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EFFECTS OF ORGANIC ADDITIVES AND NAPHTHALENE ACETID ACID (NAA) APPLICATION ON THE *IN VITRO* GROWTH OF BLACK ORCHID HYBRID (*COELOGYNE PANDURATA* LINDLEY)

SRI HARTATI[1]; RETNA BANDRIYATI ARNIPUTRI[1]; LILI ANATUS SOLIAH[1]; ONGKO CAHYONO[2]
[1] *Universitas Sebelas Maret, Faculty of Agriculture, Department of Agrotechnology, Surakarta 57126, Central Java, Indonesia*
[2] *Universitas Tunas Pembangunan, Faculty of Agriculture, Department of Agrotechnology, Surakarta 57138, Central Java, Indonesia*

Abstract

Hartati, S., R. B. Arniputri, L. A. Soliah and O. Cahyono, 2017. Effects of organic additives and naphthalene acetid acid (NAA) application on the *in vitro* growth of Black orchid hybrid (*Coelogyne pandurata* Lindley). *Bulg. J. Agric. Sci.*, 23 (6): 951–957

Black orchid (*Coelogyne pandurata*), Bornean native beautiful orchid, which is currently threatened with extinction, was successfully crossed with *Coelogyne rumphii*. The hybrids will be multiplicated using *in vitro* propagation technique. To produce plants with good results both quantitatively and qualitatively it is necessary to add the growth regulator substances and organic additive compound into the culture medium.

This study aim to get the best medium composition that is able to optimize the growth protocol for the hybrids of *Coelogyne pandurata* and *Coelogyne rumphii*. The experiment was arranged in a factorial design based on completely randomized design with two factors and five replications. The first factor was the concentration of Naphthalene Acetic Acid /NAA (0, 1, 3 and 5 ppm). The second factor was the organic additivess (without any organic additives, coconut water was 250 ml L^{-1}, banana 150 g L^{-1}, potato 200 g L^{-1} and sweet potato 150 g L^{-1}).

The results showed that the addition of 3 ppm NAA capable of accelerating time root emergence. The organic materials affect all of parameter significantly. The combination of 3 ppm NAA with organic coconut water can accelerate the emergence of shoots of 9.8 days after planting (DAP), combination of 1 ppm NAA with coconut water can stimulate the multiplication of shoots as many as 6.29 shoots and combination of 3 ppm NAA with sweet potatoes can increase the number of roots as many as 23.5 roots

Key words: genetic diversity; *in vitro*; composition medium; *Coelogyne pandurata; Coelogyne rumphii*

Introduction

Orchidaceae has the most plentiful members, 30 000 – 35 000 orchid species, compared to other flowering plants (Singh et al., 2007). Among the genus of Orchidaceae family, *Coelogyne* Lindl has region of spread mostly in Asian region such as India, China, Indonesia (Kalimantan and Sumatra) and Himalaya (Devi et al., 2012). There is a rare and very exciting species of *Coelogyne* Lindl protected by the government of Indonesia is named black orchid (*Coelogyne pandurata* Lindley.).

Martin et al. (2005) succeeded in establishing a large-scale *in vitro* propagation protocol for Dendrobium hybrids, two valuable commercial cultivars of cut flower through shoot multiplication using flower stalk node explants and protocorm-like-bodies formation (PLBs).

**Corresponding author*: tatik_oc@yahoo.com

Attempts to reproduce orchids result from crosses which have the same characters as its parent in bulk can be done by tissue culture system. Tissue culture is a series of procedures to maintain and grow a plant cell. The advantages of orchid tissue culture are capable of producing seeds of orchids from crosses in large quantities and in a short time. Seeds of orchid hybrid from tissue culture are of good quality with a uniform color of the flowers when handled using proper cultivation technology. Tissue culture can be used for conservation of orchids that is almost extinct in the wild.

The existence of *Coelogyne pandurata* Lindley and *Coelogyne rumphii* in the wild are facing a serious threat of extinction due to excessive exploitation without being offset by adequate conservation efforts (Pant, 2013).

