Micropropagation of *Vanda tricolor* Lindl. var. *suavis* with various concentrations of organic growth supplements

M Tuwo, A I Latunra and E T Ana

Department of Biology, Faculty of Mathematics and Natural Science, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia

Email: mustikatuwo@gmail.com

Abstract. Plant propagation through in vitro culture is increasingly being used to produce hybrid orchids. Plant tissue culture provides a good alternative to produce plants in large numbers in a short time. The provision of organic growth supplements in tissue culture media plays an important role as a substitute for growth regulators in stimulating the growth of explants. In this study, young coconut water, banana extract (cv. ambon), and tomato extract were used to stimulate the growth of the *Vanda tricolor* Lindl. Var *suavis* protocorm. Data were analyzed using a completely randomized design (CRD) with five replications using the Kruskal-Wallis test and continued with the Mann-Whitney test if there was a significant difference between each treatment and its concentration. Parameters observed were the percentage of the number of shoots and the number of leaves. The results showed that there was a significant difference between the treatments given to the number of leaves. Mann-Whitney further test results on the number of leaves showed a significant difference in the banana extract treatment and the control treatment.

1. Introduction

Indonesia is known as a mega-biodiversity country and is one of the centers of biodiversity in the world, one of which is orchids [1]. Orchids belong to the family Orchidaceae which consists of 800 genera. There are about 26,000 species of orchids in the world. Around 5,000 to 6,000 species are found in Indonesia, and most of them are endemic orchids. Orchid species *Vanda tricolor* Lindl. var. *suavis* is most in demand because of the beauty of its flowers [2]; besides that, it has very fragrant flower buds with three distinctive colors [3]. Orchid plants are still the prima donna of consumers, so efforts are needed to increase the number of seedlings. Plant tissue culture technology is able to produce a large number of seeds, the same properties as the parent in a relatively short time [4]. Tissue culture has been widely applied to orchids, especially in the genus *Vanda* [5–13].

The composition of the media is one of the critical success factors in plant tissue culture. The growth medium can be modified by adding organic growth supplements to increase shoot growth in vitro. The addition of organic growth supplements helps the process of plant growth and development. The use of organic growth supplements origin from plant extracts has been widely carried out by researchers [14–20]. This research was conducted to obtain an organic growth supplement that can increase the protocorm growth of the *Vanda tricolor* Lindl. var. *suavis* in vitro.
2. Materials and methods

The plant material used in this study was the protocorm of *Vanda tricolor* Lindl. var. *suavis* aged three months obtained from the Tissue Culture Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin. Protocorm was grown on MS media and modified media with the addition of organic growth supplements, i.e., of young coconut water, banana extract (cv. ambon), and tomato extract (Table 1).

All cultures were incubated in a culture room at a temperature of 26 ± 2°C with a relative humidity of 55 ± 5% and were exposed to a 16 h photoperiod. The number of shoots and leaves on each explant was recorded. Observation of protocorm is carried out every week for two months. Data analysis was carried out using a non-parametric test through the Kruskal-Wallis test to see a significant difference between the treatments given. If there is a significant difference, then proceed with the Mann-Whitney test to find out which type of treatment is significantly different.

### Table 1. Organic growth supplements treatment.

| Organic growth supplements | Concentration |
|---------------------------|---------------|
|                           | 0             | 1             | 2             | 3             | 4             | 5             |
| Coconut water             | MS            | MS            | MS            | MS            | MS            | MS            |
|                           | (MS+50 ml/L)  | (MS+100 ml/L) | (MS+150 ml/L) | (MS+200 ml/L) | (MS+250 ml/L) |               |
| Banana extract (cv. Ambon)| MS            | MS            | MS            | MS            | MS            | MS            |
|                           | (MS+50 g/L)   | (MS+100 g/L)  | (MS+150 g/L)  | (MS+200 g/L)  | (MS+250 g/L)  |               |
| Tomato extract            | MS            | MS            | MS            | MS            | MS            | MS            |
|                           | (MS+50 g/L)   | (MS+100 g/L)  | (MS+150 g/L)  | (MS+200 g/L)  | (MS+250 g/L)  |               |

3. Results and discussion

The orchid protocorm used in this study was a 3-month-old protocorm origin from *Vanda tricolor* Lindl. var. *suavis*. The organic growth supplements are given young coconut water with a concentration of 50 ml/L, 100 ml/L, 150 ml/L, 200 ml/L, and 250 ml/L, banana extract (cv. ambon), and tomato extract with a concentration of 50 g/L, 100 g/L, 150 g/L, 200 g/L, and 250 g/L. The protocorm was then observed for eight weeks after planting (WAP) (Table 1).

