Jurnal Natural
Vol. 22, (2) 2022
DOI 10.24815/jn.v22i2.25778
pISSN 1411-8513
eISSN 2541-4062
ORIGINAL RESEARCH

Utilization of shallot extract and application of indole acetic acid to *Chrysanthemum sp.* in vitro culture

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Abstract. *Chrysanthemum sp.* is a plant that has the potential to be developed in Indonesia, apart from being an ornamental flower, it can also be used as a herbal medicine. One of the efforts to develop chrysanthemum can be done in vitro by using organic growth regulators such as shallot extract and indole acetic acid (IAA). The research aims to identify the effect of shallot extract and IAA on the growth of *Chrysanthemum sp*. The research was conducted at UPT Central Horticulture Seed Center, Medan Johor, in December 2019 - January 2020. The research used a factorial Completely Randomized Design (CRD) with 2 factors, namely the first factor was shallot extract which consisted of 4 levels, namely 0, 35, 70, and 105 g/L water. The second factor was IAA which consisted of 4 levels, namely 0.6, 0.9, 1.2, and 1.5 mg/L water. The results showed that the application of shallot extract had no significant effect on the growth of chrysanthemum, but the application of IAA had a significant effect on the number of leaves and plant height with the best concentration at 0.6 mg/L water. The interaction of the two treatments also had no significant effect on the growth of chrysanthemum.

Keywords: *Chrysanthemum sp.*, shallot extract, indole acetic acid, in vitro

INTRODUCTION

Chrysanthemum is an ornamental flower plant in the form of shrubs originating from the mainland of China. Chrysanthemum originating from mainland China, known as *Chrysanthemum indicum* (yellow), *Chrysanthemum morifolium* (purple), *Chrysanthemum daisy* (round). Apart from being a beautiful ornamental plant, chrysanthemum can also be used as an herbal medicinal plant. Chrysanthemum usually contains antioxidants that are able to absorb toxins in the body, but their use is not yet popular as medicine [1].

The production of chrysanthemums in Indonesia is starting to increase from year to year. This increase in production shows that Indonesia has business potential for the development of chrysanthemum plants. The chrysanthemum business in Indonesia has considerable export opportunities along with the increasing demand for chrysanthemums, the population and changes in people's lifestyles.

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Received: April 2022 | Revised: June 2022 | Accepted: June 2022

Chrysanthemum exports are carried out to several countries including Japan, Saudi Arabia, Kuwait, Pakistan and the United Arab Emirates [2]. However, the availability of chrysanthemum seeds is limited. This can be overcome through propagation by in vitro culture. In vitro culture has enormous potential in plant breeding programs and the provision of quality seeds and seedlings [3].

Various types of growth media that can be used in in vitro culture, one of which is Murashige and Skoog (MS) media. According to [4], the media commonly used to grow chrysanthemums is Murashige and Skoog (MS) media. MS media is a medium with complete nutritional content. Murashige and Skoog (MS) media contains macro nutrients, micro nutrients, vitamins, carbohydrates, amino acids, and growth regulators [5].

Growth regulators have a function to stimulate germination, root and shoot growth [6]. The use of growth regulators depends on the purpose of the in vitro culture [7]. The growth regulators used in this study were shallot extract and Indole Acetic Acid (IAA). Shallots contain compounds that can provide fertility for plants so that they can accelerate the growth of flowers and fruit. These compounds are also very good

for plants because they can trigger root growth which will trigger increased plant stem growth of rose [8]. In addition, IAA is a natural auxin group of phytohormones that is active in small amounts. At sufficient concentrations the IAA hormone will cause cell elongation and enlargement, as well as change gene expression rapidly, as a constituent of cell walls so that it will affect the development of a plant [9].

Information on the use of shallot extract combined with IAA on plant growth in vitro is still limited, so it is necessary to conduct a study that aims to determine the effect of using shallot extract and IAA on the growth of chrysanthemum by in vitro.

METHODOLOGY

Explant material used

Explant material is taken from healthy shoots, free from dwarf diseases and viruses, then explants are taken from plant parts age 7 month that are growing fast, for example, young shoots, both shoots, axillary shoots, then washed thoroughly and cut off the shoots, then the explants were dipped in a mixture of mankozeb of 2 g/L during 1 hour and the last washed with sterile water.

Media preparation

The media used in this research is MS media. To facilitate this work, stock solutions are prepared with predetermined solution and vitamin compositions. All these solutions are separated from each other. After mixing the solution, the pH was measured from 5.5 to 5.8. Then mixed agar and heated until boiling. After that, the application of shallot extract and indole acetic acid as a plant growth regulator with a concentration according to the treatment. Then put it in a culture bottle and cover it with aluminum foil.

