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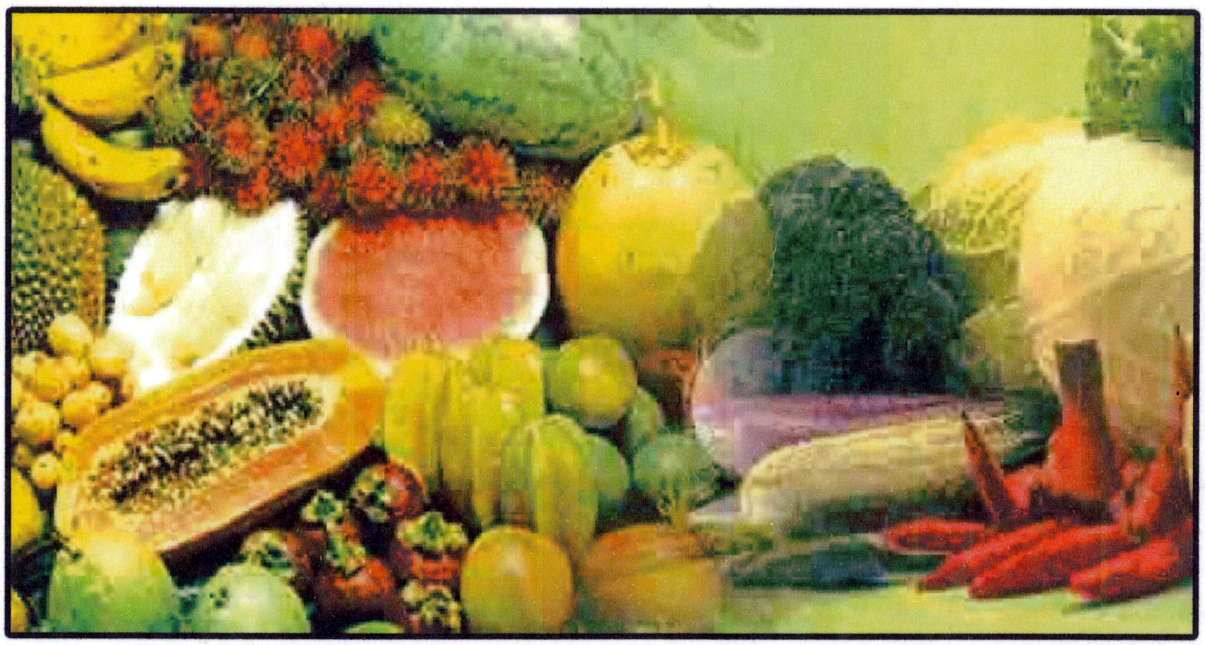

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THE EFFECT OF NITROGEN SOURCES AND TYPES OF MEDIUM SUBCULTURE ON *Brassolaeliocattleya* (Blc.) AMY WAKASUGI SHOOTS GROWTH

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ABSTRACT

The objectives of experiment were to obtain nitrogen sources and types of medium subcultures suitable for Blc. Amy Wakasugi shoots growth. The experiment was conducted in laboratory tissue culture Leaf Cycle (LC) Nursery, on Jalan Pelda Suryanta-Limusnunggal no. 121-122 Cibeureum hilir, Sukabumi which lasted from August-November 2009. The experimental design was arranged in a Completely Randomized Design (CRD), which consists of 8 treatments consisted of pure VW medium, medium 50% VW, VW 50% + Gaviota medium and Gaviota medium where each medium was added nitrogen in different sources NH_4NO_3 and $NH4(SO_4)_2$. The results of the experiment showed that there were significant effect of the addition of nitrogen into the subculture media on the number of leaves (8^{th} week after planting (wap), length of leaf (8^{th} wap, 12^{th} wap, 16^{th} wap), and high of shoot (8^{th} wap) but no effect on the number of leaves and high of shoots at the age of 12^{th} wap and 16^{th} wap. The addition of nitrogen also affected the number of shoots, length of root, dry weight of roots, dry weight of shoots and total dry weight but did not affect the number of roots. Medium subculture Went & Vacin 50% + Gaviota with NH_4NO_3 gave the best result on the number of leaves, height of shoots, length of roots, dry weight of shoots, dry weight of roots and total dry weight.

