J. Appl. Environ. Biol. Sci., 5(6)105-110, 2015

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

In Vitro Clonal Propagation of *Vanilla planifolia* Andrews Using Microshoot-derived Node Explants

Nurul Jadid[1§], Tutik Nurhidayati[1], Priyono[2]
[1]Laboratory of Plant Bioscience and Technology, Biology Department, Institut Teknologi Sepuluh Nopember, Indonesia
[2]Indonesian Coffee and Cocoa Research Institute

Received: February 11, 2015
Accepted: April 27, 2015

ABSTRACT

An *in vitro* clonal propagation for *Vanilla planifolia* was developed using microshoot-derived node explants. The explants were placed on Murashige and Skoog (MS) modified medium supplemented with a combination of Indole-3-acetic acid (IAA) and Kinetin (KIN) at concentrations of 0; 0,25; 0,5; 0,75; and 1 ppm. Shoot and root proliferation, as well as their elongation rates were analyzed statistically using Non Parametric test with Kruskal-Wallis. The inclusion of KIN alone (0,75 ppm) in MS modified medium resulted in highest shoot proliferation and elongation rate: 1.63 new shoots per explant with an average shoot length of 1.01 cm. Meanwhile, the best root proliferation and elongation were obtained in the MS modified medium containing 0.5 ppm IAA which produced 0.8 roots per explant with an average root length of 0.66 cm.
KEYWORDS-*Vanilla planifolia*, Microshoot-derived node, Indole-3-acetic acid, Kinetin.

INTRODUCTION

Vanilla planifolia Andrews (vanilla), which belongs to the orchid family (Orchidaceae) comprises more than 110 species. This climbing orchid vine is also known as *V. fragrans* Salisb and is native to Central America. However, they are now commonly distributed and cultivated in tropical regions including India, Madagaskar and Indonesia. Together with other commercially cultivated *Vanilla* species (*V. tahitensis* and *V. pompona*), *V. planifolia* is considered as the natural source of economically important flavoring substance, vanillin ($C_3H_8O_3$). It has been extracted and used in flavoring cakes, chocolate and some beverages. In addition, it serves also as the main component in cosmetics and perfume industry. It is also widely used in pharmaceuticals due to its antioxidant properties. Consequently, the market demand for natural vanillin is increasing significantly[1].

Vanilla is a perennial climbing orchid with sessile leaves and succulent green stems, producing aerial roots (velamen roots) at the nodes[2]. Their seeds do not usually germinate. Thus, vanilla is propagated mainly by stem cuttings, which possess slow rate of multiplication. Moreover, cuttings of 60 – 120 cm long per stem could interrupt the growth of the mother plant. These problems offer many challenges to produce enough vanilla vines to satisfy an increasing market demand of natural vanillin. Therefore, simple and efficient techniques are obviously required for mass and rapid production of vanilla. The *in vitro* plant propagation is reported to be the suitable method in vanilla mass multiplication. Shoot tip and axillary buds have been frequently used in vanilla tissue culture making the cultivated plants are genotypically identical. However, the choice of medium as well as the type of plant growth regulators (PGRs) used might be crucial in *in vitro* plant propagation. Previous report showed that shoot elongation on vanilla tissue culture (placed on MS medium with 0,5 ppm of 6-Benzylaminopurin (BAP)) could be seen after 5-6 week of treatment[3]. Moreover, the addition of BAP and KIN on Vanilla tissue culture derived from nodes on MS modified medium (at 0-1 ppm level of concentrations) has been reported to increase shoot multiplication[4]. On the other hand, a balance between auxin and cytokinin concentration is often required for the formation of adventitious shoot and root meristems[5]. Here, we investigated the effect of two types of PGRs, Indole Acetic Acid (IAA) and KIN on *in vitro* culture of vanilla using microshoot-derived node explants.