Preparation of planting materials

Sterilization was carried out in laminar air flow cabinet by inserting chrysanthemum explant into an erlenmeyer containing 75% alcohol.

Planting of explants

The explant used was nodes taken from plantlets by cutting with sterile scissors 1 node in 1 bottle. After cutting the explant, and after explant preparation, it is ready to be planted vertically.

Maintenance

Explant sterilization was carried out by spraying alcohol into the explant bottle 2 times a week. If contaminated plants are found, they are immediately removed from the culture room, with a room temperature of 20ºC.

Data analysis

This research used a Factorial Completely Randomized Design (CRD) with 2 factors and 3 replications, the first factor was the shallot exctract concentration (B) with 4 levels, consisting of B_1 = 0 g/L water, B_2 = 35 g/L water, B_3 = 70 g/L water, B_4 = 105 g/L water. The second factor is Indole Acetic Acid concentration (I) with 4 levels, consisting of I_1 = 0,6 mg/L water, I_2 = 0,9 mg/L water, I_3 = 1,2 mg/L water, I_4 = 1,5 mg/L water.

The parameters observed: the percentage of live explants, number of shoots, number of leaves, plantlet height and number of roots.

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 23.0. The analysis of variance (ANOVA) procedure for a factorial experiment was used to test for a significant effect of treatments, followed by Duncan Multiple Range Test (DMRT) for comparisons of different means of different treatments.

RESULTS AND DISCUSSION

Percentage of live explants

The results of variance analysis showed that the application of shallot extract, indole acetic acid and the interaction of the two treatments had no significant effect on the percentage of live explants. The mean percentage of live explants can be seen in Table 1.

Table 1. Percentage of live explants aged 5 Week After Planting (WAP)

Shallot extract	Indole Acetic Acid				Mean
	I_1	I_2	I_3	I_4	
	----------%----------				
B_1	100	100	100	100	100
B_2	100	100	100	100	100
B_3	100	100	100	100	100
B_4	100	100	100	100	100
Mean	100	100	100	100	

Based on Table 1, it can be seen that the percentage of live explants with application of shallot extract and IAA was 100% in all treatments.

The application of shallot extract and IAA at various concentrations had no significant effect on the percentage of explants survival. This is due to the balance of exogenous growth regulators with endogenous hormones from

explants so that they can support the growth of explants. [10] stated that auxins and cytokinins work together to create optimum conditions for explant growth. The interaction between endogenous hormones and the given growth regulators will be able to support the survival of explants. In addition, [11] explained that the high percentage of live explants was also due to the fact that the composition of the substance in the media was suitable to support the life of explants.

Number of shoots

The results of variance analysis showed that the application of shallot extract, indole acetic acid and the interaction of the two treatments had no significant effect on the number of shoots. The mean number of shoots can be seen in Table 2.

Table 2. Number of shoots aged 5 Week After Planting (WAP)

Shallot extract	Indole Acetic Acid				Mean	St. Dev
	I_1	I_2	I_3	I_4		
	----------shoot----------					
B_1	0.83	0.66	0.5	0.33	0.58	0.21
B_2	0.5	0.5	0.66	0.33	0.5	0.13
B_3	0.83	0.83	0.33	0.5	0.62	0.25
B_4	0.66	0.83	0.33	0.5	0.58	0.21
Mean	0.7	0.7	0.45	0.41		
St. Dev	0.16	0.16	0.16	0.10		

Based on Table 2, it can be seen that the number of shoots varied with the application of shallot extract and IAA. The highest number of shoots with the application of shallot extract was found at shallot extract concentration of 105 g/L was 0.62 shoots and the application of IAA was found at concentration of 0.6 mg/L and 0.9 mg/L was 0.70 shoots. The best interaction treatment at shallot extract concentration of 0 g/L and IAA concentration of 0.6 mg/L, shallot extract concentration of 70 g/L and IAA concentration of 0.6 mg/L, shallot extract concentration of 70 g/L and IAA concentration of 0.9 mg/L, shallot extract concentration of 105 g/L and IAA concentration of 0.9 mg/L was 0.83 shoots, but statistically not significantly different.

