

Micro Cuttings Propagation Of Pitcher Plant (*Nepenthes spp*) On Various Media And *Indole Acetic Acid* (IAA) By *In Vitro*

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Abstract

Pitcher plants is a unique type of ornamental plant and is one of the typical insectivorous plants of the tropics. Propagation of pitcher ornamental plants can be done through in vitro culture using young plant parts such as young leaves and stems. The study aimed to examine the interaction between media types and NAA concentrations to obtain the right media types and IAA concentrations to support the growth of pitcher plants micro-cuttings in vitro. Research is a laboratory experiment arranged in a Complete Randomized Design with two factors. The first factor is the treatment of various media, namely MS media, MS media with vitamin B5, and ½ MS media with vitamin B5. The second factor is the IAA concentration, which is 0.5 ppm, 1 ppm, and 1.5 ppm. The observed data were analyzed with ANOVA (Analysis of Variant) at the real level of 5% and further tested with Duncan's Multiple Range Test (DMRT) at the level of 5%. The results showed an interaction between MS media treatment and an IAA concentration of 1 ppm on the height parameter of the plantlet. Using MS media and MS media with vitamin B5 gives good results on the parameters when shoots appear, the number of shoots, and leaves. IAA 0.5 ppm and 1 ppm give the best results on the parameters at the emergence of shoots and the number of bags of nepenthes.

Keywords: pitcher plant, media sort, IAA, *in vitro*.

1. INTRODUCTION

Pitcher plant is an ornamental plant that grows in several forests of Indonesia. This plant is called an ornamental plant because it has a unique pouch resulting from leaf modification due to nutrient deficiency. The pitcher plant at the tips of the leaves have a fairly high aesthetic value, so they have the potential to be developed into ornamental plants. Sari (2009) reported that in addition to having a fairly high aesthetic value, pitcher water is also used by some Indonesian people as an eye medicine, while the stem of the pitcher plants

functions as a rope to bind. The presence of pitcher plant, including rare species protected by the state and the world, international trade in plants from natural habitats must be strictly controlled and only allowed for certain non-commercial purposes with special permits.

One of the modern cultivation technologies used is tissue culture techniques or *in vitro* plant cultivation. Through the cultivation of plant tissue culture techniques, Pitcher plant can be propagated rapidly in large and uniform quantities. In *in vitro* culture techniques use planting material (explants) in meristem or young tissue still actively dividing. The meristem tissue is part of the plant that is free from diseases and viruses. Shoots are the meristem tissue of plants most often used in *in vitro culture* (axillary buds, apical buds, or shoots and tuberous buds) (Roosel *et al.*, 2001). Vitamins often added in tissue culture media are required in small quantities. In addition, the media aims to spur plant growth. Some vitamins that can be used are thiamine, nicotinic acid, and pyridoxine (Purbaningsih, 1995).

IAA is a synthetic hormone from the auxin group used for the vegetative propagation of plants. It is also used for plant tissue culture. The hormone IAA is like other auxins that can be toxic to plants in high concentrations. The function of IAA is to stimulate cell elongation, stimulate cambium activity, stimulate stem stabbing, stimulate parthenocarpy, and stimulate apical dominance.

2. RESEARCH METHODS

The research was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Yogyakarta. This study is a laboratory experiment prepared with a factorial Complete Randomized Design (RAL). The first factor is the media type consisting of M1 = MS Media, M2 = MS Media with Vitamin B5, and M3 = 1/2 MS Media with Vitamin B5. The second factor is the concentration of NAA consisting of N1 = 0.5 ppm, N2 = 1 ppm and N3 = 1.5 ppm. The data were analyzed for diversity at the real level of 5% and further tested with *Duncan's Multiple Range Test* (DMRT) at the level of 5%.

2.1 Execution Levels

The explants used are sterile plantlets. The plantlets from the culture bottle are removed with a sterile scalpel and, one by one, placed on a petri dish with a diameter of 10 cm. The plantlets are sorted one by one and cut into pieces with a size of 2 cm, after which they are planted in each culture bottle containing a predetermined treatment medium.

3. RESULTS AND DISCUSSION

The average parameters of when shoots appear, the number of shoots, leaves, and the number of bags are presented in Table 1.

Table 1. Average parameters of When Emerging Shoots (day), The Number of Shoots, The Number of Leaves (strands), and the Number of Bags.

Treatment	Parameter				
		When Emerging Shoots (day)	The Number of Shoots	The Number of Leaves (strands)	The Number of Bags
Media Kinds					
M1 (MS)		17,85b	2,15a	9,40a	4,07a
M2 (MS+ Vit B5)		19,52b	1,93a	9,81a	4,26a
M3 (1/2 MS+VitB5)		24,37a	1,04b	5,88b	5,15a
IAA					
N1 (0.5 ppm)		20,11q	1,78p	8,93p	4,78p
N2 (1 ppm)		19,48q	1,59p	8,30p	5,00p
N3 (1.5 ppm)		22,15p	1,74p	7,89p	3,70q
Interaction		(-)	(-)	(-)	(-)

Remarks: The average followed by the same letter notation in the row and column showed no real difference at the DMRT level of 5%. A (-) sign indicates no interaction.

