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by Tri Mulyaningsih

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ICST 2018

3rd International Conference on Science and Technology

PROCEEDINGS

Mataram, December 10, 2018

INSTITUTE FOR RESEARCH AND COMMUNITY SERVICES
UNIVERSITY OF MATARAM

PROCEEDING

The 3rd International Conference on Science and Technology (ICST 2018)

"Emerging Sciences and Technology for Human Prosperity and Health"

Mataram, 10th December 2018

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Preface of the Proceeding of
The 3rd International Conference on Science and Technology 2018

Bismillaahirrahmaanirrahiim
Assalaamu'alaikumwarahmatullaahwabarakaatuh.
Peace be upon us.

Praise always we pray to God Almighty for giving us the abundance of grace, guidance and inayah, so that we all could meet in Lombok, a beautiful island "the Island of Thousand Mosques" in West Nusa Tenggara Province. Our Lombok island known to its many natural and cultural diversity where you can enjoy cuisines, beaches, waterfalls, mountain, traditional villages and handicraft of many ethnics in this Island.

On behalf the Committee, I would like to thank you all attendee of the "3rd International Conference on Science and Technology (ICST) 2018" on December 10th 2018 and shared impressive ideas, knowledge and experiences through the article to build network for possible future collaboration, therefore the proceedings can be realized.

This proceeding published articles from presenters that came from various universities and research institutions in Indonesia and from overseas (Philippine, Australia, Thailand). Research papers already reviewed on the basis of a full length manuscript that accepted based on quality, originality and relevance.

At this moment, the organizing committee would like to express our gratitude to the all keynote speakers and presenters who have submitted for article and also to all participants to share their acknowledged works, your effort and contribution to the conference are absolutely valuable. Our special gratitude also goes to the Rector of the University of Mataram and Head of Institute for Research and Community Services) University of Mataram, West Nusa Tenggara, Lombok, Indonesia, who have been highly supporting the conference.

Last but not least, I would like to thank the organizing committee as well as all other supporters and participants, as without their effort, commitment and hard work, the publication of this proceeding will be hardly achieved. Critics and suggestions on the improvement of this proceeding will be highly appreciated. Hopefully the next ICST can be better prepared based on this recommendation.

Wassalamu'alaikum warohmatullahi wabarakatuh.

Chairman of 3rd ICST 2018

Dr.rer.nat. Lalu Rudyat Telly Savalas, M.Si.

TABLE OF CONTENT

3rd International Conference on Science and Technology
ICST 2018
Mataram, December 10, 2018

IN VITRO REGENERATION OF AGARWOOD PLANT *(Aquilaria filarial)*

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Abstract. Aquilaria filaria is one of the species producing fragrant oleoresin agarwood, which is endemic to Eastern of Indonesia. In 2004, CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) incorporated A. filaria as a vulnerable species due to the declining population of this tree in its native habitat. Plant in vitro culture is only a strategy to propagate plant material in prolific rates for in situ and ex situ germplasm preservation in a range of environmental condition. This research aims to study de novo regeneration of shoot and root in plant tissue culture of A. filaria for in vitro preservation of this vulnerable plant species. In vitro cultivation conducted in Murashige and Skoog (MS) medium containing phytohormone auxin: indole-3-butyric acid (IBA), α-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) and cytokinin: 6-benzylaminopurine (BAP) and kinetin. The result reveals that leaf is a suitable material for callus induction. High proliferating callus is induced in medium containing combination of IBA (2 mg/l) or NAA (0.2 – 1 mg/l) with cytokinin BAP (0.5 – 1 mg/l). A. filaria plant callus regenerate shoot primordia by an addition of single BAP (0.5 mg/l) or combination with IBA or NAA (0.2 mg/l) into the culture medium. Rooting of shoot cultures was achieved in medium with addition of a sole phytohormone IBA (1 – 2 mg/l) or NAA (0.2 – 2 mg/l). The result of this study provides basis for in vitro propagation of A. filaria and conservation of agarwood germplasm.

Keywords: germplasm conservation, plant tissue culture, phytohormone

1. Introduction

The plant genus of Aquilaria are well known for their capability to produce agarwood, a priceless non-timber forest commodity from South East Asia. Agarwood is a fragrant oleoresin formed inside the wood of the tree [1]. Besides its traditional application as medicine and in religious ceremonies of Asian and Middle Eastern countries, agarwood oleoresin is an important material for modern perfumery industry [2]. From the ancient time up to date, agarwood remains as an economical commodity exported from

Southeast Asian to Far East, Middle East and European countries as well as USA [3,4]. Recently, the price over the top grade of agarwood oil has exceeded USD 30.000 per kilogram [5].

