

The Effect of Foliar Fertilizers as Substitution Media on The Growth of *Chrysanthemum morifolium* Shoot by In Vitro Culture

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Article History: Received : 23 November 25, Final Revision: 2 December 25, Accepted: 15 January 25, Online Publication: 30 January 25

ABSTRACT

The production of chrysanthemum plants in large quantities can be obtained by in vitro growth of chrysanthemums. The growth media that is often used is MS media which has a relatively high price. Therefore, a substitution medium that has a relatively affordable price is needed in the form of foliar fertilizer media with coconut water and ambon bananas added. This study aims to examine the best medium that can be used for in vitro chrysanthemum growth. The research was carried out in June-September 2020 at the In Vitro Culture Laboratory, Faculty of Agriculture, University of Muhammadiyah Yogyakarta. The research was carried out by a single-factor experimental method with 7 treatments and each had 5 replicates and each replicate consisted of 3 samples arranged with a Complete Random Design (RAL). The treatment that was experimented with was the use of foliar fertilizers in the form of Growmore and Hyponex Green with the addition of coconut water and bananas. The concentrations of Growmore foliar fertilizer given were 3 g/l, 4 g/l, and 5 g/l. The concentrations of Green Hyponex foliar fertilizer given were 3 g/l, 4 g/l, and 5 g/l. The results of the research showed that foliar fertilizer added with coconut water and banana ambon could be used as a substitution medium for MS media in chrysanthemum growth in *vitro*. The use of Growmore 4 g/l foliar fertilizer shows the best hail against the tall growth of chrysanthemum shoots in vitro

Keywords: *Chrysanthemum*, *foliar fertilizer*, *tissue culture*, *shoot growth*

INTRODUCTION

Krisan (*Chrysanthemum morifolium* Ramat) is one type of ornamental plant that is loved by many people. This is because chrysanthemums have very beautiful and attractive flowers so they are often used as ornamental plants in the form of *pot-plant* or as cut flowers to be used as flower arrangements and room decorations on important days (Purwanto & Tri, 2013). Chrysanthemums are

not only in demand by local and foreign communities, with the demand level reaching 59.11 tons in 2018 (BPS, 2018)

The high demand for chrysanthemums will be met if propagation is done properly and correctly. Chrysanthemums can be propagated both generatively and vegetatively. Propagation of chrysanthemum plants generatively is rarely

carried out because it has a low success rate and the seedlings obtained are heterozygous. Vegetative propagation of chrysanthemums is often carried out by shoot cuttings, but this type of propagation has not been able to meet the demand for chrysanthemums due to the limited availability of mother plants and the constraint on the decline in seedling quality in line with the increase in the age of the mother plant (Rukmana and Mulyana, 1997).

One of the efforts to overcome the obstacles to chrysanthemum propagation is *in vitro* cultural propagation. *In vitro culture* is a technique of isolating plant parts to be grown in glass bottles containing complete nutrients in a sterile environment (Oman, 2018). *In vitro* propagation since 40 years ago and over the past 20 years has helped researchers to produce plants on a large scale, one of which is the propagation of chrysanthemum plants (BBPP Lembang, 2017).

In vitro culture, the propagation medium that is often used to grow chrysanthemums *in vitro* is *Murashige and Skoog* (MS) media (Dixon, 1985 in Kristiati, et al., 2016) which is supplemented with growth regulators (ZPT) in the form of cytokinins and auxins. The use of auxins (NAA or IBA) along with cytokinins (BAP) can spur the growth of explants, especially in the intensive formation of leaves, buds and internodes (Gunawan, 1988). However, to make MS media requires a relatively expensive cost, so other alternative media that has a more affordable price is needed.

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As for alternative media as a source of vitamins and ZPT, bananas and coconut water can be used. The vitamins contained in 100 g of bananas are B1 2%, B2 5%, B3 4% and B6 28% (Suryati and Supriyadi, 2008). In addition, according to Sampath *et al.* (2012), in 100 grams of bananas ambon contain vitamin C of 72 mg. Based on the results of Hermawati's (2013) research, the addition of 50 g/l of ambon banana extract to MS media as a source of vitamins in the growth of chrysanthemums (*Chrysanthemum indicum* L.) turned out to have a better effect on the parameters of the number of shoots, the number of leaves, the number of roots and the height of the stem, compared to the addition of ambon bananas with concentrations of 25 g/l, 75 g/l, 100 g/l, and 125 g/l. The content of hormones or ZPT in coconut water is in the form of cytokinins (5.8 mg/l), auxins (0.07 mg/l), and a small amount of gibberellin (Morel, 1974 in Bey et al., 2006). Based on the results of the research of Indriani et al. (2014), the addition of 10% coconut water to the growth of chrysanthemum buds (*Chrysanthemum indicum* L.) showed optimal results from applying coconut water with concentrations of 5%, 15%, 20%, and 25% on explant height, number of buds, and number of leaves.

