±1.12^{b}	31.00±0.89^{c}	1.92±0.06^{c}	94.8±0.66^{b}	37.2±0.66^{d}	1.62±0.07^{c}
10.0	-	-	97.80±0.58^{a}	49.60±0.87^{a}	2.58±0.07^{a}	98.8±0.37^{a}	50.8±0.80^{b}	2.30±0.04^{a}
12.5	-	-	65.80±0.58^{e}	24.6±1.02^{d}	1.62±0.03^{d}	92.4±0.74^{c}	31.8±0.66^{e}	1.40±0.04^{d}
-	0.5	-	41.20±0.86^{g}	11.80±0.58^{g}	1.48±0.12^{d}	46.2±1.15^{f}	22.0±0.89^{g}	1.12±0.07^{e}
-	1.0	-	83.80±0.58^{d}	17.80±0.37^{e}	1.64±0.06^{d}	65.4±0.97^{e}	18.0±0.44^{h}	1.38±0.03^{c}
-	2.5	-	85.40±0.92^{d}	30.80±0.66^{c}	1.82±0.03^{c}	84.4±1.24^{d}	42.4±0.87^{c}	1.62±0.03^{d}
-	5.0	-	98.20±0.37^{a}	41.40±0.74^{b}	2.28±0.06^{b}	98.8±0.37^{a}	58.8±1.11^{a}	2.06±0.50^{b}
-	10.0	-	0.00±0.00^{j}	0.00±0.00^{i}	0.00±0.00^{h}	0.00±0.00^{i}	0.00±0.00^{j}	0.00±0.00^{h}
-	-	0.5	0.00±0.00^{j}	0.00±0.00^{i}	0.00±0.00^{h}	0.00±0.00^{i}	0.00±0.00^{j}	0.00±0.00^{h}
-	-	1.0	0.00±0.00^{j}	0.00±0.00^{i}	0.00±0.00^{h}	0.00±0.00^{i}	0.00±0.00^{j}	0.00±0.00^{h}
-	-	2.5	0.00±0.00^{j}	0.00±0.00^{i}	0.00±0.00^{h}	0.00±0.00^{i}	0.00±0.00^{j}	0.00±0.00^{h}
-	-	5.0	20.0±0.44^{h}	12.40±1.07^{fg}	1.00±0.04^{f}	31.20±1.01^{g}	16.8±0.96^{h}	0.86±0.50^{f}
-	-	10.0	9.40±0.50^{i}	7.20±0.91^{h}	0.70±0.05^{g}	12.00±0.44^{h}	10.4±0.87i	0.64±0.74^{g}

Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments.
Mean value followed by the same letter are not significantly different according to Duncan's Test at 5% probability.

Table 3: Effect of combination media on nodal and shoot tip explants of *P. minima* via. shoot proliferation after 6 weeks of culture

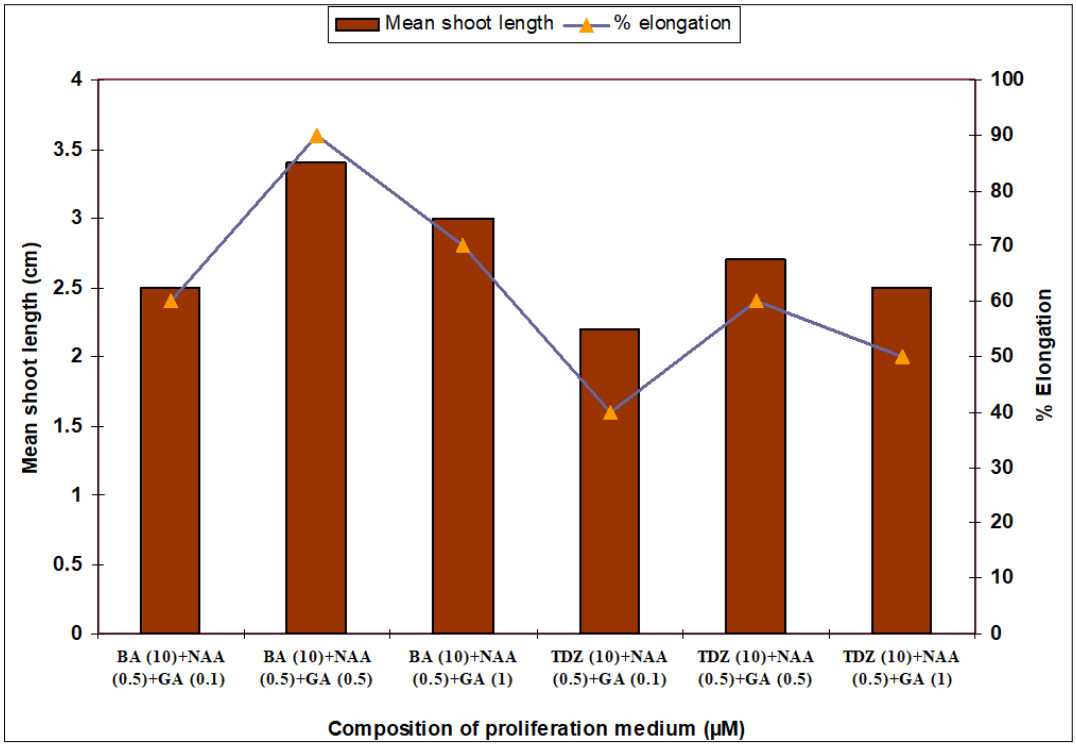
PGR (µM)			Nodal Explant			Shoot TIP Expant		
BA	TDZ	NAA	% Response	Mean no. of shoots/explant	Mean shoot length (cm)	% Response	Mean no. of shoots/explant	Mean shoot length (cm)
5.0	-	0.5	79.60±0.60^{c}	47.6±0.87^{e}	0.82±0.03^{d}	80.2±0.73^{b}	56.2±0.86^{c}	0.70±0.05^{f}
5.0	-	1.0	71.20±2.05^{d}	40.0±0.44^{fg}	1.00±0.04^{cd}	70.4±0.81^{c}	48.2±0.86^{d}	0.74±0.02^{ef}
10.0	-	0.5	98.60±0.50^{a}	72.2±0.96^{a}	1.62±0.08^{a}	89.8±1.15^{a}	77.4±0.50^{a}	1.42±0.03^{a}
10.0	-	1.0	89.0±0.70^{b}	53.2±0.66^{d}	1.12±0.05^{bc}	80.2±1.35^{b}	60.8±0.91^{b}	0.84±0.06^{def}
-	2.5	0.5	79.6±0.60^{c}	42.0±2.00^{f}	1.12±0.10^{bc}	70.4±0.87^{c}	57.2±1.28^{c}	1.06±0.02^{bc}
-	2.5	1.0	69.0±0.70^{d}	37.0±1.51^{g}	1.06±0.05^{bc}	69.6±0.87^{c}	43.8±1.15^{e}	0.98±0.07^{c}
-	5.0	0.5	88.4±0.74^{b}	63.2±1.90^{b}	1.22±0.03^{b}	92.4±0.67^{a}	80.0±0.89^{a}	1.14±0.04^{bd}
-	5.0	1.0	70.0±0.70^{d}	57.0±1.14^{c}	1.08±0.06^{bc}	90.0±1.09^{a}	61.6±0.74^{b}	0.88±0.03^{de}

Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments.
Mean value followed by the same letter are not significantly different according to Duncan's Test at 5% probability.

Table 4: Effect of different auxins either singly or in combination on *in vitro* root induction of microshoots of *P. minima* after 4 weeks of culture Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments.

Pgr(M)			Rooting		
Iba	Iaa	NAA	% Response	Mean No. of roots/shoot	Remark
0.5	-	-	80.6±0.74^{a}	9.2±0.58^{d}	Short and fibrous
1.0	-	-	69.8±0.58^{c}	11.6±0.74^{c}	Short and fibrous
-	0.5	-	69.6±0.60^{c}	8.2±0.37^{de}	Long and thin
-	1.0	-	60.2±1.01^{e}	6.8±0.58^{e}	Long and thin
-	-	0.5	70.6±0.92^{c}	14.6±0.50^{b}	Callusing at shoot base
-	-	1.0	59.4±0.50^{e}	8.4±1.07^{de}	Rooting with callus formation
0.5	0.5	-	66.2±0.73^{d}	8.8±0.37^{d}	Long and fibrous
0.5	1.0	-	74.0±0.44^{b}	11.0±0.54^{c}	Long and fibrous
1.0	0.5	-	80.4±0.74^{a}	17.6±0.60^{a}	Long and fibrous
1.0	1.0	-	80.0±1.04^{a}	12.8±0.37^{c}	Long and fibrous

Mean value followed by the same letter are not significantly different according to Duncan's Test at 5% probability.



Graph 1: Effect of various compositions of proliferation and elongation medium on mean shoot length and % shoot elongation of *P. mininma*.

Fig 1: A. Axillary Shoot regeneration through nodal explants of *P. minima* on MS medium + BA (10.0 µM) after 2 weeks of culture; B. Axillary Shoot regeneration through shoot tip explants on MS medium + TDZ (5.0 µM) after 2 weeks of culture; C. Adventitious shoot regeneration through nodal explants of *P. minima* on MS medium + BA (10.0 µM) after 2 weeks of culture; D. Adventitious shoot regeneration through shoot tip explants on MS medium + TDZ (5.0 µM) after 2 weeks of culture; E. High frequency shoot regeneration and elongation through nodal explants on MS medium + BA (10.0 µM) + NAA (0.5 µM) + GAA (0.5 µM) after 4 weeks of culture; F. High frequency shoot regeneration and elongation through shoot tip explants on MS medium + BA (10.0 µM) + NAA (0.5 µM) + GAA (0.5 µM) after 4 weeks of culture G. *In vitro* rooting of microshoot in half-strength MS medium IAA (0.5 µM)+ IBA (1.0 µM) – after 4 weeks of culture; H. Acclimatized plantlets of *P. minima* after 4 weeks of transfer to soilrite; I. Hardened plantlets of *P. minima* after 8 weeks of transfer to garden soil.

Conclusion

Present study provides an efficient high frequency regeneration protocol of *P. minima*. BA and TDZ showed considerable effect on axillary and adventitious shoot initiation and proliferation from both nodal and shoot tip explants. TDZ (5.0 µM) along with NAA (0.5 µM) proved to be of great significance where high frequency shoot regeneration was obtained. Further proper shoot elongation and proliferation was observed on medium containing BA (10.0 µM) + NAA (0.5 µM) + GA (0.5 µM). Microshoots rooted directly on IBA and IAA, while through callus mediated on NAA containing half-strength MS media. Rooted plantlets were transferred to field

conditions after proper hardening. Acclimatized plantlets were found to be phenotypically similar to the parent plant.

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