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Regeneration of *Hydnocarpus kurzi* (King) Warb. - A Red-listed Medicinal Plant

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Abstract

About 85% of the cultures regenerated showing four shoots per culture when explants of apical and axillary buds of young sprouts from naturally grown *Hydnocarpus kurzi* were cultured in MS supplemented with 2.5 mg/l BA + 0.5 mg/l NAA. Repeated subcultures, in the same medium, resulted in rapid shoot multiplication with eight shoots per culture. Addition of 15% (v/v) coconut water to the medium increased the number of shoot(s) up to 15 per culture. *In vitro* raised shoots rooted on half strength MS with 1.0 mg/l IBA + 1.0 mg/l NAA. For acclimation and transplantation the plants in the rooting culture vessels were kept in normal room temperature for seven days before transplanting in pots, where the plantlets were reared for three weeks. The survival rate of mature regenerants was found to be 75 - 80%.

Introduction

Hydnocarpus kurzi (King) Warb. is an evergreen, dioecious tree belonging to the family Flacourtiaceae. It is a native of Bangladesh, India and Myanmar. In Bangladesh the plant used to grow wild in Chittagong and Sylhet forests but now it is rarely found in those areas. A few trees are confined in reserve forests of Sylhet and in the National Botanical Garden, Dhaka. Its seeds yield an oil, known as chaulmoogra oil. It contains glycerides of cyclopentenyl fatty acids like hydnocarpic acid, chaulmoogric acid, gorlic acid, oleic acid and palmitic acid. Seed oil is highly esteemed as a cure for leprosy. It is also used for many other skin diseases (Ghani 1998). It may be mentioned here that chaulmoogra oil was the only effective medicine for the treatment of leprosy until the production of sulfur drugs in 1946 (Khan et al. 2001).

The plant is grown from seeds. Since the seeds are highly recalcitrant and harvested for its therapeutic uses and with the accelerating destruction of natural resources in the tropics the plant is naturally going to be threatened. In

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Bangladesh it has been red-listed (Khan et al. 2001). So it has become clear that the exploitation of this medicinal plant must be accompanied by conservation measures. Otherwise it will become depleted as resources or may even face extinction. Thus, it is considered to be important to regenerate this affected plant artificially, besides having beneficial effects on the ecosystems would also ensure sustainable uses as well as conservation of this medicinal plant.

This study was undertaken with a view to developing a protocol for regeneration of this important medicinal plant through *in vitro* culture, namely the plant tissue culture for *en masse* production of this material which according to Khan (2001) is now considered to be an endangered species because of its over exploitation for its medicinal properties against leprosy. Apart from mass propagation, tissue culture has two distinct advantages: (a) first it yields genetically identical material for further field culture as well as conservation (Hamann 1991; Wawrosch and Kopp 1999) and (b) secondly genetically uniform material will have identical phytochemical profiles (Roja and Heble 1993).

Materials and Methods

Healthy twigs of *Hydnocarpus kurzi* were collected from the National Botanical Garden, Mirpur, Dhaka, Bangladesh. Terminal shoot tips and stem nodes each with a single axillary bud were used as explants. Standard surface sterilization and inoculation methods as described earlier by Hassan and Roy (2005) were followed.

MS basal medium was used for shoot proliferation and adventitious shoot regeneration. Half strength MS was used for *in vitro* rooting. All media were supplemented with 30 g/l sucrose, 7 g/l agar (Difco) and dispensed into 25´150 mm culture tubes, 100 ml or 250 ml conical flasks. pH of the medium was adjusted to 5.8 before autoclaving at 121^{o}C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2^{o}C under a photon flux density of about 3 mmol m^{-2} s^{-2} emitted from cool white fluorescent tube lamps.

Shoot proliferation from shoot tips and nodal explants was obtained in two separate sets of experiments. In the first set 0 - 2.5 mg/l BA and 0 - 2.5 mg/l Kn were added to MS in different tubes to select the best cytokinin concentration for shoot induction. In the second set, combinations of BA-Kn and BA-NAA were assessed for shoot multiplication. For a large amount of shoot proliferation, coconut water was also added to the media. The number of explants showing shoot proliferation was recorded after three weeks of inoculation.

