Silver nitrate influences in vitro root formation in Decalepis hamiltonii Wight & Arn.

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Effects of AgNO₃ on root formation were examined in vitro using axillary bud cultures of Decalepis hamiltonii. In the case of a medium comprising of MS salts with indole 3-acetic acid (IAA) (0.5 mg L⁻¹), poor rooting was observed and root emergence did not occur until after 25 days. The resultant roots were stunted. Addition of 40 μM AgNO₃ improved root initiation and elongation. The promotive effects of AgNO₃ on rooting may result from inhibition of ethylene action. Upon addition of ethephon to the rooting medium, excessive callusing was observed in explants in all the treatments. Addition of 40 μM AgNO₃ to ethephon-containing medium resulted in improvement in root initiation and elongation. Ethylene production was monitored in all the treatments with IAA/AgNO₃/ethephon and it was observed that the treatment with IAA (0.5 mg L⁻¹) alone showed a greater increase in ethylene production when compared with AgNO₃, ethephon and their combinations.

The role of ethylene in plant tissue culture is not clear, although it can change the organogenic capacity of explants in vitro. Inhibition of ethylene action enhanced plant regeneration from callus of Nicottiana plumbaginifolia and Triticum aestivum. Ethylene promoted shoot formation in several species, including petunia and peach rootstocks, while it inhibited shoot in several cruciferae species and the silk tree. Ethylene appeared to inhibit adventitious root formation from pea cuttings and Prunus avium shoot cultures, whereas it

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was found to stimulate root formation from Norway spruce hypocotyl segments\textsuperscript{10}. The promotive effect of ethylene on rooting is mediated by auxins in sunflower hypocotyls\textsuperscript{11}. Auxin promotes ethylene production\textsuperscript{12}. Since auxin and ethylene cause a number of similar responses and also auxin is capable of promoting ethylene production, many responses previously attributed to auxin may have been due to ethylene production in response to auxin treatments. Higher rates of ethylene production are often associated with the tissues that contain higher amounts of auxin\textsuperscript{13}. Ma et al.\textsuperscript{14} demonstrated that the use of ethylene inhibitors such as AgNO\textsubscript{3} and CoCl\textsubscript{2} may promote root formation in shoot cultures of apple. In our earlier communication\textsuperscript{15}, we demonstrated the promotive effect of AgNO\textsubscript{3} on shoot morphogenesis and in vitro flowering in both normal and Agrobacterium rhizogenes-induced genetically altered shoot cultures of Cichorium intybus\textsuperscript{16}.

Induction of rooting is an important step in the propagation of a plant species\textsuperscript{17}. The classical root induction method uses a shock of high auxin concentration; however the roots that emerge are stunted and malformed\textsuperscript{18}. It has been shown that interaction of auxins with thiol compounds also stimulates rooting in apple shoot cultures\textsuperscript{19}.

Decalepis hamiltonii Wight & Arn. (Swallow root), a monogeneric 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\textsuperscript{19}. The roots of D. hamiltonii are used as a flavouring principle\textsuperscript{20}, appetite and blood purifier\textsuperscript{21} and preservative\textsuperscript{22}. 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. commun., Botanical Survey of India, Calcutta), calls for immediate conservation measures. George et al.\textsuperscript{23} were able to regenerate plantlets of D. hamiltonii W&A from leaf callus. In the earlier reports by George et al.\textsuperscript{24}, it was observed that these 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)\textsuperscript{24,25}. The supercritical extracts of these roots proved to be potent antimicrobial agents\textsuperscript{26}. In the present work we describe the use of AgNO\textsubscript{3}, a potent ethylene action inhibitor, for promoting in vitro rooting in the woody climber D. hamiltonii.