In addition, tissue culture method was also beneficial for growing plants that are difficult to propagate using seeds, including orchid. The percentage of germination of orchid seeds *in vivo* is low because it contains very little of food reserves even nothing. Orchid seed germination and development is highly dependent on the symbiosis with fungi.

The studies of micro propagation on orchid under different treatments on the culture media have been reported by several previous researchers: media treatment on seed of *Coelogyne* (Sungkumlong et al., 2008) on leaf of *Paphiopedilum* (Chen et al., 2004) , micropropagation transparent L (Sunitibala et al., 2009), Dendrobium temperature treatments on PLBs of *Teixeira Cymbidium* (Jaime et al., 2014), Large-scale *in vitro* propagation protocol for *Dendrobium* (Martin et al., 2005), root induction *Dendrobium* (Rafique et al., 2012), *in vitro* regeneration *Coelogyne nervosa* (Shibu et al., 2014), Dendrobium micropropagation (Jaime et al., 2015)

The content of tissue culture medium has significant effect on the growth and development of seeds and explants of orchids. Tuhuteru et al. (2012) stated that addition of coconut water could influence the growth of *Dendrobium anosmum* shoots.

Knudson C medium is a commonly used medium for tissue culture of orchids. The medium was first formulated by Lewis Knudson in 1949. Attempts to optimize the growth of orchids and enhance the qualitative and quantitative product can be done by modifying Knudson C medium through the addition of nutrition (organic additives) and a growth regulator.

This study aimed to assess the effect of the addition of plant growth regulators (Naphthalene Acetic Acid) and organic additives (coconut water, 'pisang ambon' bannana, potatoes and sweet potatoes) into Knudson C medium for increasing the growth of hybrids of *Coelogyne pandurata* and *Coelogyne rumphii*.

Material and Methods

The materials used in this study, were: the plantlet orchid hybrids of *Coelogyne pandurata* x *Coelogyne rumphii*, medium Knudson C, organic additives (coconut water, banana, potatoes and sweet potato), Naphthalene Acetic Acid.

The experiment was arranged in a factorial design based on completely randomized design with two factors and five replications. The first factor was the concentration of Naphthalene Acetic Acid (0, 1, 3 and 5 ppm). The second factor was the organic additivess (without any organic additives, coconut water was 250 ml L^{-1}, banana 150 g L^{-1}, potato 200 g L^{-1} and sweet potato 150 g L^{-1}). 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

Plant tissue culture is a plant propagation technique performed on sterile conditions or in a controlled environment, to produce clones of plants. In this process, tissues or cells, either as suspensions or solids are maintained at conditions conducive to their growth and multiplication. Plant tissue culture is developed based on the fact that many plant cells have the ability to regenerate whole plants (Idowu et al., 2009).

This technique is suitable to reproduce orchids from crosses that are difficult to do through generative way. To optimize growth and increase the production of crossbred orchids *C. pandurata* Lindley and *C. rumphii* qualitatively and quantitatively can be done by modifying the Knudson C medium through the addition of plant growth regulator and organic compounds. The addition of growth regulator and organic compounds can stimulate the growth and development of cross-orchid.

The effect of NAA and organic additives on the time of shoot emergence

The emergence of shoots is marked with a mass of white-greenish ± 1–2 mm on the top surface of the plantlets. In this study we found that the addition of NAA and organic additives affects the rate at which shoot appear. The data in Figure 1 shows that the treatment combination of NAA 3 ppm and coconut water of 250 ml l^{-1} resulted in the fastest shoot-out time of 9.8 days. However increasing concentrations of NAA up to 5 ppm tends to inhibit the emergence of shoots. Therefore the time of shoot emergence becomes 15.2 days. This is indicated by the quadratic regression model is $y = 1.607x^2 – 9.068x + 20.85$ with a coefficient of determination $R^2 = 0.881$.

