

Effects of Organic Additives and Naphthalene Acetic Acid (NAA) Application on the In Vitro Growth of *Dendrobium liniale* X *Dendrobium biggibum*

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Abstract

The research aimed to study the influence of the application of NAA concentration, the addition of organic materials and a combination of both on media Knudson C towards the growth of orchid plantlet (♀) *Dendrobium liniale* x (♂) *Dendrobium biggibum*. The purpose of using media Knudson C in culture in vitro is to optimize the growth of orchid plantlet *Dendrobium liniale* and *Dendrobium biggibum*. The experiments were carried out in the Laboratory of Plant Physiology and Biotechnology Faculty of Agriculture, Sebelas Maret University Surakarta. The research used Complete Randomized Design (CRD) with 2 treatment factors, factor I was the concentration of NAA (0 ppm, 1 ppm, 3 ppm and 5 ppm); and factor II was the organic materials (coconut water, banana, potatoes and sweet potatoes). In total there were 20 treatment combinations. Each combination was repeated four times. The results showed that the combination of sweet potato and NAA 5 ppm produced the following characteristics: the fastest root growth, most root length, and the largest number of leaves; while the combination of banana extracts and NAA 5 ppm produced a number of roots, the shoots appear most rapidly, and the highest number of shoots

Keywords: genetic diversity; tissue culture; medium;

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Introduction

Orchids are very common ornamental plants in the world. Current motives for planting orchids instead of just being just a hobby, it became an international business which shares the 8% of the world's floriculture and trade; hence, it has the potential to change the economic landscape of a country (1). However, currently the sustainability of Orchid diversity is threatened. In an effort to overcome the risk of extinction of the orchids, in vitro techniques have been frequently used (2). In vitro conservation is an effort to produce a large number of plants for reintroduction into the wild (3). According to (4), the quality improvement effort of orchids can be done through genetic

improvement programs such as crossbreeding; whereas for the population expansion can be done through in vitro culture. In vitro culture techniques provide a practical system for cloning, rapid mass multiplication of rare, endangered, horticultural, and therapeutically important genotypes (5). New type of crossbred orchid have great natural diversity, which gives the opportunity to choose the best derivative and then propagated in bulk (6).

The successful use of the tissue culture method is very dependent on the media type. While the problems of in vitro culture at this time is for instance, the need of chemicals which cost is not cheap; it is hence, important to look for

alternate media which are affordable with high quality as planting materials. One source of natural coconut water is PGR. According to (7) coconut water has been identified to contain many organic compounds and hormones that can stimulate and enhance the growth of orchid seeds in vitro. In this study, coconut water was also used to accelerate the emergence of roots and stimulate leaf formation in orchid plantlets from a cross of *Dendrobium lineare* and *Dendrobium biggibum* in vitro. The utilization of coconut water and other organic materials in this study was expected to accelerate the appearance of roots. Organic materials such as coconut water and potatoes provide the best results at high accretion plantlets; and coconut water stimulates the formation of leaves on orchid plantlet growth in vitro. Bananas also contain growth hormones such as auxin and expected to increase the growth of plants. Potatoes contain elements which required by explants in tissue culture. These elements are calcium, phosphorus, iron, vitamin B1, vitamin B2, vitamin C and niacin. On the other hand, the sweet potatoes are a source of carbohydrates, protein and contains vitamin A, vitamin C and other nutrient elements. Hence, these materials can be used as an alternative medium in tissue culture through modifications of the culture media.

The use of PGR also had an effect in repairing the quality and quantity of orchids. This is in accordance with (8) which stated that to produce plants with good results both quantitatively and qualitatively, it is necessary to add growth regulators and organic additive compounds to the culture media. In tissue culture there are two classes of substances as very important growth regulator i.e., auxin and cytokinin. According to (9), individual treatment of cytokinin, auxins and natural supplements is the most important factor to promote and enhance plant development of shoot regeneration or protocorms like body (PLBs). NAA (Naphthalene Acetic Acid) which is a type of synthetic auxin, used to increase the ratio of the growth of plant roots in culture in vitro. This will encourage the formation of new roots at a certain concentration lapse (10). This research aims to obtain the optimum organic material composition for the growth of plantlet orchids as the results of crosses between *D. lineale* and *D. biggibum*, obtain the correct concentration of NAA on the crossbred orchid plantlet growth and get the most appropriate combination of organic matter composition and concentration of NAA for the growth of these orchid plantlets. The novelty obtained in this study compared to previous research is the use of Knudson C media to

stimulate the growth of plantlet orchid of *Dendrobium lineare* and *Dendrobium biggibum* grown by culture in vitro with the application of NAA concentration and the addition of organic materials which includes coconut water, banana, potatoes, and sweet potatoes and a combination of both on media Knudson C towards growth plantlet orchid.

