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In vitro Shoot Multiplication in *Physalis minima* var. Indica L

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Apical shoot buds were taken from *in vitro* germinated seedlings of *Physalis minima.* var. indica L. These were placed aseptically on MS (Murashige and Skoog,1962),B5 (Gamborg,1968)basal medium and supplemented with auxins and cytokinins alone. Maximum healthy shoot buds(100 percent) were obtained on MS medium supplemented with 0.5 mgl^{-1}BAP (6- benzyl amino purine) but length of shoot buds was more on (1.0,2.0 mgl^{-1}) Kinetin. Multiplied shoots were transferred for rooting on MS and B5 basal medium and supplemented with auxins (IAA or IBA). Rooting was achieved in 100 percent explants on MS medium supplemented with all the levels of IAA and IBA.Higher root length (11.5+0.50cm) was observed in shoots incubated on 0.5mgl^{-1} IAA.

Key words: *Physalis minima,* Apical shoot buds, Growth regulators.

Physalis minima var. indica L. is a medicinally important plant, belongs to the family *Solanaceae*. It is found throughout India growing in waste lands as weeds and it is found in tropical Africa , Asia and Australia. It is known as Country gooseberry, Chirpoti, bladder cherry and tulatipati.Different types of Physalins were isolated from *Physalis minima.*Whole plant is useful in burning sensation, hepatitis, splenomegaly, ascites, ulcer, sexual weakness and cough. The fruit is said to be appetizer, bitter, diuretic, laxative and tonic.The juice of the leaves, mixed with mustard oil and water, has been used as a remedy for earache.The decoction of the whole plant is taken orally to treat cancer and the leaves are used as a poultice for ulcer.The leaves are crushed and applied over snakebite site[1]. Keeping in view its medicinal value, the present study was undertaken to develop an efficient protocol for plant regeneration through micropropagation.

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MATERIALS AND METHODS

Apical shoot bud explants of *P. minima.* var. indica L. were collected from NATP garden of College of Agriculture, S.K. Rajasthan Agricultural University, Bikaner. All the empty glassware and instruments to be used for media preparation, inoculation and sub culturing were autoclaved at 121^0 C and 1.1 kg/cm^2 pressure for 20 minutes. Explants were first treated with a detergent (Clextron 0.5 percent, CDH) for 10 – 15 minutes and washed with tap water. The explants were dipped for 1-2 seconds in 70 percent ethanol and then immediately immersed in sterilized water. This pre-treated explant material was surface sterilized with 0.1 percent (w/v) aqueous solution of $HgCl_2$ (E. Merck -India) for 3 – 4 minutes and were washed several times with sterilized distilled water. The process of pre-treatment and surface sterilization was carried out under aseptic condition in laminar air flow bench. The surface sterilized explants were inoculated on MS and B5 media[2,3]supplemented with plant growth regulators for shoot multiplication of*P. minima.* Cultures were incubated in 16h photo-period at 1000 lux light intensity and 25+2^{0}C temperature.

Establishment of culture

After approximately 10-15 days of inoculation, these cultures were then transferred to jars/tubes containing fresh medium. Aseptically amplified shoots produced in cultures were rooted by transferring on rooting medium.

RESULTS

Effect of basal medium

When apical shoot bud explants of *P. minima* were inoculated on MS basal medium, single shoot was induced in most of the cultures (80 percent) with mean shoot length of 1.4+0.02cm(fig-1). Root formation was not noticed.Shoot formation was not noticed on basal B5medium (table-1).

