

Substitution Media by Utilizing Banana Peel for In Vitro Culture of *Chrysanthemum morifolium*

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ABSTRACT

The use of organic matter in the in vitro culture medium can reduce costs for micropropagation and multiplication of *Chrysanthemum* in mass production. This study aims to examine the use of banana peel waste in the in vitro culture of *Chrysanthemum*. The research used an experimental method with single factor and eight treatments with 3 samples and 3 replications. The treatments were: (1) MS medium + BAP 1 ppm + NAA 0.5 ppm, (2) Foliar fertilizer 3 g/L + coconut water 150 mL/L, (3) banana endocarp puree 50 g/L, (4) banana endocarp puree 100 g/L, (5) banana exocarp puree 50 g/L, (6) banana exocarp puree 100 g/L, (7) banana peel puree 100 g/L, and (8) banana peel puree 100 g/L. All banana peel puree treatments were combined with foliar fertilizer 3g/L + coconut water 150 mL/L. The explant used a single nodus of *Chrysanthemum* with one leaf resulting from the previous in vitro culture. This study used *Chrysanthemum* varieties Naweswari and Tadasita from Ornamental Plants Research Center, West Java, Indonesia. The results showed that foliar fertilizer medium added by banana peel puree and coconut water has the good potential to be used as a substitution medium in the in vitro culture of *Chrysanthemum* varieties Naweswari and Tadasita. The banana peel puree 50 g/L in the foliar fertilizer medium shows the best growth in *Chrysanthemum* in vitro culture in both Naweswari and Tadasita varieties.

Keywords: *Tissue Culture, Alternative Medium, Chrysanthemum plant, Banana Skin*

INTRODUCTION

Ornamental plants as agricultural commodities that have high economic value have been cultivated commercially for a long time. The beauty and attractiveness of ornamental plants is the reason why the demand is quite high. Various types of ornamental plants that have commercial value in Indonesia include orchids, *Chrysanthemums*, roses, carnations, anthuriums, gladiolus, gerbera,

amaryllis, tuberose, daisies, and jasmine (Widyawan and Prahastuti, 1994).

Chrysanthemum morifolium (*Chrysanthemum morifolium*) is one of the important ornamental plants, both as cut flowers and potted flowers. *Chrysanthemum* production shows an increase every year. In 2010, the production of *Chrysanthemum* in Indonesia was even the highest compared to other ornamental plants. The

production of this plant reached 185,232,970 stalks (Central Bureau of Statistics, 2010). The production carried out is an effort to meet the demand for *Chrysanthemum* which is quite high. The demand for *Chrysanthemum* is the highest among other cut flowers because it has a beautiful crown shape and color, besides that this flower has a fairly cheap price compared to other ornamental plants (Widyawan and Prahastuti, 1994). *Chrysanthemum* (*Chrysanthemum morifolium*) is one type of ornamental plant that is favored because of the beauty of its flowers, both in the form of cut flowers and potted plants. This type of ornamental plant has been developed and cultivated by flower farmers. The development of *Chrysanthemum* cultivation in equatorial areas, such as Indonesia, requires additional light to maintain the vegetative phase of the plant so that it can grow strong to support its flowers (Berlindaswati, 2000).

Chrysanthemum is generally propagated generatively and vegetatively. Sexual propagation of *Chrysanthemum* is rarely done because it is difficult and heterozygous (the offspring are not the same as the parent). In addition, generative propagation requires a long time and special handling. Vegetative propagation is generally through shoot cuttings, tillers and in vitro culture. The problem faced is that the demand for *Chrysanthemum* plants is not comparable to the parent plant preparation. The number of mother plants are not sufficient to meet the availability of seeds for *Chrysanthemum* propagation. Another factor are the problem of seedling degeneration, namely the decline in seed quality in line with the increasing age of the parent plant, and the low quality of the seeds produced. This is because *Chrysanthemum* plants are propagated by shoot cuttings and tillers. To avoid or reduce seedling degeneration, producers are required to be able to apply appropriate propagation techniques to overcome this problem (Rukmana and Mulyana, 1997). Therefore, to obtain plants that have high productivity in a relatively short time, it is necessary to carry out vegetative propagation so that the seeds can be used sustainably. Yusnita (2003) explains that the use of in vitro culture techniques that have been carried out so far is considered effective

enough to develop quality and uniform seeds on various types of plants. Propagation by means of in vitro culture is expected to produce superior and uniform quality of *Chrysanthemum* seeds, resistant to disease, high production rates and relatively shorter time compared to conventional propagation.