The ecological niche of *Aquilaria filaria* is in the low land forests of the island of Celebes, Moluccas and West Papua, near the eastern region of Indonesia. *A. filaria* is one of the major resources of agarwood exported from Indonesia along with *A. malaccensis* which is distributed in the western region [6]. In fact, Indonesia is the largest exporting countries of agarwood, accounting for 80% of the global supply [1]. However, the tendency of agarwood export quota from *A. filaria* is on the decline from 250 ton in 1999 to 60 ton in 2008 because of the reduction of its population in the natural habitat [6,7]. One reason for the rapid depletion of *A. filaria* in nature is the excessive logging of the tree from the past to harvest the oleoresin. Long maturation time is necessary for the formation of refined agarwood in nature. High grade agarwood is likely to be formed in about 50 years old trees [8]. Therefore, the Government of Indonesia has endorsed the incorporation of *A. filaria* as vulnerable species and it was included Appendix II of CITES [9].

As included in Appendix II of CITES, *in situ* and *ex situ* preservation of *A. filaria* has become increasingly relevant. However, plant preservation via seed propagation stands face problems of seasonal production, recalcitrant seeds or short time of viability, seed deterioration and pathogen infected seeds [10,11]. Biotechnological approaches by plant tissue cultures offer an excellent technique for large-scale regeneration within a short period of time using small pieces of plant (explant), which cannot be obtained by seed propagation [12]. In addition, plant in vitro cultures are beneficial for reintroduction, study, demonstration and commercial application [13].

Su and Zhang [14] reveal that phytohormone is the main regulator of plant regeneration. Of the two methods of *in vitro* plant regeneration, direct regeneration is when organ regenerated directly from plant tissue while indirect regeneration is organogenesis from induced plant callus. Plant callus, meristematic cells similar to the root tip, can be induced from explants of basal or aerial organ of the plant [15]. Regeneration via callus culture is also important for further biotechnological applications.

Until now, there is no available information on *in vitro* propagation of *A. filaria*, nor indirect shoot and root regeneration from callus cultures of this agarwood producing species. This research aims to study regeneration of shoot and root under *in vitro* cultures of *A. filaria*.

2. Materials and Methods

2.1 Plant Materials

Sterile *in vitro* seedlings of *Aquilaria filaria* were obtained from BPPT (Badan Pengkajian dan Penerapan Teknologi), Serpong, Indonesia. *A. filaria* mature fruit, yellowish color, were surface sterilized with sodium hypochlorite (NaOCl) 3% and rinsed with sterile water three times. The fruits were opened and five seeds were transferred into 25 ml Murashige and Skoog (MS) agar medium without additional phytohormone (MS-0). *In vitro* seed cultures were incubated at 27°C under continuous light until the plantlet had 3 to 5 leaves. Plantlet leaves were used as plant explants material for *in vitro Aquilaria filaria* callus, shoot and root induction.

Callus Induction

Induction of callus from *A. filaria* was aimed to produce callus for vegetative regeneration of *A. filaria* shoots or roots. Callus regeneration was initiated from approximately 0.5 cm^2 of cut-leaf materials. The leaves of explants were overlaid in 90 mm petridishes containing 25 ml MS agar medium supplemented by 20 g/l of sucrose and phytohormone auxin, cytokinin or combination of both. Auxin type used was indole-3-butyric acid (IBA), α-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) in the range of concentration 0.2 to 2 mg/l. Cytokinin types used were 6-benzylaminopurine (BAP) and kinetin in concentration of 0.5 to 1 mg/l. Each petridish contained five explant leaves. Control treatment was made without supplementation of phytohormone in MS medium. All treatments were made in triplicates. The plant cultures were incubated for four weeks at 27°C in dark.

Control cultures were made in medium without additional phytohormones. Fresh callus were weighted after harvesting to know the best medium for callus induction.

