



Genetic Variability and Heritability of *in vitro* Chrysanthemum on Various Combination of Plant Growth Regulators

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Abstract

Heritability is a measure that describes the magnitude of a genetic influence on a character rather than an environmental factor. The higher the heritability value of a character, the higher the genetic variability. The aim of the study was to know the interaction between the combination of growth regulators and chrysanthemum varieties that gave the best chrysanthemum growth *in vitro* and analyzed the heritability of various chrysanthemum characters. The research was carried out at the *In vitro* Laboratory of Agronomy Department, Faculty of Agriculture, Hasanuddin University, Makassar from May to September 2016. The study used a separate plot design in the group. The main plot was a combination of growth regulator consisting of 200 ml Coconut Water, 0.5 ppm BAP + Coconut Water 100 ml, BAP 1 ppm + Coconut Water 100 ml, 2,4-D 0.5 ppm + Coconut Water 200 ml, 2,4-D 0.5 ppm + BAP 0.5 ppm + Coconut Water 100 ml, 2,4-D 0.5 ppm + BAP 1 ppm + Coconut Water 100 ml. The subplots included 8 varieties of chrysanthemum (Fiji Pink, Marimar, Pasopati, Salzieta, Solida Pelangi, Limeron, Arosuka Pelangi, Yellow Tumohon). The basic media used was MS medium. The results showed that adding 200 ml young coconut to the culture media gave the best *in-vitro* chrysanthemum growth in terms of shoot and root formation, shoot count, root number, and shoot height in all chrysanthemum varieties; as well as all observed characters have high heritability and genetic variability

Keywords: chrysanthemum, genetic variability, growth regulators, heritability, varieties

A. Introduction

Chrysanthemum is one of the most popular ornamental plants in Indonesia. Chrysanthemum species are characterized by being decorative with beautiful colorful flowers. It has medical benefits so it is usually included in traditional medicine; moreover, it produces material which can be used as an insecticide. Worldwide, chrysanthemum varieties are highly valued as cut flowers. In general, the demand for chrysanthemums has been increased over the last years because of the availability of alternative choices and varieties of chrysanthemum; each variety has a flower with specific characteristics in terms of shape, size, color and their distinctive aroma.

There is an increase in the total harvest of chrysanthemum flower cuts from 2011 till 2015; in the same time increase in production and productivity of chrysanthemum plants were achieved. Based on data obtained from agricultural department (2015) about the production rate of chrysanthemum in South Sulawesi, in 2011 it reached 51.557 stems, in 2012 it became 54.198 stems, in 2013 the production increased heavily to reach 565.956 stems, and in 2014 the total production increased to be 640.222 stems.

The continuous increase in demand for chrysanthemums flowers raises the necessity to strive for a better system of cultivation for achieving a higher productivity of flowers and high quality seeds. Cultivars revealed that conventional propagation method of chrysanthemums is not recommended because it is time consuming and the plant would be more susceptible to diseases and pests infestations.

Vegetative propagation of chrysanthemum is rarely done because of its heterozygous nature, so the progeny seeds will not be the same as the parent seeds. Another alternative method to get chrysanthemum propagation in high scale and short time is the in-vitro propagation method.

For optimum results, the basic media and growth regulator are very important factors (Purnamaningsih & Lestari, 1998). The combination of basic media and proper growth regulator will increase the activity of cell division organogenesis stage. Growth regulators include auxin and cytokinin groups. In general, auxin is used for induction of callus formation, suspension culture and roots through the stimulation of cell division and the elongation of cambium tissue (Pierik, 1987).

The combination of cytokines with auxin can stimulate the morphogenesis process in bud formation (Flick, Evans, & Sharp, 1993). In common rue plants, the formation of adventitious shoots from the stem can be obtained using the following media: MS + BA 1.5 mg/l + 2.4-D 0.3 mg/l (Lestari & Husni, 1997).