For *in vitro* rooting, individual shoots (3 - 5 cm) were cut from the proliferated shoots in culture tubes and implanted on to half strength MS with different concentrations and combinations of NAA, IBA and IAA.

The rooted plants were taken out from the culture tubes, washed to free the agar gel with autoclaved water and transplanted into a specially prepared sterile rooting medium containing a mixture of vermiculite, sand and soil in the ratio of 1 : 2 : 2. Plastic pots were used for transplanting the seedlings. For acclimation, the potted plantlets were kept in a polychamber at 80% relative humidity, at 32 ± 2°C under a 12 h photoperiod. The plants were given fertilizer with one-eighth MS macronutrients biweekly. Established plants were transplanted into the earthen pots under natural conditions.

Results and Discussion

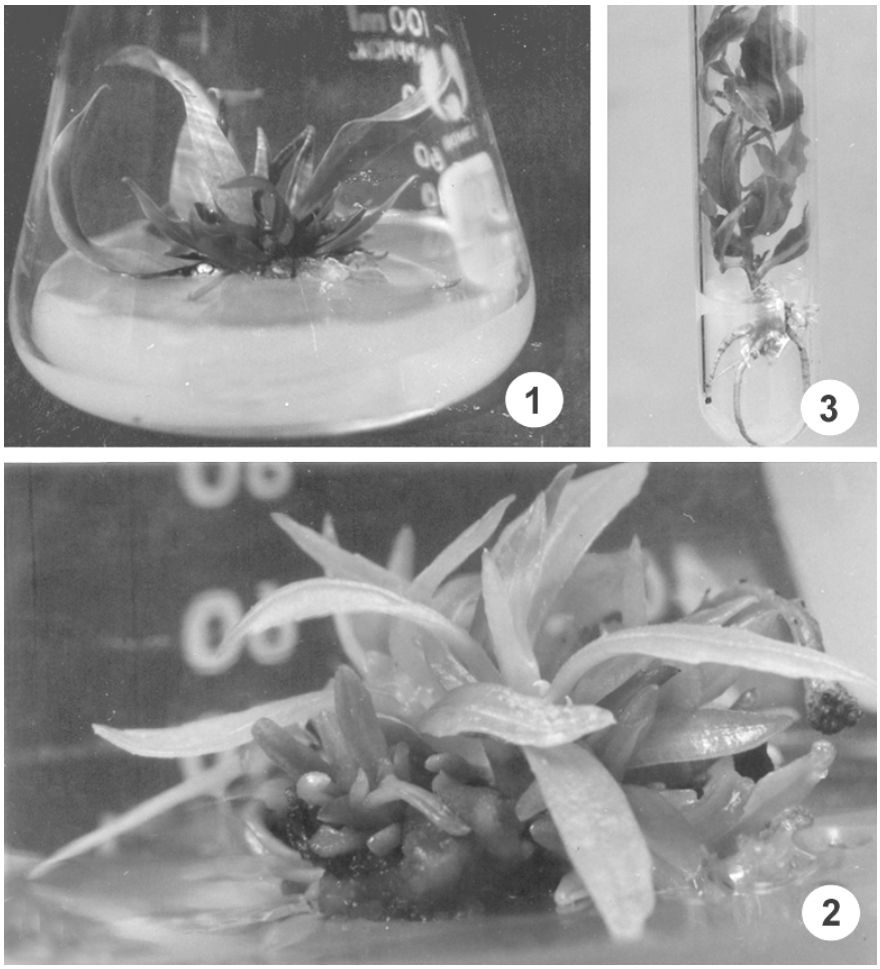
Shoot induction was not found in MS even after four weeks of culture. Explants produced two to three shoots within three-four weeks after inoculation on MS containing BA alone but the number of shoots increased up to four when the explants were cultured in MS with 2.5 mg/l BAP + 0.5 mg/l NAA (Table 1, Fig. 1). Both the explants (shoot tips and nodal explants) responded identically in the same medium. Kn alone and KN-NAA combinations were not found suitable for shoot induction.

