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RESEARCH ARTICLE

AN EFFICIENT IN VITRO REGENERATION OF MULTIPLE SHOOTS FROM LEAF EXPLANT OF PASSIFLORA CAERULEA L. AN IMPORTANT MEDICINAL PLANT

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ABSTRACT

An efficient *in vitro* protocol was established to produce multiple shoots from leaf explants of *Passiflora caerulea* L. on Murashige and skoog (MS) basal medium complemented with auxins and Cytokinins. Different concentrations of Benzyl amino purine (1.0 to 4.0 mg l^{-1}) were assessed for multiple shoot production. Large number of (more than 100) multiple shoots per explants were induced on medium containing 3 mg l^{-1} BAP. Rooting was achieved on half or full strength MS medium with 0.5-1.0 mg l^{-1} IBA (Indole-3-butyric acid) in 28 days. Regenerated plantlets acclimatized and transferred to soil showed normal morphological characteristics. Survival of transplants was 95-100 % under green house condition.

INTRODUCTION

Passiflora caerulea L. is a Perennial climber grows over large trees and bushes, commonly called blue passion vine belongs to the family Passifloraceae. Flowers are famous for being very elaborate and complex, by having radiating filaments of the corolla (corona), nodding, versatile anthers, and three radiating style branches; the design and colors have been used to symbolize Christ's Passion. Leaves palmate, 3-7 lobed and alternate and opposed by tendril. Flowers are bluish, purple colored, auxiliary and very attractive (Fig-A). A native of Brazil and introduced into Britain in 17th century, is the most vigorous and tender species having traditional use of its fruit as a sedative and anxiolytic (Hickey and king, 1988; Kirtikar and Basu, 1975 and Rendle, 1959). It was used medicinally in Uruguay but no details are available (Watt and Breyer-Brandwijk, 1962). In West Indies, Mexico, Netherlands and South America, the root has been used as a sedative and vermifuge. In Italy, the plant has been used as an anti-spasmodic and sedative. In Mauritius, a tincture and an extract of the plant had been used as a remedy for insomnia due to various nervous conditions but not due to pain. The root has been used as a diuretic and a decoction of leaves as an emetic. In Argentine folk medicine, the aerial parts are used as mild anti-microbial agents in diseases like catarrh and pneumonia (Anesini and Perez, 1933). Since the plant has high medicinal value, considering the above facts, present work has been taken up to conserve and propagate the plant in large number.

MATERIALS AND METHODS

Passiflora caerulea was collected from Lalbagh, Bangalore, Karnataka, India and maintained in the departmental garden, University of Mysore, as stock plant. Leaf were collected from healthy young branches and cut to approximately 1 cm. Explants were washed thoroughly under running tap water to remove the traces of dust and surface sterilized with 0.1% (w/v) $HgCl_2$ for 3 – 5 minutes and then rinsed with sterile distilled water. The leaf explants were inoculated on Murashige and Skoog's medium supplemented with various concentrations of Auxins and Cytokinins. The explants were inoculated in such a way that either the adaxial or abaxial surface was touching the agar medium. BAP was used for multiple shoot formation. Plantlets of 8-10cm length were harvested and transferred to half or full strength MS medium fortified with 0.25 to 3.0 mgl^{-1} IBA and NAA (Naphthalene acetic acid) for rooting. All cultures were maintained under cool florescent light at 25± 2^0 C with 16/8 hour light and dark

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conditions. Rooted plants were removed from the culture media, washed thoroughly with water and transferred to small pots containing vermiculite, yard manure and soil(1:1:1) for hardening and kept in the green house.

RESULTS AND DISCUSSION

This study was undertaken to develop an efficient protocol for regeneration of *P. caerulea*. by examining the *in vitro* response of leaf cultures. In the present investigation, a high frequency of multiple shoots were induced directly from the leaf explants by addition of 3 mg l^{-1} BAP to the culture media(Fig-1B). Under these conditions, shoot development was not accompanied by callus formation. The balance between cytokinins and auxins is known to affect *in vitro* developmental patterns and influence organogenic development in tissue cultures. Addition of cytokinin and auxin concentrations leading to efficient *in vitro* organogenesis where the addition of BAP in combination with NAA to the culture media leads to multiple shoots as well as roots formation but the number of shoots with formation of roots were not satisfactory.

Table 1 Effect of BAP alone or in combination with NAA for multiple shoots induction from leaf explants of Passiflora ceurulea after 28 days

Growth Regulators	% of Response explant	Number of shoot / Number of roots	(in cm) (mg l^{-1}) shoot length
BAP			
0	00	00 ± 00	- 00 ± 00
0.5	30	24.2±0.66	- 1.4±0.24
1	69	54.6±0.8	- 1.4±0.55
1.5	69	59.6±0.82	- 2.1 ± 1.3
2	86	81.8±0.86	- 4.8 ± 1.2
2.5	89	90.0±0.31	- 4.5 ± 1.3
3	94	98.4±1.21	- 5.8 ± 1.1
3.5	94	84.2±0.86	- 5.5 ± 1.1
4	94	77.8±0.80	- 4.7 ± 1.3
BAP+ NAA			root length
0.5 +0.5	15	7.47 ± 1.35	1.2±0.24 3.05 ± 0.23
1.0+0.5	54	11.4±0.55	1.8±0.24 4.05 ± 1.31
2.0+0.5	70	43.8±1.24	0.4±0.55 9.11 ± 1.56
3.0+0.5	72	50.2±1.15	0.4±0.55 12.05 ± 1.78
0.5 +1.0	11	6 ± 00	0.4±0.00 8.47 ± 1.35
1.0+1.0	20	1.6±0.55	1.0±0.00 2.8±0.45
2.0+1.0	36	28.0±0.70	1.2±0.24 2.6±0.55
3.0+1.0	69	45.4±0.40	1.4±0.55 8.27 ± 1.2

