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Plant regeneration through callus induction from nodal segments of *Polygonum hydropiper* L., an important medicinal plant

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Abstract:

The investigation was carried out to observed callus induction and subsequent regeneration potentiality of hydropepper (Polygonum hydropiper) from nodal segments of mature plants. Greenish friable callus was achieved from nodal segment on MS medium supplemented with 5.0mgl^{-1}2,4-D within two weeks of inoculation. The callus produced large number of shoots when cultured on MS medium with 5.0 mgl^{-1}2,4- D +0.2 mgl^{-1}Kn within 10 days of culture. In vitro raised shoots were rooted on MS medium with 1.0 mgl^{-1}IBA within 15 days of culture. In vitro grown plantlets with well root system were successfully established in natural condition through successive phages of acclimatization. The survival rate of plantlets was found to be 100% in the natural condition.

Key words: Polygonum, Callus, regeneration, nodal segments.

1. Introduction

Hydropepper *(Polygonum hydropiper)* is an erect, stout annual Nepalese herb, naturalized in Bangladesh, with narrow lanceolate leaves and pinkish white small flowers in racemes, found to grow commonly in damp places (Datta *et al*, 2000a). The whole plant has been found to contain a flavones and flavonoid glycosides, such as quercetin galactosides, a sesquiterpene acid, viscosumic acid, oxymethyl- anthraquinones and polygonic acid (Datta *et al*, 2000b). The plants also have some insecticidal properties (Kundu *et al*, 2007). 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. In nature, propagation through seed is unreliable due to poor seed quality, erratic germination and seedling mortality as under natural field conditions. Extensive and destructive harvesting of

plants by the pharmaceutical industries for procurement of naturally occurring secondary metabolites (Flavonoid and Flavones) from the plant and insufficient attempts to either allow its replenishment or its cultivation have led to the depletion of the natural plant population. *In vitro* propagation has proven as a potential technique for mass scale production of medicinal plant species (Azad *et al*, 2005; Hassan and Roy, 2005). So far our knowledge goes, no report has been publish on *in vitro* propagation of hydropepper. The protocol provides rapid proliferation of shoots from nodal segments derived callus with comparatively a reduced requirement of plant growth regulators and successful acclimatization of plants in the soil.

2. Materials and methods

2.1 Materials

2.1.1 Plant material

For the present investigation, the nodal explants of hydropepper from mature plants were collected from Rajshahi University Campus.

2.2 Methods

2.2.1 Sterilization

Explants were surface sterilized with 0.1% (W/V) $HgCl_2$ for 10 minutes. Finally, the explants were washed thoroughly with autoclave-distilled water for several times to remove traces of $HgCl_2$.

2.2.2 Inoculation and callus induction

Nodal segments of hydropepper were cut into appropriate size of 1.0cm and cultured on MS medium (Murashige and Skoog, 1962) supplemented with different concentrations and combinations of auxin (2,4-D, NAA) and cytokinin (BAP, Kn). Throughout the experiments full strength MS medium with 3% (W/V) sucrose and gelled with 0.8% (W/V) agar. The pH of all media was adjusted to 5.8 prior to autoclaving (21 min). The cultures were incubated in a culture room at 25±2°C with

a photoperiod of 16 hour at 3000-lux light intensity.

2.2.3 Shoot induction and elongation

Once the callus developed, they were further cultured for regeneration and elongation in the medium having different concentrations and combinations of auxin and cytokinin.

2.2.4 Root induction and elongation

Elongated shoots were rooted on MS medium supplemented with different concentrations of auxin (NAA and IBA) singly. The experiment was laid out in Complete Randomized Design (CRD) with 10 replications.

2.2.5 Acclimatization

Well developed plantlets were planted in plastic pots containing a potting mixture of sand, soil and farmyard manure (1:2:2). The potted plantlets were covered by transparent polythene sheet to maintain high humidity and within 15-20 days new leaves were emerged from the plantlets that resumed new growth. Plantlets were gradually exposed to the normal conditions and transferred to the natural condition.

3. Results and discussion

3.1 Callus induction and maturation

Callus induction was observed within 10 to 18 days of culture from the cut surface of the nodal explants (Table 1; Fig. 1. A- B). The highest percentage of callus induction from nodal explants was 96.6% onto the medium consisting of 5.0 mgl^{-1} 2,4-D followed by 80.0% on the medium having 4.5 mgl^{-1} 2,4-D. On the left hand the lowest percentage of callus induction was 13.3% on MS medium having 0.1 mgl^{-1} BAP+1.0 mgl^{-1}NAA. In these treatments the induced calli were greenish in color and structurally nodular (Fig. 1. C). Similar results were reported in *Polygonum multiflorum* (Lin *et al*, 2003), *Withania somnifera* (Rani and Grover, 1999) and *Physalis pubescens* (Rao *et al*, 2004), which support our present investigation findings.