Effect of cytokinins alone (BAP/Kn)

When apical shoot bud explants of *P. minima* were incubated on MS and B5 medium supplemented with cytokinins BAP/Kn (0.1,0.5, 1.0, 2.0 mg/l) added singly. Shoot formation was not noticed on B5medium supplemented with cytokinins. Multiple shoot buds were induced in 100 percent cultures on both the level of cytokinins added in MS medium. The induction of multiple shoot buds took place within 15-18 days. Highest shoot buds (100 percent) were observed on 0.5mgl^{-1}BAP. Shoot length was 2.4+0.07cm (fig-2). Whereas, on KN maximum shoot buds were observed on 1.0,2.0 mgl^{-1}.Length of shoot buds was more on 1.0 mgl^{-1}Kn(3.0+0.02cm),2.0 mgl^{-1} KN (3.6+0.25 cm). As compared to KN growth of shoot buds was slightly less on BAP, but health of shoots was better on MS medium supplemented with BAP after 30 days of incubation(table-1).

Table 1. Effect of different concentrations of cytokinins added singly in MS medium micropropagation of *P. minima*

Cytokinins (mgl^{-1})	Shoot frequency (%)	Shoot no./explant	Shoot length (cm)
Basal MS medium	80	1.9±0.09	1.4±0.02
BAP			
0.1	70	4.0±1.15	2.2±0.01
0.5	100	4.5±0.11	2.4±0.07
1.0	100	4.9±0.01	2.0±0.01
2.0	40	4.2±0.27	1.0±0.02
Kn			
0.1	20	2.0±0.10	1.0±0.03
0.5	90	4.0±0.10	2.3±0.07
1.0	100	60.0±0.21	3.0±0.02
2.0	100	6.50±0.02	3.6±0.25

Days of observation –After 30 days, Values represent mean of three replicates

Effect of auxins alone (NAA/IAA)

When apical shoot bud explant of *P. minima* were incubated on MS and B5 medium supplemented with IAA NAA (0.1,0.5, 1.0, 2.0 mgl^{-}

Table 2. Effect of different concentrations of auxins added singly in MS medium on micropropagation of *P. minima*

Cytokinins (mgl^{-1})	Shoot frequency (%)	Shoot no./explant	Shoot length (cm)
IAA			
0.1	40	0.61±0.52	0.47±0.95
0.5	40	0.22±0.40	0.43±0.81
1.0	100	2.0±0.30	1.8±0.02
2.0	40	0.25±0.50	0.20±0.40
NAA			
0.1	100	1.9±0.30	1.1±0.06
0.5	80	1.4±0.01	1.1±0.06
1.0	60	0.75±0.90	0.90±1.14
2.0	20	0.5±1.1	0.65±1.30

Days of observation –After 30 days, Values represent mean of three replicates

Table 3. Influence of MS medium concentration on rooting of *P. minima* culture derived shoots

Growth regulator (mgL^{-1})	Percentage of shoots rooted (%)	Root length (cm)
Basal MS medium	40	0.25±0.37
Basal B5 medium	0.00	0.00
IAA		
0.1	100	9.2±0.38
0.5	100	11.5±0.50
1.0	100	5.92±0.31
IBA		
0.1	100	4.2±0.23
0.5	100	6.1±0.32
1.0	100	1.2±0.21

Days of observation –After 30 days, Values represent mean of three replicates

^{1}) added singly,shoot formation was not noticed on B5medium supplemented with auxins. On MS, highest shoot buds were observed on 1.0 mgl^{-1}IAA, shoot length was 1.8+0.02 cm (fig-3). Further higher level of auxin (IAA 2.0mgl^{-1}) were found to be inhibitory for the growth of apical shoot explants into shoots as only 40 percent cultures responded on 2.0 $mgl^{-1}$and only shoots were very small in size (0.3-0.4cm) .

