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Multiplication of Dendrobium Sp Orchid Somatic Embries Using In-Vitro Concentrations of $MgSO_4$ and Myo-Inositol in Murashige and Skoog Media

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Abstract: Dendrobium Sp orchid is one type of orchid that occupies the top position in ornamental plants. This study aims to determine the administration of various concentrations of magnesium sulfate ($MgSO_4$) and Myo-inositol to Dendrobium Sp orchid explants on Murashige and Skoog media. The design used in this study was a factorial complete randomized design (CRD) consisting of 2 treatment levels (M= $MgSO_4$ and Y= Myo-inositol) with 3 replications. Namely: M0 (without $MgSO_4$), M1 ($MgSO_4$ 350 mg/l), M2 ($MgSO_4$ 370 mg/l), M3 ($MgSO_4$ 390 mg/l), and Y0 (Without Myo-inositol), Y1 (Myo-inositol 50 mg/l), Y2 (Myo-inositol 100 mg/l) , Y3 (Myo-inositol 150 mg/l). Based on the results of the study, giving various concentrations of magnesium sulfate ($MgSO_4$) alone had a significant effect on all observed parameters, where the best treatment was found in M3 with an average number of shoots 4.04, shoot height 1.08 cm, number of leaves 7.89 fruit, number of roots 6.64 fruit and root length 1.55 cm in Dendrobium Sp. For the treatment of various Myo-inositol concentrations, a single significant effect on all observed parameters, where the best treatment was found in Y3 with an average number of shoots 4.03, shoot height 1.25 cm, number of leaves 7.81, number of roots 4.96 and root length 1.48 in Dendrobium Sp. In interaction, the administration of $MgSO_4$ and Myo-inisitol had a significant effect on all observation parameters, the best treatment was M3Y3 (390 mg/l $MgSO_4$ and 150 mg/l MS Myo-inisitol) namely the number of shoots 4.33, the height of the shoots 1.68 cm, the number of leaves is 8.89, the number of roots is 7.22 cm and the root length is 2.02 cm.

Keywords: Dendrobium sp; In-vitro; Media MS; $MgSO_4$; Myo-inositol.

Introduction

Indonesia is known as a country that has many species of natural orchids. It is estimated that half of these species are found in Papua, while another 2,000 species are found in Kalimantan and the rest are spread across other islands in Indonesia (Arobaya et al., 2022). Orchid plants (Orchidaceae) include 25,000–30,000 species and constitute 10% of the world's flowering plants (Evans et al., 2023). Orchids have a high economic value when compared to other ornamental plants, both for cut flowers and for potted flowers (Janakiram & Baskaran, 2018). Besides being suitable for living orchids, Indonesia's tropical climate also has the potential to produce quality natural orchids (Semiarti et al., 2020).

The Dendrobium Sp orchid is a cut flower and is the most popular and most widely traded orchid in Southeast Asian countries (Akter et al., 2007; Ketsa & Warrington, 2023). According to Pammai et al. (2022),

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this type of orchid has beautiful flower bunches, various colors, sizes and flower shapes and a relatively long blooming period, from several weeks to several months.

Orchids generally reproduce in a generative way, but this method experiences many obstacles because it is very dependent on the presence of mycorrhizal fungi (Puri et al., 2022). This is because orchid seeds do not have endosperm, so they need to be propagated using in-vitro culture. Yusnita (2003) suggests that tissue culture techniques are plant propagation techniques by growing plant parts, whether in the form of cells, tissues or organs under aseptic conditions in-vitro (Lone et al., 2020). This technique is characterized by aseptic culture conditions, the use of artificial culture media with complete nutritional content and ZPT (growth regulators) (Kasutjianingati et al., 2022), as well as culture room conditions with controlled temperature and lighting. The success of plant propagation with tissue culture is largely determined by the media used, one of which is Murashige and Skoog (MS) media. The Murashige and skoog (MS) media is a very widely used medium because it contains complete macro and micro nutrients so that it can be used for various plant species (Madzikane et al., 2022; Phillips & Garda, 2019).

Orchid in vitro culture usually uses media supplemented with activated charcoal or carbon (Nguyen et al., 2022; Wijaya et al., 2023) which can absorb toxic compounds in the media or absorb inhibitory compounds secreted by plantlets, stabilize the pH of the media, stimulate root growth by reducing the amount of light entering the media, and stimulate morphogenesis (Chin et al., 2019). In addition, activated charcoal can reduce the browning of the media due to high heat during the sterilization process (Sparjanbabu et al., 2019).

