J. Phytol. Res. **15** (2) : 197-200, 2002

MICROPROPAGATION OF A RARE MEDICINAL PLANT SPECIES - *POLYGONUM MICROCEPHALLUM* D. DON., THROUGH HIGH FREQUENCY SHOOT MULTIPLICATION

JHUMUR DAS and P. J. HANDIQUE*

Department of Biotechnology, Gauhati University, Guwahati - 781014, Assam, India.

*e-mail : pjhandique@sify.com

An *in vitro* regeneration protocol was developed for a rare medicinal plant *Polygonum microcephallum* D. Don. through the nodal explant culture. Shoot regeneration and multiplication is achieved in high frequency on MS medium supplemented with a combination of 3 mg/l BAP and 0.1 mg/l IAA. Microshoots of varied length were successfully rooted in MS medium containing 0.1 mg/l - 0.3 mg/l IAA or IBA. Rooted plants were acclimatized in green house for I month and then was transferred to field condition.

Keywords : High Frequency Shooting; Micropropagation, *Polygonum microcephallum*; Regeneration.

Introduction

Polygonum microcephallum D. Don., (Family : Polygonaceae), locally known as "Madhusuleng" in Assam, is a glabrous herb attaining a height of 3 feet with woody, perennial root stock. Leaves are 3-5 inch long, ovate-lanceolate, abruptly narrowed to the winged petiole, which is auricled at the base. Flowers are in small heads, white in colour, on slender peduncles[1]. The species is widely used in traditional medicinal practices of Assam (India). Cooked aerial portion is eaten in the treatment of pox[2]. Young tops are used for imparting flavour[3]. These are sold as vegetables in rural markets of North East India. The species is indigenous to the North East India but not common in distribution. Small population is occasionally found in household cultivation. Considering the utilization of the species and its increasing rarity due to habitat destruction, an attempt has been made to standardize a protocol for micropropagation of *Polygonum microcephallum*.

Material and Methods

A few young plants of *Polygonum microcephallum* were collected from a natural habitat near Jorhat district (Assam), potted and maintained in the green house. Nodal stem segments (1 - 1.5 cm long) were used as explants to initiate the culture. Expanded leaves were removed and the stem segments were emersed in 100 ml of tap water containing 1-2 drops of Tween 20 (10%) for 5 minutes and later washed thoroughly in running tap water for 30 minutes. Surface sterilization was done in 70% ethanol for 1 minute followed by 0.1% $HgCl_2$ for 10 minutes. These explants were then thoroughly rinsed with double distilled sterile water for 5 minutes and implanted vertically into the nutrient medium. Murashige and Skoog (MS) basal medium[4] with 3% sucrose, 100 mg/l myo - inositol and 0.8% agar was used. MS medium was supplemented with Benzyl adenopurine (BAP), Kinetin (KN) and Indole acetic acid (IAA) in various combinations. The pH of the medium was adjusted to 5.8 before dispensing in 150 x 25 mm culture tubes. Media were autoclaved at 1.06 Kg/cm^2 and 121°C for 15 minutes. For each treatment, 25 replicates were used and each experiment was repeated twice. Cultures were maintained at 25 ± 2 °C under fluorescent light of about 3000 lux with 16 hr. photoperiod. The cultures were maintained by repeated subculture at an interval of 2 weeks and subsequently observations were recorded. *In vitro* grown shoots (3 - 4 cm in length) were cultured on rooting medium to induce root. Rooted shoots were removed from culture tube and washed free of agar. The roots were dipped in 0.2% bavistin fungicide for 5 minutes and plantlets were potted in a sterilized mixture of garden soil, sand and compost (2:1:1). They were