MATERIALS AND METHODS

The research was carried out by laboratory experiment method, using a single-factor design of 7 treatments arranged in a complete randomized design (RAL). Each treatment consisted of 5 replicates and each replicate consisted of 3

samples. The treatments tried were: (1) Medium MS + BAP 2 mg/l + NAA 0.5 mg/l, (2) Growmore 3 g/l + Coconut Water 100 ml/l + Banana Ambon 50 g/l, (3) Growmore 4 g/l + Coconut Water 100 ml/l + Banana Ambon 50 g/l, (4) Growmore 5 g/l

+ Coconut Water 100 ml/l + Banana Ambon 50 g/l, (5) Green Hyponex 3 g/l + Coconut Water 100 ml/l + Ambon Banana 50 g/l, (6) Green Hyponex 4 g/l + Coconut Water 100 ml/l + Ambon Banana 50 g/l, and (7) Green Hyponex 5 g/l + Coconut Water 100 ml/l + Ambon Banana 50 g/l.

Preparation.

Materials and tools used include: sterile Naweswari Agrihorti variety chrysanthemum ksplan, Ambon bananas, coconut water, detergent, 70% alcohol, betadin, aquades, Growmore foliar fertilizer (32-10-10), Hyponex Green foliar fertilizer (20-20-20) MS, BAP, NAA, commercial agar, aluminum foil, tissues, umbrella paper, plastic wrap, rubber, spiritus, label paper, sterile aquades, and pH sticks. The tools used include culture bottles, *petridishes*, *laminar air flow*, autoclaves, dissecting kits, analytical scales, droppers, and *timers*. Sterilization is carried out in three ways, namely wet sterilization, incineration sterilization and UV sterilization. Wet sterilization is carried out by inserting tools that have been wrapped in umbrella paper, namely culture bottles, tweezers, scalpels, aluminum foil, petridis and Erlenmeyer and medium into the autoclave at a temperature of 121°C with a pressure of 1 atm for 1 hour. Incineration sterilization is carried out in the LAF when the appliance will be used by soaking in a 70% alcohol solution and burning it every time it will be used. UV sterilization is carried out by inserting sterile tools into the LAF and turning on the UV lamp for 1 hour and turning on the blower 10 minutes before the activity starts.

Medium Manufacturing.

MS medium is carried out by inserting the available MS medium and BAP growth regulator 2 mg/l and NAA 0.5 mg/l, then adding granulated sugar 30 g/l and agar 7 g/l and pH fixing at pH 6-6.5. The media solution is stirred and heated to a boil, then distributed to each bottle of 20 ml. The preparation of substitution media was carried out by weighing growmore and hyponex leaf fertilizers according to

the treatment, then in each treatment 150 ml/l coconut water, 150 g/l ambon banana pulp, 30 g/l granulated sugar, and agar 7 g/l and pH fixation at pH 6-6.5 was added. The media solution is stirred and heated to a boil, then distributed to each bottle of 20 ml. MS medium and substitution medium are sterilized in an autoclave at a pressure of 1 atm, temperature 121°C for 20 min.

Inoculation

Inoculation is carried out in LAF (*Laminar Air Flow*) using pieces of young shoots of 1 book. Sterile chrysanthemum plants are taken aseptically with the help of sterile tweezers, placed in a petridish containing an antiseptic solution of 3 drops and cut the stem 1 cm long (1 book). The explant is then drained on filter paper and then inoculated into a culture bottle that already contains medium according to the treatment. The bottle is then covered with aluminum foil tied with rubber and then coated with plastic wrap. After that, each bottle is labeled according to the treatment and stored in the incubation room.

Observance.

Bottles that have been planted with explant are placed on culture racks in an incubation chamber with a temperature setting of 25-28°C and UV light of 100-400 ft-c (1000-4000 lux). This was done to determine the response of explants to several concentrations of growth regulators to their formation for 6-8 weeks.