Material and Methods

The material used were the Orchid explant as results of crosses between (φ) *Dendrobium lineale* \times (δ) *Dendrobium biggibum* (with the number of leaves 2-4 strands and plant height between 2-5 cm), Knudson C media, extract banana 150 g/l, potato extract 200 g/l, extracts of sweet potato 150 g/l of coconut water and 250 ml/l, aquades, soap, alcohol, and NAA (1 ppm, 3 ppm and 5 ppm).

This study used a Complete Randomized Design (CRD) with 2 treatment factors, factor I was the concentration of NAA consisted of 0 ppm, 1 ppm, 3 ppm and 5 ppm. Factor II was an organic material, consisted of coconut water, banana extracts, potatoes and sweet potatoes. In total there were 20 combinations of treatments, each treatment combination was repeated four times. of the research steps were consisted of tools sterilization, making the stock solution, the creation of media, explant, planting and maintenance. The data obtained were analyzed using ANOVA, if there was a significant difference continued with Duncan Multiple Range Test level of 5% and regression test.

Results and Discussion

Tissue culture is the technique of plant propagation in sterile conditions or a controlled environment, which is often to produce clones. Tissue or cell parts of plants can be grown under conducive environment for the growth and multiplication. Cells, protoplasts, leaves and roots of plants can be used to produce new plants through tissue culture which was grown in a culture medium with a supply of nutrients and plant hormones needed (11).

Research on the culture of orchids was done by modification of Knudson C media by the addition of organic materials into media namely coconut water, extracts of banana, extract of potato and sweet potato extracts with different concentration levels of PGR. Planting material used was the result of crossbreeding between *Dendrobium lineale* \times *Dendrobium biggibum*. Treatment effects on the research variable wereshown in Table 1.

Table 1 The ANOVA results on the effects of the treatments on the growth of crossbred orchids

Research Variables	NAA	Organic Material	Interactions
When appear root	ns	ns	ns
Number of roots	*	**	*
Root length	ns	**	*
When appear shoot	ns	ns	ns
Number of shoots	*	*	*
Plant height	*	**	*
Number of leaves	ns	*	*

Description: ns= no significant effect, (*)= significant effect ($P<0.05$), (**)= highly significant effect ($P<0.01$)

Rooting time

The results of the analysis of variance showed that experimental treatments had no significant effect on the emergence of real roots. NAA treatment 5 ppm gave the appearance of the fastest roots emergence of 21.24 DAP. This is in accordance with the statement of (10) that the addition of NAA in the black Orchid (*Coelogyne pandurata* Lindl.) with a concentration of 5 ppm, gave the best rooting time. Each plant has different capabilities in absorbing PGR to stimulate its growth.

Figure 2 shows that the fastest treatment was observed on the addition of extracts of sweet potato (23.78 DAP). According to (12), sweet potatoes contain a variety of micro-nutrients such as vitamin C, substantial thiamin (vitamin B1), riboflavin (vitamin B2), folic acid and vitamin E. It also contains some elements and essential minerals such as iron, zinc, calcium, potassium, sodium, magnesium and manganese. Vitamin B1 (thiamine), Fe, Ca, niacin, riboflavin, and vitamin A found in sweet potatoes are allegedly able to stimulate the formation of root quickly (10).

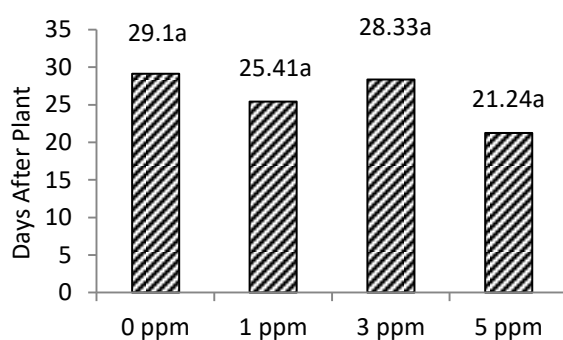


Figure 1. The influence of the concentration of NAA on the average rooting time.