Table 2 shows no significant effect on the application of shallot extract and IAA with various concentrations on the number of shoots. This is presumably due to the very significant difference in the concentration of auxin and cytokinin compounds. Differences in the concentration of auxin and cytokinin compounds greatly affect shoot growth. It is known that shallot extract has a fairly high auxin content which is combined with IAA which contains auxin. Auxin compounds play a role in spurring the process of elongation and development of cells, while cytokinin compounds play a role in spurring shoot growth. Differences in the concentration of auxin and cytokinin compounds that are not suitable cause inhibition of shoot growth. According to [12] organic growth regulators contain different auxin and cytokinin hormones. If the concentration of auxin is greater than that of cytokinins then callus will be formed, whereas if the concentration of cytokinins is greater than the concentration of auxin then it is not callus that is formed, but shoots.

Number of leaves

The results of variance analysis showed that the application of indole acetic acid had a significant effect on the number of leaves, but the application of shallot extract and the interaction of the two treatments had no significant effect on the number of leaves. The mean number of leaves can be seen in Table 3.

Table 3. Number of leaves aged 5 Week After Planting (WAP)

Shallot extract	Indole Acetic Acid				Mean	St. Dev
	I_1	I_2	I_3	I_4		
	----------blade----------					
B_1	14.5	16.33	9.33	13.75	13.47	2.57
B_2	12.33	10.83	11.83	9.33	11.08	1.15
B_3	12.5	8.16	11.5	7.16	9.83	2.23
B_4	15.83	12.83	9.33	8.66	11.66	2.88
Mean	13.79 a	12.04ab	10.50bc	9.72c		
St. Dev	1.68	3.44	1.35	2.83		

Note: Numbers followed by different letters in the same column are significantly different according to DMRT at 1%.

Based on Table 3, it can be seen that the highest number of leaves was found in IAA concentration of 0.6 mg/L was 13.79 blade which was not significantly different with IAA concentration of 0.9 mg/L , was 12.04 blade, but significantly different with IAA concentration of 1.2 mg/L treatment was 10.50 blade and IAA concentration of 1.5 mg/L was 9.72 blade. The difference number of leaves at each treatment can be seen in Figure 1.

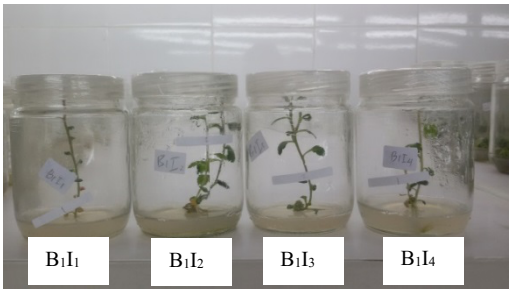

Figure 1. The number of leaves difference formed in each treatment

The relationship between application of IAA concentration on the number of leaves can be seen in Figure 2.

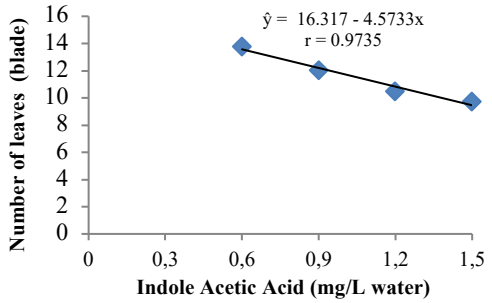

Figure 2. The relationship between IAA concentration on the number of leaves

Figure 2 shows a negative linear graph with the equation = 16,317 - 4.5733x and r = 0.9735 which shows the higher the dose of IAA given, the lower the number of leaves. The number of leaves aged 5 MST showed that the best concentration of IAA was 0.6 mg/L water in stimulating the formation of chrysanthemum leaves. It is suspected that giving too high a concentration can inhibit the plant itself from growing, because basically plants already have endogenous auxins, namely auxins that are processed by the plant cells to grow. Giving auxin to a plant must be in accordance with the levels required by the plant, because giving auxin that is too low or too high will also affect plant growth. This is in accordance with [13] that the balance of growth regulators added and processed by plant cells naturally determines plant growth. According to [14] also causes that the concentration of IAA is too high, causing plants to synthesize other growth regulators, namely ethylene, which has the opposite effect on IAA.

Plantlet height

The results of variance analysis showed that the application of indole acetic acid had a significant effect on the plantlet height, but the application of shallot extract and the interaction of the two treatments had no significant effect on the plant height. The mean plantlet height can be seen in Table 4.

