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Utilization of Moringa Leaves to Improve the Quality of Orchid Explants Through Tissue Culture Techniques

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Abstract: Moringa (Moringa oleifera) is a tropical plant that grows and develops in tropical areas such as Indonesia. The use of Moringa plants in Indonesia is currently still limited. One of the ingredients in Moringa leaves is zeatin. This study aims to determine the effect of adding organic matter of Moringa leaf extract to tissue culture media on the growth of orchids. The research was conducted at the Orchid Plant Tissue Culture Laboratory, PT. Archipelago Orchid Award, Sumber Petung Hamlet, Sempu Village, Ngancar District, Kediri Regency, East Java. The research was carried out from November 2018 to January 2019. The research method used was an experimental method in the laboratory, using a Completely Randomized Design with two factors, the first factor is the basic type of media, namely MS media (M1) and VW media (M2). The second factor is the addition of the concentration of Moringa leaf extract, namely K1 = 0 grams of Moringa leaf extract, K2 = 50 grams of Moringa leaf extract, K3 = 100 grams of Moringa leaf extract, K4 = 150 grams of Moringa leaf extract, K5 = 200 grams of Moringa leaf extract. Based on these treatments, 10 treatment combinations were obtained. The experiment was repeated 3 times, then each replication consisted of 4 samples, so there were 120 bottles. Each bottle contains 1 explant. Parameters observed were the percentage of live explants, contamination, and browning. In addition, observations were also made on the number of shoots, number of leaves, plantlet height, and number of roots. The results showed that the addition of Moringa leaf extract to the basic tissue culture media affected the growth of Dendrobium orchid explants. The addition of 150 g/l Moringa leaf extract on VW base media showed the best results in the number of shoots, plant height, and number of roots.

Keywords: orchid Explants, moringa leaves, tissue culture. Zeatin

1. Introduction

Moringa (*Moringa oleifera*) is a tropical plant that grows and develops in tropical areas such as Indonesia. Moringa plants are shrubs that can grow to a height of 7-11 meters in the tropics and subtropics (Muzzazinah, n.d.). Morphologically, sengon plants have woody stems, erect, dirty white, thin skin, rough surface, simpodial branching, upright or oblique branch directions, tend to grow straight and elongated. Has compound leaves, long-stemmed, arranged alternately (alternate), odd leaf offspring, leaf blade when young is light green when mature is dark green, leaf shape oval, 1-2 cm long, thin limp, tip and base growing (obtusus), flat edge, pinnate bone arrangement, smooth top and bottom surfaces. It thrives from the lowlands to an altitude of 1,000 meters above sea level and can live on all types of soil except heavy loam soils with neutral to slightly acidic soil pH (Isnan & Muin, 2017). Moringa plants are resistant to

drought with tolerance to drought up to 6 months and are easy to breed and do not require intensive care (Budiman & Samani, 2021).

The use of Moringa plants in Indonesia is currently still limited. People generally use Moringa leaves as a complement in cooking, as a yard fence plant, or allowed to grow wild in the natural surroundings (Dewi, 2016). Some areas in Indonesia also commonly use Moringa leaves to bathe corpses, remove amulets, and as animal feed (Augustien & Suhardjono, 2016). In addition, the Moringa plant has the potential to be used in the food and cosmetic industry because of its nutritional content and complex compounds (Soeprajitno et al., 2019).

In addition to the complex nutritional content, Moringa leaves also contain cytokinins and zeatins (Nager *et al.*, 1982). Cytokinins are plant hormones that function to regulate cell division, organ formation, cell and organ enlargement, prevention of chlorophyll damage, chloroplast formation, stomata opening and closing, and development of shoots and shoots (Harjadi, 2009). Zeatin is a strong anti-oxidant with anti-aging properties (Indonesian Center for Moringa Plant Information and Development, 2010).