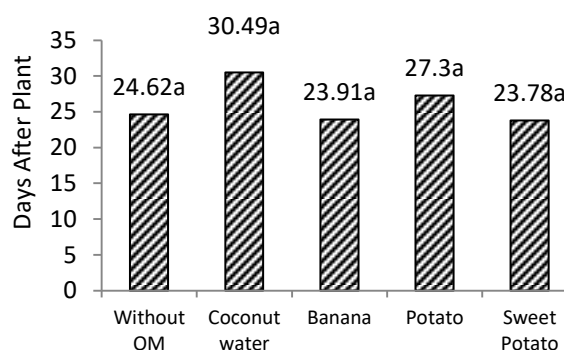


Figure 2. The influence of organic material on the average rooting time.

Number of Roots

The results of the analysis in Table 1 shows that the interaction between the two factors gave different effects towards the number of real roots. Combination treatment of NAA 5 ppm and the addition of banana extract produced the highest amount of root of 35.25 (Figure 3). The combination of both of these treatments was able to give positive results on the number of roots compared with the other treatment combinations. This is not in accordance with the opinion of (10) on his research of the black Orchid (*Coelogyne pandurata* Lindl.) and media Vacint & Went (VW) that the number of roots of the highest average obtained at treatment with the addition of extracts of sweet potato 150 g/L and NAA a 5 ppm. This shows that the needs of nutrients from each plant is different. It depends on the condition of the plant physiological response against different treatments.

The results of the research conducted by (13) shows that NAA has an important role in the formation of roots while cytokinin only to functioned to stimulate the formation of buds and not for rooting. It was suspected that the deposits of thiamin in banana can accelerate cell division in the meristem of the root. According to

(14) thiamin is very essential for in-vitro plantlet although the required amount is small. The addition thiamin in media culture can stimulate the growth of explant and increase the growth of the root. This was confirmed by (15)

which stated that the addition of a banana on the KC media was able to spur the growth of *P. fuscata* better than other media, mainly on the life percentage, the length of the leaf, as well as the number and the length of the root.

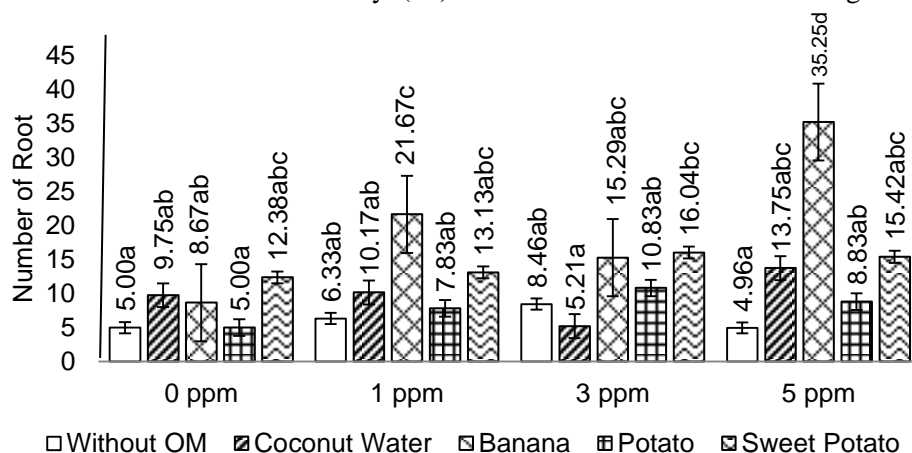


Figure 3. The effect of the concentration of NAA and types of organic material on the number of roots

Root Length

F test analysis results showed that interaction between both treatments gave significant difference. The combination of NAA 5 ppm and sweet potatoes extract 150 g/l was able to stimulate root enlargement. The research results of (16) showed that the sweet potato is rich in starch, protein, fat and fiber. Upon research (17) mentioned that sweet potato contains some nutrients among them there are carbohydrates, fiber, carotene, thiamine, riboflavin, niacin, potassium, zinc, calcium, iron, vitamins A and C and high in protein. It also contains natrium, zinc, magnesium and manganese. According to (18) these elements play a role in the formation

of root hairs and roots lengthening. It is also supported by the opinion of (19) as saying that thiamine on sweet potato was able to stimulate cell division in the area of rooting.