MATERIALS AND METHODS

Plant Material Preparation

Vanilla plants were obtained from the Indonesian Coffee and Cocoa Research Institute (ICCRI) collection. Selected nodal explants harvested from greenhouse were used as primary explants. Nodes were excised and washed under running tap water for 30 minutes. Subsequently, the isolated explants were disinfected with a surfactant solution (1 gr/l detergent), with one-two drops of Tween 20^R for 10-15 minutes and then washed under tap water for 45 minutes. Explants were then submerged in 1 gr/l Streptomycine for 0,5-1 hours followed by three-five times rinses using sterile distilled water. Subsequently, explants were submerged in 20 % clorox^R for 15-25 minutes. Finally, primary explants were rinsed four to five times with sterilized distilled water.

Development of secondary explants

Sterilized primary nodal explants obtained from the previous steps were then placed on modified Murashige and Skoog (MS) medium[6] for inducing new shoot formation for approximately 2 weeks. Nodes formed from the new shoots were then excised and placed on MS medium for stem elongation purposes and production of new microshoot-derived node explants. These explants were then used as secondary explants. Nodes from this secondary explants were excised and placed on MS 0 medium for 2 weeks then subcultured into 15 ml/flask modified MS medium supplemented with

§Corresponding author: Nurul Jadid, Laboratory of Plant Bioscience and Technology, Biology Department, Institut Teknologi Sepuluh Nopember, Indonesia. nuruljadid@bio.its.ac.id

different concentration of IAA (0, 0,25, 0,5, 0,75, 1 ppm) in combination with KIN (0; 0,25; 0,5; 0,75; 1 ppm). Two explants were placed horizontally in each flask culture. The experiments were performed for 1 month under 16/8 h photoperiod at a temperature of 25-27 ^{0}C.

Statistical Analysis

About 25 treatments were performed in this research, where each was carried out two times. The parameters used in this research were percentage of organogenesis responses, number of shoot, shoot length, number of root and root length. All data were analyzed descriptively using non-parametric test with Kruskal-Wallis.

RESULTS AND DISCUSSION

Organogenesis Responses

Organogenesis responses are showed in all treated microshoot-derived node explants. Shoot formation was observed in all treated explants, while some explants only developed adventitious root in their early developmental stages. Moreover, development of shoots as well as adventitious roots in some cases is also observed (Table 1). The overall results showed that auxin (IAA) alone is capable of inducing root formation. However, treatment with 0,5 ppm IAA alone displayed highest percentage of explants forming adventitious roots (50%), compared with other results obtained with either 0,25; 0,75 or 1 ppm of IAA alone. In contrast, treatment of explants using 0,75 ppm IAA and 1 ppm KIN successfully induces the formation of adventitious shoots (highest percentage, 95%), compared with other treatments. However, explants treated with 0 ppm IAA and 0,75 ppm KIN displayed the best organogenesis responses. All explants formed new adventitious shoots and roots (86,6% formed adventitious shoot only and 17,4 % formed adventitious roots) (Table 1). This result showed that 0,75 ppm KIN could efficiently induce loss of apical dominance that might blocked the growth of axillary and adventitious shoot in Vanilla tissue culture. In addition, the explants might contain sufficient amount of endogenous auxin to react synergistically with KIN to induce the development of adventitious shoots. In the other hand, a bizarre morphology of adventitious shoot was observed in explants treated with zero concentration of both IAA and KIN. The adventitious shoot produced in this treatment tended to be swollen and lack of chlorophyll (Figure 5A). This might explain the importance role of cytokinin in promoting chlorophyll biosynthesis[7].

