



Original Research Article

Development of Callus Biomass in Medicinally Important Plant *Podophyllum hexandrum* (Royle) through Micro-propagation Technology

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Abstract	Keywords
<i>Podophyllum hexandrum</i> (Berberidaceae) is an important medicinal plant. Callus biomass initiated from leaf, stem and rhizome explants using 0.5 to 3.0 mg l ⁻¹ BA (6-benzylaminopurine) with 1.5 to 3.0 mg l ⁻¹ NAA (naphthalene acetic acid) in different combinations was measured. In leaf explant higher biomass was noticed in 1.5:1.5 mg l ⁻¹ followed by 2.5:2.0 mg l ⁻¹ combination of BA and IAA. While 2.0 (BA):2.5 (NAA) mg l ⁻¹ gave maximum biomass in stem and rhizome explant.	Growth regulator Biomass Callus <i>Podophyllum hexandrum</i>

Introduction

Plant-based remedies have always been an integral part of traditional medicine throughout the world. The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics has highlighted the need for conservation and propagation of medicinal plant. Tissue engineering methods provides efficient techniques for rapid and large scale propagation of medicinal plants and *in vitro* conservation of germplasms. Plant tissue culture also represents one way of possible recovery of endangered and over harvested medicinal plants as well as provides methods for extraction of medicinally important products from them. Considerable attention has now been given to the conservation and multiplication of Himalayan threatened medicinal plant species

(Arumugam and Bhojwani, 1990; Giri et al., 1993; Mathur, 1993; Sulaiman, 1994).

Podophyllum hexandrum Royle (Berberidaceae) also known as the Indian podophyllum is a perennial herb, growing on the lower slopes of the Himalayas covers forest region of Afghanistan eastwards to Central China (Chatterji, 1952; Fu, 1992). Moreover, its rhizomes, leaves and stem contain vital phenylpropanoid compound which act as antitumor compound (Kamil and Dewick, 1986; Arumugam and Bhojwani, 1994). Predominantly, it also contains podophyllotoxin, precursor of etoposide and teniposide that are used for the treatment of lung and testicular cancer (Stahelin and Warburg, 1991), leukemia and rheumatoid arthritis (Lerndal and Svensson, 2000).

Moreover, *P. hexandrum* has been noted as a critically endangered species of Indian Himalayan region (Nadeem et al., 2000). Standardization of an efficient direct *in vitro* multiplication through tissue culture is a crucial prerequisite for its conservation. Previously, shoot regeneration and somatic embryogenesis from different explants of other medicinal plants have also been reported (Tiwari et al., 1998) Earlier, Arumugam and Bhojwani (1990) reported the *in vitro* multiplication of *Podophyllum* plantlets. Limited availability of *Podophyllum* sp. is due to its long juvenile phase and poor fruit setting ability as well as the time consuming collection of the plants also results in shortage of its vital resins. Therefore, production of *Podophyllum* sp. using tissue culture techniques considered as attractive technology for its mass cultivation (Yousefzadi et al., 2010).

Tissue culture technique can play an important role in the multiplication as well as germplasm conservation of the plant. Standardization of an efficient direct *in vitro* multiplication is a crucial prerequisite for this. Shoot regeneration and somatic embryogenesis from different explants of Brahmi (*Bacopa monnieri* L.) have been reported by Tiwari et al. (1998). Patra et al. (1998) had successfully regenerated *Centella asiatica* from callus cultures. Previously also, Arumugam and Bhojwani (1990) reported the *in vitro* multiplication of *Podophyllum* plantlets via somatic embryogenesis. Limited availability of *Podophyllum* sp. is due to its long juvenile phase and poor fruit setting ability as well as the time consuming collection of the plants also results in shortage of its vital resins. Therefore, production of *Podophyllum* sp. using tissue culture techniques considered as attractive technology for mass cultivation (Yousefzadi et al., 2010). Due to high

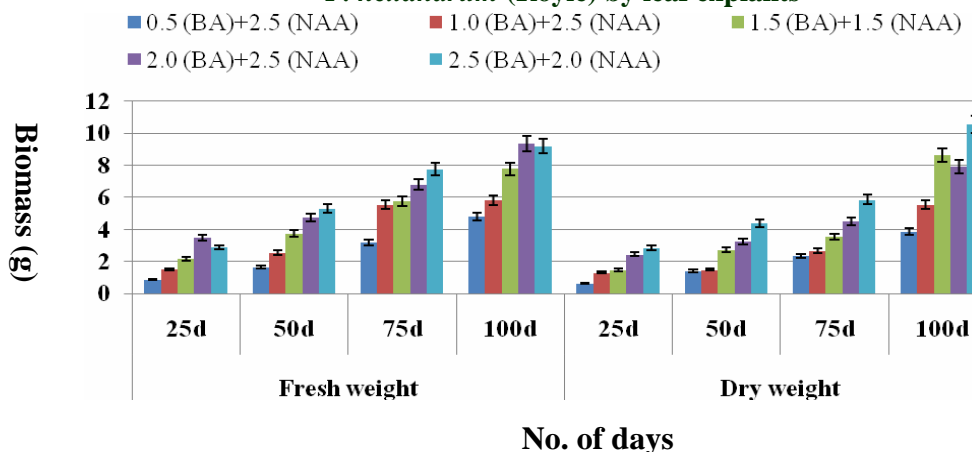
medicinal importance the plant population is declining as a result of its over-exploitation. Callus biomass studies of *P. hexandrum* are in scarce. Therefore, the present investigation deals with optimization of different explants via callusing of *P. hexandrum* to obtain its maximum biomass.

