Volume 59(1):55-58, 2015
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Helicteres isora L: regeneration through meristem culture

Harshal A Deshpande and Sanjivani R Bhalsing*

Department of Biotechnology, School of Life Sciences, North Maharashtra University, Jalgaon – 425001, India

ABSTRACT *Helicteres isora* Linn (Sterculiaceae) is an endangered plant species of medicinal importance. Rapid regeneration of this plant was achieved via shoot tip explants with highest response of shoot induction (100%) on MS basal medium supplemented with 12 µM 6-benzylaminopurine (BAP) and 16 µM kinetin (Kin) alone. This combination of phytohormones was found suitable for complete plant regeneration through meristem culture. The developed method can be successfully employed for large-scale regeneration and conservation of this medicinal plant.
Acta Biol Szeged 59(1):55-58 (2015)

KEY WORDS

Helicteres isora
in vitro regeneration
medicinal plant
meristem

Introduction

Medicinal plants synthesize a diverse array of bioactive molecules making them a rich source for conventional medicines and pharmacopoeial drugs (Deshpande and Bhalsing 2013, 2014a). *Helicteres isora* (Sterculiaceae) commonly known East Indian screw tree or Red isora is one of the important medicinal plant species known as a source of diosgenin (Deshpande and Bhalsing 2014b). This plant finds its use in traditional medicine as all the parts of the plant are used in Ayurveda; the root juice and bark of *H. isora* is claimed to be useful e.g. in diarrhea, stomach affections, intestinal infections and also as a urinary astringent (Shriram and Shitole 2008). The natural revival of *H. isora* is through seeds, however, their cultivation rate and poor seed dormancy are crucial factors for its propagation (Nikam et al. 2009). This plant is now listed as one of the endangered medicinally important plant species of India (Khandesh region of Maharashtra) and it is on the verge of extinction (Bagul and Yadav 2007). In view of the dilemma of conventional propagation and high demand of planting material, the large scale multiplication of this medicinal species can be met economically and efficiently in short span of time by *in vitro* propagation. *In vitro* propagation has been successfully applied for the conservation of a large number of endangered plant species existing in threatened habitats and thereby greatly enhancing the scope and potentiality of mass propagation by exploiting the regenerative behavior in a wide range of selected horticultural and agricultural plants including the medicinal ones (Bhalsing et al. 2000; Deshpande and Bhalsing 2014b). Unfortunately, only limited numbers of established protocols are available for plant regeneration for this immensely valuable endangered medicinal plant. In the present communication, we report rapid regeneration from shoot tip explants of *H. isora* at high frequency on MS basal medium.

Submitted Jan 1, 2015; Accepted May 6, 2015
*Corresponding author. E-mail: bhalsingsr@gmail.com

Materials and Methods

The plantlets of *H. isora* were directly obtained from Dr. Punjabrao Deshmukh Agricultural University, Akola, India. They were authenticated by an expert taxonomist from Dr. Baba Saheb Ambedkar Marathwada University, Aurangabad (BAMU) by depositing a voucher specimen (Specimen nr.: HI 01743) at BAMU Herbarium. Tender shoots of *H. isora* from the obtained plantlets served as explants. These shoot tip explants were washed thoroughly under running tap water for 15-20 minutes and treated with 1% Tween 80 (Hi Media, Mumbai) for 10 minutes followed by repeated rinsing with distilled water. The explants were then excised and surface sterilized initially with 70% (v/v) ethanol for 30 s and subsequently in 0.1% (w/v) $HgCl_2$ solution for 60 sec followed by 3-5 washes with sterilized distilled water to remove the traces of $HgCl_2$ solution under aseptic conditions in laminar air flow hood. Finally, the sterilized shoots were treated with the antifungal agent Bavistin Df (BASF Pvt. Ltd., India) in 0.4 g/l concentration and blotted dry with sterile paper towels (Deshpande et al. 2010).

