

## Regeneration and mass multiplication of *Vanilla planifolia* Andr. – a tropical orchid

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**A high frequency, simple and rapid regeneration protocol was developed from shoot tip and nodal explants of *Vanilla planifolia* on Murashige and Skoog's medium supplemented with 6-benzyl amino purine and coconut water.**

**Keywords:** Mass multiplication, regeneration, *Vanilla planifolia*.

*VANILLA* is an important orchid and offers excellent scope for cultivation in the tropical high-rainfall regions of southern India. In the global trade there is a growing demand for natural *Vanilla* essence flavour<sup>1</sup>. Vanillin, *Vanilla* essence, extracted from the beans is mainly used in flavouring ice creams, soft drinks, condiments, in cosmetics and perfumery industry<sup>1</sup>. The world production of *Vanilla* beans is estimated to be around 3500 tones per annum. The Malagasy Republic grows 70–80% of the world's *Vanilla* crop. The world trade is around Rs 300 crores annually, amounting to nearly 7% of the total value of the spice trade<sup>2</sup>.

Generally, *Vanilla* is propagated by stem-cuttings. However, this method of propagation is not economical and is time consuming. Collection of stem-cuttings from the mother plants leads to arrest of growth, development and yield<sup>3,4</sup>. In order to meet the demand for propagules, the rapid regeneration of this species is essential. The conventional method of propagation through cuttings will not solve the problem. Micropropagation of *Vanilla planifolia* has been reported through callus culture<sup>5</sup>, protocorm, root tips<sup>6</sup> and axillary bud explants<sup>7–9</sup>. Even though few reports are available on *in vitro* propagation, the protocols are complicated. Here, we report a simple, economical, rapidly multiplying and highly reproducible protocol for large-scale micropropagation.

Shoot tips and nodal explants excised from greenhouse-grown vines were used as explants. The explants were pretreated with 5% labolene solution followed by 0.1% bavistin solution for 15 min. The buds were washed several times with sterile distilled water. Surface disinfestations were effected with 70% ethanol for 30 s and 0.12% mercuric chloride for 10 min followed by four rinses in sterile distilled water. The washed explants were inoculated aseptically on Murashige and Skoog's (MS) medium.

Basal medium<sup>10</sup> containing 30 g/l sucrose and gelled with 0.8% agar (Himedia, India) was supplemented with 6-benzyl amino purine (BAP) and coconut water (CW) in various combinations, as shown in Table 1. The pH of the medium was adjusted to  $5.9 \pm 0.1$  before pouring into the culture bottle. The medium was autoclaved at 121°C for 20 min. All cultures were incubated at  $24 \pm 2^\circ\text{C}$  under a 12 h photoperiod using cool, white fluorescent light.

For rooting of shoots, well-grown shoots were separated and transferred to MS medium containing the same concentration of BAP (1.0 mg/l) and CW (150 ml/l).

Regenerated shoots with well-developed roots were transferred to plastic pots containing hardening media (decomposed coir waste:perlite:compost:: 1:1:1). The pots were kept inside the shade house and high humidity maintained by providing fogger system for 10 days; then the rooted plants were transferred to poly bags. The plantlets were exposed to natural environmental conditions.

Explants numbering 20 were inoculated on MS medium with various combinations of BAP and CW. Among the various treatments, effective results were obtained from combinations given in Table 1. Explants cultured on all the media induced a single shoot within 8–10 days. Shoot proliferation was best in BAP + CW (1.0 mg/l + 150 ml/l), followed by another combination of BAP + CW (1.5 mg/l + 150 ml/l) 20 days after subculture. Other combinations of MS with BAP and CW were also effective, but not at the level of the previous combinations.

Subculturing of the shoots for multiplication on the same medium induced multiple shoots. After two or three subcultures clump formation occurs. The proliferating axillary buds were well-defined, pale green and 0.5 to 1 cm long with bulbous base and pointed tips. A three-fold increase in multiplication was seen by 4–5 weeks. Further transfer in the same medium resulted in three- to four-fold ratio at every subculture cycle.

Explants cultured for 10–12 weeks in MS + BAP + CW (1.0 mg/l, 150 ml/l) medium, which had attained the stage of vigorous proliferation, consisting of 9–10 shoots, were divided into smaller clumps. Each clump consisting of 5–6 shoots was transferred to the same medium. On the same medium, dwarf shoots recovered to normal growth with more number of axillary shoots. At the same time, 2–3 vigorously growing shoots from each clump elongated with expanded leaves by three weeks (Figure 1). These shoots had healthy transfer in the same medium and gave rise to a three- to four-fold increase in proliferating clumps and 3–4 elongated shoots with root initials from each clump. The elongated shoots were excised and cultured separately in the same fresh medium to encourage formation of long shoots, broad leaves and basal roots. Basal tufts of rooting were observed in 100% of the transferred shoots. Proliferating clumps were transferred to fresh medium for further multiplication. The elongated shoots with roots (about 8–9 cm) were transferred to primary hardening. The well-developed, healthy *in vitro*

