Callogenesis and Plant Regeneration of *Jasminum sambac* L. (Jasmine)

Reza Farzinebrahimi [1], Kamaludin Rashid [2], Rosna Mat Taha [3] and Khairul Anwar Ishak [4]

***Abstract*—** In the present study, regeneration of *J. sambac* var. Maid of Orleans through direct and indirect organogenesis has been investigated. In direct organogenesis, shoot development from young stems (each contained one nodal part) cultured in MS media supplemented with a combination of BAP (3.0 mg L^{-1}) and NAA (1 mg L^{-1}) showed 20% shoot regeneration.

For indirect organogenesis, among different explants only young stems inoculated in MS media supplemented with 2, 4-D (0.5 mg L^{-1}) yielded callus. In order to identify embryogenic cells in callus, double staining test was carried out, and embryogenic callus was detected in all formed callus, in MS media supplemented with different concentrations of 2, 4-D. Further studies on cell suspension culture by using liquid MS media supplemented with various concentrations of 2, 4-D (0.5 mg^{-1}) has also been done. Different colors of callus were obtained in the present study including green, yellowish and whitish. However, the entire callus did not show any development of organogenesis.

***Keywords*—** Callogenesis, Regeneration, *Jasminum Sambac*, Jasmine.

I. Introduction

Plant tissue culture offers an alternative method for the conservation of rare, valuable and endangered plant genotypes. This technique can successfully preserve the plant species, which do not produce seeds or some of seeds that cannot be stored for an extended period. However, *in vitro* methods can be applied for preservation of vegetative tissues, to keep the genetic background [1].

Jasminum sambac L. (family: Oleaceae) is a genus containing approximately 600 species of small trees and shrubs.

Reza Farzinebrahimi[1] is with the [1] Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, 50603 Malaysia (corresponding author's phone: 0060173030586; e-mail: rfebrahimi@siswa.um.edu.my).

Kamaludin Rashid[2] is with Biology Division, Centre For Foundation Studies, University of Malaya, Kuala Lumpur, 50603 Malaysia (e-mail: kamalrashid@um.edu.my).

Rosna Mat Taha[3] is with the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, 50603 Malaysia (e-mail: rosna@um.edu.my).

Khairul Anwar Ishak[4] was graduated from University of Malaya, Kuala Lumpur, 50603 Malaysia (e-mail: kamalrashid@um.edu.my).

Based on old Chinese books, the origin of *J. sambac* was from Eastern south and Southeast Asia. The ten species of this genus, including *J. sambac* were cultivated into Arabia and Persia (Iran) in gardens under the common name "sambac" in the 18th century and was introduced to Europe as ornamentals [2]. According to its high medicinal value, *J. sambac* is one of the most cultivated species in many countries in Asia including Thailand. There are plenty of secondary metabolic activities such as anti-bacterial, anti-cancer, anti-tumor, anti-diabetic, anti-acne, anti-oxidant and anti-stress from this plant that had been reported [3]. The phytoconstituents of this plant contain iridoidal glycosides, linalyl 6-O-malonyl-β-D-glucopyranoside, β-primeveroside, 2-phenylethyl β-primeveroside, β-rutinoside, dotriacontanoic acid, dotriacontanol, oleanolic acid, daucosterol, and hesperidin [4]. Furthermore, flower of *J. sambac* displayed the efficacy to suppress puerperal lactation [5] and the essential oil was determined to possess antibacterial activity [6].

There are numerous cultivars of *J. sambac* on the basis form of flower bud, petal shape and number of whorls [3]. The variety, Maid of Orleans is the national flower of Philippine (Sampaguita) and cultivar of *J. sambac* that possesses flowers with a single layer of five or more oval shaped petals [7]. The goal of the present study was to develop an efficient regeneration protocol for direct and indirect organogenesis of *J. sambac* var. 'Maid of Orleans' and to examine callogenesis using various hormones and explants.

II. Materials and Methods

A. Plant Materials

The mother plants (8- month- old) were bought from a nursery (Ipoh-Malaysia). The fresh and young leaves, stems and petioles were surface sterilized by using calcium hypochlorite containing, ethanol and distilled water [8].

