International Conferences on Agriculture

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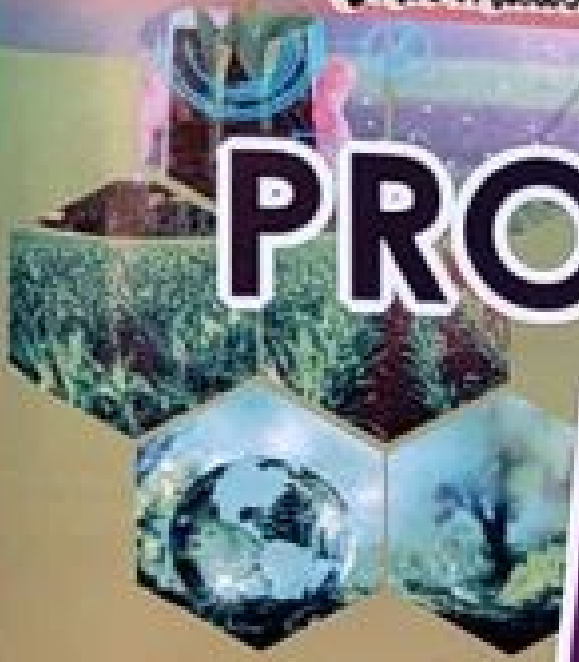

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PREFACE

Praise and gratitude to the presence of Allah SWT who has given His grace and guidance to us all requires proceedings on the results of the "International Conference on Agriculture in 2019" with the theme "Reshaping Agriculture in Disruption Era" can be changed.

The 2019 International Conference on Agriculture held by the Faculty of Agriculture of the Universitas Pembangunan Nasional "Veteran" Jawa Timur is expected to accommodate all the participants involved to jointly address various issues so that it will increase the transfer of knowledge and increase research in agriculture in agricultural change in the current disruption era. This is in accordance with the theme of the activity " Reshaping Agriculture in Disruption Era ".

The Committee thank you profusely for the trust and full support of the Rector of the Universitas Pembangunan Nasional "Veteran" Jawa Timur and the Dean of the Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur. The committee also supports thanks and gives high respect to the keynote speakers, invited speakers, moderators and all participants who have been invited to attend and actively participate in the International Conference on Agriculture in 2019. The award was also conveyed to "the e-ASIA" for its support. Then high appreciation also for all members of the committee who with dedication and hard efforts to make this activity run successfully.

We apologize for all the deficiencies, both in terms of the implementation of activities and in the preparation of this proceeding. We hope this activity can benefit us all.

Dr. Ir. Tri Mujoko, M.P.

Chair of International Conferences on Agriculture 2019

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Liquid Organic Fertilizer and Corn Extract as an Alternative Medium and Cytokinins Enhancing The Multiplication of Vanda Tricolor

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Abstract

Vanda tricolor is an endemic orchid that grows on the slopes of Mount Merapi, Yogyakarta Indonesia. This orchid has white-brown flowers with purplish red spots. The existence of *Vanda tricolor* is becoming scarce due to eruption and exploitation outside its habitat. Micropropagation is used as a method of propagation of this orchid, but the cost of chemicals used is expensive. Therefore substitution of the medium and cytokinins need to be done. This study aims to obtain a substitution medium and cytokinin for the multiplication of *Vanda tricolor* orchids. The study used a single factor completely randomized design, consisting of 6 treatments: leaf fertilizer medium, liquid organic fertilizer medium and MS medium added with 2 mg L^{-1} Thidiazuron and 100 ml L^{-1} corn extract. The results showed that the liquid organic fertilizer medium supplemented with 100 ml L^{-1} corn extract can be a substitution of the medium and cytokinins for multiplication of *Vanda tricolor* orchid shoots indicated by the parameters of increasing the number of leaves (1.15 leaves).

Keywords: Orchid, Liquid organic fertilizer, Leaf fertilizer, Corn extract, Cytokinins

Introduction

Vanda tricolor orchid is an endemic orchid on the slopes of Mount Merapi. This orchid has white flowers with reddish purple spots that live epiphytically and are often found on tree trunks in the Merapi Mountain forest area. However, the eruption of Merapi, which caused bursts of hot clouds, ignited forest fires on the slopes of the mountain, thus scorching 80% of habitat and threatening the preservation of these orchids. In addition, the population of this orchid is also reduced due to exploitation by people who bring the Vanda tricolor out of its natural habitat just to collect or sell it outside the area (Metusala, 2006).