Table 1. Organogenesis responses on *in vitro* culture of vanilla (*Vanilla planifolia)* using microshoot-derived node explants

Treatments	% explant formed root	% explant formed shoot	% explant formed shoot and root
I_0K_0 (0 ppm IAA + 0 ppm Kinetin)	0.0	88.8	6.3
I_0K_1 (0 ppm IAA + 0.25 ppm Kinetin)	5.0	52.8	37.2*
I_0K_2 (0 ppm IAA + 0.5 ppm Kinetin)	5.6	61.1	16.7
I_0K_3 (0 ppm IAA + 0.75 ppm Kinetin	0.0	82.6	17.4
I_0K_4 (0 ppm IAA + 1 ppm Kinetin)	0.0	66.3	5.0
I_1K_0 (0.25 ppm IAA + 0 ppm Kinetin)	36.3	11.3	31.3
I_1K_1 (0.25 ppm IAA + 0.25 ppm Kinetin)	15.0	25.0	10.0
I_1K_2 (0.25 ppm IAA + 0.5 ppm Kinetin)	10.0	50.0	25.0
I_1K_3 (0.25 ppm IAA + 0.75 ppm Kinetin)	12.5	47.2	16.7
I_1K_4 (0.25 ppm IAA + 1 ppm Kinetin)	0.0	70.0	10.0
I_2K_0 (0.5 ppm IAA + 0 ppm Kinetin)	50.0*	10.0	10.0
I_2K_1 (0.5 ppm IAA + 0.25 ppm Kinetin)	30.0	11.3	30.0
I_2K_2 (0.5 ppm IAA + 0.5 ppm Kinetin)	15.0	46.7	31.1
I_2K_3 (0.5 ppm IAA + 0.75 ppm Kinetin)	0.0	60.0	27.8
I_2K_4 (0.5 ppm IAA + 1 ppm Kinetin)	15.0	65.0	0.0
I_3K_0 (0.75 ppm IAA + 0 ppm Kinetin)	25.0	10.0	15.0
I_3K_1 (0.75 ppm IAA + 0.25 ppm Kinetin)	15.0	45.0	30.0
I_3K_2 (0.75 ppm IAA + 0.5 ppm Kinetin)	0.0	48.3	36.1
I_3K_3 (0.75 ppm IAA + 0.75 ppm Kinetin)	0.0	78.9	21.1
I_3K_4 (0.75 ppm IAA + 1 ppm Kinetin)	0.0	95.0*	0.0
I_4K_0 (1 ppm IAA + 0 ppm Kinetin)	40.0	15.0	15.0
I_4K_1 (1 ppm IAA + 0.25 ppm Kinetin)	5.0	47.8	21.1
I_4K_2 (1 ppm IAA + 0.5 ppm Kinetin)	15.0	50.0	10.0
I_4K_3 (1 ppm IAA + 0.75 ppm Kinetin)	10.0	58.3	16.1
I_4K_4 (1 ppm IAA + 1 ppm Kinetin)	0.0	48.8	30.0

***Highest percentage**

Shoot Multiplication and Elongation

The microshoot-derived nodes were initially cultured horizontally. Number of new adventitious shoots that were produced by explants placed on MS modified medium with KIN (0,25-0,75 ppm) increased significantly. All explants exhibited direct shoot organogenesis, which means that adventitious shoots are produced directly from the explants, not from an intermediary callus[8]. This is advantageous since we could accelerate the time needed to multiply the number of shoot formation. In addition, indirect shoot organogenesis is highly subjected to somaclonal variation[9]. Based on the results, a modified MS medium supplemented with 0 ppm IAA and 0,75 ppm KIN was the most suitable medium to be used on shoot multiplication in Vanilla tissue culture. The explants grown in this medium produced an average of 1,63 shoot per explant (Figure 1). Cytokinins are widely known as shoot-promoting agent in many *in vitro* cultured organs. Similar to our findings, many researchers have reported the use of cytokinins, including KIN for inducing shoot formation and multiplication[10,11,12]. However, the optimum concentration of cytokinins is species-dependent[13].

Meanwhile, a high level concentration of KIN (1 ppm), either in combination with IAA or alone did not significantly induced shoot multiplication. This observation indicates that KIN possess an adverse effect when used at a higher-than-optimal concentration. Higher concentration of KIN beyond its optimum level was reported to induce necrosis and reduction in shoot formation[14].

