

## Role of growth regulators on *in vitro* callus induction and direct regeneration in *Physalis minima* Linn.

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**ABSTRACT.** Suitable protocol for induction of callus and regeneration was developed from different explants viz., node, stem and leaves in *Physalis minima*. MS basal medium supplemented with various concentrations (1.0-4.0mg/l) of auxins like 2,4-Dichlorophenoxy acetic acid (2,4-D),  $\alpha$ -naphthalene acetic acid (NAA) and Indole- 3-acetic acid (IAA) and cytokinins (0.5-1.5mg/l) like BAP or Kn were used. All the three explants responded for induction of callus, however stem explants were found superior, followed by node and leaf. Callus induction was observed in all the auxins and combination of growth regulators used with varied mass (2010 $\pm$ 1.10) and highest percentage of callus induction was observed from stem at 2.0mg/l 2,4-D (90%) followed by NAA (70%) and IAA (50%). Organogenesis was induced when nodal explants were transferred on MS medium supplemented with 2,4-D and Kn at various concentrations, maximum being on 2.0mg/l 2,4-D + 1.0mg/l Kn (90%). Regenerated shoots were elongated on 0.5mg/l GA<sub>3</sub>. The shoots were subsequently rooted on MS + 1.0mg/l IBA (95%) medium. Rooted shoots were hardened and acclimatized, later they were transferred to polycups containing soil, cocopeat and sand in the ratio 1:2:1.

### 1. INTRODUCTION

*Physalis minima* L. belongs to the family Solanaceae. It is a small herbaceous plant grows as weed in crop fields, which is commonly known by several names, like native goose berry, Wild cape goose berry and Pygmy ground cherry. It is an annual herb, 20-50cm high at its maturity. Typical *Physalis* growth pattern and shape with some branching and pretty yellow flowers borne at nodes, fruits ripen in a couple of months and are enclosed in a green-yellow papery calyx which protects them from pests (tradewindsfruit.com). Geographically they are distributed in East Asia, China, Himalayas and in some places of Australia (Prasad *et al.*, 2009). The technique of plant tissue culture at present is attracting worldwide attention, because plant cells are able to synthesize specific compounds, especially various secondary metabolites useful as medicines and food additives (Sheeba *et al.*, 2013). Solanaceous plants are able to synthesize a wide range of alkaloids with interesting pharmaceutical activities (Yamada and Tabata, 1997; Alireza *et al.*, 2006). This species is used medicinally as tonic, diuretic, laxative, anti-inflammation, enlargement of the spleen and abdominal troubles and as a helpful remedy in ulceration of the bladder, fruit of this plant are used to cure spleen disorders, the leaves are crushed and applied over snakebite site (Mungole *et al.*, 2011).

### 2. MATERIALS AND METHODS

#### Plant Material

*Physalis minima* L. were collected from different areas around Gulbarga. In the present investigations explants like node, stem and leaves were used. They were soaked in solution containing Bavistin (1.0% w/v, a fungicide) and tween 20 for 15- 20mn and then the explants were washed under running tap water and rinsed thrice with distilled water. The explants were then

sterilized with 70% Ethyl alcohol for 3-4mn followed by washing with double distilled water and then surface sterilized with 0.1% (w/v) mercuric chloride for 3-4mn and subsequently washed thoroughly in sterile distilled water to remove the traces of mercuric chloride. They were then inoculated on MS medium (Murashige and Skoog, 1962) supplemented with various auxins viz., 2,4-Dichloro phenoxyacetic acid (2,4-D) (1.0-4.0mg/l),  $\alpha$ -naphthalene acetic acid (NAA) (1.0-4.0mg/l) and Indole- 3-acetic acid (IAA) (1.0-4.0mg/l) alone or supplemented with cytokinins like 6-Benzyl amino purine (BAP) (0.5-1.5mg/l) and Kinetin (Kn) (0.5-1.5mg/l). The pH of the medium was adjusted to 5.6-5.8 prior to autoclaving at 121°C for 20mn. The cultures were maintained at 25±1°C with 16/8 hr photoperiod at 40µm m<sup>-2</sup> s<sup>-1</sup> provided by cool white fluorescent tubes. The callus was maintained by regular sub-culture at 4 weeks of interval on fresh medium with the same composition or on regeneration media. The data was subjected to statistical analysis.