Conservation efforts on the Vanda tricolor have been carried out by the Natural Resources Coordinating Board by giving these plants to farmer groups around the Mount Merapi area. However, the maintenance and conventional method of plant propagation carried out by farmer groups have not been able to increase the population of Vanda tricolor. According to Metusala (2006), as many as 80 orchid plants have been given, remaining 36 plants after 1 year.

One alternative propagation technique that can be done is through in vitro culture. In vitro culture can be used for propagation of orchid plants in a relatively short time. Through this method, hundreds of true to type orchids can be obtained that have relatively uniform growth (Sandra, 2003). However, the medium and growth regulators used in in vitro culture are quite expensive, so the use of nutritional sources and growth regulators from natural ingredients needs to be done.

Young corn is a natural source containing cytokinins. The results of the study of Setiawati et al (2016) showed the use of corn extract at a concentration of 100ml / L can stimulate the growth of Dendrobium sp orchid sprouts, which is indicated by the parameters of the time the buds appear, number of shoots, and shoot length with optimal results. In addition to natural growth regulating agents, the substitution of expensive synthetic mediums also needs to be done for orchid multiplication. Liquid fertilizer and organic fertilizer is an alternative medium that can be used for multiplication, because it is cheaper when compared to in vitro culture media such as New Dogashima Medium. While the results of Wangi's research (2009) showed that the use of Growmore foliar fertilizer with the addition of banana extract 0 and 50g / L was able to produce callus on orchid Dendrodium sp. Nurika's research results (2016) also showed that the use of 3ml / L liquid organic fertilizer produced the best growth in the Vanda tricolor subculture.

Rineksane et al (2019a) results show that Benzyl Amino Purine 0.5 mgL-1 which is supplemented on New Dogashima Medium enhances the multiplication of Vanda tricolor orchids as shown by the parameters of the diameters of PLBs (1.83 mm) and the number of shoots (2.6 shoots). While Rineksane et al (2019b) also stated that New Dogashima Medium supplemented with coconut water 150 ml L-1 + banana extract 150 g L-1 could replace the role of synthetic growth regulators in the multiplication of Vanda tricolor as indicated by the parameters of number of shoots (6.66 shoots), the percentage of explants sprouting (46.6%) and increase in number of leaves (3.26 leaves).

This study aims to obtain a substitution medium and cytokinin for the multiplication of *Vanda tricolor* orchids.

Material and Method

The study was conducted using an experimental method in the laboratory with a single factor experimental design, arranged in a Completely Randomized Design consisting of 6 treatments namely 3g / L foliar fertilizer + Thidiazuron (TDZ) 2mg / L, 3g / L foliar fertilizer + 100ml corn extract / L, Liquid Organic fertilizer 3ml / L + Thidiazuron 2mg / L, Liquid Organic Fertilizer 3ml / L + corn extract 100ml / L, Murashige and Skoog (MS) medium 4,43g + Thidiazuron 2mg / L and MS medium 4,43g + corn extract 100ml / L. Each treatment was supplemented with activated charcoal 0.2 g / L. Each treatment was repeated 3 times, each replication consisted of 3 samples, each sample consisted of 1 explant so that the total treatment unit was 54 bottles.

Preparation of Corn Extract

Corn extract is made by blending young corn until it is extracted and then taken according to the treatment that is 100ml / L for 200 ml solution.

Medium Preparation

The medium is made as much as 200 ml for each treatment. Each treatment contained MS medium, liquid organic fertilizer, leaf fertilizer added by Thidiazuron or corn extract and sucrose, phytagel, ppm, activated charcoal and distilled water so that the volume of the medium became 200 ml.

