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# *IN VITRO* GERMINATION AND ITS SUBSEQUENT GROWTH OF AN ORCHID OF *Vanda tricolor* Lindl. var. *suavis* FROM BALI ON COMPLEX ADDITIVES ENRICHED MEDIUM

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## ABSTRACTS

*Vanda tricolor* Lindl. var. suavis is an Indonesian wild orchid that has been rare in nature, so it needs attention to take care and conserve them. The objective of the research was to investigate the effect of coconut water and / or tomato juice on the growth of protocorm of *V. tricolor* Lindl. var *suavis* from Bali grown *in vitro*. The experiment was laid out in the factorial design, with two factors (coconut water/CW and tomato juice/TJ), each contained three concentration (CW: 0, 100, and 200 cc $L^{-1}$; TJ: 0, 100 and 200 g $L^{-1}$) resulted in nine combination of treatments and replicated four times. The results showed that tomato juice with concentration of 100 $gL^{-1}$ or 200 $gL^{-1}$ promotes growth of protocorms of *Vanda tricolor* Lindl. var. *suavis* from Bali regardless the presence of coconut water.

Keywords: coconut water, *in-vitro,* protocorms, tomato juice, *Vanda tricolor*

## INTRODUCTION

*Vanda tricolor* Lindl. var. *suavis* is an Indonesian wild orchid which well grown in some islands in Indonesia, i.e. Sulawesi, Java (including Mount of Merapi in Yogyakarta) and Bali. Gardiner (2007) reported that this species has been rare in nature due to over gathering and/or natural disaster such as volcano eruption. Therefore, conservation of this species is an urgent need.

*V. tricolor* Lindl. var. *suavis* has fragrant flower with white perianth, red spots and purple *labellum*. This orchid species is easily pollinated to gain a capsule which contained huge number of seeds. Like commonly orchid species, the seed must be sown *in vitro* under aseptic culture in the laboratory. It has been known that complex additives like coconut water, tomato juice, potato extract and banana pulp can be used as source of organic nutrients and can promote *in vitro* growth of several plant species including orchid. The use of complex additives for promoting growth of plant *in vitro* is more environmental friendly compared to the use of chemical substances like plant growth regulator. George *et al*. (2008) proposed that the amount of these substances required for successful culture varies with the species and genotype.

Dwiyani *et al*. (2012) found that seeds of *V. tricolor*Lindl. var. *suavis* from Bali were absolutely required tomato extract for their germination. The research also concluded that tomato extract was required as a trigger for seed germination. However, no information was available regarding organic compound added in media for subsequent growth of seeds of this species after germinating.

The current research provides information about the growth of protocorms (germinated seeds) of *V. tricolor* Lindl. var *suavis* from Bali in the New Phalaenopsis (NP) medium (Islam *et al*., 1998) added with coconut water and/or tomato juice. The objective of the research was to investigate the effect of coconut water and / or tomato juice on the growth of protocorm of *V. tricolor* Lindl. var *suavis* from Bali grown in vitro.

## MATERIALS AND METHODS

The experiment was carried out at The Plant Tissue Culture Laboratory, Faculty of Agriculture, Udayana University, Denpasar, Bali, Indonesia during the period of January to June 2013.

A capsule of *V. tricolor* Lindl. var *suavis* from Bali, Indonesia (in specific area in Bali

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named Bedugul) was used as planted material. A capsule was collected 7 months after artificial selfing. Organic substances sources were coconat water (CW) from mature yellow coconut (in Bali, named "Kelapa Gading") and tomato juice (TJ) from mature red tomato of "Intan" variety from Bali.

The experiment was laid out in the factorial design, with two factors (coconut water/CW and tomato juice/TJ), each contained three concentration (CW: 0, 100, and 200 cc $L^{-1}$; TJ: 0, 100 and 200 g $L^{-1}$) resulted in nine combination of treatments. These were replicated four times, therefore thirty six glass vials were used. Treatments were named according to the type of organic substance and their concentration, *i.e.* CW0 = 0 cc $L^{-1}$ of coconut water, CW100 = 100 cc $L^{-1}$ of coconut water, CW200 = 200 cc $L^{-1}$ of coconut water, TJ0 = 0 g $L^{-1}$ tomato juice, TJ100 = 100 g $L^{-1}$ tomato juice and TJ200 = 200 g $L^{-1}$ tomato juice.

