Influence of agar concentration and liquid medium on in vitro propagation of *Boswellia serrata* Roxb.

R K Suthar, N Habibi and S D Purohit*

Plant Biotechnology Laboratory, Department of Botany, Mohanlal Sukhadia University
Udaipur 313001, India

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Reduced concentration of agar than normal and its complete elimination favoured both shoot multiplication and rooting during micropropagation of *Boswellia serrata* Roxb. There was overall increase in dry and fresh wt and chlorophyll contents in such shoots. Liquid medium devoid of agar proved to be the best for the micropropagation of *B. serrata*.

**Keywords:** *Boswellia serrata* Roxb., chlorophyll content, gel strength, in vitro rooting, liquid medium, shoot multiplication

**Introduction**

Interaction between *in vitro* raised plantlets with the gelling agent in culture medium is a dynamic process and the changes in gel consistency affect the regeneration of plants or tissues. Traditionally, agar (0.6-0.8%) is added to the culture medium to increase its viscosity. As a result of which plant tissues and organs remain above the surface of the nutrient medium. Increasing agar strength beyond a critical limit has been demonstrated to inhibit organogenesis and shoot growth and reduce the water availability to the cultures. Recent reports have suggested that low concentration of agar provides a poorly gelled medium that facilitates adequate contact between the plant tissue and the medium and better diffusion of medium constituents, resulting in better growth and their subsequent rooting. Methods of *in vitro* propagation in liquid medium have also been attempted where agar was completely omitted from the medium. By using liquid medium instead of gelled medium, propagation is accelerated, culture transfer frequencies may be decreased, labour is less intensive and cost of production is reduced.

*Boswellia serrata* Roxb. (*Family: Burseraceae*), commonly known as ‘Salar’, is an important tree species of Aravallis in Rajasthan, India, valued for its immense medicinal properties. The plant, an important source of oleo-gum-resin commonly known as Indian olibanum, is credited for its astringent, stimulant, expectorant, diuretic, antipyretic and antiseptic properties, and also reported to be useful in ulcers, goiter, piles diarrhea, etc. Micropropagation protocol for its multiplication has been developed and reported earlier. The present studies deal with the improvement of protocol by manipulating agar concentration in the medium and by using liquid culture system.

**Materials and Methods**

*In vitro* shoot cultures were established using cotyledonary node explants as per the protocol of Purohit and co workers. *In vitro* multiplying shoot clusters (3-4 shoots), harvested aseptically, were used as the explants for further studies. In order to study the effect of different agar concentrations on shoot multiplication, the standard shoot multiplication medium (MS salts + 0.5 mg L⁻¹ BAP + 0.05 mg L⁻¹ NAA) was modified using different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0% w/v) of agar (Hi Media Make). In case of liquid medium, borosilicate glass beads ca. 10 mm diameter were used as support matrix. All the cultures were maintained under controlled culture-room conditions having controlled temperature (28±2°C), light (45 µmol m⁻² s⁻¹ for 16 h/d provided by white fluorescent tubes, Philips make) and air humidity (50-60%). Shoot clusters were sub-cultured every 2 wk without harvesting the shoots and were maintained for 42 d under same incubation conditions.

For each treatment, six replicates were used and each experiment was repeated thrice. The shoot
cultures grown on both agar-gelled and liquid media were assessed and compared for their in vitro growth in terms of various growth parameters like rate of shoot multiplication, average shoot length, number of leaves per cluster, per cent water content, total fresh and dry wt, etc. Biochemical characteristics, such as, chlorophyll a, b and total chlorophyll were also recorded as per the method described by Arnon.

To find out the effect of different concentrations of agar on in vitro rooting, its concentration was varied from 0.0 to 1.0% (w/v) in standard rooting medium containing 0.5 mg L\(^{-1}\) IBA + 0.25 mg L\(^{-1}\) NAA + antioxidants solution (PVP 50 mg L\(^{-1}\) + ascorbic acid 100 mg L\(^{-1}\) + citric acid 10 mg L\(^{-1}\)). For this purpose, the shoots of uniform length (3-4 cm) were harvested aseptically from in vitro multiplying cultures and inoculated on the standard rooting medium. In case of 0.2% agar and medium without it (liquid medium), filter paper bridges were used as support matrix. Agar at 0.6% concentration was treated as control.

The experiments were conducted in a completely randomized design (CRD). Data were analyzed by a single factor ANOVA and presented as mean ± SD along with level of significance.

