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In vitro propagation of nodal and shoot tip explants of *Passiflora foetida* L. An exotic medicinal plant

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ABSTRACT

Passiflora foetida L.(Passifloraceae) is an angiosperm plant with high medicinal value. The present study was developed an efficient micropropagation protocol by using node and shoot tip explants of Passiflora. Explants are surface sterilized and inoculated into culture medium with different concentrations of growth regulators. The MS medium supplemented with the hormone 6 – Benzyl adenine at the concentration of 1.5 mg/l was found to be ideal for optimal growth of the inoculated shoot and nodal cultures (100%) and MS medium supplemented with 1.0 mg/l IBA hormones was best suited for induction of roots in vitro (90%). The rate of successful acclimatization was about 78% in the Sand: Soil: Compost (at the ratio of 1:1:1) of the hardening process.

Keywords: *Passiflora foetida* L, MS medium, Growth regulators, Multiple shoots, *In vitro* roots.

INTRODUCTION

P. foetida L. (Passifloraceae) commonly known as Passion fruit is an exotic, fast-growing perennial creeping and spreading vine [*Mossukkattan, Poonaipudukku* (in Tamil) and *stinking passion flower* (in English)], found in riverbeds, dry forest floors, way side thickets, covering the top of thorny shrubs and also growing near hamlets (Komathi et al.(2011). The specific name, *foetida* means “stinking” in Latin and refers to the strong aroma emitted by damaged foliage (Flavia Guzzo et al. 2004). The plant is widely distributed in worldwide, especially dominant in Southeast Asia and it consider as a weed in the Pacific Region, West Africa and Central America. The leaves are three- to five-lobed and viscid – hairy. When crushed, these leaves are emitting pungent odor that some people consider as unpleasant. The flowers are white to pale cream colored, about 5 – 6 cm diameter. The fruit is globose, 2–3 cm diameter, yellowish-orange to red when ripe, and has numerous black seeds embedded in the pulp; the fruit are eaten and the seeds are dispersed by birds (Ulmer and McDougal 2004).

P. foetida has quick and effective action in burn wounds and is recommended by Brazilian drugs centre as an antirheumatic (Emim Baby et al. 2010). It is used as a bactericide, antidysentric and antilithic and used for medicinal purposes as a sedative, as well as a food source. It is used for mood disorders (depression, anxiety, and stress) (Rasool et al. 2011). Young leaves and plant tips are edible. The dried leaves are used in Vietnamese folk medicine to relieve sleeping problems. The whole plant is used in the treatment of insomnia and anxiety. In India, the plant is traditionally used for diarrhea, throat and ear infections, liver disorders, tumors, itches, fever, skin diseases and for wound dressing (Ingale et al. 2010). Aqueous extracts of leaves or whole plants have been used as remedy for colic, colds, diarrhea, asthma, and sleeping problems (Joy 2010). Considering the above views, the present study was designed to produce a reliable protocol for *in vitro* propagation of node and shoot tip explants of *P. foetida.*

MATERIALS AND METHODS

Source of plant materials

The explant of *P. foetida* was collected during the month of February, in Botanical garden, Periyar University campus, Salem, Tamil Nadu and it was authenticated by Dr. D. Natarajan Assistant Professor, and the voucher specimen was deposited in Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem.

Explant selection and sterilization

The auxiliary buds of *P. foetida* were removed (from healthy &young plant parts) and nodal and shoot tip explants were dissected using a sterile surgical blade or scissors. The explant segments were used for inoculation purpose. The explants were processed initially by washing with running tap water for about 10 – 15 minutes, to remove the dust particles. The explants were treated with a solution containing 1-3 drops of Tween 20 or commercial detergents (teepol) for 10 minutes. Then, it was washed thoroughly with sterile distilled water. The explants were treated with 5% Bavistin solution for 7 minutes and again washed with sterile distilled water. Later, the explants were surface sterilized with a solution containing 0.1%$HgCl_2$ for 3 – 10 minutes. Then, the explants were finally rinsed thoroughly with sterile distilled water about 3 – 5 times for removal of any chemicals present on the surface of the explants.

