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Plant Regeneration in *Physalis pubescens* L. and Its Induced Mutant

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Abstract

Suitable protocols for callus induction and regeneration were developed for leaf and nodal segments of *Physalis pubescens* L. and its flowerless mutant. Per cent callus induction and regeneration were higher in the young leaves followed by mature leaves of the control and its mutant in that order in any combination of the media. MS medium containing 2.0 mg/l and 2.5 mg/l 2,4-D are the optimum concentration for induction of callus from leaf and stem segments, respectively. Optimum medium for callus induction of nodal explants is MS + 2.5 mg/l 2,4-D + 2.0 mg/l IAA. The per cent callus induction was higher in the explants of stem and node of the control over those of the respective organs of the mutant. Nevertheless MS basal medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA is the optimum for regeneration of callus induced from leaf and nodal segments. Regeneration could not be obtained from the callus of stem of either control or mutant in any combination of MS medium. Probable role of the mutagen ethyl methane sulphonate (EMS) either in callus induction or regeneration of calli of the mutant is suggested.

Introduction

A repeatable regeneration protocol of economically important crop plants is mandatory for the improvement of the crop through genetic transformation. In economically important Solanaceae members like tomato (Moghaieb et al. 1999) and *Withania somnifera* (Rani and Grover 1999), various explants viz., hypocotyl, cotyledons, axilary buds and shoot tips, respectively have been employed to obtain somatic cultures. To facilitate such cultures several suitable protocols have to be developed (Bright and Jones 1985).

Physalis pubescens L., a member of Solanaceae is an economically and medicinally important taxon (minor crop plant). In view of its importance some

mutants were obtained through mutagenesis for their utilisation in the breeding programmes. One of the mutants was very robust vegetatively and without flowers. To sustain this mutant an attempt was made to maintain it through *in vitro* cultures along with the control. *In vitro* response of the mutant and that of the control is documented in this communication.

