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In vitro Propagation of *Polygonum hydropiper* L. from Shoot Tips

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Abstract

An efficient protocol for plant regeneration through multiple shoots induction from shoot tips of *Polygonum hydropiper* (L.) was established. The highest percentage (96.6) of multiple shoot induction and number of shoots (9.0) per culture were found on MS supplemented with 2.0 mg/l Kn. The induced shoots were excised and inoculated on to MS contains different concentrations of IBA or NAA for rooting. The highest percentage (90.0) of root induction and the highest number of roots per shoot (12.0) was found on MS having 1.0 mg/l IBA. Well rooted plantlets were acclimated properly and transplanted in the soil under natural condition, where cent per cent plantlets survived and grew successfully.

Introduction

Polygonum hydropiper L. commonly known as 'Bishkanthali' in Bangladesh is an important medicinal plant belonging to Polygonaceae. It is an erect, stout annual Nepalese herb, naturalized in Bangladesh, with narrow lanceolate leaves and pinkish white small flowers in racemes (Datta et al. 2000), common in moist grounds, blooming from June to July (Sharma 2003). The plant leaves yield an essential oil contains oxymethyl-anthraquinones, polygonic acid and catchins-dihydroflavonol 4-reductase, flavanon 3–hydroxylase (Furukawa et al. 2002). It also contains about 7.5% protein, 1.9% fat, 8% carbohydrate and 2% ash (Raid 1977). The plant has insecticidal properties (Mollah and Islam 2005, Kundu et al. 2007), antibacterial (Hoque et al. 1989) and antifungal (Haraguchi et al. 1993). The whole plant, either on its own or mixed with other herbs, is decocted and used in the treatment of a wide range of ailments including diarrhoea, dyspepsia, itching skin, excessive menstrual bleeding and haemorrhoids (Chevallier 1996). The plant also possesses bitter, stimulant, tonic, diuretic, carminative, anthelmintic, emmeragogue, haemostatic and lithotripter properties (Sharma 2003).

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Tissue culture protocols have been extensively used for the *in vitro* propagation, germplasm conservation and production of pharmaceutically important bioactive compounds (Nalawade et al. 2003, Phatak and Heble 2002). Genetically homogenous plants with uniform contents of secondary metabolites can be obtained by *in vitro* propagation of plants either by somatic embryogenesis or shoot organogenesis (Lin et al. 2003). To date, however, there are no reports on the rapid *in vitro* propagation system for *P. hydropiper* from shoot tips would help in conserving the germplasm and allowing the commercial cultivation of this medicinally important species. This investigation deals with the standardization of a protocol for *in vitro* propagation through multiple shoot proliferation. The protocol provides rapid proliferation of multiple shoots from shoot tips with comparatively a reduced requirement of plant growth regulators and successful acclimation of plants in the natural condition. The performance of regenerated plants was also evaluated in the field condition.

Materials and Methods

The shoot tips of *Polygonum hydropiper* L. from mature plants were collected from the Rajshahi University Campus, Rajshahi, Bangladesh. They were washed first under running tap water for 30 minutes and treated with 1% Tween-80 for ten min followed by repeated rinsing with autoclave-distilled water. Further sterilization was done under aseptic conditions in a Laminar Airflow Hood. Explants were surface sterilized with 0.1% (w/v) $HgCl_2$ for ten min. Finally, the explants were washed thoroughly with autoclave-distilled water for several times to remove traces of $HgCl_2$. The shoot tips were cut into 1 cm pieces and cultured on MS with different hormonal supplements. Throughout the experiments MS with 3% (w/v) sucrose and gelled with 0.8% (w/v) agar was used. The pH of all media (supplemented with respective growth regulators) was adjusted to 5.8 with 1N NaOH or 1N HCl prior to autoclaving (21 min at 121ºC). The cultures were incubated in a culture room at 25 ± 2ºC with a photoperiod of 16 hr at 3000-lux light intensity provided by cool white fluorescent tubes. In this investigation, the basal medium was supplemented with different concentrations and combinations of auxin and cytokinin for multiple shoot induction. Root induction from the shoots was achieved on MS supplemented with NAA/IBA at different concentrations. After 35 days, well-rooted plantlets were obtained. Subsequently, the plantlets were removed from the culture vessels, washed gently under running tap water and planted in plastic pots containing sterile sand, soil and humus in the ratio of 1 : 2 : 2. The potted plantlets were covered by polythene bag to maintain suitable humidity. After 30 - 35 days of bagging, plantlets were gradually exposed to the normal

conditions and transferred to the natural condition where cent per cent plants survived and grown successfully.