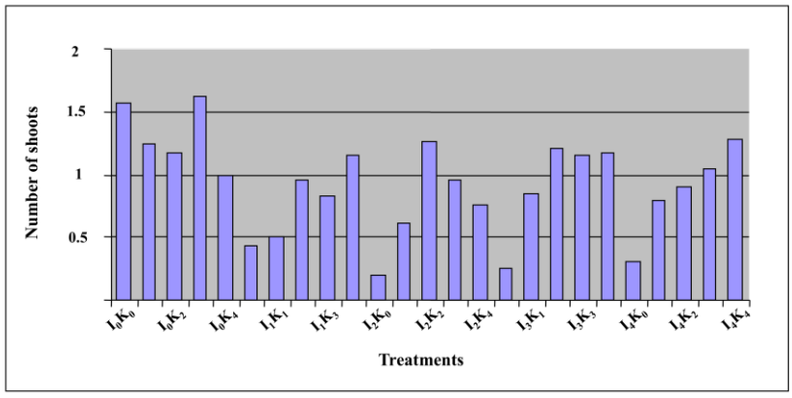

Figure 1. Effect of IAA and KIN on shoot multiplication of *in vitro* culture of vanilla (*Vanilla planifolia)* using microshoot-derived node explants

Good responses on shoot multiplication on explants that were treated on the MS modified medium, supplemented with 0 ppm IAA and 0,75 ppm Kinetin (I_0K_3) were followed by the best result in shoot elongation. The average length of shoots formed in this treatment was 1,01 cm (Figure 2). It evidenced that cytokinin is very important to encourage the growth of axillary buds and adventitious shoot, reduce apical dominance and promote cell division. Cytokinin promotes cell division activity in embryos and mature plants by altering the size and activity of meristems[15,16,17]. Furthermore, cytokinin has been reported to regulate cell cycle at both G1/S phase and G2/M phase progressions[18]. Entry into S phase requires D-cyclins accumulation, which is clearly induced by cytokinin. Hence, D-cyclins accumulation increases activity of cyclin-dependent protein kinase (CDK) enzymes that release transcription factors for genes of DNA replication[19,20]. The regulation of G2/M phase progression by cytokinin is indicated by the synthesis of this plant hormone during mitotic initiation[21].

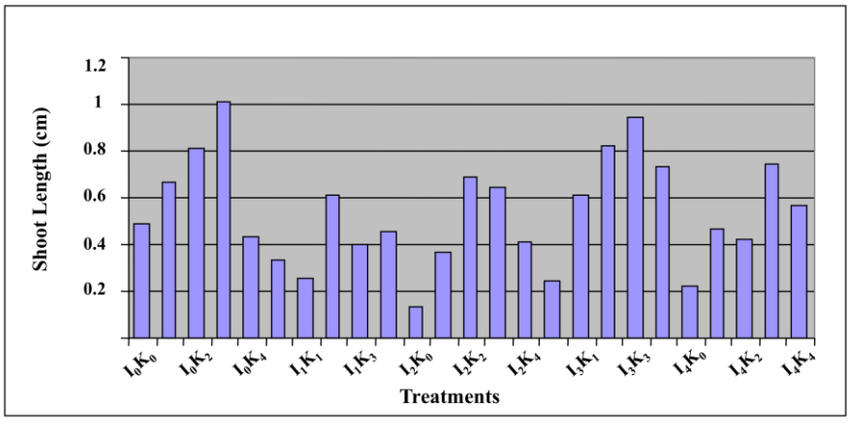

Figure 2. Effect of IAA and KIN on shoot elongation of *in vitro* culture of vanilla (*Vanilla planifolia)* using microshoot-derived node explants