Keywords: ammonium, nitrate, subculture, *Brassolaeliocattleya*

INTRODUCTION

Cattleya is one orchid genus that many favor and because the beauty named as "ratu anggrek" *(queen of orchids)*. *Cattleya* has special characteristics that differ with other genus. *Cattleya* is epifit orchid. The sizes of its flower were variated started from 3 cm to 23 cm. Thus also with the sum of flower buds variated from 1 to more than 8 per pseudobulb. Labellum or the base of a flower wider and biger also having colours that very interesting

Generally *Cattleya* grouped into *Cattleya* species (*Cattleya* nature) and *Cattleya* hybrid (crossing). One of orchid types *Cattleya* hybrid was Blc. Amy Wakasugi that is the result of hybrid between 3 different genus (trigenerik) those are genus *Brassavola x Laelia x Cattleya* = *Brassolaeliocattleya* (Blc). The interest of this orchid is the size of flower and its labellum that relatively bigger and colorful.

The seed of orchid result of hybrid is needed particular treatment in the step of germination, this case occured because the seed of orchid does not have endosperm as food supply thus need type of particular fungus to help germination naturally, assumed the fungus supply the sources of carbohydrat, auxin and vitamin for the orchid (Vij et al, 2000).

Mediums that usually uses *in vitro* culture of orchid are the mediums of Vacin and Went (VW) medium that contain the elements of macro and micro ph need by orchid for the growth. These mediums are fair for tissue culture of orchid until result plantlet (Sriyanti and Wijayani, 1992). Some cases showed that the using of VW medium 100% for the 3rd medium often still occur the forming of shoots, that's why use VW medium 50%. Modification of other culture medium can do by the addition of Gaviota manure into VW medium a half of concentration. To solve the high cost of chemical substance, nowadays has developed alternative technology that is the using of medium

with manure composition. The using of manure medium had tried by Soedjono (2005) at the step of subculture prepared acclimation and gave significant result.

The element of nitrogen hara give in form anorganic salt. The addition of nitrogen into culture medium can give in form of amonium sulphate ($(NH_4)_2SO_4$) and nitrite amonium (NH_4NO_3). Marlin's research result (2003) showed that the giving of nitrogen in subculture medium very influence the growth of ginger explant by *in vitro*. The average highest sum of shoots and roots gained at concentration 100% NH_4NO_3.

Morel's researched (1960) showed that micropropagation *in vitro* which added NH_4NO_3 help forming protokorm and proliferation of protocorm from the tip of orchid. The presence of nitrogen supply, is very support the growth and plant development. The giving of NH_4NO_3 is very need in forming plant organs such as roots and shoots (Marlin, 2003).

Based on the case, the giving of nitrogen hara to stimulate the growth of orchid is needed. The process will develop to form a normal plantlet if culture to the right medium. By the presence of forming perfect stem and roots can strengthen plantlet thus able to adapt in the domain of *ex vitro*. The aims did this research were to gain nitrogen source and subculture medium type the better for the growth of orchid shoots Blc. Amy Wakasugi.

MATERIALS AND METHOD

This experiment conducted from August to November 2009 at Laboratorium LC Nursery Jln. Pelda Suryanta-Limusnunggal no. 121-122 Cibeureum Hilir, Sukabumi.

The materials used in this research were Vacin & Went (VW) media 100%, Vacin & Went (VW) 50%, manure of Gaviota. Shoots used were orchid shoots Blc. Amy Wakasugi from 2nd subculture bottle with pure VW aged less than 6 months of seed result from the seed by height less than 1 cm and has not had roots, tomato juice, bananan juice, potato extract, Topsoil®, the source of nitrogen that is amonium nitrate (NH_4NO_3) and amonium sulphat ($(NH_4)_2SO_4$), alcohol 70%, spirtus and cotton.

