Plant Tissue Cult. & Biotech. **27**(2): 153-159, 2017 (December)

PTC&B

In vitro Plant Regeneration of *Magnolia punduana*: An Endemic and Threatened Plant Species

Rajib Borah, Suman Kumaria[1] and Hiranjit Choudhury*

Biotechnology Laboratory, Biotechnology Unit, Department of Basic Sciences & Social Sciences, School of Technology, North-Eastern Hill University, Shillong-793022, Meghalaya, India

Key words: *Magnolia punduana*, Shoot bud formation, Plant regeneration

Abstract

Shoots were induced from axillary and nodal buds of *Magnolia punduna* on MS supplemented with 0.1 μm of BAP. Out of five basal media tested (MS, ½ MS, ¼ MS, LS and WP), MS was found to be most effective for shoot and callus initiation. Different plant growth regulators (0.1 - 1.0 μm) induced shoot formation in different proportions. The combination of 0.1 μm IBA and 0.5 μm BAP was found optimum for shoot elongation with minimal necrosis of the explants. Half strength of MS supplemented with 8.0 μm IBA was found suitable for rooting.

Introduction

Magnolia punduana Hk. f. & Th. (Synonym: *Michelia punduana*) is an endemic plant from Northeast India, which has been listed in the IUCN Red List (Wheeler and Rivers 2015) in the category data deficient. The species was classified as Vulnerable (Vulnerable B1 + 2c ver 2.3) according to IUCN Red List 2014 (IUCN Red List Category and Criteria 2014). Ramasubbu (2010) listed the tree under threat in the Wild Ornamental Plant Species. It was listed as rare by Walter and Gillet (1998) and Nayar and Sastry (1990). It is a medium size tree attaining a height of approximately 25 meters. The high quality timber of the plant has posted a serious threat for its own survivability. The exploitation of timber reached to such an extent that the species was facing the risk of extinction (Haridasan and Rao 1985). Presently, the plant is exploited heavily for timber, although it holds a great promise for phytochemical extraction from non-timber resources for its application in pharmaceuticals.

*Author for correspondence: <hiranjit_c@yahoo.com>. [1]Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong-793022, Meghalaya, India.

Over exploitation, habitat fragmentation and insufficient information on distribution demands an urgent need for conservation of this rare and important plant species to reduce the immediate threat of extermination (Iralu and Upadhaya 2015). The plant is usually propagated by seeds but the germination rate in the wild is very low. Even under laboratory conditions seeds of *M. punduana* did not show any sign of germination up to 6 months indicating the prevalence of morpho-physiological dormancy in the species (Iralu and Upadhaya 2016). The present study was carried out to investigate the induction of shoot from axillary and nodal buds and develop an efficient protocol for mass propagation of clonal planting material. This is the first report of *in vitro* plant regeneration via shoot induction in *M. punduana.*

Materials and Methods

About 5 cm long shoots with dormant axillary buds were collected from mature trees of *Magnolia punduana* growing in Cherrapunji (25º14.791′N & 91º41.657′E), Meghalaya, India. The collected shoots were immediately stored in cool-keg (at 8-10ºC) till their culturing in the laboratory. Young leaves, nodal and axillary buds were excised from 5 cm long shoot, and cleansed with liquid detergent (Labolene) under running tap water for 45 min. The explants were surface sterilized following the previously described protocol (Choudhury et al. 2008) with minor modifications and dried on sterile filter paper under aseptic conditions. Sucrose (30 g/l), agar (6 g/l) and activated charcoal (0.5 g/l) were added to different basal media and pH adjusted to 5.8 before autoclaving at 121ºC for 20 min. Cultures were maintained at 25 ± 2ºC under a 16/8 hrs photoperiod at a flux of 50 µmol $m^{-2}s^{-1}$ provided by fluorescent tubes (Philips).

Five commercially available salt media with vitamins, namely MS, ½ strength MS, ¼ strength MS, WP (Lloyd and McCown 1981), and LS (Linsmaier and Skoog 1965) were evaluated to study the effect of basal media on culture initiation from young leaf, nodal and axillary bud explants. All the media were supplemented with 0.1 µm BAP. MS selected on the basis of preliminary screening experiment as stated above was supplemented with different concentrations (0.1-1.0 µm) of IAA, 2,4-D and BAP to study the effect of plant growth regulators. The regenerated shoots were cultured on ½ MS supplemented with different concentrations (2.0-14.0 µm) of IBA for induction of roots.

Data collected include percentage of explants forming callus and shoot, and their mean number during the experimental period. Data presented in the tables were analysed for significance of variance and the differences contrasted by Tukey's test (t -test) using one-way ANOVA (Origin Pro 8) at 0.05% level.