Throughout the experiment, MS medium (Murashige and Skoog 1962) containing 3% (w/v) sucrose, 0.1% activated charcoal and 0.8% (w/v) agar (Bacteriological Grade; HI-Media, Mumbai) was used. Its pH was adjusted to 5.8 with 1 N NaOH or 1 N HCl prior to autoclaving. The medium was autoclaved under 1.04 kg/cm^2 at a temperature of 121 °C for 15 min. All growth regulators were filter-sterilized (pore size: 0.22 µm; Millipore) and added after autoclaving. On average, 20 ml media was dispensed in each glass tube (Borosil, Mumbai, India). For shoot induction, surface

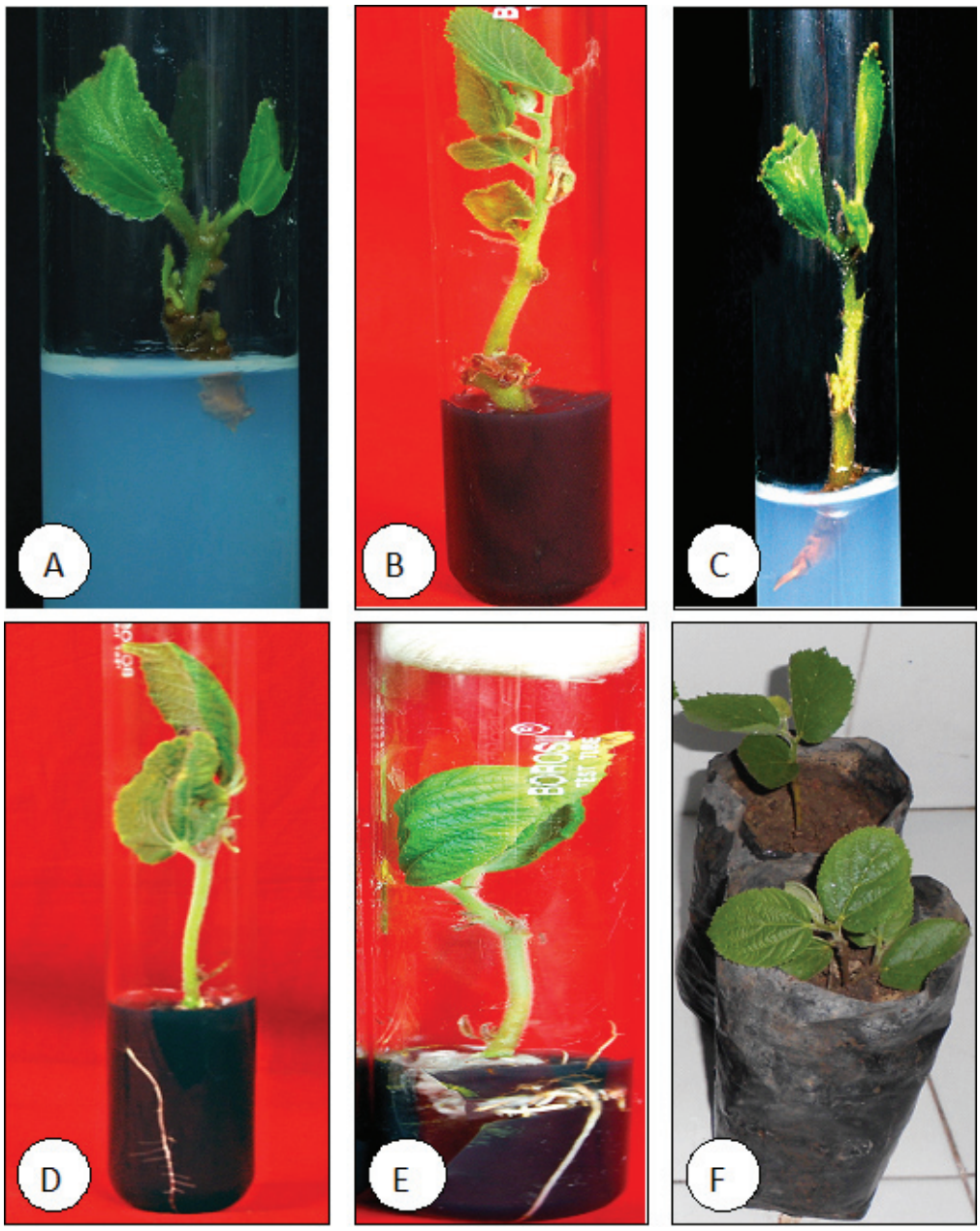

Figure 1. *In vitro* plant regeneration of *H. isora* (A) and (B) Shoot elongation within two weeks of culture on MS basal medium; (C) Root induction on half strength MS basal medium fortified with IBA at 4 µM concentration; (D) and (E) Extensive root elongation and proliferation on half-strength MS basal medium; (F) Acclimatized plantlets of *H. isora* before subsequent transfer in the field.

sterilized shoot tips of *H. isora* were inoculated aseptically on MS medium provided with different concentrations of 6 benzylaminopurine (4-20 µM) and kinetin (4-20 µM) alone. All experiments consisted of three replicates of 10 test tubes and all cultures were maintained in a sterilized culture room at 27±2 °C under a 16/8 hrs photoperiod during the experiments. The white light was provided by cool fluorescent tubes at a photon rate of 25 µmol/m^2/s light intensity (Philips, India) (Deshpande et al. 2010).