Moringa leaves have high zeatin concentrations between 5-200 μg/g leaves. Zeatin is a cytokinin hormone that has an important role in cell growth and development. According to Kurnianingsih & Sefrila (2018) zeatin can stimulate cell division, accelerate the regeneration process in plants, improve leaf growth, and accelerate the growth of young shoots. According to (Juwita, 2016) the right concentration of zeatin can increase the multiplication of explant shoots in plant propagation through tissue culture techniques.

Plant propagation through tissue culture techniques is an alternative to agricultural technology that is used to improve the quality and quantity of seeds of a plant commodity. According to George and Klar in Makkar (1996); Hastuti & Setyawan (2021) the use of tissue culture technology in plant propagation has many advantages, namely relatively little plant material, seed production can be carried out throughout the year without being influenced by season, longer seed storage period, and efficiency of nursery space.

Orchid seed propagation using tissue culture is carried out to meet the needs of the orchid market. The demand for the orchid market which tends to increase every year needs to be supported by the provision of quality orchid seeds in large quantities and in a relatively short time. The tissue culture method is an effective and efficient alternative to orchid plant propagation technology (Iswanto, 2001). This study aims to determine the effect of adding organic matter of Moringa leaf extract to tissue culture media on the growth of orchids.

2. Methodology

The research was conducted at the Orchid Plant Tissue Culture Laboratory of PT. Anugerah Anggrek Nusantara, Ngancar District, Kediri Regency, East Java. The research was carried out from November 2018 to January 2019 (Bito et al., 2021).

The tools that used during the study were laminar air flow, culture bottles, analytical scales, magnetic stirrer, hot plate, erlenmeyer, dropper pipette, measuring pipette, measuring cup, beaker, pH meter, spoon, 0.3 mm pp plastic, plastic wrap, label paper, rubber bands, kitchen utensils, stationery supplies, petri dishes, tweezers, culture spoons, sterilizers (autoclave),

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The materials that used were *Dendrobium nobile* orchid subculture explants aged 5 months after germination, MS media, VW media, distilled water, 150 ml/l coconut water, Moringa leaves according to treatment, and 70% alcohol (Dharmmesta, 2014).

The research method that used is a completely randomized design (CRD) with two factors. The first factor is the type of basic medias, namely MS media (M1) and VW media (M2). The second factor is the addition of the concentration of Moringa leaf extract, namely K1 = 0 grams of Moringa leaf extract, K2 = 50 grams of Moringa leaf extract, K3 = 100 grams of Moringa leaf extract, K4 = 150 grams of Moringa leaf extract, K5 = 200 grams of Moringa leaf extract. Based on these treatments, 10 treatment combinations were obtained. The experiment was repeated 3 times, then each replication consisted of 4 samples, so there were 120 bottles. Each bottle contains 1 explant.

The stages of the research include preparation of tools and materials, manufacture of medium, multiplication, observation and analysis.

1. Preparations

Preparation of tools and materials is done by preparing the tools and materials used. Preparation of the equipment includes sterilization of the equipment using an autoclave at a temperature of 121˚C with a pressure of 1 atm for 30 minutes. Material preparation is done by preparing explants and other materials.

2. Media Creations

Moringa leaf extract was made by preparing Moringa leaves according to the treatment, namely 0 grams, 50 grams, 100 grams, 150 grams, and 200 grams. Moringa leaves are washed in running water. Moringa leaves are then added with distilled water which has been added with coconut water and blended until smooth. The blended Moringa leaves were then filtered and prepared for solvent on MS and VW media.

The media was made using MS and VW media with the addition of Moringa leaf extract. The materials that have been prepared are then measured according to the needs of the media. Moringa leaf extract was added according to the specified concentration and then measured to pH 5.8. If the pH is < 5.8 then a few drops of 1 N NaOH are added and if the pH is > 6 then a few drops of 1 N HCl are added. Next, the solution is heated to boiling. Before pouring into bottles, the solution is poured into bottles as much as 20 ml each bottle then covered with plastic and tied with rubber. Then sterilized by autoclaving at a temperature of 121 ^{0}C and a pressure of 1 atm for 20 minutes. After that, the media was stored in the incubation room.

