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Micropropagation and estimation of biochemical constituents in *Pedalium murex* L. and *Physalis angulata* L.

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Abstract

Protocol for micropropagation of *Pedalium murex* and *Physalis angulata,* have been developed by using MS medium supplemented with different concentrations of BAP and Kn. Among the two cytokinins used in the media, better results were obtained with MS medium supplement with BAP at 1.0mg/L. Combined effect of BAP and Kn at different concentration showed better result on callus induction. Total carbohydrates, proteins, phenols and sterols were higher in *in vitro* cultured than *in vivo* cultured plants.

Keywords : biochemicals, micropropagation, *Pedalium murex, Physalis angulata,* regeneration

INTRODUCTION

Pedalium murex L. is an important medicinal plant that contains several alkaloids like Pedalitin, Diosmetin, Dinatin, Pedalin dinatin-7-glucuronide (Subramanian and Nair, 1972). Root decoction is used as an antibilious agent, while the juice of the fruit is used as an emmenagogue and to promote lochial discharge (Satyavathi *et al*., 1987). The decoction of the seeds and glycosides obtained from it showed mild diuretic activity and the alcoholic extract of the fruits reduced blood pressure in dog and rat (Haravey, 1996).

Physalis angulata is another annual medicinal herb belonging to the family Solanaceae that contains several alkaloids and physalin B as a major component along with four new components *viz*., 5b, 6b-epoxy physalin B and withaphysalin A, B and C (Glotter *et al*., 1975). Fresh ground leaves mixed with water and mustard oil is used in earache and deafness. Fruits are reported to be tonic, diuretic, and aperient. It is used in treating gonorrhea and also snakebite, malarial fever and scorpion sting (Jain and Tarafder, 1970).

The demand for such medicinal plants is increasing due to increasing utilization of natural medicinal products. So better planning of the culture methods become necessary. But literature available on acclimatization of micro propagated plants of these two medicinal plants are very meager (Ramasubbu *et al*., 2007; Saravanan *et al*., 2007). Keeping these constrains in mind, the present investigation was under taken for clonal propagation of *Pedalium murex* and *Physalis angulata* and also conduct biochemical analysis of its conventional and micro propagated clones. The present investigation has been made for developing a micro propagation protocol that would facilitate rapid multiplication of these drug yielding plants. Plant tissue and cells in the culture undergo variations, that are of particular interest because they may provide clues to the basic mechanism underlying genotypic and phenotypic stability in normal development. Such variations can also be useful in agriculture, horticulture and biochemical product developing industries (Philips *et al*., 1994). So, biochemical parameters like chlorophyll, carbohydrates, proteins, phenols and sterols of the stem and leaves of normal and regenerated plants have also been estimated in order to establish the sustainability of plants.

MATERIALS AND METHODS

Micropropagation

The explants were collected from field plants of social forestry areas, Sivakasi, Tamil Nadu, South India. The explants were pre-treated with 1% Bavistin (w/v) solution and 400 ppm chloromphenicol on a rotatary shaker and thoroughly washed with sterile distilled water for 5 minutes. A final treatment was given with 0.1% $HgCl_2$ (w/v) for 5 min. and the explants were thoroughly washed with sterile distilled water. Shoot tip explants (5-6 mm), and nodal explants (20-25 mm) were dissected out and inoculated on MS medium supplemented with various concentrations (0.5-2 mg/L) of growth regulators of Benzylaminopurine (BAP), Kinetin (Kn) and BAP combined with Kn. The cultures were maintained at 25 ± 2^0 C under a light intensity of 3000 lux provided by cool fluorescent lamps. The comparative morphogenetic response of the explants taken from *in vitro* grown explants to plant hormones was observed regularly. Size and number of callus developed individually and along with multiple shoots were also observed. For root induction, *in vitro* grown 20 days old micro shoots were aseptically transferred to MS medium supplemented with 1.0-5.0 mg/L each of IAA and IBA respectively. Plantlets with well grown roots were removed from the culture tubes. The roots were thoroughly washed with running tap water and

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the plantlets were transferred to protrays filled with coco peat + soil (1:1) and kept in a moist saturated green house having 60-80% humidity. After four weeks, the established plants were transferred to 15 cm pots containing soil with biofertilizer for further growth.