Shoot Regeneration from Callus Cultures

This experiment was aimed to induce shoot from friable callus of *A. filaria in vitro* cultures. Callus was prepared from 0.5 cm^2 of leaf explants as explained in callus induction section. Callus from leaf explants were cultured in MS medium supplemented with 20 g/l of sucrose and single phytohormone cytokinin, BAP (0.5 mg/l) or a combination of auxin, IBA or NAA (0.2 – 2 mg/l). The cultures were incubated without light at 27°C for 4 weeks for callus induction followed by 4 weeks cultivation in continuous light at similar temperature for shoot induction. Control cultures were made in medium without additional phytohormones. Then, the regeneration of shoot from callus was observed.

Root Regeneration from Shoot Cultures

Root induction was done from *A. filaria* shoot to have complete *in vitro* plant seedling which was ready for acclimatization. Approximately, 3 to 4 cm of in vitro *A. filaria* shoot was used as explant materials. In this experiment, single phytohormone NAA and IBA in range concentration of 0.2 to 2 mg/l were used to induce root formation. The explants were cultivated for 4 weeks in growth chamber at 27°C with continuous illumination and then the roots formed were evaluated. Control cultures were made in medium without additional phytohormones.

2.2 Statistical Analysis

All of the experiments were made in triplicate. Data from the experiments were analyzed by oneway ANOVA followed by LSD test at *p-value* ≤ 0.05 to determine the statistical significance of the differences between treatments. All analyses were performed using Microsoft Excel and IBM SPSS Version 16.0 for Windows.

3. Results

3.1 Callus Induction

Calli grown from *A. filaria* leaves explants were cultivated in dark. Calli observed started to emerge within one week of cultivation. The growth of callus was observed in all treatments applied, either in medium with single auxin, cytokinin or combination of both (Figure 1A). However, types, combination and concentration of phytohormones added into the culture medium affected callus proliferation. After four weeks of cultivation, there are differences in callus fresh weight obtained from various treatments applied (Figure. 1B).

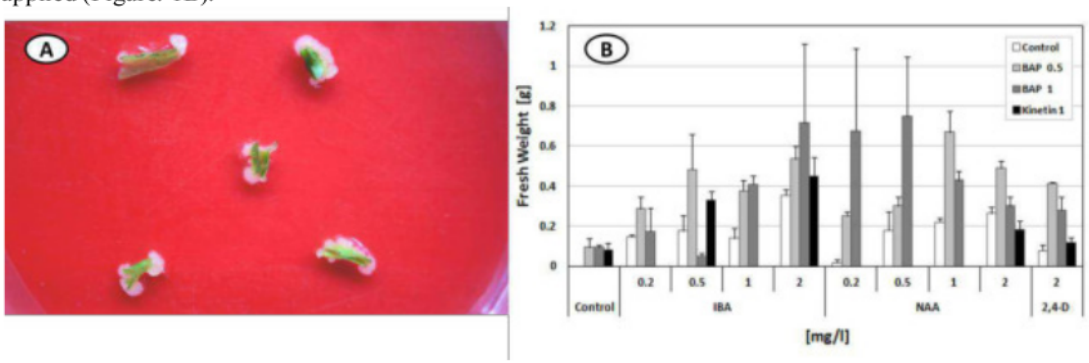

Figure 1. (A): Calli grow from *A. filaria* leaves explants cultivated in medium containing phytohormones, auxin and cytokinin. (B): Fresh weight of callus obtained from 4 weeks cultivation of *A. filaria* leaves explants in MS medium supplemented by phytohormone auxin and cytokinin.

In general, application of single auxin or cytokinin induced less of fresh callus weight compared to combination of both. Auxin types IBA and NAA induced higher callus proliferation than 2,4-D. In addition, cytokinin BAP performed better than Kinetin. Moreover, the highest fresh weight of callus was obtained from leaf explants grown in medium supplemented by combination of auxin IBA or NAA with cytokinin BAP. IBA induced more calli in higher concentration (2 mg/l), while NAA was effective in lower concentration (0.2 – 1 mg/l). In addition, combination of IBA or NAA with 0.5 to 1 mg/l of BAP promoted more callus growth. Up to 4 weeks of cultivation, the amount of fresh callus weight obtained from combinations of NAA 0.5 mg/l + 1 mg/l BAP, IBA 2 mg/l + mg/l BAP, NAA 0.2 mg/l + 1 mg/l BAP and NAA 1 mg/l + 0.5 mg/l BAP were 0.75g, 0.72g, 0.68g and 0.67g respectively. Callus induction response was 100% in almost all treatments applied.