3. Explant Multiplication

Planting is done by removing the explants in the bottle using tweezers. The explants used in this study were *Dendrobium nobile* orchid explants which were 3 months old after stocking. The explants were then cut from the bottom of the stem to the middle of the stem with a size of ± 2 cm. The explants were cut in the petridish and then were planted on the media according to the treatment. In each bottle one shoot explant was planted.

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4. Inkubation

Culture bottles that have been planted with explants are then placed on the incubation rack and arranged according to the treatment plan. The culture incubation room temperature is between 24-27 ^{0}C and the humidity is around 70% and the light intensity is 1000 lux for 24 hours every day (Zulkarnain, 2009).

Observation Variables

The observed parameters are as follows:

1. Percentage of live plantlets, contamination, and browning. This observation was carried out to see the success of orchid tissue culture based on the number of plantlets observed multiplied by 100%.
2. Number of shoots. The number of shoots was counted when new shoots began to appear after subculture either small, medium or large. Observations were made every week after planting until 12 wap.
3. Number of leaves. This observation was carried out to see the growth of the number of leaves on the plantlets during the study. It was counted from the beginning of planting or subculture by counting the total leaves in each explant that grew every week after planting until 12 wap.
4. Plantlet height. This observation was carried out to see the growth of plantlet height during the study. It was measured starting from planting or subculture using a ruler from the tip to the bottom of the plantlet root. Observations were made every 1 week until 12 wap.
5. Number of roots. This observation was carried out to see the growth of the number of plantlet roots during the study. The number of roots was counted at the end of the observation or at the age of 12 wap.

The data obtained from this study were analyzed using Analysis of Variance (ANOVA) with a significance level of =5%. If there is a significant effect on the data based on ANOVA, then a follow-up test using Duncan Multiple Range Test (DMRT) is carried out with a level of α=5%.

3. Research Results

Moringa leaves are organic materials that contain cytokinin hormones besides that Moringa leaves also contain anti-bacterial and anti-fungal compounds that can minimize contamination. In this study, Moringa leaf extract was used as a substitute for synthetic hormones which could save production costs and produce the same quality. Based on the research that has been done, Dendrobium nobile orchid explants generally showed a good growth response. This can be seen from the results of the observation parameters below.

A. Persentage of Live Planlets, Contamination, and Browning

Observations on the percentage of live, dead and contaminated plantlets were carried out from the beginning of planting to the end of planting, which was 12 WAP. Data on the percentage of live, dead and contaminated plantlets are presented in Table 4.

Table 1. Persentage of Live Planlet, Contamination, and Browning (%)

Treatment	Live Planlet (%)	Explant Contamination (%)	Eksplant Browning (%)
M1K1 (½ MS + 0 g/l moringa leave extract)	100	0	0
M1K2 (½ MS + 50 g/l moringa leave extract)	100	0	0
M1K3 (½ MS + 100 g/l moringa leave extract)	100	0	0
M1K4 (½ MS +15 0 g/l moringa leave extract)	100	0	0
M1K5 (½ MS +20 0 g/l moringa leave extract)	100	0	0
M2K1 (VW + 0 g/l moringa leave extract)	100	0	0
M2K2 (VW + 50 g/l moringa leave extract)	100	0	0

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M2K3 (VW + 100 g/l moringa leave extract)	100	0	0
M2K4 (VW + 150 g/l moringa leave extract)	100	0	0
M2K5 (VW + 200 g/l moringa leave extract)	100	0	0

1. Percentage of Live Planlets (%)

The percentage of live plantlets is the ability of planting material (eskplan) to live and grow in the treatment medium. The viability of plantlets in tissue culture is highly dependent on the state of the explants, the type and composition of the media, and the content of the given growth regulators (Miryam *et.al*., 2008). Plantlets are said to be alive if they are not contaminated or if they are able to form new roots or shoots, and do not experience permanent browning (Supriyadi, 2014).