100 percent explants responded in to shoot growth in 8-10 days on 0.1 mgl^{-1} NAA, with a mean shoot length of 1.1+0.06 cm. The percent

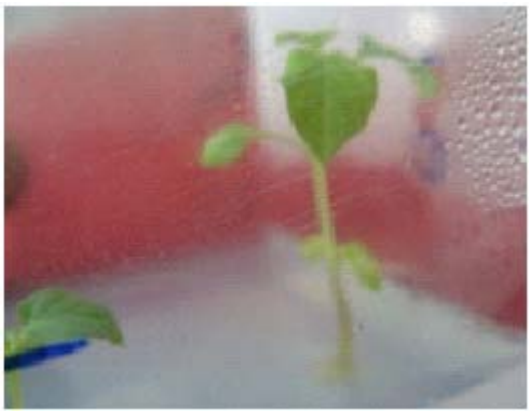

Fig. 1. Single shoot on basal MS medium

Fig. 2. Multiple shoots on MS+0.5 mgl^{-1} BAP

Fig. 3. Multiple shoot on MS+1.0mgl^{-1} IAA

Fig. 4. Rooting on MS basal medium

Fig. 5. Rooting on MS +0.5mgl^{-1}IAA

response declined to 80 on 0.5 mg/l NAA with a concomitant decline in mean shoot bud number but the mean shoot length remained same (1.1+0.06 cm).Higher level (1.0-2.0 mg/l) of NAA were not conducible for growth of apical shoot bud explants. 60 percent explants responded to form 1-2 shoots in 10-12 days on 1.0mgl^{-1} NAA with a mean shoot length of 0.90+1.14 cm . The frequency of induction declined to 20 percent on 2.0 mgl^{-1} NAA with an induction period of 10-12 days and mean shoot length was 0.65+1.30 cm (table-2).

***In vitro* rooting**

Healthy shoots induced on BAP(5.0 mg/l), Kn (1.0 mg/l) were excised and transferred on to MS and B5 basal medium and supplemented with IAA/IBA(0.1,0.5,1.0 mgl^{-1}) for root induction and were incubated under 16 hour photoperiod at 1000 lux light intensity and 25+2^{0}C temperature. Root formation was not noticed on basal B5 medium and B5 medium supplemented with auxins.About 40 percent rooting were observed on basal MS medium and root length was 0.25+0.37cm(fig-4).MS medium supplemented with both IAA and NAA (0.1, 0.5,1.0 mgl^{-1}) induced rooting in 100 percent explants in 15-18 days. Higher root length (11.5+0.50cm) was observed in shoots incubated on 0.5 mgl^{-1}IAA,which reduced to 5.92+0.31cm on 1.0 mgl^{-1} IAA (fig-5). 0.5 mgl^{-1}IBA induced a root length of 6.1+0.32 cm and smallest roots (1.2+0.21cm) were observed on 1.0 mgl^{-1}IBA (table-3).

DISCUSSION

In the present investigation,shoot formation was not noticed on basal B5mediumand B5medium supplemented with any level of auxins and cytokinins. Highest multiple healthy shoots (100 percent) were observed on MS medium supplemented with 0.5 mgl^{-1}BAP. Whereas, on Kn highest (100 percent) multiple shoot length were observed on 1.0 and 2.0 mgl^{-1}. In present study shoots were induced by culturing nodal segments and shoot tips from 15 day old seedlings of *P. minima*with 100% response. There are reports on shoot regeneration from leaf and nodal explants of *P. minima*. The frequency of induction was 86 percent.[4,5]

In solanaceae, auxins have mostly induced roots and in some cases have induced shoot elongation. In our experiments, The effect of NAA on induction of shoots from apical shoot explants was not better than IAA. Highest shoot buds were observed on 1.0 mgl^{-1}IAA, shoot length was 1.8+0.02 cm. Whereas, shoots were rooted (100 percent) using full strength MS medium supplemented with auxins (IAA/IBA) and 40 percent rooting were observed on basal MS medium but longest (11.5 +0.50cm) and healthiest roots were observed on 0.5 mgl^{-1}IAA.On 0.5 mgl^{-1} IBA roots were equally healthy but were shorter (6.1+0.32 cm) in length.

CONCLUSION

In present work a highly reproducible and efficient protocol of micropropagation of *P. minima* was developed from *in vitro* germinated seedlings. MS medium supplemented with 0.5 mgl^{-1}BAP was found to be best for multiple shoot induction and MS medium with 0.5 mgl^{-1} IAA was appropriate for rooting.

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