Full strength of NP (*New Phalaenopsis*) medium was used as basic medium added with CW and/or TJ with concentration according to the treatment. Medium were solidified with 2 g $L^{-1}$ gellan gum and autoclaved with 1.16 kg $cm^{-2}$ of pressure at 121$^{o}$C for 30 minutes.

A capsule of *V. tricolor* Lindl. var *suavis* from Bali was fire sterilized by dipping it in the 90% ethanol and then exposed it in to the fire. This procedure was done three times before putting the capsule in the laminar air flow cabinet (LAFC). In the LAFC, the capsule was sliced and opened for gathering seeds. Seeds of 0.5 g then were sown on the each treatment media containing-vial. Observation was done every 3 weeks until 24 weeks after sowing for the parameters of the number of plantlets, the number of leaves, shoot length and root length. Data was analyzed using Analysis of Variance (Steel and Torrie, 1980). The treatment means was compared based on Least Significant Difference (LSD) at 1% level of probability.

## RESULTS AND DISCUSSION

The effect of coconut water (CW), tomato juice (TJ) and their interaction can be seen at Table 1. CW had significantly effects only on the parameter of the number of plantlet, while TJ had significantly effects on all parameter observed. The effect of interaction of CW and TJ was significant only on the number of plantlet, but was not on other parameters.

Table 2 shows parameter of the number of plantlet that affected by treatments. The number of plantlet increased with the presence of TJ regardless the CW treatment. However, it did not occur for CW, the presence of CW resulted in a decrease of plantlet number in both treatment of TJ100 and TJ200.

Data indicated that TJ was required for producing plantlet (seedlings in bottle) of *V. tricolor* Lindl. var. *suavis* from Bali. Without addition of TJ, only few number of plantlets was produced (Table 2), while without addition of CW but in the presence of TJ, plantlet was produced in huge number. Plantlet number decreased with increasing of CW concentration, and it indicated that CW was less required for producing plantlets of *V. tricolor* Lindl. var. *suavis* from Bali.

Table 1. The Effect of coconut water (CW), tomato juice (TJ) and their interaction (CW x TJ) on the parameter observed at 24 weeks after seed sowing

| Parameter | CW Effect | TJ Effect | Interaction (CW x TJ) Effect |
|---|---|---|---|
| The number of plantlet | * | * | * |
| The number of leaves/plantlet | ns | * | ns |
| Root length | ns | * | ns |
| Shoot length | ns | * | ns |

Remarks: ns = non-significantl, * = significantly difference at 1% level of probability according to Analysis of Variance (Steel and Torrie, 1980).

Table 2. The effect of coconut water (CW) and tomato juice (TJ) in several concentrations on the number of plantlet at 24 weeks after seed sowing

| Treatments | CW0 | CW100 | CW200 | Mean of TJ treatment | Notation of mean differences between TJ treatment |
|---|---|---|---|---|---|
| **TJ0** | 0.00 C(a) | 0.33 C (a) | 4.33 B(a) | 1.56 | c |
| **TJ100** | 187.33 A (a) | 77.00 A (b) | 31.00 A(c) | 98.44 | a |
| **TJ200** | 19.33 B (b) | 50.67 B (a) | 25.67 A (b) | 31.89 | b |
| **Mean of CW treatment** | 68.89 | 42.67 | 20.33 | | - |
| **Notation of mean differences between CW treatment** | a | ab | b | - | - |

Remarks: The same capital letter below the value indicates no significant differences among values in the same column based on The Least Significant Difference (LSD) at 1% level of probability, while those of small letters within brackets indicates no significant differences among values for the same raw and vice versa for different letters. The same letter in the notation of mean differences indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 1% level of probability.

Table 3. The effect of coconut water (CW) and tomato juice (TJ) in several concentration on the leaf number at 24 weeks after seed sowing

| Treatments | CW0 | CW100 | CW200 | Mean of TJ treatment |
|---|---|---|---|---|
| **TJ0** | 0.01 | 0.00 | 0.02 | 0.01 b |
| **TJ100** | 19.00 | 13.00 | 11.33 | 14.44 a |
| **TJ200** | 13.00 | 11.00 | 12.33 | 12.11 a |
| **Mean of CW treatment** | 10.67 a | 8.00 a | 7.89 a | |

Remarks: The same letter behind values of mean of treatment indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 1% level of probability and vice versa for different letter.