Table 1. Effect of AgNO\textsubscript{3} (µM) on rooting of D. hamiltonii W&A shoots (values are mean ± SD, n = 5)

<table>
<thead>
<tr>
<th>Medium</th>
<th>Root emergence (days)</th>
<th>Number of roots per shoot</th>
<th>Growth rate (mm/week)</th>
<th>Rooting efficiency (%)</th>
<th>Callus formation</th>
<th>% Field survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM</td>
<td>20–25</td>
<td>2.8 ± 0.21</td>
<td>2.40 ± 0.19</td>
<td>60.8 ± 4.50</td>
<td>+++</td>
<td>45–55</td>
</tr>
<tr>
<td>*AgNO\textsubscript{3} 10 µM</td>
<td>15–17</td>
<td>2.5 ± 0.18</td>
<td>5.89 ± 0.44</td>
<td>54.6 ± 4.09</td>
<td>++</td>
<td>55</td>
</tr>
<tr>
<td>*AgNO\textsubscript{3} 20 µM</td>
<td>15–17</td>
<td>3.0 ± 0.22</td>
<td>7.50 ± 0.56</td>
<td>61.0 ± 4.50</td>
<td>++</td>
<td>55–60</td>
</tr>
<tr>
<td>*AgNO\textsubscript{3} 30 µM</td>
<td>15–17</td>
<td>4.0 ± 0.30</td>
<td>8.60 ± 0.64</td>
<td>63.9 ± 4.70</td>
<td>+</td>
<td>60</td>
</tr>
<tr>
<td>*AgNO\textsubscript{3} 40 µM</td>
<td>10–12</td>
<td>12.8 ± 0.96</td>
<td>18.90 ± 1.41</td>
<td>89.6 ± 6.72</td>
<td>+</td>
<td>80–90</td>
</tr>
<tr>
<td>*AgNO\textsubscript{3} 50 µM</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Tissue mortality</td>
<td>Nil</td>
</tr>
</tbody>
</table>

*AgNO\textsubscript{3} treatments were supplemented with RM–basal rooting medium (IAA, 0.5 mg L\textsuperscript{-1}); +, little or no callus formation; ++, good callus formation; ++++, High callus formation.

Table 2. Effect of ethephon on rooting of D. hamiltonii W&A shoots (values are mean ± SD, n = 5)

<table>
<thead>
<tr>
<th>Medium</th>
<th>Root emergence (days)</th>
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<td>+++</td>
<td>45–55</td>
</tr>
<tr>
<td>*Ethephon 8 µg/40 ml</td>
<td>15–20</td>
<td>1.2 ± 0.075</td>
<td>1.5 ± 0.11</td>
<td>20.0 ± 1.5</td>
<td>+++</td>
<td>10</td>
</tr>
<tr>
<td>*Ethephon 16 µg/40 ml</td>
<td>15–20</td>
<td>1.2 ± 0.075</td>
<td>2.1 ± 0.15</td>
<td>31.8 ± 2.36</td>
<td>++</td>
<td>15</td>
</tr>
<tr>
<td>*Ethephon 24 µg/40 ml</td>
<td>15–20</td>
<td>2 ± 0.15</td>
<td>1.8 ± 0.13</td>
<td>20.6 ± 1.5</td>
<td>+++</td>
<td>Nil</td>
</tr>
<tr>
<td>*Ethephon 32 µg/40 ml</td>
<td>15–20</td>
<td>2 ± 0.15</td>
<td>1.6 ± 0.12</td>
<td>9.8 ± 1.4</td>
<td>+++</td>
<td>Nil</td>
</tr>
<tr>
<td>*Ethephon 40 µg/40 ml</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>Nil</td>
</tr>
</tbody>
</table>

*Ethephon treatments were supplemented with RM–basal rooting medium (IAA, 0.5 mg L\textsuperscript{-1}); +, little or no callus formation; ++, good callus formation; ++++/+++++, high callus formation.
Table 3. Effect of ethylene inhibitor AgNO₃ (μM) and ethephon on root formation of *D. hamiltonii* W&A shoots (values are mean ± SD, n = 5)