Data were recorded after 4 weeks of culture. Values represent means ± SE from 5 replicates

The present investigation established a correlation between plant growth regulator BAP and NAA levels and *in vitro* regeneration. BAP is considered to be one of the most useful cytokinins for achieving the multiplication and regeneration of the plants and the application of BAP alone was found to be superior and effective for shoot initiation as found in *Centella asiatica* and *Momordica charantia* (Sammaiah *et al*., 2014) and also in *Glinus lotoides* (Teshome and Feyissa, 2015), *Abutilon indicum* (Ramar and Ayyadurai, 2015), and *Ocimum tenuiflorum* (Hegde *et al*., 2015). Results in the current study are consistent with such observations. In cultures with BAP alone, the number and length of shoots increased as the BAP concentration was increased. Thus the optimal BAP concentration for shoot proliferation and elongation was 3 mg l^{-1} (Table 1, Fig-C & D). *In vitro* regenerated shoots were excised and transferred onto half strength MS medium supplemented with different concentrations of NAA and IBA. Though roots were well established in almost all concentrations of auxins tried, more number of roots were induced in IBA than in NAA (Table-2 & Fig-E).

Table 2 Effect of various concentrations of NAA and IBA on half-strength MS medium for the induction of roots in *in vitro* derived multiple shoots of *P. caerulea*

Growth regulators (mgl^{-1})	% of response	Number of roots	Root length (in cm)
IBA			
0	0	0.0±0.00	0.0±0.00
0.25	90	1.8 ± 0.38	4.6 ± 0.8
0.5	97	3.5±0.38	6.6 ± 1.1
1	96	3.2 ± 0.38	6.6 ± 1.1
1.5	90	2.7± 0.67	5.8 ± 1.2
2	79	1.8 ± 0.38	4.6 ± 0.8
2.5	0	0.0±0.00	0.0±0.00
3.0	0.0±0.00	0.0±0.00	
NAA			
0	0	0.0±0.00	0.0±0.00
0.25	0	0.0±1.32	0.0±0.00
0.5	0	0.0±1.32	0.0±0.00
1	60	2.5± 0.67	6.2 ± 0.8
1.5	60	2.0 ± 0.16	5.1 ± 1.4
2	70	2.8 ± 0.38	5.0 ± 0.8
2.5	65	1.4±1.32	5.0 ± 0.8
3	67	0.0±0.00	0.0±0.00

Data were recorded after 4 weeks of culture. Values represent means ± SE from 5 replicates

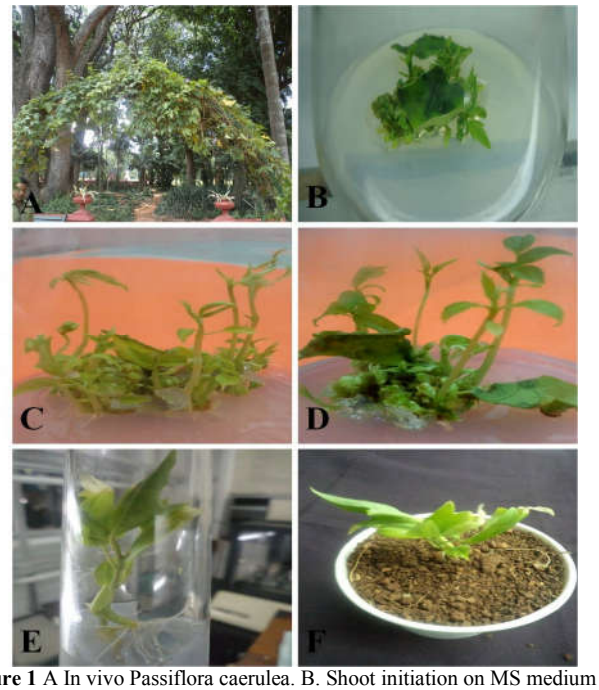

Figure 1 A In vivo Passiflora caerulea. B. Shoot initiation on MS medium at 3mg/l BAP C. Shoot Multiplication. D. Shoot proliferation and elongation. E. Rooting in half strength MS medium supplemented with 0.5mg/l IBA. F. Acclimatized plant.

These findings are similar to the observations reported in *Artemisia annua* (Ganesan and paulsamy, 2011), *Cryptolepis grandiflora* (Prema *et al*., 2013) and *Solanum hainanense* (Loc and Kiet, 2011). Regenerated plantlets were acclimatized and transferred to soil showed normal morphological characteristics (Fig -1F). Survival of transplants was 95-100 % under green house condition.

A previous study in *P. caerulea* evidenced low germination levels, slow germination speed and an imbibition period of two to five months, irrespective of the pre-treatments applied to fresh seeds, which suggested the existence of dormancy in this species (Mendiondo and García, 2003). Despite its edible (Arenas, 1983), foraging, the most important use of *P. caerulea* is medicinal (Alonso, 2004). Considering the above facts, important to conserve and propagate the plant in large number by tissue culture technique.

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