The results showed that each treatment produced a high percentage of live plantlets, namely 100%. Live plantlets were observed by looking at plantlets that did not experience contamination in the form of bacteria or fungi and plantlets that did not experience browning or browning. The high percentage value of live plantlets in this study was caused by the type of explant used, derived from the germination of orchid seeds with tissue culture. In addition, the explants used are still young so they have high regeneration power. Cells in young tissues are more actively dividing and contain relatively few contaminants (Yusnita *et al*., 2003).

According to Rifai et al., (2020) the growth and development of explants is influenced by the media used. The addition of growth regulators and the selection of quality explants affect the high ability of plantlets to survive (Ferita *et al*., 2003 in Nurjaman & Isnawan, (2020). Explants grown on media supplemented with organic growth regulators such as Moringa leaves were also capable and suitable for the growth of *Dendrobium nobile*.

Figure 1. Live explant of *Dendrobium nobile* at 12 wap

1. Percentage of Contamination (%)

The percentage of contaminated explants showed the level of contamination that occurred in all explants that were planted. The occurrence of contamination can cause the growth of the explant to be inhibited or cause the explant to die. Contaminants in the form of fungi or bacteria with explants to obtain nutrients so that the growth of the explants will be inhibited.

The level of contamination in the research was 0% (Table 1). The success in suppressing contamination in this study was due to the sterile condition of the Dendrobium nobile explant. Dendrobium nobile explants were derived from in vitro seed germination. According to Rahayu et al., (2018) explants that do not carry contaminants can minimize the occurrence of contamination in tissue culture.

2. Percentage of Browning (%)

The problem that is often faced in tissue culture propagation techniques is the occurrence of browning. Browning is a change in explant color from green to brown (Nurjaman & Isnawan, 2020). One of the main causes of browning in in vitro culture is wound from cutting tissue. Injury to plant tissue triggers stress and causes an increase in Phenylalanine Ammonia Lyase (FAL) activity which is followed by oxidase enzyme (PPO) activity and causes browning (Harianto et al., 2020). This is also supported by research by Hutami (2008) which states that browning in in vitro culture occurs due to accumulation of phenolic compounds released or synthesized by tissue and undergoes oxidation when cells are injured.

The results showed that none of the explants experienced browning. The value of browning percentage is thought to be due to the use of young tissue explants that do not contain a lot of phenolics. This is supported by George and Sherrington (1984) that browning in young tissue is less than that in old tissue. In addition, cutting that was only done on the explant roots did not cause major damage to the explant tissue.

B. Number of Shoots

The number of shoots ais the most important factor in plant multiplication in tissue culture. The success of explants in multiplication is influenced by the combination of growth stimulating substances auxin and cytokinins which can improve the efficiency of explant regeneration, depending on the concentration added.

Table 2. Average number of shoots at the age of 10,11, and 12 week after plant (wap)

Treatment	Average number of shoots		
	10 wap	11 wap	12 wap
M1K1 (½ MS + 0 g/l moringa leave extract)	2,42 a	2,42 a	2,50 a
M1K2 (½ MS + 50 g/l moringa leave extract)	4,00 b	4,25 b	4,42 b
M1K3 (½ MS + 100 g/l moringa leave extract)	4,25 b	4,42 b	4,67 b
M1K4 (½ MS +15 0 g/l moringa leave extract)	6,33 d	6,75 c	8,08 c
M1K5 (½ MS +20 0 g/l moringa leave extract)	3,83 b	4,00 b	4,25 b
M2K1 (VW + 0 g/l moringa leave extract)	3,58 b	3,82 b	4,08 a
M2K2 (VW + 50 g/l moringa leave extract)	4,33 b	4,58 a	4,83 b
M2K3 (VW + 100 g/l moringa leave extract)	4,50 c	4,67 b	5,08 b
M2K4 (VW + 150 g/l moringa leave extract)	8,00 e	9,83 d	11,7 d
M2K5 (VW + 200 g/l moringa leave extract)	4,25 b	4,42 b	4,67 b

Note: Numbers followed by different letters indicate the effect of treatment interactions according to the DMRT test at the level of α =5%.

