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Full Length Research Paper

In vitro propagation of *Hoya wightii* ssp. *palniensis* K.T. Mathew, a highly vulnerable and endemic species of Western Ghats of Tamil Nadu, India

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***In vitro* propagation of *Hoya wightii* ssp. *palniensis* (Asclepiadaceae), a highly vulnerable and endemic plant species of Western Ghats, Tamil Nadu, India was carried out. Shoot tip explants were cultured on MS medium fortified with cytokinins (KN, BA, 2-iP and TDZ) in various concentrations and in combination with auxins (IBA, IAA and NAA). High frequency of shoot bud proliferation and multiplication was observed on KN (4.65 µM) + IBA (1.47 µM). Multiple shoot induction efficiency was increased on ascorbic acid (100 mg/l) supplemented medium along with KN (4.65 µM) +IBA (1.47 µM). Rhizogenesis was observed on MS medium supplemented with IBA (0.98 µM), plantlets produced through micropropagation were hardened with the survival success of 56%. The efficient plantlet regeneration protocol developed would aid *ex situ* conservation of this vulnerable species.**

Key words: *Hoya wightii* ssp. *palniensis, in vitro* propagation, Asclepiadaceae, plant growth regulators, ascorbic acid, micropropagation.

INTRODUCTION

Hoya is a genus of 300 species of tropical climbing plants, native to south Asia (India and China), Australia and Polynesia. *Hoya* (commonly known as “wax plant” because of its waxy appearance) is of significant horticultural importance in Europe, America and Australia. *H. wightii* ssp. *palniensis*, an endemic plant species, has a very restricted distribution only in the Western Ghats of Tamil Nadu, India. Mathew (1992) and Matthew (1996) reported the presence of *H. wightii ssp. palniensis* in Pambar Shola, Tamil Nadu. It is a trailing herb hanging from a rock in hills near river streams (Figure 1A). Its highly vulnerable position is due to habitat destruction and over exploitation (Matthew, 1999a). Leaves opposite and oblong, about 2 - 4 cm long, 1.5 - 2.5 cm wide. The flowers are borne in an umbel and the flowers last about 1 week, sometimes longer. The flowers are white with red coronas. There are 7 - 12 flowers in an umbel and each are 1.5 - 2 cm in diameter (Figure 1B). Follicles elliptic-lanceolate, 5 x 0.9 cm, seeds ovate-oblong. Fruit setting (Figure 1C) was observed by the authors under green house condition at Vattakanal conservation trust nursery, Kodaikanal during August 2007.

In vitro culture has been widely used for the propagation of agricultural and horticultural crops and for the conservation of crop genetic resources (George and Sherrington, 1984). Mass propagation of plants can be obtained from a small explant shoot tip within few months. It is rapid and cost effective and helps in germplasm conservation and clonal propagation plants can be produced throughout the year. Tissue culture studies on *Hoya* species were very limited. Tube Yan et

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Abbreviations: AA, Ascorbic acid; **BA**, 6-benzyl adenine; **CH**, casein hydrolysate; **CA**, citric acid; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **KN**, kinetin; **NAA**, α- naphthalene acetic acid; **TDZ**, thidiazuron; **YE**, yeast extract; **2-iP**, 2-isopentenyladenine.

