# Light Quality Affects Shoot Multiplication of *Vanilla pompana* Schiede in Micropropagation

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## ABSTRACT

The influence of different light quality *in vitro* culture of *Vanilla pompana* Schiede to accelerate shoot induction and shoot multiplication were investigated in this study. Nodal segments of *V. pompana* with one node (12–15 mm in length) were cultured for 90 days on MS medium supplemented with 30 $gL^{-1}$ sucrose and 8 $mgL^{-1}$ BA under 7 treatments of different light qualities, including fluorescent (FF) as the control, White (WW), Red + Blue 1 : 1 (RB), Red + White 1 : 1 (RW), Blue + White 1 : 1 (BW), Blue (BB) and Red (RR) light-emitting diodes (LEDs). The results demonstrated that bud of every nodal segments regenerated shoot and continuously elongated to 0.8–2.0 cm. A combination between blue and red LEDs enhanced vegetative growth of the first regenerated shoot with increased shoot length, node number and leaf number, while monochromatic red lighting suppressed these factors. Furthermore, white LEDs resulted in the highest percentage of callus induction from nodal segment with a rapid increase for first 30 days of the culture period. Multiple shoots generated from both lateral bud of first main shoot and differentiated from callus at 90 days. The monochromatic blue LEDs promoted the greatest callus differentiation with the highest total multiple shoots. The blue (BB) and red (RR) LED light promote the highest number of multiple shoots regeneration as same as fluorescent light. These results are able to contribute to use the blue (BB) and red (RR) LED light substitution of fluorescence light for *V. pompana* micropropagation and can reduced annual electrical cost

**Keywords:** Callus induction, light emitting diode, orchid, shoot regeneration

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## INTRODUCTION

*Vanilla* is a unique member of the family Orchidaceae (Cameron, 2011). This tropical orchid is native to Mexico and Central America (Lee-Espinosa *et al.*, 2008) and commercially grown in many countries, including China, Indonesia, Madagascar, Mexico, New Guinea Papua and Turkey. *Vanilla* has a high economic value due to its ability to produce a great natural essence called vanillin, which is used as a flavour in the food industry in worldwide (Ramachandra and Ravishankar, 2000; Divakaran and Babu, 2009). However, there are three main species of *Vanilla* that important for cultivation and commerce: *Vanilla planifolia* Andrew, *Vanilla pompana* Schiede, and *Vanilla tahitensis* J.W. Moore (Ramachandra and Ravishankar, 2000). In central part of Thailand, Malachuamong *et al*. (2015) found that *V. pompana* had the great growing with the highest $CO_2$ exchange rate in year-round than other two *Vanilla* species.

Presently, tissue culture is widely used in *Vanilla* propagation instead of the more conventional method, stem cutting, as it is able to increases the rate of *Vanilla* plantlet growth, while ensuring the same quality and genetic purity (Gantait *et al.,* 2011; Zuraida *et al*., 2013). Various explants of *Vanilla* i.e. shoot apex, nodal segment and axillary bud were used to culture in *Vanilla*

micropropagation (Lee-Espinosa *et al.,* 2008; Palama *et al.,* 2010; Tan *et al.*, 2011). Plant growth regulators (PGR) such as BAP, NAA, or kinetin supplemented on MS medium are requisite for shoot multiplication of *V. planifolia* (Lee-Espinosa *et al.,* 2008; Abebe *et al*., 2009; Tan *et al.*, 2011). However, there are few studies in micropropagation of *V. pompana* even thought it was one of the main *Vanilla* species, which cultivates for the commercial production (Ramachandra and Ravishankar, 2000). Previous report by Sutthiprapa *et al.* (2016) who found that shoot multiplication of *V. pompana* was able to induce under cultured on MS medium supplemented with 8 $mgL^{-1}$ BA and 0.5 $mgL^{-1}$ NAA, but still time-consuming in the initial of shoot proliferation with a low number of shoot per explant.