**Table: 1.** Effect of auxins on induction of callus in *Physalis minima* L.

Growth Hormone	Conc. mg/l	Stem			Node			Leaf		
		Fresh Wt. (mg)	Dry Wt. (mg)	Frequency (%)	Fresh Wt. (mg)	Dry Wt. (mg)	Frequency (%)	Fresh Wt. (mg)	Dry Wt. (mg)	Frequency (%)
2,4-D	1.0	1125±1.15 <sup>c</sup>	138±0.11 <sup>c</sup>	40	1011±0.01 <sup>c</sup>	120±1.30 <sup>c</sup>	45	1002±0.00 <sup>a</sup>	115±0.00 <sup>a</sup>	40
	2.0	2010±1.10 <sup>a</sup>	428±0.11 <sup>a</sup>	90	1301±1.20 <sup>a</sup>	229±1.30 <sup>a</sup>	70	1100±1.20 <sup>b</sup>	199±1.00 <sup>a</sup>	50
	3.0	1850±1.00 <sup>b</sup>	231±1.10 <sup>b</sup>	60	1120±0.05 <sup>b</sup>	180±0.00 <sup>b</sup>	50	985±1.00 <sup>a</sup>	96±1.17 <sup>c</sup>	45
	4.0	1714±1.20 <sup>b</sup>	198±1.20 <sup>b</sup>	50	1105±1.45 <sup>c</sup>	196±0.00 <sup>b</sup>	50	890±0.12 <sup>b</sup>	81±1.21 <sup>c</sup>	40
NAA	1.0	1021±0.05 <sup>b</sup>	130±0.20 <sup>b</sup>	45	1010±1.10 <sup>c</sup>	120±1.11 <sup>c</sup>	50	1095±1.20 <sup>b</sup>	187±0.01 <sup>c</sup>	60
	2.0	1195±1.23 <sup>a</sup>	228±0.01 <sup>a</sup>	70	1098±0.20 <sup>b</sup>	190±1.23 <sup>b</sup>	55	1011±0.20 <sup>b</sup>	119±1.37 <sup>c</sup>	50
	3.0	1101±1.10 <sup>a</sup>	201±0.00 <sup>a</sup>	50	1001±1.15 <sup>b</sup>	117±1.20 <sup>c</sup>	40	986±1.25 <sup>c</sup>	96±0.10 <sup>b</sup>	45
	4.0	998±0.20 <sup>c</sup>	102±1.10 <sup>c</sup>	40	898±1.25 <sup>c</sup>	84±0.00 <sup>c</sup>	40	875±1.22 <sup>c</sup>	81±1.73 <sup>c</sup>	40
IAA	1.0	992±0.00 <sup>b</sup>	98±1.30 <sup>b</sup>	45	980±0.20 <sup>c</sup>	95±1.20 <sup>b</sup>	45	870±1.23 <sup>c</sup>	79±1.45 <sup>c</sup>	35
	2.0	1102±0.10 <sup>b</sup>	195±1.21 <sup>b</sup>	50	992±1.20 <sup>b</sup>	97±1.11 <sup>b</sup>	40	902±1.11 <sup>c</sup>	88±1.00 <sup>a</sup>	45
	3.0	1011±1.20 <sup>a</sup>	121±1.10 <sup>c</sup>	40	1060±0.22 <sup>a</sup>	170±0.10 <sup>a</sup>	30	1048±0.00 <sup>a</sup>	155±0.00 <sup>a</sup>	40
	4.0	976±0.11 <sup>b</sup>	94±1.20 <sup>c</sup>	35	901±1.11 <sup>c</sup>	88±1.76 <sup>c</sup>	35	917±1.10 <sup>c</sup>	89±1.76 <sup>c</sup>	30

Data represents average of three replicates; each replicate consists of 25 cultures. Mean ±Standard error. Mean followed by the different superscript in column are not significantly different from each other. a=P<0.05, b=P<0.01 and c=P<0.001 levels according to ANOVA.

**Table: 2.** Effect of 2,4-D (2.0mg/l) in combination with cytokinins on induction and growth of callus in *Physalis minima* L.

