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In-vitro callus induction and shoot regeneration in *Physalis minima* L

Arvind J. Mungole*[1], Vilas D. Doifode[1], Rahul B. Kamble[1] Alka Chaturvedi[1] and Prakash Zanwar[2]

[1]P.G. Department of Botany, RTM Nagpur University, Nagpur
[2]SFS Centre for Biotechnology, St. Francis De Sales College, Seminary Hills, Nagpur

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ABSTRACT

The present paper deals with In-vitro callus induction and shoot regeneration in Physalis minima (L.). Physalis minima belong to the family Solanaceae, a small herbaceous annual plant grown as weed in the crop field. Ethnobotanical information showed that this plant has tremendous medicinal value for cure out different diseases. Study includes multiple shoot cultures to regenerate whole plant from callus of apical leaf, node and root explants. The present study highlights the importance of plant tissue culture in order to be used for large-scale production of drug at cost affordable levels. This study can be utilized to develop a standard protocol to initiate multiple shoot culture of plant that may provide a good source of pharmacologically active plant constituents. Callus culture were initiated from leaf, shoot tip and root explants on Murashige and Skoog medium supplemented with different concentrations of hormone(s), alone or in combination, for rapid initiation of callus and biomass production. Shoot cultures were regenerated from these three explants and its generation capacity, length and morphology were observed.

INTRODUCTION

The capability to regenerate and propogate plants from cultures cells and tissues is one of the most exciting and useful aspects of *In-vitro* cell and tissue culture. Increasing demand of those plants, which are specially use for the food and medicine, is one of the cause of their rapid depletion from the natural habitats. Micropropogation offer a great potential for conservation and large scale multiplication of such useful species and subsequent exploitation.

Physalis minima is medicinally important plant, belong to the family Solanaceae, a small herbaceous annual plant grown as weed in the crop field. It is used as tonic, diuretic, and laxative, applied in inflammation, enlargement of the spleen and as a helpful remedy in

ulceration of the bladder. The leaves are crushed and applied over snakebite site [1]. Fruits of this plant are used to cure spleen disorders [2]. The fruit is said to be appetizer, bitter, diuretic, laxative and tonic [3, 4]. The juice of the leaves, mixed with mustard oil and water, has been used as a remedy for earache [5].

A lot of work has been done on the chemotherapeutic, bacteriostatic, bactericidal and antimicrobial activity of the plant. The antimicrobial activity of Solanaceae is well documented in the literature. These include *Capsicum annum* [6], *Withania spp.* [7].The present study was undertaken to examine the potential of different explants of plant with different concentrations of hormone(s), alone or in combination for rapid initiation of callus and biomass production. The callus obtained from these explants was chosen for shoot regeneration studies.

MATERIALS AND METHODS

Plant Material:
Physalis minima (L.) plants used in the present study were collected from the wild populations. Different explants were used for establishing callus including apical leaf, node and root explants.

Apical leaf explants:
The Apical leaf explants were cut into small pieces from the *Physalis minima* plant and wash with running water. Then explants were surface-sterilized with 0.1% (w/v) mercuric chloride for 2-3 min, followed by 70% ethyl alcohol 2-3 min, then washed 3-4 times with sterile double-distilled water and inoculated on agar-solidified MS (Murashige and Skoog) medium supplemented with different concentrations of 2,4-D, Kinetin and BAP, either alone or in combination. The pH of the medium is adjusted to 5.8 before sterilization. Cultures were maintained at 27±1°C with a photoperiod. Callus was subcultured after 22 days on the original callus-inducing medium.

Node as explants:
Nodal region as a explants of *Physalis minima* L. were used for establishing callus, it was surface sterilized similar to Apical leaves with 0.1% mercuric chloride and 70% ethyl alcohol and cultured on agar solidified MS medium supplemented with different concentrations of 2,4-D, Kinetin and BAP, alone or in combination.

