Micropropagation of vanilla (*Vanilla planifolia* Andrews.)

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*Vanilla planifolia* Andrews is an important cash crop and offers scope for cultivation in the tropical high-rainfall regions of Southern India. It is an orchid, commercially cultivated for pods (beans) from which the popular flavouring substance called vanillin is extracted. Vanillin is mainly used in flavouring ice creams, soft drinks, condiments and oleoresins. The world production of vanilla beans during the year 2001 was estimated at 4,923 metric tonnes from an area of 41,025 hectares. India ranks 6th in production in the world with 60 metric tonnes (Anon, 2002). In India, vanilla cultivation is presently feasible in Tamil Nadu, Karnataka, Kerala and the Northeast regions, Lakshadweep and the Andaman and Nicobar islands. Karnataka occupies the largest area under vanilla cultivation in India with 1,465 ha, followed by Kerala (812 ha) and Tamil Nadu (268 ha) (Anon, 2002).

Traditionally vanilla is propagated from cuttings of mature vines and raised in poly bags. However, this method of propagation is rather slow, labour intensive and time consuming. Obtaining the stem cuttings from the mother plants causes set back to their growth and yield. Moreover, the market demand for propagules is hardly met out through such cuttings. As the growers are looking out for alternate sources, micropropagated plantlets serve the purpose and are popularly used. *In vitro* multiplication of *V. planifolia* had been reported through the callus culture (Gu *et al.*, 1987), protocorms, root tips (Philip and Nainar, 1986) and auxiliary bud explants (George and Ravishankar, 1996). However, mass propagation for commercial cultivation requires a simple, economical, rapidly multiplying and highly reproducible protocol without an intervening callus or protocorm phase so as to give rise to true-to-type clones. The present study was made to establish a protocol for large scale clonal propagation of vanilla through *in vitro* culture.

Shoot tips and auxiliary buds excised from greenhouse grown vines were used as explants. The explants were pre-treated with a solution of 0.2% Carbendazim, 0.2% Streptocycline and washed with Tween-20 and sterile distilled water. The explants were surface sterilized with 0.1% HgCl₂ for 5 min. followed by 4 - 5 washes with sterile distilled water.

The explants were initially inoculated in MS (Murashige and Skoog, 1962) medium containing 3% sucrose and 0.8% agar. The medium was supplemented with various concentrations of BAP (0.5 - 2.5 mg l⁻¹) and NAA (0.5 mg l⁻¹) for multiple shoot formation. The microshoots were transferred to half strength MS medium containing NAA at various concentrations (0.25 - 1.25 mg l⁻¹) for rooting. The medium was buffered to a pH of 5.8 and dispensed in 25 x 150 mm culture tubes.
before autoclaving at 121 °C at 15 psi pressure for 20 minutes. All the cultures were maintained at 25 ± 2 °C, under 16 h photoperiod provided by white cool fluorescent light (35 EM⁻² S⁻¹) with 70% relative humidity. Each experiment was performed three times with a total number of 20 inoculated explants per treatment.

Multiple shoots developed after four weeks of culture were separated individually for further multiplication and this process was continued repeatedly every fortnight. Finally, the shoots were transferred to half strength MS medium containing various concentrations of NAA (0.25 – 1.25 mg l⁻¹) for rooting. The rooted plantlets were transferred to poly sleeves containing sand and vermiculite (1:1) and maintained in poly tunnels for hardening. The hardened plantlets were shifted to polybags containing pot mixture (FYM: Red earth : Sand).

The explants cultured in MS medium containing BAP (2.0 mg l⁻¹) and NAA (0.5 mg l⁻¹) were healthy and vigorous (Table 1). It was evident from the results that vanilla could be propagated in vitro using shoot tip and axillary bud as explants. Multiple shoot regeneration had been reported by Mary Mathew et al. (1999) using axillary nodal buds, cultured in MS + IAA (2.0 mg l⁻¹) + Kinetin (0.5 mg l⁻¹) + BAP (0.5 mg l⁻¹) + biotin (0.2 mg l⁻¹) + Ca-pantothenate (0.2 mg l⁻¹), wherein highest number of six shoots per explant of 2 cm long was obtained. Geetha and Shetty (2000) used shoot tips and nodal segments as explants. Bud elongation was noticed after 5 to 6 weeks. Induction of auxiliary proliferation occurred from the eighth week. The proliferating auxiliary buds were well defined, pale green and 0.5 to 1.0 cm long with bulbous base and pointed tips. A two fold increase in multiplication rate was observed in 8 to 10 weeks. This was supported by the findings of George and Ravishankar (1996) in *V. planifolia*. Further transfer in the MS medium supplemented with BAP (2.0 mg l⁻¹) resulted in proliferation of shoots in two fold ratio at every subculture cycle.