Observation Variables

The observation variables included: percentage of live explant, percentage of contamination, percentage of *browning*, when sprouts were growing, number of shoots, plant height, number of leaves, leaf color and when roots appeared.

Data Analysis.

The data obtained was analyzed with an *analysis of variance* at an error level of $\alpha = 5\%$. If the results show significance, further testing is carried out using the *Duncan Multiple Range Test* (DMRT) at an error level of $\alpha = 5\%$. To find out the different treatment is real.

RESULTS AND DISCUSSION

In vitro culture of chrysanthemums

The success of in vitro culture can be known from the variables of live explant percentage, browning, and contamination. The success of in vitro culture is influenced by several factors, namely: the source of explant, sterility, and the ability of the explant to grow on the culture medium. The source of the explant includes the meristematic level of the cells in the explanatory, the more meristem cells in the explanatory, the greater the growth rate. Sterility includes explant, medium, equipment, inoculation

site and environment. Planting media includes the type of media, pH, nutrient and hormone content, and the density of the planting medium.

Based on observation for 8 weeks, it can be seen that there was no contamination in all treatments. Data in Table 1. showed that in all treatments, the explant survival percentage was 98% and the browning percentage was 1.91%

Tabel 1. Effect of foliar fertilizer type and concentration on the percentage of live explants and browning on the growth yield of chrysanthemums of the Naweswari Agrihorti variety

Treatment	Percentase (%)	
	Live	Browning
MS	100	0
Growmore 3 g/l	100	0
Growmore 4 g/l	100	0
Growmore 5 g/l	100	0
Hyponex Green 3 g/l	93,33	6,67
Hyponex Green 4 g/l	100	0
Hyponex Green 5 g/l	93,3	6,67
Average	98,09	1,91

Description: MS media was added BAP 2 mg/l and NAA 0.5 mg/l, while the substitution medium was added coconut water 100 ml/l and Ambon banana 50 g/l, respectively.

One of the factors that causes the high percentage of chrysanthemum live explants is the use of appropriate media. According to Yuwono (2012), *in vitro culture media* contains five main components, namely inorganic compounds, carbon sources, vitamins, growth regulators and organic supplements. The medium used for in vitro chrysanthemum growth has contained all five main components needed for explant growth. The media used in the growth of chrysanthemums are MS media and alternative media. MS media is used

as a comparative treatment medium because MS media is a medium that is frequent and suitable for use as *in vitro* culture media on various types of plants. The MS media used in this study has nutrient content in the form of macro, micro, mineral, and vitamin elements as well as added granulated sugar (as an energy source) and growth regulators (BAP 2 ppm + NAA 0.5 ppm). The nutrient content and zpt cause chrysanthemum explants to live and grow.

In the foliar fertilizer medium treatment, data on the percentage of chrysanthemum explants with Growmore 3 g/l, Growmore 4 g/l, Growmore 5 g/l, and Hyponex Green 4 g/l treatments reached 100%, while the percentage of chrysanthemum explants with Hyponex Green 3 g/l and Hyponex Green 5 g/l treatment reached 93.33%. The percentage of chrysanthemum live explants in foliar fertilizer medium reaches a very high

percentage. This shows that the use of alternative media can be used as a substitute for MS media in *chrysanthemum* in vitro cultures. This is possible because alternative mediums also contain macro and micro nutrients such as in MS mediums, in addition to the addition of sugar, coconut water and banana pulp which contain energy sources, amino acids, vitamins, minerals, and growth regulators allow chrysanthemum explants to grow.

Growth of Chrysanthemums

Chrysanthemum explant in the form of a single book inoculated in a suitable medium, will experience bud growth on the leaf axils. In vitro chrysanthemum growth variables include: when sprouts grow, when roots grow,

shoot height, number of buds, and number of leaves. The bud growth data is found in Table 2.