Results and Discussion

For successful *in vitro* propagation, shoot tips are the preferred explants as they possess pre-existing meristem that is easily developed into shoots while maintaining clonal fidelity. *In vitro* propagation using shoot tips culture has been reported for a large number of plant species including *Penthorum chinense* (Cao et al. 2007), *Spilanthes acmella* (Haw and Keng 2003) and *Prosopis chilensis* (Caro et al. 2002). So shoot tips, each having one or more axillary buds were used in this study. Shoot proliferation could be initiated from explants on MS containing different concentrations and combinations of auxin and cytokinin. Multiple shoot initiation from shoot tips was observed within ten days of inoculation. The highest percentages of multiple shoot induction was 96.6 and 76.6 found on MS containing of 2.0 and 2.5 mg/l Kn, respectively. The highest number of shoots per axillary shoot was 9.0 followed by 7.0 on MS having 2 and 2.5 mg/l Kn, respectively (Table 1, Fig. 1A). On the other hand, the lowest percentage (13.3) of shoot 13.3 found and the lowest number of shoot (2.3) per axillary were found on MS containing of 0.5 mg/l BAP. The induced shoots were elongated in the same medium. Similar results were also reported in *Penthorum chinense* (Cao et al. 2007) and in *Bupleurum kaoi* (Chen et al. 2006).

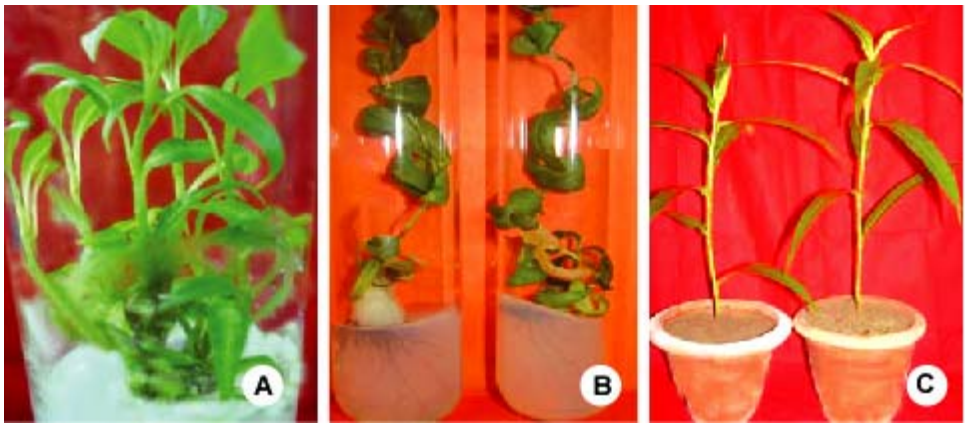

Fig. 1. A. Multiple shoots elongation on MS with 2.0 mg/l Kn. B. Initiation and elongation of roots on MS with 1.0 mg/l IBA. C. *In vitro* regenerated plantlets in natural condition.

IBA and NAA are commonly used for root induction *in vitro* (Karuppusamy et al. 2006, Cao et al. 2007, Khalafalla and Daffalla 2008). In this study, elongated shoots of *P. hydropiper* were isolated and cultured in the rooting medium with different concentrations of NAA or IBA, where root formation was observed

after two weeks of inoculation. The highest percentage of root induction (90.0) and the highest number of roots per shoot (12.0) was recorded on the medium supplemented with 1.0 mg/l IBA. On the contrary, the lowest percentage of root induction (23.3) and the lowest number of roots per shoot (2.0) were found on the

Table 1. Effect of different concentrations and combinations of BAP, Kn and NAA in MS on multiple shoot proliferation.

