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Efficient *in vitro* Multiplication Protocol for *Vanilla Planifolia* Using Nodal Segments

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Abstract

Vannila planifolia Andrews is one of the most important flavouring herbaceous perennial crop. In vanilla *in vitro* propagation study was conducted to assess the *in vitro* response of various explants in different growth regulators to identify the ideal and suitable medium for primary callus induction and to found out the response of *in vitro* regeneration of plantlets from callus tissue. Three different explants *viz.,* Nodal segment, shoot tip and aerial tip from healthy plants were undertaken for this study. Explants were inoculated in MS medium (Murashige and Skoog 1962) supplemented with BAP, IAA, D-biotin, Calcium pentothenate for primary callus initiation. The growth of multiple shoots was more vigorous on MS medium with 0.2 mg/L of KN and 0.1 mg/L of NAA. MS medium with 1.5 mg/L of IBA and 1.0 mg/L of BA gave good results for rooting. The explant nodal segment gave best response both early shoot induction and higher shooting percentage and also earlier rooting induction with rooting percentage.

Keywords: Micro propagation; Nodal segment; Shooting; Rooting.

Introduction

Vanilla Planifolia Andrews is one of the most important flavouring herbaceous perennial crop. In vanilla the method of propagation is rather slow, labour intensive and time consuming. Moreover, collection of vanilla stem cuttings leads to arrest the growth and development of the mother plants (Ayyappan, 1990). As an alternative approach is tissue culture technique, it is used to selection and rapid multiplication. In India the micro propagation technique had gained momentum to reoccupy the monopoly in vanilla tissue culture at global level. It is able to regenerate the millions of copies to ensure in a decade of time with high yielding with shorter duration, and the tissue culture plants from different explants perform uniformly. Present

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study was conducted to identify the suitable ideal medium for primary culture induction and standardize the method for *in vitro* regeneration of plantlets from callus tissue.

Materials and Methods

In vitro studies were conducted at Agricultural College & Research Institute, Madurai during 2013. The successful *in vitro* culture results depend on the interplay of the plant material (explants), medium in use and the culture environment. Two different explants namely nodal segment and shoot tip are involved in this study. The explants were surface sterilized with 0.1 percent mercuric chloride for 10 minutes followed by washed with distilled water. Then it is rinsed with 70% ethanol for 3 times then it is washed with distilled water. Now the explants were ready for inoculation. The medium contained sucrose and solidified with 0.8% agar, pH of the media was adjusted to 5.7 before autoclaving at 121°C for 20 minutes. Explants were inoculated in MS medium (Murashige and Skoog 1962) supplemented with various combinations of growth regulators for primary callus initiation. Cultures were maintained at 27 ± 2°C temperature, 75% relative humidity and with 16 hours photoperiod.

Statistical analysis

Observations *viz.*, callus induction percentage, number of shoots and roots were recorded under *in vitro* condition in the Factorial Completely randomized Block Design (FCRD). Each treatment was replicated three times and ten culture tubes constituted a replication. The percent values were transformed into corresponding arc sine values.

Treatments involved

Shooting

T_1 – Normal MS media + KN 0.2 mg/L + NAA 0.1 mg/L

T_2 – Normal MS media + KN 0.3 mg/L + NAA 0.2 mg/L

T_3 – normal MS media + KN 0.4 mg/L + NAA 0.3 mg/L

Rooting

T_4 – Normal MS media + BAP 1.5 mg/L + IBA 1.0 mg/L

T_5 – Normal MS media + BAP 2.0 mg/L + IBA 2.0 mg/L

T_6 – normal MS media + KN 2.5 mg/L + NAA 3.0 mg/L

Results and Discussion

In the present investigation growth regulator combination of normal MS media + KN 0.4 mg/L + NAA (0.3 mg/L) gave the better results for callus induction and shoot proliferation. The mean value of shooting duration was ranged from 35 to 62 days between the explants. Among the explants tested the duration for shooting was well noticed in nodal segment (Table 1). High percentage of multiple shoot induction ranged from 5 to 70 percent between the explants. High percentage of multiple shoots noticed in nodal segment (70 percent) (Table 2). In the present investigation a relatively high ratio of cytokinin 0.4 mg/L to auxin 0.2 mg/L favoured shoot formation. This is an agreement with Murashige and Skoog (1962)., Rao et al., 1992, 1999 and Mary Mathew et al. (1999).

Table 1: Duration taken for shooting

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_1	50	35
T_2	60	50
T_3	62	55
SE d	0.014	0.164
CD (0.05)	0.045	0.52

Table 2: Percentage of callus showing multiple shoots

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_1	50	35
T_2	60	50

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_3	62	55
	SE d	0.520.15
	CD (0.05)	1.450.48

The mean for rooting duration ranged from 15 to 40 days between the explants. Among the explants tested the duration for rooting in nodal segment was significantly earlier than the shoot tip. The hormonal combination T_2 – Normal MS media + BAP 2.0 mg/L + IBA (2.0 mg/L) shows better results in roots induction (Table 3). The mean value of root length was ranged from 2.2 to 7.2 cm between the explants. Among the explant long root length was observed in nodal segment at the hormonal combination of T_3 – normal MS media + KN 2.5 mg/L + NAA (3.0 mg/L). A callus with roots will almost never form shoots, as root formation within a callus masks the end of morphogenesis with no possibility of plantlet production unless shoot buds are induced (Kuruvilla 1997). A relatively high ratio of auxin to cytokinin favours root formation (Murashige and Skoog, 1962). Significant differences have been observed in the morphogenetic capacity of calli that were induced at various levels of auxin.

Table 3: Duration for rooting

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_4	28	15
T_5	34	22
T_6	–	45
SE d	0.308	0.213
CD (0.05)	0.098	0.678

Table 4: Number of roots per shoot

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_4	1.2	2.1
T_5	0.31	0.80
T_6	0.14	0.40
SE d	0.308	0.213
CD (0.05)	0.098	0.678

Conclusion

In vanilla *in vitro* propagation study was conducted to assess the *in vitro* response of various explants in different growth regulators to identify the ideal and suitable medium for primary callus induction and to found out the response of *in vitro* regeneration of plantlets from callus tissue. In the present investigation, the explant nodal segment showed 15 days earlier rooting coupled with higher number of roots/shoots (2:1) than the other explants tested under the treatment KN 0.4 mg/L with NAA 0.2 mg/L. Hence the nodal explants with this hormonal combination will enhance the *in vitro* response of vanilla.

References

1. Ayyappan P. Vanilla a money making venture for ambitious farmers. Kisan world 1990;7:24–26.
2. Kuruvilla JT, Kim K and Sagawa Y. Shoot tip culture of Vanilla. Amer. Orchid Soc. Bull. 1972;41:435–39.
3. Mary Mathew K, Rao YS, Pradip KK, et al. *In vitro* culture systems in Vanilla In: Plant Tissue culture and biotechnology, Emerging Trends (Ed) Kavikishor, P.B. Universities Press (I) ltd, Hyderabad: 1999.pp.171–79.
4. Murashige T and Skoog T. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia of Plant arum. 1962;15:473–97.
5. Rao YS, Mary Mathew K, Madhusoodanan KJ

and Naidu R. Multiple shoot regeneration in Vanilla (*Vanilla planifolia* Andrews). J. Plant. Crops. 1992;21(Supply):351–54.

6. Rao YS, Mary Mathew K, Pradip KK et al. A manual for micro propagation of selected spices. Indian Cardamom Research Institute, Spices Board, Kochi, 1999;25.