Tabel 2. Effect of various foliar fertilizers on the growth of the growth yield of chrysanthemums of the Naweswari Agrihorti variety at 8 weeks after inoculation

Treatment	Growing Up (Day 1-)		Shoots Height (cm)	Number of Shoots (shoots)	Number of Leaves (sheet)
	Shoots	root			
MS	6,00 a	-	4,30 c	4,33 a	33,13 a
Growmore 3 g/l	7,54 a	7,47 a	7,04 ab	1,20 b	11,33 b
Growmore 4 g/l	7,20 a	7,07 a	8,27 a	1,13 b	11,33 b
Growmore 5 g/l	7,80 a	6,93 a	5,69 bc	1,40 b	11,20 b
Hyponex Green 3 g/l	5,87 a	7,73 a	4,41 c	1,00 b	9,80 b
Hyponex Green 4 g/l	7,47 a	7,13 a	4,87 c	1,07 b	10,80 b
Hyponex Green 5 g/l	6, 53 a	7,93 a	4,25 c	1,53 b	11, 93 b

Description : Numbers followed by the same letter show no different results on the 5% DMRT test. MS media was added BAP 2 mg/l and NAA 0.5 mg/l, while the substitution medium was added with coconut water 100 ml/l and Ambon banana 50 g/l, respectively.

When sprouts grow and when roots are growing, they are observational indicators that show the speed of bud and root growth in chrysanthemum explants. Data in Table 2. It shows that when sprouts grow and when growing the roots of crispy explant are not affected by the type of planting medium, this shows that the use of foliar fertilizer medium with the addition of coconut water and banana pulp can replace the medium of MS+BAP 2 ppm+NAA 0.5 ppm. In Table 2. It is also known that in MS medium roots do not grow in all samples, it is possible that the administration

of NAA 0.5 ppm has not been able to stimulate the formation of roots. Higher concentrations of cytokinins than auxins make chrysanthemum roots difficult to grow because they are more likely to induce bud growth. This is in accordance with the opinion of Dwiyani (2015), the application of a higher concentration of cytokinin from auxin will encourage bud growth, while the application of auxin with a higher concentration than cytokinin will promote root growth.

The growth of chrysanthemum buds can be known from the variables of the number of junas,

the height of the shoots, and the number of leaves. Based on Table 2. It can be seen that in MS medium, the number of shoots that grow is more (4.33 buds) than the number of shoots that grow in alternative mediums. This is suspected because the MS medium is given synthetic growth regulators in the form of BAP as much as 2 ppm and NAA 0.5 ppm. BAP is a synthetic cytokinin that is often used to propagate plants in vitro. According to Arafah *et al.* (2021), BAP has high effectiveness against bud formation stimulation in plants, is more stable when used, and has resistance to oxidation. In addition, higher concentrations of cytokinins than auxins lead to bud growth. This is in accordance with Zulman's (2015) opinion, providing a higher concentration of cytokinin from auxin will encourage the growth of buds and leaves. In alternative mediums (growmore 3 g/l, 4 g/l, 5 g/l, hyponex 3 g/l, 4 g/l, and 5 g/l) the number of shoots produced did not differ significantly because the source of growth regulators for bud formation in the medium was in the form of natural ZPT found in young coconut water and Ambon bananas. The addition of young coconut water and bananas to the alternative media is the same as that it has the same effect on the formation of the number of buds. The number of shoots in the alternative medium is between 1.00 to 1.53 shoots, this is in line with what Indriani *et al.* stated. (2014) which states that the use of 100 ml coconut water can have an effective influence on the number of shoots, namely 1.27 buds. However, the addition of natural zpt has not been able to increase the number of shoots and the number of leaves as formed in MS medium, this is likely because the concentration of zpt in the natural material is too small to grow young chrysanthemum shoots.

In Table 2. It also showed that the highest chrysanthemum shoot height was in 4 g/l growmore fertilizer medium although there was no significant difference with shoots growing on 3 g/l growmore fertilizer medium. Chrysanthemum shoots can grow because the cultivated explant

absorbs the nutrients contained in the culture medium. This is in accordance with the opinion of Hartana *et al.* (1991) in Satriyas *et al.* (2017), the formation of shoots on explants requires a medium containing inorganic salt salts and growth regulators. Nutrients are needed by chrysanthemum explants to facilitate the biochemical processes that take place in plant tissues. The nutrients contained in the treatment medium include nitrogen (N) for vegetative growth, phosphorus (P) for the formation of carbohydrates, potassium (K) for cell division, iron (Fe) to form leaf green, boron (B) for remodeling sugar, and cobalt (Co) for binding nitrogen (Hendaryono and Wijayani, 1994). In addition, the presence of growth regulators, especially cytokinins in the medium, also greatly affects the growth of chrysanthemums in vitro. In alternative mediums of growmore and hyponex leaf fertilizers, young coconut water and bananas are added as a source of growth regulators, vitamins, minerals and energy sources. As stated by Kristina and Syahid (2012) that based on the chemical analysis carried out, young coconut water contains growth regulators (kinetin, zeatin, auxin); vitamins (vit. C, duck B5, riboflavin, inositol, biotin, pyridoxin, thiamin); minerals (N, P, K, Mg, Fe, Na, Mn, Zn, Ca), and sucrose. While banana pulp contains carbohydrates, vitamins, and minerals. The nutrient and zpt content contained in young coconut water and banana pulp is strongly suspected to increase the high growth of chrysanthemum buds in an alternative medium of foliar fertilizer. This is in line with what was stated by Armini, *et al.* (1992) that coconut water is an organic compound that is often used in the application of tissue culture techniques and can increase the height of plant shoots. Kristina and Syahid (2012) also mentioned that the addition of 15% young coconut water to the medium can increase the growth of temulawak in vitro.