Results and Discussion

Callus and shoot formation varied significantly depending on explant type. Leaf explant exhibited very slow response in the first week of culture followed by curling of leaf with subsequent callusing in the next two weeks (Fig. 1A-C). During maintenance, formation of occasional abnormal shoots was observed from the callus after three months of culture (Fig. 1D). Nodal segment induced shoot formation within 12 - 15 days of inoculation with sporadic callus formation under the same set of culture conditions (Fig. 1E). Shoot induction was achieved from axillary buds within 5 - 7 days of inoculation on MS supplemented with 0.1 μm of BAP (Fig. 1F). The present study revealed that the axillary bud was the best explant type to initiate shoot (Fig. 1F, H) followed by nodal explant (Fig. 1E, G). The current findings were in accordance with the report of Abdelmageed et al. (2012), Nakamura et al. (1995), Sokolov et al. (2014) and Radomir (2012) where axillary buds were used for shoot initiation in different species of *Magnolia*.

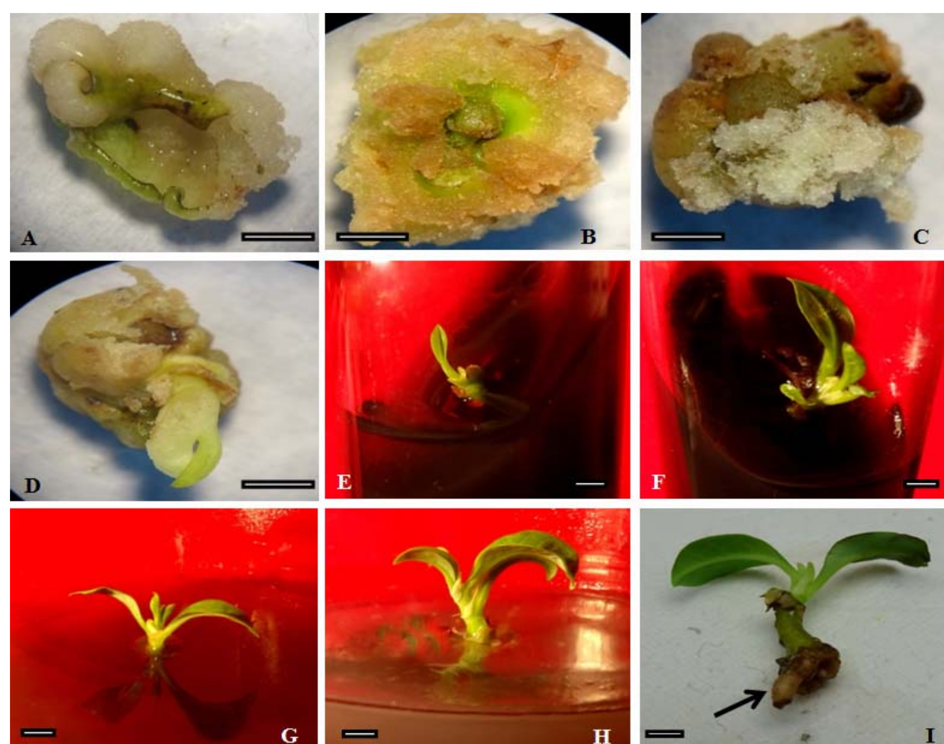

Fig.1. *In vitro* plant regeneration from various explant sources. A. Callus from leaf. B-C. Callus proliferation (after three months). D. Shoot from callus. E. Shoot from nodal segment. F. Shoot from axillary bud. G. Shoot elongation from nodal segment. H. Shoot elongation from axillary bud. I. Complete plantlet with root. Scale bars represent A-F 0.5 cm and G-I 1.0 cm. Arrow indicates root.

MS with 90% explant survivability was found to be the most effective medium for culturing axillary bud. Thus, explants from the initial screening and survival experiment were cultured on MS with different concentrations of IAA , 2,4-D and BAP to study the effect of plant growth regulators. The results revealed that BAP at 0.5 μm was most effective in shoot induction (82.3%) whereas IAA at 0.5 μm and 2,4-D at 1.0 μm, respectively were most suitable in callus induction (Table 1). The present findings are in accordance with the report of Thomas and Maseena (2006) who inferred that proper auxin/cytokinin ratio was very critical for initiation of different response in a culture medium. It was also established that auxin and cytokinin in different concentrations were strongly recommended for healthy proliferation of *Magnolia* species (Biedermann 1987, Kamenicka and Lanakova 2000, Parris et al. 2012, Radomir 2012).