Based on Table 2, it can be seen that the addition of 150 g/l moringa leave extract in VW media showed the best results compared to other concentrations. This can be caused because Moringa leaves contain zeatin, which is a group of cytokinin hormones. According to Krishnamoorthy (1981); Herry Setyawan et al., (2019) the addition of cytokinins with the right concentration can increase shoot multiplication in tissue culture.

The results showed that at a concentration of moringa leave extract 200 g/l produced fewer tillers than the concentration of moringa leave extract 50, 100, and 150 g/l but produced a higher number of tillers than the control medium, this was because the higher The higher the concentration of moringa leave extract, the higher the cytokine hormone content, a high concentration of ZPT will be toxic or toxic to the explants which results in a decrease in the number of tillers and even the explants turn brown and die. According to

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(Aliseda-Llera, 1997) administration of high exogenous cytokinins no longer has a good effect or even inhibits growth because the concentration of cytokinins becomes excessive.

Moringa leave extract concentration of 50 and 100 g/l showed a better number of tillers than the concentration of moringa leave extract of 200 g/l or 0 g/l. According to Mawadah *et al*. (2018) which stated that the combination of 100 g/l sprout extract plus 100 g/l moringa leave extract had the potential to have better growth compared to control media seen from the addition of the number of leaves and the number of roots.Konsentrasi moringa leave extract 50 dan 100 g/l menunjukan jumlah anakan yang lebih bagus dibandingkan degan konsentrasi moringa leave extract 200 g/l atau 0 g/l.

Figure 2. Number of plantlets on media ½ MS at the age of 12 wap

C. Number of Leaves

The results showed that the moringa leave extract treatment did not have a significant effect on the growth of the number of leaves of orchid explants. Figure 4 shows that ½ MS media produced a higher number of leaves than VW media. This is because the ½ MS medium contains a high chemical composition of NH_4NO_3 compared to the composition of VW media. NH_4NO_3 is a nitrate salt of ammonium cation which functions as a nitrogen-rich fertilizer. The macro nutrient that is very important in tissue culture is Nitogen (N) (Yusnita & Sc, 2003).

