In vitro propagation of Decalepis hamiltonii Wight & Arn., an endangered shrub, through axillary bud cultures

One of the essential requirements for the successful application of plant propagation technology to agriculture is the capacity to regenerate elite plantlets. During the past decade, the demand for these plants has witnessed a steep rise. To meet the ever-growing commercial requirements, the realization of in vitro multiplication of a large number of clonal plants with the improved characteristics has been gaining significance. Decalepis hamiltonii Wight & Arn. (Swallow root), a monogenic climbing shrub native of the Deccan Peninsula and forest areas of Western Ghats finds use as a culinary spice due to its high-priced aromatic roots. The roots of D. hamiltonii are used as a flavouring principle, appetizer, blood purifier and preservative. Of late, the highly aromatic roots have been subjected to over-exploitation by destructive harvesting that has endangered the survival of this plant in its wild habitat. Moreover, the absence of any organized cultivation of this plant (M. Sanjappa, pers. comm., Botanical Survey of India, Calcutta), calls for immediate conservation measures. George et al. were able to regenerate plantlets of D. hamiltonii W&A from leaf callus. In the earlier reports by George et al., it was observed that the aromatic roots of D. hamiltonii proved to be a potent bioinsecticide on storage pests at lethal and sub-lethal levels (Indian Patent No. 1301/Del/98). The supercritcal extracts of these roots proved to be potent antimicrobial agents. Induction of rooting is an important step in plant propagation. The classical root induction method uses a shock of high auxin concentration; however, the roots are stunted and malformed. It has been shown that interaction of auxins with thiol compounds also stimulates rooting in apple shoot cultures. Ma et al. demonstrated that the use of ethylene inhibitors such as AgNO₃ and CoCl₂ might promote root formation in shoot cultures of apple. We demonstrated the promotive effect of AgNO₃ on shoot morphogenesis and in vitro flowering in both normal and Agrobacterium rhizogenes-induced genetically altered shoot cultures of Cichorium intybus.

There are no reports on the micropropagation studies of D. hamiltonii. The present communication reports the clonal propagation of D. hamiltonii and use of AgNO₃ as supplement for in vitro rooting in D. hamiltonii axillary bud cultures.

Healthy plants of D. hamiltonii W&A were collected from Gumball forest ranges located between 11 to 13 N and 77 to 78 E in BR hills, Mysore district, India. Axillary buds of D. hamiltonii were washed under running tap water to remove soil and other superficial contamination. Single bud explants (1 cm each) (upper portion) were washed with Tween-20 (5% v/v) for 5 min followed by thorough washing under running tap water for 15 min. The explants were surface sterilized with 0.15% (w/v) mercuric chloride for 3 to 5 min and later rinsed 4 or 5 times with sterile distilled water. For all the experiments, MS², with 100 mg/l of myo-inositol was used. The pH was adjusted to 5.8 ± 0.2 using 1N HCl/1N NaOH before adding 0.8% (w/v) of agar (Himedia, Mumbai). The medium was subsequently autoclaved under 15 psi at a temperature of 121°C for 15 min. Explants were cultured in conical flasks (150 ml) covered with non-absorbent cotton plugs and kept in controlled conditions of temperature (25 ± 2°C) and light (45 μmol m⁻² s⁻¹) for 16 h day using fluorescent lights (Philips India Ltd) and 60 to 70% relative humidity.

Various growth regulators, viz. 1-naphthaleneacetic acid (NAA) (0.05–2.5 mg/l) and 6-benzylaminopurine (BAP) (0.05–2.5 mg/l) were tried individually or in combination to obtain the most suitable growth hormone level for the proliferation of shoots in established explants. Experiments were performed with a minimum of five replicates and repeated twice. Observations were recorded after an interval of four weeks. The basal rooting medium (RM) contained MS salts with 0.5 mg/l of indoleacetic acid (IAA) supplemented with the similar sucrose and agar concentration as given for shoot multiplication medium. Ethylene action inhibitor AgNO₃ (SRL India Ltd) was filter sterilized using 0.22 μm filters (Sartorius Ltd). AgNO₃ was incorporated into the culture medium at a concentration range of 10 to 50 μM, respectively, per 40 ml of culture medium. Shoots measuring 3 to 4 cm from 4 week cultures were taken and the top 2 cm was cut and transferred to the RM. The first set of treatments had exogenous supplementation of AgNO₃ (10–50 μM) to the RM. The experiment was repeated twice with five replicates each. The duration of root emergence was recorded for each treatment, to calculate specific growth rate expressed as mm growth/week. Rooting efficiency was calculated as the percentage of shoots producing roots after 4 weeks of culture in all the treat-

Figure 1. Effect of growth regulators on explant response in Decalepis hamiltonii.
Rooted plantlets were removed from the medium, freed of agar by washing in running water and planted in sand–compost mixture (1:2) at about 80% relative humidity under the polyethylene hoods in the greenhouse. The plantlets were hardened for 20 days and then transplanted to the field.