**Results and Discussion**

In vitro cultured shoots of *B. serrata* grew much faster on reduced agar or complete absence of agar in the medium (Figs 1 & 2). In the present investigation, the shoot cultures grown on standard shoot multiplication medium gelled with 0.8% agar (control) multiplied at a rate of 2.2-folds, producing an average 11.3 shoots with 76.3 leaves per cluster after 42 d (Table 1). The shoots produced on this medium measured 2.51±0.49 cm in length with a total 3.08±1.49 g fresh and 0.31±0.10 g dry wt. Liquid medium (without agar) or very weakly gelled medium (0.2% agar) produced more than 20 shoots (approx. 2.0 times higher than the control). The average number of shoots at 0.4 and 0.6% of agar in the medium was ca. 12. The maximum shoot length (2.83±0.15 cm) was recorded on the liquid medium. Such shoots also produced highest number of leaves (126) per cluster with a total biomass reaching up to 8.14±0.16 g. The number of leaves recorded on 0.2, 0.4 and 0.6% agar was almost comparable to control; however, the fresh and dry wt accumulation was twice to that of fresh wt recorded for control. The per cent moisture content ranged between 90-93% but no symptoms of hyperhydricity were noticed at any of
Higher chlorophyll accumulation was reported in B. serrata cultured on MS medium containing 0.5 mg L\(^{-1}\) BAP and 0.05 mg L\(^{-1}\) NAA (observations were recorded after 42 d).

![Table 1—Effect of agar concentrations on in vitro shoot multiplication and chlorophyll content in B. serrata cultured on MS medium containing 0.5 mg L\(^{-1}\) BAP and 0.05 mg L\(^{-1}\) NAA (observations were recorded after 42 d)](image1)

<table>
<thead>
<tr>
<th>Agar conc. (%)</th>
<th>No. of shoots ±SD</th>
<th>Shoot length (cm) ±SD</th>
<th>No. of leaves ±SD</th>
<th>Fresh wt (g) ±SD</th>
<th>Dry wt (g) ±SD</th>
<th>% water content</th>
<th>Chlorophyll contents (mg g(^{-1}) fresh tissue) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>9.00± ± 1.67</td>
<td>2.37± ± 0.36</td>
<td>51.50± ± 10.60</td>
<td>2.73± ± 1.71</td>
<td>0.24± ± 0.09</td>
<td>91.21</td>
<td>0.577± ± 0.01, 0.466± ± 0.01, 1.043± ± 0.01</td>
</tr>
<tr>
<td>0.8</td>
<td>11.33± ± 2.42</td>
<td>2.51± ± 0.49</td>
<td>76.30± ± 6.38</td>
<td>3.08± ± 1.49</td>
<td>0.31± ± 0.10</td>
<td>90.07</td>
<td>0.602± ± 0.01, 0.412± ± 0.01, 1.041± ± 0.03</td>
</tr>
<tr>
<td>0.6</td>
<td>12.50± ± 3.15</td>
<td>2.70± ± 0.47</td>
<td>73.50± ± 20.87</td>
<td>5.31± ± 1.12</td>
<td>0.43± ± 0.11</td>
<td>91.98</td>
<td>0.464± ± 0.00, 0.315± ± 0.01, 0.778± ± 0.02</td>
</tr>
<tr>
<td>0.4</td>
<td>12.00± ± 0.89</td>
<td>2.37± ± 0.47</td>
<td>72.00± ± 7.77</td>
<td>5.76± ± 0.18</td>
<td>0.43± ± 0.03</td>
<td>92.48</td>
<td>0.656± ± 0.01, 0.415± ± 0.02, 1.071± ± 0.01</td>
</tr>
<tr>
<td>0.2</td>
<td>24.00± ± 2.00</td>
<td>2.20± ± 0.29</td>
<td>78.00± ± 5.80</td>
<td>6.74± ± 0.57</td>
<td>0.49± ± 0.03</td>
<td>92.73</td>
<td>0.600± ± 0.00, 0.436± ± 0.02, 1.033± ± 0.00</td>
</tr>
<tr>
<td>0.00*</td>
<td>21.00± ± 3.03</td>
<td>2.83± ± 0.15</td>
<td>126.00± ± 10.66</td>
<td>8.14± ± 0.16</td>
<td>0.57± ± 0.05</td>
<td>92.96</td>
<td>0.686± ± 0.07, 0.536± ± 0.04, 1.185± ± 0.02</td>
</tr>
</tbody>
</table>

*All values are the means ± SD; means followed by different letters differ significantly at 5%.