Tissue culture techniques generally have obstacles from the root induction process (Cardoso et al., 2019). This is caused by a lack of magnesium in the media. Magnesium is given to the media usually in the form of $MgSO_4$ (Wilks et al., 2019). Where the main function of $MgSO_4$ is to stimulate root growth, the formation of carbohydrates, fats, and oils (Safitri, 2020). The micro propagation technique which is a form of application of tissue culture techniques and aims at plant propagation has been proven to be suitable for the propagation of orchids including dendrobium. To make optimal use of this technique, it is necessary to master the right conditions for the growth and development of orchids in vitro. One of them is the use of culture media with the right components and is able to stimulate the multiplication of protocormlike bodies (PLB) or shoots. According to Yusnita (2003) in MS basic medium, 370 mg/l standard $MgSO_4$ was used, while in this study the $MgSO_4$ concentrations to be given were 0 mg/l, 350 mg/l, 370 mg/l and 370 mg/l.

Inositol is a part of polyhydroxylated cycloalkanes, commonly known as cyclitol. Inositol or cyclohexane-1,2,3,4,5,6-hexol is a chemical compound with the formula $C_6H_{12}O_6$ or $(-CHOH)_6$ which is present in nine stereoisomers (Alamgir & Alamgir, 2018). Of the nine geometric isomers, myoinositol is most abundant in biological systems and functions as an essential metabolite. Myo-inositol, meso-inositol, or i-inositol are often used in culture media to improve growth and morphogenesis (Kabylbekova et al., 2020; Toma et al., 2012). For this reason, myoinositol is considered a class of plant vitamins. Myoinositol also plays a role in the biosynthetic pathway of D-galacturonic acid which produces vitamin C and pectin and its incorporation in phosphoinositides and phosphotidyl inositol which play a role in cell division. In addition, myoinositol functions to stimulate cell growth (PDR Network 2009). Myoinositol is a cyclic compound having six carbons and six hydroxyl groups with a structure similar to that of glucose. According to Yusnita (2003) in MS basic media, 100 mg/l of myoinisitol was administered, while in this study the myoinisitol concentrations to be administered were 0 mg/l, 50 mg/l, 100 mg/l and 150 mg/l. Single administration of myoinositol had a significant effect on all observation parameters with the best treatment A2 (administration of 50 mg/l myoinositol), namely shoot emergence age 20.25 days, number of shoots 2.11, shoot height 2.32 cm, number of roots 3.00 pieces, root fresh weight 26.39 mg.

The purpose of this study was to determine "Multiplication of Dendrobium Sp orchid somatic embryos by administering concentrations of $MgSO_4$ and Myo-inositol on Murashige and Skoog Media In-Vitro".

Method

This research has been carried out in the tissue culture laboratory. UPT seeding and seed certification Service for Food Crops and Horticulture Riau Province, Jalan Kaharudin Nasution No. 33 Simpang Tiga Village, Bukit Raya District, Pekanbaru City. This research was conducted for approximately 4 months, starting from September 2021 to January 2022. The tools used in this study were laminar air flow cabinets, measuring cups, beakers, petridishes, pipettes, autoclaves, analytical balances, erlenmayer, magnetic stirrers, glass stirrers, tweezers, scarpels, spirit lamps, hand sprayers, pH meters, knives, culture bottles, gas stoves, measuring flasks, test tubes, plastic rubber, scissors, aluminum foil, stationery and washing equipment that support activities in tissue culture research.

The materials used in this study were Dendrobium Sp Orchid explants, chemicals Sucrose and Nicotinic acid, MS media, alcohol, agar powder, sterile distilled water, detergent, twin, fungicide, rubber bands, label paper and other materials that support the manufacture of planting media. plant tissue isolation method.

The design used in this study was a factorial Completely Randomized Design (CRD) consisting of two factors, namely $MgSO_4$ and Myoinositol. The first factor was giving $MgSO_4$ (factor A) and Myoinositol (factor B) (Rahmi, 2022). $MgSO_4$ administration consisted of 4 treatment levels and Myo-inositol administration consisted of 4 treatment levels, so there were 16 treatment combinations with 3 replications. Thus this study consisted of 48 experimental units (bottles). Each experimental unit consisted of 1 culture bottle, each of which consisted of 4 explants. The treatments were: $MgSO_4$ (Factor A) consisted of 4 levels, namely: M0: $MgSO_4$ 0 mg/l, M1: $MgSO_4$ 350 mg/l, M2: $MgSO_4$ 370 mg/l, M3: $MgSO_4$ 390 mg/l. Myoinositol application (Factor B) consists of 4 levels: Y0 : Myoinositol 0 mg/l, Y1: Myoinositol 50 mg/l, Y2 : Myoinositol 100 mg/l, Y3 : Myoinositol 150 mg/l.

Result and Discussion

Number of Shoots (Fruits)

Based on the results of observations on the parameter of the number of shoots of Dendrobium sp orchid explants, after analysis (appendix 5) showed that the treatment of $MgSO_4$ and myo-inositol alone had a significant effect on the number of shoots of Dendrobium sp orchids, and in terms of the interaction of $MgSO_4$ and myo- inositol also had a significant effect on the number of explant shoots of Dendrobium Sp. Further test results are significantly different to be honest at the level of 5% as seen in Table 1.