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Table 1. Effect of different concentrations and combinations of auxin and cytokinin in callus induction.

Growth regulators	Days of callus initiation	% of culture response (M±SE)	Fresh wt. of callus (g)	Nature of callus
BAP+NAA				
0.1+1.0	13-18	13.3±0.7	1.8±0.8	Greenish, friable
0.3+2.0	**10-14**	**63.3±1.2**	**2.0±1.0**	Greenish, friable
0.5+3.0	16-18	23.3±0.7	1.6±0.6	Greenish, friable
2,4-D				
3.5	14-16	36.6±0.9	1.8±0.8	Greenish, friable
4.0	13-14	46.6±0.4	1.9±1.1	Greenish, friable
4.5	12-15	80.0±0.8	1.9±0.9	Greenish, friable
5.0	**10-15**	**96.6±0.6**	**2.02±1.2**	Greenish, friable
5.5	13-16	73.3±1.3	2.0±1.0	Greenish, friable
6.0	15-18	26.6±1.4	1.8±0.9	Greenish, friable
2,4 -D+BAP				
5.0+0.1	11-18	26.6±0.6	1.7±1.7	Greenish, friable
5.0+0.2	13-19	36.6±1.4	2.0±1.0	Greenish, friable
5.0+0.3	**12-16**	**70.0±0.0**	**2.0±1.0**	Greenish, friable
5.0+0.4	14-19	50.0±0.4	1.6±1.1	Greenish, friable
5.0+0.5	12-16	50.0±0.4	1.6±0.9	Greenish, friable

Note: The experiments were repeated thrice, each experiment consisting of 10 replicates.

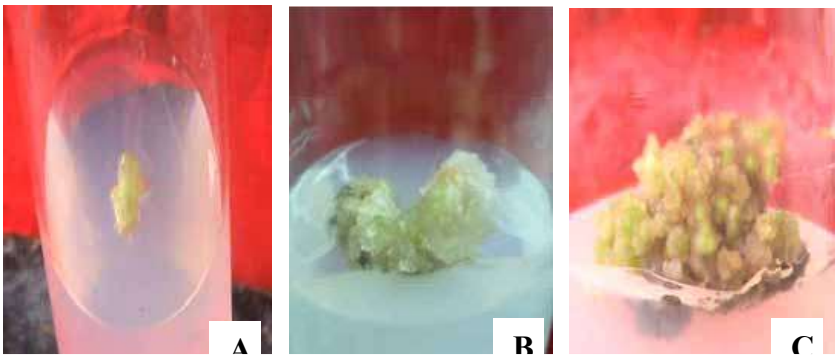

Fig. 1. *In vitro* callus induction from nodal segments of hydropepper.

A. Explant inoculation, B. Initiation of callus (10 days) and C. Mature callus (35

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3.2 Shoot induction and elongation

The highest percentage of shoot induction was 96.6% on the medium consisting of 5.0 mgl^{-1} 2,4-D+0.2 mgl^{-1}Kn followed by 83.3% on the medium consisting of 0.3 mgl^{-1} BAP+2.0 mgl^{-1} NAA+0.1 mgl^{-1} Kn (Table 2). The highest number of shoots per callus was 8.0 in MS medium having 5.0 mgl^{-1} 2,4-D+0.2 mgl^{-1}Kn (Fig.2.A) followed by 6.0 in MS medium having 4.5 mgl^{-1} 2,4-D+0.2 mgl^{-1}Kn. On the contrary, the lowest percentage of shoot induction was 13.3% in the medium having 0.5 mgl^{-1} BAP+3.0 mgl^{-1} NAA+0.1 mgl^{-1} Kn and the lowest number of shoot induction was 2.0 in the medium having 0.1 mgl^{-1} BAP+1.0 mgl^{-1}NAA+0.1 mgl^{-1} Kn. Thus 5.0 mgl^{-1} 2,4-D+0.2 mgl^{-1} Kn was found to be an ideal treatment for shoot induction as well as elongation. Similar results were found in several medicinal plant species including *Withania somnifera* (Rani and Grover, 1999), *Polygonum multiflorum* (Lin *et al*, 2003), *Physalis pubescens* (Rao *et al*, 2004) and *Acmella calva* (Senthilkumar *et al*, 2007). This results support our present investigations.