According Figure 4, it can be seen that the response of long roots dueto the application of NAA gave varied results. The length of the roots was highest on the NAA 5 ppm. That is because the ability of plant absorbed PGR varies, as claimed by (20) that the response to the additional substance to control growing (PGR) is affected by the difference of the phases of growth, physiological conditions, the ability of the plant to absorb PGR and the fluctuation of endogenous hormones.

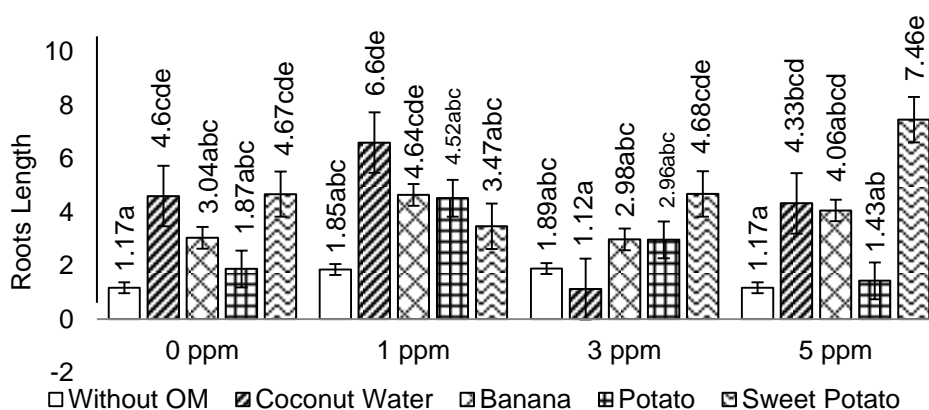


Figure 4. The effect of the concentration of NAA and types of organic materials on the length of the root

Days to shoot

The results of the analysis in Table 1 shows that the treatment of the NAA and organic

materials, as well as the interaction between the two gives non-significant effect on orchid crossbred between *D. Liniale* and *D. Biggibum*. (21) revealed that the quality, concentration and

characteristics of plant growth regulator auxin affects the effectiveness of shoot regeneration. Figure 5 shows that NAA treatment 1 ppm gave the quickest days to shoots which was 36.4 DAP. In accordance with the opinion of (22) that a low concentration of auxin in plants can help the regeneration of shoots. (23) reported similar finding that administering exogenous auxin increases resistance against time, and also influence the formation of buds. However, this research was enriched with an increase in the concentration of NAA, the influence of resistance on the days to shoot varies. It is possible that the explant have simply different contents of the endogenous auxin. The uniformity of plants' size and manner in taking the explants is not related with the endogenous plant hormones content so that the addition of exogenous auxin into media culture will give rise to a varied responses. This means that the addition of the NAA was not able to accelerate the days to shoots. (24) stated that the endogenous Auxin on explants have been able to promote the formation of buds, so the required auxin is not too high.

Figure 6 shows that the addition of sweet potato extract treatment on media culture provided the fastest response on the variable of days to shoots which was 34.13 DAP. It is presumed that hormones auxin and cytokinin on the spur the emergence of sweet potato shoots quickly on orchid media results crosses *D. liniale* > < *D. biggibum*. Sweet potatoes contain carbohydrates which can be used for the process of metabolism and biosynthesis of endogenous hormones in hormone auxin, cytokinin and gibberellin (14).

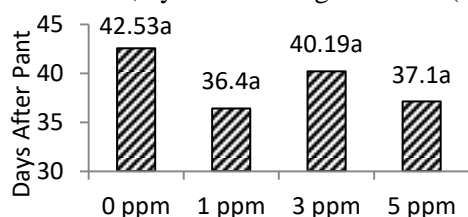


Figure 5. The effect of the concentration of NAA on the days to shoot

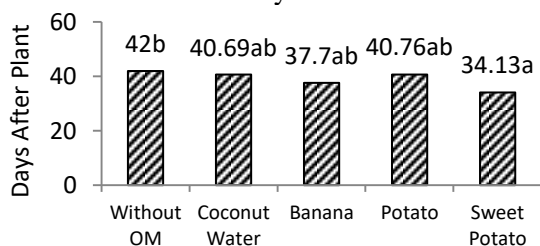


Figure 6. The effect of the types of organic material on the days to shoot

Number of Shoots

The results of the analysis of variance showed that treatment combination of showed different results on the number of shoots. NAA treatment combination 5 ppm and extract banana was able to generate the highest number of shoots that is 7.25. For the formation of new shoots, the plant requires nitrogen (N), potassium (K), sulfur (S), iron (Fe) and zinc (Zn). Elements of N, S, Fe and thiamine can stimulate cell division, thus increasing the growth side shoots.