The elongated shoots (4 to 5 cm long) were excised and cultured separately in half strength MS medium supplemented with 1.0 mg l⁻¹ NAA to encourage formation of long shoots, broad leaves and basal roots. The highest number of roots per shoot with a higher shoot growth were obtained on medium containing 1.0 mg l⁻¹ NAA (Table 2). George and Ravishankar (1996) reported that the multiple shoots of vanilla were transferred to the MS medium supplemented with 0.5 mg l⁻¹ NAA for rooting. Giridhar et al. (2001) reported that 100% explants rooted in the NAA containing medium. The elongated shoots attained a length of 8 to 10 cm in about two weeks and were ready to be transplanted. Hence, the present investigation is of importance in demonstrating efficient multiplication and defining the rate of multiplication of shoots at the rate of 40 shoots from a single axillary bud explant over a period of 140 days in in vitro propagation of *V. planifolia*.

### Table 1. Effect of growth regulators on multiple shoot induction of Vanilla

<table>
<thead>
<tr>
<th>Concentration of Growth Regulators (mg l⁻¹) in MS medium</th>
<th>Survival Percentage</th>
<th>Days taken for shooting</th>
<th>No. of shoots per explant</th>
<th>Length of shoots (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>NAA</td>
<td>0.5</td>
<td>61.44</td>
<td>38.13</td>
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<tr>
<td>1.0</td>
<td>0.5</td>
<td>70.88</td>
<td>37.69</td>
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<td>1.5</td>
<td>0.5</td>
<td>78.94</td>
<td>35.25</td>
<td>4.38</td>
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<td>2.0</td>
<td>0.5</td>
<td>90.50</td>
<td>29.94</td>
<td>6.05</td>
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<tr>
<td>2.5</td>
<td>0.5</td>
<td>84.00</td>
<td>33.94</td>
<td>5.22</td>
</tr>
<tr>
<td>SEd</td>
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<td>1.56</td>
<td>1.07</td>
<td>0.52</td>
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<tr>
<td>CD (P=0.05)</td>
<td></td>
<td>3.32</td>
<td>2.28</td>
<td>1.16</td>
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</table>

### Table 2. Effect of growth regulators on rooting of Vanilla

<table>
<thead>
<tr>
<th>Concentration of Growth Regulator (mg l⁻¹) in MS medium</th>
<th>Survival percentage</th>
<th>Days taken for rooting</th>
<th>No. of roots per plant</th>
<th>Length of roots (cm)</th>
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</thead>
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<td>NAA</td>
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<td>0.25</td>
<td>74.13</td>
<td>20.12</td>
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<td>0.50</td>
<td>83.69</td>
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<td>0.75</td>
<td>78.63</td>
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<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>93.63</td>
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<td>1.25</td>
<td>89.44</td>
<td>18.30</td>
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<td></td>
<td></td>
<td>SEd</td>
<td>1.3</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD (P=0.05)</td>
<td>2.78</td>
<td>1.25</td>
</tr>
</tbody>
</table>
Protocol for micropropagation of Vanilla planifolia Andrews

Explant
(Surface sterilization)

MS medium with BAP 2.0 mg l⁻¹ + NAA 0.5 mg l⁻¹
4 weeks
Bud break, initial auxillary proliferation
Injure shoot tip
Subculture for 8 weeks at 4 week intervals
3 fold increase
MS medium with BAP 2.0 mg l⁻¹
(Subculture every 4 weeks)

Proliferating clumps
(MS medium + BAP 2.0 mg l⁻¹)

Elongated shoots
MS medium + BAP 2.0 mg l⁻¹
2 weeks
Rooted plantlets
½MS medium + BAP 0.5 mg l⁻¹

Poly tunnels with 100 % RH
4 weeks acclimatization
Transferred to soil
(40 plants/auxillary bud explant)

References


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