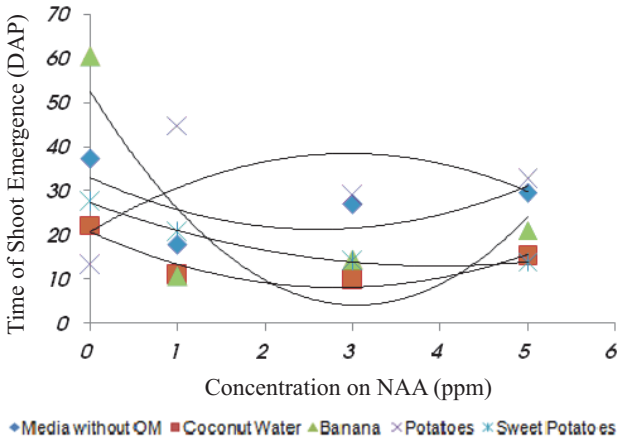

Fig. 1. Regression analysis the time of shoot emergence of *C. pandurata* x *C. rumphii* on the NAA concentration and organic additives treatments

y(media without organic additives) = $1.721x^2 – 8.976x + 33.04$; $R^2 = 0.419$
y(coconut water) = $1.607x^2 – 9.068x + 20.85$; $R^2 = 0.881$
y(banana) = $5.213x^2 – 31.72x + 52.38$; $R^2 = 0.740$
y(potatoes) = $–2{,}038x^2 + 12{,}01x + 20{,}74$; $R^2 = 0{,}307$
y(sweet potatoes) = $0.904x^2 – 7.204x + 27.33$; $R^2 = 0.998$

Addition of 3 ppm NAA and coconut water is found to be a combination of auxin and organic material that is beneficial to the growth of shoots. NAA has a significant effect speeding the shoot emergence due to insufficient endogenous auxin in the shoot regeneration process so that requires additional exogenous auxin. Pant and Thapa (2012) found in their study that the concentrations and types of auxin affect the efficacy of shoot emergence of *Dendrobium primulinum* Lindl.

Coconut water has better effect than other organic additives treatments. This is presumably because coconut water contains plant growth regulators. This finding corresponds to the study of Prades et al. (2011). They stated that coconut water could function as plant growth regulators because it contain cytokinins and other phytohormones which have different function in plants including cell division, seed germination, tissue differentiation and so for stimulating the growth of shoots. It also contain not only macro elements (P, K, Ca, Mg, and S) but also contain some trace element such as Na, Cl, Mn, Al, Zn, Fe, and Cu which stimulates the growth of shoots.

The effect of NAA and organic additives on the number of shoots

The number of shoots may be indicated as a success in multiplication. The more shoots that are formed the easier the cell multiplication. This study showed that the addition of NAA and organic additives into Kudson C medium affected the number of shoots formed. The data in Figure 2, regression analysis, showed that the combination of NAA addition treatment of 1 ppm concentration and coconut water addition treatment resulted in the highest number of shoots that are formed, which are 6.29 shoots. The regression analysis also indicated that adding more NAA would decrease the number of shoots. This is indicated by the quadratic regression model is y= $-0.409x^2+2.579x +2.645$, the coefficient of determination $R^2 = 0.688$.

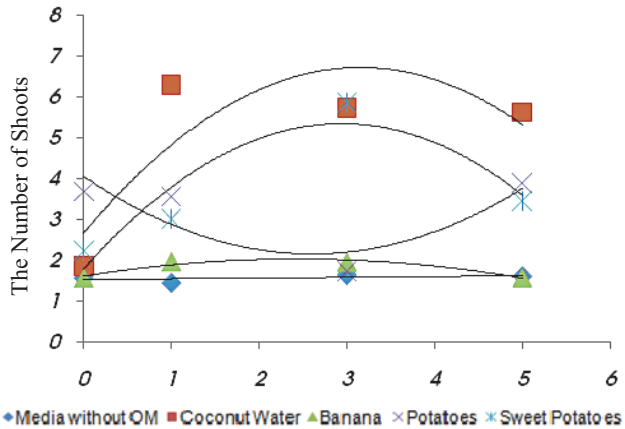

Fig. 2. Regression analysis the average of shoot number of *C. pandurata* x *C. rumphii* on the NAA concentration and organic additives treatments

y(media without organic additives) = $0.000x^2 + 0.016x + 1.532$; $R^2 = 0.318$
y(coconut water) = $–0.409x^2 + 2.579x + 2.645$; $R^2 = 0.688$
y(banana) = $–0.073x^2 + 0.357x + 1.603$; $R^2 = 0.925$
y(potatoes) = $0.281x^2 – 1.466x + 4.050$; $R^2 = 0.718$
y(sweet potatoes) = $–0.408x^2 + 2.404x + 1.790$; $R^2 = 0.851$