Shoot and Root Regeneration

The successful mass propagation by plant tissue culture rely on the capability of plant callus to differentiate into shoot and root. Four week-old dark-cultivated leaf explants with growing callus were subjected to illumination for stimulation of shoot regeneration. Leaf callus cultivated in lower ratio of auxin to cytokinin, from white callus in dark cultivation developed to become green under light cultivation (Figure 2A). Callus in higher ratio of auxin to cytokinin in the beginning of culture under illumination turned from white callus to become yellow (Figure 2B), however gradually changed to become green. After 6 weeks of cultivation under illumination, development of nodular embryonic structures (NES) which served as shoot primordia from leaves callus was observed. NES were observed in treatment medium of single cytokinin, BAP (0.5 mg/l) and combination with auxin, IBA or NAA (0.2 – 2 mg/l). In general, IBA or NAA had no differential effects in the number of NES observed. However, the concentration of each phytohormone influenced regeneration of NES from callus. The highest number NES was observed in medium containing BAP 0.5mg/l + 0.5mg/l NAA and BAP 0.5mg/l + 0.2mg/l BAP, with average number of NES per explant of 6.4 and 5.8, respectively. Two types of NES regenerated from the leaf callus: translucent node type (Figure 2C) and compact node type (Figure 2D).

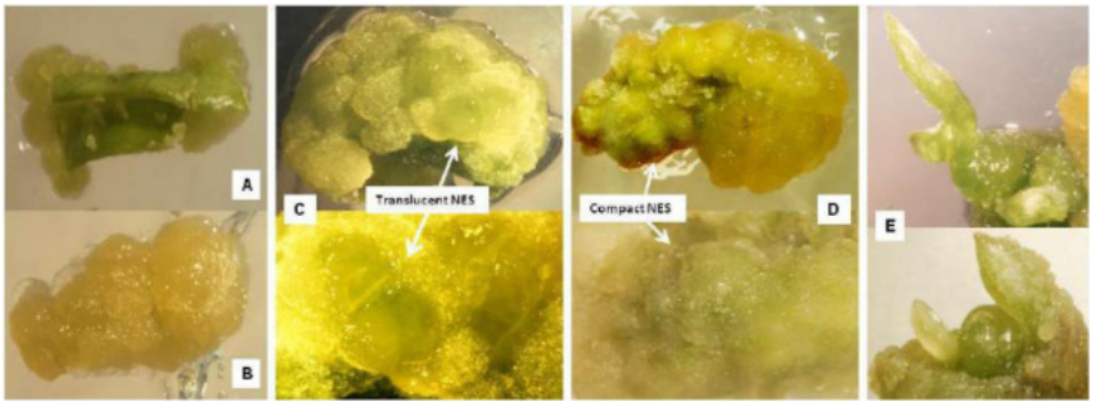

Figure 2. Regeneration of *A. filaria* callus from leaves explants. (A): Higher cytokinin than auxin, callus turn green. (B): Cytokinin lower than auxin callus turns golden yellow. (C): Translucent type of Nodular Embryonic Structure (NES). (D): Compact type of NES. (E): Shoot bud regenerate from *A. filaria* callus.

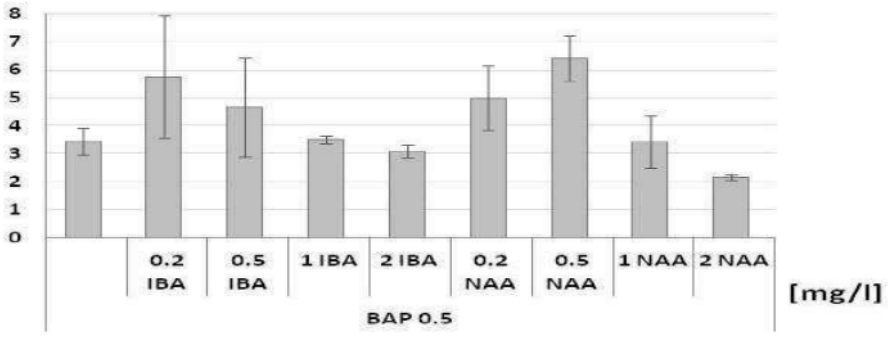

Figure 3. Number of Nodular Embryonic Structure (NES) per explants regenerate from callus derived leaf after 6 weeks of cultivation under illumination.