Inoculation

Explant inoculated in a medium bottle according to treatment. Each bottle contains one explant. After planting, the culture bottles are labeled according to the treatment and planting date and stored in the incubation room. The incubation chamber temperature of culture between 24-27°C and humidity ranges from 70% and the light intensity of 1000 lux for 24 hours every day. The parameters observed included the percentage of viable explants, the percentage of contaminant explants, the percentage of browning explants, the percentage of vitrified explants, the percentage of callus explants, shoot height and number of leaves. Observation data were analyzed using Analysis of Variance at the a = 5% error level, if there was a real difference between the treatments tested then a further test was performed using the DMRT (Duncan's Multiple Range Test) at the α = 5% level.

Research Findings and Discussion

The Percentage of Viable, Contamination, Browning and Vitrification Explant

The success of in vitro culture is determined by the percentage of viable explants, contamination, browning and vitrification. In addition, the compatibility between the medium and explants also influences the success of in vitro culture techniques. Percentage of viable explants, percentage of contamination explants, percentage of browning explants, and percentage of vitrified explants of Vanda tricolor are presented in Table 1.

The results of the analysis in Table 1 show that the percentage of viable explants in all types of medium (foliar fertilizer, liquid organic fertilizer, MS) supplemented with TDZ produced a higher percentage of viable explants as compared to the medium supplemented with corn extract. This is because explants in each medium with the addition of corn extract have increased browning. Corn extract that is mechanically damaged during the manufacturing process can spur damage to the integrity of plant tissue. Browning in the treatment of this medium is due to the enzyme activity that occurs in the fresh material of corn extract. Preparation of corn extract to substitute synthetic plant growth regulator through the process of cutting corn kernels causing mechanical damage. This mechanical damage can spur plant tissue integrity. This causes the enzyme to be in contact with the substrate which is usually an amino acid tyrosine and phenolic components such as catechins, caffeic acid and

chlorogene acid so that the phenolic substrate in plants will be hydroxylated to 3,4-dihydroxyphenylalanine (dopa) and oxidized to quinone by the enzyme phenolase (phenolase) (Blackweel. 2012).

Tabel 1. Effect of medium and cytokin substitution on the percentage of viable explants, contamination, browning, and vitrification of Vanda tricolor at 8 weeks after planting

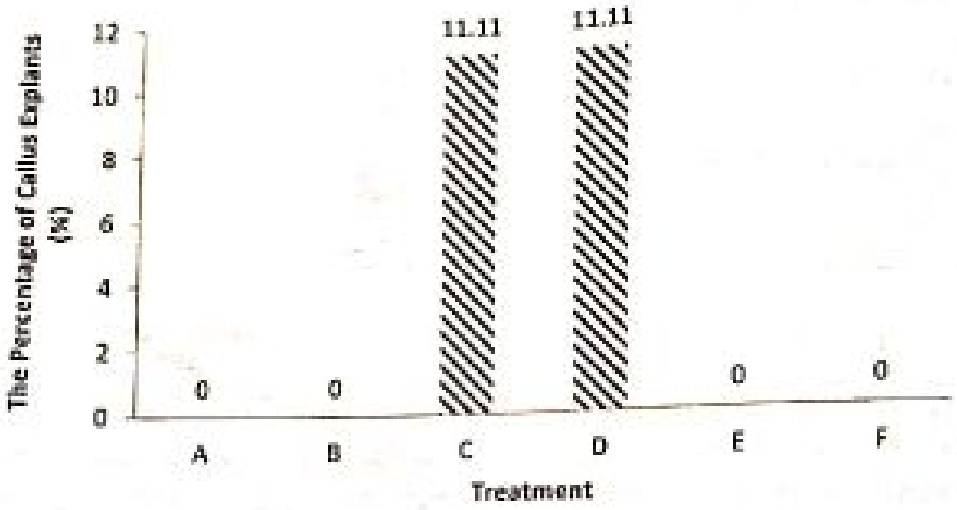
Treatment	Viable Explant (%)	Browning Explant (%)	Contamination Explant (%)	Vitrification Explant (%)
Foliar Fertilizer + Thidiazuron	55,6	44,4	0	33,3
Foliar Fertilizer + Corn Extract	22,23	77,67	0	33,3
Liquid Organic Fertilizer + TDZ	100	0	0	0
Liquid Organic Fertilizer + Corn Extract	88,9	22,2	0	11,1
MS + Thidiazuron	55,53	44,47	0	33,3
MS + Corn Extract	33,3	66,67	0	11,1