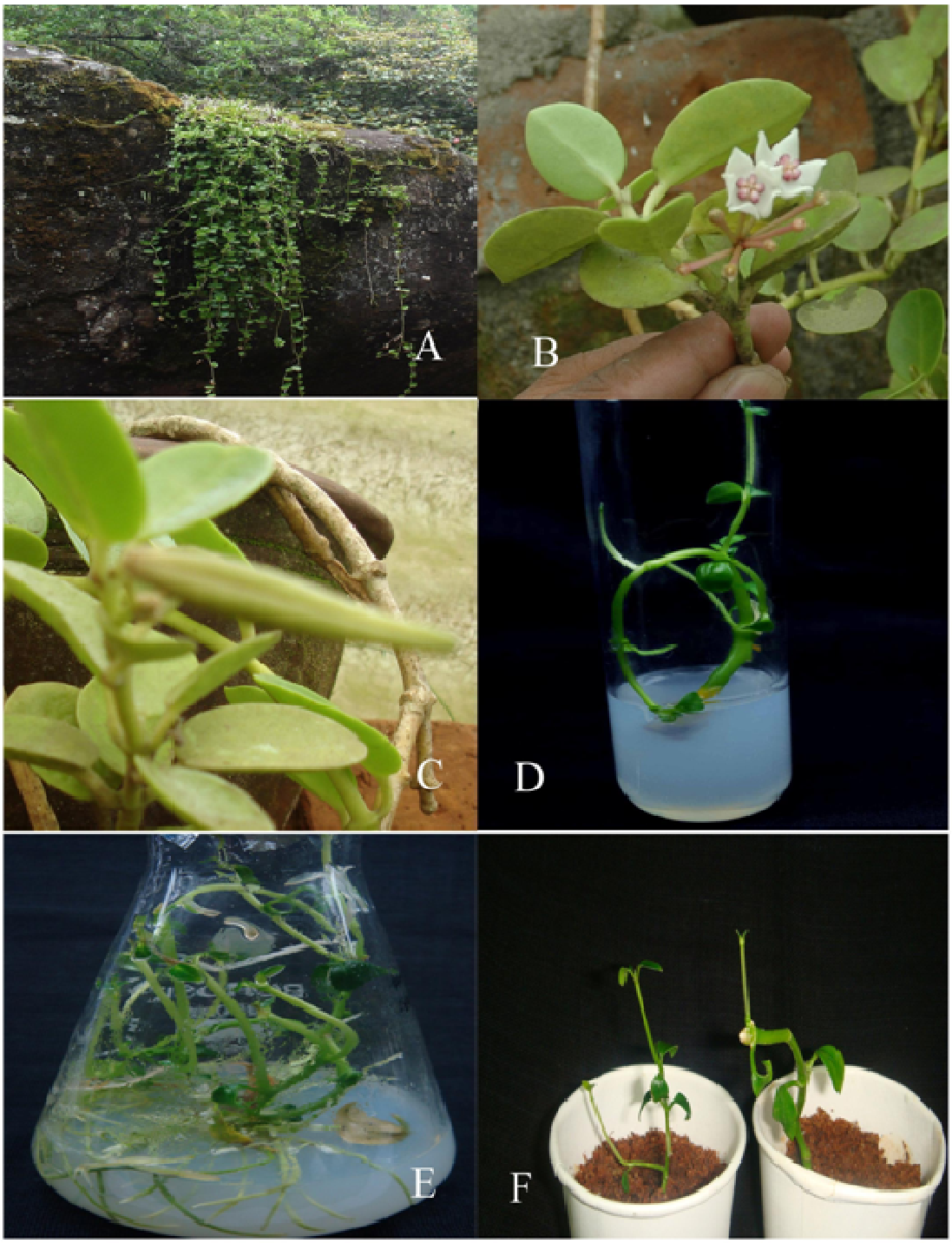

Figure 1. Micropropagation of *Hoya wightii* ssp. *Palniensis*. (A) *Hoya wightii* ssp. *Palniensis*- habitat; (B) Flower; (C) Follicle; (D) Shoot multiplication on MS+KN (1.0 mg); (E) Shoot multiplication MS +KN (1.0) +IBA (0.3) + AA)100 mg/l); (F) Hardened plants.

al. (2007) reported *in vitro* organogenesis of *Hoya kerrii* from leaf explants. Maraffa et al. (1981) worked on the asexual embryogenesis in cultured leaf sections of *Hoya carnosa* “Compacta” and *H.carnosa* “Rubra”. Besides being a highly vulnerable plant, *H. wightii* ssp. *palniensis* has never been subjected to *in vitro* studies. Here, we established a simple protocol for clonal propagation from shoot tip explant of *H. wightii* ssp. *palniensis*. These results could serve as a guide for future regeneration study of this vulnerable plant.

MATERIALS AND METHODS

Plant material

The plants of *H. wightii* ssp. *palniensis* were collected from Pambar Shola in the Western Ghats of Tamil Nadu, India and maintained in earthen pots in the glass house of Bharathidasan University, Tiruchirappalli under controlled conditions (Temp. 26 ± 2°C and RH 70%). Explants were collected from these plants after stabilization.

