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Original Research Article

In Vitro Regeneration of *Physalis maxima* (Mill) an Important Medicinal Plant

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Physalis maxima, BAP, Nodal and Leaf explant

A B S T R A C T

An efficient regeneration protocol was developed for *Physalis maxima* (Mill) an important medicinal plant. Cali and shoots were regeneration from different explants like nodal and leaf. The explant tested with MS medium supplemented with B5 Vitamins and combination of plant growth regulators in different concentrations. The shoots were obtained from the nodal explants in BAP combination with Naphthalene acetic acid (NAA) and Gibberellic acid three (GA3). The highest numbers of multiple shoots were obtained from the nodal callus at BAP (2.0 mg/l) + NAA (1.5 mg/l) + GA3 (0.5 mg/l). The leaf explants were tested with different concentration of BAP, IAA & KIN. The large numbers of shoots were observed in BAP (2.5 mg/l) + IAA (1 mg/l) + KIN (1 mg/l). The regenerated rooted planets were hardened in the greenhouse and transferred to soil. This new protocol was standardized for easy mass propagation of *Physalis maxima* using nodal and leaf explants.

Introduction

A *Physalis maxima* (Mill) belongs to the family Solanaceae, a small herbaceous annual plant grown in the crop field. Small delicate, erect annual pubescent and 2.9 meters tall. Ethno- botanical information showed that this plant has tremendous medicinal value for cure out different diseases. It is used as tonic, diuretic and laxative in inflammation, enlargement of the spleen and as s helpful remedy in ulceration of the bladder, the leaves are crushed and applied over snakebite site. Fruits of this plant are used to cure spleen disorders. 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.

Plants are also the source of many modern medicines. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances. The most popular analgesic, aspirin, was originally derived from species of *Salix* and *Spiraea* and some of the most valuable anti-cancer agents such as paclitaxel and Vinblastine are derived solely from plant sources (Katzung

BG 1995). Plants are the traditional source of many chemicals used as pharmaceutical. Most valuable Phytochemical are products of plant secondary metabolism. Biotechnological tools are important for multiplication and genetic enhancement of the medicinal plants by adopting techniques such as *in – vitro* regeneration and genetic transformations. *In vitro* propagation of plants holds tremendous potential for the production of high- quality plant based medicines. Numerous factors are reported to influence the success of *in – vitro* propagation of different medicinal plants Hu CY and Wang PJ (1983), Hussey G (1980), Short KC and Roberts AV (1991). A few scientists have reported *in vitro* tissue culture protocols with different combinations of plant growth hormones Purohit M *et, al.* (1995), Ekiert H and Gomolka E (2000), Pande D, *et, al*. (2000).

The present investigation was carried out to develop a simple and efficient protocol for rapid Micropropagation and conservation of *Physalis maxima*. This is an alternative and cost effective method to improve the crop production of medicinal plant.

Materials and Methods

Physalis maxima (Mill) were collected from the river of kavery, Tiruchirappalli, Tamilnadu. The explants were taken from 4-5 months old plants. All the explants were Washed thoroughly with running tap water for 20 min, then they were cleaned with liquid detergent Tween 20 (1%v/v) for 5-10 min and rinsed with sterile double distilled water, they were then surface sterilized with 0.01% HgCl2 (w/v) solution for 2 min and again washed well in distilled water three – four times to remove all traces of Hgcl2. Surface sterilized explants were aseptically inoculated in MS medium Murashige T and Skoog F (1962). Supplemented with B5 vitamins Gamborg OL *et al.,* (1968) 30 % sucrose and solidified with 0.8% agar. The MS with different concentration of BAP (0.5-4.0 mg/l), IAA (0.5-1.5 mg/l), NAA (0.5-2.0 mg/l), GA3 (0.5-2.5 mg/l) and KIN (0-1.5 mg/l) were used. PH was adjusted to 5.8 ± 0.1 with 0.1 N NaOH or 0.1 N HCl before autoclaving at 121° C and 15 lb for 20 min. All cultures were maintained at 22 ± 1 ° C less than 16 hrs photoperiod at a photosynthetic flux of 12.6 µmol2/s, which was provided by cool daylight fluorescent lamps. For hardening-off, 7 to 8 weeks old rooted shoot lets were removed from the culture flacks. After freeing the agar with the running water they were transferred in to small polythene bags containing sterilized cow – dung, sand and red soil (1: 2: 3) and kept in a mist house. After acclimation in the house for 2 months, they were transferred to green house.

Result and Discussion

The different explants like nodal and leaves from *Physalis maxima* were used at different concentration of BAP in combination with IAA, IBA, 2,4-D, NAA, GA3 and KIN. The maximum number of multiple shoots were obtained from nodal explant of *Physalis maxima* at BAP (2.0 mg/l) + NAA (1.5 mg/l) + GA3 (0.5 mg/l) showed the mean vale 15.3 is the best response (Table - 1) (fig. 1, 2. A & B). The large numbers of multiple shoots were obtained from leaf explant of *Physalis maxima* at NAP (2.0 mg/l) + IAA (1 mg/l) +KIN (1mg/l showed the mean vale 13.5 is best response (Table-2) (Fig. 3, 4. C & D).

