Agrotech Journal
Url: http://usnsj.id/index.php/ATJ
Email: editor.atj@usnsj.com

Early Growth of Chrysanthenum Explants Due to Various Concentration of Coconut Water During In Vitro Propagation

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ARTICLE INFO

e-ISSN: 2548-5148
p-ISSN: 2548-5121
Vol. 8 No. 1, June 2023
URL: http://dx.doi.org/10.31327/atj.v8i1.1988

Abstract

A study aimed to determine the effect of coconut water on the growth of Chrysanthemum explants was conducted. The study was carried out in the form of factorial experiments at Tissue Culture Laboratory, Department of Agronomy, Faculty of Agriculture, Hasanuddin University from December 2022 to March 2023. Media used was Murashige and Skoog (MS) added with three dose of coconut water, 100, 200, and 300 ml, respectively. Plantlets of five varieties of Chrysanthemum, Pinka Pinky, White Bakardy, Maruta, Kineta, and Dera obtained from Tissue Culture Laboratory, Horticultural Plant Seed Center, Bonto-bonto District, Gowa Regency. The explants were planted in Murashige and Skoog (MS) media containing the coconut water according to the treatments. Each combination of treatment was repeated three times. Results show that addition of 100 ml coconut water into the MS media was the best dose for plantlets growth indicated by earliest shoot emergence (9.04 days) and plantlet formation (9.25 days), and highest average number of leaves (15.00 leaves). The coconut water treatment of 200 ml showed the highest average number of roots (14.67 roots). Variation in responses between varieties were observed.

Keywords: *Chrysanthemum morifolium*, coconut water, in vitro, variety, auxins, cytokinins

A. Introduction

Chrysanthemum (*Chrysantemun morifolium*) is an ornamental plant that has high economic value and has been very popular in Indonesia for the last two decades. Demand for chrysanthemums tends to increase every year. Chrysanthemum is the second largest traded cut flower in the world after roses. The demand for cut flowers and potted chrysanthemum plants in the both domestic and the international market is increasing from year to year. The development of chrysanthemum production in Indonesia has declined during the 2018-2022 period with an average decline of 13.35% per year (Indonesian Statistics Bureau, 2022). The large number of requests for chrysanthemum plants is not proportional to the parent plant preparations and the flower quality desired by the market. The number of mother plants is not enough to meet the availability of seeds for chrysanthemum propagation. The method that can be used to overcome the problem of availability of chrysanthemum seeds is by in vitro propagation. In vitro plant propagation method large quantities of chrysanthemum seeds can be produced in a short time.

The success of plant production using the tissue culture method is determined by the composition of the nutrients in the media. The medium used for in vitro propagation consists of macro and micro nutrients, vitamins, and hormones. One of the organic materials that can be added in tissue culture media is coconut water. Coconut water contains a complete chemical and nutritional composition such as hormones, macronutrients, micronutrients, vitamins, minerals and amino acids (Tuyekar, Tawade, Singh, Wagh, Vidhate, Yevale, Gaikwad & Kale, 2021) which have a positive effect on plant growth when applied to the plants. Some of the macronutrients contained in coconut water such as sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), protein, sugar, and are supported by two hormones, namely auxins and cytokinins (Aishwarya, Prashanth, Seenivasan, & Saida, 2022), that play a role in supporting cell division and plant growth.

Previous studies showed that the use of coconut water in the media can increase the growth of plants when added in to the basic growth media. The organic compound has been used in the in vitro propagation of different types of plants such as garlic (Nandariyah, Mahmudah, Arniputri & Sakya, 2021), orchids (Pratama & Nilahayati, 2018), mustard greens (Musawira, 2017). The best organogenesis of chrysanthemum in vitro for the formation of the number of shoots, shoot height and number of leaves was obtained on MS media + 200 ml of coconut water, but the response varied between varieties (Solihah, Supriyatna, & Adawiah, 2021).

B. Methology

The study was conducted at Tissue Culture Laboratory, Department of Agronomy, Faculty of Agriculture, Hasanuddin University from December 2022 to March 2023. A Split Plot Design

was employed with coconut water as the main plot and Chrysanthemum variety as the Subplots. Three doses of coconut water of 100 ml, 200 ml, and 300 ml were added in to the Murashige and Skoog (MS) media as the basic media for the in vitro propagation. Five varieties of Chrysanthemum of Pinka Pinky, White Bakardy, Maruta, Kineta, and Dera were used. A total of 15 treatment combination was obtained. Each treatment combination consisted of two bottles with four explants planted in each culture bottle and repeated three times resulted in a total of 360 explants.