Naphthalene Acetic Acid compounds can be given in medium culture at lower concentrations. The addition of NAA in the low concentration of 1 ppm into the culture medium is optimum for growth of shoots of the orchid of *C. rumphii* x *C. pandurata*. The study by Agarwal (2015) showed that callus induction required a high concentration of auxin in plants, but for regeneration of shoots required a low concentration of auxin. Moreover Tuhuteru et al. (2012) stated that the higher concentration of cytokinins in coconut water than that of auxin in the explant, affects the nucleic acids and also influences the protein synthesis and regulator of enzyme activity. Therefore the process of cell division tends to lead to the shoot formation.

The effect of NAA and organic additives on the time of root emergence

The time of root emergence is an important factor in the growth of the plant because the plant will more easily absorb the nutrients that are contained in the culture medium. The formation of the roots is characterized by their greenish-white mass (± 2 mm) on the surface of bottom plantlets (base of the stem). From regression analysis showed that the addition of NAA up to 3 ppm was able to speed up the emergence of the roots (Figure 3). This concentration is the best for the root emergence time which for only 42.24 days. However increasing the concentration of NAA up to 5 ppm tends to slow down the time of root emergence. This is indicated by the quadratic regression model is $y= 0.695x^2-4.468x+48.92$, with a coefficient of determination $R^2 = 0.969$.

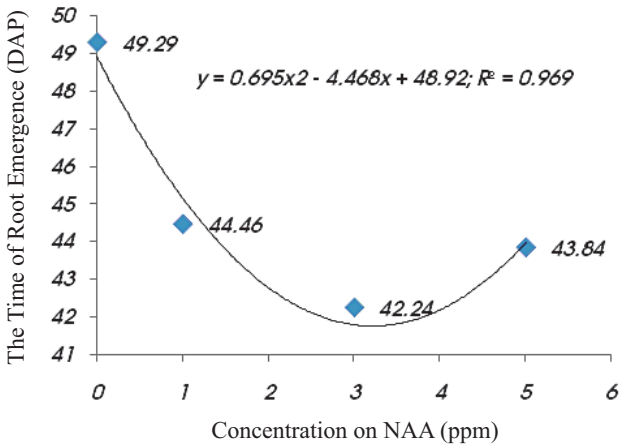

Fig. 3. Effect of NAA concentration on the time of root emergence of *C. pandurata x C. rumphii*

Growth regulators (example auxins, cytokinins and gibberellins plant hormones) play important roles in the growth and differentiation of cultured cells and tissues. NAA have been reported to encourage plant rooting *in vitro* (Hussein, 2012).

The addition of organic additives (coconut water, bananas, potatoes and sweet potatoes) shows the same effect on the time of emergence of roots (Figure 4). These four types of organic additives are capable of generating root emergence faster than the media without any organic additives addition. This indicates that when the organic additives is added into the culture medium containing auxin, it is capable of stimulating the emergence of roots. Naturally some explants produce auxin in sufficient quantities, but to support the growth they need additional auxin from outside, namely from the addition of plant growth regulator and organic compounds.

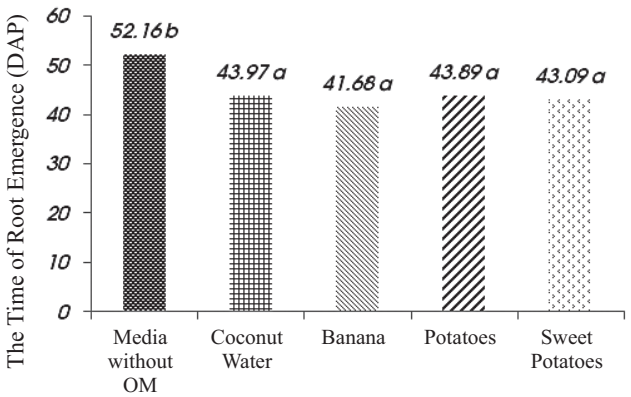

Fig. 4. Effect of organic additives on the time of root emergence (DAP) of *C. pandurata x C. rumphii*

The effect of NAA and organic additives on the roots number

The number of roots that are formed during culture medium phase is very important for the life of orchid explan, because this will cause the absorption of nutrients from the culutre medium to be more optimal. The more number of roots formed the more optimal the nutrient absorption.