The results of the study (Table 1) showed that there are no contamination (0%) in all treatments, this was due to several factors, namely the explants used were sterile explants, sterilization of materials and mediums were appropriate so that reduce the risk of contamination. In addition, each medium was supplemented with PPM (Plant Preservative Mixure) which minimize the occurrence of contamination. This is in accordance with the statement of Sharaf and Weathers (2006), PPM is one of the biocide ingredients in liquid culture including Isotiazolone which can inhibit microbes and fungi.

Explants are categorized as vitrified if its chlorophyll loss so the leaves and stems appear to be transparent and brittle white. Based on the data from Table 1, vitrification that occurred in this study was low, namely between 11.1 - 33.3%. Vitrification is generally associated with browning explants. Vitrification usually occurs 2-4 weeks after browning explants occured. However, a small portion of explants can be vitrified directly where the green colored explants will turn transparent white. Vitrification can be caused by several factors such as the type of explant used, high relative humidity, low light intensity and imbalance of micro elements and hormones. In this study vitrified explants showed abnormal growth, the color of the leaves and stems became transparent white, the plants produced were stunted and looked fragile. According to Santoso and Nursandi (2002) vitrification occurs due to failure or an obstacle in the process of cell wall formation (parenchymal tissue) and obstacles in the process of lignin formation. This causes plant growth to be inhibited.

The Percentage of Callus Explant

The ability of explants in forming callus in the treatment medium with the addition of plant growth regulator can be determined through the percentage of callus explants. The greater the percentage of callus explants showed that the response of Vanda tricolor orchid explants to plant growth regulator in the medium is better. Based on the results of the study, plant growth regulator has varied effects on callus formation in Vanda tricolor orchid explants. A graph of percentage of explant callus at the end of the observation (8 weeks) is presented in Figure 1.



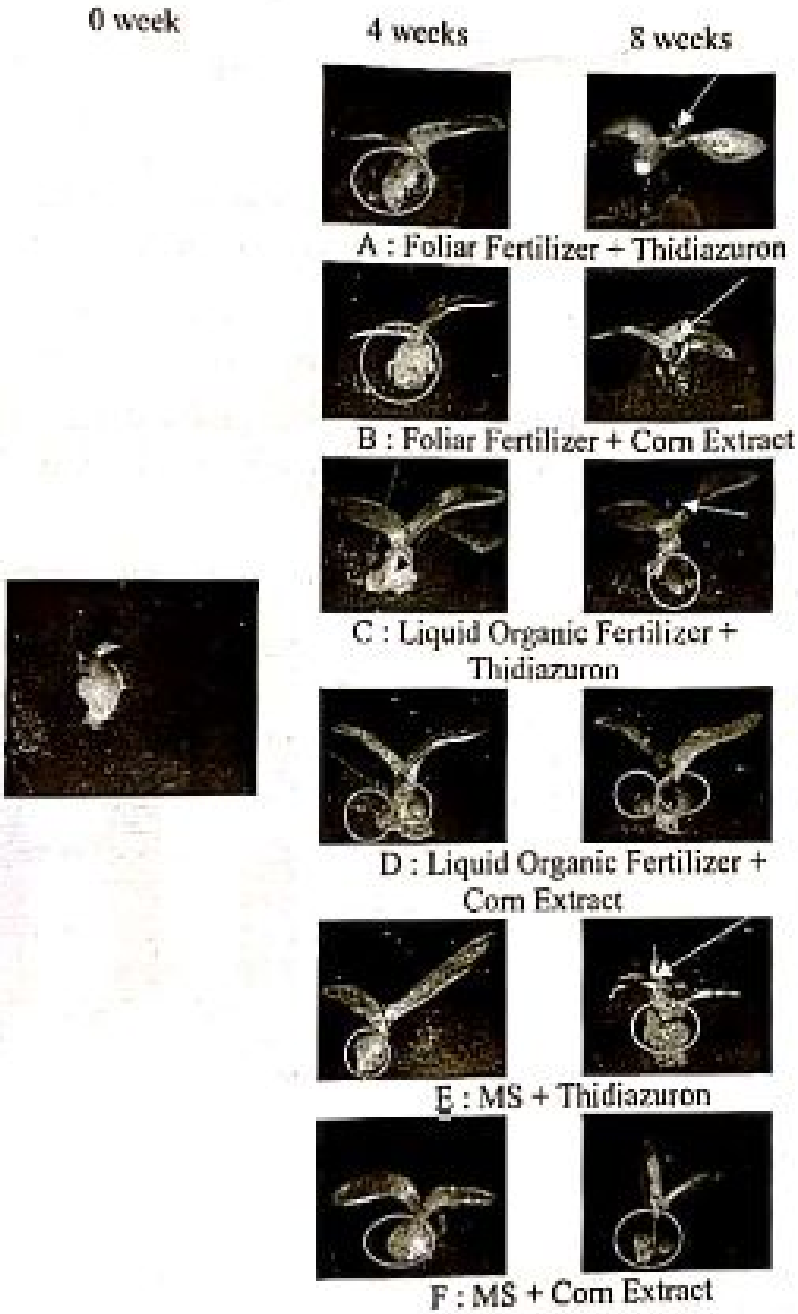
A : Foliar Fertilizer + Thidiazuron
B : Foliar Fertilizer + Corn Extract
C : Liquid Organic Fertilizer + TDZ
D : Liquid Organic Fertilizer + Corn Extract
E : MS + Thidiazuron
F : MS + Corn Extract