Banana extracts known to contain elements of potassium (K), phosphorus (P) and iron (Fe) thus providing a positive influence against the growth of shoots. A high concentration of NAA can stimulate the growth of new shoots. NAA and coconut water treatment produces an average number of 5.08 shoots. (25) stated that coconut water contains plant growth regulators such as cytokines and micro elements (Fe, Mn, Cu, Zn, Mo, B, Cl and Ni), which stimulates the growth of shoots. According to (10) at his experiments the concentration of NAA to 20 ppm still allowed an increased number of shoots for some organic media treatment. This is allegedly due to the amount of explants in one bottle (more than two explants) that can potentially reduce poisoning due to the high concentration of NAA. This may also occur in the experiment on the concentration of NAA is highest that is 5 ppm. Good root growth only occurs if the growth medium can support the needs of the roots (26).

Heigh of Plantlet

The analysis results (Table 1), found that the combination of two treatments of NAA and organic matter provided a significantly different effect on plant height ($P < 0.05$). Figure 8 shows plantlets produced on the highest combination treatment of NAA 1 ppm and addition of organic matter yams. According to (27), a high concentration of auxin can be inhibiting because of the competition in the placement of the position of the recipient cells. It is not in accordance with the opinion of (14) which showed that the growth of *Dendrobium* in the media with the addition of potatoes was higher, and lower growth in the media with the addition of a banana. It shows that the nutritional needs of each plant species in very specific culture media differed even in the same family.

Number of Leaves

The results of the analysis in Table 1 indicated that treatments of organic materials and a combination of two different treatments gave

significant effects on the number of leaves ($P < 0.05$). Average number of leaves was the highest on the treatment combination 5 ppm NAA with the addition of extracts of sweet potato with average of 5.09. NAA treatment 0 ppm with the addition of potato extract obtained average number of leaves as 5.04. According to (28), the addition of potato on plantlet regeneration is proven useful as in the addition of coconut water. While the combination treatment NAA 5 ppm and the addition of coconut water obtained average number of leaves of 5. Coconut water is the liquid endosperm containing amino acids, organic acids, nucleic acids, vitamins, carbohydrates, growth hormone (auxin and cytokinin), minerals and other substances that can improve the quality of growth plantlets (29). It is thought that the physiological condition of the plant will give a different response against

different treatments given. This is in accordance with the opinion of (11) in (10) who said that variations of the response to the giving substance to control growing (PGR) is affected by the difference of the phases of growth, physiological conditions, the ability of the plant to absorb PGR, as well as fluctuations in endogenous hormones. hence, allegedly due to fluctuations in the addition of the number of leaves.

Sweet potatoes provide the highest influence on the number of leaves with an average of 5.4. This is allegedly because sweet potatoes contain the required elements such as vitamin C, thiamin (vitamin B1), riboflavin (vitamin B2), niacin, pantothenic acid (vitamin (B5), Pyridoxine (vitamin B6), folic acid and vitamin E also) which promoted the addition of a number of leaves (30).

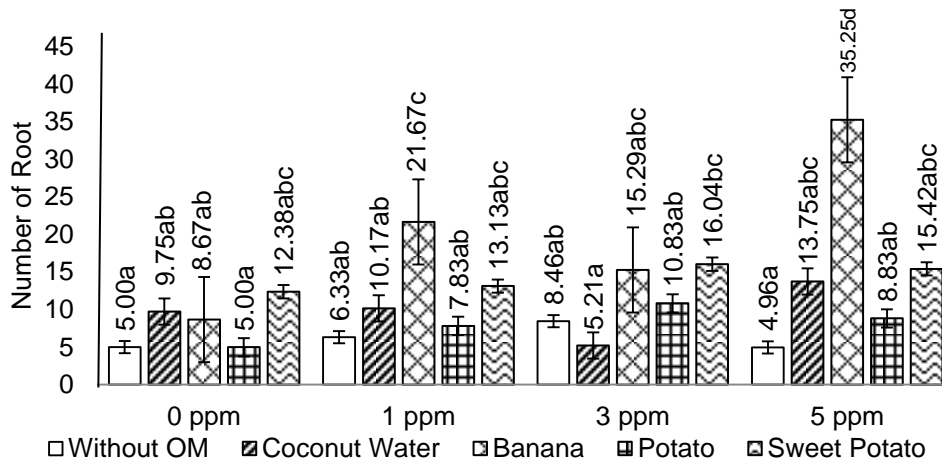


Figure 7. The influence of the concentration of NAA and types of organic material on the average number of shoots

