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# Growth and development of vanda (Vanda sanderiana) explants in vitro on the effect of extracts of organic matter

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Abstract: This study aims to determine the appropriate extract of organic matter to be substituted on MS media, to obtain Vanda orchid Vanda sanderiana plantlets ready for acclimatization. The study consisted of 2 experiments, the first experiment, factorial Completely Randomized Design (CRD), the first factor was tomato extract, consisting of 3 levels (tomato extract 50 ml/l; tomato extract 100 ml/l, and tomato extract 150 ml/l) and the second factor consists of 3 levels of bean sprouts extract (100 ml/l bean sprouts extract, 150 ml/l bean sprouts extract, 200 ml/l bean sprouts extract). The second experiment compared the best or most efficient composition of the first experiment with BAP media (3 ppm), using the T-test. In the first experiment the growth variables (plant height and number of leaves) of all treatments were not significantly different, but the highest number of shoots was obtained at 50 ml/l tomato extract and 100 ml/l bean sprouts extract (6 shoots), the other 1-2 shoots. This treatment in the second experiment was proven to show a better plant height, leaf number and number of shoots than plant growth in BAP media (3 ppm).

### 1. Introduction

Orchid plants (*Orchidaceae*) have high economic value. Indonesia has 5000 species, one of which is the Vanda orchid [1]. The market demand for orchids is increasing, but the development of orchid production in Indonesia tends to be slow [2]. Based on data from BPS (the Central Bureau of Statistics), orchid production in Indonesia has fluctuated, in 2013 orchid production was 20,777,071 stalks, 2014 (19,739,627 stalks), 2015 (21,514,789 stalks), 2016 (19,978,078 stalks), and in 2017 as many as 20,045,577 stalks [3]. The solution to increasing Vanda orchid production can be done by propagation using tissue culture techniques.

Success in tissue culture depends on the response of plants to several factors, including culture media, aseptic and accentic environmental conditions. In culture media, the most important role is growth regulators, capable of stimulating, inhibiting and changing plant physiological processes. So far, in business calculations, the main obstacle is the price of growth regulators is too expensive, so it is necessary to find a replacement solution for organic compounds.

Tomato fruit (Solanum lycopersicum) is predicted to contain auxin hormone which functions to accelerate shoot growth in the process of cell division. According to [4] the auxin content in tomato extract can stimulate organogenesis, somatic embryogenesis and shoot growth in micropropagation in various plant species, but it needs to be balanced with the provision of synthetic cytokinins in the form of BAP. Auxin functions to influence cell elongation, differentiated tissue and initiation of root

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formation. According to [5], a concentration of 150g/l bean sprouts extract gave the best results on the growth of orchid cultures. Based on this background, the main objective of the experiment is to solve the difficulty solution of orchid plant developers, especially Vanda sp, in terms of utilizing the abundant and cheap organic material found around us as a substitute for synthetic growth regulators, which are difficult to find in the market apart from being expensive.

### 2. Material and methods

The experiment was carried out at the Jember State Polytechnic tissue culture laboratory. The planting material is the explant produced by the Vanda Sanderiana (hybrid) orchid propagation in the previous experiment. Materials for planting media MS (Murashige and Skoog), BAP, bean sprouts extract and tomato extract, sugar, white swallow agar.

The study was conducted in 2 experiments. The first experiment used a factorial Completely Randomized Design (CRD) consisting of 2 factors. The first factor is tomato extract, consisting of 3 levels (A1 = tomato extract 50 ml/l, A2 = tomato extract 100 ml/l, A3 = tomato extract 150 ml/l). The second factor is bean sprouts extract, consisting of 3 levels (T1 = 100 ml/l bean sprouts extract, T2 = 150 ml/l bean sprouts extract, T3 = 200 ml/l bean sprouts extract). Data were analysed using the F test at 5% with the BNT test. The second experiment compared the best or efficient composition of growth and development of vanda orchids with 3 ppm BAP with T-test. The study was started by preparing all the explants from the previous study, then before entering the treatment media all the explants were subcultured into MS0 to normalize the conditions for 3-4 weeks while preparing the treatment media. Furthermore, after the experiment was running, growth observations were carried out on growth variables consisting of shoot height, number of leaves and number of shoots that appeared during the experiment period.