Figure 1. Effect of Medium and Cytokin Substitution on The Percentage of Callus Explants on Vanda Tricolor Explants at 8 Weeks After Planting

Callus formation occurred in several treatments at the 4th week, namely the treatment of 3ml / L Liquid Organic Fertilizer which was supplemented with TDZ and corn extract. It is suspected that Liquid Organic Fertilizer medium is able to induce callus because it contains plant growth regulator, namely auxin, and cytokinin. Auxin and cytokinin are two types of plant growth regulators which are often used to induce plant morphogenetics (Zulkarnain, 2007).

All explants that were inoculated in the foliar fertilizer medium have not formed callus. Explants in foliar fertilizer and MS medium only swell leading to callus formation. This swelling indicates that the cells in the explants are enlarged. However,

the enlargement of the cell did not continue to the cell division which then forms a callus. After the swelling explants, it should produce callus. This is in line with the statement of Rineksane and Sukarjan (2015) that the time needed in culture propagation in vitro on Vanda tricolor Lindl. Suavis varieties are quite slow, both in their growth and formation of callus and shoots. Dwiyani (2013) also stated that one of the weaknesses in the cultivation of the Vanda genus orchid is a long vegetative period, so it requires a relatively long time to flowering.



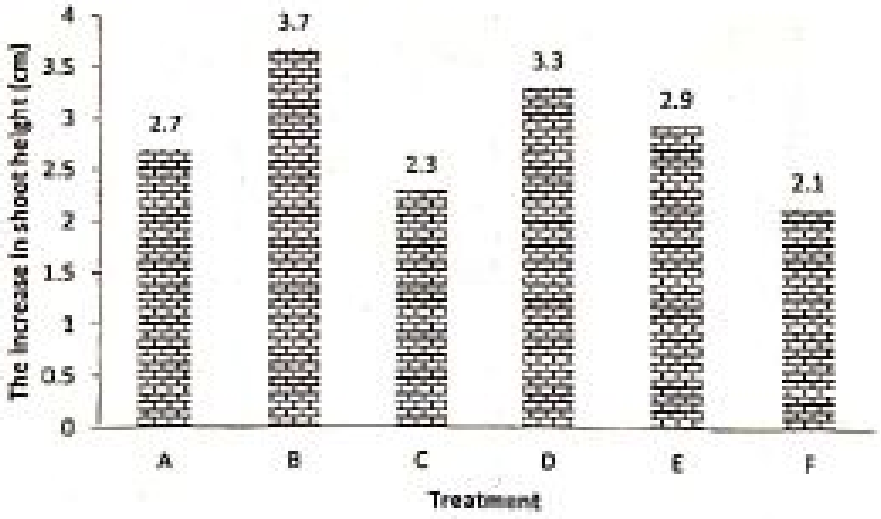
: Callus Formation
: Leaves Formation
: Root Formation
: Swollen Explant

