Micropropagation of an important medicinal plant *Catharanthus roseus* by using coconut water instead of synthetic plant growth regulators

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**ABSTRACT**

The present in vitro study was conducted for micropropagation of *Catharanthus roseus* by using coconut water. The coconut water was used in five different concentrations i.e T1 (3%), T2 (6%), T3 (9%), T4 (12%) and T5 (15%) v/v in 1L MS medium instead of synthetic plant growth regulators (PGRs). The results showed that in treatment (T4) 80% cultured explant segments showed shoot emergence and the mean number of leaves (4.80) no affected the growth rate of *Catharanthus roseus* plantlets was suppressed. It has been concluded that the treatment (T4) 12% coconut water showed the best growth rate of *Catharanthus roseus* plantlets. The plant growth hormones present in coconut water affected the in vitro micropropagation of *Catharanthus roseus* significantly, therefore, it can be used instead of synthetic plant growth regulators (PGRs) in MS media for micropropagation of *Catharanthus roseus*.

1. INTRODUCTION

*Catharanthus roseus* (L.) is a high value medicinal plant belongs to family Apocynaceae. It has two common cultivars on the basis of flower color i.e Rosea (pink flowers) and Alba (white flowers)¹². *Catharanthus roseus* has anticancer activities due to the presence of more than 400 alkaloids among which actineo plastidemeric, Vinblastin, Vincrestine, Vindesine, Vindelene Tabersonine present in aerial parts while ajmalicine, vinceine, vineamine, raubasin, reserpine and catharanthe are present in basal stem and roots¹³. Methanolic crude extracts of *Catharanthus* have anticancer activity against numerous cell types especially against the multidrug resistant tumor types²⁴. Some alkaloids are used for the treatment of childhood leukemia, Hodgkin’s disease, testicular cancer, diabetes, blood asthma, constipation and menstrual problem⁶.

Nowadays, plant tissue culture is widely used technology for large scale plant multiplication²⁵. Medicinal plants are the greatest source of valuable pharmaceutical drugs⁸. On commercial basis large scale production is required which can be achieved through in vitro culture technique for mass multiplication of valuable medicinal plants²⁶,²⁰. The induction of multiple shoots in *Catharanthus roseus* from nodal segments was achieved on MS medium supplemented with 0.5 mg/l BAP ± 1mg/l NAA¹⁶.

Attempts were made to find comparatively cheaper micro-propagation procedures by adopting low cost substituent in the culture medium. Many natural complex additives like coconut water, banana pulp, tomato juice, slap honey and beef extract were tested as growth factor substitute for the synthetic growth regulators. The use of coconut water as a plant growth regulator gives a better response on plant tissue culture³.

Coconut water (CW) is the liquid endosperm of *Cocos nucifera* L used as a supplement in tissue culture media because it contained phytohormones especially cytokinins, indole-3-acetic acid (IAA)²⁷, trans-zeatin²⁸ and gibberellins⁹.

CW supplementation highly influenced the shoot bud initiation and multiplication in in vitro regeneration studies on *Cyamopsis tetragonolobus*¹⁷. It was reported that coconut water induced the callus growth and somatic embryogenesis of *Phoenix dactylifera* (Date palm)⁴. Supplementation of coconut water produces a high number of shoots in in vitro culture of orchid shoots (*Dendrobium Sp*)¹⁰.

Due to the expensiveness of the pharmaceutically important alkaloids of *Catharanthus roseus* the interest in searching alternative ways of its production has been increased. Through tissue culture the medicinal plants can be produced rapidly throughout the year without depleting the natural resources. Plant tissue culture is an expensive technique for micropropagation of plants particularly due to use of expensive plant growth regulators. As Pakistan is an
underdeveloped country so the use of such expensive growth hormones for in vitro culture is not economical. Our study has been focused to evaluate the effect of various concentrations of coconut water on in vitro growth and micropropagation of Catharanthus roseus instead of using expensive synthetic plant growth hormones.

2. MATERIALS AND METHODS

The experiment was conducted in potato tissue culture laboratory at Hazara Agriculture Research Station (HARS), Abbottabad in 2017.

The coconut water was extracted aseptically from Coconut fruits by drilling holes through two of the micropylres and then heated at 60°C for 10 minutes with continuous stirring to precipitate out the undesirable proteins, fats and other materials and then filtered. MS medium\(^{18}\) containing 1 mg/l-1Ca-pentothenate, 100mg/l Myoinositol , 30g/l sucrose and 6g/l agar was prepared and coconut water (CW) (v/v) was added as (T\(_1\)) 3%, (T\(_2\)) 6%, (T\(_3\)) 9%, (T\(_4\)) 12% and (T\(_5\)) 15%. The MS medium with no coconut water served as control (T\(_0\)) 0%. The pH of the media was adjusted to 5.8 with either 1 N NaOH or 1 N HCl solution prior to autoclaving. Each treatment was replicated 15 times.