**CD= Critical difference; CV= Coefficient of variation

![Table 2—Effect of agar concentrations on in vitro rooting in B. serrata on standard rooting medium (observations were recorded after 21 d)](image2)

<table>
<thead>
<tr>
<th>Agar conc. (%)</th>
<th>No. of roots ±SD</th>
<th>Root length (cm) ±SD</th>
<th>Shoot length (cm) ±SD</th>
<th>No. of leaves/shoot ±SD</th>
<th>% rooting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>2.30± ± 0.95</td>
<td>1.60± ± 0.52</td>
<td>3.07± ± 0.44</td>
<td>3.00± ± 0.67</td>
<td>45</td>
</tr>
<tr>
<td>0.8</td>
<td>2.61± ± 0.79</td>
<td>3.45± ± 0.70</td>
<td>3.75± ± 0.54</td>
<td>3.25± ± 0.92</td>
<td>50</td>
</tr>
<tr>
<td>0.6</td>
<td>2.80± ± 0.79</td>
<td>3.45± ± 0.76</td>
<td>3.80± ± 0.89</td>
<td>4.10± ± 0.99</td>
<td>65</td>
</tr>
<tr>
<td>0.4</td>
<td>3.80± ± 0.78</td>
<td>2.80± ± 0.86</td>
<td>3.70± ± 0.54</td>
<td>4.00± ± 0.82</td>
<td>70</td>
</tr>
<tr>
<td>0.2</td>
<td>3.80± ± 0.95</td>
<td>3.65± ± 0.67</td>
<td>3.55± ± 0.50</td>
<td>4.50± ± 1.08</td>
<td>70</td>
</tr>
<tr>
<td>0.0 (liquid)</td>
<td>5.70± ± 1.07</td>
<td>4.30± ± 0.67</td>
<td>4.65± ± 0.67</td>
<td>5.10± ± 1.37</td>
<td>75</td>
</tr>
</tbody>
</table>

*Means followed by different letters differ significantly at 5%; CD= Critical difference, CV= Coefficient of variation.

The concentrations of agar. Low agar levels and liquid culture medium have been reported to promote shoot proliferation in several culture systems on account of faster uptake of BAP and better absorption of water by plants implanted on softer gels. Higher (1.18 mg g\(^{-1}\) fresh wt) total chlorophyll contents were also recorded in the shoot clusters grown on liquid medium in comparison to control (1.04 mg g\(^{-1}\) fresh wt). No significant differences in the chlorophyll contents were recorded at any other concentrations. Higher chlorophyll accumulation was reported in *Chlorophyllum borivilianum*, *Terminalia bellerica* and *Feronia limonia* due to better growth performance of culture in liquid medium.

On the contrary, increasing the concentration of agar to 1.0% in the medium caused significant reduction in overall growth of shoot cultures of *B. serrata* (Table 1). The rate of shoot multiplication declined to 1.8-fold and the total number of leaves (51.5) per shoot cluster was also reduced. The fresh and dry wt recorded were also lesser than the control. Such shoots were short as compared to control. The number of shoots and fresh and dry wt decreased with increasing the agar strength in rose\(^{1}\) and sweet cherry rootstock\(^{2}\), possibly due to reduced water availability.

In *B. serrata* when shoots were rooted on standard rooting medium gelled with 0.6% agar (control), on an average of 2.80±0.79 roots per shoot were produced (length 3.80±0.89 cm) with an average root length of 3.45±0.76 cm (Table 2). At 0.4 and 0.2% agar concentrations, average 3.8 roots were induced with a root length of 2.8±0.86 and 3.65±0.67 cm, respectively (Fig 3). However, the best rooting response (75%) was obtained when shoots were rooted on medium without agar (liquid) with a maximum of 5.7 roots per shoot with average length of 4.3 cm. Maximum shoot length and leaves per shoots was also recorded on this medium. Reduction in all the growth parameters were recorded on the medium gelled with higher concentrations (0.8 & 1.0% w/v) of agar. There are many examples where better shoot growth and rooting have been observed in...
liquid medium\textsuperscript{13,14}. The filter paper support provided in liquid medium gave better anchorage owing to its porosity that facilitated increased absorption throughout its surface area\textsuperscript{15}.

Lowering of agar concentration and use of liquid media that gave better micropropagation protocol of \textit{B. serrata} is a good alternative. Further studies on transfer of shoots rooted on liquid medium to \textit{ex vitro} conditions and establishment in field is underway.

\textbf{References}