Light emitting diodes (LEDs) are often used as an alternative light source in tissue culture with various species of plants (Ramírez-Mosqueda *et al.*, 2017). LEDs able to control the spectral composition and adjust the light intensity to higher levels with lower radiant heat output than commercial fluorescent tubes, leading to a longer lifespan. This reduces the cost of production and energy needed to provide a strong alternative for horticultural lighting. Numerous previous studies use LEDs as a light source, especially blue, red, and white light in both monochromatic and light combinations to stimulate the growth and development of various orchid species *in vitro* propagation. Red LEDs was reported to promote shoot formation in *Dendrobium kingianum* (Habiba *et al*., 2014) and protocorm-like bodies (PLBs) in *Oncidium* (Mengxi *et al.,* 2011) and *Oreorchis* (Bae *et al.,* 2014). Meanwhile, blue LEDs are able to enhance the highest stem node elongation in *Paphiopedilum* (Luan *et al.,* 2015) and produce PLBs in *Cymbidium* (Nahar *et al.,* 2016) and *Dendrobium* (Lin *et al.,* 2011). Moreover, the synergistic effect of blue and red light in proper combination showed efficiently enhanced growth of *Calanthe* hybrids plantlets *in vitro* (Baque *et al*., 2011) and promoted the leaf growth of *Cymbidium* (Tanaka *et al*., 1998) and *Doritaenopsis* (Shin *et al*., 2008). LEDs were previously reported in *V. planifolia* that showed the mixture of blue plus red LEDs stimulated shoot elongation in multiplication phase (Bello-Bello *et al*., 2016; Ramírez-Mosqueda *et al*., 2017) and increased shoot number in *V. tahitensis* 'Tahiti' (Sirirukwongsa *et al*., 2015). Most of previous studies suggested that LEDs showed a high potential to promote shoot proliferation in numerous genus of orchids in the micropropagation. Thus, LEDs possibly enhances the higher efficiency tissue culture of *V. pompana*. This study aims to explore the effect of different colors and combinations of LEDs on shoot regeneration and multiplication of *V. pompana* in tissue culture, with the aim of rapid increasing their growth and development through practices that can contribute to improve the micropropagation of this species.

## MATERIALS AND METHODS

### Plant Material

This experiment used *V. Pompana in vitro* plantlets and was established in Rapee Sagarik Orchid Garden, Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok (Figure 1). Vials containing the plantlets were cleaned using sterilized cloths soaked in 70% ethanol and then placed inside a laminar flow hood. Nodal segments with one node each (12 to 15 mm in length) were excised using a sterilized scalpel and used in experiment.

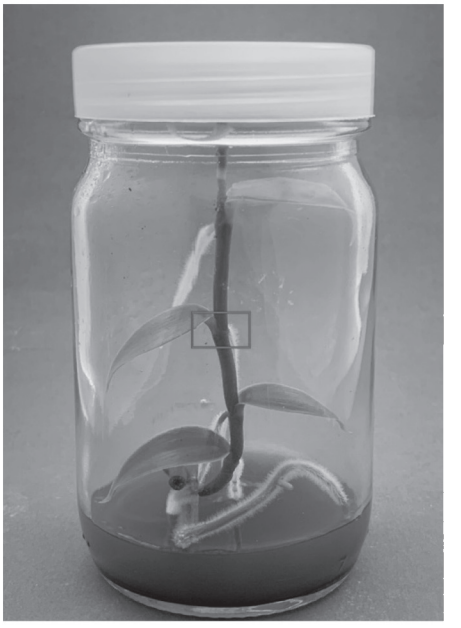

**Figure 1** *Vanilla pompana in vitro* plantlet for using as plant material in this the experiment, *red-edge square* indicates nodal segment as explant

**Shoot Induction and Multiplication**

The nodal explant with one node was transferred in a vial containing 25 ml MS (Murashige and Skoog, 1962) solid media supplemented with 8 $mgL^{-1}$ benzyladenine (BA), 30 $gL^{-1}$ sucrose and 2.4 $gL^{-1}$ Gelrite agar to induce and multiply shoots during November 2015 to March 2016. The pH of the medium was adjusted to 5.8 before autoclaving. Culture conditions were maintained at a temperature of 25 ± 1°C and relative humidity (RH) 60–70% under a 12 h photoperiod.

