JABS
Journal of Applied Biological Sciences 7 (3): 75-78, 2013
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

High Frequency Plant Regeneration of Dwarf Hygro (*Hygrophila polysperma* [Roxb.] T. Anderson) on Liquid Culture

Ayşegül ÇINAR[1] Mehmet KARATAŞ[1*] Muhammad AASIM[1]

[1]Department of Biology, Kamil Ozdag Faculty of Science, Karamanoglu Mehmetbey University, Yunus Emre Campus, 70200, Karaman, Turkey

*Corresponding author:
Email: : mkaratas@kmu.edu.tr

Received: July 31, 2013
Accepted: September 25, 2013

Abstract

Dwarf hygro (*Hygrophila polysperma* [Roxb.] T. Anderson) is one of the popular aquatic plant of European countries. It is well-known medicinal plant in Indian states of Karnataka and West Bengal, and has been the part of Ayurvedic Medicine. The study presents direct organogenesis from shoot tip and 1st nodal meristem explant on liquid MS medium supplemented with 0.10-0.80 mg/l BA. Cent percent shoot regeneration without callusing was obtained from both explants after 6 weeks of culture. Maximum number of 25.33 and 21.67 shoots were recorded from shoot tip explant and 1st nodal meristem respectively on 0.10 mg/l BA containing medium. Shoot tip explants were further sub-cultured for 8 weeks and 87.5-100.0 % shoot regeneration frequency with 11.02-15.91 more shoots per explants and 0.51-1.61 cm longer shoots were recorded. However, total number of shoots per explant ranged 26.02-36.91. In vitro regenerated shoots were successfully rooted on solidified MS rooting medium containing 1.0 mg/l IBA. Rooted plantlets were acclimatized in aquariums containing tap water and sand where all plants survived.

Key Words: Dwarf hygro, *in vitro*, liquid medium, nodal meristem, shoot tip

INTRODUCTION

Aquatic plants are the most important and primary unit of water environment that produce organic matter and oxygen for aquatic organisms. [1,2]. Additionally, some aquatic plants has ability to accumulate relatively large amounts of heavy metals or contaminants (phytoremediation) [3-5] or used as bio-monitor for monitoring of pollution (bio-monitoring) in aquatic ecosystem [6,7].

Tropical and subtropical regions are the natural habitats of aquarium plants [8]. However, aquatic plants show distribution all over the world due to rapid increase in the demand of aquarium plants. Most European counties like Holland, France, Czech Republic, Germany, Hungary, Switzerland, Austria, Turkey, Latvia, and Estonia are the leading countries that are spending millions of Euros for the import of aquatic plants. The most imported aquatic plant that are used in aquarium industry are *Egeria densa, Cabomba caroliniana, Hygrophila polysperma, Vallisneria spiralis, Echinodorus bleheri, Vallisneria americana,Najas marina,* and *Hygrophila difformis* [9].

Dwarf hygro (*Hygrophila polysperma* [Roxb.] T. Anderson), also known as dwarf hygrophila, Miramar weed or Indian weed belongs to Acanthus family. This aquatic plant is native to India and Malaysia and was introduced to USA as Eastern Ludwiga [10], in 1945 [11]. Dwarf hygro is an important ornamental plant in aquarium industry [12] and medicinal aquatic plant. The whole plant is used as an ingredient of Ayurvedic Medicine used for facial paralysis, stiff-neck, hemiplegia, and noise in the ears with headache [13] and seeds has also been used for remedies in India [14]. The plant is enlisted in the medicinal plant in Indian states of West Bengal [15] and Karnataka [16]. Besides that, it is a good source of bio-indicator for alg control along with Indian ferns [17].

Although, *in vitro* shoot regeneration of some plants of genus *Hygrophila* like *H. pogonocalyx, H. spinosa, H. Auriculata, H. stricta* has been reported. However, there is only single published report on adventitious shoot regeneration [18] of dwarf hygro using leaf explant. The present study was intended to check the organogenesis potential of meristem explants of dwarf hygro on liquid MS medium.