### 3. Result and discussion

### 3.1 Plant height

The results of the analysis of 9 media compositions from two factors of organic matter treatment (tomatoes and bean sprouts) provided statistically insignificant information on the variable measuring shoot height until 69 days after planting (DAP). This means that the composition of MS media with 50 ml/l tomato extract substitution + 100 ml/l bean sprouts extract becomes the composition of choice for maintenance media prior to acclimatization. This is in line with the statements of [6] on the results of in vitro growth of Cattleya orchids which were carried out up to 11 weeks after planting by giving tomatoes at 100, 150 and 200 g/l treatments and [7] to Black orchid (*Coelogyne pandurata* Lindl.) (Figure 1). 3,2 Number of leaves

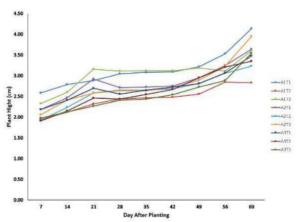
The response of the explants to the number of leaves was in line with the growth of plant height, the composition being compared showed no significant difference, means for the variable number of leaves (69 DAP), the recommended composition is also on the economic composition, namely the composition of MS media with 50 ml/l tomato extract substitution + 100 ml/l bean sprouts extract (Figure 2).

3.3 Number of shoots.

The response of explants to the number of shoots was also in line with the growth of plant vigour (plant height and number of leaves), the composition being compared showed no significant difference. If you look at Figure 3.3, the number of shoots in the composition of the A1T1 (composition 50 ml/1 tomato extract substitution  $\pm 100$  ml/1 bean sprouts extract) graph shows a tendency for the number of shoots to be higher (see Figure 3)

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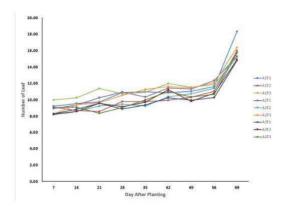
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Information:

A1T1 = 50ml/l tomato extract + 100ml/l bean sprouts extract, A1T2 = 50ml/l tomato extract + 150ml/l bean sprouts extract, A1T3 = 50ml/l tomato extract + 200ml/l bean sprouts extract, A2T1 = 100ml/l tomato extract + 100ml/l bean sprouts extract, A2T2 = 100ml/l tomato extract + 150ml/l bean sprouts extract, A2T3 = 100ml/l tomato extract + 200ml/l bean sprouts extract, A3T1 = 150ml/l tomato extract + 100ml/l bean sprouts extract, A3T1 = 150ml/l tomato extract + 100ml/l bean sprouts extract, A3T2 = 150ml/l tomato extract + 150ml/l bean sprouts extract, A3T3 = 150ml/l tomato extract + 200ml/l bean sprouts extract. DAP = Days after plant

Figure 1. Growth response to the plant height



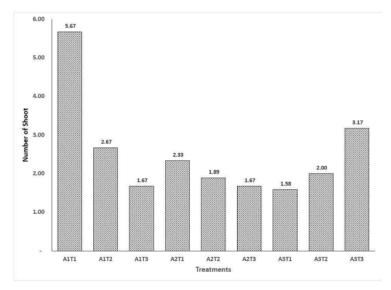
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A1T1 = 50ml/l tomato extract + 100ml/l bean sprouts extract, A1T2 = 50ml/l tomato extract + 150ml/l bean sprouts extract, A1T3 = 50ml/l tomato extract + 200ml/l bean sprouts extract, A2T1 = 100ml/l tomato extract + 100ml/l bean sprouts extract, A2T2 = 100ml/l tomato extract + 150ml/l bean sprouts extract, A2T3 = 100ml/l tomato extract + 200ml/l bean sprouts extract, A3T1 = 150ml/l tomato extract + 100ml/l bean sprouts extract, A3T2 = -150ml/l tomato extract + 150ml/l bean sprouts extract, A3T3 = 150ml/l tomato extract + 200ml/l bean sprouts extract, DAP = Days after planting

