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In vitro propagation of *Vanilla tahitensis* Moore

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Abstract

A commercially viable protocol for mass propagation of *Vanilla tahitensis*, a cultivated species of vanilla, was standardized. A multiplication ratio of 1 : 4.7 was observed over a culture period of 60–70 days on benzylaminopurine ($1mgl^{-1}$) and α-naphthaleneacetic acid ($0.1mgl^{-1}$).

Key words : micropropagation, plantlets, *Vanilla tahitensis.*

Abbreviations:

BAP : 6-Benzylaminopurine

IAA : Indole-3-butyric acid

KN : Kinetin

MS : Murashige & Skoog medium

NAA : α-Naphthaleneacetic acid

Vanilla planifolia Andrews is the most commonly cultivated species of vanilla of commerce apart from *V. pompona* Schiede and *V. tahitensis* Moore. *V. tahitensis* commonly called 'Tahiti vanilla', indigenous to Tahiti Island is of recent introduction to India and no data on its field performance is available. Hence, *en masse* production of planting materials is a pre-requisite for establishment of plantations.

In India, little variation is observed in *V. planifolia* since it has a narrow genetic base (Ravindran *et al.* 1996). Introduction of plant material which is predominantly vegetatively propagated is hardly likely to exhibit any variability (Purseglove *et al.* 1988). This would probably be one of the reasons for the limited work done on vanilla improvement by breeding. This paper presents a micropropagation protocol for *V. tahitensis* that may enable mass production of plantlets for establishment of plantations and their utilization in breeding programmes of vanilla.

Nodal segments were excised from vines of *V. tahitensis* raised at the Germplasm Repository of Indian Cardamom Research Institute, Spices Board, Kerala, India. Disinfection and surface sterilization of explants was performed as described earlier (Mary *et al.* 1999). MS basal salts (Murashige & Skoog 1962) supplemented with BAP, IAA or NAA was used for culture experiments. Sucrose was added at 3% and agar at 0.65% and the pH was adjusted to 5.8. Cultures were incubated at 25 ± 2°C and 16 h photoperiod at an intensity of 2500 lux.

Proliferated buds from established primary cultures were subcultured on the same medium for multiplication. For further shoot multiplication, well developed shoot buds were transferred to elongation and rooting media. MS basal salts were supplemented with KN and or NAA as growth regulators.

The micropropagated plants were maintained under intermittent mist for 2–3 weeks in a glasshouse prior to transfer to the greenhouse. For greenhouse planting, a mixture of garden soil and sand (1:1) and soilrite mixture was used. Data for