Table 1. Effect of growth regulators in MS on morphogenetic response of *Hydnocarpus kurzi* shoot tip and nodal segment explants.*

Growth regulators (mg/l)		Shoot tips		Nodal segments	
BAP	NAA	% of explants forming shoots	Mean No. of shoot/explant	% of explants forming shoots	Mean No. of shoot/explant
0.0	0.0	-	-	-	-
0.5	0.0	42 (3.3)	1.6 (0.4)	41 (4.3)	1.8 (0.6)
1.0	0.0	43 (3.6)	1.8 (0.5)	42 (3.8)	1.6 (0.8)
1.5	0.0	46 (4.4)	2.8 (0.5)	48 (3.9)	3.1 (0.7)
2.0	0.0	40 (4.3)	1.4 (0.5)	40 (3.8)	1.6 (0.6)
2.5	0.0	40 (3.5)	1.2 (0.7)	40 (3.8)	1.4 (0.8)
0.5	0.1	59 (5.9)	2.6 (0.9)	60 (4.8)	2.9 (0.7)
0.5	0.2	62 (5.3)	2.5 (0.6)	64 (4.9)	2.4 (0.6)
1.0	0.2	67 (5.1)	3.1 (0.2)	65 (5.3)	3.5 (0.4)
1.0	0.5	70 (5.9)	3.5 (0.4)	73 (6.9)	3.2 (0.5)
1.5	0.2	62 (5.3)	3.1 (0.2)	63 (5.0)	3.3 (0.3)
1.5	0.5	66 (4.1)	3.5 (0.5)	68 (4.9)	3.4 (0.4)
1.5	1.0	59 (4.8)	3.3 (0.3)	57 (4.2)	3.1 (0.4)
2.0	0.5	75 (4.9)	3.4 (0.4)	68 (4.8)	3.6 (0.5)
2.0	1.0	61 (4.7)	3.6 (0.4)	65 (4.9)	3.3 (0.6)
2.5	0.5	**92 (5.9)**	**4.4 (0.3)**	**93 (5.1)**	**4.0 (0.3)**
2.5	1.0	67 (4.5)	2.4 (0.3)	66 (4.9)	2.6 (0.4)

*Results are mean (± SE) of three experiments, each with 15 replications.

Newly initiated shoots were separated and subcultured repeatedly in fresh MS with 2.5 mg/l BA + 0.5 mg/l NAA, where the number of shoots increased up to eight per culture. For further modification of the medium, coconut water (CW) was added. Addition of 15% CW was found to increase the number of shoots up to 15 per culture (Fig. 2). Thus, the medium determined for shoot multiplication was MS + 2.5 mg/l BA + 0.5 mg/l NAA + 15% CW.



Figs. 1 - 3 : *In vitro* regeneration of *Hydnocarpus kurzi* from shoot tip and nodal explants. 1. Multiple shoot induction in MS with 2.5 mg/l BA + 0.5 mg/l NAA in four weeks of culture. 2. Enhanced shoot proliferation when subcultured in MS + 2.5 mg/l BA + 0.5 mg/l NAA + 15% CM. 3. Rooting of *in vitro* regenerated shoots cultured in half strength MS with 1.0 mg/l NAA + 1.0 mg/l IBA in four weeks of culture.

Mass propagation of plant species through *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture

technology. Recently there has been much progress in *in vitro* culture technology for conservation of genetic resources and clonal improvement and has been described in different reports (Barz et al. 1977; Datta and Datta 1985; Kukreja et al. 1989; Jusekutty et al. 1993; Maskay 1996; Wawrosch and Kopp 1999). Rapid shoot regeneration has been achieved in a wide range of species. The initial explants in the above material were taken from normal aerial shoots of field grown herbaceous medicinal plant species (Jaiswal et al. 1989; Mathur et al. 1993;

Table 2. Effect of auxin(s) on root induction in regenerated shoots of *Hydnocarpus kurzi* cultured on half strength MS.*

