

# Growth, Morphogenesis and Acclimatization of Several Cultivated *chrysanthemum* Varieties on Modified Murashige and Skoog Media

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**Abstract:** The need for quality chrysanthemum seedling has increased with the promotion of chrysanthemum development in Tomohon for export plans. Seedling propagation through tissue culture is a technology that really supports the supply of these seedling. This technology requires media, include Murashige and Skoog (MS) media. But this media is expensive, therefore need to lookfor alternative inexpensive materials that can substitute some MS media. The purpose of this study was to find out how much MS media can be substituted with coconut water (CW) and complementary fertilizers (CF) which resulted in growth and morphogenesis that were not different from 100% MS. The research was carried out using RAL: Factor Coconut water 3 levels namely: 0, 20 and 40%; The second factor Complementary fertilizer 0, and 2g/l. It consists of 5 replications and each replication consists of 2 bottles so there were 210 experimental units. The results showed that the use of 20% coconut water combined with complementary fertilizers on chrysanthemum varieties Suryandari; show significantly better growth than Jayanti variety and Riri variety. The use of 40% coconut water without supplementary fertilizers showed significantly better growth for each variety compare to 100% MS as well as if additional fertilizers were added. The best growth was found in the treatment of 20% coconut water on the Suryandari variety combine with complementary fertilizer; also in 40% coconut water, without or in combination with complementary fertilizer of the suryandari variety. And treatment 40% coconut water combine with CF of Riri Variety.

**Keywords:** Chrysanthemum seedling, Coconut water, Murashige and Skoog, Complimentary fertilizer

## 1. Introduction

Tomohon is a proven city of floriculture. This is clear with the implementation of TIFF (Tomohon International Flower Festival) since 2008 held  $\pm$  2 annually which starting in 2015 is held every year by the Ministry of Tourism of the Republic of Indonesia.

One type of floricultural plant that is widely cultivated and liked by the public is chrysanthemum. Chrysanthemum is an interesting type of flower because of the variety of shapes, colors and types so that it can be used for various decoration needs, display flowers, board flowers and others. With the increasing demand for chrysanthemum flowers, cultivation should be increased, including one of its activities is the provision of quality seedling.

Provision of chrysanthemum seedling by farmers in Tomohon is conventional, namely using continuous cuttings taken from mother plants which are brooded repeatedly which causes degeneration and decreases the quality of the cut flowers produced (Istianingrum et al., 2013). One way to produce quality seeds from mother plants is by rejuvenating or improving their quality at the tissue culture technology. Rejuvenation is an important step carried out to restore the regeneration capacity of explants using cytokinins (Tunggadewi, 2010). In tissue culture, the media is one of the factors that determine the growth of the culture and the media that is widely used is Murashige and Skoog's (MS) (1962).. The price of MS media is quite expensive (example is the price at the Tissue Culture Laboratory in Malang) Rp. 20,000/liter. Therefore, when organizing tissue

culture on a large scale that uses up to thousands of liters of media, it is necessary to look for alternative inexpensive materials that can substitute some of the MS media. Mandang (1995) found that substitution of MS media with coconut water up to 40% resulted in better growth of chrysanthemums compared to those cultured at 100% MS. Growth response to media treatment may differ depending on the genetic characteristics of each variety used.

The use of Liquid Complementary Fertilizer (LCF) can also encourage plant growth in tissue culture. The research of Inkiriwang, Mandang and Runtunuwu (2016) found that the use of 50% MS plus 30% coconut water and 1.5 g/l PPC produced the best growth compared to that grown at 100% MS for *Dendrobium sp.* This is because PPC also contains N, P, K and Mg as well as micro elements such as Mn, Bo, Cu, Co and Zn as well as vitamins which are components that promote plant growth. There are also many complementary fertilizers (CF) on the market which contain various mineral elements that support growth and are cheap, such as CF growth more. Growth more contains macro and micro nutrients needed for plant growth. Therefore, it can be tried to be a substitute material for some MS media. The purpose of this study was to find out how much MS media could be substituted with coconut water and complementary fertilizer (CF) which resulted in growth and morphogenesis that were not different from 100% MS and to determine the success rate at the acclimatization stage.

## 2. Materials and Methods

This research was conducted in the Biotechnology Laboratory Faculty of Agriculture's Sam Ratulangi University from May 2022 to September 2022.

### Tools and Materials

The plant materials to be used are three chrysanthemum varieties: Suryandari, Jayanti and Riri. Coconut water comes from Balitpalma gardens from coconuts aged 10 months after pollination, complementary fertilizer. The media used is MS. Tools to be used: laminar air flow (LAF), sterilization tools, autoclaves, culture bottles, planting tools, pots.

