Effect of auxins on berberine synthesis in cell suspension culture of
Coscinium fenestratum (Gaertn.) Colebr—A critically endangered
medicinal liana of Western Ghats

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Cell suspension culture of critically endangered Coscinium fenestratum was established from young leaf segments on
WPM supplemented with auxins. Effect of 2,4-D, IAA, IBA and NAA was examined on cell growth and berberine
production. Berberine was synthesized and released continuously into the liquid medium. Presence of 2,4-D stimulated
cell growth, but was not inhibitory on berberine synthesis. On the contrary, NAA stimulated berberine biosynthesis, but was not
favourable for cell growth. Among the auxins tested, highest yield of berberine (5.79 mg/30 ml; 4.14 times to that of
control) was obtained with 4 mg/l of NAA, while the best cell growth (214.43 mg dry wt., 1.96 times to that of control) was
observed in the presence of 2 mg/l of 2,4-D. IAA and IBA were not favourable for cell growth and berberine synthesis.

Keywords: Auxins, Berberine synthesis, Cell suspension culture, Coscinium fenestratum
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Coscinium fenestratum (Gaertn.) Colebr. is a critically
endangered dioecious medicinal liana found in the
Western Ghats, India. The stem of this plant is used
as a medicine in ophthalmopathy, inflammations,
wounds, ulcers, skin diseases, abdominal disorders,
jaundice, diabetes, fever, general debility, thread
worm infection and tetanus. In nature, this species
takes at least 15 years to flower and bear fruit.
Destructive collection from the forest for its stem and
root, even before flowering and fruiting has resulted
in their dwindling population and retarding the
evolutionary process in this species. Active principle
of this plant has been identified as berberine (C_{16}H_{18}NO_{2}Cl) including other minor isoquinoline
alkaloids. Berberine has demonstrated significant
activity against bacteria, protozoans, fungi, intestinal
parasites and chlamydia. Berberine is also found
useful in bacterial diarrhoea, inflammations, thrombocytopenia, ventricular tachyarrhythmias, ocular
trachoma infections and sudden coronary death after
myocardial ischemic damage. A significant
antitumour property was observed for berberine in human malignant brain tumour, oesophageal cancer,
leukemic and colon cancers. In vitro syntheses of
berberine have been reported in various plants such as
Thalictrum minus, Coptis japonica and Berberis
sp. Berberine producing callus and cell suspension
cultures have been reported in C. fenestratum from
petiole segments, while other explants such as leaf,
nodal and internodal segments died due to cell
browning and subsequent death of the explant.

The aim of the present work was to analyze the effect of
auxins on cell growth and berberine biosynthesis in
cultured cells of C. fenestratum derived from leaf
segments.

Materials and Methods

Young leaf segments derived from a 10 year old C.
fenestratum grown in the greenhouse conditions were
thoroughly washed with running tap water in a two
way flask for 2 hr and sterilization was carried out
with a solution of HgCl₂ (0.1%) for 7 min. Finally
they were washed thrice with sterile double distilled
water. Cultures were initiated on Woody Plant
Medium (WPM) containing 3% (w/v) sucrose; 2
mg/l 2,4-dichlorophenoxy acetic acid (2,4-D); and 0.2
mg/l, 6-benzyladenine (BA). The pH of the medium
was adjusted to 5.7, gelled with 0.7% agar (w/v) and
poured into tubes. The cultures were incubated at
23±1°C under dark and transferred to fresh medium
on every 3rd day for the first three subcultures, then

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after 7 days in order to eliminate phenolics released into the medium.

Powdery yellow colored callus mass after 120 days of growth was used for the initiation of cell suspension cultures. Callus (2 g) was transferred to 100 ml of liquid WPM containing 2 mg/l of 2,4-D and 0.2 mg/l of BA in a 500 ml flask and incubated on an orbital shaker (110 rpm) under a 12 hr photoperiod (light intensity of 100 μmol m⁻² s⁻¹). Subculturing was done every week in order to avoid alkaloid released into the medium. After 5th subculture, 0.7 g of fresh weight of cells on the day 6 of incubation were supplemented with various concentrations of cultured cells and released into the liquid medium growth and berberine accumulated were calculated into the medium. After 5h, subculture, 0.7 g of fresh weight of cells on the day 6 of incubation were filtered and inoculated into 30 ml of liquid medium supplemented with various concentrations of 2,4-D, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA). Cell growth and berberine accumulated were calculated after incubation of 21 days. Berberine produced by cultured cells and released into the liquid medium were separated and analysed as described elsewhere. Quantitative analysis of berberine (intracellular and released into the medium) was done by HPLC by isocratic elution through C18 reverse phase column, solvents: water/acetonitrile/trifluoro acetic acid 60:40:0.1, flow rate was 1.0 ml min⁻¹ and the effluent was monitored at 345 nm. Reference sample of berberine was obtained from Sigma Chemical Company, USA. For estimation of growth and alkaloid production, cell growth and berberine synthesized were analysed after 3 days intervals for 21 days. All the experiments were done in 7 replications and the results were analysed by ANOVA and means were compared by Duncan’s multiple range test.

