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Submission date: 16-May-2023 02:55PM (UTC+0700)

Submission ID: 2094470516

File name: Artikel_ProSIDing_Internasional_IOP_ICOFA_202_Hanif.pdf (553.11K)

Word count: 2224

Character count: 11881

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To cite this article: R Firgiyanto *et al* 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1168** 012008

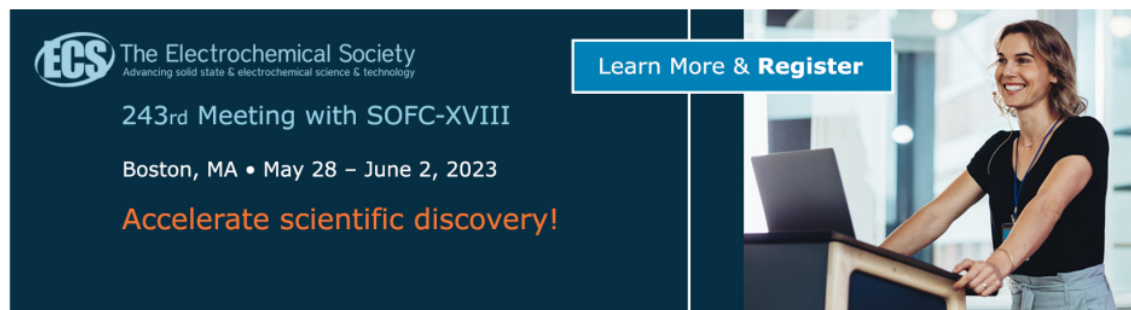
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Effect of modified murashige and skoog medium on chrysanthemum tissue culture

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Abstract.

Propagation of chrysanthemum through tissue culture can produce superior plants in large and uniform quantities and sterile cultures (therstock) can be obtained so that they can be used as material for further propagation. This research was carried out at Tissue Culture Laboratory, Jember State Polytechnic from April to November 2022. The research design used was a Factorial Completely Randomized Design (CRD). The first factor is the variety which consists of 3 cultivar (Maruta Agrihort, Pinka Pinki and Suciono). The second factor was in vitro culture media consisting of 5 levels (control, MS+BA media 2.0 mg/L and IAA 1.0 mg/L, MS+BA 1.0 mg/L and IAA 1.0 mg/L media, MS+ banana extract media, MS+ coconut water media). So there were 15 treatment combinations with 3 replications for a total of 45 experimental units. Parameters observed included plant height, the day the shoots appear, the day the roots appear, number of leaves and number of roots. The conclusion this research is addition of organic matter to MS media was able to increase the growth of explants compared to MS media with the addition of synthetic PGR and the combination of MS media + coconut water had an average optimal growth yield on all parameters.

1. Introduction

Chrysanthemum is a popular flower commodity today, because it has advantages, among others, has a variety of shapes and types. Its also become popular flower in Indonesia because it can easily propagated and need cheap cost. For the East Java region, chrysanthemum production centers located around Malang and Batu which produce most of the chrysanthemum in the East Java region with a total of 121,181,724 stalks in 2020 [1]. Along with the increase in population, the demand for chrysanthemum cut flowers is increasing every year, so agribusiness opportunities need to be developed.

The main problem of chrysanthemum production in the Tefa Highlands is the supply of chrysanthemum vegetative seeds which still have to be imported from Malang. The adaptive chrysanthemum cultivar that suitable for medium highland especially in the Polije Highlands Tefa are still not known. To overcome the problem it needed to select and producing the suitable cultivar. The availability of seeds can be overcome by vegetative seed production from mass production of mother plant with in vitro culture propagated using stem cuttings method. Tissue culture propagation method has a lot of advantage including can produce new plants in a fairly short time, in large quantities, uniform, free of pathogens and have the same character as the parent. The use of appropriate culture



media can streamline chrysanthemum tissue culture. Some materials used in MS media to form shoot formation include the addition of growth regulators [2] [3], compound fertilizers [4], and organic materials such as the addition of bananas [5] or coconut water [6].

In vitro culture media is a determining factor for the success of chrysanthemum tissue culture. The basic media that used for in vitro culture of chrysanthemum is MS0 media (MS media without addition material). The use of MS0 media produced less shoot when compared to media that had been enriched with other ingredients. Materials used for MS media include plant growth regulators, both inorganic and organic. The addition of organic material containing growth regulators can reduce production costs because the price of organic matter is cheaper than inorganic plant growth regulator. The use of alternative generative media (cheap media) needs to be developed to reduce the cost of producing chrysanthemum seeds because it can increase the cost efficiency of chrysanthemum tissue culture seed production. Organic materials that are widely used for tissue culture media are banana fruit, coconut water, potatoes and sweet potatoes extract. So far, coconut water is the most widely used organic material as an add-on to tissue culture media. The use of coconut water as an additive in tissue culture has been carried out on potato plant tissue cultures [7], *Cymbidium* orchids [8], and Broccoli [9].