The usage of growth regulators in tissue culture depends on the desired purpose or direction of plant growth. The growth regulator BA (benzyl adenine) is widely used to stimulate shoot bud proliferation and multiplication because of its strong, effective activity in compare with other artificial growth regulators. The addition of complex organic supplements is very important in stimulating in-vitro explant growth. Coconut water is considered as a complete organic compound that contains a variety of important nutrients which help in the growth of plant cultures, it is expected that adding coconut water to the culture can be helpful in the in-vitro propagation of chrysanthemum (Yusnida, Syafii & Sutrisna, 2006). Kristina & Syahid (2012) revealed that every 1 liter of coconut water contains 273.62 mg ZPT kinetin (cytokinin) and several other minerals (Zaer & Mapes, 1982).

Based on the abovementioned information, a study was conducted regarding the in-vitro growth of different types of chrysanthemum using different combinations of growth regulators. Through this research, it is expected to determine the proper combination of growth regulators for the tested chrysanthemum varieties regarding the production of high quality seeds.

B. Methodology

This research was carried out at the Plant Tissue Culture Laboratory, plant culture division, Faculty of Agriculture, Hasanuddin University, Makassar from May till September 2016.

The tools which were used in this research include analytical scale, culture bottles, autoclave, graduated beaker glass, measuring cylinders, erlenmeyer flasks, funnels, drop pipettes, wash

squeeze bottle, pH meters, electric heater, handsprayers, Laminar Air Flow Cabinet (L AFC), forceps, benzene, scalpel, scissors, petri dishes and matches.

Materials include MS media (Appendix Table 1), sucrose, agar-agar, coconut water, aquabides, 2,4 Dichlorophenoxyacetic Acid (2,4 D), Benzyl Amino Purine (BAP), alcohol 96%, alcohol 70%, eight varieties of chrysanthemums explants, aluminum foil, rubber bands, plastic wrapping, spiritus lamp and tissue.

This research was carried out based on split plot design employed in a Randomized complete block design with the main plot composed of 6 combinations of media; MS + 200 ml Coconut Water, MS + BAP 0.5 ppm + 100 ml Coconut Water, MS + BAP 1 ppm + Coconut Water 100 ml, MS + 2,4-D 0, 5 ppm + 200 ml Coconut Water, MS + 2,4-D 0.5 ppm + BAP 0.5 ppm + 100 ml Coconut Water, MS + 2,4-D 0.5 ppm + BAP 1 ppm + Coconut Water 100 ml. While the sub-plots included 8 varieties of chrysanthemums Fiji Pink, Marimar, Pasopati, Salzieta, Solida Pelangi, Limeron, Arosuka Pelangi, Tumohon Kuning. The total treatment combinations were 48. Each treatment combination had 3 replications, each replication included 2 units so the total experimental unites was 288.

Preparation

All the dissection tools (forceps, scaples, scissors and knives), culture bottles and petri dishes, were washed with soap, then rinsed with running water and then autoclaved for sterilization at 121°C and pressure of 17-20 psi for 1 hour.

Stock solutions of MS media (1000 ml) and hormone stock solutions (50 ml) (2, 4-D and BAP) were prepared. For culture media preparation, stock MS media was mixed with other components including sucrose, agar-agar, 2,4 D and BAP hormones, and coconut water according to a predetermined concentration. The next step was to adjust the pH of the culture media using a pH meter until reaching 5.8. Then in the final, aqua bides was added to a total volume of 1000 ml. The media was heated over the electric heater meanwhile it was stirred up to near the boiling point 98°C. After that the media was poured into the culture bottles (± 20) each and covered with aluminum foil. After that, the media was sterilized in autoclave at a pressure of 15 psi for 10 minutes.

Incubation room was sterilized by cleaning and spraying formaldehyde into every corner of the room then the room temperature was adjusted at 24°C. In addition, 40 watts of lighting was also provided.

Culturing

Plant culturing was carried out in Laminar Air Flow Cabinet (L AFC). Chrysanthemum explants were cut ± 2 cm above petri dish using culture scissors and forceps. Then the explants were carefully transferred using sterile forceps and cultured in each treatment media.

Incubation

Maintenance was carried out by spraying 70% alcohol in culture bottles to avoid contamination in each treatment. Spraying with alcohol was performed every day. The contaminated bottles were immediately removed and discarded from the incubation chamber.