The recent study showed that the addition of 3 ppm NAA and sweet potato additives can produce the largest number of roots, as many as 23.5 (Figure 5). The quadratic regression

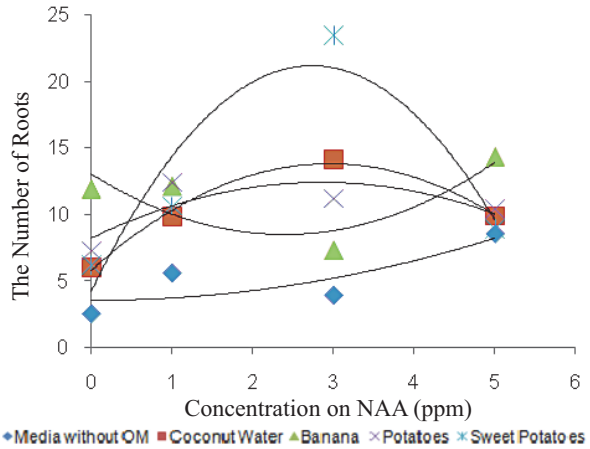

Fig. 5 Regression analysis the average of roots number of *C. pandurata* and x *C. rumphii* on the concentration NAA and organic additives

y(media without organic matter) = $0.19x^2 – 0.019x + 3.538$; $R^2 = 0.687$

y(coconut water) = $-0.918x^2 + 5.434x + 5.751$; $R^2 = 0.988$

y(banana) = $0.800x^2 – 3.830x + 13.02$; $R^2 = 0.694$

y(potatoes) = $–0.511x^2 + 2.923x + 8.215$; $R^2 = 0.614$

y(sweet potatoes) = $–2.266x^2 + 12.42x + 4.151$; $R^2 = 0.863$

model, $y = -2.266x^2 + 12.42x + 4.151$, the coefficient of determination $R^2 = 0.863$, indicates that increasing the level of NAA from 3 ppm to 5 ppm will not followed by an increase in the number of root, even would reduce the root number. This result confirmed that NAA in certain level of application will stimulate root enlargement and differentation as reported by previous studies (Bhowjwani and Radzan, 1983; Pant and Thapa, 2012; Baker et al., 2014). In addition to hormone auxin, root growth is also supported by the supply of elements needed to promote the number of root explants from organic additives. Our study showed that addition of sweet potato as organic additive is better to pormote roots. Similar result also reported by Untari and Puspitaningtyas (2006).

The effect of NAA and organic additives on the root length

Different from other parametres, the addition of NAA into the culture medium did not affect significantly the root length. However the root length was influenced by addition of organic additives. In this study we found that the addition of sweet potato has the best effect to the root length, followed by treatment of coconut water. While the treatments of banana and potato did not have significant effect (Figure 6). The addition of coconut water and sweet potatoes are capable of lengthening root better than other organic additivess indicates that the sweet potato and coconut water supplied nutrients and vitamins which are suitable for root growth. Molnár et al., (2011) stated that the coconut water is the colorless liquid endosperm that contains a number of amino acids, organic acids, nucleic acids, several vitamins, sugars and sugars alcohols, plant hormones (auxins, cytokinins), minerals, and other unidentified substances that can function as growth promoting qualities. Moreover the coconut water contains auxin and thiamine are capable of accelerating cell division in the meristem of the root (Wetter and Constabel,1991). Untari and Puspitaningtyas (2006) also reported that sweet potato additive was also capable of lenghtening root of *Coelogyne pandurata* under *in vitro* culture.