**Light Quality and Treatments**

Light emitting diodes (LEDs) treatments included

1) WW: white LED (WW)
2) RB: red and blue (1 : 1) LED
3) RW: red and white (1 : 1) (RW) LED
4) BW: blue and white (1 : 1) LED
5) BB: blue LED
6) RR: red LED
7) FF: fluorescent lamps (FF) were used as the control with a wavelength 395–700 nm

The supplement light was done above the cultures and the PPFD (photosynthetic photon flux density) was adjusted to 40 µmol $m^{-2}s^{-1}$ (on top of the culture vessel) with a 12 h photoperiod.

**Data Collection**

After culture, growth and development parameters of nodal segment explant include first regenerated shoot, node number, leaf number, multiple shoots number, and callus induction (%), which were collected at 30 days intervals for 3 months (90 days).

**Experimental Design and Data Analysis**

The experiment was designed in a complete randomized design (CRD) with 7 treatments and 4 replications (5 explants per replication). Data were analyzed by analysis of variance (ANOVA) and the differences among the treatments were analyzed by Duncan multiple range test ($P < 0.05$)

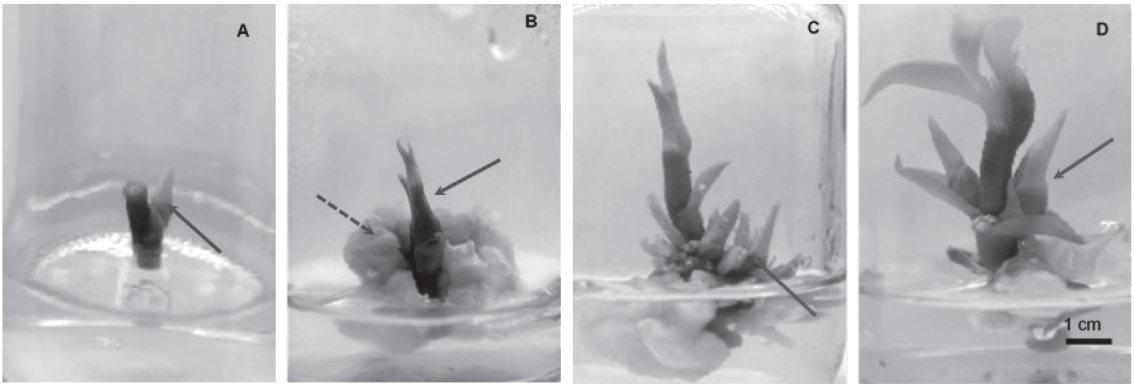


**Figure 2** Growth development pattern of nodal segment *in vitro* culture of *V. pompana*: (A) Bud formation after 2 weeks of culture, (B) First regenerated shoot (arrow) and callus formation (dashed arrow) after 30 days culture, (C) Clumps of multiple shoots from callus after 90 days culture (D) Regeneration of multiple shoots from lateral bud after 90 days culture. *Bar represented 1 cm*

## RESULTS AND DISCUSSION

### Growth Development Pattern of Nodal Segment *in vitro* Culture

The nodal segment with 1.2–1.5 cm in length of *V. pompana* was cultured on MS basal media supplemented with 30 $gL^{-1}$ sucrose and 8 $mgL^{-1}$ benzyladenin (BA) under different qualities of light in the initiation of culture. The results showed that the first shoot regeneration (main shoot) from bud of every nodal segments was observed around 2 weeks after culture (Figure 2A) and continuously elongated to 0.8–2.0 cm with the increasing of internode length and the development of first juvenile leaf after 30 days of culture in all treatments (Figure 2B). Interestingly, the callus formation was also observed under all light conditions within 1 month, except in BB and BW that occurred in the 2nd month of culture. The callus which was observed in this study showed yellow compact callus with both nodular and irregular form (Figure 2B). Multiple shoots were regenerated from callus within a culture period of 90 days (Figure 2C). Meanwhile, the multiple shoots also regenerated from the lateral bud of the first regenerated shoot (Figure 2D). Our observations differed from the report in *V. planifolia*, which was cultured in semi-solid medium and found that the nodal segments which were incubated under the same treatments of light quality occurred only in shoot proliferation (Ramírez-Mosqueda *et al*., 2017). On the other hand, Mengxi *et al.* (2011) reported that the different spectra (red, blue, yellow, green and florescence) were able to induce PLBs from shoot tips that were cultured in solid medium of *Oncidium* 'Gower Ramsey' within 1 month. Thus, the difference patterns of growth and development possibly depend on the type of medium and plant species.