Table 1. The average number of shoots of Dendrobium Sp orchid explants with the administration of Magnesium Sulfate ($MgSO_4$) and Myo-inositol (Fruits)

Factor M	Factor Y				Average M
	Y0	Y1	Y2	Y3	
M0	2.33d	2.89cd	3.22c	3.22c	3.22c
M1	3.44bc	4.00bc	4.11b	3.44bc	3.70bc
M2	3.33bc	3.67bc	4.11b	4.33ab	3.80ab
M3	3.44bc	4.04b	4.33ab	5.11a	4.04a
Average Y	3.17c	3.75b	3.81b	4.03a	
KK= 7.32%		BNJ M = 0.30	BNJ Y = 0.30		BNJ MY = 0.80

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different according to the Significant Difference Advanced Test (BNJ) at the 5% level

The data in Table 1 can be seen that giving. The $MgSO_4$ with the best treatment was found in M3 ($MgSO_4$ 390 mg/l MS medium), namely with the number of shoots of 4.04, the results of the follow-up test were significantly different at the 5% level, indicating that the M3 treatment was not significantly different from M1 (3, 70 units), but significantly different from M2 (3.80 units) and M0 (3.22 units).

M3 treatment with concentrations of $MgSO_4$ (390 mg/l to MS media) gave the best results compared to concentrations given to treatments M1, M2, M0. This is because M3 treatment with $MgSO_4$ concentration (390 mg/l to MS media) is the best concentration to be given to Dendrobium Sp. orchid explants. This is because magnesium sulfur plays a role in the formation of fat and oil compounds, helps starch translocation and distribution of phosphorus in plants, and is an activator for various types of plant enzymes, it is necessary to add $MgSO_4$, the addition of magnesium sulfur in the media must be sufficient to meet the basic energy requirements for cell division and growth of new shoots. This indicates that the concentration of $MgSO_4$ is one of the factors controlling shoot induction and growth (Sharavdorj et al., 2022). The elements magnesium and sulfur contained in $MgSO_4$ are macro nutrients needed in large quantities by plants. The role of magnesium sulfur is a stabilizer for ribosomes and nucleic acids based on the formula of the standard concentration of $MgSO_4$ 370 mg/l in basic MS media which is useful for protein.

The M0 treatment (0 mg/l $MgSO_4$) produced the least number of shoots, the condition of the plants that were not given magnesium sulfur was that the plants would look stunted or did not develop, this was because $MgSO_4$ was not given not suitable, so the growth of the number of shoots was low, because magnesium and sulfate play a very important role as an essential molecular component in plant cells (Ramage & Williams, 2002). Magnesium is primarily required for osmotic balance and the opening and closing of stomata. Magnesium and phosphorus are cofactors in the phosphorylation reaction, and magnesium is the central molecule of chlorophyll. Sulfur is required for the conversion of nitrate to certain amino acids and is involved in the production of chlorophyll. Calcium is necessary for cell wall synthesis, because calcium pectate is stored in the middle lamella, and also plays an important role as a second messenger in regulating cellular processes. Therefore, deficiency or toxicity of these nutrients will produce symptoms such as stunted growth, hyperhydricity, and chlorosis (Bošnjak et al., 2021; Poothong & Reed, 2015).

The different results were obtained, in the MS medium used for the nutrient $MgSO_4$ as much as 350 mg/l, $MgSO_4$ administration to the basic media produced a number of shoots of 8.66 plants. Orchid Dendrobium Sp. Whereas in this study the administration of 390 mg/l $MgSO_4$ resulted in a total of 4.04 shoots. This is due to the different concentration of $MgSO_4$ given, the resulting response is also different.

Based on the table above, it shows that the administration of Myo-inositol has a significant effect on the parameters of the number of shoots of Dendrobium Sp orchid explants with the best treatment found in the Y3 treatment (150 mg/l of Myo-inositol in MS media) namely 4.03 fruit, from the different test results then the honest significant difference (BNJ) at the 5% level showed that the Y3 treatment was significantly different from Y2 (100 mg/l Myo-inositol administration), namely 3.81 fruits, but not significantly different from Y1 treatment (50 mg/l Myo-inositol administration) ke) which is 3.75 fruit and Y0 (without Myo-inositol) which is 3.17 fruit.

The results of the Y3 treatment average (Giving Myo-inositol with a concentration of 150 mg/l MS media) were able to produce a faster number of shoots compared to other treatments. Because Myo-inositol has an effect on controlling the auxin hormone. Inositol conjugated with IAA functions as a storage or transport of auxin and can regulate the availability of IAA during plantlet growth, so the administration of myo-inositol is suitable for the needs of Dendrobium Sp orchid plant explants on MS media. This is caused by the multiplication process of explant shoots that play a role is the interaction between the hormones auxin and cytokinin, this interaction of hormones spurs the formation of new shoots, but the hormones auxin and cytokinin cannot work alone but must involve vitamins as storage and distribution of these hormones. , In plant tissue growth, cytokinins together with auxin provide an interacting influence on the differentiation of plant tissues (Hendaryono & Wijayani, 1994). At effective concentrations it will encourage the formation of shoots, on the other hand if the auxin is relatively high from cytokinins it will lead to the formation of roots.