Surface sterilization

Shoot tip explants (2 cm length) were washed under running tap water for 30 min and surface sterilized with 70% ethanol for 30 s, rinsed with sterile distilled water and treated with 0.1% $HgCl_2$ for 3 min with a final rinse in sterile distilled water thrice for 5 min. These sterilized explants were inoculated on culture medium.

Influence of different cytokinins

After surface sterilization, the explants were inoculated on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) fortified with various concentrations of cytokinins individually [BA (2.22 - 17.76 µM), KN (2.32 - 27.84 µM), 2-iP (2.46 - 19.68 µM) and TDZ (0.22 - 1.8 µM)] for shoot bud proliferation. The culture medium was supplemented with 3% sucrose (w/v) and solidifying agent 0.8% agar (Bacterial grade, Himedia, India). 8 weeks later, the shoot regeneration frequency was observed. Effect of the optimal cytokinin in combination with a different range of auxins [IAA (0.57 - 1.71 µM), NAA (0.54 - 1.62 µM) and IBA (0.49 - 1.47 µM)] concentrations was tested for shoot multiplication.

Effect of supplements

Organic supplements like casein hydrolysate and yeast extract, antioxidants namely citric acid and ascorbic acid: 50, 100 and 150 mg/l (w/v) were supplemented to the shoot multiplication medium (cytokinin-auxin optimal combination) to assess the enhancement of shoot multiplication rate.

Rooting and transplantation of the regenerated plantlet

The individual shoots regenerated from shoot explant were transferred to rooting medium consisting of MS medium + auxins [IAA (0.05 - 1.71 µM), IBA (0.04 - 1.47 µM) and NAA (0.05 - 1.62 µM). The rooted healthy plantlets were washed off adhering agar in sterile distilled water and were transferred to paper cups (2.5 cm diameter) containing red soil mixed with sand and coconut coir (1:1:1) under controlled growth chamber conditions of 26 ± 2°C, 16 h photoperiod, 80 - 85% relative humidity and 60 $\mu mol^{-2}s^{-1}$ light intensity. The potted plants were irrigated with MS basal salt solutions every 4 days for 3 weeks. The plants were then transferred to glass house.

Culture conditions

The pH of the medium was adjusted to 5.7 with 0.1 N NaOH or 0.1 N HCl after addition of the growth regulators, prior to the addition of 0.8% agar (Himedia, India). The medium was autoclaved at 121°C, for 30 min.

All the cultures were maintained in sterilized culture room at 26 ± 2°C, under 16/8 h light regime provided by cool white fluorescent light (60 $\mu mol^{-2}s^{-1}$ light intensity) and with 55 - 60% relative humidity.

Experimental design and data collection

All the experiments were conducted as a randomized complete design. For each experiment, a minimum of 10 replicates were taken and repeated thrice. Observations of the culture were made every week and data related to shoot multiplication (frequency of response, number of shoots and shoot length) were collected 8 weeks after culture. Comparisons between treatments were made with Duncan's new multiple range test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

H. wightii ssp. *palniensis* has inherently poor seed setting nature and the only earlier instance of follicle known is on AS 1422 (SHC) of 1914 (Matthew, 1999b). Though the present authors have observed fruit setting in Vattakanal trust recently, no comprehensive reports are available to date regarding the information on seed setting and germination of this species. Their preference to narrow habitat (Pambar ravine) also might be a reason for its disappearance (Matthew, 1999b). Thus, the species remains highly vulnerable and its distribution is confined to a very small region in the Western Ghats. An earnest effort on conservation of this sparsely distributed species through *in vitro* propagation is the need of the hour. Our study, therefore, focused on the development of complete plantlet with well developed shoots and roots from shoot tip explants.

Influence of cytokinins on shoot multiplication

Shoot tip proliferation was observed at all individual concentrations of BA, KN, 2-iP and TDZ but showed varied response with respect to number of shoots obtained per explant and their shoot length (Table 1). The explants responded well (100%) and produced increased number of shoots (3.08 ± 0.05) on MS medium supplemented with KN (4.65 µM) (Figure 1D) whereas, TDZ resulted in a significantly low shoot multiplication (6.1%). The average number of shoots per explant was significantly reduced with high concentrations of 2-iP when compared to KN (Table 1). Our study showed that the highest number of shoots was formed on MS medium supplemented with KN (4.65 µM) among the cytokinins and increasing or decreasing their concentration reduced the frequency and number of shoots produced. KN induced multiple adventitious shoots as was reported in another *Asclepiadaceous* member *Gymnema sylvestre* (Komalavalli and Rao, 2000). Shoots developed on medium supplemented with TDZ (0.67 µM) showing higher shoot length (6.8 ± 0.2 cm) than other cytokinins used in our study. TDZ showed negligible effects on shoot bud proliferation and multiplication, though TDZ is

Table 1. Shoot multiplication from shoot tip explant of *Hoya wightii* ssp. *palniensis* on MS medium supplemented with different cytokinins[1].