Biochemical analysis

Stem and leaf of both normal, regenerated plants and callus tissues (ca. 300µg) of both the plants were collected separately and washed thoroughly with distilled water. The quantitative estimation of total chlorophyll content was done by the method suggested by Arnon (1949). Carbohydrates contents of both normal and regenerated plants were estimated by the standard method (Jayaraman, 1981). Protein contents were analyzed quantitatively by the method of Lowry *et al.* (1951). Total phenols content were extracted with 80% methanol from dry leaves of naturally grown plants and clones obtained through tissue culture. 0.5 ml of extract was added with 0.5ml of Folin Ciocalteu reagent with vigorous shaking. After 3 min., anhydrous sodium carbonate reagent was added and incubated at 30C for 1hr as suggested by Swain and Hills (1959). After incubation, absorbance was recorded at 720nm and phenols content was estimated from reference curve of gallic acid as standard. For the extraction of free sterols, leaf tissues were extracted by the method of Folch *et al.* (1957). The tissue after extraction was transformed to air tight glass topered flask containing chloroform: methanol (2:1) mixture. The contents after shaking for three hours were filtered through sintered glass funnel. To one fifth of chloroform: methanol extract, solution of 0.9% NaCl was added and shaken in a separatory funnel to form separate layers. The lower layer containing pure lipid fraction was removed and free sterols were estimated by method of Zlatkis *et al*. (1953). Each experiment was replicated 5 times and repeated twice.

RESULTS AND DISCUSSION

Shoot tip and nodal explants of *Pedalium murex L.* and *Physalis angulata* L. were tried with MS medium supplemented with BAP and Kn individually and combined for direct regeneration for both shoot tip and nodal explants. Shoot tip responded well at 1.0 mg/l of BAP in *Pedalium murex* L. and *Physalis angulata* L. (Figure 1a and d) with mean values of 12.71± 1.32 mm and 11.27±0.87 mm shoots (Table 1). The higher concentration of BAP was noticed to inhibit the formation of shoots and the shoots so formed were short and thick. These results corroborate with earlier findings of Rahman and Lau (1996) in which BAP at lower concentration supplemented with full strength MS medium encouraged the multiple shoot induction in flowering plants. Multiple shoots were also induced from shoot tip and nodal explants on MS medium supplemented with different concentration of Kn (0.1-2.0 mg/l). Number of multiple shoots were higher on medium containing 1.0 mg/l of Kn, which decreased with further increase in the concentration of Kn. The combined effect of BAP and Kinetin revealed that, lower concentration showed best response for both nodal and shoots tip explants of *Pedalium murex* L. and *Physalis angulata* L. Callus was also obtained from shoot tip and nodal explants on MS medium supplemented with higher concentration of BAP and Kn (Fig. 1b). The callus was transferred to MS medium for the further development. The results obtained were similar to the finding of Suri *et al.* (1999) in *Curculico orchioides.* Several plantlets were propagated *in vitro* and it was observed that, number of shoots that could regenerate roots were comparatively more prevalent in shoot tip explants than that of the nodal explants. Root generation can be induced by means of different concentration of plant growth regulators (IAA and IBA). In the present investigation, the shoots were separated and transferred to MS medium containing different concentrations (1.0-5.0 Mg/L) of rooting hormone and maximum root induction was observed when shoots were treated with high concentration (5mg/l) of IBA (Fig. 1c). Similar method of rooting by means of IBA at higher concentration was reported by Purohit and Ashis (1996) also. The higher concentration of auxins were earlier reported to enhance markedly the root formation in MS medium (Gaur *et al.,* 1995). The completed plantlets, thus formed, were hardened in green house and transferred to pots where they showed 50% survival.