Table 4. The effect of coconut water (CW) and tomato juice (TJ) in several concentration on the length of shoot (cm) at 24 weeks after seed sowing

| Treatments | CW0 | CW100 | CW200 | Mean of TJ treatment |
|---|---|---|---|---|
| **TJ0** | 0.00 | 1.53 | 3.03 | 1.52 b |
| **TJ100** | 10.07 | 7.77 | 7.87 | 8.57 a |
| **TJ200** | 9.87 | 4.43 | 7.90 | 7.40 a |
| **Mean of CW treatment** | 6.64 a | 4.58 a | 6.27 a | |

Remarks: The same letter behind values of mean of treatment indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 1% level of probability and vice versa for different letter

Table 5. The effect of coconut water (CW) and tomato juice (TJ) in several concentrations on the length of root t 24 weeks after seed sowing

| Treatments | CW0 | CW100 | CW200 | Mean of TJ treatment |
|---|---|---|---|---|
| **TJ0** | 0.00 | 0.00 | 0.00 | 0.00 b |
| **TJ100** | 7.33 | 3.20 | 5.70 | 5.41 a |
| **TJ200** | 5.40 | 3.90 | 6.33 | 5.21 a |
| **Mean of CW treatment** | 4.24 a | 2.37 a | 4.01 a | |

Remarks: The same letter behind values of mean of treatment indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 1% level of probability and vice versa for different letter.