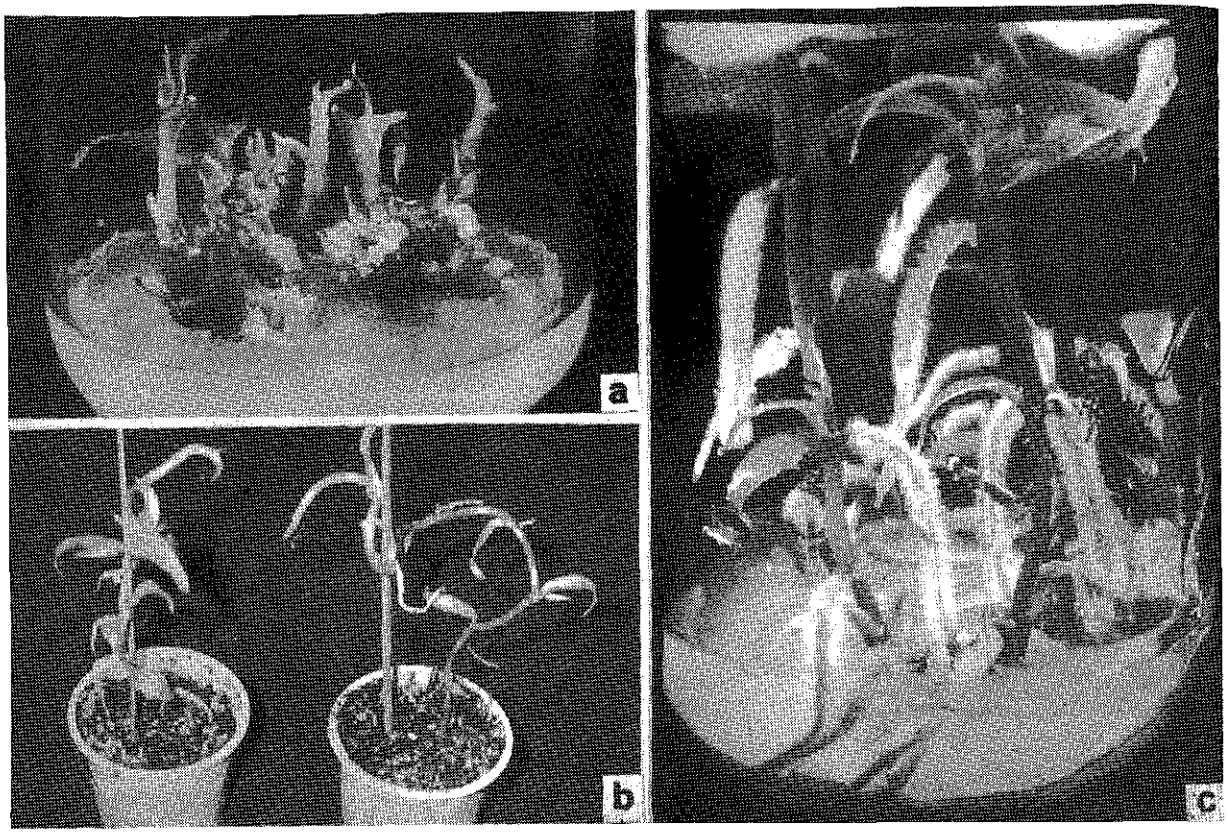

Fig 1 Micropropagation of *Vanilla tahitensis*
a. *In vitro* multiple shoot formation b. Rooted plantlets c. Hardened plant

shoot multiplication and percentages of rooting and hardening were recorded.

Primary cultures of *V. tahitensis* were established within 5–6 weeks of culture initiation. Among the various growth regulators tested, MS with BAP at 1mgl^{-1} and NAA at 0.1 mgl^{-1} proved most suitable for shoot multiplication and for further growth and development of shoots (Table 1). IAA was the most suitable auxin for *in vitro* multiplication of *V. planifolia* (Mary *et al.* 1999; Rao *et al.* 1999) whereas NAA was best suited for *V. tahitensis*. NAA when used in *V. planifolia* cultures promote excessive aerial root formation in multiplication subculture cycles, whereas in *V. tahitensis* this was not observed. Repeated sub culture on the aforesaid medium resulted in production of a large number of buds with an average multiplication ratio of 1:4.7 at each cycle (Fig 1 a) over a culture period of 60–70 days (Table 1). The shoots were separated from multiple shoot clumps and cultured on

Table 1. Multiple shoot regeneration in *Vanilla tahitensis*

Growth regulator (mgl^{-1})	No. of multiple shoots/explant	Shoot morphology	No. of days in culture
BAP 0.5 + NAA 0.2	2.0	Thin stemmed; rooting profuse	50–60
BAP 1.0 + NAA 0.1	4.7	Healthy, vigorously growing	60–70
BAP 1.0 + KN 1.0 + NAA 0.1	4.8	Clustered, short shoots	60–70
BAP 3.0	-	Yellowish callus; nodular protocorm like growth without much differentiation	70–90

Basal medium : MS

medium for elongation and rooting. It was noticed that simultaneous elongation and rooting occurred in 95% shoots on medium with reduced levels of BA (0.1 mgl^{-1}) and NAA (0.2 mgl^{-1}) (Fig 1b). In *V. planifolia* (Mary *et al.* 1999), KN (0.2 mgl^{-1}) was effective for shoot elongation and rooting. Plantlets attained a height of 6–7 cm with healthy leaves, aerial roots and one or two basal roots, within 65–70 days.

Subsequently, the plantlets were removed from the cultures, washed free of agar, and transferred to pots containing soil/soilrite mixture. They were transferred to a mist chamber with 80–90% relative humidity for 2 to 3 weeks and thereafter hardened in the greenhouse for 30–45 days (Fig 1c) and subsequently transplanted in the field under partial shade. The survival percentage was 70–80% showing that micropropagation technique can be effectively used for mass propagating *V. tahitensis*. The technique used is simple, efficient and reproducible and 500 plantlets were produced using the protocol described.

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