The instruments used in this research were *autoclave* (sterilized instrument), refrigerator, pHmeter, analytic weights, Erlenmeyer glasses, measurement glasses, *Laminar Air Flow* (LAF), subculture bottle, rubber shut bottle, knife, eraser, blender, petridish, pinset, scapel knife, neon lamp, shelf and spirtus lamp.

This experiment used Completely Randomized Design (CRD) consist of 8 treatments combination by 4 repetitions those were:

A : medium of Vacin & Went 100% + $(NH_4)_2SO_4$
B : medium of Vacin & Went 100% + NH_4NO_3
C : medium of Vacin & Went 50% + $(NH_4)_2SO_4$
D : medium of Vacin & Went 50% + NH_4NO_3
E : medium of & Went 50% + pupuk Gaviota + $(NH_4)_2SO_4$
F : medium of Vacin & Went 50% + pupuk Gaviota + NH_4NO_3
G : medium of Gaviota + $(NH_4)_2SO_4$
H : medium of Gaviota + NH_4NO_3.

The observation did started at the age of 4, 8, 12 and 16 weeks after planting (WAPt) for the addition of shoot height, the sum of leaves and length of leaves, while for the sum of shoots, stem diameter, the sum of roots, length of roots, roots dry weight, budding leaf dry weight and total dry weight did after plantlet destructed to the age of 16 WAP

RESULTS AND DISCUSSION

The Addition of sum and length of leaves at 8, 12 and 16 WAP

Table 1 showed that the source of nitrogen and type media of subculture effect toward the addition sum of leaves at the age of 8 mst however it was not significant effect at the age of 12 WAP and 16 WAP.

Table 1. The Effect Sources of Nitrogen and Type of Subculture Medium Toward the Addition Sum of Leaves at the Age of 8 , 12 and 16 WAP.

Treatments	The Addition Sum of Leaves (pieces)		
	8 mst	12 mst	16 mst
A (medium of VW 100% + $(NH_4)_2SO_4$)	0,384 a	0,705 ab	0,737 ab
B (medium of VW 100% + NH_4NO_3)	0,517 a	0,604 ab	0,632 ab
C (medium of VW 50% + $(NH_4)_2SO_4$)	0,449 a	0,732 ab	0,733 ab
D (medium of VW 50% + NH_4NO_3)	0,528 a	0,854 ab	0,972 ab
E (medium of VW 50% + Gaviota + $(NH_4)_2SO_4$)	0,406 a	0,416 a	0,587 a
F (medium of VW 50% + Gaviota + NH_4NO_3)	0,750 b	1,195 b	1,063 b
G (medium of Gaviota + $(NH_4)_2SO_4$)	0,521 a	0,569 ab	0,619 ab
H (medium of Gaviota + NH_4NO_3)	0,351 a	0,613 ab	0,625 ab

Information: Averaged scores followed by the same letter was not significant effect based on Duncan's Double Range Test at standard 5%
WAP ; /weeks after planting

At the age of 8 WAP, the sources of nitrogen and type of subculture medium was significant effect toward the addition sum of leaves. Medium of F which the content of nutrient elements decreased become the half of concentration (VW 50%) added by Gaviot manure and nitrogen with source of NH_4NO_3 (Ammonium nitrate) resulting the addition sum of leaves that was significant effect with other treatments.

Ammonium nitrate is a nitrogen source that provides the two forms of inorganic nitrogen is ammonium ion (NH4 +) and nitrate (NO3-). Ammonium nitrate containing N-total is available for 35% of the N-ammonium in the form of 17.5% and the form of N-nitrate by 17.5%, while ammonium sulphate containing only the N-total is 21.2% which is in the form of ammonium 21.2%. The growth of plant cells occurs by giving NH4 + but would be better with the addition of NO3-(Torres, 1989).

The F treatment also contains Gaviota Manure. Gaviota Manure used in the experiment contains a total of N-21% consisting of 4.2% N-ammonium, N-nitrate 4.2% and 12.6% of organic nitrogen in the form of urea.

