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Induction of shoot and root from nodes of *Kadsura heteroclita*

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Abstract Results described on shoot and root induction from node of medicinal plant *Kadsura heteroclita*. The sterilized nodes were used as explants and cultured on solid synthetic medium, Murashige and Skoog (MS) medium supplemented with single or combined Plant Growth Regulators (PGRs) of 6-benzylaminopurine (BAP), *meta*-Topolin (*m*T), Indole-3-butyric acid (IBA),1-Naphthaleneacetic acid (NAA) and Gibberellin (GA_3) were applied. The best result for shoot induction was shown on the medium supplemented with 0.5 mg/L of *m*T after 8 weeks. Transferred shoot to MS medium supplemented with combination PGRs of 0.5 mg/L of *m*T and 0.5, 1.0, 1.5 and 2.0 mg/L of IBA NAA or GA_3 after 16 weeks revealed that the explant were developed to multiple shoots which averaged number of shoot were 3 shoots/explant. The roots were not perfectly exhibited like sore on medium supplemented with 0.5 mg/L of *m*T and 1.5 mg/L of GA_3. The high concentration of Auxin inhibited growth roots. The root induction was done by cutting the shoot and cultured on MS medium supplemented with combination PGRs of 0.5 mg/L of *m*T and 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L of IBA after 4 weeks found that root length was approximately 2 mm on 0.5 mg/L of *m*T and 0.05 mg/L of IBA.

Keyword: Multiple shoot, Plant regeneration, *Kadsura heteroclita*

Introduction

The genus of Schisandra, Schisandraceae are used in traditional Chinese and Thai medicine also while the western medicine was used for therapeutic methods (Hancke et al., 1999) since the past the plants have been used for long time to activate blood and resolve stasis, active qi circulation to relieve pain, dispel wind and eliminate dampness (Liu *et al.*, 2012). There for Schisandra is an important medicinal plant, but no more report of it by tissue culture and were not had report of morphogenic capabilities *in vitro* too (Smiskova *et al.*, 2005). This family is supported with three genera, *Schisandra* Michx., *Kadsura* Kaempf. Ex Juss. and *Illicium* L. (Li, 2003). There are 25 species in *Schisandra*, 22 in *Kadsura* 16 species were found in Asia, and 8 species of them existed in

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the south and southeast of China (Wu *et al.,* 2008) (Saunders, 1998) and 42 in *Illicium* (Smith AC, 1947) were widely to distributed in East and Southeast Asia and some time was found in North America (Krussmann, 1978). They are primitive dicotyledon, vines glabrous woody with undifferentiated embryo, seed germination need to had cyclic stratification to break dormancy, and ratio of germination is low (Saunders, 2000). In the past of Thailand *Kadsura* is 1 of 9 conservation plants were precious and become extinct under the studied of Plant Genetic Conservation Project Under The Royal initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG), this organization want to protection, planting, preservation, conservation and utilization of plant genetic to develop the personal and plant genetics resources for the keeping of plant varieties, and for the development to be helpful for the farmer and business section of the country (Plant Genetic Conservation Project Office, 1996). The aim of this experiments were induce shoot and root induction from node of *Kadsura heteroclite* being the choices to leave out the problem of low germination rate and have been to origin of biochemical, molecular or breeding and biotechnology in *Kadsura* on the future.

Materials and methods

Plant materials

Plant Genetic Conservation Project Under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG) at Lampang province, Thailand under RSPG at Royal Chitralada Projects Bangkok, Thailand offered the young plants of *Kadsura heteroclite*, nodes was an explant of this experiment.

Sterilization explant

Selected young node from plant was cut and washed by water and detergents to remove the dust and made it clean on the surface, sterilized by 1 min by rinsing in 70% (V/V) ethanol, shook on 250 rpm for 30 min of mix solution of 0.2% (W/V) mercuric chloride ($HgCl_2$), 0.1% (V/V) Cefotaxime (Nida Pharma incorporation, Thailand), 0.1% (V/V) Antibiotic Antimycotic Solution [100X] (Sigma) and 0.1% (V/V). Preservative for plant tissue culture media active (PPM) was then shook on 250 rpm for 10 min which in sterile distilled water with 0.1% (V/V), Cefotaxime, 0.1% (V/V), antibiotic Antimycotic solution [100X]. The 0.1% (V/V) PPM was moved for shaking on 250 rpm for 10 min in sterilized distilled water with 0.1% (V/V). Antibiotic antimycotic solution [100X] and 0.1% (V/V) PPM were finally washed in

sterile distilled water in 250 rpm for 5 min, and surface dried with sterilized absorbent paper before culture on medium.