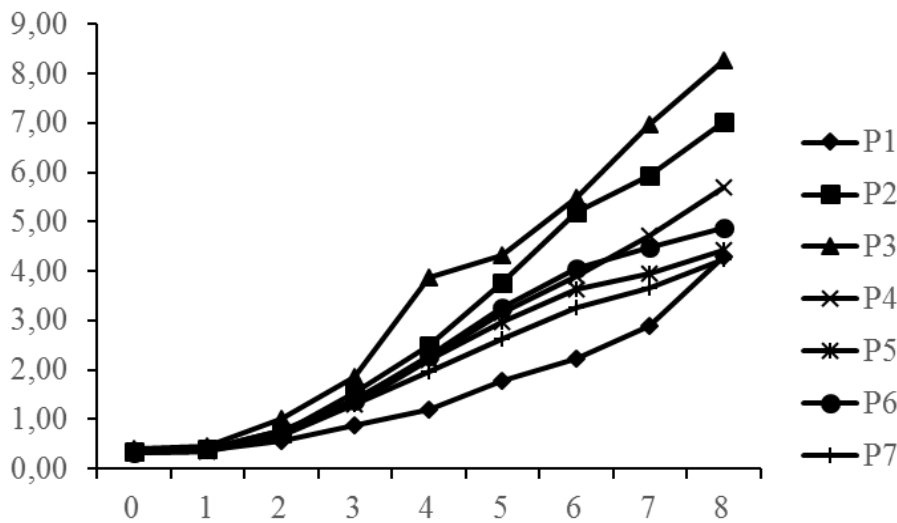


Figure. 1. Graph of Plant Height growth rate of Naweswari Agrihorti chrysanthemums based on certain treatments from week 1 to week 8 after inoculation

Based on the graph of the high growth rate of the chrysanthemum growth plant (Figure 1.) shows that the chrysanthemum explant provides growth for 8 weeks after planting. In the graph, it can be seen that in the 2nd to 8th week the highest growth of chrysanthemums is shown by the Growmore 4 g/l treatment, while the lowest growth in the 2nd to 7th week is shown by the MS treatment.

The nutrients contained in the substitution medium used came from Gromore and Hyponex Green leaf fertilizers, while in MS media came from macro stock and micro stock. Macro and micro nutrients contained in foliar fertilizer have an important role for the growth of explants by stimulating various hormones contained in chrysanthemum explants. The availability of macronutrients in the form of nitrogen,

phosphorus, and potassium can meet the needs of young plants that are still actively organizing new cells. Nitrogen is needed by plants for the formation of amino acids and proteins as well as the formation of nucleic acids as the building blocks of cells and cell walls. Potassium plays an important role in maintaining plant turgor and maintaining the osmotic potential of cells (Okorie *et al.*, 2015). Potassium in foliar fertilizer is needed by plants as activators of various kinds of enzymes in meristematic tissue. Phosphorus functions in energy storage and transfer through the formation of adenosine diphosphate (ADP) and adenosine triphosphate (ATP) compounds. Therefore, the application of nutrients into the growing medium is suspected to provide normal growth in chrysanthemum explants.