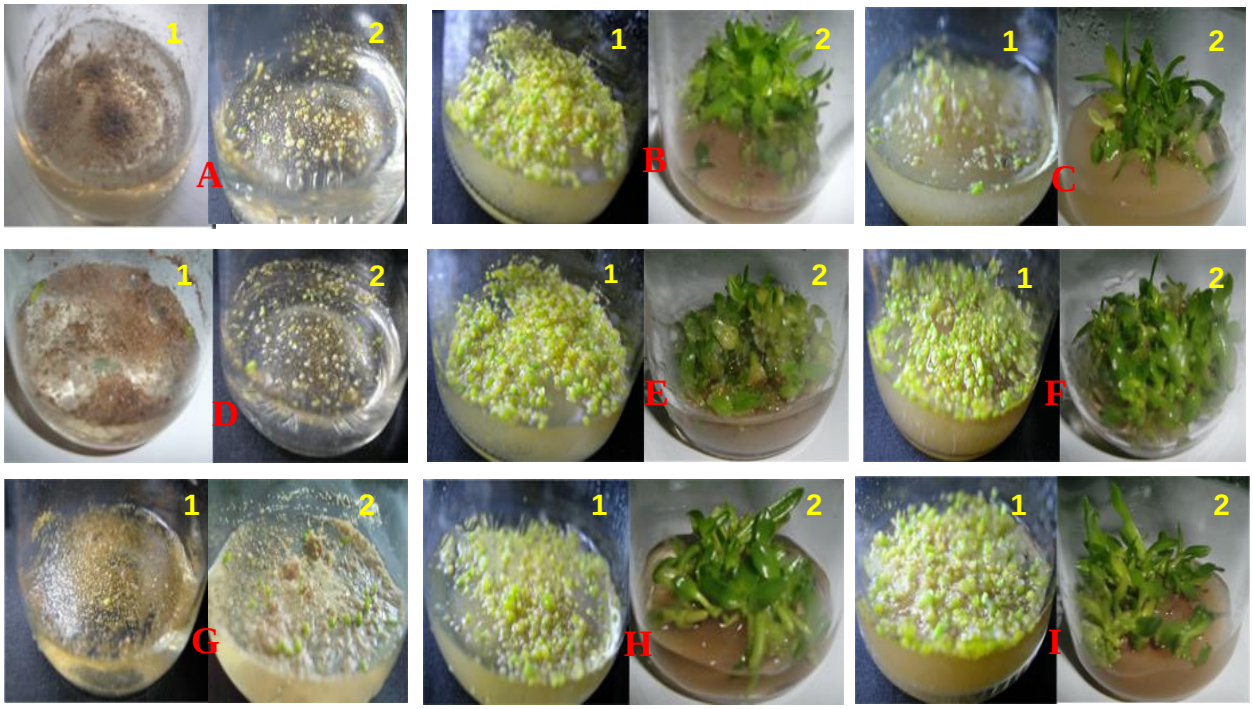


Figure 1. Growth of protocorms of *V. tricolor* orchid on the *New Phalaenopsis* medium added with complex additives (tomato juice/TJ and coconut water/CW) (A=TJ0+CW0 ; B=TJ100+CW0; C=TJ200+CW0; D=TJ0+CW100; E=TJ100+CW100; F=TJ200+CW100; G=TJ0+CW200; H=TJ100+CW200; I = TJ200+CW200; TJ=Tomato Juice; CW = Coconut water; The number behind TJ and CW indicates concentration (see Material and Methods); 1=8 weeks after seed sowing; 2=24 weeks after seed sowing ; Bottom diameter of culture bottle = 4.5 cm)

Table 3, 4 and 5 shows the effect of CW and TJ on the number of leaves, shoot length and root length, respectively. Treatment of CW did not statistically affect the number of leaves, shoot length and root length, while TJ treatment affected those of parameters. Treatment of TJ100 resulted in highest value of leaf number, shoot length and root length even there was no significant different with TJ200.

Data at Table 3, 4 and 5 indicated that TJ promotes growth of protocorms and subsequent regeneration of *V. tricolor*, from Bali, while CW did not affect the growth, even inhibited them. This is in agreement with Arditti (1992) who proposed that for in vitro growth of PLBs and seedling of an orchid, some complex additives were satisfactory, while some were unsatisfactory and even inhibitory.

Goncalves *et al*. (2012) found that the highest number of seedlings of *Laelia purpurata* orchid occurred in Knudson C (KC) medium containing 90 g $L^{-1}$ banana pulp, while the seedling number of *Encyclia* orchid was high in KC medium containing coconut water. Other research by Nambiar *et al*. (2012) investigated that the best organic additive for proliferation of PLBs of *Dendrobium* Alya Pink was coconut water compared to banana and tomato homogenate. Aktar *et al*. (2008) also found that the highest values of all parameters in *Dendrobium* orchid were gained with interaction of ½ MS medium with Sabri banana pulp. The current research found that the best growth of protocorms and seedlings of *V. tricolor* was gained with addition of tomato juice. All data above indicated that the need of types of organic additives for growth of orchid seedlings was species-specific.

Figure 1 shows the effect of CW and TJ on the growth of protocorms and their subsequent growth. The important role of TJ in promoting growth of orchid seedling is clear enough. No growth of seeds shown without the presence of TJ, while the growth was still occurred when CW was absence. In the concentration of 100 or 200 g $L^{-1}$ of TJ with or without CW, growth was better.

Gnasekaran *et al*. (2012) reported that protocorm like bodies (PLBs) of Vanda Kasem's Delight orchid grown on Vacine and Went (VW) medium added with tomato extract was remained green, viable and appeared to be very fresh. Proliferation rate of the PLBs was also higher compared to those of plbs grown on VW added with papaya extract. Those results was in agreement with the results of the current research which found that tomato juice promoted the growth of protocorms of *Vanda tricolor* orchid. Gnasekaran *et al*. (2012) concluded that the presence of easily absorbed sugar like glucose and fructose and the presence of strong antioxidant in tomato fruit played an integral role in the proliferation and the production of healthy PLBs of V. Kasem's Delight orchid. However, we believe that the effects of tomato in promoting growth of protocorms of *V. tricolor* orchid in the current research and also in increasing proliferation rate of V. Kasem's Delight PLBs in those of Gnasekaran research is more affected by antioxidant properties of tomato. We proposed that sugar content in tomato fruit has little role in affecting growth of protocorm or PLBs of Vanda genus. As it has been shown in Table 6, CW and TJ used in the current research contains total sugar in the relatively equal amounts, but TJ contains vitamin C and total carotene in huge number that do not presence in CW, indicating that antioxidant properties of tomato such as vitamin C and carotene plays an important role in promoting growth of protocorm of *V. tricolor* orchid.

