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EFFECT OF COCONUT WATER AND MONOSODIUM GLUTAMATE ON CHRYSANTHEMUM EXPLANT GROWTH IN VITRO

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Abstract:
This study aims to determine the effect of coconut water and MSG on the growth of chrysanthemum explants in vitro. This study is held in the Tissue Culture Lab of the Faculty of Agriculture of Universitas Klabat from September 2019 to January 2020. This study uses a completely randomized factorial design with two treatment factors, namely coconut water (0%, 15%, and 30%) and MSG (0 ppm, 1 ppm, and 2 ppm). The variables observed were the number of shoots, plant heights, and the number of leaves. Observations were made each week for 8 weeks after the explants were planted (MSK). Coconut's water dosage that gives the highest number of shoots is 15%, while the dosage for the tallest plant heights and the most leaves is 30%. The dosage of MSG that gives the best result for the number of shoots is 2 ppm, and 1 ppm for plant heights and the number of leaves. The best combination of coconut water and MSG for the number of shoots is 15% coconut water and 0 ppm MSG; for plant heights, 30% coconut water and 2 ppm MSG; and for the number of leaves, 15% coconut water and 0 ppm MSG.

Keywords: Coconut water, explants, chrysanthemum, MSG

INTRODUCTION

Chrysanthemum or chrysanthemum means golden flower. In Greek chrysos means gold and anthemon means flower, another name for chrysanthemum is chrysanthemum (Hall for Ornamental Plant Research, 2019). Chrysanthemum is one of the popular ornamental plants used as cut flowers and potted plants and is an important commodity in the world ornamental plant trade.

Indonesia's national chrysanthemum production in the 2014 – 2018 period increased by 14.26% or an average of 3.57% per year, although in 2016 there was a decrease of 2.17%. The largest chrysanthemum harvested area in Indonesia is East Java Province with an area of 5,791,735 m2 in 2018, which is 52.15% of the national harvested area, with chrysanthemum production of 137,886,801 stalks with a productivity of 23.81 stalks/m2. Although North Sulawesi had a harvested area of 66.008 m2 in 2018, which is 0.59% of the national harvested area, productivity can reach 71.97 stalks/m2 with a total harvest of 4,750,606 stalks (Central Bureau of Statistics, 2019).

Chrysanthemum occupies the first position in export value ahead of roses and orchids. In 2018, the export volume of chrysanthemum increased from 49.52 tons to 59.11 tons and the FOB (Free On Board) value increased from US$ 699,176 to US$ 817.208. The only importing country for chrysanthemum from Indonesia in 2018 was Japan, although in 2017 it was Japan and Kuwait. The largest chrysanthemum exporting areas are East Java (38.37%), West Java (28.25%) and Central Java (26.50%) (Central Bureau of Statistics, 2019). One of the largest centers of chrysanthemum outside Java is North Sulawesi, where the center of chrysanthemum production in North Sulawesi is the Tomohon area. North Sulawesi is not yet a chrysanthemum exporting area. The flower market share is still absorbed by the local market, but Tomohon has the opportunity to become an exporter of chrysanthemum flowers, especially to Singapore (Annonimous, 2018).

The productivity and demand for chrysanthemums continues to increase from year to year, thus requiring the availability of new high-yielding varieties and quality seeds on an ongoing basis. The development of chrysanthemum can have a positive impact on the economy in rural areas, especially on increasing the income of farmers and the people involved in its development.

Flower farmers in Tomohon usually cultivate local varieties of chrysanthemum, namely the kulo and riri chrysanthemum varieties, but they also cultivate other varieties of chrysanthemum whose seeds are taken from the island of Java. So the current problem is that the procurement of chrysanthemum seeds still depends on seed producers from outside the city of Tomohon.

The chrysanthemum we know today is a complex hybrid plant, which when grown from seed will result in genetic segregation into individual plants that have very diverse colors and shapes (House of Ornamental Plant Research, 2019). Therefore, the propagation of chrysanthemum is mostly done vegetatively by using cuttings. The disadvantage of using cuttings in chrysanthemum propagation is that it cannot produce large amounts of plants in a short time. Another way of vegetative propagation is by tissue culture. Chrysanthemum plant propagation through tissue culture will be able to produce large numbers of plants in a short time.