MATERIAL AND METHODS

The plants of dwarf hygro were taken from local aquarium of Karaman province of Turkey. 4-5 cm long twigs with 5-6 nodes were cleaned under tap water for 5 min followed by surface sterilization with 24% hydrogen peroxide by continuous stirring for 10 min. Thereafter, plants were washed thrice with sterilized distilled water by continuous stirring for 5 min each in order to remove contaminants. After sterilization, sterilized twigs were cultured on Murashige and Skoog [19] medium containing

3% sucrose, solidified with 0.65 % agar and without any growth regulators for 2 weeks. The medium was also provided with 500 mg/l broad spectrum antibiotic (Duocid) in order to inhibit bacterial contamination. After two weeks of culture, shoot tips and 1st nodal meristems were isolated from sterile twigs and cultured on liquid MS medium supplemented with sucrose (3%) and 0.10-0.80 mg/l 6-Benzylaminopurine (BA) in Magenta GA7 vessels.

After 6 weeks of culture, data regarding frequency of shoot regeneration (%) and shoots per explants (Table 1, Column A) were scored and subjected to statistical analysis. Thereafter, only shoot tip explants were transferred to new liquid culture medium with 0.10-0.80 mg/l BA for 8 weeks and data regarding shoot regeneration frequency, shoots per explant (Table 2, Column B) and mean shoot length were scored again and statistically analyzed. Whereas, data regarding total number of regenerated shoots from shoot tip explants (Table 3, Column C) were also subjected to statistical analysis.

In vitro regenerated shoots from (A) and (B) were transferred to MS medium supplemented with 1.00 mg/l IBA (Indole-3-butyric acid) for rooting for 4 weeks. Rooted plantlets were taken from Magenta vessels and washed carefully under tap water for removing agar without damaging the roots. For acclimatization, rooted plants were shifted to aquariums provided with sand and tap water. The pH of solidified and liquid media were adjusted to 5.6-5.8 prior to autoclaving (120°C for 21 min, 118 kPa atmospheric pressure). All cultures (shoot regeneration and rooting) were cultured under 16 h light photoperiod (1500 lux) using white Light Emitting Diode (LED) lights.

Each treatment was replicated 6 times in both shoot regeneration and rooting experiments and repeated twice. Statistical analysis was done by One Way ANOVA using SPSS 20 for Windows. Post hoc test was accomplished using Duncan's test. Data were given in percentages and before statistical analysis, they were subjected to arcsine transformation [20].

RESULTS

The present study depict organogenesis of shoot tips and 1st nodal meristem explants cultured on liquid MS medium provided by 0.10-0.80 mg/l BA for multiple shoot induction. Direct shoot organogenesis initiated on both the explant after 10 days culture inoculation in the medium. Multiple shoots were recorded on both explants after 3 weeks of culture. However, no callus was observed on both explants on all culture medium tested. After 6 weeks of culture (Figure 1a,b) data regarding frequency of shoot regeneration and number of shoots per explants of both explants were scored and subjected to statistical analysis. The result of analysis of variance showed that shoots per explants were significant (p<0.05) and recorded 100.0 %. Whereas, shoot regeneration frequency of both explants was found insignificant (p<0.05).

Maximum number of 25.33 shoots from shoot tip and 21.67 shoots from 1st nodal meristem explant were scored on MS medium with 0.10 mg/l BA. The mean number of shoots were found significant (p<0.05) and ranged 15.0-25.33 and 15.0-21.67 shoots per explant on shoot tip and 1st nodal meristem respectively (Table 3).