Growth Hormone	Conc. mg/l	Stem		
		Fresh Wt. (mg)	Dry Wt. (mg)	Frequency (%)
2,4-D+BAP	2.0+0.5	1923±0.10 <sup>b</sup>	252±1.00 <sup>b</sup>	70
	2.0+1.0	1852±1.15 <sup>b</sup>	235±1.25 <sup>b</sup>	80
	2.0+1.5	1620±1.00 <sup>a</sup>	201±0.00 <sup>a</sup>	60
2,4-D+Kn	2.0+0.5	3580±0.00 <sup>a</sup>	595±1.12 <sup>b</sup>	90
	2.0+1.0	2310±1.00 <sup>c</sup>	445±0.00 <sup>c</sup>	80
	3.0+1.5	2110±1.20 <sup>c</sup>	430±0.10 <sup>c</sup>	70

Data represents average of three replicates; each replicate consists of 25 cultures. Mean  $\pm$ Standard error. Mean followed by the different superscript in column are not significantly different from each other. a=P<0.05, b=P<0.01 and c=P<0.001 levels according to ANOVA.

**Table: 3.** Effect of 2,4-D (2.0mg/l) in combination with Kn on direct regeneration of multiple shoots in *Physalis minima* L.

Growth Hormone	Conc. (mg/l)	Node	
		No. of Multiple Shoots	Frequency (%)
2,4-D+Kn	2.0+0.5	3.10 $\pm$ 0.17 <sup>b</sup>	70
	2.0+1.0	7.26 $\pm$ 0.60 <sup>a</sup>	95
	2.0+1.5	3.20 $\pm$ 0.25 <sup>b</sup>	70

Data represents average of three replicates; each replicate consists of 25 cultures. Mean  $\pm$ Standard error. Mean followed by the different superscript in column are not significantly different from each other. a=P<0.05, b=P<0.01 and c=P<0.001 levels according to ANOVA.

**Table: 4.** Effect of IBA on root induction from *in vitro* raised shoots of *Physalis minima* L. after 30 days of culture.

Growth Hormone	Conc. (mg/l)	No. of roots	Frequency (%)
IBA	1.0	15.20 $\pm$ 0.30 <sup>a</sup>	95
	2.0	8.35 $\pm$ 0.11 <sup>c</sup>	70
	3.0	4.64 $\pm$ 0.28 <sup>c</sup>	70

Data represents average of three replicates; each replicate consists of 25 cultures. Mean  $\pm$ Standard error. Mean followed by the different superscript in column are not significantly different from each other. a=P<0.05, b=P<0.01 and c=P<0.001 levels according to ANOVA.

### 3. RESULTS AND DISCUSSION

The induction of callus and regeneration of plant depends on explants and influenced by the concentrations and combinations of growth regulators in the medium. Different explants were cultured on MS medium supplemented with various auxins and cytokinins in varied combinations to assess the morphogenetic potential of the explants.

### 4. CALLUS INDUCTION

In the present investigations node, stem and leaf explants were inoculated on MS medium fortified with different concentrations of 2,4-D, NAA and IAA alone or in combination with BAP or Kn for callus induction. All the three explants responded for callus induction with varying degree, stem explants proved best followed by node and leaf. Highest frequency (90% ; 18 days) was noticed in stem explants followed by node (70% ; 20 days ) and leaf (50% ; 25 days).

Among the three auxins tested the frequency of callus induction was maximum on MS medium supplemented with 2.0mg/l 2,4-D, followed by NAA and IAA. From the data presented in Table-1, it can be noticed that with an increase in the concentration of auxins there was an increase in the frequency and growth of the callus upto 2.0mg/l and at 4.0mg/l the frequency and growth of callus decreased. Maximum frequency (90%) of callus induction and highest biomass (2010 $\pm$ 1.10mg) was observed on MS medium supplemented with 2.0mg/l 2,4-D when stem were used as explants followed by nodal explants (1301 $\pm$ 1.20mg) with 70% frequency and leaf explants (1100 $\pm$ 1.20mg) with 50% frequency (Table-1; Plate-I a). However other auxins viz., NAA and IAA showed poor response with respect to induction and further growth of callus at all the

concentrations used (1.0-4.0mg/l). Further enhancement of callus formation was observed when MS medium was supplemented with 2.0mg/l 2,4-D with 0.5mg/l Kn with a frequency of callus induction being 90% and biomass of  $3580 \pm 50$ mg (Table-2; Plate-I b).

In the present studies, an efficient protocol has been standardized for callus induction in stem explants of *Physalis minima*. Results obtained from the present study revealed that the response of explants varies with different hormonal concentrations for callus induction. In the present research work, MS medium supplemented with 2,4-D alone and in combination with Kn was effective to produce callus. In earlier reports (Mungole *et al.*, 2011 and Sheeba *et al.*, 2013) the same combinations of growth regulators and explants proved to be efficient for callogenesis in this species. Our results obtained are in agreement with earlier reports.