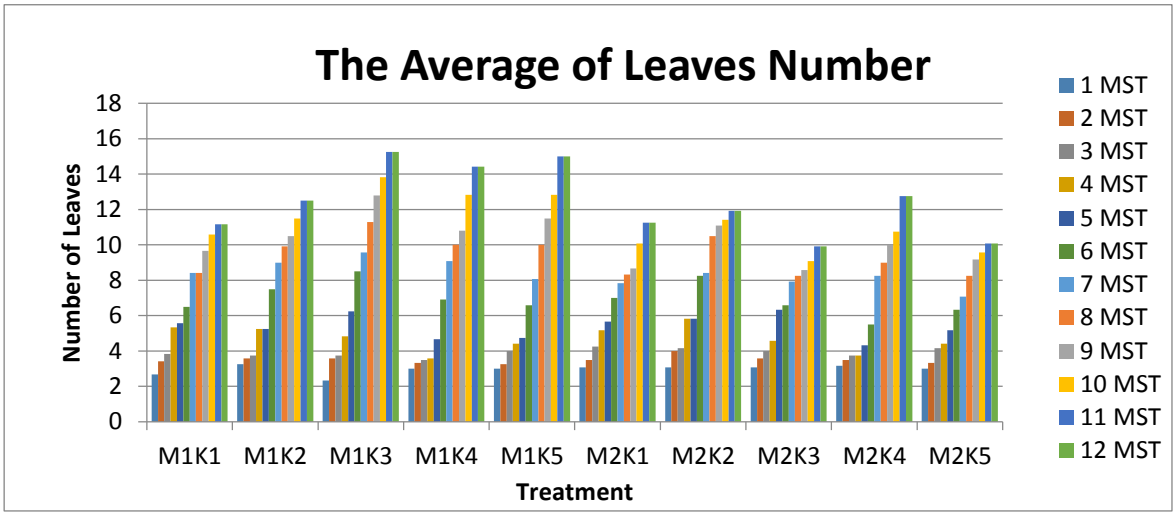

Figure 4. Grafik leaves number at the age 1-12 wap

½ MS media provides nitrogen in the form of NH_4NO_3 salt which is good as a source of N. In addition, NH_4NO_3 also plays a role in lowering the pH of the growing media (Sari & Setiawan, 2021), so that the MS media showed a higher number of leaves than the VW. MS media added with complete fertilizer can be used as an alternative medium for orchid growth because the media can increase the number of leaves and plantlet height (Dewi, 2016).

D. Planlet Height

The results of the observations showed that at the age of 3-7 wap there was a significantly different interaction. However, in the following week there was no significant difference. The addition of moringa leave extract on VW media resulted in the best plant height but not significantly different from ½ MS media. The basic media with the addition of moringa leave extract had no significant effect on the tallest plants. This can be caused by the combination of media used to play a role in the formation of new shoots and roots.

Table 3. Average Plant Height at Age 3, 4, 5, 6, and 7 wap

Treatment	Average Plant Height (cm)				
	3 wap	4 wap	5 wap	6 wap	7 wap
M1K1 (½ MS + 0 g/l moringa leave extract)	1,64 a	1,78 b	1,83 b	1,92 b	2,02 b
M1K2 (½ MS + 50 g/l moringa leave extract)	1,68 a	1,83 c	1,88 b	1,94 b	2,03 b
M1K3 (½ MS + 100 g/l moringa leave extract)	1,53 a	1,55 a	1,63 a	1,63 a	1,71 a
M1K4 (½ MS +15 0 g/l moringa leave extract)	1,59 a	1,68 ab	1,71 a	1,76 a	1,79 a
M1K5 (½ MS +20 0 g/l moringa leave extract)	1,68 a	1,71 b	1,72 a	1,74 a	1,82 a
M2K1 (VW + 0 g/l moringa leave extract)	1,68 a	1,85 c	1,91 b	1,98 b	2,06 b
M2K2 (VW + 50 g/l moringa leave extract)	1,68 a	1,88 c	1,92 b	1,99 b	2,06 b
M2K3 (VW + 100 g/l moringa leave extract)	1,76 b	1,87 c	1,90 b	1,93 b	1,98 b
M2K4 (VW + 150 g/l moringa leave extract)	1,59 a	1,64 ab	1,68 a	1,72 a	1,82 a

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M2K5 (VW + 200 g/l moringa leave extract)	1,74 b	1,88 c	1,88 b	1,89 b	1,93 b

Note: Numbers followed by different letters indicate the effect of treatment interactions according to the DMRT test at the level of α =5%.

Explant height was caused by two processes, namely cell division and elongation. Both of these processes occur in the meristem tissue, namely at the point of growth of the stem so that the plant grows larger and is positively correlated in determining crop yields (Untari & Puspitaningtyas, 2006).

E. Number of Roots

Table 4. Average of Number of Roots at Age 12 wap.

Treatment	Average of Number of Roots
M1K1 (½ MS + 0 g/l moringa leave extract)	8,67 a
M1K2 (½ MS + 50 g/l moringa leave extract)	9,50 a
M1K3 (½ MS + 100 g/l moringa leave extract)	10,20 a
M1K4 (½ MS +15 0 g/l moringa leave extract)	13,20 c
M1K5 (½ MS +20 0 g/l moringa leave extract)	10,70 b
M2K1 (VW + 0 g/l moringa leave extract)	8,83 a
M2K2 (VW + 50 g/l moringa leave extract)	10,10 a
M2K3 (VW + 100 g/l moringa leave extract)	11,10 b
M2K4 (VW + 150 g/l moringa leave extract)	19,70 d
M2K5 (VW + 200 g/l moringa leave extract)	10,80 b

Note: Numbers followed by different letters indicate the effect of treatment interactions according to the DMRT test at the level of α =5%.