Figure 2. Development of Callus Vanda Tricolor Orchid on Medium Fertilizer Leaves, Liquid Organic Fertilizer and MS + (Thidiazuron, and Corn Extract) at 0, 4, and 8 Weeks

The data on Figure 2 showed the formation of callus in the treatment of Liquid Organic Fertilizer 3ml / L + TDZ 2mg / L and Liquid Organic Fertilizer 3ml / L + corn extract 100ml / L at 0, 4, and 8 weeks. It is thought that corn extract contains the hormones cytokinin and auxin which can stimulate cell division and cell division. In accordance with the statement of Natsir (2002) that the addition of auxin causes callus growth from explants, while the cytokinins which supplemented can encourage the process of cell division. Explants in the foliar fertilizer 3g / L and MS medium only swell leading to callus formation, which means the cell is enlarged. The results of observations of shoot development in several treatments also showed root growth at the fourth week. This is due to balanced cytokinin and auxin content in the medium, so growth leads to the formation of shoots and roots.

Shoot Height

Shoot height is one of the growth indicators that aims to determine the response of Vanda tricolor shoot explants to the treatment of medium and cytokinins. The result of analysis of shoot height is presented in Figure 3.



A : Foliar Fertilizer + Thidiazuron
B : Foliar Fertilizer + Corn Extract
C : Liquid Organic Fertilizer + TDZ
D : Liquid Organic Fertilizer + Corn Extract
E : MS + Thidiazuron
F : MS + Corn Extract

Figure 3. Influence of Medium Composition of Foliar Fertilizer, Liquid Organic Fertilizer and MS + (Thidiazuron, and Corn Extract) on Height Addition of Vanda Tricolor Explants at 8 Weeks.

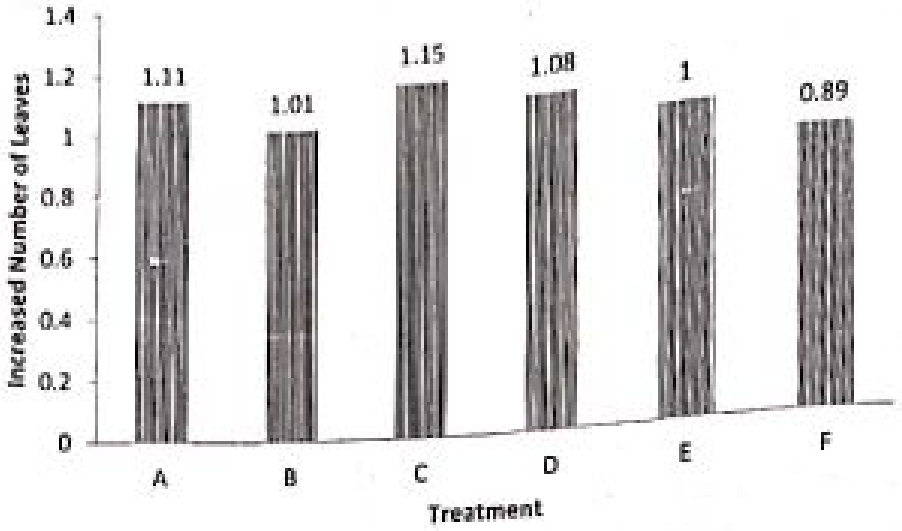
The results of analysis of variance showed that the use of foliar fertilizer, liquid organic fertilizer and MS medium did not significantly influence the height of Vanda tricolor shoots at 8 weeks (Figure 3). However, the use of foliar and liquid organic fertilizer with the addition of corn extract encourages the increase in shoot height. This is presumably because foliar and liquid organic fertilizers contain high Nitrogen elements which are able to synergize with protein in corn extract. Setiawati et al (2016) also mentioned that corn endosperm contains a lot of carbohydrates and protein. Nitrogen elements functions to arrange amino acids (proteins), nucleic acids, nucleotides and chlorophyll in plants, so that in the presence of Nitrogen, plants will accelerate plant growth (height, number of tillers, number of branches).