Hormone preparation: Based on preliminary *in vitro* hormones optimization and effect of auxin/cytokinin ratio to plant regeneration [9], for callus initiation, high concentration of auxins and low concentration of cytokinins were used. A combination of NAA (α-Naphthalene acetic acid) and BAP (6-Benzylaminopurine) as high cytokinin to auxin (3 mg L^{-1} BAP, 1 mg L^{-1} NAA) and high auxin to cytokinin (3 mg L^{-1} NAA, 1 mg L^{-1} BAP) for direct

http://dx.doi.org/10.15242/IICBE.C814027

organogenesis was applied. To achieve indirect organogenesis 2, 4-D (2, 4 Dichlorophenoxyacetic acid) from 0 to 2 mg L^{-1} with 0.5 increment was used.

B. Media preparation

MS basal media [10] included 30 g sucrose, 5 g L^{-1} gelrite supplemented with two different combinations of NAA/BAP and five different concentrations of 2, 4-D was used as inoculation medium. All media were adjusted to pH 5.8 with 1 N, KOH and autoclaved at 121 °C for 20 min and was dispensed into 60 mm specimen containers under aseptic condition. The sterilized explants were cut and were cultured onto Murashige and Skoog (MS) medium supplemented with various concentrations of hormones with 16 hours light and 8 hours dark conditions in a sterile container.

The 2-month callus formed *in vitro* was tested by double staining method adopted from [11] to obtain embryogenic cells. The callus (20 g) established *in vitro* (MS supplemented with 0.5 mg L^{-1} of 2, 4-D) was weighed out under laminar flow cabinet and was cultured on liquid MS media supplemented with different concentrations of 2, 4-D (0 to 2 mg L^{-1} with 0.5 increment). The cultures were maintained on a horizontal shaker (120 rpm) in a growth chamber at 24°C. All experimental units were monitored every day to record the contamination of explants. The regular subcultures were carried out at the intervals of two weeks into the same media and concentration of the hormone.

C. Statistical analysis

Callus induction experiment was arranged as RCBD (Randomized Completely Block Design) with 10 replications. Mean comparison was performed using Duncan multiple range tests. The data were subjected to normality test using one sample Kolmogorove-Smirnove. Before ANOVA, the percentage data were arcsine transformation to normalize the distribution. All data analysis was done using SPSS.

III. Results

The different types of explants showed various responses to media and hormones. After two weeks, the highest amount (89%) of greenish friable calli was obtained from stem explants cultured on MS medium supplemented with a combination of 3 mg L^{-1} BAP and 1 mg L^{-1} NAA. The compact green-yellowish calli formed from petiole (64%) and leaf (58%) explants on the same concentration of hormones, respectively (Fig. 1).

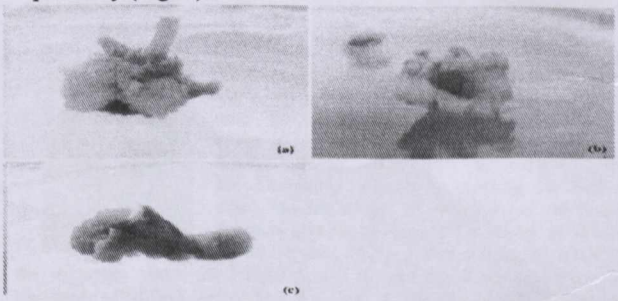

Figure 1: Callus formation from stem (a), petiole (b) and leaf (c) explants of *J.sambac* on MS media supplemented with high ratio of BAP and NAA

Shoot was formed from stem explants cultured on MS media with addition of 3 mg L^{-1} BAP and 1 mg L^{-1} NAA as a combination of high ratio cytokinin to auxin (Fig. 2).

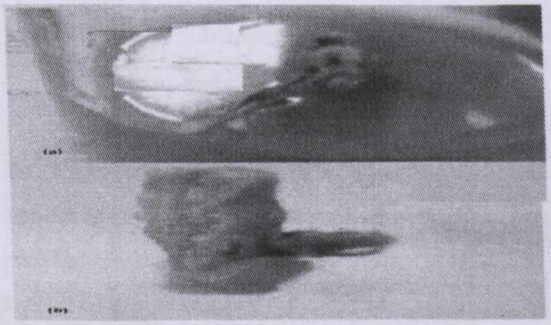

Figure 2: Shoot formation from stem explant of *J. sambac* on MS media supplemented with high ratio of BAP to NAA.