Culture bottles and planting tools were sterilized using autoclave set to temperature of 121 °C at 17.5 psi (pound per square inch) for 1 h. Media was prepared by mixing all stock solution (A, B, C, D, E, F, G and H) components sequentially one by one while stirring. The mix then added with 100 ml of coconut water, 20 grams of sugar, and the dose of coconut water according to the treatment. Sterile distilled water was added until the total volume reached to 1,000 ml. pH of the media was adjusted to 5.8 by adding NaOH or HCl before poured to a saucepan and add 7 gr of agar. The media is heated over an electric stove while stirring until it approaches the boiling point of 98°C. After the media boils, the media is removed and poured into culture bottles as much as 25 ml each. Furthermore, the culture bottle was covered with aluminum foil and tied with a rubber band. Media was sterilized using autoclave at temperature of 121 °C at 17.5 psi (pound per square inch) for 15 minutes

Source of explants was obtained from Tissue Culture Laboratory, Horticultural Plant Seed Center, Bonto-bonto District, Gowa Regency. Planting was conducted in a laminar air flow cabinet (LAF) that previously sterilized with alcohol 70% and UV light turned on for one hour. Plantlets of each variety were cut and sub cultured into the MS media contained coconut water according to the treatment levels. Plantlets were cut including two nodes with one node was immersed into the media.

Observation was made on growth of the explants as response to different coconut water and variety treatments. Data was then analyzed using a two-way analysis of variance (ANOVA). If there is a significant effect of treatments, a Tukey's further test was carried out at confidence level of 95% to test the difference between averages.

C. Result

Time to root emergence

Analysis of variance showed that the coconut water treatment did not have any significant effect on the time of root emergence of Chrysanthemum explants. No differences between the coconut water concentration of 100, 200, and 300 ml added to the MS media used, were observed. The parameter only significantly varied between the varieties. Table 1 shows time to roots emergence on the explants of Chrysanthemum. Kineta variety showed earliest root emergence of 5.24 days and was not significantly different to Dera and Maruta. Longest root emergence time of 7.71 days was observed in White Bakardy variety that was not different significantly to Pinky Pink variety (6.55 days). Variation detected in the recent study among the varieties of the chrysanthemum explants can be caused by the high degree of heterozygosity in the plant (Nasri, Zakizadeh, Vafaee, & Mozafari (2021). Variation also found the previous studies such as in Solihah et al. (2021).

Table 1. Average time to root emergence (days) of Chrysanthemum explants in different coconut water treatments.

Variety	Media			Mean	Tukey's [V] 0.05
	MS + 100ml Coconut Water	MS + 200ml Coconut Water	MS + 300ml Coconut Water		
Pinka Pinky	6.17	6.67	6.81	6.55ab	
White Bakardy	6.33	8.29	8.50	7.71a	
Maruta	6.00	5.79	6.51	6.10bc	1.24
Kineta	4.75	5.54	5.42	**5.24c**	
Dera	4.69	5.83	6.33	5.62bc	
Mean	5.59	6.42	6.71	6.24	

Numbers followed by the same letter mean not significantly different based on Tukey's test at level of confidence of 95% (α=0.05).

Time to Shoot Emergence

Based on analysis of variance, shoot emergence parameter on Chrysanthemum explants was significantly affected by the coconut water treatment and variety. Addition of 100 ml of coconut water into MS media resulted in earliest time to shoot emergence of 9.04 days in explant of White Bakardy variety (Table 2). Use of higher dose of coconut water of 200 ml and 300 ml resulted in earlier time to shoot emergence in variety of Maruta (9.58 days) and White Bakardy (9.87 days), respectively. The latest shoot emergence time was observed in Kineta variety (13.50 days) with 300 ml of coconut water in the MS media. Shoot formation on explants during in vitro propagation is regulated by the division and development of cells in the meristematic tissue of the plant. Coconut water in the media contain a complete composition of nutrients to allow the process. In addition, content of auxin and cytokine in the organic compound also stimulate the cell division and development (Aishwarya et al., 2022). In the recent study, a varied responses in the variety to increased coconut water doses were observed, in general higher doses of coconut water delay shoot emergence.