Figure 1 shows the protocorm growth of orchids grown in the MS0 treatment (control) and the addition of banana extract. *Shoot Apical Meristem* (SAM) was formed faster in the banana extract treatment at 3 WAP, compared to the control treatment, young coconut water and tomato extract formed at 4 WAP. SAM and absorbing hair are parameters to determine the protocorm growth rate. *Shoot Apical Meristem* (SAM) is the initial structure to form leaves, while absorbing hair is a hair-like structure that absorbs nutrients from its environment [21]. The protocorm will then differentiate into an organ to form leaf and root initiation, which will later form a perfect new individual [22]. Protocorm explants are also widely used as propagation material [23]. According to [19], banana extract (cv. ambon) contains growth regulators auxin and gibberellin, which can stimulate cell enlargement and elongation. In addition, banana extract (cv. ambon) also contains more phosphorus (P) than young coconut water, which is 27.00 mg which plays a role in root development. The banana extract also contains vitamin B complex, namely Pyridoxine (B6) 0.51 mg, Riboflavin (B2) 0.07 mg, and Thiamine (B1) 0.04 mg, which play a role in triggering the emergence of roots faster than other treatments [24].
Figure 1. The growth of *Vanda tricolor* Lindl. var. *suavis* protocorm on MS0 treatment (control) and organic growth supplements of banana extract (cv. ambon). (Bar 0.1cm). SAM = shoot apical meristem; flp = first leaf promordia; ah = absorbing hair; slp = second leaf promordia; WAP = week after planting.

The first leaf primordia formed faster in the young coconut water and tomato extract treatment which was 6 WAP, while the banana extract treatment was 8 WAP. Tomato extract contains growth regulators gibberellin, auxin, and cytokinin, which play a role in stimulating growth [25]. Tomato extract contains low cytokinin, namely in 1000 g of tomato extract contained 0.15 g of BAP. Cytokinins will decrease as the tomatoes ripen [16]. The second leaf primordia formed faster in the young coconut water treatment at 8 WAP, while for tomato and banana extract treatments at 11 and 12 WAP, respectively. Absorbing hair was also formed faster in coconut water treatment and banana extract, namely 7 MST, while the tomato extract was 8 MST. Young coconut water contains growth regulators of the cytokinin groups such as kinetin (273.62 mg/L) and zeatin (290.47 mg/L), auxin, and gibberellin [26]. Cytokinins will stimulate cells to divide rapidly, while auxin is active in stimulating cell elongation in the tip meristem, and gibberellin increases height gain [26]. In addition, young coconut water contains nitrogen (N) 43.00 mg/L, which stimulates the number of leaves and tillers [19]. Nitrogen in sufficient quantities is able to accelerate plant growth, especially in leaves [27]. Giving young coconut water alone on growth media is also able to increase the number of leaves up to 4-5 leaves [14] [28]. Calcium (Ca) 24.67 mg/L plays a role in the growth of root hairs and root elongation. Phosphorus (P) (13.17 mg/L) functions in stimulating root growth and fertilization [29].

Data on the number of shoots and number of leaves obtained were analyzed using a non-parametric test through the Kruskal-Wallis test to see a significant difference between the treatment given to the number of shoots and the number of leaves. If P<0.05, it indicates that there is a significant difference between the treatments, whereas if P>0.05, it shows that there is no significant difference.

| Table 2. Kruskal-Wallis test results based on treatment |
|-------------------------------------------------------|
| Number of shoots | Number of leaves |
| Chi-Square       | 5.556            | 8.589          |
| Df               | 3                | 3              |
| Asymp.Sig.       | 0.135            | 0.035          |
Based on the results of the Kruskal-Wallis test (table 2), there was no significant difference between the treatments given to the number of shots indicated by a significant value of 0.135 (P>0.05), while for the number of leaves with a significant value of 0.035 (P <0.05) which means that there is a significant difference between the treatments so that the number of leaves can be further tested through the Mann-Whitney test to see which types of treatments are significantly different.