Root explants:
Root explants of *Physalis minima* were surface-sterilized with 0.1% (w/v) mercuric chloride for 2-3 min, followed by 70% ethyl alcohol 2-3 min, then washed 3-4 times with sterile double-distilled water and inoculated on agar-solidified MS [8] medium supplemented with different concentrations of 2,4-D, Kinetin and BAP, either alone or in combination. The pH of the medium is adjusted to 5.8 before sterilization. Cultures were maintained at 27±1°C with a photoperiod.

Callus obtained from various explants was used for shoot culture on MS medium supplemented with different concentrations of Kinetin and BAP. In this, 30 tubes were inoculated with desired explants i.e. apical leaf, nodal region and root explants for callus induction and incubated under optimal conditions as defined above. The experiment was terminated after an interval of 30 days, fresh weight and dry weight of calli was determined. In another set of experiments where the

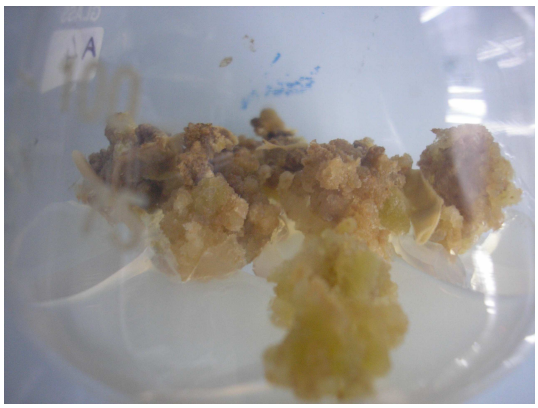
shoot regeneration capacity was determined, the callus obtained from each explants was inoculated into the tubes containing MS medium supplemented with Kinetin and BAP in different concentrations. At least 30 tubes were inoculated and incubated under optimal condition as defined above. After 30 days, the experiment was terminated and shoot generation capacity, its length and morphology were recorded.

RESULT AND DISCUSSION

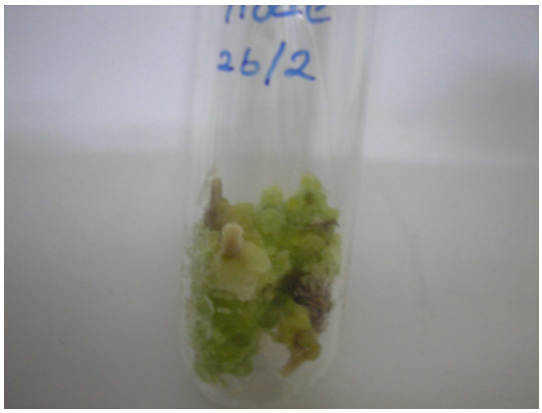
Initially explants from apical leaf, node and root, were cultured on MS medium with different concentration of 2, 4-d, kinetin and BAP alone or in combination for callusing. The best result in term of percentage response of callus induction (90%) and nature of callus obtained on 2, 4-D (0.4 mg/l) in case of apical leaf after 12 days. Callus obtained from these explants was greenish-yellowish and very soft in nature (Fig. 1a). MS medium frequently used for micropropogation in large number of plants [9]. It was reported that the *Terminalia bellerica* culture grew better on MS medium [10].

Similarly according [11, 12] the medium for *in-vitro* multiplication of *Drosera* plant is MS medium. MS medium was also reported as a superior medium for the micropropagation of *Coptis teeta* [13]. In addition of this several workers reported importance of auxins for the production of callus. [14-17]. Percentage of callus induction response followed by same explants (60%) on medium supplemented with 2, 4-D (0.3mg/l) and nature of callus was greenish-yellowish. In term of callus biomass(MS medium with different concentration of 2,4-D, kinetin and BAP alone) the highest fresh weight (768 mg) and dry weight (164 mg) obtained from apical leaf explant on same hormonal concentration i.e., 2,4-D (0.4mg/l) and followed by the fresh weight (455 mg) and dry weight (78 mg) on same concentration (Table 1) 30% of the node explant successfully produced calli with the same concentration of 2, 4-D (0.4 mg/l) after 18 days. Nature of callus was greenish and hard (Fig 1-b).