Materials and methods

P. hexandrum explants (leaf, stem and rhizome) collected from the Division of Medicinal and Aromatic Plants (MAP), UHF, Bharsar, Pauri, India. Initially the plant material were washed with tap water followed by washing with 1% (v/v) Labolene detergent for 15 minutes and then in running tap water for 30 minutes. The explants were surface sterilized with an aqueous solution of 0.1% HgCl₂ (w/v) for 2-3 minute. The explants are then rinsed several times with sterilized double distilled water. Damage parts were aseptically trimmed with sterilized surgical blade. All the explants were separately inoculated in the MS basal medium (Murashige and Skoog, 1962) fortified with growth regulators such as auxins [indole-3-acetic acid (IAA), naphthalene acetic acid (NAA)] and cytokinin (6-benzylaminopurine-BA) at varying concentration.

The pH of all the media is adjusted to 5.8±0.1 and it autoclaved. All the cultures were incubated at 24°C±2°C and 60% relative humidity with 16:8 hrs light: dark photoperiod. After different intervals i.e. after 30 days and then at the interval of 25 days onwards up to 100 days after inoculation, fresh and dry biomasses were enumerated. All the treatments were conducted in triplicates and the data are tested statically.

Fig.1 Influence of growth regulator (BA and NAA) on the fresh and dry biomass of *P. hexandrum* (Royle) by leaf explants

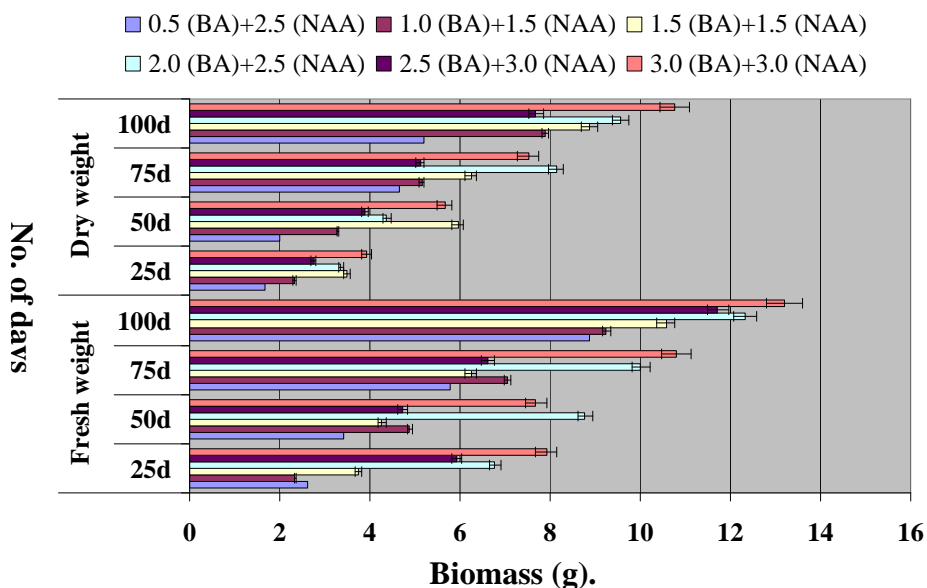


Results and discussion

In leaf explants initially at 25 day stage callus biomass on fresh weight basis was found to be lower in 0.5: 2.5 mg^l⁻¹ (0.89 ± 0.33) and 1.0: 2.5 mg^l⁻¹ (1.54 ± 0.22) of BA and NAA. Higher biomass was noticed in 2.0: 2.5 mg^l⁻¹ (3.52 ± 1.12) followed by 2.5: 2.0 mg^l⁻¹ (2.90 ± 0.88) combinations of BA and NAA at this stage.

Maximum callus biomass on dry weight basis at the end of study was observed in 2.5:2.0 mg^l⁻¹ combination instead of 2.0:2.5 mg^l⁻¹ combination in which fresh weight biomass was highest reflecting the variation in the moisture content (Fig. 1). Next higher value was recorded in 1.5: 1.5 mg^l⁻¹ (8.65 ± 1.56) combinations. In stem explants 0.5 to 3.0 mg^l⁻¹ of BA and 1.5 to 2.5 mg^l⁻¹ NAA was tried for callus induction.