Table 2. Effect of different concentrations and combinations of auxin and cytokinin in MS medium for shoot proliferation.

Growth regulators (mgl^{-1})	% of culture response (M±SE)	No. of shoots /callus (cm), (M±SE)	Shoot length (cm) (M±SE)
BAP+NAA+Kn			
0.1+1.0+0.1	23.3±0.4	2.0±1.1	1.8±1.1
0.3+2.0+0.1	**83.3±0.7**	**4.0±1.0**	**4.0±1.0**
0.5+3.0+0.1	13.3±0.7	3.0±1.0	3.0±1.0

2,4-D+Kn			
3.5+0.2	23.3±0.6	2.6±0.6	2.6±0.8
4.0+0.2	46.6±1.4	4.0±1.0	3.0±1.0
4.5+0.2	73.3±0.7	6.0±1.0	3.6±1.6
5.0+0.2	**96.6±0.4**	**8.0±1.0**	**4.0±1.0**
5.5+0.2	60.0±1.4	4.0±1.0	3.8±1.2
6.0+0.2	26.6±0.8	3.0±1.0	3.0±1.0
2,4 –D+BAP+Kn			
5.0+0.1+0.3	23.3±0.7	2.6±0.6	2.5±1.3
5.0+0.2+0.3	46.6±1.4	3.2±1.3	2.9±1.1
5.0+0.3+0.3	**70.0±0.0**	**4.0±1.0**	**3.9±1.4**
5.0+0.4+0.3	60.0±0.0	2.6±1.3	3.5±1.1
5.0+0.5+0.3	26.6±0.4	2.0±1.0	3.0±1.0

Note: The experiments were repeated thrice, each experiment consisting of 10 replicates.

3.3 Root induction and elongation

The highest percentage of root induction was 93.3% on the medium consisting of 1.0 mgl^{-1} IBA followed by 80.0% on the medium having 2.0 mgl^{-1} NAA (Fig. 2. B). The highest number of roots per shoot was 9.0 in the MS medium having 1.0 mgl^{-1} IBA (Fig. 2.C) followed by 7.0 in the MS medium having 2.0 mgl^{-1}NAA (Table 3). On the left hand, the lowest percentage of root induction was 23.3% in the medium having 3.0 mgl^{-1} NAA and the lowest number of root induction was 2.0 in the medium having 0.5 mgl^{-1} NAA. Thus 1.0 mgl^{-1}IBA was found to be an ideal treatment for root induction as well as shoot elongation. After 30 days, well-rooted plantlets were achieved. Biswas *et al*, (2007) in *Abrus precatorius*, Chaplot *et al*, (2006) and Verma *et al*, (2002) in *Plumbaga zelanica* reported similar results for root induction. Pillai and Gangaprasad (2018) also found the same result on ½ strength MS medium supplemented with 0.2mgL-1IAA for rooting which support our present findings.

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Table 3. Effect of different concentrations of NAA and IBA on root induction from callus derived shoots.

Growth regulators (mgl^{-1})	% of culture response (M±SE)	No. of roots/nodal segment (M±SE)	Root length (cm) (M±SE)
NAA			
0.5	26.6±1.4	2.7±0.6	2.0±1.0
1.0	46.6±1.4	3.8±0.8	2.9±0.9
1.5	73.3±0.8	4.6±0.6	3.6±1.3
2.0	**80.0±1.4**	**7.0±0.3**	**4.7±0.9**
2.5	43.3±0.7	5.0±1.0	4.6±1.4
3.0	23.3±0.7	3.0±1.0	3.0±1.0
IBA			
0.1	46.6±1.4	4.0±1.0	3.4±0.6
0.5	66.6±1.4	5.9±0.9	4.7±0.7
1.0	**93.3±0.9**	**9.0±1.0**	**5.0±1.0**
1.5	53.3±1.3	7.0±1.0	4.3±1.3
2.0	36.6±2.1	5.6±1.2	3.8±0.8
2.5	26.6±1.6	4.2±0.9	2.8±1.1

Note: The experiments were repeated thrice, each experiment consisting of 10 replicates.

3.4 Acclimatization

The well rooted plantlets were planted in small pots containing potting mixture of sterile sand, soil and farmyard manure in the ratio of 1:2:2 (Fig. 2. D). The potted plantlets were covered by transparent polythene sheet to maintain high humidity and within 15-20 days new leaves were

emerged from the plantlets that resumed new growth. After 50-55 days, the plants were transplanted in the natural condition, where 100% plants were survived and grown successfully. The same results were found by Sarker *et al*, (2015) in *Citrus aurantifollia* plant. Kaushal *et al*, (2014), Deshpande and Bhalsing (2015) and Haque *et al*, (2013) also reported the similar survival rate of hardening for different plantlets which support our present findings.