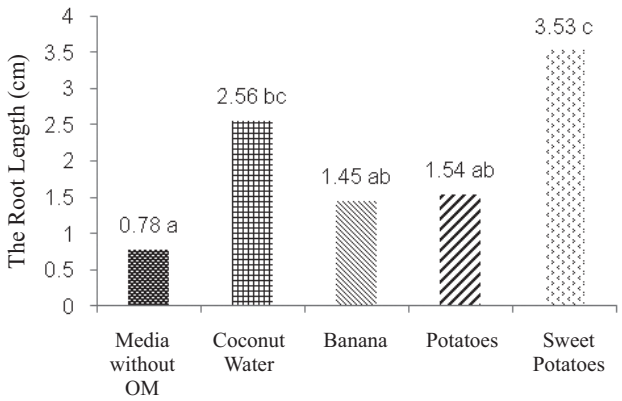

Fig. 6. The effect of organic additives on the root length of *C. pandurata* x *C. rumphii*

The effect of NAA and organic additives on the increase of plantlet height

The increase in plantlet height is caused by two processes: cell division and elongation that occur in the meristem network, at the growing point of the stem. These processes need suppy of carbohydrates and other elements for energy. This study showed that the addition of organic additives, that are coconut water, potatoes and sweet potatoes, affected significantly on plantlet height increment, while banana additive did not affect significantly. The highest plantlet was found at the treatment of coconut water which was 1.83 cm followed by sweet potato and potato which were 1.8 cm and 1.54 cm respectively (Figure 7).

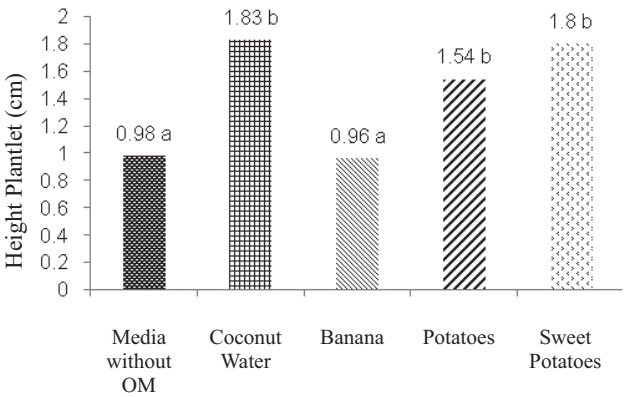

Fig. 7. Effect of organic additives on the increase of plantlet height of *C. pandurata* x *C. rumphii*

This demonstrates that the three types of organic additives added into the culture medium containing a carbohydrate that have positive effect on the growth of crossbred plantlet. Gauchan (2012) states that the concentration and type of exogenous carbon source added to the medium affected the growth and multiplication of plantlet *in vitro*. Gnasekaran et al. (2010) found that organic extract additives contain carbohydrates, protein, fat, vitamins, phenols, amino acids, fiber, hormones, sterols and organic acids at various levels. Thus, an extract being stimulatory or inhibitory towards the PLB proliferation is dependent upon the composition and its level in the extract.

The effect of NAA and organic additives on the number of leaves

Leaves are plant organs to perform photosynthesis. The more the number of leaves the more photosynthate produced so that the growth of the plant will be better. The formation of leaves is influenced by many factors such as the availability of nutrients in the media.

This study found that the addition of NAA into the culture medium did not affect significantly the number of leaves. However the leave number was influenced by addition of organic additives. We found in this study that the addition of coconut water has the best effect to the leave number, 9.92 leaves. This is followed by treatment of sweet potato, 6.45 leaves and potato, 6.1 leaves (Figure 8). The addition of coconut water performed the best result in increasing the number of leaves may be caused by its element contents. The coconut water is the liquid endosperm containing amino acids, organic acids, nucleic acids, vitamins, carbohydrates, growth hormone (auxin and cytokinins), minerals and other substances that can improve the quality of growth plantlets (Molnár et al., 2011). The concentration of plant growth hormones (auxin, cytokinin, gibberelins) present in coconut water affected the leaf number and fresh weight of the plantlets (Muhammad et al., 2015). Zeatin compound belonging to the class of cytokinins are able to stimulate the occurrence of organogenesis that can accelerate the growth of leaves Salisbury and Ross (1993) these elements play a role in the formation of root hairs and lengthening roots.