Media preparation

The MS (Murashige and Skoog 1962) medium was prepared by adding the aliquots of macro, micro nutrients, vitamins and amino acids. Along with inositol, carbon source (3% sucrose) and gelling agents (0.8 % agar or clarigel) was added and finally the addition of phytohormones like auxins and cytokinins (such as 2, 4 – Dichlorophenoxy acetic acid, 1- Napthalene acetic acid, and 6 – Benzyl adenine, Adenine sulphate, Kinetin etc) to the shooting and rooting medium. The pH of the medium was adjusted to 5.6 - 5.8 using 0.1N NaOH and 0.1N HCL. Then, the liquid medium was dispersed equally in culture tube (each 10 ml) was air tight closed with cotton plug. Then, the medium was autoclaved at 15 lbs or 121 ºC for 20 minutes. The mouth of culture tube was sealed tightly after autoclaved, left undistributed to cool the culture and labeling on the cotton plug. The culture test tubes were being kept under the sterile conditions under laminar air flow (Tissue Culture Chamber).

Explants Inoculation and Incubation

The explants were transferred to the cutting board and trimmed to remove the dead cells from the cutting edges in order to eliminate the contamination and inoculated healthy explants into the MS medium supplemented with specific cytokinins and auxins. The inoculated culture test tubes were incubated photoperiod of 16/8hrs light and dark conditions at 24±2ºC. Cool-white fluorescent lights of 3000 lux provided light conditions; observation and recording were made every day starting from sixth day of shoot initiation. The responses of tissue/explants were carefully observed for parameters of Morphogenic changes, i.e., new leaves and shoot induction and multiple shoot formation.

Multiple Shoots Formation

Shoot tips were established on basal salt medium (MS medium) supplemented with 30 g/l sucrose, 8g/l agar, and the medium containing 1.5 mg/L 6- Benzyl adenine (6 - BA) induced multiple shoots (1-2) with maximum percentage of responding cultures (95%). Shoot tip and inter nodal segments (1cm) were sub cultured on media similar to those used for initiation.

In-vitro rooting

The shoots developed in the culture tubes were maintained for 4 – 5 weeks and monitored for the continuous elongation of the shoots upto the desired level. After, the formation of multiple shoots, 3 – 5 cm length of cultures were dissected and inoculated in a medium containing ½ strength MS medium by eliminating the vitamins and amino acids and full strength MS medium supplemented with various concentration of IBA for root formation. Then, the cultures were incubated as similar to earlier steps.

Acclimatization

Rooted shoots are removed from culture and placed in the polycups containing sand: soil: compost at the ratio of 1:1:1. The humidity must be gradually reduced over time because tissue cultured plants are extremely susceptible to

wilting. This was initially treated with ½ MS stock solution and gradually adding sterile and normal water and the survival rates were monitored.

RESULTS

Shoot Proliferation

Shoot tip and nodal explants of *P. foetida* showed regenerated *in-vitro* cultures after 6 days inoculated on MS medium fortified with different concentrations of 6 - BA (MS basal, 0.5, 1,0, 1.5 & 2.0 mg l^{-1}) alone and combination with KIN (1.5 B + 0.5mg l^{-1}K). It was recorded that the growth of shoot (length upto 5 - 6 cm long) within 3–6 weeks periods. The MS medium supplemented with BA (1.5 mg l^{-1}) alone developed more number of multiple shoots (3.16 ±0.6) developed within 2 – 3 weeks emerged directly from the explants. Out of these shoots, about 80% are continued to elongation. The response was good at 1.5 mg l^{-1} and 2.0 mg l^{-1} 6 - BA at the rate of 100 % and 95 % respectively. A few multiple shoots were developed at 1.0 mg l^{-1} 6 - BA alone and combination of 6 – BA with Kin at the concentration of 1.5 + 0.5 mg l^{-1}. Overall, the response was moderate and lack of multiple shoot formation in other concentrations of medium. Auxiliary buds were isolated and repeatedly subcultured on the same hormone for continuous elongation and multiplication (Table 1; Figure 1) of shoots.

Root Proliferation

For root induction, the shoots excised from the cultures (2 – 3cm length) were transferred to the ½ and full strength MS medium containing different concentrations of IBA (0.4 – 2.0 mg l^{-1}), and maximum number of roots were formed in full strength MS medium (after 15 days of incubation). The MS medium supplemented with the concentration of 1.0 mg l^{-1} IBA produced more roots at the rate of 90 % and the maximum roots length was about 4±0 cm (Table 2). Whereas, the rooting response of other concentrations of IBA (0.5, 1.5, 2.0 mg l^{-1}) produced 3.0 ± 0, and 2.0 ± 0 CM at the rates of 80, 70, 60 % respectively. But, the MS basal medium which produced the fewer number of roots which limits to elongate after certain length of roots. The well developed roots were observed at 80% (after 4 weeks of incubation) in higher concentration of medium under controlled conditions (Figure. 1).