As shown in Fig. 3, calli were formed (94%) and 28% on liquid media supplemented with 0.5 and 2 mg L^{-1} of 2, 4-D, respectively.

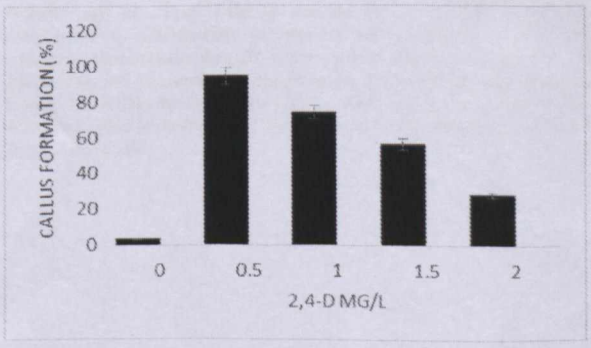

Figure 3: Callus formation of *J. sambac* on MS liquid media and various concentrations of 2, 4-D.

According to double staining test, the embryogenic cells observed in callus formed in MS medium supplemented with auxin.

IV. Discussion

Effects of explants and different ratios of auxin/cytokinin to callus formation was reported by [9, 12], in the present study, stem explant inoculated on MS media with addition of a high ratio of BAP to NAA gave the better response to callus formation compared to other explants and PGR's ratio. However, [13, 14] reported the same results of shoot and root formation when different explants cultured on MS media containing different 2,4-D/Zeatin and IBA/BAP. The double staining test and callus formation in liquid media supported our previous results of embryogenic cells from callogenesis of *G. jasminoides* by 2,4-D [15] and [7].

ACKNOWLEDGMENT

The authors gratefully acknowledge the support of this research by University of Malaya and Bright Sparks Unit of University Malaya, Kuala Lumpur, Malaysia.

References

[1] A. Hussain, Ahmed, Q. I., Nazir, H., and Ullah, I.,"Plant Tissue Culture: Current Status and Opportunities", in *Recent Advances in Plant in vitro Culture*A. LEVA, Place, 2012, pp.

[2] A. K. Singh,"Jasmine", in *Flower Crops: Cultivation and Management*, New India Publishing, 2006, pp. 190-206.

[3] S. Sabharwal, Sudan, S., and Ranjan, V.,"*Jasminum Sambac* Linn (Motia): A Review," *International Journal of Pharmaceutical Research and Bio-Science*.Vol. 2, no. 5, pp. 108-130, 2013.

[4] P. Kunhachan, Banchonglikitkul, C., Kajsongkram, T., Khayungarnnawee, A., and Leelamanit, W.,"Chemical Composition, Toxicity and Vasodilatation Effect of the Flowers Extract of *Jasminum sambac* (L.) Ait. "G. Duke of Tuscany"," *Evidence-Based Complementary and Alternative Medicine*.Vol. 2012, no. pp. 2012.

[5] P. Shrivastav, George, K., Balasubramaniam, N., Jasper, M. P., Thomas, M., and Kanagasabhapathy, A. S.,"Suppression of puerperal lactation using jasmine flowers (*Jasminum sambac*)," *The Australian and New Zealand Journal of Obstetrics and Gynaecology*.Vol. 28, no. 1, pp. 68–71, 1988.

[6] C. C. Rath, Devi, S., Dash, S. K., and Mishra, R.,"Antibacterial potential assessment of Jasmine essential oil against *E. coli*," *Indian Journal of Pharmaceutical Sciences*.Vol. 70, no. 2, pp. 238-241, 2008.

[7] C. Fernando, Sanchez, J., Dante, S., and Caroline, P. K.,"Production Management Practices of Jasmine (*Jasminum sambac* [L.] Aiton) in The Philippines," *International society for Southeast Asian agricultural sciences*.Vol. 16, no. 2, pp. 126-136, 2010.

[8] R. Farzinebrahimi, Taha, R. M., and Fadaie Nasab, M.,"*In Vitro* Plant Regeneration, Antioxidant and Antibacterial Studies on Broccoli, *Brassica oleracea* var. italica," *Pakistan Journal of Botany*.Vol. 44, no. 6, pp. 2117-2122, 2012.