Figure 2. Growth response to the number of leaf

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ation: A1T1 = 50ml/l tomato extract + 100ml/l bean sprouts extract, A1T2 = 50ml/l tomato extract + 150ml/l bean sprouts extract, A1T3 = 50ml/l tomato extract + 200ml/l bean sprouts extract, A2T1 = 100ml/l tomato extract + 100ml/l bean sprouts extract, A2T2 = 100ml/l tomato extract + 150ml/l bean sprouts extract, A2T3 = 100ml/l tomato extract + 200ml/l bean sprouts extract, A3T1 = 150ml/l tomato extract + 100ml/l bean sprouts extract, A3T2 = 150ml/l tomato extract + 150ml/l bean sprouts extract, A3T3 = 150ml/l tomato extract + 200ml/l bean sprouts extract, A3T3 = 150ml/l tomato extract + 200ml/l bean sprouts extract, A3T3 = 150ml/l tomato extract + 200ml/l bean sprouts extract.

Figure 3. Growth response to the number of shoot

The purpose of the utilization of the two organic materials (tomatoes and bean sprouts) is that the explants are able to respond to the hormone content in the extracts of the ingredients (tomatoes and bean sprouts). The hope is that the auxins from the bean sprouts extract can interact with the cytokinins in the explant. Sprouts also contain the amino acid tryptophan which is an important organic substance in auxin biosynthesis, besides that it also contains minerals such as calcium, iron, magnesium, phosphorus and zinc which play a role in shoot formation. According [8] [9], the use of tomato extract to accelerate the growth of orchid shoots requires additional cytokinins sourced from BAP (3 ppm). In this experiment, after the results of growth and development of the A1T1 composition explants were compared with BAP treatment (3 ppm) (Table 1), the results were statistically not significantly different, thus it can be recommended that the composition of A1T1 is an economical composition of organic material for invitro orchid culture.

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**Table 1.** Table of responses to the growth and development of hybrid Vanda orchids on A1T1 treatment (50 ml tomato extract + 100 ml bean sprouts extract) and BAP (3 ppm).

DAP	A1T1	BAP	d and a d
	Plant Height		t-stat
7	0.86	0.52	3.40**
14	0.93	0.55	7.23**
21	0.94	0.44	7.00**
28	0.96	0.38	8.63**
35	0.96	0.46	7.21**
42	0.96	0.51	5.77**
49	1.00	0.49	6.37**
56	1.07	0.37	8.33**
69	1.27	0.44	6.93**
	Number of	of Leaves	
7	3.07	1.98	8.37**
14	3.18	2.40	5.57**
21	3.11	2.27	3.81**
28	3.40	2.44	3.31**
35	3.40	2.49	2.60**
42	3.38	2.42	3.00**
49	3.42	2.69	2.14*
56	3.51	2.76	2.02*
69	5.71	2.96	2.57**
	Number o	of Shoots	
69	0.51	0.64	$0.42^{\rm ns}$

Note: ns: Not significant, \*Significant difference at  $\alpha$  0.05, \*\*Highly significant difference at  $\alpha$  0.01; A1T1 = 50ml/l tomato extract + 100ml/l bean sprouts extract; BAP = 3 ppm; DAP = Days after planting

### 4. Conclusion

The growth response of Vanda hybrid orchid explants to the substitution treatment of organic matter of tomato extract and bean sprouts extract into tissue culture media (MS) showed non significant difference. The composition of A1Ti ( $50 \, \text{ml/l}$  tomato extract +  $100 \, \text{ml/l}$  bean sprouts extract) is the most economical composition in supporting the growth and development of Vanda hybrid orchid explants ready for acclimatization.

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