Auxin(s) (mg/l)	% of shoots rooted (± SE)	Days required for root induction (± SE)
IBA 0.5	-	-
IBA 1.0	-	-
IBA 1.5	-	-
IBA 2.0	-	-
IBA 2.5	-	-
IBA 0.5 + NAA 0.5	50 (4.2)	26 (1.8)
IBA 1.0 + NAA 0.5	71 (4.2)	25 (1.6)
IBA 1.0 + NAA 1.0	**90 (4.1)**	**23 (1.7)**
IBA 1.5 + NAA 1.0	80 (3.9)	24 (2.6)
IBA 2.0 + NAA 1.0	50 (1.2)	24 (1.9)
IBA 2.0 + NAA 2.0	45 (3.1)	25 (1.8)
IBA 2.5 + NAA 2.0	44 (4.2)	24 (2.1)
IBA 0.5 + IAA 0.5	37 (3.9)	24 (1.7)
IBA 1.0 + IAA 0.5	30 (3.3)	23 (1.8)
IBA 1.0 + IAA 1.0	20 (1.0)	18 (2.8)
IBA 1.5 + IAA 1.0	14 (1.2)	18 (1.8)
IBA 2.0 + IAA 1.0	18 (1.2)	20 (2.1)
IBA 2.0 + IAA 2.0	22 (2.2)	23 (1.9)
IBA 2.5 + IAA 2.0	23 (3.8)	25 (2.4)
NAA 0.5 + IAA 0.5	31 (4.3)	21 (3.7)
NAA 1.0 + IAA 0.5	30 (0.0)	21 (2.6)
NAA 1.0 + IAA 1.0	28 (2.7)	21 (2.1)
NAA 1.5 + IAA 1.0	23 (4.5)	24 (2.0)

*Data were recorded after four weeks of culture. Results are mean (± SE) of 15 replications.

Maskay 1996; Rai 2002; Hall and Camper 2002; Hassan and Roy 2005). In the present investigation, regeneration of shoots took place directly in explants comprising shoot tips and nodal segments of *Hydnocarpus kurzi*. The most suitable medium was found to be MS + 2.5 mg/l BAP + 0.5 mg/l NAA. Repeated subcultures of explants on fresh shoot proliferation medium helped achieving continuous supply of healthy shoot buds and shoots at least for five to ten

subculture cycles. For further shoot multiplication and growth, addition of CW in the nutrient medium was found to be effective. Addition of 15% CW to the medium increased the number of shoots up to 15 per culture. Positive effect of CW in nutrient medium is also reported by Roy et al. (1998) and Hassan and Roy (2005). CW of the green nut is very effective in providing an undefined mixture of organic nutrients and growth factors (Gamborg and Phillips 1995).

About 90 per cent of the regenerated shoots rooted, when the excised shoots were cultured individually on root induction medium. The rooting medium consisted of half strength MS supplemented with 1.0 mg/l IBA and 1.0 mg/l NAA (Table 2). Roots initiated by the end of the third week of culture (Fig. 3) and on the week following lateral roots developed from the primary roots. For rooting use of auxin(s) singly or in combination was also reported by Sahoo and Chand (1998); Ajith and Seeni (1998); Rai (2002).

After four - six weeks in the rooting medium the rooted shoots were transferred to pots. Plantlets transferred directly from the rooting medium to the pot under natural conditions did not survive. On the other hand, about 75 to 80 per cent of the transplanted plants survived if they were hardened by keeping them in the rooting culture tubes at normal room temperature for seven days. Before transplanting into pots, plantlets were grown for three weeks at 30 ± 2°C and light (2000 lux) in a chamber with 80% humidity. During this period shoots elongated, leaves expanded and turned deep green, giving the regenerated population of plants a healthy look.

After three weeks, plants were transferred to an open place and gradually acclimated to outdoor conditions, where 90% of them survived. The technique described here appears to be readily adaptable for large scale clonal propagation and plantation of *Hydnocarpus kurzi*. Moreover, by further improving the protocols for clonal propagation of elite plants of this material, it is possible to achieve a tenfold increase of the products per unit area of cultivation.