In this case it is known that shoot will more quickly absorb ion NO_3 - compared to NH4 + because of the media set at pH 5.6 (acidic conditions), except that nitrate has high mobility so that their absorption by plants would be better if compared with ammonium. In the F nitrogen treatment in the form of nitrate supplied not only from raw media (VW 50%), but also from Gaviota and ammonium nitrate manure were added to the media.

From Table 2 can be seen that at the age of eight mst, 12 mst and 16 mst treatment D produced an average of the longest leaves length increment compared with other treatments.

Table 2 Showed the effect of adding nitrogen into the subculture media on the length of leaves at the age of 8 mst, 12 and 16 WAP

Treatments	The Increasing Length of Leaves (cm)		
	8 mst	12 mst	16 mst
A (medium of VW 100% + $(NH_4)_2SO_4$)	0,121 ab	0,111 ab	0,127 ab
B (medium of VW 100% + NH_4NO_3)	0,170 abc	0,248 cd	0,250 ab
C (medium of VW 50% + $(NH_4)_2SO_4$)	0,197 bc	0,236 bcd	0,212 bcd
D (medium of VW 50% + NH_4NO_3)	0,239 c	0,287 d	0,279 d
E (medium of VW 50% + Gaviota + $(NH_4)_2SO_4$)	0,142 ab	0,126 abc	0,156 abc
F (medium of VW 50% + Gaviota + NH_4NO_3)	0,175 abc	0,170 abcd	0,172 abc
G (medium of Gaviota + $(NH_4)_2SO_4$)	0,143 ab	0,119 abc	0,116 a
H (medium of Gaviota + NH_4NO_3)	0,101 a	0,099 a	0,129 ab

Information: The average value is followed by the same letter were not significantly different according to Duncan multiple range test at standard 5% WAP: weeks after planting

Medium VW 50% with ammonium nitrate nitrogen source (treatment D) to produce the best leaves of the length that is equal to 0.239 cm at age 8 mst, 0.287 cm at the age of 12 mst and 0.279 cm at the age of 16 mst. This is caused by nitrogen content in this medium is better for growth long-leaf. In treatment D, nitrogen was given in the form of ammonium nitrate (NH4NO3). Ammonium nitrate will affect the pH of the medium that will determine the availability of nutrients. Addition of ammonium nitrate and together can overcome the increase or decrease the pH of media. The presence of nitrogen supply in the form of NH4NO3 strongly support the growth of plantlets (Marlin, 2003). Nitrogen is a major component of culture media, and the type of nitrogen source significantly affected the growth and morphogenesis of plant tissue culture (Gamborg et al., 1981).

The addition of height at the age of 8, 12 and 16 WAP

The source of nitrogen and type medium of subculture was significant effect toward the increasing height of shoots at the age of 8 WAP but was not significant effect at the age of 12 and 16 WAP (Table 3)

Table 3. Effect Sources of Nitrogen and Types of Medium Subculture toward the increasing height of shoot at the age of 8, 12 and 16 WAP

The increasing of height	The Increasing Height (cm)		
	8 mst	12 mst	16 mst
A (medium of VW 100% + $(NH_4)_2SO_4$)	0,130 bc	0,093 ab	0,105 a
B (medium of VW 100% + NH_4NO_3)	0,074 a	0,145 ab	0,139 a
C (medium of VW 50% + $(NH_4)_2SO_4$)	0,060 a	0,101 ab	0,123 a
D (medium of VW 50% + NH_4NO_3)	0,082 ab	0,098 ab	0,101 a
E (medium of VW 50% + Gaviota + $(NH_4)_2SO_4$)	0,073 a	0,090 ab	0,106 a
F (medium of VW 50% + Gaviota + NH_4NO_3)	0,145 c	0,111 b	0,128 a
G (medium of Gaviota + $(NH_4)_2SO_4$)	0,057 a	0,062 ab	0,088 a
H (medium of Gaviota + NH_4NO_3)	0,076 a	0,045 a	0,076 a