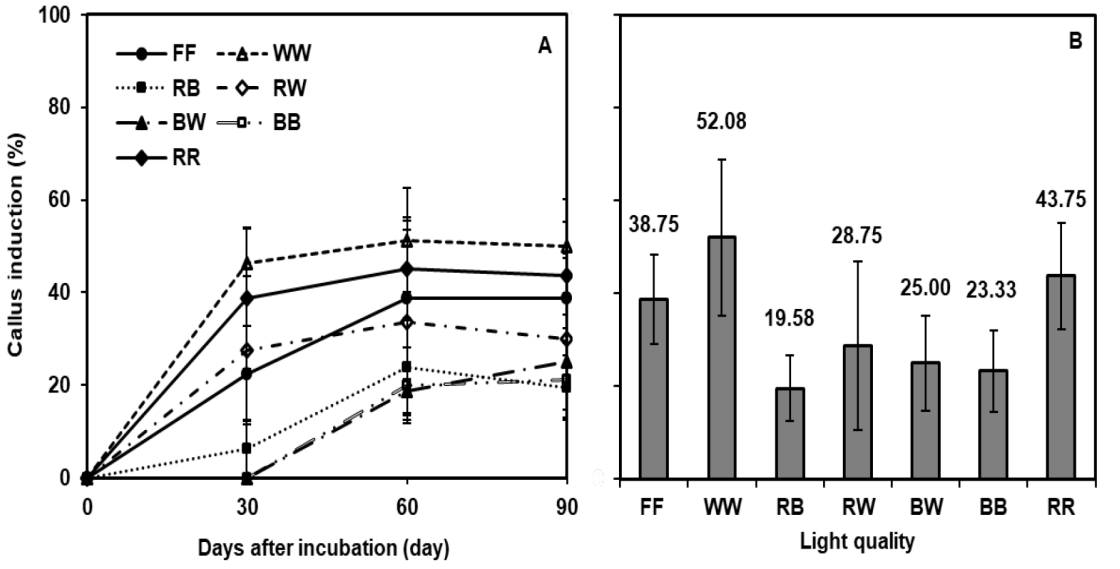


**Figure 3** Callus formation of *Vanilla. pompana in vitro* culture: (A) Percentage of callus induction in 30 days interval for 90 days after culture, (B) Percentage of callus induction after 90 days of culture

**Effect of Light Quality on First Regenerated Shoot and Callus Induction**

According to Table 1, the different LED colors had a significant influence on the growth and development of shoot regeneration of *V. pompana in vitro* culture. The growth of the first regenerated shoot was better under RB culture, especially shoot length (4.45 ± 0.67 cm) and leaf number (4.11 ± 0.34 leaves), which was 1.7 times significantly higher than the RR treatment. RR gave the lowest shoot length (2.58 ± 0.22 cm), node number (2.38 ± 0.29 nodes) and leaf number (2.55 ± 0.28 leaves) after a 90-day culture period. Meanwhile, the growth of the first regenerated shoot in other lighting conditions showed the similarity between the treatments. These results were in agreement with the previous reports in *V. planifolia in vitro* culture under LEDs. The combination between blue and red (1 : 1) stimulated the longest shoot in the proliferation phase, while monochromatic red LEDs reduced shoot height in the plantlet stage (Bello-Bello *et al*., 2016; Ramírez-Mosqueda *et al*., 2017). These results were also supported by Baque *et al*. (2011), who found that shoot length was highest in *Calanthe* hybrids under a mixture of red plus blue LEDs. Monochromatic red in this study displayed an inhibitory effect on the elongation of shoots. In contrast, numerous previous reports showed that red light is the most effective to promote stem elongation in *Oncidium*, which contrast with our study (Chung *et al*., 2010; Mengxi *et al*., 2011). Therefore, the response to difference light quality was dependent on each plant species, development stage and explant.