## 5. SHOOT REGENERATION

Investigations were also carried out to study the effect of auxin and cytokinins (BAP and Kn) for induction of shoots directly from stem, node and leaf explants. Regeneration was achieved only from the nodal explant within 15-20 days on MS medium containing 2,4-D and Kn. On the other hand stem and leaf explants completely failed to regenerate in all the combinations and concentrations of 2,4-D with Kinetin or BAP tested. Regeneration was observed on MS medium supplemented with 2.0mg/l 2,4-D and different concentrations of BAP and Kn (0.5-1.5mg/l). From the result it is clear that among the cytokinin used Kn was found more effective for regeneration where as BAP failed to induce regeneration (Data not shown). Maximum number of multiple shoots were induced when the nodal explants were cultured on MS medium supplemented with 1.0mg/l Kn + 2.0mg/l 2,4-D ( $7.26 \pm 0.60$ ) with 95% frequency with an increase in the concentration of Kn decrease in the frequency and number of multiple shoots was noticed (Table-3; Plate-II a & b).

## 6. RHIZOGENESIS

For the induction of roots, 30 days old 4-5cm long shoots were separated carefully and were transferred to IBA at (1.0-3.0mg/l). From the data presented in Table-4 it is clear that the frequency of rooting (95%) and the number of roots ( $15.20 \pm 0.30$ ) was highest on medium supplemented with 1.0mg/l IBA. The roots were thick and elongated (Table-4; Plate-II c). Rooted plantlets were transferred to half strength MS medium for two weeks for hardening. The shoots become strong and thick on this medium. After hardening the shoots were transferred to pots containing sterile vermiculate and later established in soil in the greenhouse, where 50% of them survived and resumed growth (Plate-II d).

## 7. DISCUSSION

In the present studies, an efficient protocol has been standardized for callus induction and regeneration from stem and nodal explants of *Physalis minima*. Different explants have been used for callus induction in this species viz, leaf (Sheeba *et al.*, 2013) stem (Sipahimalani *et al.*, 1981) leaves and stem (Saripalli *et al.*, 2013; Jualang Azlan and Marziah, 2013). In the present investigations stem pieces were found to be best source of explant for callus induction followed by nodal and leaf explants, the results are in agreement with that of Julanga Azlan and Marziah, 2015 who reported that stem is a better explant than others, however contrary to our results Sheeba *et al.*, reported leaves as source of explant, however they have only used leaves not compared with other explants.

In *P. minima* auxins like 2,4-D, NAA and IAA alone or in combination with cytokinins for induction of callus (Sipahimalani *et al.*, 1981; Sheeba *et al.*, 2013; Saripalli *et al.*, 2013 and Julanga Azlan and Marziah, 2013). Generally the media supplemented with high auxin and low cytokinin concentrations promotes cell proliferation resulting in callus formation (Chawla and Arora, 2005). There are contradictory reports regarding the requirement of growth regulator for induction of callus, as observed in the present investigation. Julanga Azlan and Marziah, (2013) reported that