The *in vitro* regenerated plantlets were hardened in plastic polycups containing a mixture of sterile garden soil: peat moss: sand (1:2:1; v/v/v) irrigated after every 24 h with diluted MS basal salts since it increased survival rate and promoted the growth of plantlets. These pots were maintained

Table 1. Effect of various concentrations of BAP and Kin on micropropagation of *H. isora* through shoot tip cultures.

BAP (µM)	Kin (µM)	Frequency of caulogenesis (%)
4	--	20±10
8	--	35±13
12	--	99±2
16	--	40±4
20	--	00
--	4	8±2
--	8	36±4
--	12	60±2
--	16	99±0.5
--	20	29±3

Each experiment was done in triplicate and number of explants per replicates was 10. The values are mean of three replicates ±SD

at 25±2 °C in 16/8 hrs photoperiod with light intensity of 25 µmol/m^2/s provided by white, fluorescent tubes for two weeks in the culture room. Finally, the plantlets were kept in greenhouse at 80-90% relative humidity 28±2 °C before subsequent transfer to field.

Results and Discussion

Shoot tips isolated from authenticated plantlets of *H. isora* were cultured on MS basal medium fortified with BAP (4-20 µM) and kinetin (4-20 µM) alone. Initially the explants treated with BAP produced globular mass of cells at the basal part of the shoot tip. Shoot induction and differentiation was visually observed within 3-4 weeks of culture. Maximum frequency of caulogenesis was achieved on MS basal medium fortified with 12 µM BAP alone (Fig. 1a). In the medium fortified with kinetin, extensive shooting was observed at 16 µM concentration. (Fig. 1b and Table 1). The healthy elongated shoots (3-4 cm) produced *in vitro* were rooted after four weeks of culture on the half-strength MS medium supplemented with phytohormones with various concentrations of auxins: IBA (4-12 µM), IAA (4-12 µM) and NAA (4-12 µM). In the present study, we observed that MS medium supplemented with 4 µM IBA alone gives 90% of rooting frequency responsible for inducing roots, while other concentrations studied (8 µM and 12 µM) gave low frequency of root induction (40% and 10%, respectively) (Fig. 1c). Rooting response of IBA has been reported for several medicinal plants like *Rauwolfia tetraphylla* L (Faisal et al. 2012) and *Adhatoda vasica* Ness (Nath and Buragohain 2005). Rooting frequency with IAA was insignificant (20%) followed by NAA (10%) at all concentrations studied here. The auxins were inconsequentially effective for root proliferation in our study. Different combinations of Kin combined with BAP used for rooting were studied in other plant species like *Kaempferia galangal* (Bhattacharya and Sen 2013), *Eryngium foetidum* (Chandrika et al. 2011) and *Matthiola incana* (Afshin et al. 2011). However, a combined effect of different concentrations of Kin and BAP were also employed in order to investigate the root proliferation efficiency of regenerated shoots. The regenerated shoots with induced roots were further cultured on half strength MS basal medium fortified with different concentrations of Kin in combination with 12 µM BAP for six weeks in our study. We found that half strength MS basal medium fortified with 16 µM Kin in combination with 12 µM BAP was the best for effective root proliferation and elongation (Table 2). BAP applied alone at 12 µM proved to be more effective than other combinations studied. Hence we conclude that Kin along with BAP not only favors shooting but also favored extensive pro-

Table 2. Effect of various phytohormones on rhizogenesis frequency in *H. isora*.