### Methods

The study was conducted using a Completely Randomized Design (CRD). Coconut water (CW) with 3 levels, namely: (0% = A0; 20% = A1 and 40% = A3); CF (Complimentary Fertilizer): 0 (P0) and 2 g/l (P1) and Varieties: Suryandari (S), Jayanti (J) and Riri. So there are 21 treatments:

- 1) MS 100% Suryandari (MS 100 S)
- 2) MS 100% Jemani (MS 100 J)
- 3) MS 100% Riri (MS 100 R)
- 4) A0P0S: 50% MS medium without CW without CF var. Suryandari
- 5) A0P0J: 50% MS medium without CW, without CF var. jemani
- 6) A0P0R: 50% MS medium without CW without CF var. Riri
- 7) A0P1S: 50% MS medium without CW + CF var. Suryandari
- 8) A0P1J: 50% MS medium without CW + CF var. jemani
- 9) A0P1R: 50% MS medium without CW +CF var. Riri
- 10) A1P0S: Medium MS 50% + CW 20% without CF var. Suryandari
- 11) A1P0J: Medium MS 50% + CW 20% without CF var. Jemani
- 12) A1P0R: Medium MS 50% + CW 20% without CF var. Riri
- 13) A1P1S: MS medium 50% + CW 20% + CF var. Suryandari
- 14) A1P1J: Media MS 50% + CW 20%+ CF var. jemani
- 15) A1P1R: Medium MS 50% + CW 20%+CF var. Riri
- 16) A2P0S: Media MS 50% + CW 40%+ CF var. Suryandari
- 17) A2P0J: Media MS 50% + CW 40%+ CF var. Jemani
- 18) A2P0R: Media MS 50% + CW 40%+ CF var. Riri
- 19) A2P1S: Media MS 50% + CW 40%+ CF var. Suryandari
- 20) A2P1J: Media MS 50% + CW 40%+ CF var. jemani
- 21) A2P1R: Medium MS 50% + CW 40%+ CF var. Riri

It consists of 5 replications and each replication consists of 2 bottles so that a total of 210 bottles.

### Variables observed

Shoot height; Number of shoot; Shoot wet weight; final pH culture media; percentage of live plants at acclimatization.

### Data analysis

Data were analyzed by analysis of variance and if there was a significant effect it was continued with the smallest significant difference test.

## 3. Results and Discussion Plant

### Height

Chrysanthemum plant height *in vitro* is presented in Table 1.

**Table 1:** Average Plant Height, Fresh Weight of Shoots and Number of Chrysanthemums

Treatments	Plant Height (Cm)	Final fresh(wet) weight (g)	Number of Shoot
MS100 S	9.45 <sup>l</sup>	1.22 <sup>cde</sup>	1 <sup>a</sup>
MS100 J	7.15 <sup>efg</sup>	0.94 <sup>abc</sup>	1 <sup>a</sup>
MS100 R	5.93 <sup>cd</sup>	0.6 <sup>a</sup>	1 <sup>a</sup>
AOPO S	7.90 <sup>fghi</sup>	0.97 <sup>bc</sup>	1 <sup>a</sup>
AOPO J	8.03 <sup>ghi</sup>	0.86 <sup>ab</sup>	1 <sup>a</sup>
AOPO R	8.40 <sup>hi</sup>	0.70 <sup>ab</sup>	1 <sup>a</sup>
AOP1 S	5.96 <sup>cd</sup>	0.99 <sup>bc</sup>	1 <sup>a</sup>
AOP1 J	6.08 <sup>cd</sup>	0.78 <sup>ab</sup>	1 <sup>a</sup>
AOP1 R	6.55 <sup>de</sup>	0.72 <sup>ab</sup>	1 <sup>a</sup>
A1PO S	8.71 <sup>ij</sup>	1.48 <sup>def</sup>	1.33 <sup>a</sup>
A1PO J	8.65 <sup>ij</sup>	1.49 <sup>def</sup>	1 <sup>a</sup>
A1PO R	6.74 <sup>de</sup>	1.14 <sup>c</sup>	1 <sup>a</sup>
A1P1 S	7.46 <sup>efgh</sup>	2.02 <sup>gh</sup>	1 <sup>a</sup>
A1P1 J	7.35 <sup>efg</sup>	1.38 <sup>ef</sup>	1 <sup>a</sup>
A1P1 R	7.13 <sup>efg</sup>	1.62 <sup>f</sup>	1 <sup>a</sup>
A2PO S	8.60 <sup>ij</sup>	2.26 <sup>h</sup>	1 <sup>a</sup>
A2PO J	6.98 <sup>ef</sup>	1.54 <sup>f</sup>	1 <sup>a</sup>
A2PO R	7.81 <sup>fghi</sup>	1.74 <sup>fg</sup>	1 <sup>a</sup>
A2P1 S	4.00 <sup>a</sup>	1.67 <sup>f</sup>	1 <sup>a</sup>
A2P1 J	5.25 <sup>bc</sup>	1.46 <sup>def</sup>	1 <sup>a</sup>
A2P1 R	4.23 <sup>ab</sup>	2.18 <sup>h</sup>	1.67 <sup>b</sup>
BNT0.05	1.03	1.26	0.33