Table1: *In-vitro* shoot formation of Shoot tip and Nodal explants of *P. foetida* L.

Hormones (mg/l)	Type of Explants		Regenerative Response (days)	Growth Rate (%)	Rate of Multiple Shoots (%)	Elongation (cm)
	Shoot tip	Nodal				
MS basal	+	+	12	60	1.8 ± 3.2	3.7 ± 0
0.5	-	+	12	65	2.0 ± 1.8	3.9 ± 0
1.0	-	+	8	75	2.1 ± 0.8	4.2 ± 0
1.5	+	+	6	100	3.6 ± 0.6	7.3 ± 0
2.0	-	+	7	95	3.1 ± 2.1	5.8 ± 0
1.5B + 0.5K	+	+	6	85	2.1 ± 0.1	4.5 ± 0

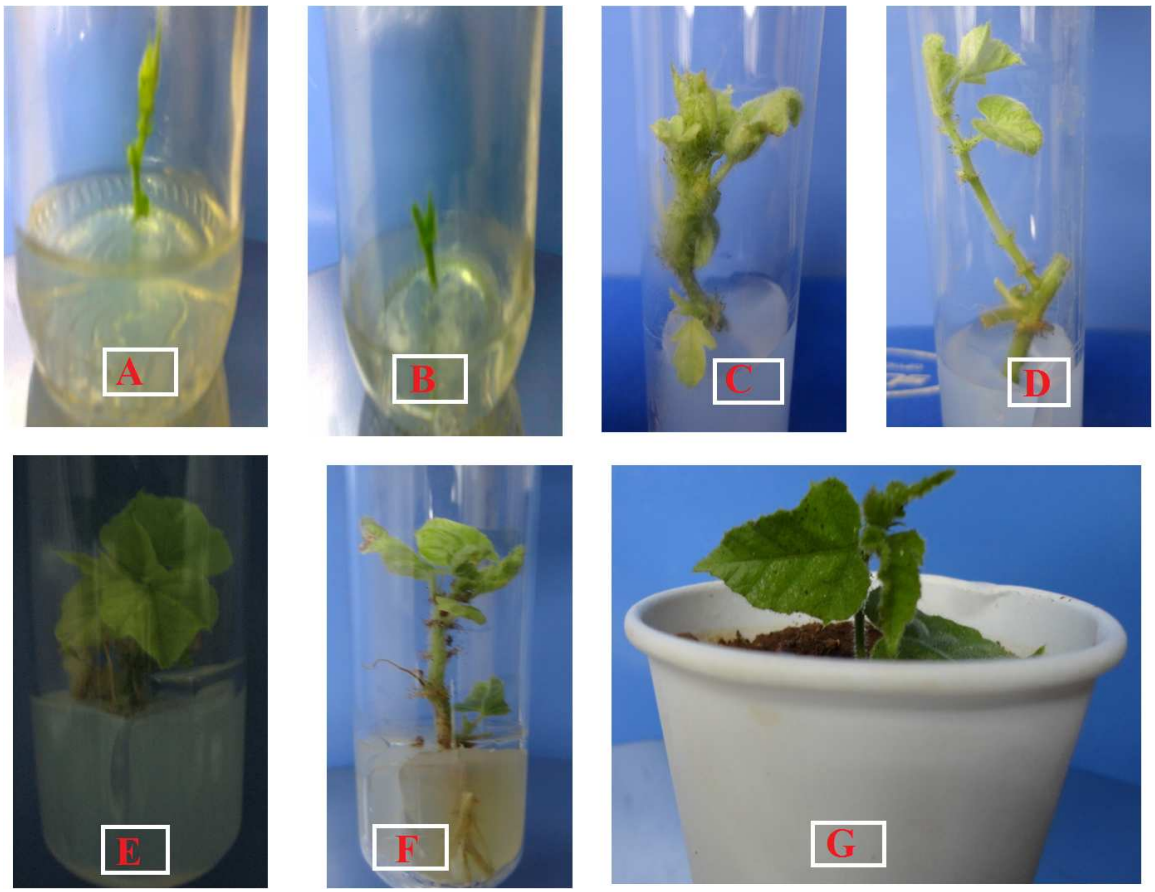
- *= Slow response, + = Well growth . Values are given as the mean ±SE. n = 14 explants*

Table: 2 *In-vitro* Root Development from regenerated shoots of *P. foetida* L.