Growth regulators (mg/l)	% of culture response ($\bar{x} \pm SE$)	No. of shoots/ culture ($\bar{x} \pm SE$)	Shoot length (cm) ($\bar{x} \pm SE$)
BAP			
0.5	13.3 ± 2.3	2.3 ± 0.2	4.4 ± 0.8
1.0	26.6 ± 2.0	2.6 ± 0.3	5.3 ± 0.3
1.5	46.6 ± 1.4	3.6 ± 0.4	5.3 ± 0.3
2.0	**56.6 ± 2.2**	**4.0 ± 1.0**	**5.6 ± 0.6**
2.5	43.3 ± 1.9	3.3 ± 0.5	5.0 ± 1.0
3.0	26.6 ± 0.9	2.6 ± 0.6	4.3 ± 0.6
BAP + NAA			
0.5 + 0.2	26.6 ± 0.9	3.5 ± 1.2	5.2 ± 0.9
1.0 + 0.2	46.6 ± 1.4	4.0 ± 1.0	6.0 ± 1.0
1.5 + 0.2	46.6 ± 1.4	4.6 ± 0.9	6.8 ± 0.8
2.0 + 0.2	**70.0 ± 0.0**	**6.0 ± 1.0**	**8.2 ± 1.2**
2.5 + 0.2	46.6 ± 1.4	5.2 ± 1.4	8.0 ± 1.0
3.0 + 0.2	23.0 ± 1.0	3.0 ± 1.0	6.7 ± 0.7
Kn			
0.5	26.6 ± 0.9	4.0 ± 1.0	4.4 ± 0.8
1.0	46.6 ± 1.4	4.0 ± 1.0	5.3 ± 1.3
1.5	60.0 ± 1.0	6.6 ± 0.6	7.8 ± 1.3
2.0	**96.6 ± 0.6**	**9.0 ± 0.5**	**8.5 ± 1.2**
2.5	76.6 ± 2.1	7.0 ± 1.0	7.6 ± 0.8
3.0	46.6 ± 1.4	4.9 ± 0.9	5.0 ± 1.0

The experiments were repeated thrice, each experiment consisting of ten replicates.

medium consisting of 3.0 mg/l NAA (Table 2). In the treatments of NAA, the induced roots were short and thick. On the other hand, in the treatments of IBA, the induced roots were long and thin (Fig. B). Smilar result has been reported for *in vitro* rooting of shoots in several medicinal plants species like *Polygonum multiflorumr* (Lin et al. 2003), *Solanum trilobatum* (Jawahar et al. 2004), *Plumbaga zeylanica* (Chaplot et al. 2006) and *Cassia alata* (Hasan et al. 2008).

The ultimate success of *in vitro* propagation lies in successful establishment of plants in the soil. Normally, in the absence of greenhouse facilities *in vitro* plantlets loose tremendous amount of water through leaf surfaces with poorly deposited cuticular wax and poorly developed or non-active stomatal system (Wardley et al. 1983). In the present investigation, well-rooted plantlets were

Table 2. Effect of different concentrations of NAA and IBA on rooting.

Growth regulators (mg/l)	% of culture response ($\bar{x} \pm SE$)	No. of roots/ shoot ($\bar{x} \pm SE$)	Root length (cm) ($\bar{x} \pm SE$)	Nature of roots
NAA				
0.5	30.0 ± 0.0	2.5 ± 0.9	2.0 ± 1.0	Short, thick
1.0	43.3 ± 1.9	4.0 ± 1.0	2.7 ± 0.8	"
1.5	66.6 ± 2.0	4.2 ± 0.7	3.0 ± 1.0	"
2.0	**80.0 ± 0.0**	**10.0 ± 1.0**	**3.5 ± 1.3**	"
2.5	46.6 ± 1.4	5.9 ± 1.4	3.2 ± 1.3	"
3.0	23.3 ± 1.2	2.0 ± 1.0	2.1 ± 0.9	"
IBA				
0.1	56.6 ± 2.2	4.0 ± 1.0	4.0 ± 1.0	Long, thin
0.5	73.3 ± 1.6	6.4 ± 1.2	5.0 ± 1.0	"
1.0	**90.0 ± 0.0**	**12.0 ± 1.0**	**6.8 ± 1.6**	"
1.5	66.6 ± 1.0	6.9 ± 1.5	5.0 ± 1.0	"
2.0	43.3 ± 1.9	5.0 ± 1.0	4.3 ± 0.6	"
2.5	26.6 ± 0.9	4.2 ± 1.6	3.9 ± 0.9	"

The experiments were repeated thrice, each experiment consisting of ten replicates.

gradually acclimated and transplanted in the natural condition (Fig. 1C), where cent per cent plants were survived. In the present study, the high survival rate of *in vitro* plantlets of *P. hydropiper* indicates that this procedure could be easily adopted for large scale multiplication and cultivation. The *in vitro* propagated plantlets resembled the general growth and phenotypically indistinguishable from donor plants.

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References

Cao H, Yang J, Peng ZS, Kang CY, Chen DC, Gong ZC and **Tan X** (2007) Micropropagation of Penthorum chinense through axillary bud. In Vitro Cell Dev. Biol. Plant. **43**: 149-153.