<table>
<thead>
<tr>
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<td>60.8 ± 4.50</td>
<td>+++</td>
<td>45.55</td>
</tr>
<tr>
<td>Ethephon + AgNO₃ 8° 40 μM</td>
<td>10-15</td>
<td>2.09 ± 0.15</td>
<td>2.50 ± 0.18</td>
<td>29.0 ± 2.1</td>
<td>++</td>
<td>50</td>
</tr>
<tr>
<td>Ethephon + AgNO₃ 16° 40 μM</td>
<td>10-12</td>
<td>8.56 ± 0.64</td>
<td>2.86 ± 0.96</td>
<td>68.0 ± 5.1</td>
<td>+</td>
<td>75</td>
</tr>
<tr>
<td>Ethephon + AgNO₃ 24° 40 μM</td>
<td>10-15</td>
<td>3.10 ± 0.23</td>
<td>2.69 ± 0.20</td>
<td>35.8 ± 2.6</td>
<td>++</td>
<td>55</td>
</tr>
<tr>
<td>Ethephon + AgNO₃ 32° 40 μM</td>
<td>10-15</td>
<td>3.56 ± 0.16</td>
<td>2.09 ± 0.15</td>
<td>32.6 ± 2.4</td>
<td>++</td>
<td>55</td>
</tr>
<tr>
<td>Ethephon + AgNO₃ 40° 40 μM</td>
<td>15-20</td>
<td>1.06 ± 0.07</td>
<td>1.86 ± 0.13</td>
<td>28.6 ± 2.1</td>
<td>+++</td>
<td>45</td>
</tr>
</tbody>
</table>

*Ethephon and AgNO₃ treatments were supplemented with RM-basal rooting medium (IAA, 0.5 mg l⁻¹); "Ethephon added to the medium (8-40 μg/40 ml); +, little or no callus formation; ++, good callus formation; +++/++++, high callus formation.*

later rinsed 4 to 5 times with sterile distilled water. Shoot maintenance medium contained MS (Murashige and Skoog) salts ², 30 gm l⁻¹ sucrose, 8 gm l⁻¹ of agar agar (Hi Media, Mumbai) and 2 mg l⁻¹ of benzylaminopurine (BAP). The basal rooting medium (RM) comprised MS salts with 0.5 mg l⁻¹ of indole 3-acetic acid (IAA) supplemented with a similar sucrose and agar concentration as given for the shoot maintenance medium. All media were adjusted to pH 5.8 with NaOH (1 N) and autoclaved at 1.1 kg cm⁻² (121°C) for 15 min. Aqueous solutions of the ethylene precursor-ethephon (2-chloroethyl-phosphonic acid) (40% v/v) (SRL India Ltd) and the ethylene action inhibitor AgNO₃ (SRL India Ltd), were filtered sterilized using 0.22 μm filters, (Sartorious Ltd). Ethephon and AgNO₃ were incorporated into the culture medium at a concentration range of 8 to 40 μg (10 to 50 μM), respectively per 40 ml of culture medium. Shoot cultures established from the axillary buds of *D. hamiltonii* have been maintained by subculturing every 4 weeks from axillary buds for approximately two years. These cultures were incubated at 25°C with a 16-h photoperiod (30 μ mole m⁻² s⁻¹). Under these conditions, 8 to 10 shoots were produced per culture. Shoots measuring 3 to 4 cm from 4-week culture were taken and the top 2-cm was cut and transferred to the basal RM. The first set of treatments had exogenous supplementation of AgNO₃ (10 to 50 μM) to the RM, whereas the second set had ethephon added at 8 to 40 μg/40 ml to the RM. In the final treatment it was planned to add the best working concentration of AgNO₃ and ethephon supplementation to the RM. In each experiment, there were 5 replicates of one shoot of 2 cm per container for each concentration of AgNO₃ or ethephon. 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 treatments. Ethylene in the culture vessel was measured periodically in all the treatments. The serum stopper of each flask in which explants were grown was removed. The flasks were allowed to stand for 1 h in a laminar flow hood with a constant airflow (0.48 m s⁻¹), and then the flasks were resealed with the serum stopper. Cultures were allowed to stand in the hood and at the end of 3 h, a 5 ml gas sample was withdrawn using a pressure lock syringe (Merck India Ltd) and assayed by a gas chromatograph (Shimadzu Ltd, Japan). The conditions for GC were as described by Chi et al.²⁸