Information: The average value is followed by the same letter were not significantly different according to Duncan multiple range test at standard 5% WAP: weeks after planting

Table 3 showed that at age of 8 WAP,. F Treatment which was the medium treatment of VW Gaviota 50% added by Gaviota manure with nitrogen sources in the form of ammonium nitrate

produced the addition of better shoot height as many as 0.145 cm. This is apparently due to the nitrogen content contained in the medium consisting of ammonium (NH4 +), nitrate (NO3-), and urea. Urea manure supplied through Gaviota manure, Gaviota manure containing 12.6% urea. N elements which absorbed by plant useful in the division and enlargement of cells that occurred in the apical meristem allowing height-added plant.

In culture medium, the supply of N derived from nitrate, ammonium salts, amino acids and organic complexes. Nitrate N is a good source of N because it can be absorbed and metabolized by cells. Setyamidjaja (1986), stated that the element nitrogen plays an important role in stimulating vegetative growth by adding the plant height and sum of of leaves. This nitrogen composes amino acid tryptophan as the main basic ingredients that play a role in cell elongation at the meristematic tissue.

The sum of shoots at the age of 16 mst

Nitrogen sources and the type of subculture medium gave significant effect toward the sum of shoot plantlet (Table 4)

Table 4. The Effect of Nitrogen Sources and Types of Medium Subculture Toward the Sum of Shoots at the Age of 16 WAP

Treatments	Sum of Shoots
A (medium of VW 100% + $(NH_4)_2SO_4$)	1,792 ab
B (medium of VW 100% + NH_4NO_3)	1,806 ab
C (medium of VW 50% + $(NH_4)_2SO_4$)	2,606 d
D (medium of VW 50% + NH_4NO_3)	2,250 cd
E (medium of VW 50% + Gaviota + $(NH_4)_2SO_4$)	1,639 a
F (medium of VW 50% + Gaviota + NH_4NO_3)	1,964 bc
G (medium of Gaviota + $(NH_4)_2SO_4$)	1,949 abc
H (medium of Gaviota + NH_4NO_3)	1,781 ab

Information: The average value followed by the same letter were not significantly different according to Duncan multiple range test at standard 5% WAP: weeks after planting

Table 4 showed that the sum of shoots in treatment C produced the highest sum of shoots. Treatment C, which represented 50% VW medium with a nitrogen source in the form (¬ NH4) 2SO4 to produce an average of the highest sum of shoots that is as many as 2.606. This was caused by nitrogen sources in the form of ammonium sulphate to supply two different nutrient elements are nitrogen (ammonia) and sulfur (sulphate). Media composition is thought to further stimulate the formation of the shoot. But the appearance of buds are less well roset with short segments. This is caused by the content of NH4 + (ammonium) cause the media pH became lower so that the absorption of some macro elements are blocked, and due to inhibition of absorption of some elements to stimulate the growth of shoots with short parts.

Roots dry weight, budding leaf dry weight and total dry weight of plantlet at the age of 16 WAP

The source of nitrogen and type of subculture medium was significant effect toward roots dry weight, budding leaf dry weight was significant effect toward the roots dry weight, and total dry weight of plantlet at the age of 16 WAPt (Table 5).

Table 5. The Effect of Nitrogen Sources and Types of Media Subculture of Root Dry Weight, Budding Leaf Dry Weight and Total Dry Weight of Plantlets at the Age 16 WAP

Treatments	Roots dry weight (mg)	Budding Leaf dry weight (mg)	Total dry weight of plantlet (mg)
A (medium of VW 100% + $(NH_4)_2SO_4$)	1,028 ab	1,768 a	2,796 a
B (medium of VW 100% + NH_4NO_3)	2,325 cde	3,006 bcd	5,331 bcd
C (medium of VW 50% + $(NH_4)_2SO_4$)	1,512 abc	2,286 ab	3,798 ab
D (medium of VW 50% + NH_4NO_3)	2,779 de	3,327 cd	6,212 cd
E (medium of VW 50% + Gaviota + $(NH_4)_2SO_4$)	1,019 a	1,914 a	2,933 a
F (medium of VW 50% + Gaviota + NH_4NO_3)	2,885 e	3,600 d	6,380 d
G (medium of Gaviota + $(NH_4)_2SO_4$)	1,867 bcd	2,545 abc	4,412 abc
H (medium of Gaviota + NH_4NO_3)	1,792 abc	2,306 ab	4,097 ab