Note: Numbers followed by the same letters are not different based on the LSD test at the 5% level

From Table 1, it can be seen that the highest plants were found in MS 100% of the Suryandari variety significantly different from the other two varieties; but not different from the height of the plants in the media given coconut water varieties Suryandari and Jemani. The average height at ½ MS (A0) did not differ from the MS 100 treatment for Jayanti and Riri varieties. It is suspected that the need for growth of chrysanthemum *in vitro* was sufficient with ½ MS medium. The use of 20% coconut water without the addition of fertilizer is significantly better than 100% MS except for the Riri variety, and on average the growth is better than that added with CF. This shows that the use of coconut water is enough to encourage high growth without the need for the addition of CF. The addition of 40% coconut water (A2) without CF show high growth P0 was relatively the same as MS 100 for each variety.

### Wet (Fresh) Weight of Shoots

The average fresh weight of chrysanthemum shoots on ½ MS media (A0) without and with fertilizer showed no

difference with 100% MS treatment. In media with 20% coconut water with and without CF the wet weight was higher than MS100 except Suryandari. In the media combination of 40% coconut water without and in combination with fertilizer the wet weight of shoots was greater than that of 100% MS for each variety. It is suspected that the absorption of nutrients from the media with coconut water is better because the media does not experience a significant decrease in pH, which ranges from 5.0 to 6.0. The efficiency of nutrient use by the tissue for N and P increases if the media added coconut water. In addition, it was also found that the sucrose and starch content was low in Chrysanthemum tissue that grows on media that is added coconut water. This shows that the process of tissue metabolism is more active and growth is better. In addition, it was found that the leaf chlorophyll content was higher in plant tissue (Mandang, 1995; Mandang et al, 2017).

#### Number of Shoots

The average number of shoots (Table 1) did not differ except for the A2P1R treatments. this is because the media is not added cytokinins so there is no increase in the number of shoots.

#### Medium Acidity

Media acidity is presented in Table 2.

**Table 2:** Acidity (pH) of the media after 12 weeks of culture

Treatments	Final pH medium
MS100 S	4.2
MS100 J	4.0
MS100 R	4.0
AOPO S	4.3
AOPO J	3.2
AOPO R	3.2
AOP1 S	3.4
AOP1 J	3.2
AOP1 R	3.3
A1PO S	4.3
A1PO J	5.0
A1PO R	5.2
A1P1 S	5.7
A1P1 J	4.5
A1P1 R	5.0
A2PO S	6.1
A2PO J	5.6
A2PO R	5.5
A2P1 S	5.0
A2P1 J	5.0
A2P1 R	5.0

From the table it can be seen that the pH of the media in 100% MS media ranged from 4.0 to 4.2; on ½ MS media 3.2 to 3.4; in the media given 20% coconut water, the pH ranged from 4.3 to 5.7 while those given 40% coconut water ranged from 5.0 to 6.1. So it is clear that the final pH of the media without coconut water was lower while that which was given coconut water was higher. This can be caused because coconut water contains amino acids and organic acids which can neutralize the decrease in the pH of the media. Mandang (1995) found that the use of coconut water increased the buffer capacity of the media.

#### Acclimatization

Acclimatization has been carried out by removing the plants from the bottles and planting them in the media in small plastic pots. Plants in pots that have just been moved are each covered with a plastic lid which aims to suppress transpiration. After 20 days, live plants were observed (Table 3).

**Table 3:** Plants that live at 20 days acclimatization

Treatments	Plant live (%)
MS100 S	100
MS100 J	100
MS100 R	100
AOPO S	100
AOPO J	66.6
AOPO R	50
AOP1 S	100
AOP1 J	100
AOP1 R	100
A1PO S	100
A1PO J	66.6
A1PO R	50
A1P1 S	100
A1P1 J	100
A1P1 R	66.6
A2PO S	100
A2PO J	100
A2PO R	100
A2P1 S	100
A2P1 J	100
A2P1 R	100

At the age of 20 days the new plants begin to open the plastic cover. During 7 days the plant died almost 50%. After 3 weeks after opening the lid, only 20% of the plants that survive those are plants that grow on media that is treated with coconut water.

### 3. Conclusion

From the results of research and discussion it can be concluded:

- The growth of *in vitro* chrysanthemum varieties Suryandari, Jemani and Riri was better on 50% MS medium supplemented with 20% and 40% coconut water. So there is a saving in the use of MS media up to 50%. Best growth of Suryandari on media added 20 CW combine with CF and on treatment 40% CW without CF, and Riri Variety on 40% CW combine with CF.
- The pH of the media that added coconut water decreased very little at the end of the culture. So CW as buffer capacity of media.
- Addition of complementary fertilizer is needed if coconut water is 20% and is not needed if 40% coconut water is added to the media.
- Plants that survived an average of 100% acclimatization after opening the lid were one week old and about 50% acclimatized grew on media with coconut water on average.

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