The explants of Catharanthus roseus were collected from Department of Botany, Government Post Graduate College, Abbottabad and identified with the help of Flora of Pakistan.\(^{19}\) After removing the leaves the shoots were washed under running tap water for 15 minutes, later washed with a mild detergent followed by rinsing thoroughly with distilled water for 5-6 times. After rinsing the explants were cut in to nodal segments and were surface sterilized in 0.1% (w/v) mercuric chloride for 3 minutes, washed with sterilized distilled water 3-4 times. Further the explants were treated with 0.1% (w/v) fungicide (Ridomil) for 1 min then washed thrice with sterilized distilled water.

The nodal segments were trimmed at both ends after sterilization in order to expose fresh tissues and were inoculated aseptically on media in the test tubes under sterilized conditions in laminar flow cabinet. All cultured test tubes were incubated at 18-20°C with 16 hours light and 8 hours dark in the growth chamber.

The Data were recorded after 30, 50 and 60 days of culturing for various growth parameters including root and shoot emergence, shoot length, number of nodes and number of leaves. The Data were analyzed by using computer software Statistix 8.1 and Least significance difference test (LSD) at 95% level of significance was performed to assess significant difference between various treatments. The Experiment was designed as Complete Randomized Design (CRD).

3. RESULTS AND DISCUSSION

The data revealed that different concentrations of coconut water showed great variation on the in-vitro growth of Catharanthus roseus as compared to control.

Shoot Emergence: Data regarding number of days to shoot emergence percentage (Table 1) showed that after 5 days of culturing the shoot emergence was observed in all the five treatments except control (T\(_0\)). The maximum shoot emergence was recorded in treatment T\(_4\) (60%) and T\(_3\) (50%) in which CW was added @ 12% and 9% while after 15 days a significant increase (P ≤ 0.05) in the shoot emergence percentage was recorded (Table 1) (Figure 1). Whereas in the control no emergence was observed even after 15 days of culturing.

Number of leaves: The data collected after 30 days showed that all the five treatments are significantly different (P ≤ 0.05) regarding number of leaves (Table 2) (Figure 2). The maximum average number of leaves was observed in treatment T\(_4\) (3.80) followed by T\(_3\) (2.40) and T\(_2\) (1.80). The minimum mean value for number of leaves was observed in treatment T\(_1\) (1.60) and T\(_5\) (1.10) in which 3% and 15% CW was added (Table 2). After 50 days of culturing a significant increase in average number of leaves between treatments was recorded (Table 3).

<table>
<thead>
<tr>
<th>Table 1. Shoot emergence percentage in the cultured nodal segment of Catharanthus roseus</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of days</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>5Days</td>
</tr>
<tr>
<td>15 Days</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Means in a row followed by different letters are significantly different at P ≤ 0.05
Figure 1. Treatment T1, T2, T3, and T4 showing shoot emergence of Catharanthus roseus after 15 days of culturing.

No of Nodes: Statistical analysis after 30 days of culturing revealed that the highest mean value (2.00) for number of nodes among the treatments was recorded in T4 (Table 2) followed by treatment T3 (1.20) and T2 (1.00). The minimum number of nodes was observed in treatment T1 (0.80) and T5 (0.70) in which 3% and 15% CW was added. A similar trend was observed after 50 days (Table 3).

Figure 2. Treatment T3 and T4 showing number of leaves after 20 days of culturing.

Table 2. Comparison of growth rate of Catharanthus roseus on MS media at different concentrations of coconut water after 30 days of culturing

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot Length (cm)</th>
<th>Average no of leaves</th>
<th>Average no of nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1.06 bc</td>
<td>1.60 bc</td>
<td>0.80 b</td>
</tr>
<tr>
<td>T2</td>
<td>1.24 bc</td>
<td>1.80 bc</td>
<td>1.00 b</td>
</tr>
<tr>
<td>T3</td>
<td>1.80 b</td>
<td>2.40 b</td>
<td>1.20 b</td>
</tr>
<tr>
<td>T4</td>
<td>2.70 a</td>
<td>3.80 a</td>
<td>2.00 a</td>
</tr>
<tr>
<td>T5</td>
<td>0.68 cd</td>
<td>1.10 cd</td>
<td>0.70 b</td>
</tr>
<tr>
<td>T0</td>
<td>0.00 d</td>
<td>0.00 d</td>
<td>0.00 c</td>
</tr>
</tbody>
</table>

Means in a column followed by different letters are significantly different at P ≤ 0.05