In order to have complete plantlet organogenesis, shoot explants of *A. filaria* were stimulated for rooting (Figure 4). It can be seen from the graph (Figure 4A) that NAA or IBA were effective in inducing rooting of *A. filaria* shoot explants. However, root regeneration from shoot cultures was most effective at higher concentrations of NAA. After 4 weeks of cultivation, application of 2 mg/l of NAA induced on average more than 2.5 healthy roots per explant with a response frequency of 100%.

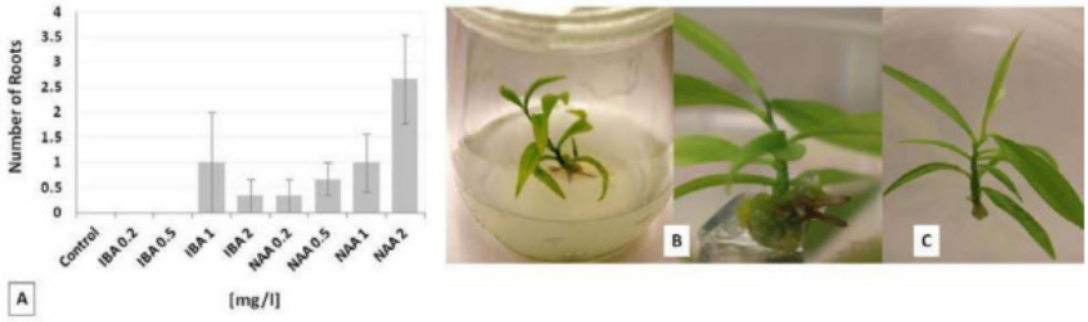

Figure 4. Root regeneration from shoot cultures of *A. filaria*. (A): The number of root emerge from shoot cultures of *A. filaria* after 4 weeks of cultivation in MS medium containing auxin (NAA or IBA) 0.5 – 2 mg/l. (B): Roots grow from shoot explants of *A. filaria* after 4 weeks of cultivation in MS medium containing auxin (NAA or IBA) 0.5 – 2 mg/l. (C): No root grows from control treatment without addition of phytohormone (control treatment).

4. Discussion

4.1 Callus Induction

Some authors have illustrated the method of callus induction from leaves or nodal explants of some *Aquilaria* species, those are *A. sinensis* from Taiwan, *A. crassna* from Thailand and Vietnam, and *A. malacensis* Lam. and *A. agallocha* Roxb. from India by phytohormone auxin, 2,4-D or NAA, and cytokinin BAP. Moreover, it was reported that Murashige and Skoog (MS) medium was better than Woody Plant Medium (WPM) for cultivation of callus [16,17,18,19]. Consistent with these studies, calli proliferated from leaf explants of *A. filaria* in all hormone-treatment medium (Figure 1). Additionally, high fresh weight of callus was obtained from explants cultivated in MS medium supplied by auxin IBA (2 mg/l) or NAA (0.2 – 1 mg/l) combined with cytokinin BAP (0.5 – 1 mg/l).

In vitro callus proliferation is controlled by auxin-rich callus induction media (CIM) in synergistic effect with an addition of cytokinin [20]. In regard to this study, application of auxin and cytokinin in combination into growth medium produced callus more than that of single phytohormone. In addition, *A. filaria* callus emerged in higher amounts in sole medium of auxin compared to medium with cytokinin individually. High callus fresh weight obtained in this study by ratio of auxin IBA (2 mg/l) to cytokinin BAP (1 mg/l) is also in agreement to previous studies. Even though in application of lower auxin to cytokinin, NAA (0.2 – 0.5 mg/l) to BAP (1 mg/l) ratio, high callus fresh weight was achieved. The reasons might be due to the influence of endogenous phytohormone within explants, type of auxin and cytokinin and source of explants [21, 22, 23].

4.2 Shoot and Root Regeneration in Shoot Cultures

In general, cytokinin-rich shoot induction medium (SIM) is used for shoot induction [24]. Consistent with this research, medium with single BAP (0.5 mg/l) and combination with less auxin NAA or IBA (0.2 mg/l) promoted development of shoot primordia from leaf callus. Debnath *et al.* [25] also found that 0.2 mg/l BAP induced 18-fold shoot buds from nodal segment and shoot tip of *A. agallocha*.