Observed parameters

Shoot and root initiation in each treatment media. Its were determined by counting the days from culturing until the first appearance of the shoot. Total number of roots and shoots in each treatment media that ware calculated in the end of the experiment. Height of shoots (cm) in each treatment media that was measured from the base of the leaf till the final height of growth in the end of the experiment.

Data Analysis

Data obtained from the observations were analyzed using one way Anova employed in a Randomized complete block design (RCB). Significantly different variables were then analyzed using LSD analysis ($\alpha = 0.05$).

Heritability

According to Stansfield (1983) the predicted value of heritability and its criteria were calculated using the following formula:

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100\%$$

Heritability value criteria:

- $h^2 > 0.5$: high heritability
- $0.2 < h^2 < 0.5$: medium heritability
- $h^2 < 0.2$: low heritability

C. Result and Discussion

Analysis of variance showed that the interaction between varieties and planting media had a very significant effect on all observed parameters. Interactions that occur between varieties and media have an effect on the growth of explants.

1. Shoot Initiation

Table 1. Average of shooting initiation rate (days) in each treatment media and Chrysanthemum varieties

Varities	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	7.67 ^b _q	4.75 ^a _p	7.67 ^b _q	9.00 ^b _q	8.08 ^b _q	7.33 ^b _{pq}	1.72
V2	5.75 ^a _{pq}	5.58 ^a _p	5.50 ^a _{pq}	6.42 ^{ab} _p	7.58 ^b _{pq}	6.42 ^{ab} _p	
V3	7.17 ^{ab} _q	8.58 ^b _q	6.50 ^a _{pq}	21.08 ^c _s	10.08 ^b _r	10.17 ^b _q	
V4	7.67 ^{ab} _q	7.50 ^{ab} _q	6.33 ^a _{pq}	6.33 ^a _p	7.92 ^{ab} _q	9.00 ^b _q	
V5	4.75 ^a _p	6.08 ^{ab} _{pq}	5.42 ^a _p	5.83 ^{ab} _p	6.08 ^{ab} _p	7.50 ^b _{pq}	
V6	6.17 ^a _{pq}	6.00 ^a _{pq}	6.08 ^a _{pq}	13.42 ^b _r	13.00 ^b _r	7.17 ^a _p	
V7	5.17 ^a _p	4.75 ^a _p	6.00 ^{ab} _{pq}	9.00 ^c _q	7.75 ^{bc} _{pq}	7.17 ^b _p	
V8	5.58 ^a _{pq}	7.42 ^b _q	7.17 ^{ab} _q	7.50 ^b _{pq}	8.67 ^b _{qr}	7.75 ^b _{pq}	
NPm LSD		1,72					

Note: Means in the same column (abc) with different super script and in the same row (pqr) with different sub script are significantly different ($P < 0.05$).

Results in table 1 revealed that there were interaction between the media type and the plant variety regarding the shoot initiation period as the following; there were an interaction between M1 media (MS+coconut water 200 ml) and V5 (Solida Pelangi) and V7 (Arosuka Pelangi) varieties, between M2 media (MS+ BAP 0,05 ppm+coconut water 100 ml) and V1 (Fiji Pink), V2 (Marimar), and V7 (Arosuka Pelangi) varieties, between M3 media (MS+BAP 1 ppm+coconut water 100 ml) and V5 (Solida Pelangi) variety, between M4 media (MS+2,4 D 0.05 ppm+coconut water 200 ml) and V4 (Salzieta) variety and between M6 media (MS+2,4 D 0.05 ppm+BAP 1 ppm+coconut water 100 ml) and V6 (Limeron) variety producing the fastest shoot initiation which were not significantly different from the interactions between M1 media with V2, V3, V4, V6, V8 varieties, between M2 media with V4, V5, V6 varieties, between M3 media with V2, V3, V4, V6, V7, V8 varieties, between M4 media with V2 and V5 varieties, between M5 media with V4 and V5 varieties, between M6 media with V2 variety but significantly different from other treatment interactions.

Results in table 2 revealed the presence of interaction between M1 media (MS + coconut water 200 ml) with V6 (Limeron) and V7 (Arosuka Pelangi) varieties, between M4 media (MS+2,4 D 0.05 ppm+coconut water 200 ml) with V3 (Pasopati) variety which were significantly different from other treatment interactions.