Shoot Length: The statistical analysis of the data after 30 days of culturing showed that the highest mean shoot length was observed in treatment T4 (2.70cm) followed by treatment T3 (1.80cm) and T2 (1.24cm). The lowest mean value among the treatments for shoot length was observed in treatment T5 (0.68). In control no shoot growth was observed. A similar trend in shoot growth was recorded even after 50 days (Table 3) (Figure 3).
Figure 3. Treatment T1, T2, T3, and T4 showing Shoot development of Catharanthus roseus

Table 3. Comparison of Growth rate of Catharanthus roseus on MS media at different concentration of coconut water after 50 days of culturing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot Length(cm)</th>
<th>Average no of leaves</th>
<th>Average no of nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1.46 bc</td>
<td>2.70 bc</td>
<td>1.40 b</td>
</tr>
<tr>
<td>T2</td>
<td>1.59 bc</td>
<td>2.80 bc</td>
<td>1.50 b</td>
</tr>
<tr>
<td>T3</td>
<td>2.23 ab</td>
<td>3.60 ab</td>
<td>1.80 ab</td>
</tr>
<tr>
<td>T4</td>
<td>3.15 a</td>
<td>4.80 a</td>
<td>2.60 a</td>
</tr>
<tr>
<td>T5</td>
<td>0.90 cd</td>
<td>1.60 cd</td>
<td>0.90 c</td>
</tr>
<tr>
<td>T0</td>
<td>0.00 d</td>
<td>0.00 d</td>
<td>0.00 c</td>
</tr>
</tbody>
</table>

Means in a column followed by different letters are significantly different at P ≤ 0.05

Root emergence: After 60 days of culturing, the best rooting response (Figure 4) was observed in treatment T4 (60%) (Figure 5a) followed by T3 (30%) in which 12% and 9% coconut water (CW) was added while the rest of the treatments failed to induce rooting of the regenerated shoots. In vitro rooted plantlets were transplanted in the green house where their leaves expanded and plants grow quickly (Figure 5b).
Discussion: In the present study coconut water was used instead of plant growth regulators for the micropropagation of Catharanthus roseas. Results showed that shoots were emerged after 5 days of culturing in every treatment except control. It might be due to presence of phytohormones cytokinin that stimulate in vitro growth of plants. Cytokinins are a major group of phytohormones that play significant role in plant growth and development e.g cell division, formation and activity of shoot meristem. It was demonstrated that the addition of coconut water enhanced axillary shoots proliferation along with BA in Faidherbia albida.

Comparatively maximum shoot emergence was recorded in treatment T1 (80%) in which 12 % coconut water was added while at higher and lower concentrations the shoot emergence and growth was suppressed. It was reported that Dendrobium needed medium doses (10-15%) of coconut water for improvement of shoot and leave number, and the higher concentration of coconut water causes the shoots to be stunted and eventually died. It was also observed that the emerged shoots were stunted and eventually died in treatment T3 in which CW at 15% concentration was used. The type and concentration of plant growth regulators (PGR) is a component of tissue culture medium that determines the success of tissue culture.

The maximum root emergence % was observed in treatment T4 (60%) followed by treatment (T3) in which 30% rooting occurred. This may be due to presence of auxin in coconut water. Auxin plays a major role in formation of main root, lateral and adventitious roots, and possible reason for rapid root initiation in treatment (T4) is may be that 12% coconut water concentration resulted in optimum auxin level in the medium that causes rooting of Catharanthus roseus. The physiological changes of rooting are correlated with changes in auxin concentration.

The data revealed that among the treatments the highest shoot length was observed in treatment T4 (3.15cm). Shoot length can be affected by presence of Gibberellin in coconut water because it is also reported that in vitro addition of GA3 (0.5mg/L) combined with low cytokinin concentration was effective in shoot growth of potato and GA1 and GA3 were successfully detected and quantified in coconut water.

Regarding number of leaves the significant increase in the number of leaves (4.80) were found in treatment (T4) in 50 days old plantlets of Catharanthus roseas. Higher concentration of coconut water (100 ml 1\(^{-1}\)) decreased all the growth and morphological features, as well as induced abnormal plantlets growth in Calanthe hybrids. The leaves arise from buds and it was reported that cytokinins are usually known to make promotion of buds formations in many in vitro cultured organs.

4. CONCLUSION AND RECOMMENDATIONS

It has been concluded from the present study that 12 % coconut water was most suitable for in vitro micropropagation of Catharanthus roseus which showed the maximum number of nodes, leaves, shoot length and highest percentage of root/shoot emergence.

Therefore, fresh coconut water extracted from coconut fruit can be used instead of synthetic plant growth regulators (PGR) in tissue culture of Catharanthus roseus and further research is needed to test its efficacy against micropropagation of other medicinal plants as a cost effective substitute for expensive synthetic plant growth hormones.

REFERENCES