Caro LA, Lindstrom PA, Echenique CV and **Hernandez LF** (2002) Micropropagation of *Prosopis chilensis* (Mol.) Stuntz from young and mature plants. Biocell **26**: 25-33.

Chaplot BB, Dave AM and **Jasrai YT** (2006) A valued medicinal plant - Chitrak (*Plumbaga zeylanica* Linn.): Successful plant regeneration through various explants and field performance. Plant Tissue Cult. & Biotech. **16**(2): 77-84.

Chen U, Hsia C, Yeh M, Agrawal DC and **Tsay H** (2006) *In vitro* micropropagation and *ex vitro* acclimation of *Bupleurum kaoi* - An endangered medicinal plant native to Taiwan. In Vitro Cell Dev. Biol. Plant. **42**: 128-133.

Chevallier A (1996) The Encyclopedia of Medicinal Plants. Dorling Kindersley, London. p. 185.

Datta BK, Datta SK and **Sarker SD** (2000) Quercetin 3-O-(6′-galloyl)-beta-D-galactoside from *Polygonum viscosum* (Polygonaceae). Biochem. Syst. Ecol. **28**: 805-807.

Furukawa T, Eshima A, Kouya M, Takio S, Takano H and **One K** (2002) Coordinate expression of genes involved in catchin biosynthesis in *Polygonum hydropiper* cells. Plant Cell Rep. **21**: 385-389.

Haraguchi H, Matsuda R and **Hashimoto K** (1993) HPLC determination of sesquiterpene dialdihydes and antifungal activity from *Polygonum hydropiper.* J. Agric. Food Chem. **41**(1)**:** 5-7.

Hasan MF, Das R, Rahman MS, Hossain MS and **Rahman M** (2008) Micropropagation from shoot tips and nodal segments of *Cassia alata* L. Intl. J. Bio-Res. **4**(4): 70-74.

Haw AB and **Keng CL** (2003) Micropropagation of *Spilanthes acmella* L.- A bio-insecticide plant, through proliferation of multiple shoots. J. Appl. Hort. **5**: 65-68.

Hoque MM, Hasan MA and **Khan MR** (1989) Screening of plants available in *Polygonum* L. Bangladesh J. Bot. **18**: 141-146.

Jawahar M, Rebert GA and **Jeyaseelan M** (2004) Rapid proliferation of multiple shoots in *Solanum trilobatum* L. Plant Tissue Cult. **14**(2): 107-112.

Karuppusamy S, Kiranmai C, Aruna V and **Pullaiah T** (2006) Micropropagation of *Vanasusha peduta* - An endangered medicinal plant of South India. Plant Tissue Cult. & Biotech. **16**(2)**:** 85-94.

Khalafalla MM and **Daffalla HM** (2008) *In vitro* micropropagation and micrografting on gum Arabic tree (*Acacia senegal* (L.) Wild.) Int. J. Sustain. Crop Prod. **3**(1): 19-27.

Kundu BR, Ara R, Begum MM and **Sarkar ZI** (2007) Effect of Bishkatali, *Polygonum hydropiper* L. plant extracts against the red flour bettle, *Tribolium castaneum* Herbst. Univ. J. Zool. Rajshahi Univ. **26**: 93-97.

Lin LC, Nalawade SM, Mulabagal V, Yeh MS and **Tsay HS** (2003) Micropropagation of *Polygonum multiflorum* THUNB and quantitative analysis of the anthraquinones, emodin and physcion formed in *in vitro* propagated shoots and plants. Biol. Pharm. Bull. **26**: 1467-1471.

Mollah JU and **Islam W** (2005) Effect of *Polygonum hydropiper* L. extracts on the oviposition and egg viability of *Callosobruchus cinensis* F. (Coleoptera: Bruchidae). J. Bio-Sci. **12**: 101-109.

Nalawade SM, Sagar AP, Lee CY, Kao CL and **Tsay HS** (2003) Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. Bot. Bull. Acad. Sin. **44**: 79-98.

Phatak SV and **Heble MR** (2002) Organogenesis and terpenoid from plant tissue culture. Oxford: Claredon Press, pp. 1-21.

Raid BE (1977) Famine foods of the Chiu-Huang Pen-ts'ao. Southern Materials Center, Taipei.

Sharma R (2003) Medicinal plants of India-An encyclopedia. Daya Publishing House, Delhi-110035. pp. 46-47.

Wardley K, Dobbs EB and **Short KC** (1983) *In vitro* acclimatization of aseptically cultured plantlets to humidity. J. Amer. Soc. Hort. Sci. **108**: 386-389.