In high cytokinin to auxin ratio medium, white callus turned green under light cultivation, whilst in higher ratio of auxin than cytokinin (2 : 1), yellow callus was observed, which finally become necrotic. Kobayashi *et al.* [26] by studying detached roots of *Arabidopsis* explained that cytokinin has positive regulation of chlorophyll biosynthesis and chloroplast biogenesis conversely to auxin, which promotes negative regulation. However, beside its negative effect, certain amount of auxin is needed for shoot regeneration. Furthermore, it has been proved that interaction between auxin and cytokinin occur during *de novo* shoot regeneration [14, 27, 28].

Root induction from shoot cultures was achieved in medium containing single auxin. Unlike shoot regeneration in callus culture, root regeneration from shoot cultures of *A. filaria* was more effectively induced by NAA (0.2 – 2 mg/l), compared to IBA (1 – 2 mg/l). He *et al.* [29] shows similar result of rooting stimulation from shoot of *A. agallocha* by NAA. However, stimulation of root regeneration from *Aquilaria crassna* shoot culture showed IBA more effective in root regeneration than NAA, while no root growth took place from *A. malacensis* shoot cultures [30]. It is suggested that different species of *Aquilaria* may give specific responses to certain auxin types.

5. Conclusion

In situ and *ex situ* preservation of *A. filaria* requires appropriate method for propagation. The results from this research provide basic method for *in vitro* micropropagation of *A. filaria*. *In vitro* regeneration of A. *filaria* can be done from the induction of callus culture followed by regeneration of shoot from callus and root from shoot culture. Calli was induced from leaf explants in auxin-rich medium combined with low or equal concentration of cytokinin. Conversely, medium for shoot induction is sole cytokinin or combined with lower concentrations of auxin. Rooting from shoot culture was achieved only in medium containing single auxin.

References

[1] Jensen. 2009. Valuation of Non-Timber Forest Products Value Chains. Forest Policy and Economics 11:34–41.

[2] Akter, S., Islam, M.T., Zulkefeli, M., Khan, M.S.I. 2013. Agarwood Production - A Multidisciplinary Field to Be Explored in Bangladesh. International Journal of Pharmaceutical and Life Sciences. Volume 2, 1: 22-32.

[3] Antonopoulou, M., Compton, J., Perry, L.S., Al-Mubarak, R., 2010. The Trade and Use of Agarwood (Oudh) in the United Arab Emirates. A report Commissioned by The CITES Secretariat. TRAFFIC Southeast Asia. 56pp.

[4] Wyn, L.T. and Anak, N.A., 2010. Wood for the trees: A Review of The Agarwood (Gaharu) Trade In Malaysia. A report Commissioned by The CITES Secretariat. TRAFFIC Southeast Asia. 117pp.
[5] Government of India Ministry of Environment and Forests, 2014. Draft Policy for Sustainable Utilisation of Agar Wood. New Delhi.
[6] Patmomihardjo, T. and Semiadi, G. 2008. Case Study On NDF of Agarwood (*Aquilaria spp.* and Gyrinops spp.) in Indonesia. Presented at International Expert Workshop on CITES Non-Detriment Findings. Cancun, Quintana Roo, Mexico. 17 – 22 November 2008.
[7] Samedi. 2006. Proceeding CITES Agarwood Experts Group Meeting. Kuala Lumpur, Malaysia, 14-17 November 2006.
[8] Damania, A. B. 2001. Agarwood: The History of the Prized Incense Tree. Asian Agri-History Vol. 5, No.4: 321-330.
[9] CITES. 2014. Online accessed: http://www.cites.org.
[10] Johnson, K. A. 2002. In Vitro Conservation Including Rare and Endangered Plants, Heritage Plants and Important Agricultural Plants. *In* The Importance of Plant Tissue Culture and Biotechnology in Plant Sciences A. Taji and R. Williams (eds). University of New England Publications Unit. Australia
[11] Belokurova, V.B. 2010, Methods of Biotechnology in System of Efforts Aimed at Plant Biodiversity Preservation (Review). Cytology and Genetics. Vol. 44, No. 3, pp. 174–185.
[12] Rai, M. K. 2010. Review: Biotechnological Strategies for Conservation of Rare and Endangered Medicinal Plants. Biodiversitas. Volume 11, Number 3, Pages: 157-166.
[13] Pence, V.C. 2011. Evaluating Costs for The In Vitro Propagation And Preservation Of Endangered Plants. In Vitro Cell.Dev.Biol.—Plant (2011) 47:176–187.
[14] Su, Y. H. and Zhang, X. S., 2014. The Hormonal Control of Regeneration in Plants. Current Topics in Developmental Biology. Volume 108. Pp: 35-69.
[15] Sugimoto, K., Jiao, Y., and Meyerowitz, E.M. 2010. Arabidopsis Regeneration from Multiple Tissues Occurs via a Root Development Pathway. Developmental Cell 18: 463–471.
[16] Okudera, Y. and Ito, M. 2009. Production of Agarwood Fragrant Constituent in Aquilaria Calli and Cells Suspension Cultures. Plant Biotechnology 26: 307-315.
[17] Saika, M., Shrivastava, K. and Singh, S.S. 2012. An Efficient Protocol For Callus Induction In *Aquilaria malaccensis* Lam. Using Leaf Explants at Varied Concentrations of Sucrose. International Journal of Plant Research 2012, 2(6): 188-194.
[18] Saika, M., Shrivastava, K. and Singh, S.S. 2013. Effect of Culture Media and Growth Hormones on Callus Induction in *Aquilaria malaccensis* Lam., a Medicinally and Commercially Important Tree Specias of North East India. Asian Journal of Biological Sciences 6 (2): 96-105.
[19] Debnath, B., 2013. In vitro response, growth and maintenance of callus of aquilaria agallocha Roxb. (Thymelaeaceae). Bioscience Discovery: An International Journal of Life Sciences. Vol. 4 Issue 2, p155.
[20] Ikeuchi, M., Sugimoto, K. and Iwase, A. 2013. Plant Callus: Mechanisms of Induction and Repression. The Plant Cell, Vol. 25: 3159–3173.
[21] Jimenez, V. M. and Bangerth, F. 2001. Endogenous hormone concentrations and embryogenic callus development in wheat. *Plant Cell, Tissue and Organ Culture* 67: 37–46.
[22] Yancheva, S. D., Golubowicz, S., Fisher, E., Lev-Yadun, S., Flaishman, M.A. 2003. Auxin Type And Timing Of Application Determine The Activation Of The Developmental Program During In Vitro Organogenesis In Apple. Plant Science 165: 299-309.