**Table 1** First regenerated shoot size and multiple shoots number of *Vanilla pompana in vitro* culture after 90 days culture under different quality of light

| Light treatment | First regenerated shoot | | | Multiple shoots regeneration | | |
|---|---|---|---|---|---|---|
| | Shoot length (cm) | Node number | Leaf number | Regeneration from callus | Regeneration from lateral bud | Total Multiple shoots number |
| FF | 3.37 ± 0.24$^{ab}$ | 3.45 ± 0.23$^{ab}$ | 3.60 ± 0.26$^{ab}$ | 13.13 ± 1.91$^{a}$ | 1.50 ± 0.87$^{b}$ | 14.63 ± 1.23$^{ab}$ |
| WW | 3.58 ± 0.51$^{ab}$ | 3.83 ± 0.60$^{a}$ | 4.03 ± 0.68$^{ab}$ | 5.75 ± 4.01$^{ab}$ | 2.50 ± 0.96$^{ab}$ | 8.25 ± 4.77$^{ab}$ |
| RB | 4.45 ± 0.67$^{a}$ | 4.17 ± 0.30$^{a}$ | 4.11 ± 0.34$^{ab}$ | 2.50 ± 1.50$^{b}$ | 1.92 ± 1.08$^{b}$ | 4.42 ± 2.29$^{b}$ |
| RW | 3.81 ± 0.61$^{ab}$ | 3.74 ± 0.46$^{a}$ | 4.48 ± 0.36$^{ab}$ | 4.63 ± 4.63$^{ab}$ | 3.00 ± 0.87$^{ab}$ | 7.63 ± 4.60$^{ab}$ |
| BW | 3.41 ± 0.38$^{ab}$ | 3.59 ± 0.41$^{ab}$ | 5.13 ± 1.32$^{a}$ | 5.75 ± 3.38$^{ab}$ | 1.13 ± 0.52$^{b}$ | 6.88 ± 3.08$^{ab}$ |
| BB | 3.01 ± 0.53$^{ab}$ | 3.24 ± 0.49$^{ab}$ | 3.99 ± 0.61$^{ab}$ | 14.58 ± 0.68$^{a}$ | 1.50 ± 0.50$^{b}$ | 16.08 ± 0.72$^{a}$ |
| RR | 2.58 ± 0.22$^{b}$ | 2.38 ± 0.29$^{b}$ | 2.55 ± 0.28$^{b}$ | 9.00 ± 3.08$^{ab}$ | 5.25 ± 1.45$^{a}$ | 14.25 ± 3.33$^{ab}$ |

**Note:** Results represent mean ± SE of four replications. Values denoted by different letters differ significantly at $P < 0.05$ level

The findings of this study indicated that the combined blue spectrum with the other lights accelerated growth of first regenerated shoot of *V. pompana*, whereas monochromatic red suppressed them. The combination between blue and red could promote *Vanilla* growth in this study that was similar with the report of Wang *et al*. (2016) who found that the mixture of blue and red LED increased photosynthesis performance as opposed to monochromic blue or red, which increases with the increasing of blue light fraction. Moreover, leaf photosynthesis pigments including chlorophyll a and chlorophyll b were accelerated under a mixture of blue and red light in *Doritaenopsis* (Shin *et al*., 2008) and *V. planifolia* (Ramírez-Mosqueda *et al*., 2017).

The callus formation was clearly observed with difference in each light condition. The percentage of callus formation in the first 30 days of culture period was highest under WW and RR, 46.3% and 38.8%, respectively. Conversely, callus formation was induced less than 10% under RB, while callus formation did not occur altogether under BB and BW. Callus formation in all light conditions gradually increased until 60 days and remained constant until 90 days (Figure 3A). The percentage of callus formation exhibited the highest under WW and RR at 90 days of culture, which account for 52.08% and 43.75%, respectively (Figure 3B). Our results are consistent with the report in *Anthurium*, which found that the highest callus formation was induced under monochromatic red spectrum (Budiarto, 2010). In addition, red LEDs were reported to enhance the highest protocorm-like body (PLBs) induction rate and PLBs proliferation on *Oncidium 'Gower Ramsey* from shoot tip which is in agreement with our observation (Mengxi *et al.,* 2011). In contrast to these results, it has been previously reported that blue LEDs promoted the highest number of PLBs of *Dendrobium kingianum* in 4 weeks of culture, whereas white LEDs reduced the number of PLBs (Habiba *et al.,* 2014). This finding suggested that combination between blue and red LEDs was appropriated for vegetative growth promoting of first regenerated shoot, while white LEDs and red LEDs were suitable for callus induction of *V. pompana*.