Response of both explants to different concentrations of BA was same and each increase in BA concentration resulted in decrease number of shoots per explant. The regenerated shoots were isolated under aseptic conditions and transferred to rooting medium. Whereas, shoot tips explant were again cultured on same liquid mediums for further 8 weeks. Shoot buds sprouted after one week of the culture, (Figure 2a) and it was clearly observed within 2 weeks (Figure 2b).

Figure 1. *In vitro* multiple shoot regeneration of *H. polysperma* in liquid medium from (a) shoot tip and (b) 1st nodal meristem explants after 6 weeks of culture

Table 1. Effect of various concentration of BA on multiple shoot regeneration of *H. polysperma*

BA (mg/l)	Mean number of shoots per explant	
	Shoot tip (A)	1th nodal meristem
0.10	25.33[a]	21.67[a]
0.20	21.00[ab]	17.67[ab]
0.40	20.00[ab]	14.67[b]
0.60	17.00[b]	15.67[b]
0.80	15.00[b]	15.00[b]

Values in a column followed by different letters are significantly different (p<0.05) according to Duncan's Multiple range test

Table 2. Multiple shoot regeneration of shoot tip of *H. polysperma* on various concentration of BA after 8 weeks of culture

BA (mg/l)	Shoot regeneration frequency (%)	Shoots Per explant (B)	Shoot length (cm)
0.10	100.00[ns]	11.58[ns]	0.61[b]
0.20	91.66	15.91	1.61[a]
0.40	95.83	13.83	0.63[b]
0.60	87.50	13.41	0.51[b]
0.80	87.50	11.02	0.61[b]

Values in a column followed by different letters are significantly different (p<0.05) according to Duncan's Multiple range test

Table 3. Total number of shoots obtained from shoot tip of *H. polysperma* on various concentration of BA.

BA (mg/l)	Total number of shoots per explants		
	Before Sub-culturing (A)	After Sub-culturing (B)	Total number of shoots per explant A + B = (C)
0.10	25.33[a]	11.58[ns]	36.91[a]
0.20	21.00[ab]	15.91	36.91[a]
0.40	20.00[ab]	13.83	33.83[b]
0.60	17.00[b]	13.41	30.41[c]
0.80	15.00[b]	11.02	26.02[d]

Values in a column followed by different letters are significantly different (p<0.05) according to Duncan's Multiple range test

After 8 weeks of culture, analysis of variance results showed that BA concentrations had insignificant effects (p<0.05) on shoot regeneration frequency and shoots per explants. Whereas, BA concentrations had significant

effects ($p<0.05$) on mean shoot length. Shoot regeneration frequency ranged 87.5-100.0 % (Table 2) with maximum shoot regenration frequency at 0.10 mg/l BA. Although, number of explants were found insignificant, an addition of 11.02-15.91 (Table 2) shoots per explants were obtained after 4 weeks. Maximum of 15.91 shoots per explants were obtained on MS medium containing 0.20 mg/l BA followed by 13.82 and 13.41 shoots per plant on MS medium containing 0.40 and 0.60 mg/l BA respectively. The maximum length (1.61 cm) was obtained on MS medium containing 0.20 mg/l BA that shoot length ranged 0.51-1.61 cm.

Total number of shoots obtained from first (A) and second culture (B), from shoot tip explant (Table 3) were subjected to statistical analysis, it showed significance ($p<0.05$) to BA concentrations and ranged 26.02-36.91 shoots per explants (Table 3, Column C). The concentration 0.10 and 0.20 mg/l BA with MS medium were produced maximum number of shoots in the present study (Table 3). Shoots per explant were ranged 26.02-36.91. It was interestingly noted that each increase in BA concentrations was inhibitory that resulted reduction shoots per plant.

All regenerated shoots from both explants were separated under sterile conditions and rooted on MS medium containing 1.0 mg/l IBA. Irrespective of smaller shoots than 1.0 cm, all shoots rooted well and successfully acclimatized in aquariums containing tap water and sand.