The treatment of Myo-inositol (Y0) resulted in the least number of shoots, this was because there was no addition of Myo-inositol Myoinositol played a role in controlling the auxin hormone. Inositol conjugated with IAA functions as a storage or transport of auxin and can regulate the availability of IAA during plantlet growth, inositol is also often used in culture media to improve growth and morphogenesis. The impact on plants that lack myo-inositol is that plants will not grow properly due to a lack of vitamins, and myo-inositol plays an important role in plant growth. For this reason, myoinositol is considered a class of plant vitamins. In addition, myoinositol functions to stimulate cell growth (Hegeman et al., 2001).

The results of this study when compared with research conducted by (Heriansyah et al, 2014), different results were obtained, concluding that the administration of 50 mg/l Myo-inositol into MS media had a significant effect on the growth of the number of shoots of Dendrobium Sp orchid explants with an average -the average number of shoots was 2.11, whereas in this study the administration of the same concentration of 50 mg/l Myo-inositol into MS media produced a better number of shoots of 3.75.

Based on table 4 the results of the analysis of variance showed that the interactive treatment of the administration of Magnesium Sulfate ($MgSO_4$) and Myo-inositol had a significant effect on the number of shoots on the Dendrobium Sp. orchid explants. The treatment combination that produced the highest mean value was in the M3Y3 treatment, where M3 (390 mg/l $MgSO_4$ administration) functioned to provide macro nutrients which play a very important role for plants. While Y3 (Myo-inositol 150 mg/l) plays a role in controlling the auxin hormone. Inositol conjugated with IAA functions as a storage or transport of auxin and can regulate the availability of IAA during plantlet growth.

The M0Y0 treatment was the lowest in terms of the number of shoots with an average number of shoots of 3.17, this was due to the absence of $MgSO_4$ and myo-inositol in the media. This causes the growth of the number of M0Y0 shoots to be less when compared to M3Y3. If a deficiency of $MgSO_4$ can cause stunted shoot growth.

Shoot Height (cm)

Based on the results of observations on the parameters of shoot height of Dendrobium sp orchids, after analysis showed that the treatment of $MgSO_4$ and myo-inositol alone had a significant effect on shoot height of Dendrobium sp orchids, and interactively administration of $MgSO_4$ and mio-inositol also had a significant effect on shoot height of Dendrobium Sp. orchid plant explants. The results of the follow-up test were honest significant differences (BNJ) at the 5% level.

Table 2. Average shoot height of Dendrobium Sp orchid explants treated with Magnesium Sulfate ($MgSO_4$) and Myo-inositol (cm)

Factor M	Factor Y				Average M
	Y0	Y1	Y2	Y3	
M0	0.07d	0.53d	0.77c	0.84c	0.76 d
M1	0.34d	0.82c	0.83c	1.20b	0.87 c
M2	0.86c	0.87c	0.90c	1.27b	0.96 b
M3	0.88c	1.07bc	1.08bc	1.68a	1.08 a
Average Y	0.78b	0.80 b	0.83 b	1.25 a	
KK = 7.73 %		BNJ M = 0.08	BNJ Y = 0.08	BNJ MY = 0.21	

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different according to the Significant Difference Advanced Test (BNJ) at the 5% level.

The data in Table 2 can be seen that the administration of $MgSO_4$ with the best treatment found in M3 (Giving $MgSO_4$ 390 mg/l MS media) namely with a shoot height of 1.08 cm, the results of the follow-up test were significantly different (BNJ) at the 5% level indicating that the M3 treatment was significantly different from M2 (0.96 cm), M1 (0.87 cm) and M0 (0.76 cm).

M3 treatment with $MgSO_4$ concentration (390 mg/l to MS media) gave the best results compared to M1, M2 and M0 concentrations, this was because M3 treatment with $MgSO_4$ concentration (390 mg/l to MS media) was the lowest concentration suitable to be given to Dendrobium Sp. orchid explants. The elements magnesium and sulfur contained in $MgSO_4$ are macro nutrients needed in large quantities for plants, one of which is in the growth of shoot height. Magnesium sulfur plays an important role in the growth of shoot height (Yusnita 2010).

The M0 treatment (administration of $MgSO_4$ mg/l) produced the least shoot height, this was because there was no $MgSO_4$ administration into the MS medium resulting in a low shoot height, because a plant must be given nutrients in sufficient quantities and according to the dose it needs, then the plant will grow well, according to the opinion of Bohn et al. 2004), magnesium and sulfate play an important role as essential molecular components in plant cells.