2. Roots Initiation

Table 2. Average of root initiation rate (days)

Varietes	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	10.25 ^a _{qr}	90.00 ^c _t	90.00 ^c _p	27.67 ^b _s	90.00 ^c _q	90.00 ^c _p	2.42
V2	12.17 ^a _r	42.50 ^c _s	90.00 ^d _p	37.33 ^b _u	35.08 ^b _p	90.00 ^d _p	
V3	10.75 ^a _{qr}	90.00 ^b _t	90.00 ^b _p	11.17 ^a _p	90.00 ^b _q	90.00 ^b _p	
V4	9.50 ^a _q	22.67 ^b _q	90.00 ^c _p	23.58 ^b _r	90.00 ^c _q	90.00 ^c _p	
V5	9.67 ^a _q	90.00 ^c _t	90.00 ^c _p	31.08 ^b _t	90.00 ^c _q	90.00 ^c _p	
V6	5.08 ^a _p	10.58 ^b _p	90.00 ^c _p	8.75 ^b _p	90.00 ^c _q	90.00 ^c _p	
V7	5.83 ^a _p	37.67 ^c _r	90.00 ^d _p	19.08 ^b _q	90.00 ^d _q	90.00 ^d _p	
V8	12.42 ^a _r	90.00 ^c _t	90.00 ^c _p	27.50 ^b _s	90.00 ^c _q	90.00 ^c _p	
Npm LSD			2.33				

Note: -Means in the same column (abcd) with different super script and in the same row (pqrstu) with different sub script are significantly different (P < 0.05), Number 90 means there is no root formed.

3. Total number of roots

Table 3. Average of total number of roots

Varieties	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	12.25 ^a _q	0.00 ^c _s	0.00 ^c _p	5.33 ^b _r	0.00 ^c _q	0.00 ^c _p	0.70
V2	8.92 ^a _s	1.83 ^c _r	0.00 ^d _p	3.17 ^b _s	3.67 ^b _p	0.00 ^d _p	
V3	7.50 ^a _t	0.00 ^c _s	0.00 ^c _p	3.33 ^b _s	0.00 ^c _q	0.00 ^c _p	
V4	12.75 ^a _q	2.08 ^c _r	0.00 ^d _p	7.08 ^b _p	0.00 ^d _q	0.00 ^d _p	
V5	11.17 ^a _r	0.00 ^c _s	0.00 ^c _p	3.75 ^b _s	0.00 ^c _q	0.00 ^c _p	
V6	10.50 ^a _r	7.42 ^b _p	0.00 ^c _p	6.17 ^b _q	0.00 ^c _q	0.00 ^c _p	
V7	14.17 ^a _p	3.00 ^c _q	0.00 ^d _p	5.08 ^b _r	0.00 ^d _q	0.00 ^d _p	
V8	12.33 ^a _q	0.00 ^c _s	0.00 ^c _p	7.17 ^b _p	0.00 ^c _q	0.00 ^c _p	
Npm LSD			0.68				

Note: - Means in the same column (abcd) with different super script and in the same row (pqrst) with different sub script are significantly different (P < 0.05)

Results in table 3 revealed the presence of interactions between M1 media (MS + coconut water 200 ml) and V7 (Arosuka Pelangi) variety producing the highest number of total roots that was significantly different from other treatment interactions.

4. Total number of shoots

Results in table 4 revealed interactions between M2 media (MS+BAP 0.05 ppm+coconut water 100 ml) and V3 (Pasopati) variety, between M3 media (MS+BAP 1 ppm+ coconut water 100 ml) and V2 (Marimar) variety, between M5 media (MS+ 2.4 D 0.05 ppm+ BAP 1

ppm+coconut water 100 ml) and V3 (Pasopati) variety producing the highest total number of shootings that were significantly different from other treatment interactions.