Figure 2. *In vitro* shoot regeneration from shoot tip explants of *H. polysperma* in liquid medium after (a) 1 weeks, (b) 2 weeks, and (c) 8 weeks of subculture

DISCUSSION

The study presents the organogenesis of shoot tip and 1st nodal meristem explants of dwarf hygro on liquid MS medium containing different concnetrations of BA. Liquid culture for multiple shoot regeneration of other aquatic plants like *Trapa japonica* Flerov [21], *Lemna gibba* var. *Hurfeish* and *Spirodela punctata* [22] and *Ludwigia repens* [23] has been reported. In this study, direct organogenesis from both explants used in the study were recorded without callus induction and has been reported in dwarf hygro [18] and water hyssop [24].

In the present study, BA concentrations showed insignificant effects on shoot regeneration frequency and both explants responded well in liquid culture and induced cent percent shoot regeneration. Karataş et al. [24] also reported cent percent shoot regeneration from leaf explant of water hyssop BA-NAA containing medium. Contrarily, 62.50–100.00% shoot regeneration frequency on solidified MS medium containing 0.10-1.60 mg/l TDZ of leaf explant of dwarf hygro [18]. Whereas, shoot regeneration frequency of 30.0-95.0 % and 50.0-95.0 % of water hyssop [25] cultured on BA and TDZ respectively was reported.

On the other hand, shoots per explant number also revealed the greater response of both explants. However, shoot tip explant was more responsive to all concentrations of BA and induced more shoots than 1st nodal meristem explant. Öztürk [26] obtained maximum shoots per explants from 1st nodal explant cultured on liquid MS medium containing TDZ-NAA compared to shoot tip meristem, leaf, and petiole explants of *Hygrophila difformis.* The present study further revealed that low concentration of BA in the culture medium induced more number of shoots and decreased with increased BA concentrations. Cytokinin at higher concentrations inhibit the shoot induction [27]. Karatas et al. [24] reported decreased number of shoots with increase of BA-NAA concentration in *Bacopa monnieri*. Sharma et al. [28] also indicated relatively low BA requirement for maximum number of shoots per explants of *Bacopa monnieri.*

After 6 weeks of culture, regenerated shoots were isolated and it was found that shoot tip explant has still number of shoot buds which can produce more shoots. Therefore, they were sub-cultured on same media for further eight weeks and data scored was analyzed and results showed insignificant effects of sub-culturing on shoot regeneration frequency which was recorded 87.5-100.0%. Although, explants induced shoots after sub-culturing that ranged 11.02-15.91 shoots per explants but were found insignificant. Positive effects of subculturing on shoot induction has been reported by Tiwari et al., [29] and Banerjee and Shrivastava, [30]. However, when total number of shoots per explants were analyzed, they were found significant and lower concentrations of 0.10 and 0.20 mg/l BA gave maximum number of shoots per explant in agreement with Sharma et al. [28].

The MS medium with 0.20 mg/l BA was superior over the tested medium that resulted more longer shoots in the present study. Karataş et al., [18], also obtained relatively shorter shoots ranged 0.42-0.70 cm and 0.35-0.43 cm from leaf explant of dwarf hygro cultured on Kin and TDZ alone. Contrarily, increased BA and TDZ concentration promoted shoot length from leaf explant of *B. monnieri* [25]

Regenerated shoots before (A) and after sub-culturing (B) were rooted successfully on 1.0 mg/l IBA in line with Karatas et al. [18] in dwarf hygro. Thereafter, rooted plantlets were successfully acclimatized in aquarium where plants survived and continue their growth without showing any negative signs of necrosis. Successful acclimatization of aquatic plants in aquariums has been reported previously in *Nymphoides indica* [31], *Rotala macrandra* [32], *Veronica anagallis-aquatica* [33], *Cryptocoryne wendtii* ve *Cryptocoryne beckettii* [34], *H. polysperma* [18], and *B. monnieri* [24].

