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IN VITRO PROPAGATION OF VANILLA (*Vanilla planifolia* Andr.) ON DIFFERENT CONCENTRATION OF CYTOKININS

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ABSTRACT/ABSTRAK

Micropropagation of vanilla was conducted through excised apical shoot tip and nodal segment as explants to determine the best concentration of growth hormone of cytokinin on multiple shoots production and regeneration. The explants were cultured on MS medium augmented with different concentration of N6-Benzyladenin and Kinetin. Sub culture was done four weeks after inoculation to regenerate moreshoots on the medium containing the same concentrationof plant growth regulators as inoculation medium. After 60 days of cultured, multiple regenerated shoots were elongated and formed leaves and roots on the fresh medium following the second subcultured. Data were recorded at 90 days of cultures. The two cytokinins tested induced bud break to different degrees and among the various concentrations tested, N6-Benzyladeninat 1.5 mg.l^{-1} gave the highest mean shoot number (3.27/explant) and leaves (3.80/shoot) differentiation. While the lowest number of shoots and leaves were found on MS medium fortified with 1.0 mg.l^{-1} Kinetin. All cultures produced roots and the highest number of roots formed on the medium containing 2 mg.l^{-1} Kinetin.

Mikropropagasi tanaman vanili menggunakan tunas apikal dan nodal sebagai eksplan dilakukan untuk menentukan konsentrasi dan kombinasi hormone tumbuh yang menghasilkan multiplikasi terbaik secara in vitro. Eksplan ditumbuhkan secara aseptik pada medium Murashige and Skoog yang dilengkapi dengan N6-Benzyladenin dan Kinetin pada berbagai konsentrasi yang berbeda. Subkultur dilakukan 4 minggu setelah penanaman untuk multiplikasi tunas tunas menggunakan media dan kandungan zat pengatur tumbuh yang sama dengan media penanaman. Setelah 60 hari, eksplan beregenerasi membentuk tunas, berelongasi menghasilkan daun dan akar setelah dilakukan sub kultur kedua. Pengambilan data dilakukan 90 hari setelah penanaman; jumlah tunas tertinggi (3.27/eksplan) dan jumlah daun terbanyak (3.80/tunas) diperoleh pada media MS yang mengandung 1.5 mg.l^{-1}N6-Benzyladenindan yang terendah dihasilkan dalam media yang dilengkapi 1.0 mg.l^{-1} Kinetin. Semua eksplan berakar dalam media tumbuh, namun rata-rata jumlah akar terbanyak dihasilkan pada media yang diberi Kinetin 2 mg.l^{-1}..

INTRODUCTION

Vanilla, a vine climbing orchid is native to Central America but distributed and cultivated in tropical regions such as inMadagascar, India, Philippine and Indonesia. Of these, production vanilla in Indonesia and Madagascarare more obvious and contribute to 90% of the world production (Sujatha and Bhat, 2010). Vanilla is a high value export crop due to its beans that contain sweet scent, aroma and pleasant of vanillin (vanilla essence). Vanillin is extracted from the cured beans and widely used for flavoring cakes, sweets, chocolates, ice creams, beverages, yoghurts, soft drinks and liquors. It is widely used also in scenting tobacco, perfumery and pharmaceuticals due to its antioxidant properties (Chandran &Puthur, 2009; Morwal et al., 2015). Consequently, the market demand for natural vanillin is increasing significantly although there is a competition from synthetic vanilla due to its cheap price.The increased of health awareness and a restriction use of synthetic vanillin in precious food products due to concerns about safety strengthen the demand for vanilla beans and the market price of high-quality natural vanillin has increased steadily over recent years.

Vanilla is a perennial climbing orchid with sessile leaves and succulent green stems, producing aerial roots at the nodes. Their seeds do not usually germinate; the plant is propagated mainly by stem cuttings, which possess slow rate of multiplication. Another problem of this vegetative method is removal of many cuttings can injure the mother plant, resulting in retarding growth and reduction in yield.Therefore, an alternative method of propagation is needed to solve these problems and to produce enough vanilla vines that can fulfill the increasing market demand of natural vanillin.

The *in vitro* plant propagation is reported to be the suitable method in vanilla mass multiplication as it ensures arapid rate of multiplication. It is not season dependent and requires only a limited quantity of plant tissue as a source of initial explants. Tissue culture techniques also ensure production of virus free propagules (Morwal et al., 2015). Shoot tip and axillary buds have been frequently used in vanilla tissue culture making the cultivated plants are genotypically identical (Divakaran *et al*., 2006; Palama *et al*., 2010; Neelannavar, 2011). To achieve anoptimal number of propagules, the explants were incubated at the medium containing a combination of plant growth regulators (PGRs) and used a different combination medium for rooting (Mushimiyimana, 2011, Tan *et al.,* 2011, Jadid *et al*., 2015). To develop an efficient propagation, this paper reports a micropropagation system for*V. planifolia* using shoot tip and axillary bud cultured on the medium containing a single PGR.

MATERIALS AND METHODS

Plant material

Stem node sections with dormant axillary buds were excised from1-year-old field-grown plants of *V. planifolia.* These sections, measuring 1.5–2.0 cm, eachwith one dormant bud, were washed with liquid detergent and then rinsed in running tap water. The cleaned explants were surface sterilized in a laminar air flow cabinet with 5% of sodium chloride for 15 min and then two rinses in sterile distilled water. The explants were sterilized further by immersing in 70% alcohol for 15 min. The sterilized explants were rinses three times in sterile distilled water before being inoculated on the growth medium.