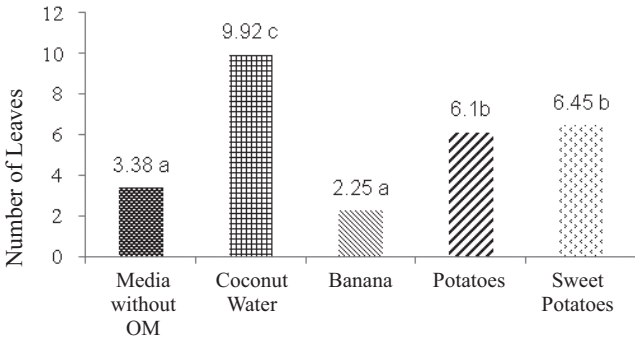

Fig. 8. The effect of organic additives on the leaf number of *C. pandurata* x *C. rumphii*

Conclusion

The study concluded that the addition of 3 ppm NAA capable of accelerating time root emergence. The organic materials affect all of parameter significantly. The combination of 3 ppm NAA with organic coconut water can accelerate the emergence of shoots of 9.8 days after planting (DAP), combination of 1 ppm NAA with coconut water can stimulate the multiplication of shoots as many as 6.29 shoots and combination of 3 ppm NAA with sweet potatoes can increase the number of roots as many as 23.5 roots

References

Agarwal, M., 2015. Tissue culture of *Momordica charantia* L.: a review. *J Plant Sciences*, **3**(1): 24-32. DOI: 10.11648/j.jps.s.2015030101.14

Baker, A., K. Behzad, N. Ghorbanali and N. Naser, 2014. Micropropagation of Orchis Catasetum – A Rare And Endangered Orchid. *Acta Sci. Pol., Hortorum Cultus*, **13**(2): 197-205.

Bhojwani, S.S. and M.K. Radzan, 1983. Plant Tissue Culture: Theory and Practice. New York (US): *Elvisier Science Publishing Company*.

Chen, T.Y., J.T. Chen and W.C. Chang, 2004. Plant regeneration through direct shootbud formation from leaf .cultures of Paphiopedilum orchids. *Plant Cell Tissue Org. Cult.*, 76: 11-15.

Devi, B.C., B.S. Shibu and P.S. Wesly, 2012. *In vitro* regeneration of *Coelogyne stricta* direct somatic embryogenesis. *Journal Tropical Medicine Plants*, **13**(2): 153-161.

Gauchan, D.P., 2012. Effect of different sugars on shoot regeneration of maize. *Kathmandu University J Sci Eng Technol*, **8**(1): 119-124.
http:// ku.edu.np/kuset/vol8no1/15dhurvaGauchan.pdf

Gnasekaran, P., X. Rathinam, U.R. Sinniah and S. Subramaniam, 2010. A study on the use of organic additive on the protocorm like bodies (PLBs) growth of *Phalaenopsis violaceae* orchid. *J Phytol.*, **2**(1): 029-033.
http:// journal-phytology.com. ISSN: 2075-6240

Hussein, N., 2012. Effects of nutrient media constituents on growth and development of banana (*Musa spp*.) shoot tips cultured *in vitro*. *African J Biotech*., **11**(37): 9001-9006. DOI: 10. 5897/AJB11.4173.

Idowu, P.E., D.O. Ibitoye and O.T. Ademoyegun, 2009. Tissue culture as a plant production technique for horticultural crops. *African J Biotech.,* **8**(16): 3782-3788.
http://academicjournals.org/article/article1379924819Akin-Idowuet al.pdf.

Jaime, A. Teixeira da Silva, 2014. Successful storage of protocorm-like bodies of hybrid Cymbidium (Orchidaceae) under low temperature conditions. *Journal of Genetic Engineering and Biotechnology*, 12: 71-74.

Jaime, A. Teixeira da Silva, J.C. Cardoso, J..Dobranszki and S. Zeng, 2015. Dendrobium Micropropagation: A review. *Plant Cell Rep*, 34: 671-704. DOI 10.1007/s00299-015-1754-4.