Based on the graph of the influence of the composition of the foliar fertilizer medium, Liquid Organic Fertilizer and MS + (Thidiazuron and corn extract) on the high addition of Vanda tricolor explants at 8 weeks, it shows that leaf fertilizer and liquid organic fertilizer with the addition of corn extract can increase shoot height increase. This is thought to be in leaf fertilizers and liquid organic fertilizers containing high N elements that are able to synthesize with protein in corn extract. Setiawati et al (2016) also mentioned that in endosperm corn contains mostly carbohydrates and protein. The N element functions to arrange amino acids (proteins), nucleic acids, nucleotides, and chlorophyll in plants, so that in the presence of N, plants will accelerate plant growth (height, number of tillers, number of branches).

On the contrary the addition of corn extract in MS medium causes the increase in shoot height which is lower as compared to MS medium with the addition of TDZ. This is presumably because the MS medium contains more essential amino acids than any other medium. This is consistent with the statement of Yusnita (2004) that each plant requires a different composition of the medium for optimum growth.

Increased Number of Leaves

The growth of shoots is shown by the increase in the number of new leaves to form plantlets. The increase in the number of leaves occurs due to cell division induced by plant growth regulators.The results of the analysis of increasing number of leaves are presented in Figure 4.



A : Foliar Fertilizer + Thidiazuron
B : Foliar Fertilizer + Corn Extract
C : Liquid Organic Fertilizer + TDZ
D : Liquid Organic Fertilizer + Corn Extract
E : MS + Thidiazuron
F : MS + Corn Extract

Figure 4. The Effect of Medium Composition of Foliar Fertilizers, Liquid Organic Fertilizer and MS + (Thidiazuron, and Young Corn Extract) on The Development of The Number of Leaves in Vanda Tricolor Explants at 8 Weeks

The results of the analysis in Figure 4 showed that the use of foliar fertilizer, liquid organic fertilizer and MS medium did not significantly affect the increase in the number of leaves at 8 weeks. The number of leaves increases starting 4 weeks after planting. The number of leaves that appeared varied in different explants in each treatment. This is presumably due to the different content of endogenous hormones in each explant, so there is a difference in the explant's response to the addition of growth regulators in the medium. The data in Figure 4 showed that the increase in the number of leaves tends to be the most in Vanda tricolor explants grown in the medium of Liquid Organic Fertilizer 3ml / L + TDZ 2mg / L, as many as 1.15 strands. This is also due to the presence of Nitrogen (in the form of NH4 +), Magnesium (Mg) and Manganese (Mn) contained in the liquid organic fertilizer which functions as forming vegetative organs and the formation of chlorophyll in plants. In addition, liquid organic fertilizer contains higher nitrogen, which is 13.86%, element K in the form of K_2O is 14% and element P in the form of P_2O_5 is 8%. The composition of the liquid organic fertilizer can supply the availability of nutrients, especially the elements N, P and K so that it can meet the needs of orchids during the growth process and development of protocorm to form a complete plantlet with organs such as stems, roots and leaves. This is consistent with the statement of Wattimena (1988)

that for the formation of new shoots, plants need the elements Nitrogen (N), Potassium (K), Sulfur (S), Iron (Fe) and Zinc (Zn). Yaroslavskaya et al. (2006) states that cytokinins regulate the biogenesis and function of chloroplasts which regulate ultrastureure of chloroplasts, chloroplast enzyme activity, pigment accumulation and photosynthesis rate.

The lowest number of Vanda tricolor explant leaf was shown in Medium MS + 100ml / L Corn Extract treatment which was 0.89 strands. The data in Figure 4 showed that each treatment with the addition of corn extract always has a decrease in the number of leaves. In contrast to the TDZ medium treatment which has a higher effectiveness than corn extract. This is also supported by Khawar et al. (2003) that TDZ has a very strong influence on plant growth.

Results and Suggestions

Liquid organic fertilizer medium supplemented with corn extract can be a substitution of medium and cytokinins for multiplication of Vanda tricolor shoots.

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