Based on the table above, it shows that the administration of Myo-inositol significantly affected the parameter of shoot height of Dendrobium sp. with the best treatment found in treatment Y3 (administration of Myo-inositol 150 mg/l into MS media) namely 1.25 cm, from the results of the further difference test honest significant difference (BNJ) at the 5% level showed that treatment Y3 was significantly different from Y1 (Myo-inositol 50 mg/l) was 0.80 cm, Y2 (Myo-inositol 100 mg/l ke) was 0.83 cm and Y0 (Myo-inositol 150 mg/l) was 0.78 cm.

Y3 treatment (Giving Myo-inositol 150 mg/l MS medium) was able to produce shoot height compared to other treatments. This is because Myo-inositol at this concentration is suitable for the needs of Dendrobium Sp orchid plant explants on MS media. This is caused by the multiplication process of explant shoots that play a role is the interaction between the hormones auxin and cytokinin, this hormone interaction spurs the formation of new shoots, but the hormones auxin and cytokinin cannot work alone but must involve vitamins as storage and distribution of these hormones. In the growth of plant tissue, cytokinins together with auxin provide an interacting effect on tissue differentiation, myoinositol also plays a role in storing and distributing these hormones. This is in accordance with the opinion of Chhetri et al. (2019), which states that inositol is a carbohydrate, although it is not a sugar in general, this compound plays a role in the phosphatidylinositol signaling pathway, storage and distribution of auxins and cytokinins. At effective concentrations it will encourage the formation of shoots, on the other hand if the auxin is relatively high from cytokinins it will lead to the formation of roots.

The treatment with Myo-inositol (Y0) resulted in the lowest shoot height, this was because Myo-inositol wasnot added to the MS medium for growth of Dendrobium Sp. orchid shoot height. While Myo inositol functions for the multiplication of explant shoots whose role is the interaction between the hormones auxin and sitakinin, it is this hormone interaction that spurs the formation of new shoots, however the auxin and cytokinin hormones cannot work alone but must involve vitamins as storage and distribution of these hormones, so when this material is not added to MS media it will produce low plant height.

The results of this study when compared with research conducted by Heriansyah et al. (2014), different results were obtained, concluding that the administration of 50 mg/l Myo-inositol into MS media had a significant effect on the height growth of Dendrobium Sp orchid explants with an aver

age -the average shoot height was 2.32 cm, while in this study the administration of 150 mg/l Myo-inositol into the MS media was able to produce a shoot height of 1.25 cm, so the results of this study, even though they had used higher concentrations, the results obtained lower.

Based on the table above, the results of the analysis of variance showed that the interactive treatment of the administration of Magnesium Sulfate ($MgSO_4$) and Myo-inositol had a significant effect on the height of the shoots on the explants of Dendrobium sp. The treatment combination that produced the highest mean value was in the M3Y3 treatment, while the lowest average was in the M0Y0 treatment. The high number of shoots in the M3Y3 treatment was because the concentration of the treatment gave a good response to the Dendrobium Sp. orchid explants. Where M3 (Provision of $MgSO_4$ 390 mg/l) functions to provide macro nutrients which play a very important role for plants, while Y3 (Myo-inositol 150 mg/l).

Number of Leaves (Streams)

Based on Table 3 it can be seen that the $MgSO_4$ administration with the best treatment was found in M3 (390 mg/l MS medium $MgSO_4$ administration), namely with a total of 7.89 leaves, further test results with a significant difference (BNJ) at the 5% level showed that the M3 treatment not significantly different from M2 (7.39 strands), but significantly different from M1 and M0.

Table 3. The average number of leaves of Dendrobium Sp orchid explants with the administration of Magnesium Sulphate ($MgSO_4$) and Myo-inositol (Streams)

Factor M	Factor Y				Average M
	Y0	Y1	Y2	Y3	
M0	4.44c	4.67c	5.44c	7.00b	6.55 c
M1	6.44bc	7.33b	8.11ab	8.00ab	7.33 b
M2	7.56ab	7.33b	8.44ab	8.56ab	7.39 ab
M3	7.67ab	8.11ab	8.67ab	8.89a	7.89 a
Average Y	6.81 b	6.89 b	7.67 a	7.81 a	
KK= 6.76%		BNJ M = 0.55	BNJ Y = 0.55		BNJ MY = 1.47

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different according to the Significant Difference Advanced Test (BNJ) at the 5% level.

M3 treatment with $MgSO_4$ concentration (390 mg/l to MS media) gave the best results compared to M2, M1 and M0 concentrations, this was because M3 treatment with $MgSO_4$ concentration (370 mg/l to MS media) was the lowest concentration. suitable to be given to Dendrobium Sp. orchid explants. The elements magnesium and sulfate contained in $MgSO_4$ are macro nutrients needed in large quantities. sulfate is the main and primary source of sulfur. The integration of reduced sulfur into the amino acid cysteine (catalyzed by cysteine synthase; EC.2.5.1.47) is positioned at a critical stage in the assimilatory sulfate reduction pathway. The importance of cysteine in the zygotic embryogenesis of Arabidopsis thaliana has been reported (Xu & Møller, 2004)). Currently, in plant sulfur biomolecules, sulfur does not only serve as a structural component but is also involved in catalytic, electrochemical or characteristic functions.