[23] Welander, M. and Snygg, J.O. 1987. Effect of Applied and Endogenous Auxin on Callus and Root Formation of *In Vitro* Shoots of the Apple Rootstocks M26 and A2. *Annals of Botany* 59, 439-443, 1987.

[24] Motte, H., Vereecke, D., Geelen, D. and Werbrouck, S. 2014. The Molecular Path to In Vitro Shoot Regeneration. Biotechnology Advances 32: 107–121.

[25] Debnath, B., Sinha, S. and Sinha, R. K. 2013. In Vitro Multiplication Of Shoot Buds Of Aquilaria Agallocha Roxb. (Thymelaeaceae). CIBTech Journal of Biotechnology Vol. 2 (2), pp.7-10.

[26] Kobayashi K., Baba, S., Obayashi, T., Sato, M., Toyooka, K., Keraenen, M., Aro, EM., Fukaki, H., Ohta, H., Sugimoto. K., and Masuda, T. 2012. Regulation of Root Greening by Light and Auxin/Cytokinin Signaling in Arabidopsis. The Plant Cell, Vol. 24: 1081–1095.

[27] Kakani, A. and Peng, Z. 2011. ARR5 and ARR6 Mediate Tissue Specific Cross-talk between Auxin and Cytokinin in *Arabidopsis*. American Journal of Plant Sciences 2: 549-553.

[28] Cheng, Z.J., Wang, L., Sun, W., Zhang, Y., Zhou, C., Su, Y.H., Wei Li, Sun, T.T., Zhao X.Y., Li, X.G., Cheng, Y., Zhao, Y., Xie, Q., and Zhang, X.S. 2013. Pattern of Auxin and Cytokinin Responses for Shoot Meristem Induction Results from the Regulation of Cytokinin Biosynthesis by AUXIN RESPONSE FACTOR3. Plant Physiology, Vol. 161, pp. 240–251.

[29] He, M.L. Qi, S-Y & Hu, L-J. 2005. Rapid in vitro propagation of medicinally important *Aquilaria agallocha*. Journal of Zhejiang University Science 8: 849-852.

[30] Tiengtum, P. 1995. In Vitro Culture of Agarwood Trees (*Aquilaria* spp.). Kasetsart Univ., Bangkok (Thailand). Graduate School.81 pp.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14

PAGE 15