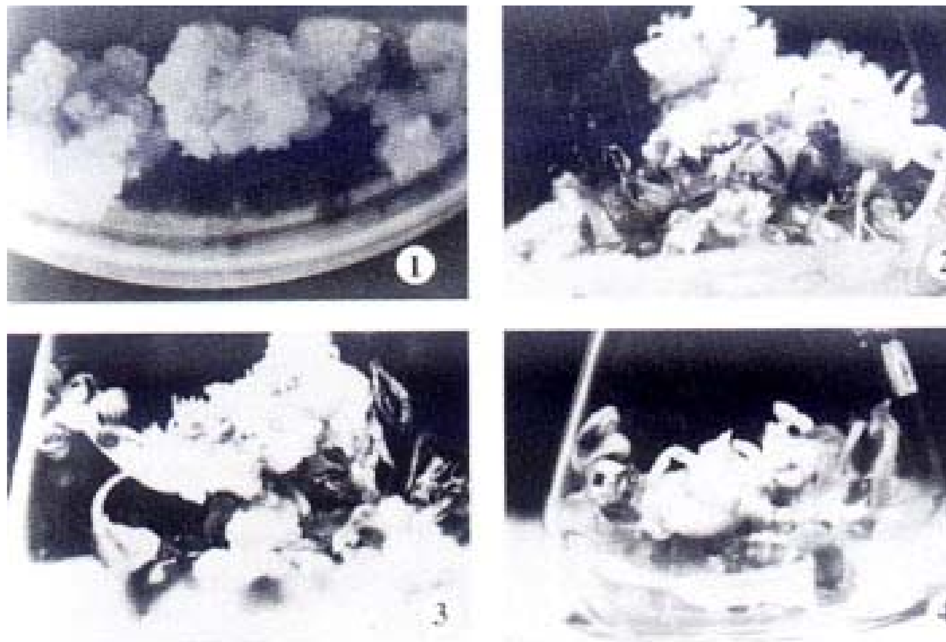
Materials and Methods

The genetic stocks of *Physalis pubescents* L., obtained from Royal Botanical Gardens, Kew, U.K. were selfed and used in the current experiment. A flowerless mutant was recovered and isolated in one treated M_1 line (0.3%/16 h EMS). The leaf, stem and nodal segments of 1 to 1.5 cm length of both control and the mutant were initially washed with double distilled water and subsequently surface sterilized with 0.1% $HgCl_2$ for 6 to 8 minutes and washed three to four times with sterilized double distilled water. Sterilized segments were then inoculated on MS medium solidified with 0.8% agar and supplemented with different concentrations and combinations of both auxins and cytokinins. The media used were viz., MS + 2.0 mg/l 2,4-D; MS + 2.5 mg/l 2.4-D; MS + 2.0 mg/l 2,4-D + 2.0 mg/l IAA; MS + 2.5 mg/l 2,4-D + 2.0 mg/l IAA; MS + 5.0 mg/l 2,4-D for callus induction and MS + 2.0 mg/l BAP + 0.5 mg/l Kn; MS + 0.5 mg/l BAP + 5.0 mg/l NAA; MS + 2.0 mg/l BAP + 0.5 mg/l NAA and MS + 5.0 mg/l BAP + 0.5 mg/l NAA for regeneration. The pH of the medium was adjusted to 5.8 prior to autoclaving. The cultures were maintained at 26 ± 2_C, 8/6 day light photoperiod by cool fluorescent lamp at 1500 lux. Cultrues were subcultured at an interval of three weeks. Observations were later recorded. Differentiated shoots from the above cultures were transferred to MS basal medium supplemented with 0.5 mg/l NAA for root induction. Plantlets were removed from culture tubes and their roots were washed in running water, transferred to pots containing sterile sand and soil mixture and covered with plastic bags to maintain humidity following the method adopted by Rani and Grover (1999). The culture experiments was repeated twice. Young leaves of mutant were not cultured because mutant could not be identified at seedling stage due to lack of specific marker.

Results and Discussion

Shoot regeneration from leaf explants: Difference in callusing ability among explants of young and mature leaves of the control and the mature leaves of the mutant was discernible when the respective explants were cultured on MS basal medium supplemented with different concentrations of 2,4-D and IAA. Callus

was visible after five days in young; seven days in mature leaves of the control and nine days in the mutant after inoculation. The calli derived from these three sources were white to pale white, compact and organized in nature (Fig. 1). Among the several combinations of growth regulators used per cent callus induction was found to be higher on MS medium supplemented with 2.0 mg/l 2,4-D than in others tested (Table 1). The present finding revealed that per cent callus induction was higher in the young leaves and relatively lower in the mature leaves of the control. Similar results were reported in *Physalis peruviana* (Torres et al. 1988), *Physalis minima* (Bapat and Rao 1977) and *Withania somnifera* (Rani and Grover 1999). Nevertheless, per cent callus



Figs. 1-4: Regeneration of different explants of control and mutant. 1. Callus from mature leaves of the control. 2. Differentiation of callus of mature leaves of control. 3. Plantlet differentiation from 30 days old callus of nodes of control. 4. Plantlet formation from 40 days old callus of node of mutant.

induction was higehr in the mature leaves of the control over those of the mutant. Regeneration of plants from white compact callus was obtained in about 45 days when subcultured on MS + 2.0 mg/l BAP + 0.5 mg/l NAA medium (Fig. 2). Plantlet formation was highest in the calli of young leaves of control and least in the calli of mature leaves of mutant (Table 2). So far no report exists either on callusing or regeneraiton potential of the mutants of any of the members of *Physalis*, and the present finding is the first report on this taxon.

Table 1. Effect of different concentrations of 2,4-D alone and in combination with IAA for callus induction in control and mutant plants of leaf, stem and node of *Physalis pubescens* L.