Plant growth regulator[2] (µM)	Shoot length (cm)	No.of shoots/explant	Frequency percentage	Percentage of basal callusing
BA				
2.22	3.1 ± 0.1^{jk}	2.6 ± 0.2^{b}	85.7 ± 1.7^{b}	0
4.44	2.7 ± 0.4^{m}	2.4 ± 0.5^{c}	42.8 ± 3.5^{h}	0
6.66	2.5 ± 0.4^{n}	1.2 ± 0.4^{i}	40.0 ± 2.6^{hi}	0
8.87	2.1 ± 0.2^{pq}	1.0 ± 0.1^{j}	40.0 ± 1.2^{hi}	0
13.32	2.1 ± 0.1^{pq}	1.0 ± 0.3^{j}	71.4 ± 1.6^{e}	57.1 ± 1.2^{d}
17.76	2.7 ± 0.1^{m}	1.6 ± 0.1^{g}	83.3 ± 1.3^{bc}	66.6 ± 2.1^{c}
KN				
2.32	5.0 ± 0.7^{e}	1.8 ± 0.04^{f}	66.6 ± 2.0^{f}	0
4.65	5.3 ± 0.1^{cd}	3.8 ± 0.5^{a}	100.0 ± 0.0^{a}	0
6.96	5.3 ± 0.11^{c}	3.8 ± 0.1^{a}	85.7 ± 1.9^{b}	0
9.29	2.7 ± 0.3^{m}	2.2 ± 0.4^{d}	71.4 ± 1.4^{e}	18.0 ± 1.3^{h}
13.95	2.4 ± 0.2^{no}	1.4 ± 0.3^{h}	83.3 ± 1.6^{bc}	51.1 ± 1.1^{de}
27.84	2.2 ± 0.2^{p}	1.4 ± 0.1^{h}	85.7 ± 1.5^{b}	66.6 ± 1.0^{c}
2-ip				
2.46	2.6 ± 0.3^{n}	1.6 ± 0.7^{g}	81.1 ± 2.1^{cd}	0
4.92	3.6 ± 0.2^{gh}	1.8 ± 0.1^{f}	57.1 ± 1.1^{g}	27.1 ± 0.2^{g}
7.38	3.6 ± 0.1^{gh}	2.44 ± 0.2^{c}	28.5 ± 1.0^{kl}	40.0 ± 1.1^{f}
9.84	5.7 ± 0.7^{b}	1.8 ± 0.7^{f}	22.7 ± 1.6^{m}	40.0 ± 2.0^{f}
14.76	4.1 ± 0.5^{f}	1.8 ± 0.1^{f}	18.0 ± 2.0^{n}	75.0 ± 1.2^{b}
19.68	3.7 ± 0.14^{g}	1.6 ± 0.8^{g}	18.6 ± 1.7^{n}	100.0^{a}
TDZ				
0.22	3.2 ± 0.12^{j}	1.0 ± 0.5^{j}	31.2 ± 1.20^{jk}	0
0.45	3.5 ± 0.30^{hi}	1.2 ± 0.1^{i}	31.2 ± 1.17^{jk}	0
0.67	6.8 ± 0.24^{a}	2.0 ± 0.4^{e}	32.0 ± 1.0^{j}	0
0.90	6.8 ± 0.10^{a}	1.8 ± 0.1^{f}	32.0 ± 1.2^{j}	40 ± 0.6^{f}
1.35	3.0 ± 0.04^{kl}	1.4 ± 0.7^{h}	12.0 ± 1.2^{o}	100 ± 0.0^{a}
1.80	2.7 ± 0.09^{m}	1.4 ± 0.1^{h}	6.1 ± 1.7^{p}	100 ± 0.0^{a}