Fig.2 Influence of growth regulator (BA and NAA) on the fresh and dry biomass of *P. hexadrum* (Royle) by rhizome explants



Initial observation of fresh weight revealed the lowest value in 0.5: 2.5 mg^l⁻¹ combinations of BA and NAA (Table 1). Highest biomass was in 2.0: 2.5 mg^l⁻¹ (11.73 ± 2.51) followed by 2.5:2.0 mg^l⁻¹ (10.99 ± 2.51) of BA and NAA combination. 1.5: 1.5 mg^l⁻¹ combinations of these chemicals were found better than lower concentrations of NAA of fresh weight basis.

Rhizome also used as explants for callus induction. Concentration and combination used in initiating callus from rhizome explants were similar to those used in stem explants. BA and NAA at 1.5:1.5 mg^l⁻¹ responded most favorably in this explants during the entire period of observation (Fig. 2). 1.5: 1.5 mg^l⁻¹ and 3.0:3.0 mg^l⁻¹ of BA and NAA also exhibited better response of callusing and callus proliferation. Highest and lowest values on dry weight basis were in the same combinations as observed on fresh weight basis. Second highest values was however, noticed in 2.0:2.5 mg^l⁻¹ combination of

BA and NAA reflecting lower moisture content in the callus as compared to callus of 2.5: 3.0 mg^l⁻¹ combination. Best callusing with respect to concentrations of growth regulators of all three explants were clearly demonstrated in Fig. 3.

Physiological age of the explants for inducing the callus is an important factor determining the morphogenetic response (Snehlata, 2002). Callus biomass measured on fresh and dry weight basis at the interval of 25 days up to 100 days in different explants exhibited that 3.0: 3.0 mg^l⁻¹ of BA and NAA gave best callus mass and thus maximum fresh weight value in the leaf and rhizome explants (Table 1 and Fig. 2). Higher concentrations of BA and NAA reflected similar findings in *Roylea elegans* (Snehlata, 2002). In stem explants 2.0: 2.5 mg^l⁻¹ concentrations of BA and NAA reflected maximum fresh weight at the end of study period. Similar results have been depicted in *Psoralea corylifolia* leaves and stem explants (Saxena et al., 1997). Higher

concentration of BA (1.5:3.0 mg^l⁻¹) in combination with higher concentration of NAA

(1.5:3.0 mg^l⁻¹) responded towards best callusing in the present study.

Fig.3 (A) Callus formation in leaf explants of *P. hexandrum* with 2.0:2.5 mg^l⁻¹ of BA and NAA. (B) Callus formation in stem explants of *P. hexandrum* with 2.5:2.0 mg^l⁻¹ of BA and NAA. (C) Callus formation in rhizome explants of *P. hexandrum* with 3.0:3.0 mg^l⁻¹ of BA and NAA.



Table.1 Effect of growth regulator (BA and NAA) on callus biomass in *P. hexandrum* by stem explants

Concentration (MS + growth regulator) (mg ^l ⁻¹)	Fresh Biomass (g)				Dry Biomass (g)			
	Number of days (d)							
	25d	50d	75d	100d	25d	50d	75d	100d
T ₁	0.89±0.55	1.78±0.63	3.89±1.22	5.65±1.31	0.88±0.23	1.17±0.07	1.79±0.24	1.50±0.44
T ₂	2.54±0.88	3.53±1.26	6.59±1.08	8.16±0.66	1.79±0.66	2.95±0.48	3.21±0.91	4.41±1.46
T ₃	4.18±2.34	7.41±1.62	9.37±1.78	9.22±2.23	3.56±1.46	4.76±2.08	5.36±2.30	7.11±2.24
T ₄	5.39±2.66	7.19±2.45	9.67±2.12	11.73±2.51	2.56±1.11	3.82±1.26	5.27±1.64	9.25±1.53
T ₅	7.83±1.44	5.17±0.82	6.80±0.87	10.99±2.15	3.79±1.12	2.58±0.36	3.41±0.32	6.05±0.56
T ₆	5.14±3.44	8.23±3.38	9.14±3.25	10.85±2.22	2.70±0.91	2.76±0.82	3.97±2.19	8.85±3.49

T₁= 0.5 (BA) + 2.5 (NAA); T₂= 1.0 (BA) +2.5 (NAA); T₃= 1.5 (BA) + 1.5 (NAA); T₄= 2.0 (BA) + 2.5 (NAA); T₅= 2.5 (BA) + 2.0 (NAA); T₆=3.0 (BA) + 2.5); ± sd.

Conclusion

In leaf explant of *P. hexandrum*, higher biomass was noticed in 1.5: 1.5 mg^l⁻¹ followed by 2.5: 2.0 mg^l⁻¹ combinations of BA and IAA. While 2.0 (BA): 2.5 (NAA) mg^l⁻¹ gave maximum biomass in stem and rhizome explants.

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