**Effect of Light Quality on Multiple Shoot Regeneration**

The multiple shoots of *V. pompana* regenerated from the lateral bud of the first regenerated shoot and mainly differentiated from callus (Figure 4, Table 1). The number of multiple shoots from the lateral bud at 90 days in the culture period showed the significantly greatest under RR exposure (5.25 ± 1.45 shoots) in comparison with the control (1.5 ± 0.87 shoots). Meanwhile, the culture under BB and FF stimulated callus differentiation with 14.58 ± 0.68 and 13.13 ± 1.91 multiple shoots, respectively that showed 5 times higher than RB (2.5 ± 1.50 shoots). Therefore, the total multiple shoots exhibited the highest under blue LEDs (16.08 ± 0.72 shoots) (Figure 4F), following by FF (14.63 ± 1.23) (Figure 4A) and red LEDs (14.25 ± 3.33 shoots) (Figure 4G) that 2 times higher than WW and the mixture between light spectra treatments. Similar with the previous report in various orchid that blue light induced shoot regeneration in *Dendrobium officinale* (Lin *et al*., 2011) and *Paphiopedilum delenatii* (Luan *et al*., 2015). Moreover, blue light promoted the proliferation of PLBs, while red LEDs increased the number of shoot and fresh weight of PLBs of *D. kingianum* (Habiba *et al.,* 2014). In contrast with the report in *V. planifolia* that found different light spectra no effects on number of shoots in proliferation phase (Ramírez-Mosqueda *et al.,* 2017)