2. Material and Methods

2.1 Material

3 varieties of chrysanthemum (Maruta Agrihort Varieties, Pinka Pinki Varieties, Suciono Varieties), MS media, IAA, BAP, banana, coconut water, aquadest, spirtus, alcohol, oven, autoclave, laminar air flow, tissue culture planting tool set, stationery and ruler.

2.2 Methods

This study used a factorial complete randomized block design (CRD). The first factor were Chrysanthemum varieties consist of 3 variety (Maruta Agrihort Variety, Pinka Pinki Variety, Suciono Varieties). The second factor were in vitro culture media consist of 5 level (P1:MS, P2 : MS + BAP 1 mg/liter + IAA 1 mg/liter, P3 : MS + BAP 2 mg/liter + IAA 1 mg/liter, P4 : MS + banana extract, P5 : MS + coconut water).

The data obtained will be analyzed using variance (Anova), according to the design used. If in the treatment there is a significant difference in the observed variables, further tests carried out using Duncan Multiple Range Test (DMRT) at 5% significance level. Parameters that observed were plant height, the day the shoots appear, the day the roots appear, the number of leaves and the number of roots.

3. Result and Discussion

The results of the analysis of the various effects of culture media on the emergence of chrysanthemum buds showed that in vitro culture media was able to give different effects on the emergence of chrysanthemum cuttings. This result were based on the growth of the plantlet at 5 weeks after planting. Based on Figure 1 on the parameters of shoot emergence time, it can be seen that the VIP5 treatment, the combination of Maruta agrihort cultivar and modified MS media + coconut water gave significant effect, namely shoots appeared on day 6.3. This can happen because the application of coconut water on MS media can support shoot growth because coconut water contains cytokinins that can accelerate cell division and shoot development [10]. Cytokinins are very influential on metabolic processes, cytokinins play a role in the synthesis of amino acids, nucleic acids, and proteins [11].

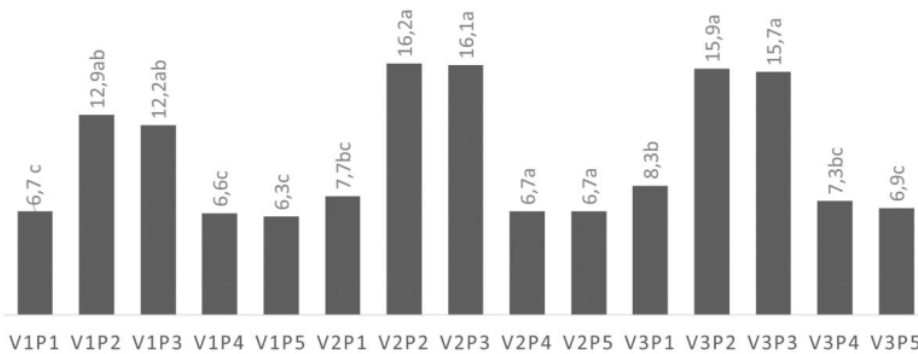


Figure 1. Diagram of shoot emergence time (days)

The parameters of root emergence, the treatment that gave the fastest root emergence was V2P5, the treatment of pinka pinki varieties and MS+coconut water media on day 8.2 (Figure 2). Root growth was strongly influenced by the optimal PGR in the media, and on modified media MS + coconut water was the right combination to meet the needs of explants to grow, especially to encourage root growth. Cytokinins and auxins contained in coconut water can play a role in the process of plant morphogenesis that stimulates the formation of roots and shoots if the media contains balanced cytokinins and auxins [12] as well as minerals and nutrients contained in MS media is a combination that is optimal enough to support growth. root.

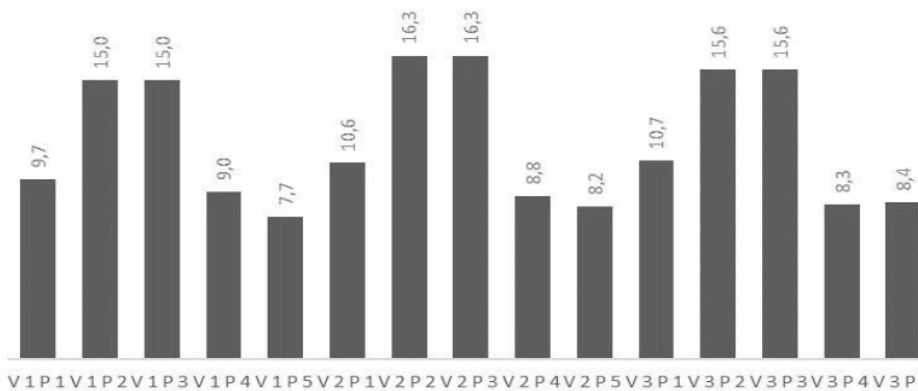


Figure 2. Diagram of root emergence time

Parameters of plant height treatment with the best results were V2P4 the pinka pinki cultivar and MS media + Ambon banana extract and V3P5 namely the Suciono cultivar and MS media + coconut water, banana extract and coconut water had their respective advantages, banana extract contains thiamin which serves to accelerate cell division in root meristems [13], the addition of coconut water on MS media can stimulate the growth of chrysanthemum explants due to the chemical content of coconut water. Cytokinins contained in coconut water will stimulate the cell division process to be faster, so that it will stimulate the plantlet growth process. The most characteristic property associated with cytokinins is the stimulation of cell division in plant tissue cultures. The discovery and separation of kinetin depends on this property and many studies on cytokinins emphasize this effect. Non-meristematic tissues separated from higher plants for in vitro growth require cytokinins [14].