The M0 treatment ($MgSO_4$ 0 mg/l) produced the least number of leaves, because in the M0 treatment there was no addition of $MgSO_4$, Magnesium Sulfate ($MgSO_4$) is a macro nutrient that explants really need for the growth process. That if a plant is not given its main food source, its growth process will be disrupted and there is no possibility of abnormal growth. Therefore, if a plant is given nutrients in sufficient quantities and according to the dosage it needs, then the plant will grow well according to the opinion of Sandra (2004), the elements magnesium and sulfur are essential nutrients needed for growth in every plant. $MgSO_4$ contains the element magnesium (Mg) which plays a role in stimulating cell division and making chlorophyll in leaves.

Based on the table above, it shows that the administration of Myo-inositol has a significant effect on the parameters of the number of leaves of Dendrobium Sp. orchid explants. with the best treatment found in treatment Y3 (administration of Myo-inositol 150 mg/l into MS media) namely 7.81 strands, from the results of the further difference test honest significant difference (BNJ) at the 5% level showed that treatment Y3 was not significantly different from Y2 (Giving Myo-inositol 50 mg/l) which was 7.67 strands, but significantly different from the Y1 treatment (giving Myo-inositol 50 mg/l ke) which was 6.89 strands and Y0 (Giving Myo-inositol 0 mg/l) ie 6.81 strands.

Y3 treatment (Giving Myo-inositol 150 mg/l MS medium) was able to produce a faster number of leaves compared to other treatments. This is because Myo-inositol at this concentration is suitable for the needs of Dendrobium Sp orchid plant explants on MS media. This is because the administration of myoinositol has a significant effect on the number of leaves of the orchid explants, this is related to the process of cell division. This process involves several vitamins, one of which is myoinositol which is conjugated with the hormone auxin which plays a role in spurring cell division. This is in accordance with the opinion of Ciarkowska et al. (2023), who explained that myoinositol plays an important role in controlling the auxin hormone. Inositol conjugated with IAA functions as a storage or transport of auxin and can regulate the availability of IAA during plantlet growth.

The treatment of Myo-inositol (Y0) resulted in the least number of leaves, this was because there was no addition of Myo-inositol to the MS medium for the growth of the number of leaves. As a result, if auxin and cytokinins are at effective concentrations, they will encourage the formation of the number of leaves, conversely, if the auxins are relatively high, the cytokinins will lead to the formation of leaves.

The obtained different results, concluding that the administration of 100 mg/l Myo-inositol into the MS base medium had a significant effect on the growth of the number of leaves of Dendrobium Sp orchid explants with an average the number of leaves was 7.25, whereas in this study the administration of 150 mg/l Myo-inositol into MS media was able to produce a total of 7.81 leaves.

Based on the table above, the results of the analysis of variance showed that the interactive treatment of the administration of Magnesium Sulfate ($MgSO_4$) and Myo-inositol had a significant effect on the number of leaves on the explants of Dendrobium sp. The treatment combination that produced the highest mean value was in the M3Y3 treatment, while the lowest average was in the M0Y0 treatment. The large number of leaves in the M3Y3 treatment was because the concentration of the treatment gave a good response to the Dendrobium Sp. orchid explants. Where M3 (Providing $MgSO_4$ 390 mg/l) functions to provide macro nutrients which play a very important role for plants. While Y3 (Myo-inositol 150 mg/l) plays a role in controlling the auxin hormone. Inositol conjugated with IAA functions as a storage or transport of auxin and can regulate the availability of IAA during plantlet growth.

Number of roots (Fruits)

The data in Table 4 can be seen that administration of $MgSO_4$ was not significantly different on the parameters of the number of roots of Dendrobium sp. This is because the concentration of the nutrient $MgSO_4$ given has not been able to give a good response to the number of roots of Dendrobium sp orchid explants. However, if seen from the average value, the number of roots produced in this study was obtained in the M3 treatment by administering a concentration of $MgSO_4$ (390 mg/l), namely 6.33 fruit, followed by M2 ($MgSO_4$ 380 mg/l), namely 5.56 fruit, M1 ($MgSO_4$ 370 mg/l) which is 5.52 fruit and M0 (without $MgSO_4$ 0 mg/l) which is 2.06 fruit. The provision of $MgSO_4$ nutrients has not been able to meet the needs of plants. Even though each plant already has endogenous ZPT, it needs to be given more nutrients so that the plant's need for the right nutrients can be fulfilled properly.

