Micropropagation and conservation of rare medicinal plant *Wattakaka volubilis* (Linn.) Stapf

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A micropropagation protocol for the important medicinal plant species *Wattakaka volubilis* was developed using shoot tip and nodal explants by culturing on Murashige-Skoog (MS) media fortified with 1.5 mg/L BAP in combination with 0.7 mg/L Kn that resulted in 5.3±1.4 multiple shoots per explant. Maximum shoot length observed was 5.8±1.1 cm on media supplemented with 1.5 mg/L BAP and 0.6 mg/L Kn. The *in vitro* raised micro shoots produced highest percentage of rooting (85%) in MS media augmented with 1.0 mg/L IBA. The hardened plantlets were acclimatized in the greenhouse conditions and then reintroduced in the Herbal garden attached to the Centre in which 76% of the plants survived.

**Keywords:** *Wattakaka volubilis*, medicinal plant, shoot segments, mass propagation

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. According to WHO, over 4.3 billion people rely upon such traditional plant based systems of medicine to provide them with primary health care¹. In India, thousands of medicinal plant species are known to have medicinal value to cure specific ailments. However, it is estimated that about 15,000 species of medicinal plants are globally threatened, the causes include loss of habitat, over harvesting and pollution. Conservation of medicinal plants is therefore important to ensure sustainable human development. In the present study, plant tissue culture was chosen to conserve the selected medicinally important plant species.

The medicinal plant, *Wattakaka volubilis* is an important member of the family Asclepiadaceae. It is a fleshy, large climber with green flowers in drooping umbels and found throughout the plains. This plant is used in the treatment of various ailments since ancient times². The literature survey revealed that among the various saponins obtained from the stem and flower of this plant, two compounds are active against Enrich’s ascites carcinoma³,⁴.

The roots contain a glucoside, which lowers carotid blood pressure in mice and dogs when administered intravenously. The extract from the root is also applied to cure snake bites and given to women to cure headache after child birth. The leaves are common ingredients of many folk and herbal medicines⁵,⁶. Leaf extract has been reported to possess pharmacological activity, including anti-inflammatory activity⁷. *W. volubilis* is used for treating rheumatic pain, cough, fever and severe cold⁸.

The healthy mother plants of *W. volubilis* were collected from Thathanuthu, Tirunelveli District of Tamilnadu and reared in the Green house attached to the Centre. The nodal and shoot tip explants were collected from the green house reared mother plant and a pinch of Bavistin was added to get rid of the fungal contaminants, washed thoroughly in running tap water for 10 min, then treated with Tween 20 for 5 min and washed five times with sterile distilled water under aseptic conditions. For surface sterilization, explants were rinsed with 0.1% aqueous HgCl₂ solution for 3 min and rinsed with sterile distilled water five times to remove traces of HgCl₂. The surface sterilized explants were excised to remove the tissues affected by HgCl₂. Then the shoot tips and nodal explants were placed on MS medium supplemented with sucrose 3% (w/v). Also polyvinylpyrrolidone (PVP) 0.1% (w/v) was added to the media for controlling the phenolic exudations from the cut ends of explants. Various concentrations of Benzyl amino purine (BAP) (0.4, 0.7, 1.0, 1.2 & 1.5 mg/L), Kn (0.1, 0.3, 0.6& 0.9 mg/L) and Naphthalene acetic acid (NAA), Indole-3-acetic acid (IAA), 2,4-Dichlorophenoxy acetic acid (2, 4-D) and Indole-3-butyric acid (IBA) (0.1, 0.3, 0.5, 1.0 and 1.5 mg/L) were used for shoot, callus and root induction. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving and then adding

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0.6% agar for preparing a solid medium. About 15 mL of the medium was dispensed in each culture tube and plugged with non-absorbent cotton plugs prior to autoclaving at 121°C for 15 min. All the cultures were maintained at 12 h photoperiod with 2000 lux light intensity at 25±2°C.

The rooted micropropagules were thoroughly washed to remove the traces of agar and planted in polycups containing a mixture of soil, sand and farmyard mixture in the ratio of 1:1:1 and covered with perforated plastic bags. Once in two days half MS liquid medium without sucrose was added and kept in the culture room for 2 wks after which they were transferred to the Greenhouse for acclimatization. The percentage of survival was recorded one month after transfer. All the experiments were repeated three times with ten replicates per treatment, observations were made regularly and the details were carefully recorded.