Fig. 2. Plant regeneration and establishment of *in vitro* grown hydropepper, A. Shoot initiation from callus (10 days), B. The inoculated single shoot for root induction, C. Root induction and elongation (35) and D. *In vitro* regenerated plantlets in field condition (60 days).

4. Conclusion

In this investigation, a reproducible protocol for plant regeneration was established through callus induction from nodal explants in hydropepper. It is expected that a standard protocol to induce callus and rapid proliferation of shoots through in vitro culture would provide a more homogeneous source of medicine.

5. Acknowledgements

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6. Author Contributions

MFH, MEKC and MMR designed the

experiments, developed the methodology and prepared the manuscript. MFH, MEKH, SA and MMR collected the data and carried out analysis. SA and MMR assisted with manuscript preparation.

7. Author statement

All authors read, reviewed, agree and approved the final manuscript

8. Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper

9. Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

10. References

[1]. Azad MAK, Yokota S, Ohkubo T, Andoh Y, Yahara S and Yoshizawa N. 2005. In vitro regeneration of medicinal woody plant *Phellodendron amurense* Rupr. through excised leaves. Plant Cell Tissue and Organ Culture, 80: 43-50.

[2]. Biswas A, Roy M, Bari Miah MA and Bhadra SK. 2007. In vitro propagation of *Abrus precatorius* L. - A Rare Medicinal Plant of Chittagong Hill Tracts. Plant Tissue Culture & Biotechnology, 17(1): 59-64.

[3]. 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 Culture & Biotechnology, 16(2): 77-84.

[4]. Datta BK, Datta SK and Sarker SD. 2000a. Biochemical Systematics and Ecology, 28: 805-807.

[5]. Datta BK, Datta SK, Rashid MA, Nash RJ and Sarker SD. 2000b. Phytochemistry. 54: 201-2005.

[6]. Deshpande HA, and Bhalsing SR. 2015. *Helicteres isora* L: regeneration through meristem culture. Acta Biol Szeged, 59(1): 55-58.

[7]. Haque ME, Rezwana D, Islam MI, and Sikdar B. 2013. In vitro regeneration in

snake gourd (*Tricosanthes anguina*) from shoot apical meristem and young leaves. J Crop and Weed. 9(1): 124-127.

[8]. Hassan AKMS and Roy SK. 2005. Micropropagation of Gloriosa superba L.through high frequency shoot proliferation. Plant Tissue Culture, 15 (1): 67-74.

[9]. Kaushal S, Sidana A, and Dev K. 2014. In vitro plant production through apical meristem culture of *Gentiana kurroo* Royle. Journal of Medicinal Plants Studies, 3(1): 4-9.

[10]. 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.

[11]. 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. Biology and Pharmacology Bulletin, 26: 1467-1471.

[12]. Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiology of Plant 15: 473-497.

[13]. Pillai A R, and Gangaprasad A. 2018. Adventitious Root Derived Callus Culture and Anthraquinone Production in *Gynochthodes umbellata* (L.) Razafim. & B. bremer (Rubiaceae). Asian Journal of Plant Science and Research, 8(1):34-39.

[14]. Rani G & Grover IS. 1999. In vitro callus induction and regeneration studies in *Withania somnifera*. Plant Cell Tissue and Organ Culture 57: 23-27.

[15]. Rao YV, Shankar AR, Lakshmi TVR and Rao KGR. 2004. Plant regeneration on *Physalis pubescens* L. and its induced mutant. Plant Tissue Culture, 14(1): 9-15.

[16]. Sarker I, Islam J, Shaekh MPE, Rahman MM, Khan H, Chowdhury

ASMHK, Mukim MSI, Islam R, and Haque R. 2015. Establishment of a Standard Protocol for in vitro Meristem Culture and Plant Regeneration of *Citrus aurantifollia*. Journal of Pharmacy and Biological Sciences, 2(10): 61-69.

[17]. Senthilkumar P, Paulsamy S, Vijayakumar KK and Kalmuthu K. 2007. In vitro Regeneration of the Medicinal Herb of Nilgiri shola, *Acmella calva* L. from leaf Derived callus. Plant Tissue Culture & Biotechnology, 17(2): 109-114.

[18]. Verma PC, Shing D, Rahman L, Gupta MM and Banerjee S. 2002. In vitro studies in *Plumbago zeylanica*: rapid micropropagation and establishment of higher plumbagin yielding hairy root culture. Journal of Plant Physiology, 159:547-552.