Multiplication is one of the stages in plant growth in vitro where there is development (differentiation) of cells into many more cells and form shoots or other organs needed (Salisbury and Ros 1995). According to Gunawan (2002) multiplication is the stage of propagation or doubling of explants grown in vitro. At this stage shoot propagation occurs by pushing lateral shoots or stimulating adventitious shoots (Yusnita, 2003). In in vitro plant culture there are three regeneration patterns, namely axillary shoot propagation, organogenesis, and embryogenesis (Yusnita, 2015). In vitro axillary shoot propagation is a method that most often used to produce true-to-type seedlings, and this method guarantees more genetic stability in the resulting regenerant plants (Kane, 2000). According to Yusnita (2015), propagation by this method is widely used because it is relatively simple, genetic aberration is very small, multiplication takes place quite quickly, and the resulting plants grow well due to rejuvenate Dixon (1985) argues that 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. In addition to MS media, it is necessary to look for other growth media, such as modified media, in order to determine which growth media is suitable for the growth of chrysanthemum cuttings.

Alternative media constituent materials can come from leaf fertilizer and banana peels because they are easy to obtain. The high potential for banana fruit production is not comparable to the potential for banana peels produced. Banana peel waste is generally not widely used and sometimes it is only thrown into garbage and becomes waste that will disturb the community nation (Wahyudi, 2011).

Based on this background, the purposed of this research are: (1) to assign the effect of addition

banana peel puree on the *Chrysanthemum* in vitro culture, and (2) to determine the most optimum

concentration of banana peel puree for the *Chrysanthemum* growth in the in vitro culture.

MATERIALS AND METHODS

Materials

In vitro culture plantlets of *Chrysanthemum morifolium* var. Naweswari and Tadasita were used as explants source. As a control medium implemented Murashige and Skoog (MS) medium added by *Benzyl Amino Purin* (BAP) 1 ppm + *Naphtaleine Acetic Acid* (NAA) 0.5 ppm, and Green Foliar Fertilizer (GFF) 3 g/L + coconut water 150 mL/L. The substitution media utilized banana peel divided into three parts: banana endocarp, banana exocarp, and banana peel (endocarp+exocarp). Each part of the banana peel weighed 50 g/L and 100 g/L. Sugar 30 g/L and agar 7 g/L were added to all treatments. Sterilization used detergent, bactericide (Agrept) 2 g/L, fungicide (Dithane) 2 g/L, alcohol 70%, and antiseptic liquid.

Methods

The research was conducted at Tissue Culture Laboratory, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, from November until January. This research methods used laboratory experiment with single factor, 8 treatments and 3 replications. Each replication consist of 3 samples. The treatments were: (1) MS medium + BAP 1 ppm + NAA 0.5 ppm, (2) Foliar fertilizer 3 g/L + coconut water 150 mL/L, (3) Inner banana peel puree 50 g/L, (4) Inner banana peel puree 100 g/L, (5) Outer banana peel puree 50 g/L, (6) Outer banana peel puree 100 g/L, (7) whole banana peel puree 100 g/L, and (8) whole banana peel puree 100 g/L. All banana peel puree treatments were combined with foliar fertilizer 3g/L + coconut water 150 mL/L. The explant used a single nodus of *Chrysanthemum* with one leaf.

Research Prosedures

Banana peels were washed with detergent, then soaked in Agrept bactericide and Dithane fungicide 2 g/L each for 12 hours. The banana peel

was rinsed with water and boiled. After boiling, the banana peel is divided into some parts: banana endocarp, banana exocarp, and banana peel (endocarp+exsocarp). Each part was crushed using a food processor, and weighed according to the treatment 50 and 100 g/L.