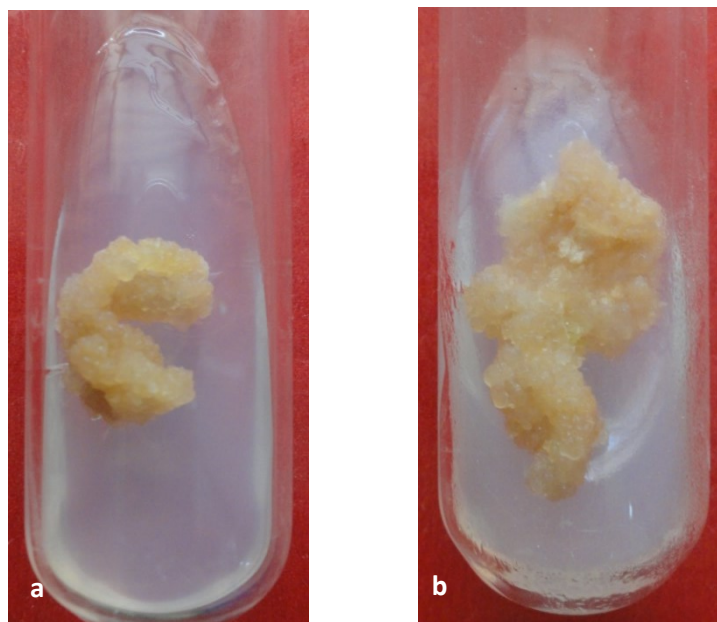
2,4-D and kinetin was best for callus induction in this species, Sipahimalani *et al.*, (1981) reported that combination of 2,4-D + BAP favoured callus formation, however they have not used kinetin in combination with 2,4-D, on the contrary Prasad *et al.*, (2009) and Saripalli *et al.*, (2013) reported combination of BAP + NAA was better for callus induction with stem explants. It is very difficult to explain the contradictory reports with respect to requirement of growth regulators using the same (stem) explants. Sheeba *et al.*, (2013) reported combination of IAA and BAP for callus induction in this species, this varied results may be due to the different explants (Leaves) by them and stem in our investigation, however again there is a contradictory report of callus induction from leaf explant (Saripalli *et al.*, 2013) who reported 1.0mg/l BAP + 0.5mg/l  $\alpha$ -NAA suitable for callus induction in this species.

The results obtained from present research work, MS medium supplemented with 2,4-D and in combination with Kn was effective to produce multiples from nodal explant.

Different explants have been used for induction of multiple shoots in this species viz, shoot tip (Intzaar *et al.*, 2013; Ramar and Ayyadurai 2014) leaf, shoot tip and nodes (Sheeba *et al.*, 2013). A single explant has been used and an attempt to compare the response of other explants is not made, however, Sheeba *et al.*, (2013) have reported that nodal explants were superior to shoot tips with respect to induction of multiple shoots, the results are in conformity with our investigations.

There are reports that the shoot regeneration using BAP and coconut water on MS medium from nodal explants in *P. minima* (Afroz *et al.*, 2009) and from leaf and nodal explants of *P. minima* were responsive to organogenesis with BAP, IAA and IBA at different hormones (Sheeba *et al.*, 2010; Ashwinin Solanki and Dipali Gupta, 2013). In the present investigation multiple shoots were induced on MS medium supplemented with 2, 4-D and kn. Highest frequency (95%) and maximum ( $7.26 \pm 0.60$ ) number of multiple shoots were recorded on medium supplemented with 1.0mg/l kn + 2.0mg/l 2,4-D.

**Plate - I**



**Plate I.** *In vitro* callus induction of *Physalis minima* L. a) Induction of callus from stem explants on MS + 2.0mg/l 2,4-D b) Induction of callus from stem explants on MS + 2.0mg/l 2,4-D +0.5mg/l Kn.

## Plate - II



**Plate II.** *In vitro* shoot induction, proliferation and acclimatization from nodal explants of *Physalis minima* L. a) Initiation of direct multiple shoots from nodal explants on MS + 2.0mg/l 2,4-D + 1.0mg/l Kn. b) Multiple shoot formation from *in vitro* derived nodal explants on MS + 2.0mg/l 2,4-D + 1.0mg/l Kn after 15 days. c) Root induction on MS + 1.0mg/l IBA. d) Acclimatized potted plant.

### There are contradictory reports

There are also reports in different species like *P. pubescens*, stating that MS basal medium supplemented with BAP and NAA is optimum for regeneration and callus induced from leaf and nodal segments (Rao *et al.*, 2004), shoots were initiated from callus obtained from the apical leaf explants only not from the root and node explants calli (Mungole *et al.*, 2011) and the maximum number of multiple shoots were achieved from nodal and stem explants on BAP, GA<sub>3</sub> and 2,4-D in *P. peruviana* by (Ramar *et al.*, 2014). This shows that organogenesis as callogenesis is also highly dependent on genotype. In our investigation organogenesis was not obtained on BAP, IAA and NAA supplemented media, however earlier reports mentioned above have BAP as suitable cytokinin for induction of shoots. Among these results nodal explants is common in all the reports only differs in hormonal concentrations. Our results are in against with these reports.

## 8. CONCLUSION

The method standardized in the present study for the production of callus and plants from internodal and nodal explants is useful for the callogenesis and multiplication as well as for the genetic manipulation. The plant is known to have important secondary metabolites Physalin, so callus can be used for secondary metabolite production and this protocol is expected to be very useful for the mass production of this species.

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