The present study emphasizes successful *in vitro* regeneration protocol of dwarf hygro on liquid MS medium. This may be helpful for fulfill of rapid increase in the demand of aquarium plants in the world.

REFERENCES

[1] Cirik Ş., Cirik S. Conk-Dalay M. 2011. Su bitkileri II (İçsu Bitkilerinin Biyolojisi, Ekolojisi, Yetiştirme Teknikleri). Ege Üniversitesi, Su Ürünleri Fakültesi Yayınları, İzmir.

[2] Oyedeji AA, Abowei JFN. 2012. The classification, distribution, control and economic importance of aquatic plants. International Journal of Fisheries and Aquatic Sciences. 1:118-128.

[3] Kanabkaew T, Puetpaiboon U. 2004. Aquatic plants for domestic wastewater treatment: Lotus (*Nelumbo nucifera*) and Hydrilla (*Hydrilla verticillata*) systems.

Songklanakarin Journal of Science *and* Technology. 26:749-756.

[4] Hegazy AK, Abdel-Ghani NT, El-Chaghaby GA. 2011. Phytoremediation of industrial wastewater potentiality by *Typha domingensis*. International Journal of Environmental Science *and* Technology. 8:639-648.

[5] Farid M, Irshad M, Fawad M, Ali Awan Z, Egrinya Eneji A, Aurangzeb N. 2013. Effect of cyclic phytoremediation with different wetland plants on municipal wastewater. International Journal of Phytoremediation. 16:572-581.

[6] Nirmal Kumar JI., Soni H. Kumar RN. 2008. Evaluation of biomonitoring approach to study lake contamination by accumulation of trace elements in selected aquatic macrophytes: A case study of kanewal community reserve, Gujarat, India. Applied Ecology and Environmental Research. 6:65-76.

[7] Skorbiłowicz E. 2009. Aquatic plants as bioindicators of contamination of upper Narew river and some of its tributaries with heavy metals. Environmental Protection Engineering. 35:65-77.

[8] Alpbaz, A., 1984. Akvaryum Tekniği ve Balıkları. Acargil Basımevi, 433 s, İzmir, Turkiye.

[9] Brunel S. 2009. Pathway analysis: Aquatic plants imported in 10 EPPO Countries. Bulletin OEPP/EPPO Bulletin. 39:201-213.

[10] Langeland KA, Burks KC. 1998. Identification and Biology of Non-Native Plants in Florida's Natural Areas. University of Florida, Gainesville, Florida.

[11] Innes WT. 1947. Hygrophila*,* A new aquarium plant. The aquarium. 16:30-1.

[12] David WH, Vernon VV, Cody JG. 2012. East Indian *Hygrophila*, *Hygrophila polysperma* (Roxb.) T. Anderson, University of Florida*,* Florida.

[13] Anonymous, 2013a. Mashabaladi Kvatha. Herbal tonics/combinations, *http://www.holisticonline.com/Herbal-Med/Remedies/hol_herbal-tonics-ashabaladi-kvatha.htm-*

[14] Bowes G. 1982. *Limnophilia sessiliflora* and *Hygrophila polysperma,* baseline physiology of the potential problem plants. Department of Botany University of Florida, Gainesville.

[15] Anonymous, 2013b. Medicinal plants of West Bengal, Envis centre on medicinal plants, *http://envis.frlht.org/checklist/WestBengal.pdf-*

[16] Anonymous, 2013c. Medicinal Plants of Karnataka, Envis centre of medicinal plants, *http://envis.frlht.org/checklist/karna.pdf*

[17] Anonymous, 2013d. FAQs on freshwater algae & their control, *file:///C:/Users/acer/Desktop/TEZZ/FAQs%20on%20Fresh water%20Algae%20and%20Their%20Control%202.htm*

[18] Karatas M, Aasim M, Çınar A, Dogan M. 2013a. Adventitious shoot regeneration from leaf explant of dwarf hygro (*Hygrophila polysperma* (Roxb.) T. Anderson). The Scientific World Journal, volume 2013, Article ID 680425, 7 pages. DOI:http://dx.doi.org/10.1155/2013/680425.