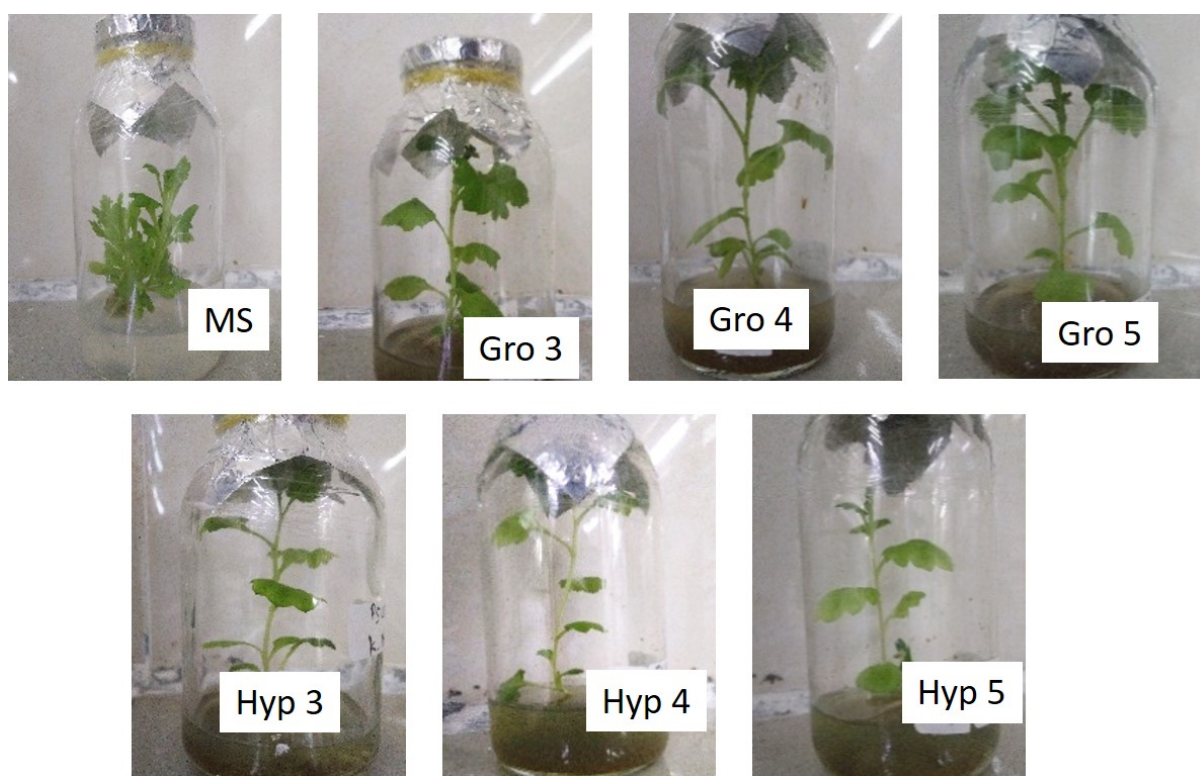


Fig. 2. The growth of shoots from the growth of chrysanthemums of the Naweswari Agrohorti variety was based on MS + BAP treatment 1 mg/l + NAA 0.5 mg/l and substitution media with the addition of coconut water 100 ml/l and Ambon banana 50 g/l.

The growth of chrysanthemum shoots at week 8 in MS medium and substitution media (growmore and hyponex foliar fertilizers) can be seen visually in Figure 2. Shoots that grow on MS media are clearly seen to have more buds than in the substitution medium of growmore and hyponex foliar fertilizer at various concentrations. However, visually, chrysanthemum plants in MS medium look dwarfed and small, the leaves, although numerous, appear small and pale green. While chrysanthemum plantlets in the substitution medium appear taller, they also have wider and older leaves. This is likely due to the different content of nutrients and growth regulators in the

medium. BAP in MS medium plays a greater role in bud propagation, so that the energy source in the medium is more used to propagate shoots so that the buds are small, while cytokinin in coconut water and bananas plays a greater role in stem lengthening and internode growth, so that the energy source in the medium is used for bud height increase and leaf enlargement. Based on these data, it can be seen that MS media can be used for multiplication (bud propagation), while foliar fertilizer medium (growmore and hyponex) with the addition of young coconut water and banana pulp can be used for sub-culture/enlargement of plantlets and preparation for acclimatization.

CONCLUSION

Foliar fertilizer medium (growmore and hyponex) with the addition of 150 ml/l of young coconut water and 150 g/l of banana pulp can be used as a substitution medium in the sub-culture/enlargement of the Nareswari variety

chrysanthemums in vitro. Growmore foliar fertilizer medium 4 g/l + young coconut water 150 ml/l + banana pulp 150 g/l produces the highest bud height.

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