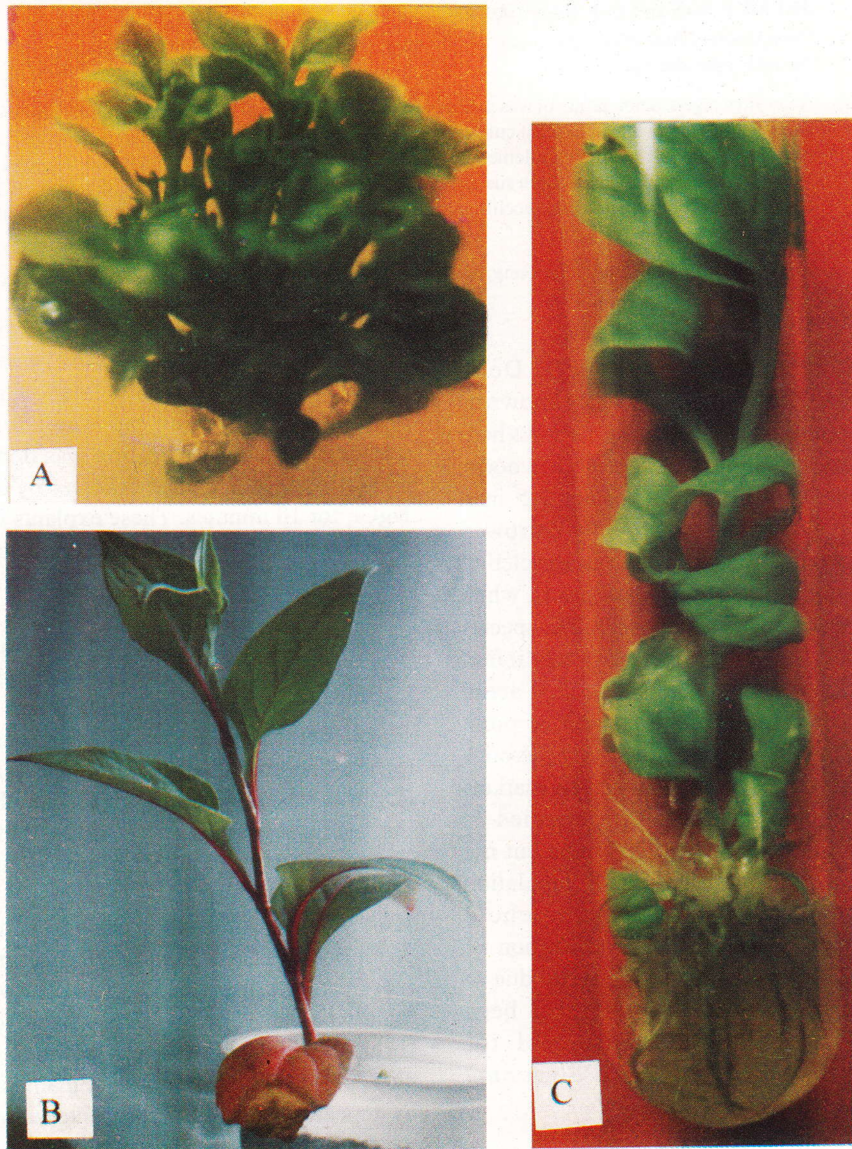

Fig. 1. Various stages of micropropagation of *Polygonum micrpcephallum* D. Don :
A. Multiple shoot development in MS Medium containing 3mg/l BAP and 0.1 mg/l IAA.
B. Rooting of microshoots in MS medium containing 0.3 mg/l IBA.
C. *In vitro* regenerated plantlet on soil pot.

irrigated with half strength MS solution for 1 week and subsequently with tap water. The potted plants were kept under shade condition inside a polythene tent to avoid desiccation and after 2 week of acclimatization they were transferred outdoor.

Results and Discussion

Initially establishment of culture was difficult due to a higher rate of fungal contamination, whcih was later eliminated by treatment of explants with 1% Bavistin for 30 minutes followed by 70% ethanol for 1 min. and 0.1% $HgCl_2$ for 10 minutes. Development of first shoot was recorded after 5 days of inoculation in 75% of the cultures in MS medium supplemented with various concentrations of growth regulators. The control also showed the development of first shoot but with no further growth. This indicates that formation and development of new shoots solely depends on the presence of optimum growth regulator levels in the medium. Therefore, different combination of BAP, KN and IAA were tried. Among this combination a maximum of 15 numbers of multiple shoots were recorded in MS medium containing 3mg/l BAP and 0.1 mg/l IAA in combination (Fig. 1A). The average number of microshoots developed in this medium was 13.2 per explant. It was observed that a cytokinin in combination with auxin gives better results than with another cytokinin in tissue regeneration of *P. microcephallum* (Table 1). A multiple number of shoot initiations was also observed in MS medium containing BAP and KN, but no further growth occurred even after subsequent sub culturing in the same medium. Decline of these cultures occurred due to browning and necrosis. The growth further occurred when shoots were transferred to the medium containing 3 mg/l BAP and 0.1 mg/l IAA. This observation supports that addition of IAA at a low concentration with a comparatively higher concentration of BAP helps in shoot multiplication and sustenance of shoot growth. Similar results were also reported in *Adhatoda beddomei*[5]. Combination of BAP and KN in lower concentration of 0.5 mg/l each gave no multiple shoots. A combination of higher concentration of BAP (2 mg/l) and lower concentration of KN (0.5 mg/l) gave 3.8

Table 1. Shoot multiplication in nodal explants of *P. microcephallum* on MS medium containing BAP, IAA and KN in various combination.

Concentration (mg/l)		% of explants with > 1 shoot per explant	*Mean number of shoot ± S.E. (after 6 weeks of culture)
BAP	**IAA**		
3	0.05	100	11.7 ± 0.40
3	0.10	100	13.2 ± 0.46
3	0.20	72.7	6.5 ± 0.22
BAP	**KN**		
0.5	0.5	72.7	6.1 ± 0.27
2.0	0.5	50	3.8 ± 0.19
0.5	2.0	20	0.25 ± 0.35
1.0	1.0	0	0
Control		0	0

* Average of 25 replicates repeated twice
S.E. = Standard errors of mean

numbers of shoots where as a lower BAP concentration (0.5 mg/l) and higher concentration of KN (2mg/l) gave a negligible average of 0.25 number of shoots per explant.

Root initiation took place after one week of culture of microshoots in MS medium containing 0.1 and 0.3 mg/l either IAA or IBA. However, IBA at a concentration of 0.3 mg/l in MS medium stimulated the development of a maximum average of 7.9 numbers of roots with a mean length of 8 cm within 3 weeks (Fig. 1B). This result is in accordance with the report in *Morus nigra*[6]. *In vitro* regenerated plantlets were acclimatized in green house for 2 weeks and then transferred to field condition as well as on big earthen pots (Fig. 1C). The survival percentage was 75.

This is the first report on *in vitro* culture and regeneration of *Polygonum microcephallum*. The protocol developed through this study can be utilized in the mass propagation programme of this species.

Acknowledgement

Financial assistance from DBT, New Delhi is duly acknowledged.

References

1. Kanjilal U N, Kanjilal P C and Das A 1940, *Flora of Assam*, Vol-IV, Shillong, pp 18-19.
2. Handique P J 1994, *Ethno-medico-botanical studies of the indigenous medicinal plants of Golaghat and Jorhat districts of Assam*, ASTEC Project Report, Guwahati, 101
3. CSIR 1992, *The Useful plants of India* (reprints), PID, New Delhi
4. Murashige T and Skoog F 1962, *Physiol. Plant* **15** 473
5. Sudha G and Seeni S 1994, *Plant Cell Report* **13** 203
6. Yadav U, Lal M and Jaiswal V S 1990, *Scientia Horticulture* **44** 61