### Results and Discussion

Different auxins tested differed for their ability for inducing cell growth and berberine synthesis. The liquid medium became yellow even at the initial stages of the culture. Presence of 2,4-D even at low concentration (0.5 mg/l) stimulated cell growth. Maximum cell growth obtained was at 2mg/l of 2,4-D (214 mg dry wt; Table 1). Presence of 2,4-D was not inhibitory to berberine synthesis. With increasing concentrations for 2,4-D amount of berberine synthesized and its release showed a concomitant

<table>
<thead>
<tr>
<th>Conc. of auxins (mg/l)</th>
<th>Cell growth (mg dry wt.)</th>
<th>Berberine released into liquid medium (mg/30ml)</th>
<th>Intracellular berberine (% of dry wt.)</th>
<th>Total berberine (mg/30ml)</th>
<th>% of berberine released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal WPM 2,4-D</td>
<td>109.71 ±3.92</td>
<td>0.70 ±0.03</td>
<td>0.59 ±0.03</td>
<td>1.40 ±0.06</td>
<td>50.02</td>
</tr>
<tr>
<td>0.5</td>
<td>142.14 ±2.97</td>
<td>1.27 ±0.07</td>
<td>0.74 ±0.02</td>
<td>2.32 ±0.10</td>
<td>54.72</td>
</tr>
<tr>
<td>1</td>
<td>161.57 ±3.01</td>
<td>1.92 ±0.09</td>
<td>0.85 ±0.01</td>
<td>3.30 ±0.10</td>
<td>58.36</td>
</tr>
<tr>
<td>2</td>
<td>214.43 ±2.07</td>
<td>2.16 ±0.17</td>
<td>0.84 ±0.05</td>
<td>3.97 ±0.23</td>
<td>54.44</td>
</tr>
<tr>
<td>4</td>
<td>200.29 ±5.55</td>
<td>2.45 ±0.21</td>
<td>0.91 ±0.04</td>
<td>4.29 ±0.26</td>
<td>57.14</td>
</tr>
<tr>
<td>6</td>
<td>181.14 ±3.37</td>
<td>2.93 ±0.14</td>
<td>0.92 ±0.06</td>
<td>4.60 ±0.18</td>
<td>63.66</td>
</tr>
<tr>
<td>IAA</td>
<td>117.14 ±3.08</td>
<td>0.74 ±0.04</td>
<td>0.59 ±0.02</td>
<td>1.43 ±0.06</td>
<td>51.45</td>
</tr>
<tr>
<td>0.5</td>
<td>148.71 ±3.15</td>
<td>1.06 ±0.04</td>
<td>0.79 ±0.03</td>
<td>2.23 ±0.07</td>
<td>47.66</td>
</tr>
<tr>
<td>6</td>
<td>153.86 ±3.79</td>
<td>1.58 ±0.10</td>
<td>0.84 ±0.04</td>
<td>2.86 ±0.11</td>
<td>55.14</td>
</tr>
<tr>
<td>IBA</td>
<td>0.5</td>
<td>102.14 ±5.75</td>
<td>0.62 ±0.04</td>
<td>0.58 ±0.02</td>
<td>1.20 ±0.07</td>
</tr>
<tr>
<td>4</td>
<td>144.57 ±5.37</td>
<td>0.97 ±0.04</td>
<td>0.78 ±0.03</td>
<td>2.10 ±0.10</td>
<td>46.15</td>
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<tr>
<td>6</td>
<td>129.29 ±3.29</td>
<td>1.27 ±0.09</td>
<td>0.84 ±0.04</td>
<td>2.36 ±0.07</td>
<td>53.82</td>
</tr>
<tr>
<td>NAA</td>
<td>0.5</td>
<td>122.29 ±6.76</td>
<td>1.12 ±0.18</td>
<td>0.77 ±0.05</td>
<td>2.06 ±0.14</td>
</tr>
<tr>
<td>1</td>
<td>124.71 ±3.50</td>
<td>1.95 ±0.27</td>
<td>1.00 ±0.08</td>
<td>3.20 ±0.23</td>
<td>60.99</td>
</tr>
<tr>
<td>2</td>
<td>134.00 ±3.53</td>
<td>4.09 ±0.46</td>
<td>1.12 ±0.09</td>
<td>5.58 ±0.35</td>
<td>73.28</td>
</tr>
<tr>
<td>4</td>
<td>109.71 ±3.53</td>
<td>4.49 ±0.11</td>
<td>1.18 ±0.09</td>
<td>5.79 ±0.86</td>
<td>77.63</td>
</tr>
</tbody>
</table>