Table 4. Plantlet height aged 5 Week After Planting (WAP)

Shallot extract	Indole Acetic Acid				Mean	St. Dev
	I_1	I_2	I_3	I_4		
	----------cm----------					
B_1	11.36	12.65	9.4	10.91	11.08	1.16
B_2	10.5	11.66	12.26	7.98	10.6	1.64
B_3	11.5	7.7	10.71	7.06	9.24	1.90
B_4	12.06	12.8	8.86	7.6	10.33	2.16
Mean	11.35a	11.20a	10.31b	8.39b		
St. Dev	0.65	2.39	1.52	1.72		

Note: Numbers followed by different letters in the same column are significantly different according to DMRT at 1%.

Based on Table 4, it can be seen that the highest plantlet height at 5 WAP was found in IAA concentration of 0.6 mg/L was 11.35 cm, which was not significantly different with IAA treatment of 0.9 mg/L was 11.20 cm, but significantly different with IAA concentration of 1.2 mg/L was 10.31 cm and IAA concentration of 1.5 mg/L was 8.39 cm. The relationship between application of IAA on the plantlet height can be seen in Figure 3.

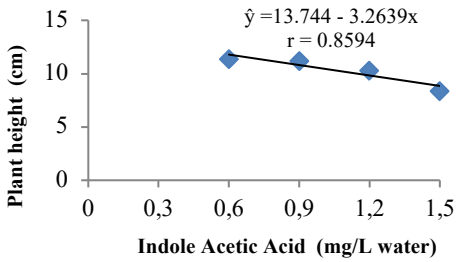

Figure 3. The relationship between IAA concentration on the plantlet length

Figure 3 shows a negative linear graph with the equation = 13.744 - 3.2639x and r = 0.8594 which shows that the higher the dose of IAA given, the lower the plantlet height. Plantlet height on chrysanthemum aged 5 WAP showed that the best concentration of IAA was given with a concentration of 0.6 mg/L water in stimulating the growth of chrysanthemum

plantlet height. It is suspected that giving too high a concentration can cause auxin not work optimally. In line with [15] which states that auxin will be able to work optimally in low concentrations, whereas in high concentrations it will inhibit plant growth. Furthermore, [16] stated that auxin causes cells in stems to release hydrogen ions throughout the cell wall which then lowers the pH and results in a decrease in cell walls and rapid plant growth.

Number of roots

The results of variance analysis showed that the application of shallot extract, indole acetic acid and the interaction of the two treatments had no significant effect on the number of roots. The mean number of roots can be seen in Table 5.

Table 5. Number of roots aged 5 Week After Planting (WAP)

Shallot extract	Indole Acetic Acid				Mean	St. Dev
	I_1	I_2	I_3	I_4		
	----------root----------					
B_1	2.16	3	3.5	2	2.66	0.61
B_2	5.16	2.83	2.33	3	3.33	1.08
B_3	1.33	3.33	2.66	1.5	2.2	0.83
B_4	5.5	4.83	2.5	2.5	3.83	1.35
Mean	3.54	3.5	2.75	2.25		
St. Dev	2.10	0.91	0.52	0.65		

Based on Table 5, it can be seen that the number of roots varied with the application of shallot extract and IAA. The highest number of roots with the application of shallot extract was found at shallot extract concentration of 105 g/L was 3.83 roots and the application of IAA was found at IAA concentration of 0.6 mg/L was 3.54 roots. The best interaction treatment at shallot extract concentration of 105 g/L and IAA concentration of 0.6 mg/L was 5.50 roots, but statistically not significantly different. The morphology of the formed roots can be seen in Figure 4.

Figure 4. Morphology of chrysanthemum root

Table 5 shows no significant effect on the application of shallot extract and IAA with various concentrations on the number of chrysanthemum roots. This is presumably due to the inappropriate concentration of auxin compounds. Auxin is a very good plant growth regulator compound to stimulate root growth if the concentration is right, improper concentration can cause poor root growth and even roots can not grow at all. Thus some types of auxin used can also inhibit root growth. According to [17], the action of auxin affects the elongation of root cells by flexing the cell wall. While at high concentrations, root growth decreased, this indicates that growth requires the right concentration of auxin. Improper concentration will not stimulate growth and will even inhibit root growth. Application of growth regulators will be efficient if applied with the right concentration [18, 19]. In addition, [20] said that endogenous and exogenous factors affect the rooting of cuttings. To accelerate the rooting of cuttings, can be do by hormones treatment. The hormone addition from the outside must pay attention to the amount and concentration in order to get a good root system.

CONCLUSION

The application of shallot extract and treatment interactions did not significantly affect the growth of chrysanthemum, but the application of IAA significantly affected the number of leaves and plantlet height of chrysanthemum. The application of IAA with a concentration of 0.6 g/L water can increase the number of leaves and plantlet height of chrysanthemum in vitro.

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