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Research Article

Comparative Studies on Callus Induction from Different Explants of *Vanilla planifolia* Andrews

***Ruby Sharma and Sunil Bora**

Department of Horticulture, College of Agriculture,

Assam Agricultural University, Jorhat 785013, Assam, India

*Corresponding author: Email: rubyagri99atgmail.com, Tel: +91-9706206595

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ABSTRACT:

An efficient callus induction protocol has been developed for *Vanilla planifolia* using young leaf segment, shoot tip and nodal segment as explant source. The explants were cultured after surface sterilization on modified MS medium supplemented with various combinations and concentrations of 2,4-D, NAA and BAP and tested for the callus development. Nodal segments proved to be the best explants as compared to young leaf segment and shoot tip. Maximum callus development was obtained on MS medium supplemented with 1.0 mgL^{-1} BAP and 2.0 mgL^{-1} NAA.

Keywords: Callus induction, *Vanilla planifolia*, NAA, BAP

[I] INTRODUCTION

Vanilla planifolia belongs to the orchidaceae family which comprises 700 genera and over 20,000 species. Vanilla is the only edible fruit that contains relevant flavour and aroma compounds [1]. It is the only orchid of significant economic importance as an edible crop and 95% of the world's traded vanilla pods are derived from *V. planifolia*. Vanilla is explored and domesticated as a value based export crop. It is cultivated for its beans containing sweet scent, aroma and pleasant flavour, which is mainly due to the presence of vanillin. Vanillin, a naturally producing aromatic compound extracted from Vanilla beans and is widely used in many industries as food, beverages, sodas, pharmaceutics, cosmetics tobacco and traditional crafts. Thus, the potential of this species may be economically exploited as aromatic plants. Plants of vanilla originated in Mexico, and in some Central American countries as Costa Rica and Honduras. However, today they are cultivated in many areas of the world. *Vanilla planifolia* grows

wild in tropical forests and was first used by the Aztec people in Mexico to flavour cocoa.

Conventionally, vanilla is propagated almost entirely by stem cutting normally 6-8 nodes (80-120 cm long, 1 cm in diameter) in length, but cuttings are more difficult to deal with during planting and are also more expensive. Vegetative growth from cutting proceeds slowly, usually taking at least one year before the plants reach the flowering/fruiting stage. Plants established from *in vitro* propagules are phenotypically more uniform and healthier, and consequently yield higher than vegetative cuttings. Therefore, micropropagation is one of the viable approach for large scale multiplication of vanilla. Callus formation can be useful for several aims such as establishment of cell suspension culture [2] [3], protoplast culture [4], induction of embryogenic callus [5] and gene transformation [6]. Moreover, shoot regeneration from callus tissue is required for regeneration of modified or genetically transformed plants. For this reason, plant regeneration of *Vanilla planifolia* from different explants is in progress. The objectives of this study were to investigate the effects of explant type and plant growth regulators on callogenesis in *Vanilla planifolia*.

[II] MATERIALS AND METHODS

The present investigation was carried out at Commercial Tissue Culture Laboratory of the Department of Agril. Biotechnology, College of Agriculture, Assam Agricultural University, Jorhat. Different explants like young leaf segment, nodal segment and shoot tip (0.5-1.0 cm) were excised from 2 year old plants of *Vanilla* for callus induction. The explants were washed thoroughly with sterilized water, then washed with 5% (v/v) detergent solution (Teepol) for 5 minutes. Then disinfected with 0.1% $HgCl_2$ for 5 minutes and finally rinsed for 3 times using sterile double distilled water to remove the sterilants. Sterilization of culture medium and instruments was done by autoclaving at 121˚ C at 15 psi pressure for 15 minutes. For callus induction, the explants were cultured on Murashige and Skoog [MS] medium supplemented with different concentrations and combinations of growth regulators **[Table-1]**. MS medium with 3% sucrose (w/v) and 0.8 % agar was used as basal culture medium. Growth regulators were added and the pH of the medium was adjusted to 5.8 with NaOH/HCl (0.1N) before autoclaving.

The chemicals and growth regulators used were analytical grade and purchased from Himedia Pvt. Ltd. Mumbai, India. The cultures were kept in dark chamber created by covering the culture shelves all around with a black cloth for callus induction, maintained at 25±2ºC temperature and 70-80% RH. The cultures without growth regulators served as control. The experiment was conducted with a total of 10 replicates per treatment and was repeated thrice. The percentage data were subjected to arcsine transformation. The data were recorded and analysed statistically in a completely randomised design followed by Duncan's multiple-range test at a significance level of $P < 0.05$.