Table 4. The average number of roots of Dendrobium sp orchid explants with the administration of Magnesium Sulphate ($MgSO_4$) and Myo-inositol (Fruits)

Factor M	Factor Y				Average M
	Y0	Y1	Y2	Y3	
M0	1.44d	1.56d	1.89cd	5.11b	2.06 b
M1	3.22cd	3.33cd	4.22bc	5.52b	5.52 b
M2	5.89b	5.89b	6.00b	6.11b	5.56 b
M3	6.22b	7.00ab	7.22a	7.22a	6.33 a
Average Y	4.47c	5.42b	4.61 ab	4.96 a	
KK = 6.40 %		BNJ M = 0.35	BNJ Y = 0.35		BNJ MY = 0.93

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different according to the Significant Difference Advanced Test (BNJ) at the 5% level

Based on the Table 4, it shows that the administration of Myo-inositol has a significant effect on the parameters of the number of roots of Dendrobium Sp. orchid explants. with the best treatment found in treatment Y3 (administering Myo-inositol 150 mg/l into MS media) namely 4.96 fruit, from the results of the further difference test honest significant difference (BNJ) at the 5% level showed that treatment Y3 was not significantly different from Y1 (Giving Myo-inositol 50 mg/l) which is 5.42 fruit, but significantly different from the Y2 treatment (giving Myo-inositol 100 mg/l ke) which is 4.61 fruit and Y0 (without Myo-inositol 0 mg/l) ie 4.47 pieces.

Y3 treatment (Giving Myo-inositol 150 mg/l MS media) was able to produce more roots compared to other treatments. This is because Myo-inositol at this concentration is suitable for the needs of Dendrobium Sp orchid plant explants on MS media. The results of the interaction between auxin and cytokinin will spur the emergence of roots on plantlets so that root formation takes place, because when auxin and cytokinins are at effective concentrations they will encourage the formation of shoots, conversely if auxin is relatively High levels of cytokinins will lead to root formation

The treatment of Myo-inositol (Y0) resulted in the least number of roots, this was because there was no addition of Myo-inositol to the MS medium to stimulate the growth of the number of roots of Dendrobium sp. As a result, if auxin and cytokinins are at effective concentrations, they will encourage the formation of shoots, conversely, if auxins are relatively high, the cytokinins will lead to root formation.

The results of this study when compared with research conducted by (Heriansyah et al., 2014)obtained different results, concluding that the administration of 50 mg/l Myo-inositol into MS media had a significant effect on the growth of the number of roots of Dendrobium Sp orchid explants with an average the average number of roots was 3.00, whereas in this study the administration of 150 mg/l Myo-inositol into MS media was able to produce a total of 4.96 roots.

Based on the table above, the results of the analysis of variance showed that the interactive treatment of the administration of Magnesium Sulfate ($MgSO_4$) and Myo-inositol had a significant effect on the number of roots in Dendrobium sp- orchid explants. The treatment combination that produced the highest score was in the M3Y3 treatment with an average of 5.89, while the lowest was in the M0Y0 treatment with an average of 1.44. The large number of roots in the M3Y3 treatment because the concentration of the treatment gave a good response to the Dendrobium Sp. orchid explants. Where M3 (Providing $MgSO_4$ 390 mg/l) functions to provide macro nutrients which play a very important role for plants.

Root length (cm)

Data on Table 5, it can be seen that $MgSO_4$ administration with the best treatment was found in M3 (390 mg/l MS medium $MgSO_4$ administration), namely with a root length of 1.55 cm. M2 (1.28 cm), M1 (1.23 cm) and M0 (1.13 cm).

Table 5. Average root length of Dendrobium sp orchid explants treated with Magnesium Sulphate ($MgSO_4$) and Myo-inositol (Cm)

Factor M	Factor Y				Average M
	Y0	Y1	Y2	Y3	
M0	0.74d	0.83d	0.88cd	1.13cd	1.13c
M1	1.19c	1.28bc	1.32bc	1.34bc	1.23 bc
M2	1.20c	1.43bc	1.44bc	1.55b	1.28 b
M3	1.31bc	1.43bc	1.69a	2.02a	1.55 a
Average Y	1.13c	1.26bc	1.33 b	1.48a	
KK = 8.77 %		BNJ M = 0.13	BNJ Y = 0.13		BNJ MY = 0.34

Note: the numbers in the row and column followed by the same lowercase letter are not significantly different according to the follow-up test of significant difference (BNJ) at the level of 5%

M3 treatment with $MgSO_4$ concentration (390 mg/l to MS media) gave the best results compared to M2, M1 and M0 concentrations, this was because M3 treatment with $MgSO_4$ concentration (390 mg/l to MS media) was the lowest concentration. suitable to be given to Dendrobium Sp. orchid explants. The provision of $MgSO_4$ nutrients is classified as a macro element that plays an important role in the growth of explants. $MgSO_4$ contains the element magnesium (Mg) which plays a role in promoting cell division and the production of chlorophyll in roots. magnesium and sulfate play important roles as essential molecular components in plant cells.