[1]Data were scored after 8 weeks of culture.
[2]Cytokinins were supplemented to MS medium. For all the parameters mean values followed by the same letter are not significantly different at P = 0.05 (Duncan's New Multiple Range Test).

known to induce cytokinin like effects in woody plants (Barrueto Cid et al., 1999; Datta et al., 2007) and in herbaceous crop species as well (Huetteman and Preece, 1993). Nevertheless, constraints such as poor elongation of shoots and inadequate rooting have been reported (Murthy and Saxena, 1998) in the conversion of TDZ-induced shoots in to complete plantlets. Moreover, it reveals the fact that role of TDZ on the shoot length was dependent on the species.

Synergistic effect of kinetin and auxins on multiple shoot formation

The effect of cytokinin on tissue or organ culture varies according to the particular compound used, the type of culture and age of explants (juvenile or mature) (Messeuger and Mele, 1987). One of the advantages of adding auxin at low concentration on the culture medium is to nullify the effect of the high concentrations of cytokinin on axillary shoot elongation (Hu and Wang, 1983). Hence, after determining the optimum level for best shoot bud multiplication with KN (4.65 µM), screening for the effect of auxins like IAA, NAA and IBA at different concentrations was done. The shoot number was increased when auxin (both NAA and IBA at 1.62 µM and 1.47 µM respectively) combined with KN (4.65 µM). A three fold enhancement in shoot formation was observed with a high concentration of KN (4.65 µM) and low concentration of IBA (1.47 µM) can be attributed to the synergistic effect of the combination (Table 2). The supply of exogenous growth regulators in the medium is essential for differentiation which otherwise did not occur. Improved shoot regeneration with cytokinin and auxin

Table 2. Influence of different concentrations of auxins in combination with KN (4.65 µM) on shoot multiplication of *H. wightii* ssp. *palniensis*.[1]

Plant growth regulator[2] (µM)	Shoot length (cm)	No. of shoots/explant	Shoot multiplication frequency (%)	% Basal callusing
IBA				
0.49	6.5 ± 0.9^{bc}	4.4 ± 0.8^{de}	40 ± 0.6^{fg}	-
0.98	7.2 ± 0. 2^{b}	6.4 ± 0.9^{bc}	58 ± 0.1^{cd}	-
1.47	9.1 ± 0.1^{a}	10.3 ± 0.1^{a}	73 ± 0.2^{a}	12.5 ± 0.4^{bc}
IAA				
0.57	4.3 ± 0.1^{de}	1.5 ± 0.5^{fg}	35 ± 0.1^{h}	-
1.14	4.3 ± 0.3^{de}	1.5 ± 0.7^{fg}	40 ± 0.3^{fg}	-
1.71	3.9 ± 0.7^{ef}	2.4 ± 0.6^{f}	42 ± 0.5^{f}	-
NAA				
0.54	2.8 ± 0.9^{g}	2.4 ± 0.1^{f}	55 ± 0.2^{de}	-
1.07	4.4 ± 0.6^{d}	4.5 ± 0.2^{d}	60 ± 0.3^{bc}	25 ± 1.4^{b}
1.62	6.5 ± 0.2^{bc}	6.5 ± 0.3^{b}	63 ± 0.1^{b}	50 ± 1.2^{a}

[1]Data were scored after 8 weeks of culture.
[2]Combinations of KN and auxins were supplemented to MS medium. For all the parameters mean values followed by the same letter are not significantly different at P=0.05 (Duncan's New Multiple Range Test).

combinations has been proved in several other species of Asclepiadaceae - *Pergularia pallida* (Bapat et al., 1986), *Gymnema elegans* (Komalavalli and Rao, 1997), *Decalepis hamiltonii* (Bais et al., 2000) and *Holostemma ada-kodien* (Martin, 2002). In accordance with this, our study also exemplifies the positive effect of low concentration of auxin in combination with a cytokinin in shoot multiplication efficacy.