The method of vegetative propagation of plants through tissue culture for several species of flower plants such as orchids and chrysanthemums is more efficient than through seeds. Therefore, choosing plants that have high commercial value should be considered in plant propagation through tissue culture so that they can provide benefits (Dwimahyani and Gandanegara, 2001).

Tissue culture is a technique for clonal propagation of plants for mass propagation. The advantages of procuring seeds through tissue culture include obtaining superior plant material in large and uniform quantities, in addition to obtaining sterile cultures (mother stock) so that they can be used as material for further propagation (Lestari, 2008). Tissue culture is a method for isolating parts of plants such as a group of cells or tissues that are grown under aseptic conditions, so that these plant parts can reproduce and grow into complete plants again (Hameed N, Shabbir A, Ali A, Bajwa R., 2006).

The implementation of tissue culture techniques requires various prerequisites to support the life of the cultured tissue. The most essential thing is a sterile container and growing media. The composition of the media used in tissue culture can be different in composition. Differences in media composition may result in differences in growth and development of explants grown in vitro. Murashige and Skoog (MS) media are often used because they are sufficient to meet macro, micro and vitamin nutrients for plant growth (Marlina, 2004).

The nutrients available in the media are useful for metabolism, and the vitamins in the media are required by organisms in small amounts for regulation. In MS media, there were no growth regulators (PGR) therefore PGR was added to the media (exogenous). ZPT or plant hormones affect plant growth and development. The interaction and balance between PGR that is given in the medium (exogenous) and produced by cells endogenously determines the direction of development of a culture (Soomro R, Yasmin S, Aleem R, 2003).

To obtain optimum results, the use of appropriate growth regulators is an important factor. The combination of basic media and appropriate growth regulators will increase cell division activity in the process of morphogenesis and organogenesis.

The implementation of this tissue culture technique is still constrained by the high cost of chemicals, especially ZPT. The application of cytokinins in in vitro plant propagation can be derived from synthetic chemicals or natural materials such as coconut water. Coconut water is a natural ingredient that has cytokinin activity for cell division and encourages organ formation. The concentration of coconut water commonly used in tissue culture is 2 - 15% (Trigiano, R.N. and J.G. Dennis, 2000).

The results of the research of Prihatmanti, D. and N.A. Mattjik (2004) found that the use of natural ingredients coconut water at a concentration of 100 to 200 ml/l for shoot multiplication of Anthurium andreanum can increase the growth power of in vitro cultures. Furthermore, Bey, Y., W Syafil and Sutrisna (2006) suggested that the treatment of coconut water alone at a concentration of 250 ml/l was able to produce leaves and roots faster in in vitro culture of orchids (Phalaenopsis amabilis BL.). Katuuk (2000) also observed that by giving 250 ml/l of coconut water, it showed the most appropriate time for germination of tiger orchid seeds (Grammatohyllum scirptum) where pulp appeared more quickly in the combination treatment of Giberilin + coconut water with a combination of GA 2 ppm + water. coconut 250 ml/l, because it can exogenously affect the ratio of endogenous PGR in the seed, so that the pulp is able to grow at the right time. The results of the study (Seswita, 2010) showed that without chemical components, with the addition of coconut water at various concentrations on MS base media, it succeeded in forming shoots, leaves and roots in the multiplication of temulawak shoots.

Monosodium glutamate (MSG), also known as sodium glutamate, is the sodium salt of glutamic acid that is white at room temperature. This MSG compound is soluble in water and alcohol. Glutamic acid is an amino acid involved in the biosynthesis of other amino acids and nitrogen-rich compounds such as nucleic acids, chlorophyll, hormones and secondary metabolite products (Okoye, C.N., Ochiogu, I.S. and Onah, C.E., 2006). Nucleic acids, chlorophyll and hormones are compounds that are important for plant growth.