The M0 treatment (0 mg/l $MgSO_4$ administration) resulted in the least root length, because there was no $MgSO_4$ administration to the media, as a result if a plant is given a sufficient amount of nutrients and in accordance with the dose it needs, the plant will grow well. good and according to what we want.

If this study is compared with the research conducted by Gunawan (2021) then the results are different, in the MS medium used for the nutrient $MgSO_4$ as much as 370 mg/l, $MgSO_4$ administration to the basic media resulted in a root length of 1 .26 cm Dendrobium Sp. orchid plant Whereas in this study the administration of 390 mg/l $MgSO_4$ resulted in a root length of 1.55 cm for the Dendrobium Sp. orchid plant. This is due to the different concentration of $MgSO_4$ given, the resulting response is also different.

Based on the table above, it shows that the administration of Myo-inositol has a significant effect on the root length parameters of Dendrobium Sp. orchid explants. with the best treatment found in treatment Y3 (administering Myo-inositol 150 mg/l into MS media) namely 1.48 fruit, from the results of the further difference test honest significant difference (BNJ) at the 5% level showed that treatment Y3 was significantly different from Y1 (Myo-inositol 50 mg/l) was 1.26 cm, Y2 (Myo-inositol 100 mg/l ke) was 1.33 cm and Y0 (Myo-inositol 0 mg/l) was 1.13 cm.

Y3 treatment (Giving Myo-inositol 150 mg/l MS media) was able to produce root length faster than other treatments. This is because Myo-inositol at this concentration is suitable for the needs of Dendrobium Sp orchid plant explants on MS media. This is in accordance with the opinion of Hegeman et al. (2001) who said that myoinositol plays a role in the biosynthesis of phytic acid. Phytic acid is a form of phosphorus storage which plays a role in mRNA transport to promote root length growth.

The treatment of Myo-inositol (Y0) resulted in the least root length, this was because there was no Myo-inositol in MS media, because Myo inositol played a role in the growth of agar length, as a result if auxin and cytokinins were at effective concentrations it would encourage formation of shoots, on the other hand if auxin is relatively high than cytokinin will lead to the formation of roots.

Yielded different results, concluding that the administration of 100 mg/l Myo-inositol into MS basic media had a significant effect on the growth of root length of chrysanthemum shoot explants (Hesami et al., 2020) with an average length roots 2.34 cm, whereas in this study the administration of Myo-inositol was able to produce root lengths of 1.55 cm. this is due to the different concentrations of the Myo-inositol nutrient given, the resulting response is also different.

Based on the table above, the results of the analysis of variance showed that the interactive treatment of the administration of Magnesium Sulfate ($MgSO_4$) and Myo-inositol had a significant effect on root length in Dendrobium Sp. orchid explants. The treatment combination that produced the highest score was in the M3Y3 treatment with an average of 2.02 cm, while the lowest was in the M0Y0 treatment with an average of 0.74 cm. The number of root lengths in the M3Y3 treatment because the concentration of the treatment gave a good response to the Dendrobium Sp. orchid explants. Where M3 (Providing $MgSO_4$ 390 mg/l) functions to provide macro nutrients which play a very important role for plants. This is because the presence of magnesium plays a role in stimulating cell division and the manufacture of chlorophyll in the roots and is supported by the availability of Myo-inisitol which provides a role as a storage or transport of auxin for the growth of root length.

Conclusion

Administration of 390 mg/l of $MgSO_4$ into Ms (M3) media was the best treatment for the observed parameters and had a significant effect on the parameters of the number of shoots, shoot height, and root length with an average number of shoots 4.04, shoot height 1, 08 cm, and root length 1.55 cm, the number of leaves and the number of roots with an average number of leaves is 7.89 leaves and the number of roots is 6.64 pieces. The administration of Myo-inositol in this study also affected the parameters of the number of shoots, shoot height, number of leaves, number of roots, and root length of Dendrobium Sp orchid explants. 150 mg/l into MS medium) with an average number of shoots of 4.03 and number of leaves of 7.81, shoot height 1.25 cm, number of roots 4.96 and root length 1.48 cm. The interactive treatment of $MgSO_4$ and Myo-inisitol gave a significant effect on each parameter of observation of the growth of Dendrobium Sp orchid explants. The M3Y3 treatment (370 mg/l $MgSO_4$ and 150 mg/l MS Myo-inisitol) was the best treatment for total growth. shoots with an average number of shoots of 5.11, the M3Y3 treatment (administering $MgSO_4$ 390 mg/l and Myo-inisitol 150 mg/l MS) was the best treatment for growth in shoot height and root length with an average height of 1.68 cm and root length of 2 .02 cm, M3Y3 treatment (350 mg/l $MgSO_4$ and 150 mg/l MS Myo-inisitol), the best treatment with an average number of leaves of 8.89 leaves, the best treatment with 7.22 roots.

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Conflicts of Interest

The authors declare no conflict of interest.

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