Hormones (mg/l)	Root initiation (days)	Rate of root formation (%)	Rate of multiple roots (%)	Elongation (cm)
MS basal	30	60	1.8 ± 0	1.7 ± 0
0.5 IBA	12	80	2.0 ± 0	3.0 ± 0
1.0IBA	13	90	5.0 ± 0	4.0 ± 0
1.5IBA	16	70	4.0 ± 0	2.0 ± 0
2.0IBA	14	60	3.0 ± 0	2.0 ± 0

Values are given as the mean ±SE. n = 14 explants

Figure 1: Direct organogenesis of nodal and shoot tip explants of *Passiflora foetida L.*



Legend: A & B. Nodal & Shoot tip explants, C & D. Regenerated node & shoot tip, E. Elongation and multiple shoots, F. *In vitro* roots, G. Acclimatized plant.

DISCUSSION

Passiflora species is almost exclusively propagated through seeds (Meletti 2002), by cuttings and grafts (Silva et al. 2005). But, there is problems related to the seed physiological quantity, unequal germination that directly damages the plant germination rates (Negreiros et al. 2004). Although many reports on the successful propagation on this species results in many reliable protocols were reported. However, the reason for plant responses in micropropagation is still poorly understood. Few species are growing through somatic embryogenesis and direct organogenesis, depending on the culture conditions (Dodsworth 2009). The present study was focused on micro-propagation of *P. foetida* using shoot tip and nodal explants in modified MS medium tries to study the hormonal roles in direct regeneration of plant *invitro.*

The *in-vitro* propagation of *P. foetida* was achieved in MS medium supplemented with different concentrations of 6 - BA (1.0 – 2.0 mg/l), and multiple shoots emerged from the shoot-tip and nodal explants of *P. foetida* after 7 days of inoculation. The medium containing 1.5 mg/l of BA induced maximum number of multiple shoots with highest percentage of cultures (100%). The other concentration of BA and its combination were found to be low to moderate growth rates for shoot proliferation. Similarly Komathi et al. (2011) achieved maximum shoot tip from *P. foetida* (76%) in MS medium containing BAP and NAA at 3.0 and 0.5 mg/l and rooted in the MS medium containing the auxin, IBA and IAA alone at 1.0 mg/l. On the other hand, Isuta (2004) also reported the elongation of *Passiflora* shoots at 2.2 mg/l BAP. Yara Britto (2012) was successfully achieved indirect regeneration of the same species (*P. foetida*) from zygotic embryos. Fernando et al. (2007) developed a protocol for direct formulation of leaf primordial

without any promeristem or cell proliferation phases. These reports were similar to our studies of successfully reproducing more shoots from single node and shoot tip explants.

Also few other reports focused on observation of the indirect organogenesis from the same genus *Passiflora* by Silva et al. (2009) and reported that somatic embryogenesis from zygotic embryos of *P. cincinnata*. Pinto et al. (2010) and Reis et al. (2007) developed a reliable protocol for somatic embryogenesis in the *Passiflora*. Such kind of reports was the representation of hormonal regulation of plant growth in *In-vitro* propagation. The present work was suggests that complete reproducible protocol using the PGR 6 - Benzyl adenine and Indole 3 butyric acid in shoot and root induction in direct regeneration of *P. foetida*.
Based on our results the hormone Indole 3 Butyric acid was found to successful in *in-vitro* rooting. 1.0 mg/l IBA was more effective than other concentration of IBA in roots from shoot cultures. The percentage of rooting response was also highest (90%) in the same concentration of auxin. The well developed plantlets were transferred to polycups containing the mixture of soil, sand and compost (in the ratio of 1:1:1) for hardening.

CONCLUSION

The present study was believed that the protocol developed for the *in-vitro* propagation of *P. foetida* in MS medium supplemented with the hormone 6-Benzyl adenine at the concentration of 1.5 mg/l was found to be ideal for the growth of inoculated shoot and nodal cultures. And, similarly the MS medium supplemented with 1.0 mg/l IBA hormone was best suited for the induction of roots *in vitro* with a 78 % of successful acclimatization will support the researchers and other herbalists for mass development for their beneficial uses in traditional system.

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