Table 1. Effect of different plant growth regulators on callus and shoot induction from axillary buds of *M. punduana*.

Growth regulators	Conc. (μm)	Shoot regeneration (%)	Callusing (%)
IAA	0.10	30.6 ± 2.33[a]	33.6 ± 1.20[d]
	0.25	30.3 ± 3.17[a]	33.0 ± 1.15[d]
	0.50	**61.6 ± 2.33[b]**	**53.3 ± 1.45[e]**
	0.75	55.3 ± 2.02[b]	50.6 ± 1.76[e]
	1.00	50.3 ± 1.45[bc]	42.0 ± 1.73[f]
BAP	0.10	37.6 ± 3.48[g]	32.3 ± 1.45[j]
	0.25	41.3 ± 1.45[g]	54.3 ± 1.20[k]
	0.50	**82.3 ± 4.33[h]**	**60.6 ± 2.33[k]**
	0.75	67.6 ± 1.76[i]	49.6 ± 2.02[kl]
	1.00	58.6 ± 2.40[i]	42.3 ± 1.20[l]
2,4-D	0.10	20.6 ± 1.20[m]	31.3 ± 3.17[o]
	0.25	30.6 ± 2.33[n]	32.3 ± 2.33[o]
	0.50	32.6 ± 1.76[n]	41.6 ± 1.45[o]
	0.75	24.0 ± 2.08[mn]	55.3 ± 2.60[op]
	1.00	**21.0 ± 2.30[m]**	**75.3 ± 4.33[q]**

Data were collected after three weeks of culture. Axillary buds were cultured on MS basal medium. Experiments were performed with 100 samples per condition, distributed in 25 replicates, each replicate containing 4 explants in each vessel and repeated three times. Values represent the mean ± SE. Means

followed by the same letter within a column are not significantly different according to t-test ($p < 0.05$).

Various combinations of growth regulators were used for maintaining callus and shoot. Calli and shoots showed healthy proliferation and elongation respectively on MS supplemented with BAP in combination with 2,4-D and IBA (ranging from 0.1 - 1.5 μm). BAP and 2,4-D used in lower concentrations (1.0 μm and 1.5 μm respectively) were highly effective in maintenance and proliferation of the callus. The calli were maintained in the dark for their survival and growth. It was observed that exposure to light inhibited their proliferation and the colour changed from green to black. Further, the calli obtained in the present study were morphologically diverse with green, cream, brown and white friable type (Fig. 1A-C), which in due course of time could be manipulated to take the route of indirect organogenesis. The present study is in agreement with the report of Weisshaar and Jenkins (1998) and Wang (1985) that a dark environment is essential for healthy growth of calli. BAP (0.5 μm) in combination with IBA (0.1 μm) were found effective in elongation of shoot length with minimal necrosis of the explants (Table 2, Fig. 1G-H). The present findings conform to the study of Nakamura et al. (1995) and Sokolov et al. (2014) that BAP along with NAA or IBA is essential in shoot elongation and multiplication of Magnolia genotypes.

Table 2. Callus proliferation and shoot elongation on MS basal medium with various combinations of plant growth regulators after three months of culture.

Growth regulator	Concentration (μm)	Callus weight (gm)
BAP + 2,4-D	1.00 + 0.50	0.21 ± 0.02[a]
	1.00 + 1.00	0.74 ± 0.16[b]
	1.00 + 1.50	**2.42 ± 0.10[c]**
		Shoot length (cm)
BAP + IBA	**0.50 + 0.10**	**3.34 ± 0.07[d]**
	0.50 + 0.25	2.38 ± 0.04[e]
	0.50 + 0.50	1.39 ± 0.03[f]

Data were collected after three months of culture. The calli and shoots regenerated (as in Table 1) were cultured on MS supplemented with various combinations of plant growth regulators. Experiments were performed with 10 samples per condition and repeated thrice. Values represent the mean ± SE. Means followed by the same letter within a column are not significantly different according to t-test ($p < 0.05$).

The regenerated shoots were subjected to *in vitro* root formation. Among various concentrations of IBA used, the best rooting was observed with 8 µm of IBA on ½ MS after 22 days of culture (Fig. 1I). The result obtained conforms to the report of Chaidaroon et al. (2004) that modified USK II medium supplemented with 4 mg/l of IBA showed 90% response after 24 days of culture.

The process of hardening of plantlets through gradual acclimatization is under progress. The present study can be viewed as an effective method for micropropagation of *M. punduana.* The micropropagated plants thus obtained could be the source of biochemical as well as timber extraction. This would alleviate the pressure on natural population and indirectly help in conserving the species in the wild.