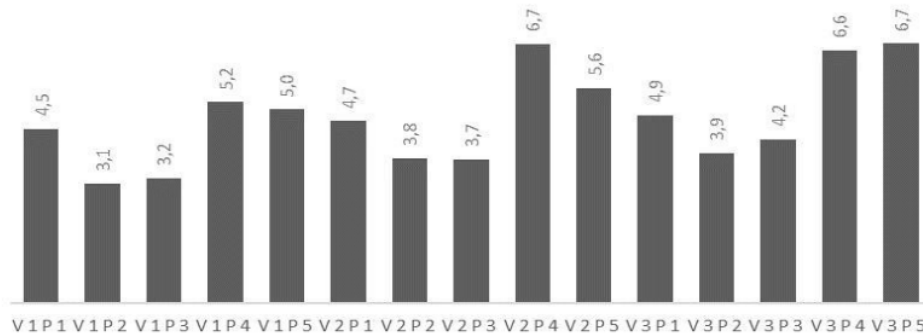


Figure 3. Diagram of the plant height

The best root number parameters were obtained in the V3P5 treatment, the treatment of the Suciono cultivar and MS+coconut water media, coconut water contains growth regulators of the auxin group with concentrations that we cannot determine, several studies have shown that the use of coconut water in tissue culture tends to result in the formation of root organ and have a significant effect on the development of the root system by producing the longest root value [15].

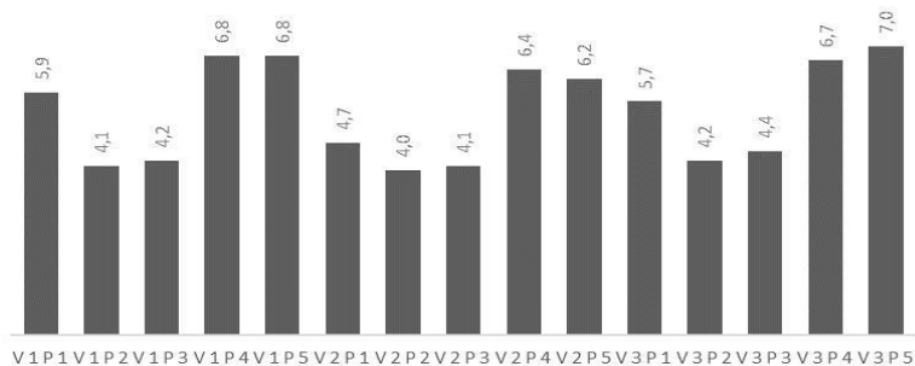


Figure 4. Diagram of the number of root

The best treatment for the number of leaves parameters were shown in the V2P5 treatment, namely the pinka pinki cultivar and MS+coconut water media (Figure 5). Explants grown on MS media with the addition of coconut water and banana extract had higher number of leaves compared to explants that grown on MS media without the addition of coconut water. This result can be done because coconut water contained carbohydrates, vitamins, minerals, and plant growth regulator such as auxins, cytokinins, and gibberellins which are useful as a driving force in tissue proliferation, facilitating metabolism and respiration. Coconut water also contains vitamin C which is useful in stimulating stem growth where there is a process of cell division, cell elongation, and new cell enlargement in plants [16]. Banana extract can significantly increased plantlet height, leaf number, and leaf size of *Dendrobium* sp orchid plantlet growth [17].

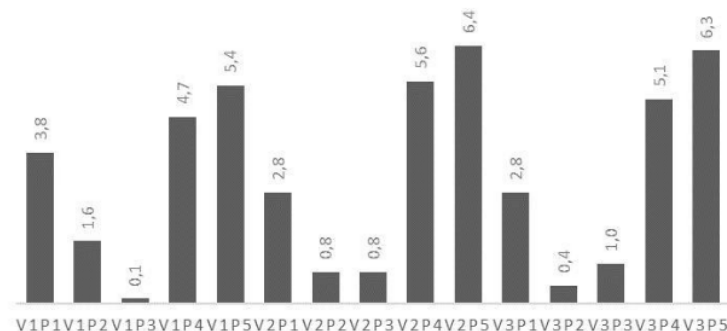


Figure 5. Diagram of the number of leaves

4. Conclusion

Based on the results of the study, it can be concluded that the addition of organic matter to MS media was able to increase the growth of explants compared to MS media with the addition of synthetic PGR. The combination of MS media + Coconut water had an average optimal growth of *Chrysanthemum* in vitro propagation on all parameters observed.

1

Acknowledgment

The authors would like to thank the financial support of PN¹ Politeknik Negeri Jember Year 2022. The authors also thank the Department of P3M for providing support and assistance in completing this research.

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