Influence of growth regulators on basal callusing

Profuse callusing was observed in higher concentrations of cytokinin along with the reduction in shoot multiplication and growth retardation (Table 1). Shoot tips cultured on MS medium fortified with 2-iP above 9.84 µM or TDZ above 0.90 µM showed a high percentage of basal callusing when compared to that of BA (13.32 µM) and KN (9.29 µM) but shoot differentiation from basal callus failed to occur. This may be due to the action of accumulated auxin at the basal cut ends which stimulates cell proliferation, especially in the presence of cytokinin (Marks and Simpson, 1994). The suppression of shoot sprouting and domination of callus growth at higher concentrations of cytokinin has also been observed in *G. sylvestre* (Komalavalli and Rao, 2000).

Effect of supplements

After selecting the optimum concentrations and combination of cytokinin-auxin for shoot multiplication, organic supplements, casein hydrolysate and yeast extract was used to improve the shoot multiplication efficiency. Casein hydrolysate is a complex source of reduced nitrogen while yeast extract is a culture media supplement because of the high concentration and quality of B vitamins present in it. Hence, their potential on shoot enhancement was analyzed. The efficiency of KN (4.65 µM) + IBA (1.47 µM) for shoot multiplication decreased significantly when it was supplemented with CH and YE (Table 3). The addition of CH and YE did not significantly improve the shoot multiplication frequency, their number or length. Increase in the concentration of CH and YE resulted in suppression of shoot multiplication. Among the two organic supplements, YE showed significantly low shoot multiplication frequency than CH. Contrary to our observation, YE and CH have been successfully used in several perennial species for shoot multiplication (Ahmad and Anis, 2005; Hussain et al., 2008; Roy et al., 1998).

Citric acid and ascorbic acid control browning of culture medium and also plays a significant role in the multiplication of shoots in many plants. Hence, their efficiency on shoot multiplication was examined. Citric acid in combination with KN (4.65 µM) + IBA (1.47 µM) enhance shoot multiplication frequency and number of shoot production per explant was lower when compared to that of AA (Figure 1E) (Table 3). Similarly, Sanjaya et al. (2005) on *Pseudoxytenthera stocksii* observed effectiveness of CA on enhancing the shoot multiplication frequency and number of shoots per explant. In our study, ascorbic acid showed affirmative effects on shoot multiplications than the cytokinin-auxin combination. The addition of exogenous ascorbic acid to plant tissue is said to increase their metabolic activity and accelerate the release of sugars for better growth and development (George, 1993). Similarly a simulative effect of ascorbic

Table 3. Multiple shoot induction from shoot tip explants of *H. wightii* ssp. *palniensis* on MS medium supplemented with KN (4.65 µM) + IBA (1.47 µM) and supplements[1].

Supplements [2] (mg/l)	Shoot length (cm)	No. of shoots/explant	Shoot multiplication frequency (%)
CH			
50	3.7 ± 0.2^{h}	2.3 ± 0.7^{hi}	25 ± 0.6^{g}
100	4.6 ± 0.9^{de}	2.5 ± 0.4^{gh}	30 ± 0.5^{f}
150	3.0 ± 0.1^{jk}	1.5 ± 0.1^{kl}	40 ± 0.3^{e}
YE			
50	2.7 ± 0.1^{l}	1.6 ± 0.4^{jk}	20 ± 0.1^{h}
100	4.5 ± 0.1^{ef}	1.6 ± 0.6^{jk}	25 ± 0.6^{g}
150	3.2 ± 0.7^{i}	1.5 ± 0.2^{kl}	25 ± 0.4^{g}
AA			
50	4.9 ± 0.5^{cd}	6.0 ± 0.5^{de}	50 ± 0.3^{c}
100	6.5 ± 0.6^{a}	6.5 ± 0.2^{d}	75 ± 0.2^{a}
150	5.0 ± 0.7^{c}	4.5 ± 0.1^{f}	60 ± 0.4^{b}
CA			
50	4.06 ± 0.2^{g}	2.00 ± 0.1^{ij}	40 ± 0.2^{e}
100	5.7 ± 0.1^{b}	4.5 ± 0.2^{f}	50 ± 0.3^{c}
150	4.8 ± 0.7^{cd}	3.0 ± 0.2^{g}	45 ± 0.4^{d}

[1]Data were scored after 8 weeks of culture.
[2]Cytokinin-auxin combination and supplements were fortified with MS medium. For all the parameters mean values followed by the same letter are not significantly different at P=0.05 (Duncan's new multiple range Test).

acid has also been reported (Neelam and Chandel, 1992; Shekhawat et al., 1993).