Table 1 shows the parameters when buds appear, the number of shoots, and the number of leaves treated with MS (M1) and MS media with vitamin B5 (M2) shows that the shoots appear faster, the number of shoots and the number of leaves are more than the 1/2 MS media with vitamin B5. This is because the pitcher plant is more responsive to both media types, giving rise to new shoots. After all, the nutrient content in complete MS media, such as potassium and ammonium nitrate content is high enough to increase cytokinin biosynthesis activity, so the explant will tend to sprout (Ridhawati, 2017). MS media (M1) and MS media with vitamin B5 (M2) are more buds than 1/2 MS media treatment with vitamin B5. This is due to the same response in producing shoots. It is suspected that the thiamin content plays a role in stimulating the shoots. This is in accordance with the opinion of Srilestari (2004) who said that thiamin functions as a coenzyme that stimulates the activity of these hormones and encourages the division of new cells. The role of thiamin as a coenzyme can improve metabolic processes so that shoots appear faster and more. It is expected that the number of leaves is also more with many shoots. According to Amalia (2013), the presence of thiamin

plays a role in optimizing respiration activity, which is important to produce energy necessary in metabolism for growth and development.

In the parameters of the number of treatment bags, IAA concentrations of 0.5 ppm and 1 ppm showed a real difference compared to IAA concentrations of 1.5 ppm. This suggests that the administration of NAA that is not balanced with endogenous cytokinins produced by the plant itself will inhibit cell elongation and the plant cannot organogenesis pitcher plant at the tips of leaves. According to Karjadi (2007), auxin in low concentrations will stimulate the enlargement and elongation of stem cells after cell division stimulated by cytokinins. However, when the concentration of auxin used is too high, it will cause inhibition of cell elongation.

The average parameters of plantlet height, fresh weight, and dry weight of plants are presented in Table 2.

Table 2. Average Parameters of Plantlet Height, Plant Fresh Weight, and Plant Dry Weight

Treatment Combination	Parameter		
	Planlet Height (cm)	Plant Fresh Weight (g)	Plant Dry Weight (g)
M1N1(MS dan IAA 0.5ppm)	5,54bc	266,33bcd	26,11cde
M1N2(MS dan IAA 1 ppm)	6,56a	403,33a	38,11ab
M1N3(MS dan IAA 1.5 ppm)	5,84b	402,89a	39,11a
M2N1(MS + Vit B5 dan IAA 0.5 ppm)	5,1bcd	312,55abc	30,22bcd
M2N2(MS + Vit B5 dan IAA 1 ppm)	5,29bcd	226,00cd	21,78def
M2N3(MS +Vit B5 dan IAA 1.5 ppm)	5,34bcd	352,55ab	32,00abc
M3N1(1/2 MS + Vit B5 dan IAA 0.5 ppm)	4,80d	199,22d	18.89ef
M3N2(1/2 MS dan IAA 1 ppm)	5,04cd	203,66d	18.89ef
M3N3(1/2 MS dan IAA 1.5 ppm)	4.07e	169,66d	16,78f
Interaction	(+)	(+)	(+)

Description: The mean followed by the same letter notation in the row and column showed no real difference at the DMRT level of 5%. A (+) sign indicates there is an interaction.

The combination of MS and IAA media treatment 1 ppm showed a synergistic relationship (interaction) on the height parameter of the plantlet. This is because MS media

contains complete macro and micronutrients needed by plantlets. The addition of IAA 1 ppm will stimulate the budding explant to develop. MS media contains complete nutrients to meet the needs of the plantlet, and IAA function affects cell elongation. This is in line with Kartiman's research (2018), which says that the increase in explant height is caused by two processes, namely cell division and elongation. Both of these processes occur in meristem tissue, namely at the stem growth point, so that plants grow larger and correlate positively in determining plant yield.

In the fresh weight parameter, the combination of various media and IAA concentration treatment shows an interaction between the two treatments. In combination with MS and IAA 1 ppm media treatment, MS and IAA 1.5 ppm media has the heaviest fresh weight compared to M1N1, M2N2, M3N1, M3N2, and M3N3 treatment combination. This shows that MS media can provide nutrients that are in accordance with the needs of the explant so that it shows an increase in volume, weight, and number of cells in the plantlet so that the weight is heavier and with the addition of IAA will stimulate the growth of shoots. The combination of MS media and IAA concentrations of 1-2 ppm synergizes to produce shoots so that their weight will increase.

In dry weight parameters, the combination of treatment of various media and IAA concentrations shows an interaction between the two treatments. Dry weight is the weight of the material after drying and is the accumulation of photosynthate. This understanding can be achieved by opening the material so that all the water evaporates, the weight of the material will decrease, and the amount of reduction is considered the difference between fresh weight and dry weight. This ratio of the final and initial weight reductions is then converted into percent and moisture content is found. Water content varies greatly in plant organs, depending on the type of plant, structure, and age of organ tissue (Ellya, 2009).

In the MS and IAA media combination, 1.5 ppm has the heaviest dry weight compared to the treatment of M1N1, M2N1, M2N2, M3N1, M3N2, and M3N3. This shows that the combination of MS and IAA 1.5 ppm media treatment increases the nutrients absorbed by the plantlet so that the dry weight of the plantlet is heavier because it contains nutrients instead of water. If the plantlet only contains high water content, then the dry weight will not differ significantly from other treatments. In addition, phytohormones work synergistically with other growth hormones in stimulating growth. IAA, a synthetic auxin, works synergistically with the plant's endogenous cytokinins. It is very important in regulating cell division, stimulating leaf growth, and can help in photosynthesis so that photosynthesis (photosynthate) is high.

4. CONCLUSION

The results showed an interaction between the combination of MS media treatment and an IAA concentration of 1 ppm on the height parameter of the plantlet. Using MS media and MS media with vitamin B5 gives good results on the parameters when shoots appear, the number of shoots, and leaves. IAA 0.5 ppm and 1 ppm give the best results on the parameters at the emergence of shoots and the number of bags of nepenthes.

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