Dwiyani (2012) found that protocorms of *V. tricolor* contains phenol compound in a greater number compared to protocorm of other orchid (*Phalaenopsis amabilis*). Arditti (1992) also proposed that during their growth, basal part of orchid seeds produced phenolic compound. Titov *et al.* (2006) proposed that oxidation of phenolic compound produced quinone that was very reactive and inhibitive for plant growth. Sukendah *et al.* (2008) proposed that the phenolic compound blocked enzyme activities and promoting death of the culture. Due to subculture was not done in this current experiment, we can conclude that the presence of antioxidant properties had important role in promoting the growth of protocorms and their subsequent growth of *V. tricolor* orchid. Tomato also contains lycopene (Gnasekaran *et al*., 2012). Lycopene is a strong antioxidant which counteracts free radical formation, hence assisting in the repair of wounded cells and helping to inhibit DNA oxidation as well (Halliwell *et al*., 1995). In this study, lycopene as well as vitamin C of tomato inhibit oxidation of phenolic compound that was secreted during growth of orchid protocorms and prevent browning in the medium, thus promoting growth of protocorms. We believe that for protocorms and protocorm like bodies of Vanda genus of orchid such as *Vanda tricolor* and *Vanda* Kasem's Delight absolutely require antioxidant for their growth. Those antioxidants can be fulfilled by adding tomato juice or tomato extracts in the medium. Arditti and Ernst (1993) also proposed that tomato fruit should be added as additive complex in to medium culture and can promote germination of orchid seeds and their subsequent growth.

Table 6. Component of Coconut water and tomato juice used in the experiment (in 100 g offresh weight)*)

| No. | Component | Coconut Water | Tomato Juice |
|---|---|---|---|
| 1. | Water (%) | 94.95 | 95.35 |
| 2. | Ash (%) | 0.55 | 0.31 |
| 3. | Lipid (%) | 0.1 | 0.5 |
| 4. | Protein (%) | 0.2 | 1.8 |
| 5. | Crude fiber (%) | - | 1.0 |
| 6. | Soluble protein (%) | 0.2 | 1.5 |
| 7. | Reduction sugar (%) | 3.0 | 3.4 ± 0.1 |
| 8. | Total sugar (%) | 3.2 ± 0.1 | 3.7 ±0.1 |
| 9. | Vitamin C (mg) | - | 42.5 ± 0.6 |
| 10. | Antioxidant (DPPH) | - | 23.8 ± 1.8 |
| 11. | Total carotene (mg) | - | 1837.2 ± 44.6 |
| 12. | P2O5 (mg) | 13.3 ± 0.9 | 132.0 ± 7.7 |
| 13. | Mg (ppm) | 58.3 ± 0.5 | 80.6 ± 0.4 |
| 14. | Mn (ppm) | 2.1 ± 0.1 | 0.3 ± 0.1 |
| 15. | Na (ppm) | 458.1 ± 3.3 | 90.2 ± 0.6 |
| 16. | K (ppm) | 2267.7 ± 4.4 | 1570.2 ± 73.1 |
| 17. | pH | 5.2 | 4.3 |

Remarks: *)Analyses was done by the laboratory of Food and Product Technology of The Faculty of Agricultural Technology-Gadjah Mada University (Indonesia)

Beside of antioxidant properties, tomato fruits contain phosporus ($P_2O_5$) in a greater number in TJ compared to CW (Table 6). George *et al.* (2008) proposed that phosphorus is a vital element in plant biochemistry. It occurs in numerous macromolecules such as nucleic acids, phospholipids and co-enzymes. Besides, plant tissue culture secrete phosphatase enzyme into the medium (Ciarrocchi *et al*., 1981) which would release phosphate ions from organic phosphates (George *et al*., 2008). In the current research, phosphorus found in a greater number in TJ might contribute in stimulating the growth of protocorms.

## CONCLUSION AND SUGGESTIONS

Coconut water had significantly effects only on the parameter of the number of plantlet, while tomato juice had significantly effects on all parameter observed. The effect of interaction of coconut water and tomato juice was significant only on the number of plantlet, but was not on other parameters. The best complex additives to promote growth of protocorms and producing plantlets of *Vanda tricolor* Lindl. var. *suavis* from Bali is tomato juice in concentration of 100 g $L^{-1}$ or 200 g $L^{-1}$.

We suggest that producing plantlets of orchid of *V. tricolor* Lindl. var. *suavis* from Bali, seeds should be sown and kept growing their subsequent growth *in vitro* in medium enriched with tomato juice regardless the presence of coconut water.

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