Acknowledgements

Laboratory facilities provided by the Plant Biotechnology Laboratory, Department of Basic Sciences & Social Sciences and Department of Botany, North-Eastern Hill University, Shillong, India are acknowledged. The authors also express their gratitude to Dr. K. Upadhaya, Assistant Professor, North-Eastern Hill University and his research team for their assistance in the field collection and statistical analysis.

References

Abdelmageed AHA, Faridah QZ, Nor SK and **Julia AA** (2012) Callus induction and plant regeneration of *Michelia champaca* (Magnoliaceae): A multipurpose tree. J. Med. Plants Res. **6**(17): 3338-3344.

Biedermann IEG (1987) Factors affecting establishment and development of *Magnolia* hybrids *in vitro*. Acta. Hort. **212**: 625-629.

Chaidaroon S, Unguichian I and **Ratnathavornkiti K** (2004) *In vitro* root initiation of Champi Sirindhorn (*Magnolia sirindhorniae* Noot. & Chalermglin). Au. J. T. 129-132.

Choudhury H, Kumaria S and **Tandon P** (2008) Induction and maturation of somatic embryos from intact megagametophyte explants in Khasi pine (*Pinus kesiya* Royle ex. Gord.). Curr. Sci. **95** (10): 1433-1438.

Haridasan K and **Rao RR** (1985) Forest Flora of Meghalaya. Vols I. Bishen Singh, Mahandrapal Singh, Dehradun, India. p. 450.

IUCN (2014) IUCN Red List of Threatened Species. Version 2014.1

Kamenicka A and **Lanakova M** (2000) Effect of medium composition and type of vessel closure on axillary shoot production of magnolia *in vitro*. Acta. Physiol. Plant. **22**(2): 129-134.

Lloyd G and **McCown B** (1981) Commercially feasible micropropagation of Mountain Laurel, *Kalmia latifolia*, by use of shoot tip culture. Combined Proceeding International Plant Propagator's Society. **30**: 421-427.

Linsmaier EM and **Skoog F** (1965) Organic growth factor requirements of tobacco tissue culture. Physiol. Plant. **18**: 100-127.

Nakamura K, Wakita Y, Yokota S, Yoshizawa N and **Idei T** (1995) Induction of multiple shoots by shoot apex culture in *Magnolia obovata* Thunb. Plant Tissue Culture Letters. **12**(1): 34-40.

Nayar MP and **Sastry ARK** (1990) Red Data Book of Indian Plants. Vol. III. Botanical Survey of India, Calcutta. pp. 243-244.

Parris JK, Touchell DH, Ranney TG and **Adelberg J** (2012) Basal salt compositions, cytokonins, and phenolic binding agents influence *in vitro* growth and *ex vitro* establishment of *Magnolia*. Ann. Hort. Sci. **47**: 1625-1629.

Radomir AM (2012) Comparative study on the *in vitro* multiplication potential of *Magnolia stellata* and *Magnolia* × *Soulangiana* species. J. Hort. Forest Biotechnol. **16**: 39-44.

Ramasubbu R (2010) Protecting the wild beauties. Science Reporter. **47**(5): 19-22.

Sokolov RS, Atanassova BY and **Iakimova ET** (2014) Physiological response of *in vitro* cultured *Magnolia* sp. to nutrient medium composition. J. Hort. Res. **22**(1): 49-61.

Thomas TD and **Maseena EA** (2006) High frequency shoot regeneration from the nodal cuttings and leaf derived callus of medicinally important *Cardiospermum halicacabum* Linn. Sci. Hortic. **108**: 332-336.

Iralu V and **Upadhaya K** (2015) Notes on *Magnolia Punduana* Hk. F. & Th. (magnoliopsida: magnoliales: magnoliaceae): an endemic and threatened tree species of north eastern India. J. Threat. Taxa. **7**(9): 7573-7576.

Iralu V and **Upadhaya K** (2016) Dormancy, storability, and germination of seeds in *Magnolia punduana* (Magnoliaceae). Bot. **94**(10): 967-973.

Walter KS and **Gillett HJ** (1998) 1997 IUCN Red List of Threatened Plants. p. 434.

Wang PJ (1985) Producing pathogen free plants using tissue culture. Int. Assoc. Plant tissue culture (IAPTC) Newslett. **46**: 2-8.

Weisshaar B and **Jenkins GI** (1998) Phenylpropanoid biosynthesis and its regulation. Curr. Opin. Plant Biol. **1**: 251-257.

Wheeler L and **Rivers MC** (2015) *Magnolia punduana*. The IUCN Red List of Threatened Species. Version 2015.2. www.iucnredlist.org. Downloaded on 21 July 2015.