
Effect of plant growth regulators on micropropagation of *Vanilla aphylla* and *Vanilla planifolia* sp. Variegata

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Abstract The plant growth regulators for propagating large number of vanillas in a short period of time was reported. Axillary buds were used from the explants of *Vanilla aphylla* and *Vanilla planifolia* sp. variegata that cultured on solid Gamborg's B5 medium supplemented with 0, 0.5, 1, 1.5 and 2 mg/L 6-benzylaminopurine (BA) for 8 weeks. The result showed that the highest average number of shoot proliferation and root induction were obtained at 1 mg/L BA of 3.2 cm. and 5.3 cm. respectively for *Vanilla aphylla*. *Vanilla planifolia* sp. variegata showed the highest shoot elongation of 1.9 cm. at 0.5 mg/L BA. Root induction was the highest of 0.9 cm. at 2 mg/L BA. After 12 weeks of culture The explants were grew the best after 12 weeks. They were transplanted into pots and covered by plastic bags for acclimatization and has maintained in greenhouse.

Keyword: Axillary bud, Shoot induction, *Vanilla aphylla*, *Vanilla planifolia* sp. variegata

Introduction

Vanilla is plant which belongs to Orchidaceae. It is a vine that needs to stick to other trees. Vanilla is normally found in the tropical area, higher than 600 m from the sea level, and it benefits from rainfall of 2000-2500 mm/year and average temperature of 27°C. The suitable area for planting vanilla is slope with mold soil, good drainage and organisms that supports proliferation of roots. Vanilla is one of the best seasoning orchid species with beautiful flowers which provide good odor. Vanilla naturally blooms after 3 years depending on size of vanilla tree and it produces the largest quantity of flowers after 7-8 years. Vanilla originates from Central America, (Mexico and Guatemala). It is cultivated in many countries especially in Madagasca. The cultivation of vanilla is supported by Thailand Institute of Scientific and Technological Research since 2002 (Chalermglin, 2015).

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There are 5 species of vanilla in Thailand *Vanilla albida*, *Vanilla aphylla*, *Vanilla borneensis Rolfe*, *Vanilla griffithii*, and *Vanilla siamensis*, but the popular species of the world are *Vanilla pompona*, *Vanilla tahaitensis*, and *Vanilla planifolia* Andrew which is the most commercial vanilla species. (Fhasaiwanmai, 2018). The vanillin from extracted vanilla pods and seeds can be used to flavor various types of beverage, bakery, dessert and cuisine etc. (Sruamsiri, 1986). Due to the terrain and climate of country and requirement of high skill for propagation in greenhouse, vanilla is hard for cultivation and needed high principal, so tissue culture is good solution of the problems and leads to propagation of vanilla in a high quantity within a short time. The purposes were to determine and optimize concentration of plant growth regulator 6-benzyladenine (BA), and to induce shoots and roots of vanilla plants.

Materials and methods

Plant Materials and Aseptic Culture

Explants were axillary buds of fully grown *Vanilla aphylla* and *Vanilla planifolia* sp. variegata which obtained from Phratamnak Suan Pathum, Bang Khayaeng, Pathum Thani province, Thailand. The axillary buds were cut in pieces for 1.5-2.0 centimeter, sterilized by washing by tap water for 10 minutes, soaking in alcohol at concentration of 95% for 1-2 minutes, then shaking in 0.15% Mercuric Chloride with 2-3 drops of tween 20 for 10 minutes and sterilized water for 5 times. The explants were then placed in Petri dishes to culture.

Culture condition

The sterilized explants were cultured on solid mediums B5 (Gamborg,1970) and supplemented with plant growth regulator 6-Benzyladenine (BAP) at concentration of 0, 0.5, 1, 1.5 and 2 mg/L to induce shoots. The cultured explants were incubated at controlled room temperature room of $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with 16 hours by cool-white fluorescent lamps. The observation was made in 4 and 8 weeks after culture and recorded the number, growth, and characteristic of shoots. The vanilla with full proliferation of shoots and roots were planted in natural condition by removing the plants from the tissue culture flasks, washing by tap water to clean out the agar, and soaking in fungicide to protect vanilla from contamination. Vanilla was planted in pots and covered by transparent plastic bags and placed in covered area to observe acclimatization.

Statistical Analysis

Data was analyzed by program SPSS (Statistical Package for the

Social Sciences) software (IBM Corporation and other(s)1989, 2017) to determine completely randomized design (CRD). The experiment contained 5 treatments and each treatment contained 5 experiment units.

Results

The axillary buds of *V. aphylla* and *V. planifolia* sp. variegata were cultured on B5 medium with supplemented with BA at concentration of 0, 0.5, 1, 1.5 and 2 mg/L to induce shoot for 8 weeks. There were shoot induction from all media at different concentrations of BA in the fourth weeks as shown in Table. 1.