Information: The average value followed by the same letter were not significantly different according to Duncan multiple range test at standard 5% WAP: weeks after planting

From Table 5 it can be concluded that F treatment produced roots dry weight, budding leaf dry weight and total dry weight of the best plantlet. This is caused by the absorption of nutrients element in the medium better than other mediums, this is proved by the high sum addition of leaves, height of plantlet, stem diameter and root length produced by the media. This is related with the effect of pH, which affect the absorption of nutrients elements in culture medium in vitro.

One nutrient that plays an important role in plantlet growth is nitrogen element. This nitrogen compose amino acid tryptophan as the main basic ingredients forming auxin that play a role in on cell elongation of meristematic tissue. Nitrogen is also the composer of adenine which is the material basis for the formation cytokinin (Kuruseng, 2008). Weight of plants reflects the increase in protoplasm, this occurs due to the size and sum of cells increases.

CONCLUSION

Conclusion

1. Sources of nitrogen and types of media subculture difference influenced toward all parameters observed except the sum of roots.
2. Medium subculture Vacin & Went 50% + Gaviota with a nitrogen source NH4NO3 are the best medium for shoot growth demonstrated by the sum of leaves, the increase in height, roots length, budding leaf dry weight, root dry weight and total dry weight.

Suggestion

1. In this experiment only observed plantlet growth until the final stage of culture, it is necessary to conduct further research on the growth at the acclimatization stage.

REFERENCES

Gamborg, O.L., Shyluk, J.P. and Shahin, E.A. 1981. Isolation, Fusion and Culture of Protoplasts. In Plant Tissue Culture: Methods and Applications in Agriculture, ed. T. A. Thorpe. Academic Press Inc., New York. p.115-180.

Kuruseng, H. 2008. *Pertumbuhan dan Produksi berbagai Varietas Tanaman Jagung pada Dua Dosis Pupuk Urea*. Jurnal Agrisistem Vol. 4 (1).

Marlin. 2003. *Regenerasi Planlet Jahe (Zingiber officinale Rosc.) dengan Pemberian Nitrogen pada Berbagai Bentuk Media Subkultur*. Jurnal Akta Agrosia 6 (1) : 12-17.

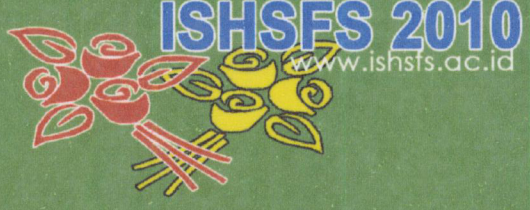
Morel, G. 1960. Tissue Culture - A New Means of Clonal Propagation in Orchids. Am. Orch. Soc.Bul.

Setyamidjaja, D. 1986. Pupuk dan Pemupukan. CV Simplex. Jakarta.

Soedjono, S. 2005. *Pengaruh Beberapa Pupuk Daun dalam Media Agar terhadap Pertumbuhan Meriklon Anggrek Dendrobium 'Walter Oumae'*. BuletinPenelitian Hortikultura XVIII (3) : 44-48.

Sriyanti, H. dan A. Wijayani. 1992. *Teknik Kultur Jaringan*. Kanisius. Yogyakarta.

Vij, S.P., Anoopama Kher and Ashish Gupta. 2000. Orchid Micropropagation. In Biotecnology in Horticulturaland Plantation Crops. Eds. K. L. Chadha, P.N. Ravindran and Leela Sahijram. Malhotra Publishing House. New Delhi.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