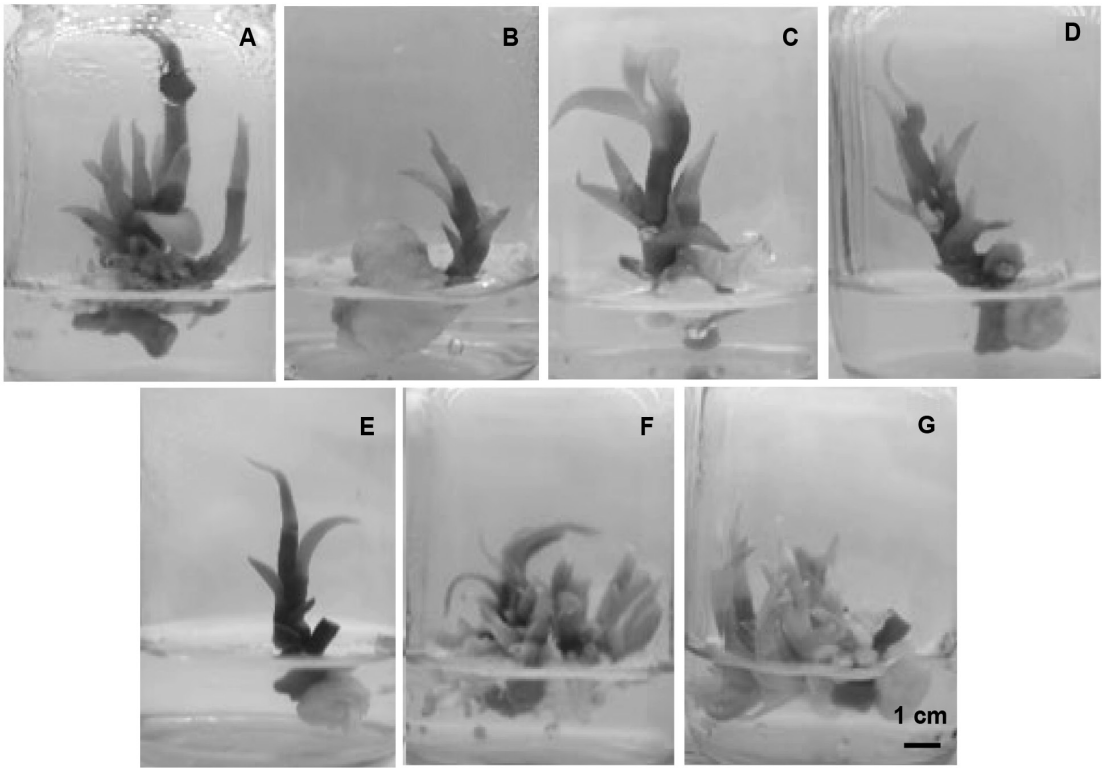


**Figure 4** Shoot growth and multiplication of *Vanilla pompana* after 90 day of culture under difference light quality: (A) Fluorescent (FF, control), (B) White (WW), (C) Red+Blue 1:1 (RB), (D) Red+White 1:1 (RW), (E) Blue+White 1:1 (BW), (F) Blue (BB) and (G) Red (RR) light-emitting diodes (LEDs). Bar represented 1 cm

Our investigation observed that the culture *of V. pompana* nodal segment in micropropagation required a different quality of light for each development stage. The combination between red and blue LEDs stimulated growth of the first regenerated shoot, but suppressed multiple shoot regeneration (Table 1, Figure 4C). Meanwhile, white LEDs promoted rapid callus formation and induced the highest percentage of callus induction, but callus differentiation had been less performed (Figure 3, Figure 4B). Thus, these results indicated that white LEDs was suitable for only the callus induction phase. Finally, blue LEDs was an appropriate light source for multiple shoot regeneration (Table 1, Figure 4F). These results demonstrated LEDs light effects on shoot regeneration and multiplication and could contribute to use LEDs as the alternative light source in different development stages of *V. pompana in vitro* culture that had lower the annual costs of electricity for lighting (Figure 5).

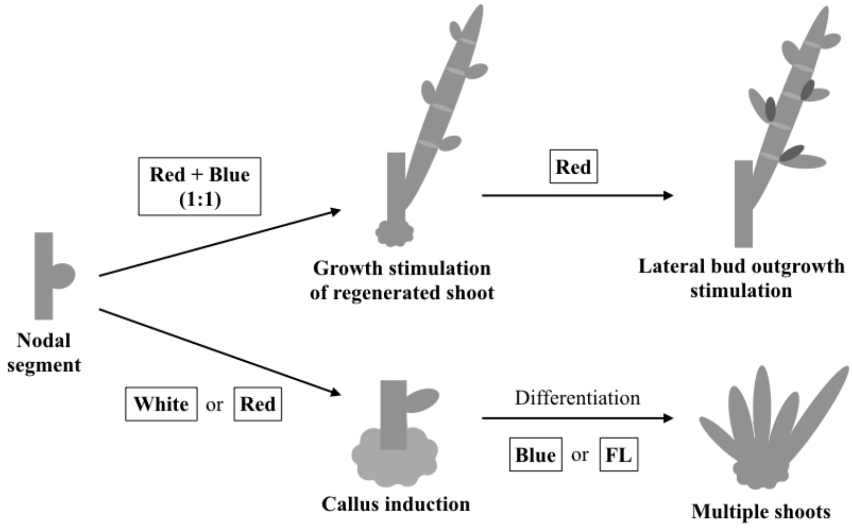


**Figure 5** The diagram of different light quality on shoot regeneration and shoot multiplication of *Vanilla pompana* nodal segment *in vitro* culture

## CONCLUSIONS

This study demonstrates that blue LED, red LED and fluorescence light were the most effective light source for promoting multiple shoot regeneration of *V. Pompano* Schied *in vitro* culture with the highest of number of total multiple shoots that contributed to 16.08 ± 0.72, 14.25 ± 3.33 and 14.63 ± 1.23 shoots/explant, respectively. Meanwhile, white and red LEDs were appropriate for rapid callus induction before induced callus differentiation by blue LEDs. In conclusion, the LED lighting seems to suitable for propagation efficiency and cost reduction in *V. pompana*.

## ACKNOWLEDGEMENT

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