Table 1. Average of number and length of shoots of *V. aphylla* and *V. planifolia* sp. variegata a in B5 medium with various concentrations BA after 4 weeks of cultivation

Species	BAP (mg/L)	Number of shoots per explant (%)	Shoot length (cm)
<i>V. aphylla</i>	0	0	0 ± 0.0
	0.5	40	0.1 ± 0.1
	1	80	1.0 ± 0.4
	1.5	60	0.4 ± 0.2
	2	60	0.6 ± 0.3
<i>V. planifolia</i> sp. variegata	0	40	0.3 ± 0.2
	0.5	40	0.9 ± 0.5
	1	40	0.2 ± 0.1
	1.5	20	0.5 ± 0.5
	2	40	0.2 ± 0.1

V. aphylla grew for four weeks showed shoot induction per axillary bud in tested BA at concentration of 1 mg/L averaged 80% of the explants, and followed at 1.5 and 2 mg/L BA. Result showed shoot induction per axillary bud was 60%. The highest shoot elongation was 1.0 cm when treated with BA at 1 mg/L concentration, and followed by 0.4 cm. and 0.6 cm. from the tested BA at 1.5 and 2 mg/L, respectively as shown in Fig. 1A. It was found that the lowest shoot elongation was obtained from B5 medium without supplemented with plant growth regulator.

V. planifolia sp. variegata in the fourth week showed approximately shoot induction per axillary bud from BA at concentration of 0, 0.5, 1 and 2 mg/L that was 40% of the explants. The highest shoot elongation was 0.9 cm. that was obtained from BA at 0.5 mg/L concentration and the lowest shoot elongation was obtained from BA at concentration of 2 mg/L as shown in Fig.1B.



Figure 1A. Shoot induction of *V. aphylla* at 1 mg/L BA after 4 weeks of culture

Figure 1B. Shoot induction of *V. planifolia* sp. variegata at 0.5 mg/L BA after 4 weeks of culture

After the first 4 weeks, *V. aphylla* and *V. planifolia* sp. variegata were cultured on the B5 medium until the eight weeks to observe shoot induction of both vanillas as shown in Table 2.

Table 2. Average of number and length of shoots of *V. aphylla* and *V. planifolia* sp. v in B5 medium with various concentrations BA after 8 weeks of cultivation

Species	BAP (mg/L)	Number of shoots per explant (%)	Shoot length (cm)
<i>V. aphylla</i>	0	0	0 ± 0.0
	0.5	60	1.8 ± 1.0
	1	80	3.2 ± 1.1
	1.5	60	0.4 ± 0.2
	2	60	0.6 ± 0.3
<i>V. planifolia</i> sp. variegata	0	40	0.3 ± 0.2
	0.5	40	1.7 ± 1.0
	1	60	1.2 ± 1.1
	1.5	20	0.5 ± 0.3
	2	40	0.2 ± 0.1

The highest shoot induction and elongation of *V. aphylla* was cultured on B5 medium supplemented with BA at 1 mg/L concentration averaged 3.2 cm., and followed by BA with 0.5, 1.5 and 2 mg/L concentrations averaged 1.8, 0.4 and 0.6 cm. respectively as shown in Fig. 2A.

The shoot induction and elongation of *V. planifolia* sp. variegata showed the highest length in B5 medium supplemented with BA at 0.5 mg/L concentration averaged 1.7 cm., and followed by BA with 0, 1, 1.5 and 2 mg/L concentrations which averaged 0.3, 1.2, 0.5 and 0.2 cm., respectively, as shown in Fig. 2B.

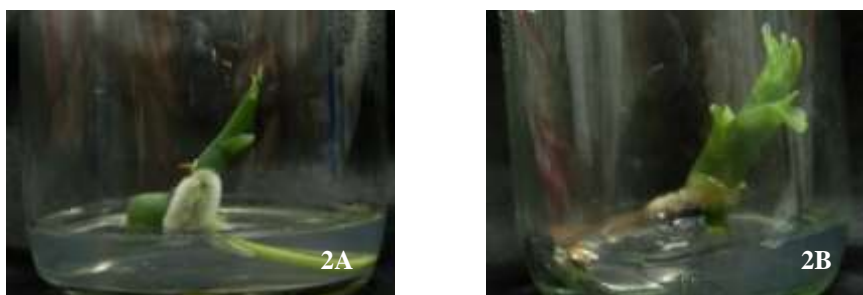


Figure 2A. The highest shoot elongation of *V. aphylla* at 1 mg/L BA after 8 weeks of culture

Figure 2B. The highest shoot induction of *V. planifolia* sp. variegata at 0.5 mg/L BA after 8 weeks of culture

Moreover, it was found that the roots of both *V. aphylla* and *V. planifolia* sp. variegata were induced in the same period of shoot induction.

The root induction for *V. aphylla* was highest when tested in BA at concentration of 1 mg/L, and followed by 0.5, 1.5 and 2 mg/L respectively. The root elongation averaged as 5.3, 2.8, 0.7 and 0.3 cm. respectively. The highest average of root induction for *V. planifolia* sp. variegata was discovered from BA at concentration of 2 mg/L at 0.9 cm and BA at concentration of 0, 0.5, 1 and 1.5 mg/L showed the same average of root induction at 0.4, 0.2, 0.3 and 0.8 respectively.