The root is the part of the plant that functions to absorb water and mineral (nutrients) and support and strengthen the establishment of plants. The number of roots is an important factor in determining the ability of explants to absorb nutrients in the culture medium. The more number of roots can optimize the absorption of nutrients in the media. The number of roots was observed to see whether there was an effect between the treatments given to the increase in the number of roots. The results of the analysis of the average number of roots at week 12.

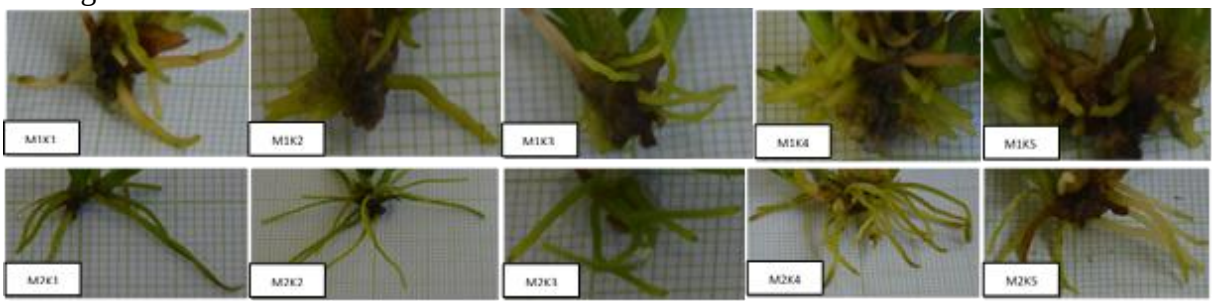

Figure 4. Number of plantlet roots at the age of 12 wap

The results showed that the addition of Moringa leaves to the growing media had a significant effect on the number of roots. Media VW+ moringa leave extract 150 g/l produced the highest number of roots compared to other treatments. This was because the composition of VW media contained high phosphorus elements compared to the composition of ½MS media. Phosphorus is needed by plants for vegetative growth.

The function of phosphorus is to accelerate root growth, strengthen plant stems, accelerate the flowering process, increase the production and ripening of fruits and seeds. According to Sutedjo (2002) Phosphorus is a growth stimulant for plant roots and is a

material for growth and formation of a number of proteins and helps assimilation and cellular respiration

Moringa leave extract which is added to the media in addition to containing the hormone cytokinin also contains high calcium. Calcium for plants acts as a binder for membrane molecules so that it can function well in cell growth and development. Calcium can also trigger the activity of some enzymes. This is in accordance with the results of research by Gardner *et.al.* (1985) that calcium is a constituent of cell walls, this element also functions in cell division and elongation. Rupawan et al., (2014) also stated that the treatment of moringa leave extract in sugarcane nurseries has increased the diameter of sugarcane roots. The increase in the number of roots can be caused by the higher metabolic activity of dividing cells (Krishnamoorthy, 1981), (Saptaria & Setyawan, 2021).

The lowest number of roots was in the control medium MS with an average number of roots of 8.67 and not significantly different from the M1K2, M1K3, M2K1, M2K2 treatments. Based on the results of this study showed that the addition of PGR in small amounts or less causes the growth of Dendrobium orchids to be less than optimal, while the addition of high concentrations can reduce the number of roots. At a concentration of 200 g/l explants were still able to take root better than the control medium, but at this concentration the number of roots decreased, with an average number of roots 10.7 on ½ MS media and 10.8 on VW media

5. Conclution

The results showed that the addition of moringa leave extract to the basic tissue culture media affected the growth of Dendrobium orchid explants. The addition of moringa leave extract as much as 150 g/l on VW base media showed the best results in the number of shoots, plant height, and number of roots (Setyawan & Nawangsari, 2021).

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