Table 4. Average of total number of shoots in each treatment media and Chrysanthemum varieties

Varieties	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	1.58 ^{bc} _p	1.67 ^b _p	2.33 ^a _q	1.08 ^c _q	1.42 ^{bc} _{pq}	1.33 ^c _p	0.27
V2	1.25 ^c _q	1.67 ^b _p	2.92 ^a _p	1.50 ^{bc} _p	1.67 ^b _p	1.17 ^c _{pq}	
V3	1.33 ^b _{pq}	1.83 ^a _p	1.83 ^a _r	1.00 ^c _q	1.67 ^a _p	1.17 ^{bc} _{pq}	
V4	1.42 ^a _{pq}	1.00 ^b _q	1.17 ^{ab} _t	1.00 ^b _q	1.00 ^b _r	1.08 ^b _{pq}	
V5	1.00 ^a _q	1.17 ^a _q	1.00 ^a _t	1.00 ^a _q	1.00 ^a _r	1.00 ^a _q	
V6	1.42 ^a _{pq}	1.17 ^{ab} _q	1.25 ^a _s	1.00 ^b _q	1.33 ^a _q	1.00 ^b _q	
V7	1.00 ^a _q	1.00 ^a _q	1.00 ^a _t	1.00 ^a _q	1.08 ^a _{qr}	1.00 ^a _q	
V8	1.42 ^a _{pq}	1.00 ^b _q	1.00 ^b _t	0.83 ^b _q	1.00 ^b _r	1.00 ^b _q	
NPm LSD			0.26				

Note: - Means in the same column (abc) with different super script and in the same row (pqrst) with different sub script are significantly different (P < 0.05).

5. Shoot Length

Table 5. Average of final shoot length in each treatment media and Chrysanthemum varieties

Varieties	Media						NPv LSD
	M1	M2	M3	M4	M5	M6	
V1	19.67 ^a _p	3.45 ^c _q	2.83 ^{cd} _q	5.87 ^b _q	2.47 ^d _{pq}	2.88 ^{cd} _p	0.65
V2	9.15 ^a _s	2.23 ^c _{rs}	2.09 ^{cd} _r	3.47 ^b _s	1.96 ^{cd} _q	1.46 ^d _q	
V3	11.42 ^a _{qr}	1.97 ^c _s	2.55 ^{bc} _{qr}	2.79 ^b _t	1.42 ^{cd} _{qr}	0.79 ^d _r	
V4	8.04 ^a _t	1.75 ^d _s	2.00 ^d _r	3.75 ^b _s	2.83 ^c _p	2.42 ^{cd} _p	
V5	12.04 ^a _q	4.96 ^c _p	4.67 ^c _p	6.04 ^b _q	1.25 ^d _r	1.17 ^d _{qr}	
V6	7.29 ^a _u	4.83 ^b _p	2.38 ^c _{qr}	4.71 ^b _r	0.67 ^d _r	0.67 ^d _r	
V7	11.21 ^a _r	2.07 ^c _{rs}	1.82 ^c _r	3.67 ^b _s	0.88 ^d _r	0.75 ^d _r	
V8	8.19 ^b _t	2.67 ^{cd} _r	2.25 ^d _{qr}	8.93 ^a _p	1.88 ^d _{qr}	3.05 ^c _p	
NPm LSD			0.67				

Note: - Means in the same column (abcd) with different super script and in the same row (pqrst) with different sub script are significantly different (P < 0.05).

Results in table 5 revealed interaction between M1 media (MS+coconut water 200 ml) and V1 (Fiji Pink) variety, between M4 media (MS + 2.4 D 0.05 ppm +coconut water 200 ml) and V8 (Tumohon Kuning) variety producing the highest shoots which are significantly different from other treatment interactions.

6. Heritability

Table 6. Heritability value in each treatment media and Chrysanthemum varieties

No	Character	h ² (%) value	Degree
1	Shoot Initiation	91.10	High
2	Root Initiation	99.04	High
3	Total number of roots	93.29	High
4	Total number of shootings	88.70	High
5	Shooting length	95.69	High

Note: $0 < h^2 \leq$ (low), $21 < h^2 \leq 50$ (medium), $51 < h^2 \leq 100$ (high)

Results of heritability analysis in table 6 showed that all the evaluated 5 parameters have high heritability scores. The highest heritability score was revealed in the plantlet formation rate parameter which has heritability score equal to 99.26 %. Such results confirm that the influence of genes on chrysanthemum plants dominates when compared with the influence of environment.