IBA (µM)	IAA (µM)	NAA (µM)	BAP (µM)	Kin (µM)	Freq. of rhizogenesis (%)
4	--	--	--	--	89±1
8	--	--	--	--	39±0.6
12	--	--	--	--	10±0.3
--	4	--	--	--	19±0.5
--	8	--	--	--	9±1
--	12	--	--	--	19±5
--	--	4	--	--	9±0.4
--	--	8	--	--	4±0.5
--	--	12	--	--	9±0.5
--	--	--	12	4	39±1
--	--	--	12	8	58±2
--	--	--	12	12	29±0.5
--	--	--	12	16	99±0.5
--	--	--	12	20	10±00

Each experiment was done in triplicate and number of explants per replicates was 10. The values are mean of three replicates ±SD

liferation and elongation of the roots (Fig. 1d and 1e).Thus, cytokinins are most effective for entire plant regeneration and thus we are firstly reporting *in vitro* regeneration of *H. isora* through direct organogenesis. The *in vitro* regenerated 6-10 weeks old plantlets derived from shoot tip culture were hardened by the procedure mentioned above. The hardened plantlets were subsequently transferred to the fields after four weeks with a survival rate of 70±5%. (Fig. 1f). Successful *ex vitro* rooting and field establishment has been reported in several other species (Bhalsing et al. 2001; Patil et al. 2009; Deshpande et al. 2010).

Conclusion

The conservation of natural resources and endangered plant species has been imposed in recent years. The present study provides a successful method for *in vitro* regeneration of *H. isora* through direct organogenesis for the first time. The developed method can be successfully employed for large-scale regeneration and conservation of this valuable medicinal plant.

Acknowledgement

Harshal A Deshpande is thankful to Shri Gyanchand Raisoni Fellowship for doctoral studies from North Maharashtra University, Jalgaon. Authors gratefully acknowledge financial assistance towards instrumentation facility at School of Life Sciences, from UGC; New Delhi under SAP-DRS Program and DST; New Delhi under DST FIST Program.

References

Afshin AH, Behzad K, Alireza T, Sahar BZ (2011) Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*). Plant Omics J 4(5):236-238.

Bagul RM, Yadav SS (2007) Threat assessment of some medicinally important plants of Satpuda Forest East Khandesh: A conservative approach. J Plant Archives 7 (1):367-370.

Bhalsing SR, Teli NP, Pawar PK, Maheshwari VL (2000) Isolation and characterization of solasodine from cultured cells of *Solanum khasianum*. J Plant Biol 27(2):185-187.

Bhalsing SR, Teli NP, Pawar PK, Baviskar MP and Maheshwari VL (2001). Tissue culture grown banana - A cost effective strategy for hardening. Physiol Mol Biol Plants 7(2):185-189.

Bhattacharya M, Sen A (2013) *In vitro* regeneration of pathogen free *Kaempferia galangal* L. - a rare medicinal plant. Res Plant Biol 3(3):24-30.

Chandrika R, Vyshali P and Kaliwal BB (2011). Rapid multiplication of mature flowering plant of *Eryngium foetidum* L. by in vitro technique. Int J Biotech Appl 3(4):114-117.

Deshpande HA, Bhalsing SR (2013) Recent advances in the phytochemistry of some medicinally important *Cassia* species: A Review. Int J Pharma Med Biol Sci 2(3):60-78.

Deshpande HA, Bhalsing SR (2014a) A review of phytochemical profile of *Desmodium gangeticum* (L.) DC: A valued endangered medicinal plant. Int J Pharma Sci Healthcare 1(4):36-48.

Deshpande HA, Bhalsing SR (2014b) Isolation and characterization of diosgenin from *in vitro* cultured tissues of *Helicteres isora*. Physiol Mol Biol Plants 20(1):89-94

Deshpande HA, Chalse MN, Bhalsing SR (2010) *Centella asiatica* Linn: plant regeneration through leaf derived callus. J Herbal Med Toxicol 4(2):119-122.

Faisal M, Ahmad N, Anis M (2012) Shoot multiplication in *Rauvolfia tetraphylla* L. using thidiazuron. Plant Cell Tiss Org Culture 80:187-190.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Phy Plant 15:473-479.

Nath S, Buragoham AK (2005) Micropropagation of *Ahatoda vasica* Nees – A woody medicinal; plant by shoot tip culture. Indian J Biotechnol 4:396-399.

Nikam TD, Mohmmad AE, Patil VA (2009) Embryogenic callus culture of *Tribulus terrestris* L - A potent source of Harmaline, Harmine and Diosgenin. Plant Biotechnol Rep 3:243-250.

Patil GS, Shimpi TS, Deshpande HA, Narkhede JD, Bhalsing SR (2009) Tissue culture studies on *Bacopa monnieri* (L) Pennell - A threatened medicinal herb. Res Anal Eval 1:29-31.

Shriram V, Shitole MG (2008) Indirect organogenesis and plant regeneration in *Helicteres isora* L, an important medicinal plant. In vitro Cell Dev Biol Plant 44(3):186-193.