MS medium was made by using a stock solution of macro and micronutrients based on the “Murashige and Skoog” formula. The MS medium was added by BAP 1 ppm + NAA 0.5 ppm, sucrose 30 g/L, and agar 7 g/L. Foliar fertilizer medium formulated using Green Foliar Fertilizer (composition of N:P:K = 32:10:10) 3 g/L added by coconut water (from green skin coconut) 150 mL/L, 30 g/L sugar, and agar 7 g/l). In this research, the treatment media were banana peel that divided into 3 parts: banana endocarp, banana exocarp, and banana peel (endocarp+exsocarp). Each part was weighted 50 g/L and 100 g/L. Each treatment was mixed with foliar fertilizer 3 g/L, coconut water 150 mL/L, sugar 30 g/L and agar 7 g/L. All pH media are arranged 6.5 – 7. The media boiled and sterilized using autoclave at 1 atm and 121°C for 20 minutes.

Inoculation was carried out in Laminar Air Flow with selected the good quality plantlets parent: having many shoots, green leaves, enough height, and rooted. After that, the plantlets were placed into the petri dish containing an antiseptic liquid and cut into 1 nodus with 1 leaf. The explant was then inoculated in each medium according to the treatments. After inoculation, all the samples were arranged in the incubation room with a controlled environment, such as temperature (20°C - 25°C), humidity (60%), light intensity, and sterility. Observations of all variables were carried out in the incubation room.

Data Analysis

Data Analysis: The data were analyzed with ANOVA (Analysis of variance) at a 5% error level. If there were significant differences, the data were analyzed using the Duncan Multiple Range Test

(DMRT) at a 5% error level. Some data is presented in graphs and images and then analyzed descriptively.

RESULT AND DISCUSSION

Growth Results of Chrysanthemum var. Naweswari and Tadasita on the in vitro culture medium

The growth data of the Naweswari variety *Chrysanthemum* on culture medium with the addition of banana peels are shown in Table 1, while

the growth of the Tadasita variety *Chrysanthemum* is shown in Table 2

Table 1. Growth Results of *Chrysanthemum* var. Naweswari on the in vitro culture medium with banana peels

Treatments	Time to grow (days)		Shoot height (cm)	Number of leaves	Number of shoots	Leaves colour (last observ)
	shoots	roots				
MS	9.67 ^a	-	2.80 ^c	8.89 ^{ab}	2.17 ^a	5 GY 7/6
FF + CW	15.44 ^c	6.56	11.08 ^a	10.11 ^{ab}	1.83 ^{ab}	5 GY 5/6
BEn50	15.11 ^c	7.11	8.73 ^{ab}	10.22 ^a	1.94 ^{ab}	5 GY 6/8
BEn100	15.11 ^c	7.11	6.23 ^{bc}	8.78 ^{ab}	1.50 ^{ab}	5 GY 6/8
BEx50	10.56 ^{ab}	7.00	11.21 ^a	9.44 ^{ab}	1.39 ^b	5 GY 6/8
BEx100	15.56 ^c	6.89	3.43 ^c	5.33 ^c	1.28 ^b	5 GY 5/8
BP50	19.89 ^d	6.67	9.59 ^{ab}	8.67 ^{ab}	2.17 ^a	5 GY 5/8
BP100	10.89 ^{ab}	6.33	6.07 ^{bc}	7.89 ^b	1.39 ^b	5 GY 6/10

Noted: MS= Murashige-Skoog medium, FF+CW= foliar fertilizer, BEn50 & BEn100 = Banane Endocarp puree 50g/l and 100 g/l, BEx 50 & BEx 100 = Banane Exocarp puree 50 g/l and 100 g/l, BP 50 & WS 100 g/l = Banana Peels 50 g/l and 100 g/l.

The data on Table 1. indicated that there is a significant effect of medium culture in the shoot height, number of leaves and number of shoots. The Banana exocarp 50 mg/L treatment tended to give higher yields but was not significantly different from the Foliar fertilization, Banana peels 50 mg/L, and Banana Endocarp 50 g/L treatments. This was due to the macronutrient content contained in the Ambon banana peel. The carbohydrate content in Ambon banana peel is thought to play a role in stimulating the growth of *Chrysanthemum* plant height. The visual appearance of the increase in height of the *Chrysanthemums* of the Naweswari Agrihorti variety can be seen in Figure 2. The content of cytokinin in coconut water is also one of the factors that affect the increase in height of *Chrysanthemum* plants. Cytokinin can increase

plant height because it has been shown to increase the rate of cell elongation (Salisbury and Ross, 1995).