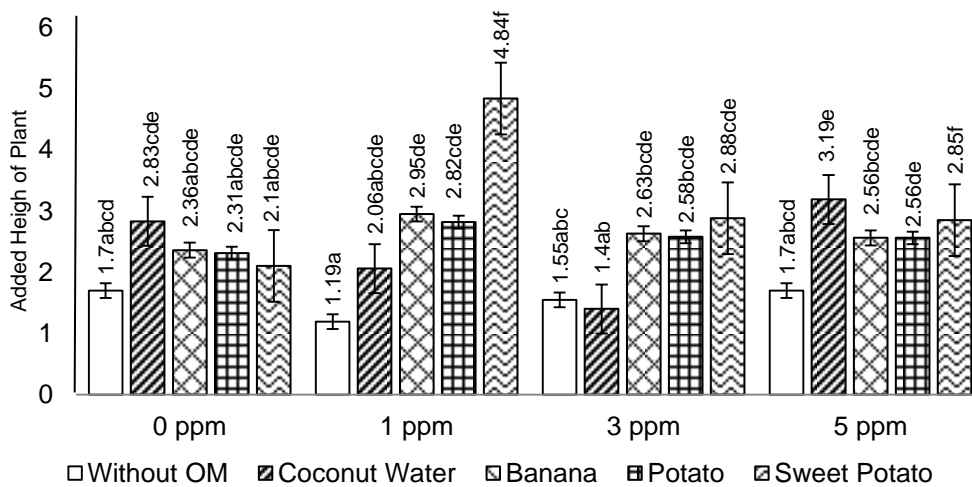


Figure 8. The influence the concentration of NAA and organic materials on the average plants height

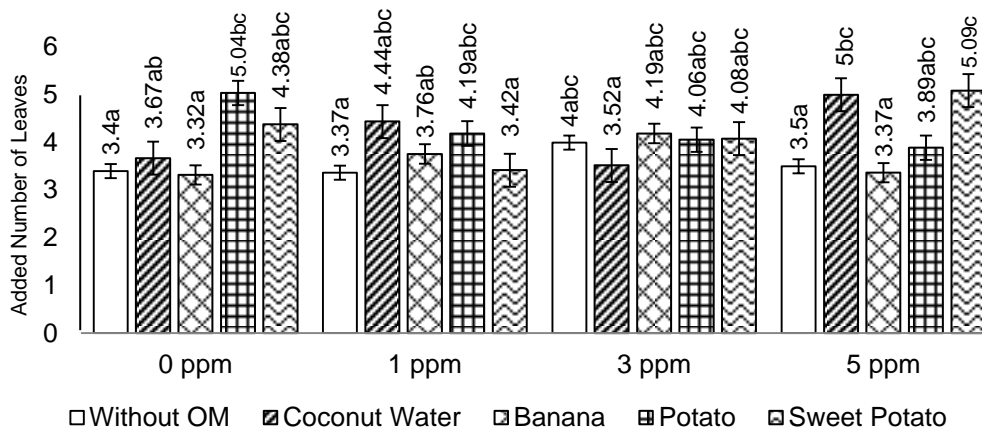


Figure 9. The influence of the concentration of NAA and organic material the average number of leaves

Conclusions

Based on the research that has been carried out, it can be concluded:

1. The addition of 3 ppm NAA was able to accelerate the emergence of the roots
2. The addition of a single organic material such as coconut water, bananas, potatoes and sweet potatoes can accelerate the appearance of roots, organic materials such as coconut water and sweet potato positive effect on root length, organic materials such as coconut water, potatoes and sweet potatoes provide the best results at high accretion plantlets and coconut water stimulates the formation of leaves
3. The combination of 3 ppm NAA with organic coconut water can accelerate the emergence of shoots, combination of 1 ppm NAA with organic coconut water can stimulate the multiplication of shoots and combination of 3 ppm NAA with sweet potatoes can increase the number of roots.

Recommendation

Based on these results it can be used to further research from the best organic materials such as coconut water to optimize growth crossbred orchid plantlets.

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