The nodal and shoot tip explants were inoculated in various concentrations and combinations of BAP, Kn and NAA. Among these, the maximum number of shoots (5.3±1.4) was developed on MS media fortified with 1.5 mg/L BAP in combination with 0.7 mg/L Kn. Maximum shoot length was observed as 5.8±1.1 cm on a medium supplemented with 1.5 mg/L BAP and 0.7 mg/L Kn (Fig. 1; Table 1). Earlier, the nodal explants produced a maximum of 4.2 shoots on MS medium fortified with 2 mg/L BA+0.1 mg/L NAA. However, in the present study, we could induce maximum of 5.3±1.4 shoots.

Similarly, Prasad developed an in vitro regeneration protocol in MS medium fortified with BAP 2 mg/L and Kn 0.1 mg/L. An efficient protocol for Withania somnifera on MS medium supplemented with 1.0 mg/L BAP and 1.0 mg/L Kn was also developed. These results revealed that BAP alone in the medium is not sufficient to induce multiple shoots indicating the necessity of using combination of cytokinins. Whereas, Sanatombi and Sharma established a protocol for Capsicum frutescens where shoot tip explants produced multiple shoot buds when cultured on MS medium containing BAP in higher concentrations.

Basu also developed a protocol for in vitro propagation by multiple shoot induction of Crataera religiosa. High frequencies of multiple shoot regeneration were achieved from apical bud on MS medium fortified with 8 mg/L BAP alone. Similarly, an excellent protocol for Solanum trilobatum on MS medium fortified with 11.1 µM Kn reported the

<table>
<thead>
<tr>
<th>S. No</th>
<th>Plant growth regulators (mg/L)</th>
<th>No. of explants inoculated</th>
<th>Percentage of response</th>
<th>Average no. of shoots proliferated per explant (Mean± SD)</th>
<th>Average shoot length (cm)</th>
</tr>
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<tbody>
<tr>
<td>01</td>
<td>0.5 BAP 0.05 Kn NAA</td>
<td>10</td>
<td>60%</td>
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<td>02</td>
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<td>85%</td>
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<tr>
<td>03</td>
<td>1.0 0.1</td>
<td>10</td>
<td>70%</td>
<td>2.8±0.7 4.8±1.2</td>
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<tr>
<td>04</td>
<td>1.2 0.5</td>
<td>10</td>
<td>80%</td>
<td>4.0±0.8 4.5±1.3</td>
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<tr>
<td>05</td>
<td>1.5 0.7</td>
<td>10</td>
<td>90%</td>
<td>5.3±1.4 5.8±1.1</td>
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<tr>
<td>06</td>
<td>1.0 0.1</td>
<td>10</td>
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<tr>
<td>07</td>
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<td>10</td>
<td>65%</td>
<td>3.5±0.9 4.0±0.8</td>
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<tr>
<td>08</td>
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<td>74%</td>
<td>3.0±1.0 3.6±0.4</td>
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<tr>
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<td>75%</td>
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<tr>
<td>10</td>
<td>1.5 1.5</td>
<td>10</td>
<td>70%</td>
<td>4.5±0.6 4.4±0.5</td>
<td></td>
</tr>
</tbody>
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Fig. 1—Micropropagation of W. volubilis: a. Habit; b & c. Shoot initiation; d & e. Multiple shoot formation and elongation; f. Root induction; & g-i. Various stages of callus formation.
production of highest number of multiple shoots\textsuperscript{14}. It was also found that addition of an auxin (NAA) along with BAP did not improve the shoot proliferation but favoured callusing at the lower end of the nodal stem explants\textsuperscript{15}.

Leaf and internodal explants were used for the purpose of callus induction. Highest percentage (90\%) of callus was observed on MS media fortified with 2.0 mg/L 2,4-D (Fig. 1; Table 2) Highest percentage of green coloured callus was observed in the combination of 1.2 mg/L Kn and 1.5 mg/L IAA. The well developed callus was inoculated in various concentrations and combinations of plant growth regulators such as 0.5, 1.5 mg/L of BAP, Kn and NAA for shoot induction and it is under observation. Godbole et al\textsuperscript{16} found that in media combinations containing different concentrations of 2,4-D and NAA, callus formation started at the cut ends within 8-10 d, and the frequency of its induction varied with concentrations of 2,4-D.

For the purpose of rooting from the well developed shoots, various concentrations and combinations of PGR such as IBA, IAA and NAA were used. Among these, maximum number of rooting was observed in MS medium supplemented with a concentration of 1.0 mg/L IBA (Fig. 1 & Table 3). A protocol for \textit{Tridax procumbens} in which excellent rooting on the excised shoots raised from secondary cultures on half strength MS medium having 1 mg/L IBA was developed\textsuperscript{17}.

### References