Culture medium and conditions

Solid synthetic Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) (Phytotech) supplemented with 30 g/L sucrose and 2.6 g/L Gellan Gum powder (Phytotech), pH was calibrated to 5.8 for shoot induction which were combined with 0.5, 1.0, 2.0, 3.0 and 5.0 mg/L of 6-benzylaminopurine (BAP) or *meta*-Topolin (*m*T) was sterilized in autoclaved at 121 ℃ for 15 min. The sterilized nodes were upper and lower surface cut before placed on medium. The temperature was 25 ℃ at 8 h dark and 16 h light were operated and recorded every 4 weeks. The it was sub-cultured after 8 weeks by transferred the shoots to MS medium supplemented with combination PGRs of 0.5 mg/L of *m*T and 0.5, 1.0, 1.5 and 2.0 mg/L of Indole-3-butyric acid (IBA), 1-Naphthaleneacetic acid (NAA) and Gibberellin (GA_3) to multiple the shoots and sub-cultured every 4 weeks. The roots induction was done by cutting the multiple shoots and cultured on MS medium supplemented with combination PGRs of 0.5 mg/L of *m*T and 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L of IBA, then sub-cultured every 4 weeks.

$$\text{Shoot induction rate (\%)} = \frac{\text{No. of explants generating shoot}}{\text{No. of total explants}} \times 100$$

Results

Result showed that the concentrations of PGRs in Cytokine's group made the highest percentage of shoots induction which treated with 0.5, 1.0, 2.0, 3.0 and 5.0 mg/L of BAP or *m*T for 4 weeks of MS medium supplemented with 0.5 mg/L *m*T that gave the best result (33.33%) (Fig 1) after 8 weeks transferred shoot to MS medium supplemented with combination PGRs of 0.5 mg/L of *m*T and 0.5, 1.0, 1.5 and 2.0 mg/L of IBA NAA or GA_3. The explant were developed to multiple shoots after 16 weeks that revealed the highest percentage of multiple shoots (50.00%) and averaged 3 shoots/explant at 1.5 mg/L of GA_3.(Fig 2, Table 1). The high concentration of Auxin inhibited rootsgrowth. The roots were induced by cultured on MS medium supplemented with combination PGRs of 0.5 mg/L of *m*T and 0.01, 0.02, 0.03, 0.04. Applying 0.05 mg/L of IBA after 4 weeks found that root length averaged approximately 2 mm in 0.5 mg/L of *m*T and 0.05 mg/L of IBA. (Fig.3, Table 1).

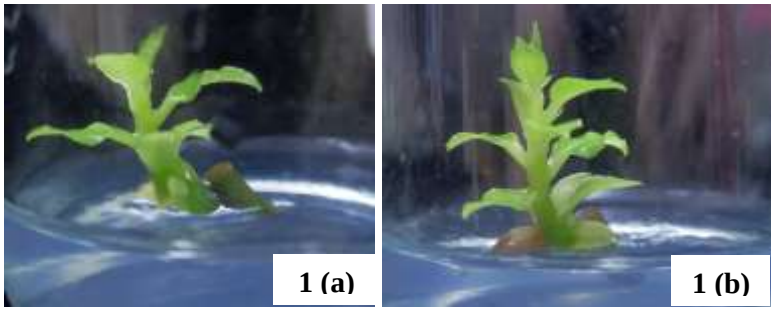

Figure 1. (a) After 4 weeks cultured node on MS medium combined with 0.5 mg/L of *m*T and (b) developed of node after 8 weeks before transferred to multiple shoot's medium

Figure 2. At 18 weeks of multiple shoot on MS medium combined with 0.5 mg/L of *m*T and 1.5 mg/L of GA_3 was gave the average number of shoot are 3 shoots per explant

Figure 3. Cut the shoot from last section cultured on MS medium supplemented with 0.5 mg/L of *m*T and 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L of IBA for root induction

Table 1. Effect of PGRs on the shoot and multiple shoots from nodes of *Kadsura heteroclita*

PGRs (mg/L)		Shoot induction (%)	Multiple shoot (%, average number of shoots)
BAP 0.5		2 (16.67)	
1		-	
2		-	
3		1 (8.33)	
5		-	
*m*T 0.5		4 (33.33)	
1		1 (8.33)	
2		3 (25.00)	
3		2 (16.67)	
5		1 (8.33)	
BAP 0.5			
Combined with	IBA 0.5		-
	1		-
	1.5		-
	2		-
	NAA 0.5		1 (25.00), 1
	1		1 (25.00), 1
	1.5		-
	2		-
	GA_3 0.5		1 (25.00), 1
	1		-
	1.5		2 (50.00), 3
	2		0

[1]/The number of total explants in shoot induction treatment are 12 explants and multiple shoots are 4 shoots.