Various studies have been conducted using coconut water as PGR added to MS media in in vitro plant propagation, but so far there has been no use of MSG added to MS media.

Based on the description above, the researchers are interested in conducting research on the effect of coconut water and MSG on the growth of chrysanthemum explants in vitro. with the title: "Effect of Coconut Water and Monosodium Glutamate on In Vitro Growth of Chrysanthemum Explants.

The problem to be formulated in this research is whether there is an effect of coconut water treatment, monosodium glutamate treatment and the effect of a combination of coconut water and monosodium glutamate treatment on the growth of chrysanthemum explants in vitro, which is described by the number of shoots, shoot height and number of leaves.

The hypothesis to be tested is that coconut water treatment has a significant effect on the growth of chrysanthemum explants in vitro, MSG treatment has a significant effect on the growth of chrysanthemum explants in

vitro, and the combination treatment of coconut water and MSG has a significant effect on the growth of chrysanthemum explants in vitro.

The following sections of this paper are structured as follows, research methods, results and discussion of research, and the last section contains conclusions and suggestions.

RESEARCH METHODS

This research was carried out at the Biotechnology Laboratory, Klabat University, Manado, North Sulawesi, starting from September 2019 to January 2020.

The materials used were chrysanthemum shoots, agar as a compactor, sucrose, coconut water, MSG, 70% and 95% alcohol and sterile aquadest, and other materials that support this research. the basic media used was MS media (Murashige and Skoog) plus several ratios of MSG and Coconut Water concentration. The tools used are glass equipment (culture bottles, measuring cups, beaker glass, erlenmeyer, and petridish), analytical balance, pH meter, autoclave, Laminar Air Flow (LAF), dissection equipment (tweezers, scissors and scalpel), stirrer, lamp spiritus, culture rack with 40 watt lamp and other tools that support this research.

Using a factorial design in a completely randomized design, namely a combination of coconut water in 3 levels and MSG in 3 levels, so that 9 treatment combinations were obtained and repeated 10 times. For treatment the concentration of Coconut Water and MSG is as follows, 0% Coconut Water + 0 ppm MSG; 0% Coconut Water + 1 ppm MSG; 0% Coconut Water + 2 ppm MSG; 15% Coconut Water + 0 ppm MSG; 15% Coconut Water + 1 ppm MSG; 15% Coconut Water + 2 ppm MSG; 30% Coconut Water + 0 ppm MSG; 30% Coconut Water + 1 ppm MSG; 30% Coconut Water + 2 ppm MSG.

The variables observed were the number of shoots counted at 1, 2, 3, 4, 5, 6, 7, and 8 weeks after culture (MSK); explant heights were measured at 1, 2, 3, 4, 5, 6, 7 and 8 MSK; and the number of leaves counted at 1, 2, 3, 4, 5, 6, 7 and 8 MSK. The data obtained in this study were processed with analysis of variance and continued with Duncan's test.

RESEARCH RESULT

Number of Shoots

The data on the average number of shoots are presented in Table 1.

Table 1. Average Number of Shoots in Various Doses of Coconut Water and MSG

Treatment	Age (MSK)					
	3	4	5	6	7	8
Coconut water						
0%	1.73a	1.50a	1.89a	1.90a	2.08a	2.42a
15%	-	2.40b	3.20b	4.00b	4.12b	4.38b
30%	1.50a	1.57a	1.75a	2.13a	2.20a	3.20ab
MSG						
0 ppm	1.33a	2.11a	2.60a	3.36a	3.37a	3.50a
1 ppm	1.33a	1.17b	1.43b	1.70b	2.16b	2.85a
2 ppm	2.00a	1.80ab	2.20ab	2.50ab	2.50b	3.45a
Combination						
K0M0	1.50a	1.60a	2.40ab	2.60b	2.83b	3.17ab
K0M1	1.25a	1.33a	1.25a	1.20a	1.33a	1.67a
K0M2	2.20a	2.23b	2.33	2.50b	2.75ab	3.13ab
K15M0	-	2.75b	3.25b	4.00c	4.17c	4.17c
K15M1	-	-	1.00a	4.00c	5.00c	5.00c
K15M2	-	1.00a	1.00a	1.20	5.00c	5.00c
K30M0	1.00a	1.20a	1.00a	1.10	4.00c	2.50a
K30M1	1.50a	1.50a	1.00a	1.75ab	2.33ab	3.23ab
K30M2	1.67a	2.00ab	2.20ab	2.50b	2.14ab	3.30ab