Table: 1. Composition of modified MS media tested for callus induction

Media	Basal media	BAP (mg/L)	NAA (mg/L)	2,4-D (mg/L)
VPC_1	MS	-	-	-
VPC_2	MS	0.5	-	1.0
VPC_3	MS	1.0	-	1.0
VPC_4	MS	0.5	-	2.0
VPC_5	MS	1.0	-	2.0
VPC_6	MS	2.0	-	2.0
VPC_7	MS	0.5	1.0	-
VPC_8	MS	1.0	1.0	-
VPC_9	MS	0.5	2.0	-
VPC_{10}	MS	1.0	2.0	-
VPC_{11}	MS	2.0	2.0	-

***(VPC = *Vanilla planifolia* Callus Induction Media)**

[III] RESULTS

Auxins and cytokinins are major growth regulators that have profound influence on various phenomena of cell division, callus induction and regeneration [7][8]. In the present study, maximum callus induction (61%) was observed in nodal segment within a minimum time period of 28.06 days, when 1.0 mgL^{-1} BAP and 2.0 mgL^{-1} NAA was used **[Table-2] [Plate-1].** The callus thus induced was found to be compact and creamy white in color with a vigorous growth **[Plate-1]**. The rest of combinations were capable of producing more or less poor results. Callus induction was not observed in VPC_1, VPC_2, VPC_3, VPC_4, VPC_5 and VPC_6 media. No callus was formed from either explant type on MS basal medium without growth regulators. It was observed that, nodal explants showed a good response (61%) for callus induction than young leaf segments (10%) whereas shoot tips did not show any response for callus induction. This difference might be due to inherent character of the explants. The growth characteristics of the induced callus were found to be medium. No organogenesis was observed in all these media.

Table 2. Effect of different modified MS media on callus induction of *Vanilla planifolia* Andrews from different explants.

a. Nodal segment

Medium	No. of explants[b] responded for callus initiation	Percentage of explants[b] responded	Days required for callus induction	Callus growth*	Morphogenic response
VPC_1	0	0.00 (0.00)	0	x	no callus induction
VPC_2	0	0.00 (0.00)	0	x	no callus induction
VPC_3	0	0.00 (0.00)	0	x	no callus induction
VPC_4	0	0.00 (0.00)	0	x	no callus induction
VPC_5	0	0.00 (0.00)	0	x	no callus induction
VPC_6	0	0.00 (0.00)	0	x	no callus induction
VPC_7	1.03^b	10.33 (18.75)	38.10^c	-	very small amount of callus was formed
VPC_8	2.07^b	20.67 (27.04)	42.13^b	++	greenish callus was formed
VPC_9	1.00^b	10.00 (18.44)	50.07^a	-	very small amount of callus was formed
VPC_{10}	6.10^a	61.00 (51.35)	28.07^e	+++	Compact and creamy white callus was formed
VPC_{11}	5.07^a	50.67 (45.38)	33.07^d	+++	Compact and creamy white callus was formed
SEd±	**0.748**		**1.805**		
CD at 5 %	**1.72**		**4.15**		

Means followed by a common letter are not significantly different at the 5% level by DMRT.
Figures in parentheses are arcsine transformation values.

b. Shoot tip

Medium	No. of explants[b] responded for callus initiation	Percentage of explants[b] responded for callus initiation	Days required for callus induction	Callus growth*	Morphogenic response
VPC_1	0	0.00 (0.00)	0	x	no callus induction
VPC_2	0	0.00 (0.00)	0	x	no callus induction
VPC_3	0	0.00 (0.00)	0	x	no callus induction