Table 2. Average time to shoot emergence (days) of Chrysanthemum explants in different coconut water treatments.

Variety (V)	Media (P)			Tukey's [V] 0.05
	MS + 100ml Coconut Water	MS + 200ml Coconut Water	MS + 300ml Coconut Water	
Pinka Pinky	10.30^{a}_{p}	10.92^{a}_{p}	10.38^{a}_{q}	
White Bakardy	$\mathbf{9.04^{b}_{p}}$	11.42^{a}_{p}	$\mathbf{9.87^{ab}_{q}}$	
Maruta	9.08^{a}_{p}	$\mathbf{9.58^{a}_{p}}$	9.92^{a}_{q}	2.02
Kineta	9.55^{b}_{p}	9.72^{b}_{p}	13.50^{a}_{p}	
Dera	9.37^{b}_{p}	11.82^{a}_{p}	10.68^{ab}_{q}	
Tukey's [P] 0.05		2.54		

Numbers followed by the same letter (a,b) in the same row and (p,q) in the same column, mean not significantly different based on Tukey's test at level of confidence of 95% (α=0.05).

Time to plantlet formation

Recent research show that plantlets formation of Chrysanthemum explants was varied between the coconut water dose and variety. Addition of 100 ml coconut water in Dera variety resulted in earliest time to plantlet formation (9.25 days) (Table 3). On the other hand, earlier time to plantlet formation was observed from the use of higher concentration of coconut water in the MS media (200 and 300 ml) in White Bakardy variety compared to other varieties. Successful of plant propagation using in vitro method depends on the composition of nutrients in the media. MS media is a basic in vitro media generally used to meet the requirement of the explants for formation of new shoots and roots on the explants. Despite the content of the basic media, presence of hormones also needed to direct the further growth of the newly formed organ. Coconut water serve to the media to provide the hormone namely auxin and cytokine (Aishwarya et al., 2022). Auxin is known to regulated the cell division and cytokine helps the plant in shoot formation, presence of both growth regulator will result in more effective growth of the planlets (Nandariyah et al., 2021).

Table 3. Average time to plantlet formation (days) of Chrysanthemum explants in different coconut water treatments.

Variety (V)	Media (P)			Tukey's [V] 0.05
	MS + 100ml Coconut Water	MS + 200ml Coconut Water	MS + 300ml Coconut Water	
Pinka Pinky	9.46^{b}_{p}	12.58^{a}_{p}	10.03^{b}_{q}	
White Bakardy	9.63^{a}_{p}	$\mathbf{9.45^{a}_{q}}$	$\mathbf{10.00^{a}_{q}}$	
Maruta	9.33^{a}_{p}	10.17^{a}_{q}	10.08^{a}_{q}	1.35
Kineta	9.55^{b}_{p}	9.75^{b}_{q}	12.50^{a}_{p}	
Dera	$\mathbf{9.25^{b}_{p}}$	9.67^{ab}_{q}	10.92^{a}_{q}	

Tukey's [P] 0.05	1.38

Numbers followed by the same letter (a,b) in the same row and (p,q) in the same column, mean not significantly different based on Tukey's test at level of confidence of 95% (α=0.05).

Number of leaves

Analysis of variance showed that number of leaves on the Chrysanthemum plantlets were differed between the concentration of coconut water added to the MS media. No significant difference were found between variety. Table 4 shows that concentration of 100 ml coconut water in the MS media resulted in highest leave numbers of Chrysanthemum plantlets of 15.00 leaves. Higher coconut water concentration in the media did not necessarily increase the number of plantlet leaves. A decreased value of the parameter observed when the concentration increased. However, a slight increase was observed in the parameter when the concentration increased to 300 ml. Organogenesis during in vitro propagation, including the formation of leaves, is mostly directed to the balance of auxins and cytokines hormones contained in the media. In this parameter, no difference found between varieties and leaves formation on the plantlets was significantly affected by the coconut water in the MS media. In addition to hormones, coconut water also contains sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), protein and sugar (Tuyekar et al., 2021). Study of Prando, Chiavazza, Faggio, & Contessa (2014) showed that the addition of 20% coconut water increased the number of adventitious shoots per explant.