The primary metabolites like carbohydrates, proteins, phenols and sterols were higher while chlorophyll pigments were lower in regenerated plants than the normal plants in both the species of the present study (Figs. 2 & 3). The amount of total chlorophyll and carbohydrates of the callus tissues were considerably lower than the normal and tissue cultured plantlets. The results are in agreement with earlier reports in other plants (Tejavathi and Rao, 1998; Mohabatra and Rath, 2005).

CONCLUSION

Present study revealed that micro propagated plants contain higher quantities of potentially active primary and secondary chemical constituents. Using the advantage of tissue culture technique the plants could be propagated at a faster rate with more concentration of potentially active secondary constituents thus increasing their commercial utilization.

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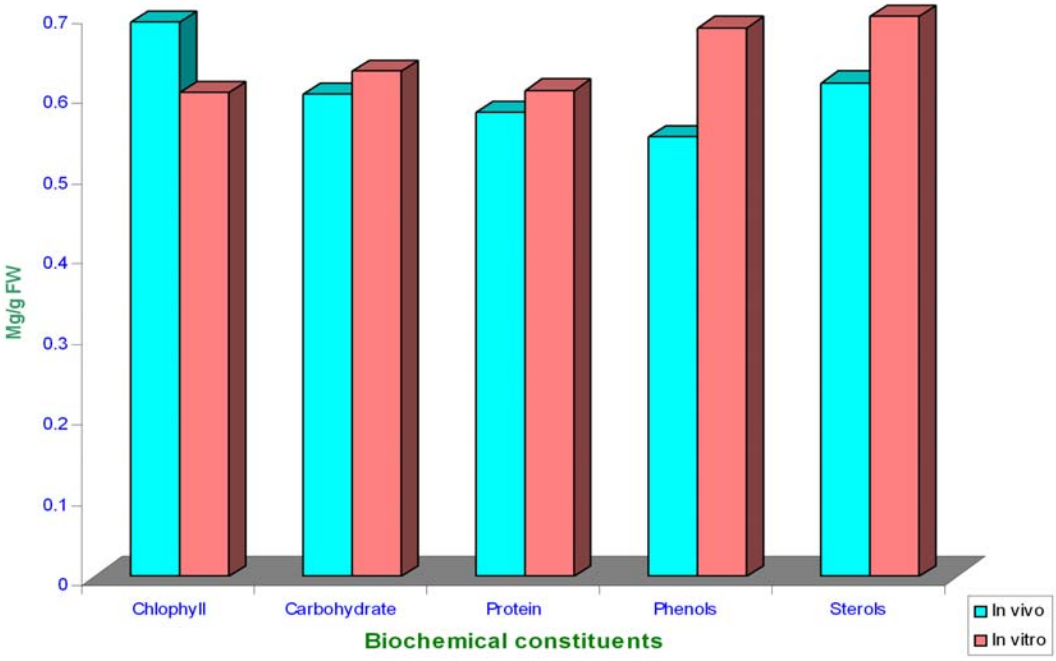

Figure 2. Biochemical variation in *in vivo* and *in vitro* plantlets of *Pedalium murex*