Table 3. Mann-Whitney test results on the number of leaves

| Treatments                                      | Sig.  |
|------------------------------------------------|-------|
| Banana extract (cv. ambon) : tomato extract    | 1.000 |
| Banana extract (cv. ambon) : young coconut water| 0.181 |
| Banana extract (cv. ambon) : control (MS0)     | 0.048 |
| Tomato extract : young coconut water            | 1.000 |
| Tomato extract : control (MS0)                  | 0.550 |
| Young coconut water : control (MS0)             | 1.000 |

Based on the results of the Mann-Whitney test (table 3), it was shown that between the banana extract (cv. ambon) and control treatments, there was a significant difference in the mean of the number of leaves indicated by the P-value <0.05, namely 0.048.

Table 4. Kruskal-Wallis test results based on the concentration

|                  | Number of shoots | Number of leaves |
|------------------|------------------|------------------|
| Chi-Square       | 3.328            | 0.272            |
| Df               | 4                | 4                |
| Asymp.Sig.       | 0.505            | 0.992            |

The effect of adding natural supplements based on concentration showed that there was no significant difference between the concentrations given with a P value> 0.05, namely 0.505 for the number of shoots and 0.992 for the number of leaves, so it cannot be further tested through the Mann-Whitney test to find out which concentrations are significantly different (Table 4).

4. Conclusion
There was a significant difference between the treatments given to the number of leaves. Mann-Whitney further test results showed that there was a significant difference between the banana extract (cv. ambon) and control treatment.

References
[1] Anggraini W 2018 Keaneekaragaman hayati dalam menunjang perekonomian masyarakat Kabupaten Oku Timur. *J. Aktual STIE Trisna Negara* 16 99–106
[2] Rupawan I M, Basri Z and Bustami M 2014 Pertumbuhan anggrek Vanda Vanda sp. pada berbagai komposisi media secara In vitro *J. Agrotekbis* 2 488–94
[3] Dwiyani R 2014 *Anggrek Vanda tricolor Lindl. var. suavis* (Denpasar: Udayana University)
[4] Hartati S, Yunus A, Cahyono O and Setyawan B A 2019 Penerapan teknik pemupukan pada aklimatisasi anggrek hasil persilangan Vanda di Kecamatan Matesih Kabupaten Karanganyar *J. community Empower. Serv.* 3 63–70
[5] Decruse S W, Gangaprasad A, Seeni S and Menon V S 2003 Micropropagation and ecorestoration of Vanda spathulata, an exquisite orchid *Plant Cell. Tissue Organ Cult.* 72 199–202
[6] Rahman M S, Hasan M F, Das R, Hossain M S and Rahman M 2009 In vitro micropropagation of orchid Vanda tessellate L. from shoot tip explant *J.bio-sci* 17 139–44
[7] Tuwo M and Indrianto A 2016 Improvement of orchid vanda hybrid (Vanda limbata Blume X
Vanda tricolor Lindl. var. suavis) by colchicines treatment in vitro Mod. Appl. Sci. 10 83–9

[8] Mondal T and Banerjee N 2017 Micropagation and in vitro conservation of threatened orchids: a brief review CIB tech J. Biotechnol. 6 1–12

[9] Baby R and Valsala P A 2019 Micropropagation protocol for Vanda hybrid ‘Sansai Blue’ Int. J. Chem. Stud. 7 2957–63

[10] Baby R, Valsala P A and Doddamani M B 2019 In vitro micropropagation protocol for Vanda hybrid ‘Dr. Anek’ Int. J. Curr. Microbiol. Appl. Sci. 8 2073–84

[11] Maharjan S, Pradhan S, Thapa B B and Pant B 2019 In vitro propagation of endangered orchid, Vanda pumila Hook.f. through protocorms culture Am. J. Plant Sci. 10 12001223

[12] Maharjan S, Thakuri L S, Thapa B B, Pant K K, Joshi G P and Pant B 2020 In vitro propagation of endangered orchid Dendrobium chryseum Rolfe from protocorm culture Nepal J. Sci. Technol. 19 39–47