Root Proliferation and Elongation

IAA is generally considered as the main auxin in most plants and controls the formation of adventitious root[22,23]. Adventitious root is a postembryonic root arising from the stem and leaves or from nonpericylce tissues in old roots[24]. In the present study, a treatment with 0,5 ppm IAA and 0 ppm KIN showed the best response in promoting adventitious root formation and proliferation. The average number of root formed in 0,5 ppm IAA alone was 0,8 roots per explants (Figure. 3). In many cases, auxins have been reported to induce adventitious roots formation[22,25,26]. Similar to our finding, addition of 0,5 ppm of IAA on *in vitro* culture of *Hypericum perforatum* L. cv. "New Stem" has been also reported as accelerator of root formation and proliferation[27]. Explants placed in the auxin-free medium were also performed adventitious roots development. This is probably due to the presence of endogenous IAA ensuring the rooting response. Natural auxin is synthesized in the apical meristematic zones and moves to the to the lower part of the plants and hence stimulating root formation[28]. In accordance with this data, polar auxin transport (PAT) has been known as a key mechanism by which auxin regulates several aspects of plant growth, including root development. In addition, recent study has revealed that sterol biosynthetic intermediate (SBI) is also involved in the regulation of auxin in modulating plant growth and development[29]. These studies reveal a complex mechanism of auxin in regulating root development.

We observed that explants treated with 0,5 ppm of IAA and 0,25 ppm KIN also exhibited positive response in adventitious root proliferation (Figure 3). This result explains that an optimal interaction between auxin and cytokinin might provide a favorable hormonal balance to the root production. Together with auxin, cytokinins play a significant role in cell division from G1/S to the G2/S stage[30].

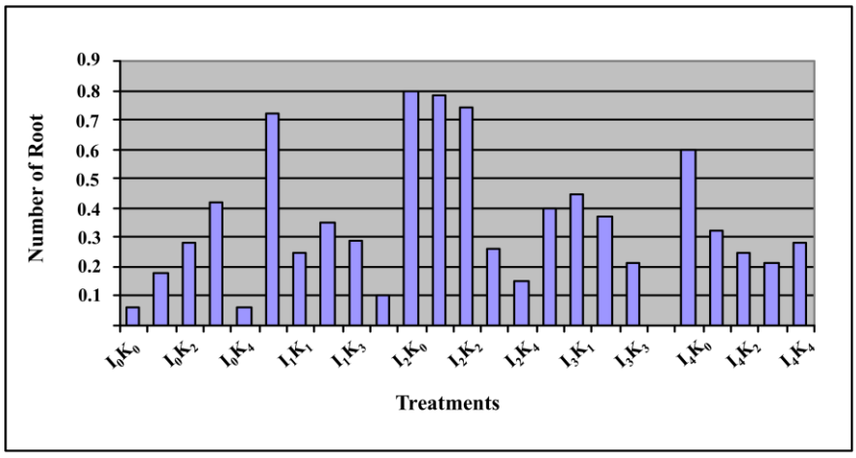

Figure 3. Effect of IAA and KIN on root proliferation of *in vitro* culture of vanilla (*Vanilla planifolia)* using microshoot-derived node explants

Treatments with IAA alone have positively promoted root elongation in all explants formed adventitious root. The best result was observed in treatment with 0,5 ppm IAA (with average length 0,66 cm) (Figure 4). 0,5 ppm IAA has been reported to induce root elongation on micropropagation of *Limonium thiniense* in MS medium[31]. Nevertheless, explants cultured in MS modified medium supplemented with 0,5 ppm IAA and 0,25 ppm KIN also displayed a good response in root elongation.