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**Table 1.** Effect of different concentrations of plant growth regulators on *in vitro* development of *Vanilla planifolia* plantlets

Concentration of BAP and CW (15%) in MS medium	Initiation			Multiplication and rooting	
	No. of explants used	Percentage of explants forming shoots	No. of shoots per explant (8–10 days)	No. of shoots per explant after 20 days	No. of elongated shoots forming root
0.5 + CW	20	80 <sup>a</sup>	1	6.70 <sup>a</sup>	3.53 <sup>a</sup>
1.0 + CW	20	100 <sup>b</sup>	1	9.43 <sup>b</sup>	4.53 <sup>b</sup>
1.5 + CW	20	75 <sup>c</sup>	1	5.40 <sup>c</sup>	2.60 <sup>c</sup>
2.0 + CW	20	70 <sup>d</sup>	1	5.50 <sup>dc</sup>	2.37 <sup>dc</sup>
2.5 + CW	20	65 <sup>e</sup>	1	3.63 <sup>e</sup>	1.40 <sup>e</sup>
3.0 + CW	20	40 <sup>f</sup>	1	1.50 <sup>f</sup>	1.57 <sup>f</sup>

Values are mean of six replicates.

Common superscript letters are not significantly at  $P < 0.05$  using DMRT analysis.



**Figure 1.** Different stages of *in vitro* propagation of *Vanilla planifolia* on MS medium. *a*, Initiation of nodal segment; *b*, Multiple shoots formation; *c*, Green coloured multiple shoots; *d*, Well-grown plants; *e*, Rooted plants and *f*, Hardened plants.

rooted plantlets were washed thoroughly in running tap water and planted in soil rite, a commercially available sterile potting mix in net pots and hardened in a shade

house under 90–95% relative humidity for 8–10 days. They were gradually transferred to plastic pots or poly bags. A survival rate of 90–95% was achieved during the

hardening. After 20–25 days, the hardened plants were transferred to field.

Thus *Vanilla* can be propagated *in vitro* using shoot tips and nodal buds as explants. Multiple shoot regeneration has been reported<sup>11</sup> using axillary nodal bud, culture in MS + IAA (2 mg/l) + kinetin (0.5 mg/l) + BAP (0.5 mg/l) + biotin (0.2 mg/l) + Ca-pantothenate (0.2 mg/l) and maximum of six shoots per explant, about 2 cm long were obtained.

Phillip and Nainar<sup>6</sup> used four different basal media, viz. Knudson, MS, Gamborg's and SH to culture stem sections and root tips. MS alone has been reported to be suitable for optimal plantlet induction and growth. It seems that for *Vanilla*, a basal medium consisting of high salt concentration such as MS is essential in the different stages of culture<sup>8</sup>. In the present study and in all the previous studies on *Vanilla* micropropagation, full strength MS basal medium had been used<sup>5,6,11,12</sup>.

Use of organic substances like d-biotin and Ca-pantothenate has been reported to enhance multiplication of *Vanilla*<sup>11</sup>. During the present study, although no comparative studies were made on addition of adjuvant, it is assumed that the presence of d-biotin and folic acid in CW might be one of the reasons for proliferation of axillary shoots and also elongation of shoots with root initials forming complete plantlets.

Previous studies on *Vanilla* micropropagation used more than one type of media for initiation, multiplication and rooting<sup>10,11,13,14</sup>. The present study reports a simple one-step protocol using MS + BAP and CW for initiation, multiplication, elongation and rooting of *Vanilla*.

The present study has revealed an efficient, simple and single medium for both primary culture and multiplication without many growth regulators and organic substances.

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## Significance of coal petrological investigations in coal bed methane exploration – Indian context

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Understanding of sorption and desorption processes of gas by coal is important in coal bed methane (CBM) estimation and determining its producibility. The results of the investigations carried out so far in Australia, on the role of coal type and rank in CBM storage and recovery are found to be inapplicable in the context of Indian coals. This is probably because the Australian Permian coals were considered as a two-component system – vitrinite- and inertinite-rich (liptinite macerals being present in negligible amount), when tested through sorption and desorption experiments. Liptinite maceral group, the third component of almost all high-volatile bituminous Permian coals of India, comprising hydrogen-rich plant parts (mostly the sporinite-spores and pollen), was not acknowledged in the model studies. Likewise, two lithotype bands – bright and dull – including bulk coal samples were tested for the preceding experiments, whereas a third lithotype band – semi-bright, the common lithotype of Permian coals was not included in such studies. Besides some general and specific comments on observations made, it is sug-

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