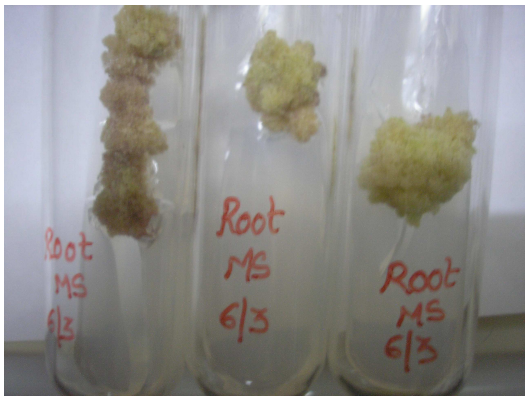
Figure 1: Growth of *Physalis minima* callus from apical leaf, node and root as explants after two week in MS media supplemented with different hormone concentrations.



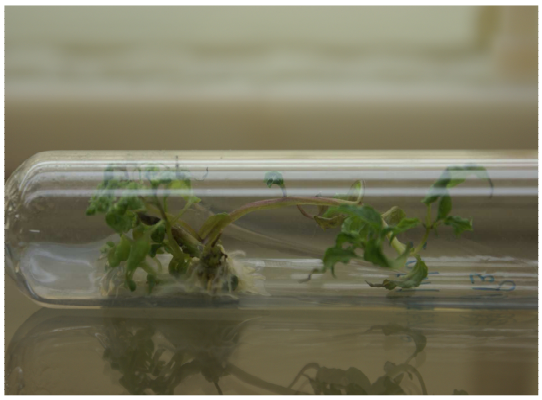
Apical leaf callus of *Physalis minima*



Node callus of *Physalis minima*



Root callus of *Physalis minima*



Regenerated *Physalis minima*

Table 1: Callus induction & biomass production for apical leaf, node and root explants of *Physalis minima* on MS medium supplemented with various hormone(s).

Source of Explant	No. of test tubes Inoculated	Growth regulators mg/l		Results	Duration in Days for callus initiation	Response in %	Fresh weight (mg)	Dry weight (mg)
		2,4-D	Kinetin					
Apical leaf	30	0.1	0	Callus	19	40	256	33
	30	0.2	0	No callus	- -	- -	- -	- -
	30	0.3	0	callus	16	60	455	78
	30	0.4	0	Callus	12	90	768	164
	30	0	0.1	Callus	17	30	156	23
	30	0	0.2	No callus	- -	- -	- -	- -
	30	0	0.3	Callus	15	55	245	41
	30	0	0.4	Callus	13	40	324	61
Node	30	0.1	0	No Callus	- -	- -	- -	- -
	30	0.2	0	No Callus	- -	- -	- -	- -
	30	0.3	0	Callus	22	20	148	20
	30	0.4	0	Callus	18	30	210	39
	30	0	0.1	No Callus	- -	- -	- -	- -
	30	0	0.2	No Callus	- -	- -	- -	- -
	30	0	0.3	No Callus	- -	- -	- -	- -
	30	0	0.4	Callus	21	30	246	38
Root	30	0.1	0	No callus	- -	- -	- -	- -
	30	0.2	0	Callus	20	20	190	33
	30	0.3	0	No callus	- -	- -	- -	- -
	30	0.4	0	No callus	- -	- -	- -	- -
	30	0	0.1	No callus	- -	- -	- -	- -
	30	0	0.2	No callus	- -	- -	- -	- -
	30	0	0.3	No callus	- -	- -	- -	- -
	30	0	0.4	No Callus	- -	- -	- -	- -

Table 2: Callus induction & biomass production for apical leaf, node and root explants of *Physalis minima* on MS medium supplemented with various combinations of hormone(s).