Table.1 Multiple shoot induction from nodal explants of *Physalis maxima* L

S. No	Different Concentration of Growth regulators.			No. of shoots/ explants Mean ± SD
	BAP	NAA	GA3	
1	0.5	0	0	13.0±0.53
2	1.0	0.5	0	13.8±0.55
3	1.5	1.0	0	12.0±0.39
4	2.0	1.5	0.5	15.3±0.51
5	2.5	1.5	1.0	11.5±0.50
6	3.0	1.5	1.5	14.5±0.83
7	3.5	1.5	2.0	9.6±0.60
8	4.0	1.5	2.0	8.7±0.76

Table.2 Multiple shoot induction from leaf explants of *Physalis maxima* L.

S. No	Different Concentration of Growth regulators.			No. of shoots/ explants Mean ± SD
	BAP	IAA	KIN	
1	0.5	0.5	0	9.8±0.61
2	1.0	0.5	0	13.8±0.55
3	1.5	0.5	0.5	11.7±0.64
4	2.0	1.0	1.0	13.5±0.46
5	2.5	1.0	1.0	12.0±0.51
6	3.0	1.5	1.5	11.6±0.52
7	3.5	1.5	1.5	11.4±0.59
8	4.0	1.5	1.5	10.7±0.56

Fig.1 A. Multiple Shoots from nodal explant

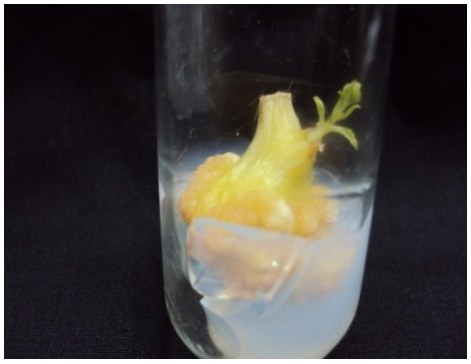

Fig.2 B. Elongated Shoots from nodal explant

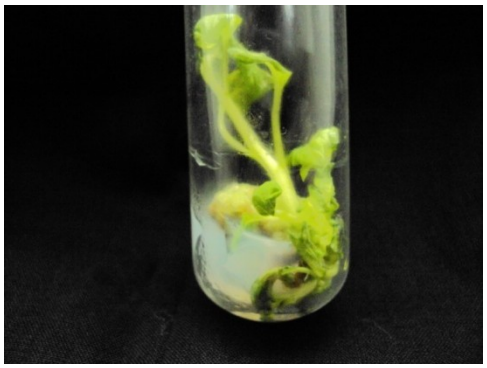

Fig.3. C. Shoot initiation from leaf explant

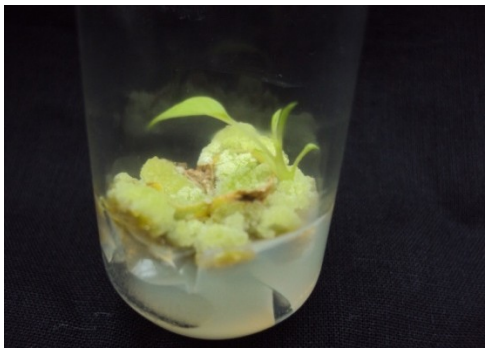

Fig.4. D. Multiple elongated shoots from leaf explant

Shoot initiation was reduced by increasing the concentration of BAP (3.0 – 4.0 mg/l) on MS Similar results have also suggested the same type of explants for propagation of other medicinal plants, such as, the maximum number of shoots was obtained from nodal explant of *Aegle marmelos* (L) at BAP (2.5 mg/l) and IAA (1.0 mg/l) Ajithkumar D and Seeni S (1998). Multiple shoots were produced from nodal explants of lime Abdul-Aziz M and Al-Bahrany (2002). Shoots multiplication of *Bupleurum distichophyllum* was achieved from the nodal and shoot tip explants of mature plants at BAP (1.0 mg/l) + NAA (0.1 mg/l). The combination of BAP and GA3 was also found to be effective for both types of explants Karuppusamy S and Pullaiah T (2007). The optimum number of shoos were obtained from nodal explant of *Adhatoda beddomei* at BAP (3.0 mg/l), 2-ip (0.5 mg/l) and IAA (1.0 mg/l) Charantharayil G *et, al.* (1994). The maximum of shoots were obtained from nodal explant of *portulace grandiflora* at BAP (4.0 mg/l) Ashok K *et al*., (2010). Shoots multiplication of *Echinacea purpurea* was achieved from the leaf explants of mature plants at BAP (4.44 µM) and NAA (0.054µM), providing high shoot regeneration frequencies (100%) associated with a high number of shoots per explant (7.7 shoots/ explant) Koroch A *et, al* (2002).Optimum number of regenerated shoots were obtained from leaf explants of Dwarfing apple rootstocks at 10 µM BAP, 1 µM NAA and 3 % sucrose Welander M and Maheswara G (1992), Bhagyalakshmi N and Singh NS (1988), Murch SJ *et, al*. (2000).

Rooted plant lets were successfully hardened and transferred in to the soil, this protocol can be used as efficient tool rapid multiplication maintenance of germplasm and conservation of this important medicinal species in natural resources.

In this present investigation, we have reported very simple and efficient protocol for plant *in vitro* Regeneration of *Physalis maxima* as compared to the methods described for other members of Solanaceae. This ensures large scale propagation to the targeted plants, which is important for the sustainable supply of plant materials to the pharmaceutical industries and for conservation of elite germplasm in nature resources. In the present investigation on *Physalis maxima* by using Nodal and Leaf explants for producing of multiple elongated shoots were observed.

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