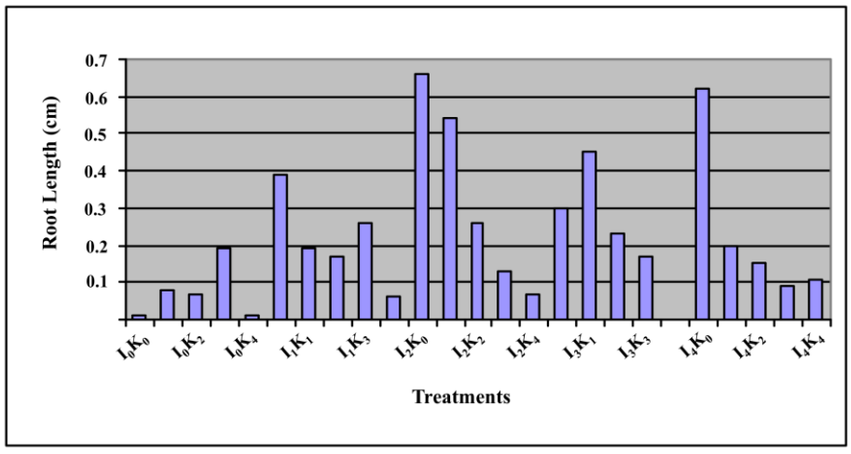

Figure 4. Effect of IAA and KIN on root elongation of *in vitro* culture of vanilla (*Vanilla planifolia)* using microshoot-derived node explants

Explants treated in MS modified medium with no addition of both IAA and KIN did not show a significant response in both root proliferation and elongation. These results explained that cell expansion located at the elongation

zone of extending root has been induced by auxin. Furthermore, various experiments showed that high auxin : cytokinin ratio promotes root formation, while a low auxin : cytokinin ratio results in shoot regeneration[32]. Auxins also interact with other plant hormone in controlling root growth. They function by modulating cellular responses to the gibberellin (GA). Besides its function in cell division and cell elongation, GA has been reported also to promote growth of root segments[33] by opposing the effect of nuclear DELLA protein growth repressors[34].

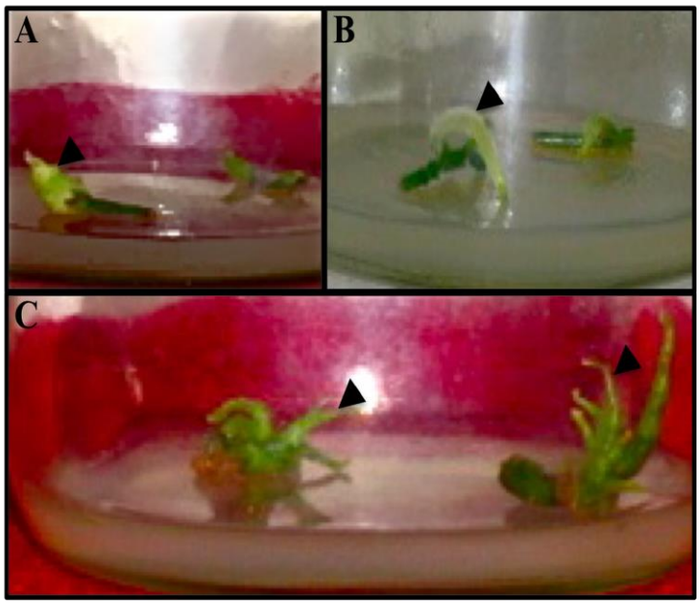

Figure 5. Responses of microshoot-derived explants from *Vanilla planifolia* Andrews treated in MS modified medium. Adventitious shoot produced on MS modified medium supplemented with 0 ppm IAA and KIN (A), Rooted explants grown on MS modified medium with 0,5 ppm IAA alone (B), Multiple adventitious shoot produced on MS modified medium supplemented 0,75 ppm KIN alone (C)

CONCLUSIONS

The inclusion of KIN alone (0, 75 ppm) in MS modified medium resulted in highest shoot proliferation and elongation rate: 1.63 new shoots per explant with an average shoot length of 1.01 cm. Meanwhile, the best root proliferation and elongation were obtained in the MS modified medium containing 0.5 ppm IAA, which produced 0.8 roots per explant with an average root length of 0.66 cm. Future study should explore further optimization of IAA and KIN concentration for boosting the *Vanilla planifolia* Andrews *in vitro* regeneration.

ACKNOWLEDGMENTS

We thank members of the plant tissue culture laboratory of the Indonesian Coffee and Cocoal research Institute (ICCRI) for productive discussion.