Source of Explant	No. of test tubes Inoculated	Growth regulators mg/l		Results	Duration in Days for callus initiation	Response in %
		2,4-D + KN	2,4-D + BAP			
Apical leaf	30	2+0.1	0	Callus	18	30
	30	2+0.2	0	Callus	16	50
	30	2+0.3	0	Callus	16	70
	30	2+0.4	0	Callus	14	65
	30	0	2+0.1	No Callus	- -	- -
	30	0	2+0.2	No callus	- -	- -
	30	0	2+0.3	No callus	- -	- -
	30	0	2+0.4	Callus	24	30
Node	30	2+0.1	0	No Callus	- -	- -
	30	2+0.2	0	No Callus	- -	- -
	30	2+0.3	0	Callus	21	20
	30	2+0.4	0	No callus	- -	- -
	30	0	2+0.1	No Callus	- -	- -
	30	0	2+0.2	No Callus	- -	- -
	30	0	2+0.3	No Callus	- -	- -
	30	0	2+0.4	Callus	24	30
Root	30	2+0.1	0	callus	24	20
	30	2+0.2	0	Callus	16	30
	30	2+0.3	0	callus	14	50
	30	2+0.4	0	callus	11	70
	30	0	2+0.1	No callus	- -	- -
	30	0	2+0.2	No callus	- -	- -
	30	0	2+0.3	No callus	- -	- -
	30	0	2+0.4	No Callus	- -	- -

Table 3: Effect of different concentration of hormone(s) on shoot regeneration and height attained from shoot-derived callus of Physalis minima after 30 days of culture.

Growth Regulators		No Of shoot per treatment	Shoot length in Cm.	Shoot morphology
Kinetin	BAP			
0.1	2	2	3.1	Thin Short
0.2	2.5	4	5.2	Thick short
0.3	3	2	3.7	Thin short
0.4	3.5	6	7.7	Green and long
2	0.1	- -	- -	- -
2.5	0.2	2	2.2	Thin and short
3	0.3	- -	- -	- -
3.5	0.4	- -	- -	- -

Percentage response of calli from root explant was observed quit low in comparison to the apical leaf and node explant in alone concentration of 2,4-D but it was observed more overall in combination of 2,4-D and kinetin. The best result in root explants (Fig 1-c) callusing (20%) was recorded on the 2, 4-D hormonal concentration (0.2) after 20 days. Use of explant from different parts of the plants viz. root, stem, apical leaf, leaf, inflorescence tip and parts of seedlings suggested that each and every parts of the plant has potential to regenerate a complete plant, it

also proves totipotency aspect of cells. This aspect has been utilized by [18] for multiplication of plants. Further studies were carried out for shoot regeneration capacity of the callus. Shoot were initiated from the callus obtained from the apical leaf explants only not from the root and node explants calli. The best result of shooting (7.7 cm.) was observed with MS medium supplemented with the combination of Kinetin (0.4 mg/l) and BAP (3.5 mg/l) after 17-19 days with good morphology (Table-3). The result is supported by the work done by, [19] and found that the combination of BAP and kinetin favors shoot proliferation. Shooting was followed by the in response to no. of shoots (4) and shoot length (5.2 cm.) was recorded in MS medium supplemented with kinetin (0.2 mg/l) and BAP (2.5 mg/l) Table-3.

CONCLUSION

From the above study, it is concluded that multiple shoot cultures of *Physalis minima* were established from single apical explants on MS medium supplemented with combination of hormones Kinetin (o.4 mg/l) and BAP (3.5 mg/l). This study aims to develop a standard protocol to initiate multiple shoot culture and standardization of media and hormonal concentration of plant that may provide a good source of pharmacologically active plant constituents.

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