Table 4. Average number of leaves (leaves) of Chrysanthemum plantlets in different coconut water treatments.

Variety (V)	Media (P)		
	MS + 100ml Coconut Water	MS + 200ml Coconut Water	MS + 300ml Coconut Water
Pinka Pinky	14.33	9.67	13.67
White Bakardy	14.33	10.67	11.33
Maruta	18.00	13.33	14.00
Kineta	14.00	14.33	13.33
Dera	14.33	12.67	13.67
Mean	**15.00a**	12.13b	13.20ab
Tukey's [P] 0.05		2.32	

Numbers followed by the same letter mean not significantly different based on Tukey's test at level of confidence of 95% (α=0.05).

Number of Roots

Results show that number of roots formed on the plantlet of Chrysanthemum was affected significantly by the coconut water and variety treatments. Highest plantlet roots number of 14.67 roots was observed in the 200 ml of coconut water in Maruta variety. In fact, almost all variety showed higher root formation the 200 ml coconut water treatment added to MS media compared to other two treatments of 100 and 300 ml of coconut water in all variety except in Kineta variety which number of roots increased with the coconut water concentration (Table 5). Root formation is one of important processes in the plant propagation using in vitro method. Roots play important role in nutrition absorption of the plantlets from the media. Initiation of root and further growth can be hastened by the addition of plant growth regulator in the media. Due to its content of different plant growth regulators, coconut water is considered as root hormone (Prando et al., 2014). The natural Auxin in coconut water help the development of the adventitious roots on the Chrysanthemum explants.

Table 5. Average number of root (roots) of Chrysanthemum plantlets in different coconut water treatments.

Variety (V)	Media (P)			Tukey's [V] 0.05
	MS + 100ml Coconut Water	MS + 200ml Coconut Water	MS + 300ml Coconut Water	

Pinka Pinky	2.81^{c}_{r}	14.27^{a}_{p}	7.10^{b}_{qr}	
White Bakardy	8.10^{b}_{pq}	12.20^{a}_{p}	10.20^{ab}_{q}	
Maruta	$\mathbf{9.38^{b}_{p}}$	$\mathbf{14.67^{a}_{p}}$	$\mathbf{14.65^{a}_{p}}$	3.36
Kineta	4.91^{b}_{qr}	5.83^{b}_{q}	10.05^{a}_{q}	
Dera	7.88^{ab}_{pq}	10.86^{a}_{p}	5.50^{b}_{c}	
Tukey's [P] 0.05		4.26		

Numbers followed by the same letter (a,b) in the same row and (p,q) in the same column, mean not significantly different based on Tukey's test at level of confidence of 95% (α=0.05).

Number of Shoots

Shoot formation in all variety of Chrysanthemum's plantlets was not significantly affected by the coconut water treatments. Number of shoots varied between varieties and ranged between 1 and 1.67 shoots Highest number of shoots was shown by White Bakardy and Kineta variety each with the use of 300 ml coconut water in the MS media (Figure 1). While the lowest shoot numbers was observed in Maruta and Kineta variety. Despite the insignificant results of the treatments, number of shoots tend to increase with the dose of coconut water in the MS media. Highest average number of shoots (1.4 shoots) was observed in the 300 ml coconut water treatment. The dose of coconut water in the recent study was higher than what use in the previous study by Musawira (2017) which was 200 ml + MS media. Nutrition in the coconut water in addition to the content of macronutrients, micronutrients, and vitamin the MS media can support the cell division in the meristematic tissue of the planlets which at the later stage forming new shoots.