Martin, K.P., J. Geervarghese, D. Joseph and J. Madassery, 2005. *In vitro* propagation of Dendrobium hybrids using flower stalk node explants. *Indian J Exp Biol.*, **43**(3): 280-285.
http://nopr.niscair.res.in/bitstream/1234 56789/23091/1/IJEB43(3) 280-285.pdf

Molnár, Z., E. Virág and V. Ördög**,** 2011. Natural substances in tissue culture media of higher plant. *J Acta Biologica Szegediensis*, **55**(1): 123-127.
http://sci.uzeged.hu/ABS/2011/Acta20HP/55123.pdf

Muhammad, K., Z. Gul, Z. Jamal, M. Ahmed, A.R. Khan and Z.U. Khan, 2015. Effect of coconut water from different fruit maturity stages, as natural substitute for synthetic PGR in *in vitro* potato micropropagation. *International J Biosciences*, **6**(2): 84-92. ISSN 2220-6655. innspub.net/wp-content/uploads/2015/01/IJB-V6No2-p84-92.pdf

Pant, B., 2013. Medicinal orchids and their uses: tissue culture a potential alternative for conservation. *African J Plant Science*, **7**(10): 448-467. DOI: 10.5897/AJPS2013.1031.

Pant, B. and D. Thapa, 2012. *In vitro* mass propagation of an epiphytic orchid *Dendrobium primulinum* Lindl. Through shoot tip culture. *Afr J Biotechnol*., **11**(42): 9970-9974. DOI: 10.5897/AJB. 3106

Prades, A., M. Dornier, N. Diop and J.P. Pain, 2011. Coconut water uses, composition and properties. *J Fruit.,* **6**(7): 87-107. DOI: 10.1051/fruits/2012002.

Rafique, R., B. Fatima, S. Mushtaq, M.S. Iqbal, M. Rasheed, M. Ali and S.Z. Ul Hasan, 2012. Effect of Indole-3-Butyric Acid (IBA) on *In vitro* root Induction in Dendrobium orchid (*Dendrobium sabin* H). *African Journal of Biotechnology*, **11**(20): 4673-4675.

Salisbury, F.B. and C.W. Ross, 1993. Plant Physiology. 4rd ed., California (US): *Wadsworth Publishing Company.*

Shibu, B.S., P.S. Wesley, S. Moin and B.C. Devi, 2014. *In vitro* regeneration *Coelogyne nervosa* A. Rich. And *Eria pseudoclavicaulis* Blatt., threatened orchid of Western Ghats, India. *Indian Journal of Experimental Biology*, 52: 658-663.

Singh, M.K., A.R. Sherpa, V. Hallan and A.A. Zaidi, 2007. A potyvirus in *Cymbidium* spp. in Northern India. *Austr. Plant Dis. Notes*, 2: 11-13.

Sinitibala, H. and R. Khisor, 2009. Micropropagation *Dendrobium transparent* L. from axenic pseudobulb segments. *Indian Journal of Biotechnology*, 8: 448-452.

Sungkumlong and C.R. Deb, 2008. Effect of different factors on immature embryo culture PLBs differentiation and RAPID mass multiplication of *Coelogyne suaveolens* (lindl) Hook. *India Journal of Experimental Biology,* **46**(4): 243-248.

Tuhuteru, S., M.L. Hehanussa and S.H.T. Raharjo, 2012. The growth and development of the orchid *Dendrobium anosmum* in medium culture *in vitro* with some coconut water concentration. *J Agrologia*, **1** (1): 1-12. ISSN 2301-7287.

Untari, R. and D.M. Puspitaningtyas, 2006. Pengaruh bahan organik dan NAA terhadap pertumbuhan Anggrek Hitam (*Coelogyne pandurata* Lindl) dalam kultur *in vitro*. *Biodiversitas*, **7**(3): 344-348.

Wetter, L.R. and L. Constabel, 1991. The method of tissue culture plants. Mathilda BW (Translator). Translating from: Bandung ITB (ID).

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