[13] Setiaji A, Annisa R R R, Santoso A G, Kinasih A and Riyadi A D 2021 Factors affecting mass propagation of Vanda orchid Vitr. cell Biol. Dev. 5 51–62

[14] Djajanegara I 2010 Pemanfaatan limbah buah pisang dan air kelapa sebagai bahan media kultur jaringan anggrek bulan Phalaenopsis amabilis tipe 229 J. Tek. Lingkung. 11 373–80

[15] Tuhuteru S, Hehanussa M L and Raharjo S H T 2012 Pertumbuhan dan perkembangan anggrek Dendrobium anosmum pada media kultur in vitro dengan beberapa konsentrasi air kelapa J. Agrol. 1 1–12

[16] Dwiyan R, Purwantoro A, Indrianto A and Semiarti E 2012 Konservasi anggrek alam Indonesia Vanda tricolor Lindl. varietas suavis melalui kultur embrio secara in vitro J. ilmu-ilmu hayati dan Fis. 14 215–2020

[17] Kaur S and Bhutani K K 2012 Organic growth supplement stimulants for in vitro multiplication of Cymbidium pendulum (Roxb.) Sw. Hort. sci. 39 34–52

[18] Serliana, Mukarlina and Elvi R P W 2017 Pertumbuhan anggrek hitam Coelogyne pandurata Lindl. secara in vitro dengan penambahan ekstrak tomat Solanum lycopersicum L. dan benzyl amino purin (BAP) J. protobiont. 6 310–25

[19] Nurfadilah, Mukarlina and Elvi R P W 2018 Multiplikasi anggrek hitam Coelogyne Pandurata Lindl. pada media murashige and skoog (MS) dengan penambahan ekstrak pisang ambon dan benzyl amino purine (BAP) J. protobiont. 7 47–53

[20] Tuwo M, Baharuddin, Latunra A I, Masniawati A and Kuswinanti T 2021 Effect of organic growth supplements on in vitro shoot regeneration of banana cv. Barangan Musa acuminate Colla J. Metamorf. J. Biol. Sci. 8 124–30

[21] Puspasari R Z, Rosyidi I N, Ningrum E F C and Semiarti E 2018 Pengaruh pepton terhadap pertumbuhan embrio anggrek Vanda tricolor Lindley var. suavis asal merapi secara in vitro J. Biol. 5 47–50

[22] Melisa A O 2018 Pemberian kombinasi 2,4-D dan kinetin terhadap induksi protokorm like bodies (PLB) anggrek Grammatophyllum scriptum secara in vitro J. Biol. Educ. 1 34–46

[23] Cardoso J C, Zanello C A and Chen J T 2020 An overview of orchid protocorm-like bodies: mass propagation, biotechnology, molecular aspects, and breeding Breed. Interbational J. Mol. Sci. 21 1–32

[24] Delviandra D, Nopsagiarti T and Haitami A 2021 Uji berbagai ekstrak pisang sebagai suplemen terhadap pertumbuhan eksplan tanaman pisang roti pada media MS J. green swarnadwipa 10 109–17

[25] Heriansyah P and Indrawanis E 2020 Uji Tingkat Kontaminasi Eksplan Anggrek Bromheadia finlysoniana L. miq Dalam Kultur In-Vitro Dengan Penambahan Ekstrak Tomat J. Agroqua Media Inf. Agron. dan Budid. Perair. 18 223–32

[26] Gusmiaty, Restu M, Larekeng S H and Setiawan E 2020 The optimization of in vitro micropropagation of betung bamboo (Dendrocalamus asper backer) by medium concentrations and plant growth regulators IOP Conf. Ser. Earth Environ. Sci. 575

[27] Batti J R, Larekeng S H, Arsyad M A, Gusmiaty and Restu M 2020 In vitro growth response
on three provenances of Jabon Merah based on auxin and cytokinin combinations *IOP Conf. Ser. Earth Environ. Sci.* **486** 1–16

[28] Monja-mio K M and Robert M L 2013 Direct somatic embryogenesis of Agave fourcroydes Lem. through thin cell layer culture 541–9

[29] Malhotra H, Vandana, Sharma S and Pandey R 2018 Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess *Plant Nutrients and Abiotic Stress Tolerance* pp 171–90