Rooting and acclimatization

Rhizogenesis, the formation of roots, is a crucial step in micropropagation for the formation of complete plantlets. Auxins induce root differentiation and their role in root development is well documented (Scott, 1972). During the study, regenerated shoots, transferred to MS medium fortified with IBA and NAA (above 0.24 and 0.27 µM) showed well developed roots when compared to IAA (above 0.28 µM). The highest rooting frequency (80%) was observed on MS medium supplemented with IBA (1.47 µM) (Table 4). Similarly, IBA has been used successfully to obtain the highest rooting frequency of *Decalepis hamiltonii* (Obul Reddy et al., 2001). Intermittent callus formation was noticed on MS medium supplemented with NAA (1.62 µM). This may be due to the residual cytokinin in shoots (Nemeth, 1979). The formation of adventitious roots was restricted only to the concentration of NAA (0.27 µM) on MS medium. The roots formed at the higher concentrations of NAA on MS medium were thick, stumpy and green in colour. These roots easily detach from the microshoots during transplantation and therefore needs further studies.

The rooted shoots were transferred to paper cups containing sand, red soil and coconut coir in the ratio of 1:1:1 (Figure 1F). Initially each pot was covered with a polythene bag to maintain high moisture. Subsequently, the moisture was reduced by the removal of polythene bags to harden the plants from which about 56% survived. These plants were successfully planted in the green house till maturity indicating the potential use of this system for *H. wightii* ssp. *palniensis in vitro* regeneration.

Conclusion

An efficient protocol was developed for micropropagation of *H. wightii* ssp. *palniensis*, a highly vulnerable plant of Western Ghats of Tamil Nadu, India. KN in combination with IBA showed adventitious shoot regeneration from shoot tip explant. This protocol can be used for the propagation and *ex situ* conservation of this vulnerable species. Application of this protocol could help in minimizing the pressure on wild populations of this highly vulnerable species and contribute to the conservation of the valuable biodiversity of the Western Ghats.

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Table 4. Effect of auxins on in *vitro* rooting of *Hoya wightii* ssp. *palniensis*[1].

Plant growth regulator[2] (µM)	Days to initiate root	Average No. of roots/microshoot	Root length (cm)	Rooting percentage
NAA				
0.05	10 - 12	4.2 ± 0.6^{b}	4.2 ± 0.6^{cd}	75 ± 0.6^{cd}
0.27	10 - 12	3.5 ± 0.1^{c}	4.5 ± 0.2^{bc}	76 ± 0.2^{bc}
0.54	10 - 12	3.1 ± 0.2^{d}	3.7 ± 0.1^{ef}	50 ± 0.5^{e}
1.07	9 - 12	2.7 ± 0.2^{e}	3.3 ± 0.3^{g}	50 ± 0.3^{e}
1.62	9 - 12	2.7 ± 0.1^{e}	2.5 ± 0.6^{h}	50 ± 0.1^{e}
IBA				
0.04	-	-	-	-
0.24	7 - 10	1.3 ± 0.1^{hi}	0.8 ± 0.1^{j}	50 ± 0.3^{e}
0.49	7 - 10	1.4 ± 0.1^{gh}	4.5 ± 0.1^{bc}	75 ± 0.1^{cd}
0.98	7 - 10	5.3 ± 0.1^{a}	4.6 ± 0.1^{b}	80 ± 0.2^{a}
1.47	7 - 10	3.1 ± 0.2^{d}	4.5 ± 0.3^{bc}	78 ± 0.3^{ab}
IAA				
0.05	-	-	-	-
0.28	7 - 9	16 ± 0.6^{fg}	3.8 ± 0.6^{e}	25 ± 0.1^{f}
0.57	7 - 9	1.7 ± 0.1^{f}	6.4 ± 0.2^{a}	50 ± 0.2^{e}
1.14	5 - 8	1.3 ± 0.1^{hi}	1.4 ± 0.1^{i}	50 ± 0.4^{e}
1.71	5 - 8	-	-	-

[1]Data were scored after 4 weeks of culture.
[2]Auxins were supplemented to MS medium. For all the parameters mean values followed by the same letter are not significantly different at P=0.05 (Duncan's New Multiple Range Test).

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