Means followed by same letters are statistically not significant at α=0.05 by Duncan’s multiple range test.
increase, even though the cell growth was at the decline. Similar results were also observed in berberine synthesizing cultures of *T. minus*. However in *T. flavum* and *T. dipterocarpum* no significant difference in berberine production was observed among the auxins. On the other hand, strong inhibiting effect is known for 2,4-D on production of secondary metabolites, such as nicotine, betacyanins, anthocyanins and stimulating effect on carotenoid biosynthesis and anthocyanin biosynthesis. In the present study, more than 3 mg of berberine accumulated in presence of 1-4 mg of 2,4-D, which was due to increase in growth of the cells and not due to increase in the rate of berberine biosynthesis.

Of the auxins used in the present experiment, NAA was found to be the best for stimulating berberine synthesis. High level of NAA was not favourable for cell growth, similar to the results obtained in petiole segment derived cell suspension cultures of *C. fenzestruum*, *T. minus* and other species of *Thalictrum*. Replacement of 2,4-D by NAA or IAA has been shown to enhance the production of anthocyanins in *Populus* and *Daucus carota*, betacyanins in suspension of *Portulaca*, Nicotine synthesis in *Nicotiana tabacum*, Shikonin in cultures of *Lithospermum erythrorhizon* and anthraquinones in *Morinda citrifolia*. In the present experiment, IAA has been not effective in inducing cell growth and berberine production. However, in cell suspension cultures of *Thalictrum rugosum*, among the auxins evaluated, IAA has been found to be the best for berberine production. IBA was also not effective in inducing cell growth and synthesis of berberine. Maximum cell growth was observed in presence of 2 mg/l of 2,4-D and maximum synthesis of berberine and release by cells were observed in the presence of 4 mg/l of NAA (Table 1). Although, the cell growth obtained in the presence of NAA in cultured cells of *C. fenzestruum* was inferior to those in 2,4-D, synthesis of berberine was remarkably stimulated. The highest yield obtained (5.79 mg/109.71 mg dry wt./30 ml in presence of 4 mg/l NAA in liquid WPM) in the present study using leaf derived cell suspension culture, even in the presence of auxin alone, was superior than the previously reported highest yield in petiole segment derived cell suspension cultures (5.4 mg/274 mg dry wt./50 ml in presence of 0.2 mg/l BA and 1.8 mg/l NAA in liquid MS medium) and inferior to production medium in shake flask cultures of *T. minus* (19.5 mg/161 mg dry wt./30 ml in presence of 1.2 mg/l BA and 18 mg/l NAA added to liquid LS medium containing 20 mM KNO₃ and 40 mM NH₄Cl). The type and concentration of growth regulator concentration was a crucial factor and altered dramatically both cell growth and secondary product formation.

Kinetics of cell growth and berberine accumulation showed that berberine was continuously synthesized and released into the liquid medium and was more or less parallel to cell growth, indicating that berberine synthesis was growth associated (Fig. 1). Growth associated berberine production has also been reported earlier in petiole derived suspension culture of this species, *T. minus* and *C. japonica*. However, an inverse relationship has also been reported between cell growth and berberine synthesis (stress related) in a cell line culture of *T. minus*.

Most of the berberine produced was released into the medium right from the beginning of the culture and continued for the whole culture period (21 days) as in *T. minus*. Release of berberine was maximum in presence of NAA and least in presence of IBA (Table 1). However, in *Coptis japonica* and *Berberis* cells under suspension do not release berberine into the medium, but accumulate berberine in vacuoles.

The present experiment demonstrated feasibility of obtaining an appreciable amount of active principle by using plant cell culture and thereby, conserving this medicinal liana. The release of berberine from cultured cells into the medium was of particular interest and provided an unique opportunity for further research.

![Fig. 1—Time course of cell growth and berberine production in cell suspension culture of *C. fenzestruum* derived from leaf segments. (Vertical bars represent standard error of 7 replications.)](image-url)
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