All the axillary buds grew in size and new shoots began to proliferate from the bases within 15 days of incubation. Of the various treatments tested (Figure 1) in MS medium, BAP (2.0 mg/l) and NAA (0.5 mg/l) resulted in maximum number of shoots per culture (12.8 ± 0.96) that were supported by maximum length of shoots (5.8 ± 0.43) and number of leaves (16.4 ± 1.23) on 30th day (Figures 1 and 2a). Extreme concentrations of BAP and NAA were not growth promoting (Figure 1). It was observed that D. hamiltonii shoot cultures grown on RM containing 0.5 mg/l IAA rooted after 20 to 25 days with little root proliferation (Table 1, Figure 2c). These were not suitable for transfer to pots. 40 µM AgNO₃ to the RM, stimulated root emergence from the cut ends of shoots after 10 to 12 days (Table 1, Figure 2a), with a very high rooting efficiency (89.6 ± 6.72%) when compared to the response in RM. The highest number of roots per shoot (12.8 ± 0.96) with a higher growth rate of 18.9 ± 1.41 mm/week was obtained from this treatment with AgNO₃ (Table 1). The treatment of shoots with 50 µM AgNO₃ inhibited root proliferation and multiplication that further led to tissue mortality after 20 days of culture (Table 1), while treatments at 10 to 30 µM AgNO₃ were sub-optimal for promoting maximum rooting (Table 1).

The present investigation demonstrated that it was possible to successfully obtain multiple shoots of D. hamiltonii from axillary bud explants with an efficient in vitro rooting protocol, thereby increasing field survival rate (Figure 2a–d). In chickory shoot cultures, AgNO₃ exhibited feedback inhibition of ethylene production and promoted polyamine biosynthesis through increased utilization of SAM (S-adenosylmethionine). Similarly, in this system AgNO₃ showed promotion of root growth and differentiation compared to treatments with either auxin or ethephon alone (data not shown). In another communication we have demonstrated that IAA-induced ethylene production was inhibitory to the rooting process in D. hamiltonii, wherein ethephon treatment promoted the ethylene production that influenced callusing in these shoots. Treatment with AgNO₃ (40 µM) restored rooting efficiency and callus formation at the cut ends of multipliled shoots (Table 1). These results demonstrated that AgNO₃ can influence root emergence and growth and can improve rooting efficiency (Figure 2b and c).

Ethylene production in plant tissue increases following application of ethephon and AgNO₃ inhibits ethylene action through the Ag⁺ ions reducing the receptor capacity to bind ethylene. In our earlier communication we have demonstrated the use of AgNO₃ for shoot multiplication and in vitro flowering, showing that it increased the pool of endogenous polyamines. AgNO₃ also
enhanced morphogenesis by increasing the endogenous pool of polyamines. The levels of ethylene can also influence callus formation\(^{1}\). Ethephon also induced callusing from root explants of the silk tree (\textit{Albizia julibrissina})\(^{1}\). We have observed differences in callus formation at the base of shoot cuttings of \textit{D. hamiltonii} in the ethephon treatments (Figure 2 e). The use of an ethylene action inhibitor, viz. AgNO\(_3\) can improve and enhance \textit{in vitro} rooting in shoot cultures of \textit{D. hamiltonii}. These regenerated multiple shoots, by repeated sub culturing, were further proliferated, rooted and hardened with well-developed rooting system for establishment under greenhouse conditions (Figure 2 d). Thus this propagation protocol will be useful to conserve this endangered plant.

\begin{table}
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\caption{Effect of AgNO\(_3\) (\textmu M) on \textit{in vitro} rooting of \textit{D. hamiltonii} W&A shoots (values are mean ± SD, \(n = 5\))}
\begin{tabular}{llllll}
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\textbf{Medium} & \textbf{Root emergence (days)} & \textbf{Number of roots per shoot} & \textbf{Growth rate (mm/week)} & \textbf{Rooting efficiency (\%)} & \textbf{Callus formation} & \textbf{% Field survival} \\
\hline
RM & 20–25 & 2.8 ± 0.21 & 2.40 ± 0.19 & 60.8 ± 4.50 & +++ & 45–55 \\
AgNO\(_3\) (10 \textmu M) & 15–17 & 2.5 ± 0.18 & 5.89 ± 0.44 & 54.6 ± 4.09 & ++ & 55 \\
AgNO\(_3\) (20 \textmu M) & 15–17 & 3.0 ± 0.22 & 7.50 ± 0.56 & 61.0 ± 4.50 & ++ & 55–60 \\
AgNO\(_3\) (30 \textmu M) & 15–17 & 4.0 ± 0.30 & 8.60 ± 0.64 & 63.9 ± 4.70 & + & 60 \\
AgNO\(_3\) (40 \textmu M) & 10–12 & 12.8 ± 0.96 & 18.90 ± 1.41 & 89.6 ± 6.72 & + & 80–90 \\
AgNO\(_3\) (50 \textmu M) & – & – & – & – & Tissue mortality & Nil \\
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