[19] Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum*,* 15:473-497.

[20] Snedecor, GW, Cochran, WG. 1967. Statistical methods. The Iowa State University Press, Iowa, USA.

[21] Hoque A, Rahman SM, Arima S, Takagi Y, 2001. Efficient *in vitro* germination and shoot proliferation of chilling-treated water chestnut (*Trapa japonica* Flerov) embryonal explants. *In vitro* Cellular & Developmental Biology – Plant. 37:369-374.

[22] Li J, Jain M, Vunsh R, Vishnevetsky J, Hanania U, Flaishman M, Perl A, Edelman M. 2004. Callus induction and regeneration in *Spirodela* and *Lemna*. Plant Cell Reports. 22:457-464.

[23] Öztürk M, Khawar KM, Atar HH, Sancak C, Özcan S. 2004. *In vitro* Micropropagation of the aquarium plant *Ludwigia repens*, Asia Pacific Journal of Molecular Biology and Biotechnology. 12:21-25.

[24] Karatas M, Aasim M, Dogan M, Khawar KM. 2013b. Adventitious shoot regeneration of the medicinal aquatic plant water hyssop (*Bacopa monnieri* L. Pennell) using different internodes. Archives of Biological Sciences, Belgrade. 65:297-303.

[25] Vijayakumar M, Vijayakumar R, Stephen R. 2010. *In vitro* propagation of *Bacopa monnieri* L. - a multipurpose medicinal plant. Indian Journal of Science and Technology, 3:781-786.

[26] Öztürk M. (2008). *In vitro* propagation of aquarium plants *Hygrophila difformis* and *Microsorium pteropus.* Ph. D. Thesis,University of Ankara, Biotechnology Institute, Ankara.

[27] Kendir H, Sahin-Demirbag N, Aasim M, Khawar KM. 2009. *In vitro* plant regeneration from Turkish Narbon Vetch (*Vicia narbonensis* L.). African Journal of Biotechnology. 8:614–618.

[28] Sharma S, Kamal B, Rathi N, Chauhan S, Jadon V, Vats N, Gehlot A, Arya S. 2010. *In vitro* rapid and mass multiplication of highly valuable medicinal plant *Bacopa monnieri* (L.). Wettst. African Journal of Biotechnology. 9:8318-8322.

[29] Tiwari V, Tiwari KN, Singh BD. 2001. Comparative studies of cytokinins on *in vitro* propagation of *Bacopa monniera*. Plant Cell Tissue Organ Culture. 66:9-16.

[30] Banerjee M. Shrivastava S. 2008. An improved protocol for *in vitro* multiplication of *Bacopa monnieri* (L.). World Journal of Microbiology & Biotechnology. 24:1355-1359.

[31] Jenks MA, Kane ME, Dennis B, McConnell, DB. 2000. Shoot organogenesis from petiole explants in the aquatic plant *Nymphoides indica.* Plant Cell, Tissue and Organ Culture. 63:1-8.

[32] Şumlu Ş. 2009. *In vitro* micropropagation and genetic transformation of aquarium plant *Rotala macrandra*. Ph. D. Thesis, University of Ankara, Graduate School of Natural and Applied Sciences, Ankara.

[33] Shahzad A, Parveen S, Fatema M, 2011. Development of a regeneration system via nodal segment culture in *Veronica anagallis-aquatica* L.-An amphibious medicinal plant. Journal of Plant Interactions. 6:61-68.

[34] Stanly C, Bhatt A. Keng CL. 2011. An efficient i*n vitro* plantlet regeneration of *Cryptocoryne wendtii* and *Cryptocoryne becketti* through shoot tip culture. Acta Physiologiae Plantarum. 33:619-624.