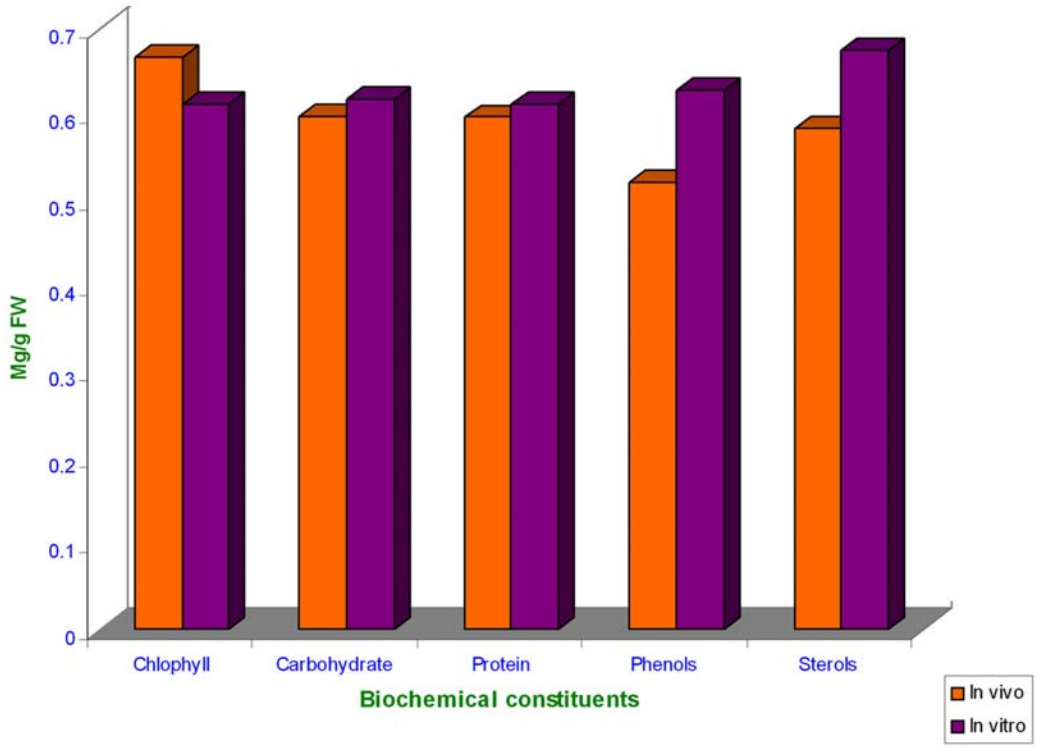

Figure 3. Biochemical variation in *in vivo* and *in vitro* plantlets of *Physalis angulata*

Table 1. Morphogenic response of shoot tip and nodal explants (growth in mm) of *Pedalium murex* and *Physalis angulata*

Growth hormones	*Pedalium murex*		*Physalis angulata*	
	Shoot tip	Nodal	Shoot tip	Nodal
BAP				
0.1 Mg/L	2.17±0.12	3.84±0.84	3.32±1.08	4.21±0.32
0.5 Mg/L	3.72±0.12	4.73±0.24	4.27±0.80	5.28±0.32
1.0 Mg/L	12.71±1.32	10.21±1.30	11.71±0.87	10.30±0.17
2.0 Mg/L	5.27±1.02	6.84±0.63	7.42±0.48	6.84±0.94
Kn				
0.1Mg/L	2.23±0.84	3.78±0.93	2.23±0.50	2.73±0.95
0.5Mg/L	3.14±0.27	4.27±0.95	4.37±0.98	4.16±0.98
1.0Mg/L	5.42±1.71	6.33±0.18	6.43±0.87	3.28±0.57
2.0Mg/L	3.18±0.29	3.98±0.89	1.43±0.68	3.28±0.56
BAP + Kn				
0.1 +0.5Mg/L	3.24±0.47^{a*}	3.78±0.70	2.87±0.28	3.18±0.43
0.5+0.5 Mg/L	5.71±0.08	2.54±0.97^{a**}	2.69±0.65^{a**}	3.48±0.98^{a**}
1.0 +0.5Mg/L	3.21±0.23	1.43±0.56^{a*}	1.57±0.57	2.27±0.39
2.0 +0.5Mg/L	1.84±0.42^{a*}	1.21±0.92^{a*}	1.35±0.49^{a*}	1.09±0.09^{a*}

± Standard error, a- Multiple shoot with callus, *- Moderate, **-good

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Figure 1. a) Multiple shoot induction by BAP (1.0 mg/L) in *Pedalium murex* b) Callus induction along with the multiple shoots c) Shoot and root inductionin *Physalis angulata* d) Well developed multiple shoots obtained from nodal explants of *P. murex*