REFERENCES

[1] Divakaran, M., K. Nirmal, B., K.V. Peter. 2006. Conservation of *Vanilla* species, *in vitro*. Scientia Horticulturae **110**: 175-180.
[2] Cahyono, B. 1996. Pembudidayaan Tanaman Panili Teknik Pengembangan Intensif-Potensi Pasar-Analisa Usaha. C.V. Aneka. Solo.
[3] Geetha, S and Sudheer, A.S. 2000. *In vitro* propagation of *Vanilla planifolia*, a tropical orchid. Curr. Sci. **79**: 886-889.
[4] Wuriyanti, A and Priyono. 2004. Pengaruh Hormon BAP Dan Kinetin Terhadap Pertumbuhan Eksplan Yang Berbeda Pada Vanili (Vanilla planifolia Andrews) Secara *In Vitro.* Jember: Universitas Jember.
[5] Hendaryono,P.D dan Ari Wijayani. 1994. *Teknik Kultur Jaringan*. Penerbit Kanisius. Jakarta.
[6] Murashige, T and Skoog, F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue culture. Physiol. Plant **15(3)**: 473-497.
[7] Thimann, K.V. 1987. Plant senescence: A proposed integration of the constituent processes. P. 1-19. Proc. X annu. Symp. Plant Physiol. **10**: 1-19.
[8] Kane, M.E., Philman, N.J., and Jenks, M.A. 1994. A laboratory excercise to demonstrate direct and indirect shoot organogenesis using internodes of *Myriophyllum aquaticum.* HortTech **4**: 317-320.