Note: The numbers in the column from the same treatment group, followed by the same notation, are not significantly different at the 5% level according to Duncan's multiple-distance test.

Treatment of coconut water on the observation of the number of shoots at 3 weeks after culture (MSK) did not show a significant effect, but the treatment of 4-8 MSK showed a significant difference. The best treatment that gave the highest number of shoots was a dose of 15% coconut water.

MSG treatment did not have a significant effect on observations 3 and 8 MSK, but had a significant effect on observations 4-7 MSK. The best treatment that gave the highest number of shoots was 0 ppm MSG.

In the combination treatment, the 3 WAP observations did not show a significant difference, but the 4-8 MSK observations showed a significant difference.

In the observations of 4 and 5 MSK, the highest number of shoots was found in K15M0 treatment, at 6 MSK the highest number of shoots was in K15M0 and K15M1. The highest number of shoots at observations 7 and 8 MSK was found in K15M1 and K15M2 treatments.

Plant height

Treatment of coconut water on plant height observations at 1 MSK did not show a significant effect, but the treatment 2-8 MSK showed a significant difference. The best treatment that gave the highest plant height was a dose of 30% coconut water (Table 2)

MSG treatment did not have a significant effect on observations 1, 2, 3 and 8 MSK, but had a significant effect on observations 4-7 MSK. The best treatment that gave the highest plant height was MSG 1 ppm.

Table 2. Average Plant Height (cm) in Various Doses of Coconut Water and MSG

Treatment	Age (MSK)							
	1	2	3	4	5	6	7	8
Coconut water								
0%	0.75a	1.42a	1.70a	2.49a	3.35a	3.91a	4.71a	5.46a
15%	1.26a	1.74b	2.27b	3.33b	4.32b	4.89b	6.05b	6.92b
30%	1.37a	2.00c	2.97c	3.95c	5.00c	5.96c	7.11c	8.06c
MSG								
0 ppm	1.12a	1.67a	2.25a	3.04a	3.93a	4.59a	5.52a	6.58a
1 ppm	1.16a	1.78a	2.37a	3.42b	4.39b	5.22b	6.22b	6.90a
2 ppm	1.09a	1.72a	2.34a	3.32b	4.35b	4.96b	6.12b	6.97a
Combination								
K0M0	0.83a	1.47a	1.78a	2.64a	3.41a	4.10ab	4.68a	5.68ab
K0M1	0.78a	1.46a	1.73a	2.52a	3.44a	3.92a	4.73a	5.38a
K0M2	0.64b	1.31a	1.58a	2.29a	3.18a	3.72a	4.71a	5.30a
K15M0	1.34cd	1.75b	2.18b	3.07b	4.07b	4.63bc	5.58b	6.27bc
K15M1	1.24c	1.72b	2.18b	3.23b	4.34b	5.04c	6.12bc	6.93cd
K15M2	1.23c	1.76b	2.44b	3.68c	4.52b	4.98c	6.40c	7.54de
K30M0	1.24c	1.79b	2.49b	3.42bc	4.32b	5.07c	6.34c	7.85e
K30M1	1.46d	2.16c	3.16c	4.46e	5.34c	6.62d	7.72d	8.32e
K30M2	1.41d	2.07c	2.95c	3.96d	5.34c	6.17d	7.23d	7.99e

Note: The numbers in the column from the same treatment group, followed by the same notation, are not significantly different at the 5% level according to Duncan's multiple-distance test.