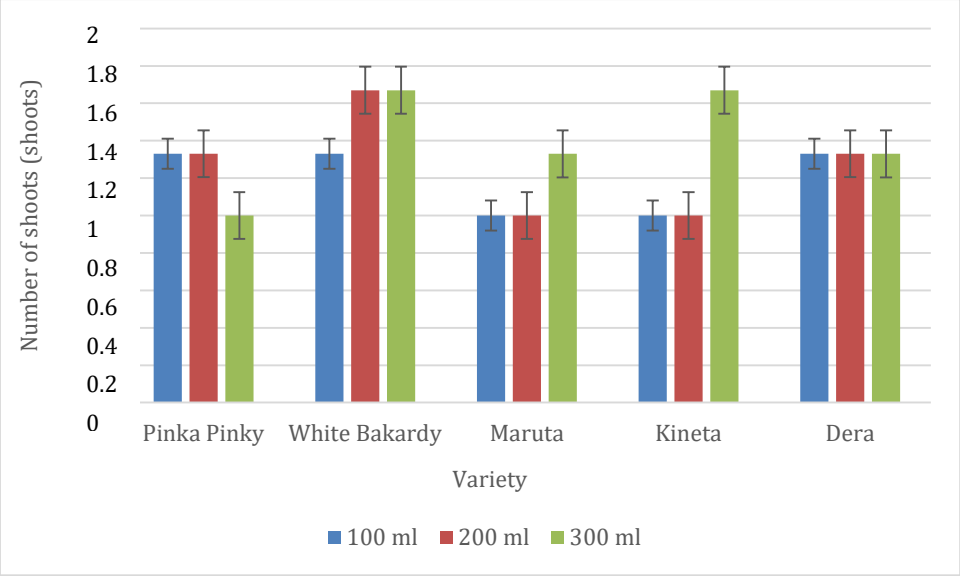

Figure 1. Average Number of Shoots (shoots) of Chrysanthemum plantlets in different coconut water treatments.

D. Conclusion

Modified MS media used by the addition of coconut water for in vitro propagation of Chrysanthemum explants can improve the early growth of explants. The best dose for plantlets growth was 100 ml indicated by earliest shoot emergence (9.04 days) and plantlet formation (9.25 days), and highest average number of leaves (15.00 leaves). The coconut water treatment of 200 ml showed the highest average number of roots (14.67 roots). Varied responses of the varieties used in this study indicated a strong genetically differences between the varieties in responding to the coconut water treatments in all parameter observed.

E. References

Aishwarya, P. Prashanth, N. Seenivasan & D. Saida, Naik (2022). Coconut water as a root hormone: Biological and chemical composition and applications. *The Pharma Innovation Journal* 11(12): 1678-1681.

Indonesian Statistics Bureau (2022). *Produksi Tanaman Florikultura (Hias) 2022*. BPS Jakarta.

Musawira (2017). *Organogenesis Berbagai Varietas Krisan (Chrysanthemum morifolium R.) pada Berbagai Kombinasi Zat Pengatur Tumbuh Secara In Vitro*. Skripsi. Fakultas Pertanian Universitas Hasanuddin.

Nasri F., H. Zakizadeh, Y. Vafaee, & A. A. Mozafari (2021). In vitro mutagenesis of Chrysanthemum morifolium cultivars using ethylmethanesulphonate (EMS) and mutation assessment by ISSR and IRAP markers. *Plant Cell, Tissue and Organ Culture* (PCTOC) 1-17. https://doi.org/10.1007/s11240-021-02163-7.

Nandariyah, L.S. Mahmudah, R.B. Arniputri & A.T. Sakya (2021). The effect of NAA and coconut water combination on garlic (Allium sativum L.) tissue culture IOP Conf. Ser.: Earth Environ. Sci. 905 012036.

Prando S.M.A., P. Chiavazza, A. Faggio, & C. Contessa (2014). Effect of coconut water and growth regulator supplements on in vitro propagation of Corylus avellana L. *Scientia Horticulturae* 171: 91-94. https://doi.org/10.1016/j.scienta.2014.03.052.

Pratama, J. & N. Nilahayati (2018). Modifikasi Media MS dengan Penambahan Air Kelapa untuk Subkultur I Anggrek Cymbidium. *Jurnal Agrium*, 15(2):96-109.

Solihah, S.F., A. Supriyatna, & A. Adawiyah (2021). Pengaruh Konsentrasi Air Kelapa (Cocos nucifera L.) Terhadap Eksplan Krisan (Chrysanthemum morifolium) Kultivar 'Xanne Agrihorti'secara In Vitro. *In Gunung Djati Conference Series*, 6, pp. 163-168.

Tuyekar S.N., B.S. Tawade, K. S. Singh, V.S. Wagh, P.K. Vidhate, R. P. Yevale, S. Gaikwad, & M.Kale (2021). An Overview on Coconut Water: As A Multipurpose Nutrition. *Int. J. Pharm. Sci. Rev. Res*., 68(2): 63-70.