D. Discussion

The research results showed that media without the addition of 2, 4-D (M1, M2, and M3) provide faster sprout growth in compare with adding 2, 4-D (M4, M5, and M6). The rapid growth of shoots in M1, M2 and M3 media were caused by the addition of coconut water and BAP as a cytokinin hormone which accelerated cell division and the growth of explants, and stimulated cytokinesis process that caused the cells to grow long and cell differentiation, such a differentiation make those cells to undergo functional specialization that initiate the organogenesis process and lead to buds/shoots formation (Suryowinoto, 1996).

The formation of shoots within short time leads to a fast shoot length, the highest shoot length produced in this investigation was found in pink Fiji variety which was cultured in M1 media that contain 200 mL⁻¹ (M1) (Table 5). It could be because the coconut water is a complex organic compound containing vitamins, ZPT, macro and micro nutrients, and high sugar content as an energy source for growth of shoots and leaves (Vigliar, Sdepanian, & Fagundes-Neto, 2006).

The highest shoot formation would lead to the formation of auxin in the tip of the shoot, then the produced auxin translocate to the base of stem, and causes auxin concentration to be increased until the stimulation of faster organogenesis process in the root area and the increased number of grown roots. In MS+air kelapa 200 mL⁻¹ (M1) the growth rate was faster and the total productivity was more (Table 2 and 3). Such results are in line with the findings of Bey, Syafii, & Sutrisna, (2006), who revealed that adding coconut water at a concentration of 250 mL⁻¹ gave faster orchid growth (*Phalaenopsis amabilis* BL.).

Nevertheless, the total number of shoots was higher in case of combining coconut water with air BAP in MS like in M3 media (Table 4). According to Parera (1997), adding 20% coconut water in the media of orchid plants lead to a significant increase in the total number of shootings if compared with other concentrations 30%, 40%, and 50%, but not significantly different with 10%.concentration. The higher the concentration of coconut water the less the number of shoots produced and this shows a quadratic tendency. Thus, decreasing the concentration of coconut water to 100 mL⁻¹ combined with BAP 1 ppm gave the highest number of shoots in Fiji Pink (V1) and Marimar (V2) varieties. Such results were in agreement with Sompotan & Tilaar (2008) who revealed that adding BAP to MS media with concentration 1 - 5 ppm lead to the growth of new shoots. Moreover, Tilaar, Rantung, & Tulung (2013) had an investigation about in-vitro propagation of kulo chrysanthemum buds explants in media containing NAA and BAP, the highest number of new shoots (8.2 shoots) were observed when adding 1 ppm BAP.

The presence of hormones in the culture media is considered as an important factor that impact on the growth rate of explants. The in-vitro organogenesis and growth rate of the

explants are usually controlled by the balance and interaction of the plants' hormones (Kasli, 2009). Basri & Muslimin (2001) revealed that part of the hormone in the culture media will infiltrate the plant cell through diffusion or active absorption, that lead to the change in the internal hormone balance.

Such a change can be obviously revealed from the results of heritability of each evaluated variable. In this research, the results revealed that all the evaluated variables have high heritability score. That led to the assumption that the growth and organogenesis rates were mainly affected by the genetics (75.16% - 99.26%) rather than the environment. therefore, chrysanthemum has a high genetic variability and incase of performing genotypic selection, and better types could be improved. Rasyad (1996) suggested that the coefficient value of genetic variability was high, so genetic factors had a great influence on traits expression compared to the environment effects.

E. Conclusion

Based on the results obtained, then the conclusion is as follows:

1. The fastest in-vitro organogenesis of chrysanthemum Fiji Pink varieties could be achieved by using the following culture media formula; MS + 2.4-D 0.5 ppm + BAP 1 ppm + coconut water 100 ml (M6), callus formation occurred in just 11.5 days.
2. The best in-vitro organogenesis of chrysanthemum for shoot, root formation, total number of shoots, total number of roots, root length, shoot height, total number of leaves and plantlet formation could be achieved by using the following culture media; MS + coconut water 200 ml (M1).
3. All the observed variables have high heritability and genetic variability.

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