It was observed that *D. hamiltonii* shoot cultures grown on RM containing 0.5 mg l⁻¹ IAA rooted after 20 to 25 days with little root proliferation (Tables 1–3). These were not suitable for transfer to pots. Supplementation of 40 μM AgNO₃ to the RM, stimulated root emergence from the cut ends of the shoots after 10 to 12 days (Table 1), with a very high rooting efficiency (89.6 ± 6.72%) when compared to the response in the RM. The highest number of roots per shoot were allowed to stand in a laminar flow hood with a constant airflow (0.48 m s⁻¹), and then the flasks were resealed with the serum stopper. Cultures were allowed to stand in the hood and at the end of 3 h, a 5 ml gas sample was withdrawn using a pressure lock syringe (Merck India Ltd) and assayed by a gas chromatograph (Shimadzu Ltd, Japan). The conditions for GC were as described by Chi et al.²⁸

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(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.

![Figure 2](image_url)  
**Figure 2.** Effect of ethephon on ethylene production by shoot cultures of *D. hamiltonii* in vitro (values are mean ± SD, n = 5).

In the case of experiments in which ethephon was supplemented at various concentrations ranging from 8 to 40 μg/40 ml to the RM (Table 2), lower levels favoured root elongation as well as higher root growth (Table 2). The treatment in which ethephon was fed at 16 μg/40 ml to the basal RM showed (Table 2) a moderate response in terms of the number of roots (2.5 ± 0.08) per shoot, root growth rate (2.5 ± 0.09 mm/week) and percentage rooting efficiency (31.8 ± 2.36), which was less compared to treatments with AgNO₃ (10–40 μM), (Tables 1 and 2).

The treatment in which AgNO₃ (40 μM) was added along with 16 μg ethephon/40 ml medium gave a better rooting response compared to single ethephon treatments (Table 2). The treatments with ethephon + AgNO₃ gave better rooting than that obtained from the basal RM alone, but were less effective compared to the AgNO₃ treatments only (Tables 1–3). Treatments with combinations of AgNO₃ (40 μM) and ethephon (8–40 μg/40 ml) showed less callusing and better rooting than single ethephon treatment as well as basal RM (Tables 1–3). Ethylene measurements in all the treatments showed that the RM, which was supplemented with 0.5 mg l⁻¹ of IAA, resulted in maximum ethylene production on day 21 (6.1 μl l⁻¹) (Figure 1) compared to the other treatments. AgNO₃-treated shoot cultures showed an increase in ethylene production with increasing concentration of AgNO₃ (Figure 1). As expected, the treatment in which ethephon was incorporated to the RM, showed an increased accumulation of ethylene compared to AgNO₃-treated shoot cultures (Figure 2). Cultures in media supplemented with the ethephon (8–40 μg/40 ml media) and AgNO₃ (40 μM) combination showed less accumulation of ethylene compared to ethephon treatment alone or the simple RM (Figure 3).
RESEARCH COMMUNICATIONS

In chicory shoot cultures, AgNO₃ exhibited feedback inhibition of ethylene production and promoted polyamine biosynthesis through increased utilization of SAM (S-adenosylmethionine)¹⁵. Similarly, in this system AgNO₃ also showed promotion of root growth and differentiation compared to treatments with either auxin or ethephon alone. IAA-induced ethylene production was inhibitory to the rooting process. Ethephon treatment promoted the ethylene production that influenced callusing in these shoots. Treatment with AgNO₃ (40 μM) restored rooting efficiency (Table 3).

These results demonstrated that AgNO₃ can influence root emergence and growth and can improve rooting efficiency (Figure 4).

Ethylene production in plant tissue increases following the application of ethephon³⁰, and AgNO₃ inhibits ethylene action³⁰ through the Ag⁺ ions, reducing the receptor capacity to bind ethylene³⁰. In our earlier communication¹⁵, we 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 formation¹. Ethephon also induced callusing from root explants of the silk tree (Abizia julibrissina)⁹. We have observed differences in callus formation at the base of shoot cuttings of D. hamiltonii in the ethephon treatments (Figure 4). The use of an ethylene action inhibitor, viz. AgNO₃ can improve and enhance in vitro rooting in shoot cultures of D. hamiltonii W&A. Thus AgNO₃ may be useful as a media supplement to develop efficient rooting and propagation protocols so as to conserve this endangered plant. The development of this effective micropropagation technology will be an important step towards its conservation.


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