After that *V. aphylla* and *V. planifolia* sp. variegata were culture on B5 medium for 12 weeks and subculture every 4 weeks to thrive both shoot and root and transfer to plantlet ex vitro environment as shown in Fig. 3A and 3B.



Figure 3A. full proliferation of shoots and roots of *V. aphylla* at 1 mg/L BA after 12 weeks of culture with subculture for every 4 weeks

Figure 3B. full proliferation of shoots and roots of *V. planifolia* sp. variegata at 0.5 mg/L BA after 12 weeks of culture with subculture for every 4 weeks

After 20 weeks of culture *V. aphylla* and *V. planifolia* sp. variegata were transferred to plantlet in pots covered by plastic bags to acclimatize vanilla with environment as shown Fig. 4A and 4B.

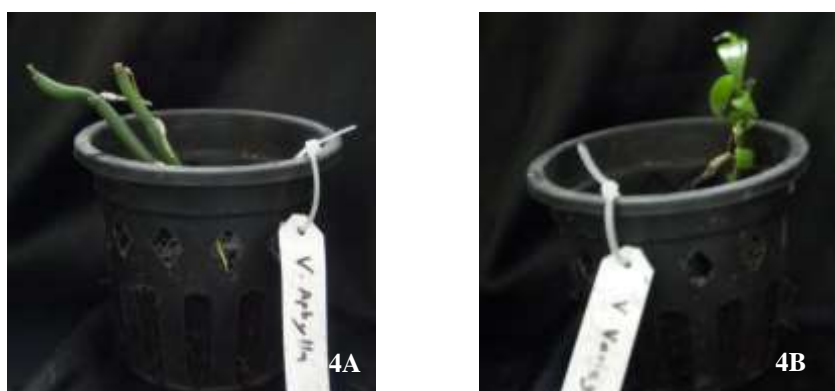


Figure 4. (A) *V. aphylla* and (B) *V. planifolia* sp. variegata were transferred to plantlet in pots covered by plastic bags after 20 weeks of culture

Discussion

From the experiment, the effect of plant growth regulators *in vitro* vanilla (*Vanilla planifolia* Andr.) revealed that Gamborg B5 medium was good to induce shoot in according with Bhattacharjee and Islam (2014) who studied *Vanda tessellate* that was induced the seeds to shoots by using combination of medium including B5. Asikin *et al.* (2014) studied micropropagation and chromosome of four endanger orchid species Indonesia by using half-strength B5 medium and combination of other medium with BA and NAA. Zhang *et al.* (2013) compared with half-strength MS, MS, Vacin and Went (VW) and Gamborg (B5) supplemented with cytokinin BA, Kin and auxin NAA. Haque and Ghosh (2017) reported the protocorm-like body (PLB) formation was from callus culture *in vitro* germinated seedlings of *Spathoglottis plicata* blume on MS medium and B5 medium supplemented with 2,4-D, NAA, KIN and aloe vera gel.

The shoot induction of *V. aphylla* was achieved by using BA at concentrations of 0.5, 1.5 and 2 mg/L that showed the same quantity and BA at 1 mg/L concentration gave the highest shoot induction along with shoot elongation with related to Zuraida *et al.* (2013) who studied half or full strength MS medium with BA and NAA to produce shoot proliferation and the result was 1 mg/L BA at half strength MS medium, indicating the highest number of shoots and maximum shoot length. Halim *et al.* (2017) used the node explants of *Vanilla planifolia* to shoot induction and found that 1 mg/L BA adding 0.5 mg/L NAA cultured on MS medium that provided 100 percent shoot regeneration. Abebe *et al.* (2009) reported the highest number of shoots and found that 1 mg/L BA combined with 0.5 mg/L Kin cultured on MS medium for 45 days. In the same manner with Tan *et al.* (2011) showed callus initiation from nodal explants at 2 mg/L NAA and 1 mg/L BA, which better than leaf explants.

V. planifolia sp. variegata cultured in BA at concentration of 1 mg/L showed the highest shoot induction, but at concentration of 0.5 mg/L showed the highest shoot elongation that similar to Biradar *et al.* (2017) who used shoot tip explants of *Vanilla planifolia* on MS medium supplemented with different concentration of BA showing averaged leaf production at BA 0.5 mg/L. The shoot induction of *V. planifolia* and *V. pilifera* were found in liquid MS medium containing with BA 0.5 mg/L for 4 weeks (Khunsri, 2009).

Furthermore, there was root induction during a period of shoot induction. The average of root induction decreased when the concentration of BA decreased inconsistent with Ayele *et al.* (2017) who suggested that vanilla explants cultured on a PGR free MS medium tended to initiate roots. BA is regarded for the most effective cytokinins to induce shoot regeneration in plant tissue culture (Baskaran and Jayabalan, 2005) Full elongation of shoots and roots of *V. aphylla* and *V. planifolia* sp. variegata after 20 weeks of culture were transferred the plantlets to pots that simulated in natural condition. This experiment is a limitation in repeated experiment due to shortage of explant of *V. planifolia* sp. variegata as a rare orchid species.

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