The treatment that gave the best effect on the number of leaves was Banana Endocarp 50 g/L, which was 10,222 strands. This is due to the content of Magnesium (Mg) in the organic material of banana peels because Mg is needed by plants in the process of photosynthesis. According to Ashihah (2020), the process of leaf formation involves minerals found in the medium, one of which is Magnesium (Mg). The element Mg is a component of the chlorophyll molecule nutrient (Sriyani, 2002). This is also following research conducted by Permatasari (2019) that the addition of banana peels with a concentration of 50 g/L obtained the highest number of leaves, namely 9,110 strands. While the

treatment with the least effect is the Banana exocarp 100 g/L treatment.

The addition of banana peels to the media of leaf fertilizer and coconut water gave significant results.

Most shoot initiation was found in the treatment of Banana peels 50g/L media and MS media with the addition of 0.5 ppm NAA and 1 ppm BAP. This is because growth driven by BAP includes faster cell division (Marlin, 2005).

Table 2. Growth Results of *Chrysanthemum* var. Tadasita on culture medium with banana peels

Treatments	Time to grow (days)		Shoot height (cm)	Number of leaves	Number of shoots	Leaves colour (last observ)
	shoots	roots				
MS	2,28 ^a	-	1,24 ^c	4,44 ^a	1,30 ^a	5 GY 6/8
FF + CW	2,01 ^a	12,44 ^d	3,17 ^{bcd}	8,33 ^a	1,38 ^a	5 GY 5/6
BEn50	2,74 ^a	10,89 ^c	4,01 ^{ab}	9,11 ^a	1,43 ^a	5 GY 4/6
BEn100	2,16 ^a	7,78 ^a	2,92 ^{cd}	7,56 ^a	1,34 ^a	5 GY 5/8
BEx50	2,28 ^a	9,33 ^b	3,13 ^{bcd}	7,56 ^a	1,53 ^a	5 GY 4/6
BEx100	2,73 ^a	8,17 ^{ab}	2,38 ^d	4,44 ^a	1,48 ^a	5 GY 7/8
BP50	2,56 ^a	7,78 ^a	4,39 ^a	7,67 ^a	1,34 ^a	5 GY 5/8
BP100	2,67 ^a	10,89 ^c	3,27 ^{bcd}	7,56 ^a	1,55 ^a	5 GY 5/6

Noted: MS= Murashige-Skoog medium, FF+CW= foliar fertilizer, BEn50 & BEn100 = Banane Endocarp puree 50g/l and 100 g/l, BEx 50 & BEx 100 = Banane Exocarp puree 50 g/l and 100 g/l, BP 50 & WS 100 g/l = Banana Peels 50 g/l and 100 g/l.

Growth Rate of *Chrysanthemum* var. Naweswari and Tadasita for 8 Weeks

The graph of growth rate of Naweswari and Tadasita variety *Chrysanthemum* on culture

medium with the addition of banana peels is shown in Figure 1.

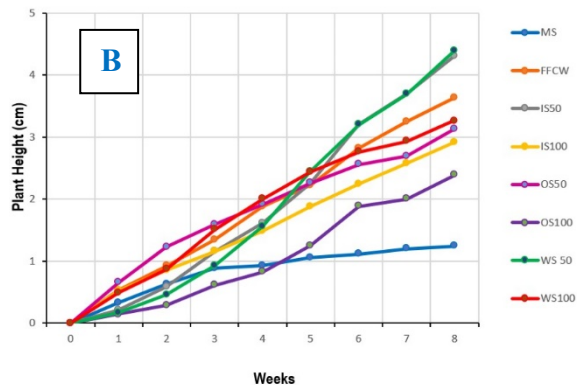
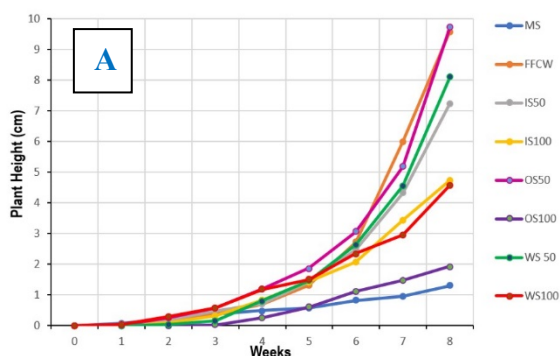


Figure 1. Growth rate of Naweswari (A) and Tadasita (B) varieties of *Chrysanthemum* on culture medium with the addition of banana peels for 8 weeks.