Calli source	MS + 2.0 mg/l 2,4-D			MS + 5.0 mg/l 2,4-D			MS + 2.0 mg/l 2,4-D + 2.0 mg/l IAA		
	No. of explants	No. callused	% callused	No. of explants	No. callused	% callused	No. of explants	No. callused	% callused
Control									
Young leaves	54	4	7.40	67	3	4.40	60	4	6.60
Mature leaves	50	3	6.00	56	2	3.50	56	3	5.30
Mutant									
Mature leaves	52	3	5.70	58	1	1.70	64	3	4.60
	MS + 2.5 mg/l 2,4-D			MS + 5.0 mg/l 2,4-D			MS + 2.5 mg/l 2,4-D + 2.0 mg/l IAA		
Control									
Stem	40	2	5	58	-	-	47	-	-
Nodes	40	2	5	52	-	-	58	3	5.1
Mutant									
Stem	45	1	2.2	54	-	-	54	-	-
Nodal region	48	1	2.1	56	-	-	58	2	3.4

Culture of stem explant: Callus is induced from the stem explants of the control and those of mutant only on MS + 2.5 mg/l 2,4-D (Table 1). Per cent callus induction was more in the control and less in the mutant (Table 1). Callus was visible after seven days in control and nine days in mutant after inoculation. The callus was white to brown, slimy/gelatinous and non-embryogenic in nature in both categories. The calli when subcultured for regeneration on the following

Table 2. Regeneration of calli in leaf, stem and node in control and mutant of *Physalis pubescens.*

Sources of explant	Media and callus induction	Plantlets produced in different media		Only roots formed MS + 0.5 mg/l BAP + 5.0 mg/l NAA
		No. of calli subcultured	MS + 2.0 mg/l BAP + 0.5 mg/l NAA	
Control				**MS + 2.0 mg/l 2,4-D**
	Young leaves	12	2 (16	-
	Mature leaves	8	1 (12.5)	-
Mutant	Mature leaves	10	1 (10)	-
	MS + 5.0 mg/l 2,4-D			
Control	Young leaves	6	-	-
	Mature leaves	2	-	-
Mutant	Mature leaves	6	-	-
	MS + 2.0 mg/l 2,4-D + 2.0 mg/l IAA			
Control	Young leaves	4	-	-
	Mature leaves	3	-	-
Mutant	Mature leaves	-	-	
	MS + 2.5 mg/l 2,4-D			
Control	Stem	5	-	-
	Nodal region	4	1 (25)	-
Mutant	Stem	2	-	-
	Nodal region	12	1 (8.33)	1 (8.30)
	MS + 000 mg/l 2,4-D			
Control	Stem	-	-	-
	Nodal region	-	-	-
	MS + 2.5 mg/l 2,4-D + 2.0 mg/l IAA			
Control	Stem	-	-	-
	Nodal region	18	-	6 (33.30)
Mutant	Stem	-	-	-
	Nodal region	6	-	1 (16.6)

media viz. MS + 2.0 mg/l BAP + 0.5 mg/l NAA; MS + 2.0 mg/l BAP + 0.5 mg/l NAA; MS + 5.0 mg/l BAP + 0.5 mg/l NAA and MS + 2.0 mg/l BAP + 0.5 mg/l Kn, these did not differentiate into plants. However, Sipahimalani et al. 1981 reported regeneration from stem explants of *P. minima*. This could probably be due to either genotypic differences between the two taxa or due to differences in ploidy level; *P. minima* being an allotetraploid and *P. pubescens* a pure diploid. Further, differences in the growth regulators could also be a probable factor.

Shoot regeneration from nodal segments: Per cent callus induction was higehr in the nodal explants of the control over those of the mutant when cultured on MS + 2.5 mg/l 2,4-D + 2.0 mg/l IAA. Callus was visible six and eight days after inoculation in control and the mutant, respectively. The calli were white in colour, compact and friable both in control and mutant. Nevertheless, the per cent callus induction was relatively high in the leaf and less in the stem compared to the nodal segments (Table 2).

The callus of either sources when subcultured on MS + 2.5 mg/l BAP + 0.5 mg/l NAA developed shoots after 45 - 50 days (Figs. 3 - 4). However, the calli subcultured on MS + 0.5 mg/l BAP + 5.0 mg/l NAA developed only roots (Table 2).

Regenerated plants were transferred first to pots and after two - three weeks, the hardened plants were shifted to field.

A survey of literature indicates that mutagen causes serious effect on hormonal imbalance resulting in physiological disorder (Behera and Patnaik 1975). In view of this, it is likely that the chemical mutagen (EMS) exerted lethal effects on induction of callus and regeneration potential on leaf, stem and nodal explants of mutant.

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