Multiple shoot initiation and Proliferation

The basal nutrient medium by Murashige and Skoog (MS) was used as culture media supplemented with 100 $mg.L^{-1}$ myoinositol, 35$g.L^{-1}$ sucrose, and 7.5 $g.L^{-1}$ bacto agar; pH was adjusted to 5.7 before autoclaving at 1 $kg.cm^{-2}$ for 30 min and 121^{o} C. For shoot induction, the bases of surface-sterilized explants were inserted into the above media augmented with different concentrations of6-benzylaminopurine (BA) or with kinetin.

The shoots obtained after 30 days were sub-cultured to a proliferation medium containing the same cytokinin concentrations used for the induction stage. The second sub-culture was performed 30 days after the first sub-culture by separating and dissecting the micro shoots with sterile dissecting needles and forceps. The separated shoots were incubated and allowed to elongate on the fresh MS media containing the same growth regulator as

proliferation media. All the culture steps were donein the sterile environment of laminar air flow cabinet. The cultures were placed at an incubated room at 23±2°C and 16/8 h photoperiod. Fifteen explants were used for each treatment and data were recorded after 90 days of culture. Significant differencesin multiple shoot differentiation among the treatments were teste dusing the ANOVA *F*-test followed by least significant test (LSD) at the 5% level.

RESULT AND DISCUSSION

Regeneration of shoots from the shoot tip and auxiliary bud cultured in the present study were occurred through direct organogenesis. Shoot initiation was observed from cultured explants after 10 days of inoculation. Response to shoot formation was varied with type of cytokinins used and also the concentration of the PGRs on the medium. The proliferation rate of explants cultured on the medium supplemented with BA was generally higher than Kinetin (Table 1). Of the various treatments tested in MS medium, BA (1.5mg.l^{-1}) resulted in an average of 3.27 shoot buds per explant after 90 daysof culture. The number of shoots obtained in this treatment was insignificantly different from other treatments at the 5% level. In contrast, shoot elongation and rooting were more pronounced in kinetin treatments (Figure 1).

BA has been considered to be one of the most effective cytokinins for the induction of shoot regeneration in vanilla plant tissue culture (Janarthanam and Seshadri 2008; Neelannavar *et al*., 2011: Tan *et al.,* 2011). Lower concentrations of BA produced more number shoots and as the concentration of BA increased, the number of shoots and number of leaves reduced. Similar results were recorded that an increase level of cytokinin had an adverse effect on shoot growth (Mushimiyimana *et al*., 2011; Jadid *et al.*, 2015; Morwal *et al.*, 2015). It evidenced that cytokinin is an essential growth hormone to induce the growth of axillary buds and adventitious shoots, reduce apical dominance and promote cell division. However, an optimal concentration is critically factor in PGRs role.

Table 1. Effect of different concentration Cytokinin in direct organogenesis of vanilla explants after 12 weeks of culture

PGRs	Days for shoots initiation	Number of shoots per explant	Number of leaves per shoot	Number of roots per shoot
BA 1 mg. l^{-1}	11.42a	2.19a	2.94a	1.35a
BA 1.5 mg. l^{-1}	9.50a	3.27a	3.80a	1.60a
BA 2 mg. l^{-1}	10.20a	3.06a	2.08a	1.47a
Kn 1 mg. l^{-1}	13.37a	1.50a	2.87a	1.50a
Kn 2 mg. l^{-1}	9.80a	2.20	3.20a	3.50a

Values are mean of five replicates. Common superscript letters are not significantly different at $P < 0.05$ using LSD analysis.

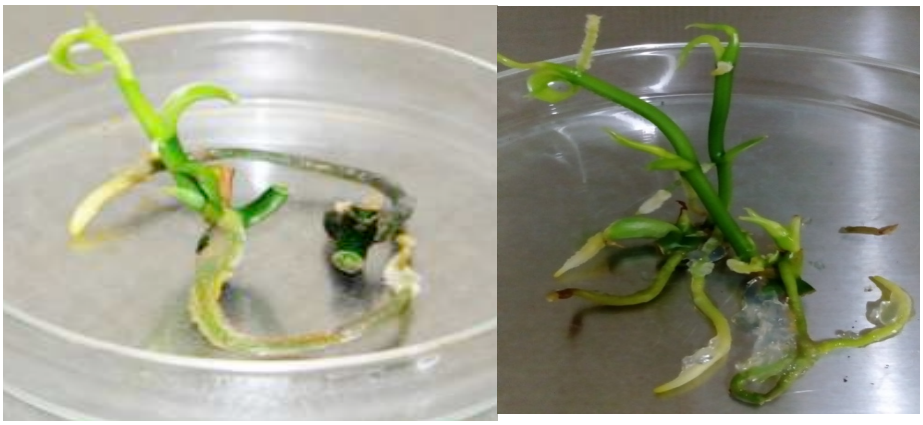

Fig. 1. Root development from elongated shoots on MS medium containing 1 mg.l^{-1} (left) and 2 mg.l^{-1}(right)

Rooting of elongated shoots was also achieved in one culture medium used in this study. This represented a prominent advantage, because in most cases of in vitro propagation, it is necessary to induce rooting through the addition of auxin (Morwal *et al.*, 2015; Jadid *et al*., 2015; Al-Dein Al-Ramamneh *et al*., 2017; Momeni *et al*., 2018). As fewer studies have shown the advanced effect of a sole PGR on in vitro propagation of different plants, the current study showed the positive effect of a single growth regulator on micropropagation of *Vanilla planifolia*.

CONCLUSION

A single growth regulator (cytokinin) used on this study produced a multiple shoots and roots of cultured vanilla. The presence of BA1.5 $mg.l^{-1}$ on MS modified medium resulted in the highest shoot proliferation and number of leaves. Meanwhile, the best root proliferation and elongation were obtained in the MS medium containing 2 $mg.l^{-1}$ Kinetin.

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