Noted: MS= Murashige-Skoog medium, FF+CW= foliar fertilizer, BEn50 & BEn100 = Banane Endocarp puree 50g/l and 100 g/l, BEx 50 & BEx 100 = Banane Exocarp puree 50 g/l and 100 g/l, BP 50 & WS 100 g/l = Banana Peels 50 g/l and 100 g/l.

Based on the growth graph of Naweswari and Tadasita, it can be seen that the shoots appear of Tadasita faster than Naweswari. In the Tadasita variety, the shoot started to grow under 1 week, meanwhile, in the Naweswari variety, the shoot started to grow between 1 and 2 weeks. However, the growth of Naweswari was higher than Tadasita at the end of the observation. The shoot height of Naweswari can reach almost 10 cm, while Tadasita is under 5 cm. It shows that the response of Naweswari in the culture media is better than Tadasita's.

On the Naweswari growth chart, the highest growth is the banana exocarp 50g/L treatment, followed by Foliar Fertilizer and banana peels 50 g/L treatments, and the lowest is in MS medium followed by banana exocarp 100 g/L. Meanwhile, on Tadasita's growth chart, the highest growth was in the banana peels 50 g/L, followed by banana endocarp 50 g/L and foliar fertilizer, and the lowest

was in MS medium followed by banana exocarp 100 g/L treatments.

Visualization of Chrysanthemum growth on the culture medium

This Figure is comparison visualization of *Chrysanthemum* between Naweswari vs Tadasita variety. In this Figure we can see that the growth of the shoot and the leaves of Naweswari is bigger than Tadasita in all treatment. Also we can see that: in the MS medium, the growth of *Chrysanthemum* was the slowest in both varieties. The appearance of both varieties are not significantly difference, but during observation for 8 weeks, it looks difference in all treatment. It may be because of genetically both of the variety is not the same. They are clone from different parents. Naweswari was breeding from Wastu Kania and Stroika, while Tadasita was breeding from Alpha and Tirta Ayuni.

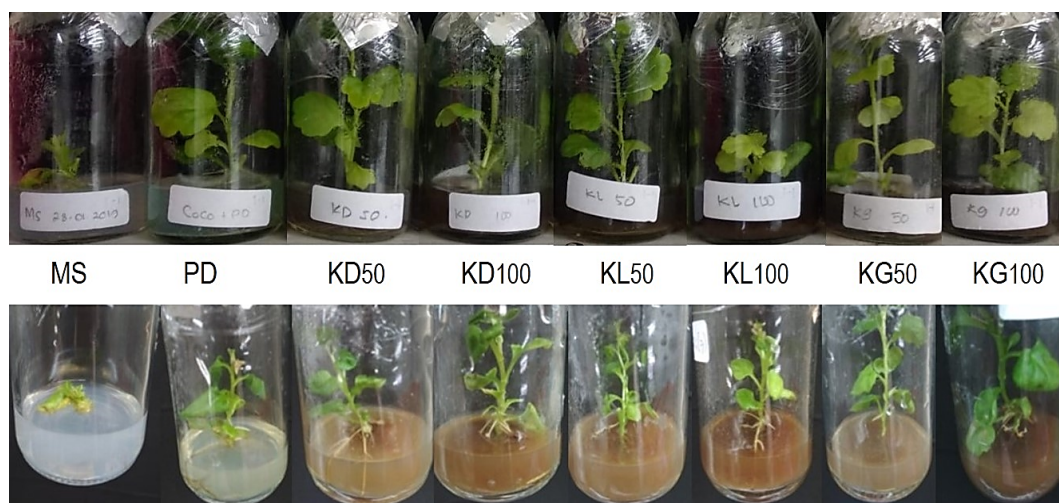


Figure 2. Visualization *Chrysanthemum* var. Naweswari and Tadasita on the culture medium with the addition of banana peels at 8th weeks

CONCLUSION

Banana peel, banana endocarp, and banana exocarp combined with foliar fertilizer + coconut water potentially used as a substitution medium for MS media in the in vitro culture of *Chrysanthemum*

var. Naweswari and Tadasita. The recommended application for in vitro culture of *Chrysanthemum* to substitute MS medium is banana peel (exocarp+endocarp) 50 g/L.

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