Discussion

The study were investigated on shoot and multiple shoots induction from node of *Kadsura heteroclite* by culturing on MS medium appended in PGRs, cytokinin. BAP and *m*T, gave a little difference in molecular structure that revealed the large effect on plant responses, originally isolated from poplar (Strnad *et al*., 1997) and closely related to BAP. The optimum percentage (33.33%) of shoot induction was on 0.5 mg/L *m*T. The application of *m*T and its derivatives resulted to control hyperhydricity, shoot-tip necrosis and delay senescence in various plant species (Mala *et al.*, 2013). Accordingly tissue culture of black pepper (*Piper Nigrum L.*) in Pakistan, the shoot regeneration was excellent on MS medium added with 0.5 mg/L of BAP (Hussani *et al*., 2011). The multiple shoots was induced on MS medium combined with 0.5 mg/L with 0.5, 1.0, 1.5 and 2.0 mg/L of IBA, NAA or GA_3. The high concentration of Auxin was inhibited the root growth at highest percentage (50.00%). The previous result was different form due to develop in other PGRs. GA_3 is widely used to break seed dormancy of various plant species. The multiple shoots were cultured on MS medium combined with 1 mg/L of TDZ or 2 mg/L of Zeatin or 2,4-D (Sun *et al.*, 2013). The roots were induced by culturing on MS medium supplemented with combination PGRs of 0.5 mg/L of

*m*T and 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L of IBA found that root length was 2 mm in 0.5 mg/L of *m*T and 0.05 mg/L of IBA that similar report by Smiskova *et al.* (2005) who used a WV5 medium combined with 1.5% sucrose, 0.1% activated carbon and 0.05 μM of IBA.

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References

Hancke, J. L., Burgos, R. A. and Ahumada, F. (1999). Schisandra chinensis (Turcz.) baill. Fitoterapia. 70:451-471.

Hussain, A., Naz, S., Nazir, H. and Shinwari, Z. K. (2011). Tissue culture of black pepper (*Piper nigrum* L.) in Pakistan. Pakistan Journal of Botany. 43:1069-1078.

Ii, A. (2003). An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society. 141:399-436.

Krussmann, G. (1978). Manual of cultivated broad - leaved trees and shrubs. S.ed. PRU-Z Portland: Timber press.

Liu, H. Qi, Y. Xu, L. Peng, Y. Zhang, B. and Xiao, P. (2012). Ethno-pharmacological investigation of Schisandraceae plants in China. China Journal Chinese Materia Medica. 37:1353-1359.

Malá, J., Máchová, P., Cvrčková, H., Karady, M., Novák, O., Mikulík, J., and Doležal, K. (2013). The role of cytokinins during micropropagation of wych elm. Biologia plantarum. 57:174-178.

Plant Genetic Conservation Project Office (1996). An origin and activities of projects. Received 30 September 2018. http://www.rspg.or.th/rspg_eng/origin.htm.

Saunders, R. M. K. (1998). Monograph of Kadsura (Schisandraceae). Systematic botany monographs. 1-106.

Saunders, R. M. K. (2000). Monograph of Schisandra (Schisandraceae). Systematic botany monographs. 58:1-146.

Smiskova, A. Vlasinova, H. and Havel, L. (2005). Somatic embryogenesis from zygotic embryos of Schisandra chinensis. Biologia Plantarium. 49:451-454.

Smith, A. C. (1947). The families Illiciaceae and Schisandraceae. Sargentia. 7:1-224.

Strnad, M., Hanuš, J., Vaněk, T., Kamínek, M., Ballantine, J. A., Fussell, B., and Hanke, D. E. (1997). Meta-topolin, a highly active aromatic cytokinin from poplar leaves (Populus× canadensis Moench., cv. Robusta). Phytochemistry. 45:213-218.

Sun, D., Lang, W. X., Li, H. B., Guo, X. W., Lian, M. L. and Piao, Z. Y. (2013). An improve method for somatic embryogenesis of Schisandra chinensis (Turcz.) Baillon. Pakistan Journal of Biological Sciences. 16:127-134.

Wu, Z. Y., Raven, P. H. and Hong, D. Y. (2008). Science Press, Beijing.for somatic embryogenesis of Schisandra chinensis (Turcz.) Baillon. Pakistan Journal of Biology Science. 16:127-134.

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