[9] Kabir, A.H., Istiak, M., M.A. Razvy., M. Bulbul Ahmed., M.F. Alam. 2008. Indirect Organogenesis and Somaclonal Variation in Four Rice Cultivars of Bangladesh. Journal of Applied Sciences research **4(4)**: 451-458.
[10] Chawla, H.S. 2009. Introduction to Plant Biotechnology, New Hampsire, Science Publisher.
[11] Hesar, A., Kaviani, B., Tarang, A.R., Bohlooli, S. 2011. Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*). Plant Omics J. **4(5)**: 236-238.
[12] Fatmawati, T.A., T. Nurhudayanti., N. Jadid. 2010. Pengaruh kombinasi zat pengatur tumbuh IAA dan BAP pada kultur jaringan tembakau *Nicotiana tabacum* L. Var Prancak 95. Skripsi Fakultas Matematika dan Ilmu Pengetahuan Alam. Institut Teknologi Sepuluh Nopember. Surabaya.
[13] Gomes, F., Simoes, M., Lopes, M.L., Canhoto, M. 2010. Effect of plant growth regulators and genotype on the micropropagation of adult trees of *Arbutus unedo* L. strawberry tree). New Biotech.
[14] Madhulatha, P., Anbalagan, M., Jayachandaran, S., Sakthivel, N. 2004. Influence of liquid pulse treatment with growth regulators on in vitro propagation of banana (*Musa* sp. AAA). Plant Cell Tissue Organ Cult**. 76**: 189-192.
[15] Medford, J.I., Horgan, R., El-Sawi, Z., Klee, H.J. 1989. Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene. Plant Cell **1**: 403-413.
[16] Werner, T., Motyka, V., Strnad, L., and Schmülling, T. 2001. Regulation of plant growth by cytokinin. Proc. Nat. Acad. Sci. USA. **98**: 10487-10492.
[17] Zhang, K., Diederich, L., and John, P.C.L. 2005. The cytokinin requirement for cell division in cultured *Nicotiana plumbaginifolia* cells can be satisfied by yeast Cdc25 protein tyrosine phosphatase. Implications for mechanisms of cytokinin response and plant development. Plant Physiol. **137**: 308-316.
[18] Guitierrez, C., Ramirez-Parra, E., Castellano, M.M., del Pozo, J.C. 2002. G(1) to S transition: more than a cell cycle engine switch. Curr. Opin. Plant Biol. **5**: 480-486.
[19] Riou-Khamlichi, C., Huntley, R., Jacqmard, A., Murray, J.A.H. 1999. Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. Science **283**: 1541-1544.
[20] Murray, J.A.H., Doonan, J., Riou-Khamlichi, C., Meijer, M., Oakenfull, E.A. 2001. G1 cyclins, cytokinins and the regulation of the G1/S transition. In: francis D (ed) The plant cell cycle and its interfaces. CRC Press, Sheffield, pp 19-41.
[21] John, P.C.L., Zhang, K., Dong, C., Diederich, L., Wightman, F. 1993. $P34^{cdc2}$ related proteins in control of cell cycle progression, the switch between division and differentiation in tissue development and stimulation of division by auxin and cytokinin. Aust J Plant Physiol. **20**: 503-526.
[22] De Klerk, G.J., Van der Krieken, W., de Jong, J.C. 1999. Review the formation of adventitious roots: new concepts, new possibilities. In Vitro Cell. Dev. Biol. Plant **35**: 189-199.
[23] Pop, T.I., Pamfil, D., Bellini, C. 2011. Auxin control in the formation of adventitious roots. Not. Bot. Hort. Agrobot. Cluj. **39(1)**: 307-316.
[24] Geiss, G., Guiterrez, L., and Bellini, C. 2009. Adventitious root formation: New insights and perspectives. In Root Development, T. Beekman, ed. (Oxford: Wiley-Blackwell) p. 376.
[25] Diaz-Sala, C., Hutchison, K.W., Golfbarb, B., Greenwood, M.S. 1996. Maturation-related loss in rooting competence by loblolly pine stem cuttings: role of polar auxin transport and tissue sensitivity. Physiol. Plant. **97**: 481-490.
[26] Goldfarb, B., Hackett, W.P., Furnier, G.R., Mohn, C.A., Plietzsch, A. 1998. Adventitious root initiation in hypocotyl and epicotyl cuttings of eastern white pine seedlings. Physiol. Plant **102**: 513-522.
[27] Cherry, M., Bhatti, S., Long, D., Sauve, R.J., and Myles, E. 2000. Effect of hormones on in vitro culture of St. John's Wort. Southern Nursery Association research Conference **45**:274-275.
[28] Hartmann, H.T., Kester, D.E., Davies, F.TJ., Geneve, R.L. 2011. Plant propagation: principles and practices (8^{th} ed). Prentice-Hall, New York, USA.
[29] Mialoundama, A.S., Jadid, N., Brunel, J., Di Pascoli, T., Heintz, D., Erhardt, M., Mutterer, J., Bergdoll, M., Ayoub, D., Van Dorsselaer, A., Rahier, A., Nkeng, P., Geoffroy, P., Miesch, M., Camara, B., Bouvier, F. 2013. *Arabidopsis* ERG28 tethers the sterol C4-demethylation complex to prevent accumulation of a biosynthetic intermediate that interferes with polar auxin transport. The Plant Cell **25**: 4879-4893.
[30] Mercier, H., Beatriz, M.S., Jane, E.K., regina, M.H., and Bruno, S. 2003. Endogenous auxin and cytokinin contents associated with shoot formation in leaves of pineapple cultured *in vitro*. Braz. J. Plant Physiol. **15(2)**: 107-112.
[31] Lledo, M.D., M.B. Crespo., and J.B. Amo-Marco. 1996. Micropropagation of *Limonium thiniense* Erben (*Plumbaginaceae*) using herbarium material. Botanic Gardens Micropropagation News **2(2)**: 18-21.
[32] Teale, W.D., F.A. Ditengou., A.D. Dovzhenko., X. Li., A.M. molendijk., B. Ruperti., I. Papanov., and K. Palme. 2008. Auxin as a model for the integration of hormonal signal processing and transduction. Molecular Plant. **1(2)**: 229-237.
[33] Inada, S and Shimmen, T. 2000. Regulation of elongation growth by gibberellin in root segments of *Lemna minor*. Plant Cell Physiol. **41**: 932-939.
[34] Fu, X and Harberd, N.P. 2003. Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. Nature **421**: 740-743.