References

Ajith KD and **Seeni S** (1998) Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr., a medicinal tree. Plant Cell Rep. **17**: 422-426.

Barz W, Reinhard E and **Zenk MH** (1977) Plant Tissue Culture and its Biotechnological Application, pp. 27-43, Springer Verlag, Berlin, New York.

Datta PC and **Datta SC** (1985) Applied Biotechnology on Medicinal, Aromatic and Timber Plants. Calcutta University, Calcutta, India.

Gamborg OL and **Phillips GC** (1995). Plant Cell, Tissue and Organ Culture (Fundamental Methods), p. 22, Springer Verlag, Berlin, Heidelberg.

Ghani A (1998) Medicinal Plants of Bangladesh (Chemical Constituents and Uses). Asiatic Society of Bangladesh, Dhaka.

Hall KC and **Camper ND** (2002) Tissue culture of goldenseal (*Hydrastis canadensis* L.). In Vitro Cell. Dev.Biol.-Plant, **38**: 293-295.

Hassan AKMS and **Roy SK** (2005) Micropropagation of *Gloriosa superba* L. through high frequency shoot proliferation. Plant Tissue Culture **15** : 55-63.

Hamann O (1991) The joint IUCN-WWF plant conservation program and its interests in medicinal plants. *In:* Akerele O, Heyood V and Synge H (eds). Conservation of Medicinal Plants. pp. 13-22, Cambridge University Press, Cambridge.

Jaiswal VS, Narayan P and **Lal M** (1989) Micropropagation of *Adhatoda vasica* Nees through nodal segment culture. *In*: Kukreja AK, Mathur AK, Ahuja PS and Thakur RS (eds.). Tissue Culture and Biotechnology of Medicinal and Aromatic Plants, pp. 7-11, Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

Jusekutty PC, Swati S and **Prathapasenan G** (1993) Direct and indirect organogenesis in *Coccinia indica*. J. Hort. Sci. **68**: 31-35.

Khan MS, Rahman MM and **Ali MA** (2001) Red data book of vascular plants of Bangladesh. pp. 68-69, Bangladesh National Herbarium, Dhaka.

Kukreja AK, Mathur AK, Ahuja PS and **Thakur RS** (1989) Tissue Culture and Biotechnology of Medicinal and Aromatic Plants. Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

Masky N (1996) Micropropagation of the threatened Nepalees medicinal plants *Swertia chirata* Buch.-Ham. Ex Wall. and *Mahonia napaulensis* DC. Mainz, Chorus Verlag, Munchen.

Mathur A, Ahuja PS and **Mathur AK** (1993) Micropropagation of *Panax quinquefolium, Rauvolfia serpentina* and some other medicinal and aromatic plants of India. *In*: Quynh NT and Uyen NV (eds.). Adapted Techniques for Commercial Crops of the Tropics. , pp. 155-173, Agricultural Publishing House, Ho Chi Minh City.

Rai VR (2002) Rapid clonal propagation of *Nothapodytes foetida* (Weight) Sleumer - a threatened medicinal tree. In Vitro Cell. Dev. Biol. Plant **38**: 347-351.

Roja G and **Heble MR** (1993) The quinoline alkaloids camptothecin and 9-methoxy camptothecine from tissue culture and mature trees of *Nothapodytes foetida.* Phytochemistry **36**: 65-66.

Roy SK, Islam MS and **Hadiuzzaman S** (1998) Micropropragation of *Elaeocarpus robustus*. Plant Cell Rep. **17**: 810-813.

Sahoo Y and **Chand PK** (1998) Micropropagation of *Vitex negundo* L. - A woody aromatic medicinal shrub through high frequency axillary shoot proliferation. Plant Cell Rep. **18**: 301-307.

Wawrosch C and **Kopp B** (1999) Application of plant tissue culture in protection and domestication of rare and endangered medicinal plants. In Vitro Cell. Dev. Biol. Plant **35**: 180-181.