In the combination treatment there were significant differences in all observations. The best plant height was in the K30M1 treatment, which was a combination of 30% coconut water and 1 ppm MSG treatment.

Number of Leaves

The data on the average number of leaves are presented in Table 3.

Table 3. Average Number of Leaves in Various Doses of Coconut Water and MSG

Treatment	Age (MSK)							
	1	2	3	4	5	6	7	8
Coconut water								
0%	1.49a	3.26a	5.33a	7.89a	10.75a	13.04a	15.18a	18.07a
15%	2.07b	4.36b	7.05b	9.69b	13.03b	15.69b	17.80b	21.68b
30%	2.10b	4.83a	7.31b	10.32b	13.56b	16.11b	18.53b	23.32b
MSG								
0 ppm	2.02a	4.22a	6.83a	9.93a	13.47a	16.52a	18.69a	22.36a
1 ppm	1.88ab	4.10a	6.64a	9.15a	12.05a	14.27b	16.90b	20.84a
2 ppm	1.77a	4.14a	6.26a	8.88a	11.84a	14.05b	15.89b	19.93a
Combination								
K0M0	1.55ab	3.35ab	5.40ab	8.45ab	12.05b	15.15bc	17.45b	20.80abc
K0M1	1.63ab	3.40ab	6.00bc	8.37ab	11.05ab	13.11ab	15.26ab	18.44ab
K0M2	1.28a	3.00a	4.56a	6.78a	9.00a	10.61a	12.56a	14.47a
K15M0	2.21cd	4.47c	8.00e	11.79d	16.16c	20.11d	22.63c	28.05d
K15M1	1.85bc	4.05bc	6.30bcd	8.35ab	11.70ab	13.90ab	15.80ab	19.10ab
K15M2	2.15cd	4.55c	6.90cde	9.05bc	11.40ab	13.30ab	15.20ab	18.20ab
K30M0	2.30d	4.85c	7.16cde	9.65bc	12.26b	14.37abc	16.05ab	18.32ab
K30M1	2.15cd	4.85c	7.60de	10.70cd	13.35bc	15.75bc	19.55bc	24.75bcd
K30M2	1.84bc	4.79b	7.15cde	10.60cd	15.17c	18.33cd	20.00bc	27.00bc

Note: The numbers in the column from the same treatment group, followed by the same notation, are not significantly different at the 5% level according to Duncan's multiple-distance test.

Treatment of coconut water on the variable number of leaves had a significant effect on all observations. The best treatment that gave the highest number of leaves was a dose of 30% coconut water.

MSG treatment did not have a significant effect on observations 2, 3, 4, 5 and 8 MSK, but had a significant effect on observations 1, 6 and 7 MSK. The best treatment that gave the highest number of leaves was MSG 1 ppm.

In the combination treatment, all observations showed that there were significant differences. The highest number of leaves in observations 1 and 2 MSK was in the K30M0 treatment, namely the 30% coconut water treatment without MSG. In the observation of 3-8 MSK, the highest number of leaves was found in the K15M0 treatment, namely the coconut water treatment without MSG.

CONCLUSION

Based on the results of the study it can be concluded:

1. Coconut water treatment had a significant effect on the number of shoots at observations 4-8 MSK, significantly affected plant height at observations 2-8 MSK, and significantly affected the number of leaves at observations 1-8 MSK.
2. The MSG treatment had a significant effect on the number of shoots at observations 3 and 8 MSK, had a significant effect on plant height at observations 4-7 MSK, and had a significant effect on the number of leaves at observations 1, 6, and 7.
3. The combination treatment of coconut water and MSG had a significant effect on the number of shoots at observations 4-8 MSK, had a significant effect on plant height and number of leaves in all observations.
4. Coconut water which gives the best number of shoots is at a dose of 15%, plant height and the best number of leaves at a dose of 30%. The best dose of MSG for the number of shoots is 2 ppm, for plant height and number of leaves 1 ppm. The best combination of coconut water and MSG for the number of shoots was K15M0, for plant height was K30M2, and the number of leaves was K15M0.

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