[9] R. L. M. Pierik,"Vegetative Propagation", in *In Vitro Culture of Higher Plants*,1st, Springer, 1997, pp. 183-229.

[10] T. Murashige, and Skoog, F.,"A revised medium for rapid growth and bioassays with tobacco tissue cultures," *Physiol. Plant*.Vol., no. 15, pp. 473-497, 1962.

[11] P. K. Gupta, and Durzan, D. J.,"Biotechnology of somatic polyembryogenesis and plantlet regeneration in *Loblolly pine*," *BioTechnology*.Vol. 5, no. 2, pp. 147- 151, 1987.

[12] P. Venkatachalam, and Jayabalan, N.,"Effect of auxins and cytokinins on efficient plant regeneration and multiple-shoot formation from cotyledons and cotyledonary-node expiants of groundnut (*Arachis hypogaea* L.) by *in vitro* culture technology," *Applied Biochemistry and Biotechnology*.Vol. 67, no. 3, pp. 237-247, 1997.

[13] A. C. G. Cunha, Ferriera, M. F., and F,"Somatic embryogenesis, organogenesis and callus growth kinetics of *flax*," *Plant Cell, Tissue and Organ Culture*.Vol. 47, no. 1, pp. 1-8, 1996.

[14] H. Turhan, Colak, C., Turkmen, O. S., and Gul, M. K., "The effects of explant type and medium on *in vitro* callus induction of linseed (*Linum usitatitum* L.)," *Research People and Actual Tasks on Multidisciplinary Science II*, Lozenec, Bulgaria,2009, pp. 200-203

[15] R. Farzinebrahimi, Taha, R. M., Kamaludin, R., and Syafawati Yaacob, J.,"The Effect of Various Media and Hormones via Suspension Culture on Secondary Metabolic Activities of (Cape Jasmine) *Gardenia jasminoides* Ellis," *The Scientific World Journal*.Vol. 2014, no. pp. 2014.

Reza Farzinebrahimi was born in Kerman, Iran in 1973. He had graduated in BSc (Horticulture) in 1995 from Islamic Azad University in Iran, MSc (Plant Biotechnology) from University of Malaya in Kuala Lumpur-Malaysia in 2009. Mr. Farzinebrahimi currently is pursuing his PhD in Plant Biotechnology at Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia that is being sponsored by the university under the Bright Sparks scholarship. Prior to joining the University of Malaya, he worked as a head of Complex of Laboratories and Workshops in Islamic Azad University, Sirjan branch-Iran from 2000 to 2006 and as research assistant in University of Malaya-Malaysia (Faculty of Science, ISB) from 2009 to 2013. He awarded a fellowship from University Putra Malaysia, Serdang-Malaysia from 2006 to 2007. To date, he had authored over 10 articles in ISI and Scopus journals and had participated in several national and international conferences.

Kamaludin A Rashid has obtained his B.Sc in Biology from National University of Malaysia (UKM) in 1981 and PhD from Southampton University, UK in 1990 on Plant Breeding and Cytogenetic. He was born in Malaysia on May 30, 1957. He is currently working as a lecturer (associate professor) at Centre for Foundation Studies in Sciences, University of Malaya, Kuala Lumpur Malaysia. He has more than 30 years of experience in teaching in Biology in University of Malaya. He has more than 10 books published and more than 20 articles published in reputable ISI-cited journals and proceedings. He also had participated in various conferences and exhibitions related to his work.

Rosna Mat Taha. was born in Malaysia on January 22, 1958. In 1984, she obtained her B.Sc. in Botany from University of Malaya, Kuala Lumpur, Malaysia and eventually completed her Ph.D in 1989 on Plant Morphogenesis from University of Wales, Cardiff, UK. She is currently working as a lecturer (professor) at Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. She has published more than 100 papers in ISI-cited journals, proceedings, book chapters and etc. Prof. Taha is also an expert in plant tissue culture, morphogenesis, biotechnology, pigment and cellular behavior studies. She has won many awards (more than 20) in her research. She is a member of European Association for Research on Plant Breeding (EUCARPIA) and International Society for Horticultural Science (ISHS). Also, she is the associate editor of Asian Journal of Biotechnology, Asian Journal of PlantSciences and Journal of Applied Sciences.