VPC_4	0	0.00 (0.00)	0	x	no callus induction
VPC_5	0	0.00 (0.00)	0	x	no callus induction
VPC_6	0	0.00 (0.00)	0	x	no callus induction
VPC_7	0	0.00 (0.00)	0	x	no callus induction
VPC_8	0	0.00 (0.00)	0	x	no callus induction
VPC_9	0	0.00 (0.00)	0	x	no callus induction
VPC_{10}	0	0.00 (0.00)	0	x	no callus induction
VPC_{11}	0	0.00 (0.00)	0	x	no callus induction
SEd±	**0.00**		**0.00**		
CD at 5 %	**0.00**		**0.00**		

c. Young leaf segment

Medium	No. of explants[b] responded for callus initiation	Percentage of explants[b] responded for callus initiation	Days required for callus induction	Callus growth*	Morphogenic response
VPC_1	0	0.00 (0.00)	0	x	no callus induction
VPC_2	0	0.00 (0.00)	0	x	no callus induction
VPC_3	0	0.00 (0.00)	0	x	no callus induction
VPC_4	0	0.00 (0.00)	0	x	no callus induction
VPC_5	0	0.00 (0.00)	0	x	no callus induction
VPC_6	0	0.00 (0.00)	0	x	no callus induction
VPC_7	0	0.00 (0.00)	0	x	no callus induction
VPC_8	0	0.00 (0.00)	0	x	no callus induction
VPC_9	0	0.00 (0.00)	0	x	no callus induction
VPC_{10}	0.6^b	6.66 (14.96)	40^a	x	Brown Callus
VPC_{11}	1.00^a	10.00 (18.435)	48^b	x	Hard compact callus
SEd±	**0.1421**		**0.492**		
CD at 5 %	**0.327**		**1.132**		

Means followed by a common letter are not significantly different at the 5% level by DMRT.
Figures in parentheses are arcsine transformation values.

[IV] DISCUSSION

In the present investigation, two different auxins were assessed on the basis of the calli induction. However our results revealed that the combination of NAA and BAP was found to be more effective than 2,4-D and BAP for callus induction. This result is in agreement with Davidonis and Knoor [9], who verified callus proliferation on medium containing NAA 2.0 mgL^{-1} +BAP 1.0 mgL^{-1}. Findings of Tan *et al.* [10], is in close agreement of our findings; they found 35% of explants forming callus when cultured on Murashige and Skoog [MS] basal

medium supplemented with 2.0 mgL^{-1} NAA and 1.0 mgL^{-1} BAP, whereas no callus formed in the presence of any concentrations of 2,4-D and BA. Similarly, Funk and Brodelius [11], obtained callus from fruit explants on MS medium supplemented with 0.54 µM NAA and 8.88 µM BA. But, our result contrasts with the studies carried out by Janarthanam and Seshadri [12], who reported that 2,4-D was more effective than NAA at inducing callus on both of these types of explants and Velankar and Heble [13],who indicates that the formation of early callus from nodal explants of *V. planifolia* cultured on MS media containing 2,4-D. However, in other reports NAA has been shown to be more effective than 2, 4-D for callus induction in orchids, such as Cymbidium [14][15] and *D. candidum* [16].

In our experiment, nodal segment found to be the best for induction of callus than young leaf segments and shoot tips. These results were in close agreement with those of Davidonis and Knoor [9], showing that the first node section was the best region for callus initiation and proliferation. Similarly, Tan *et al.* [10], used juvenile leaf and nodal segments from *V. planifolia* as explants to initiate callus and found nodal explants showed better callus initiation than juvenile leaf explants.

Plate 1. Callus formation from nodal explants on Murashige and Skoog (MS) medium containing 2.0 mg/L NAA and 1.0 mg/L BAP

But in contrast, Janarthanam and Seshadri [12], reported that 60.0% of juvenile leaf explants of *V. planifolia* produced callus, whereas only 35.0% of nodal explants formed callus when cultured on MS basal medium supplemented with 1.0 mgL^{-1} 2,4-D and 0.5 mgL^{-1} BA. Some other earlier report showed poor callusing of *V. planifolia* (Kononowicz and Janick [17]; Gu *et al.* [18]). Plant regeneration in orchids *via* an intermediary callus phase is considered to be a rather difficult morphogenic pathway. This might be due to the tendency of necrosis of orchid callus and slow rate of growth (Begum *et al.* [14]; Huan *et al.* [15]; Long *et al.* [19]).

[V] CONCLUSION

The investigation was conducted for establishment of callus initiation system for *Vanilla* using young leaf segment, shoot tip and nodal segment as explant source. From the results, it is clear that induction of callus depends upon the type of explants and growth regulators. The present study discovers nodal segment as an effective explant which expressed maximum callus at MS medium supplemented with 1.0 mgL^{-1} BAP and 2.0 mgL^{-1} NAA.

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