

Methods: Manipulation of Benzyl Acetate and Jasmone Content of *Jasminum sambac* L. Using Modified Murashige and Skoog Medium on Callus Explant

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Abstract. Jasmine (*Jasminum sambac* L.) is a flowering plant that grows in shrub form. Jasmine flowers have been extensively used as ornamental and for the production of fragrances, flowers, tea and essential oils. The amount of essential oil that can be collected from jasmine petals is very small relative to the material required, but is valued as the raw material for natural perfume and aromatherapy treatments. This study attempted to improve propagation from explant to manipulate essential jasmine oil production. Modified Murashige and Skoog (MS) medium, produced from carbohydrate precursor compounds (MS + 20 % fructose, MS + 20 % glucose and MS + 20 % sucrose), was used to produce explants from young leaves and calluses. Essential oil (benzyl acetate and jasmone) content was highest in jasmine explant calluses grown on MS + 20 % sucrose. The content of benzyl acetate reached 1.27 % and jasmone content reached 1.15 % in 12 weeks old calluses.

Keywords: Benzyl acetate, Explant, *Jasminum sambac* L., Jasmone, Precursor.

INTRODUCTION

Jasmine (*Jasminum sambac* L.) is an ornamental plant extensively used in perfumery and religious purposes, a shrub herb which produces white flowers with a very pleasant fragrance (Davallo, 2014). Jasmine essential oil produces a distinctive fragrance, used as a natural perfume and also useful for aromatherapy (Satuhu, 2004). Jasmine essential oil is used for many different applications such as industrial perfumes, soaps, cosmetics, foods, and pharmaceuticals. Until now, the production of jasmine essential oil for industrial use in Indonesia is still reliant on imported raw material (Davallo, 2014).

Only low volumes of jasmine oil can be extracted from petals. One way to increase the production of essential oils is through application of tissue culture techniques. The production of secondary metabolites through plant cell culture can improve the content of secondary metabolites more effectively than conventional production. Cell culture can result in the production of secondary metabolite compounds throughout the year under controlled environmental conditions (Taji *et al.*, 2002).

Secondary metabolite production using plant tissue culture techniques is induced with a precursor carbohydrate. Ramawat (2008) showed that secondary

metabolites induced from calluses can be improved by changing the content of the components in the tissue culture medium or by adding precursor compounds into the medium.

Carbohydrates are a necessary source of carbon in the metabolism as an energy source. The addition of a carbon source derived from precursor carbohydrates is required when using the Murashige and Skoog medium formulation (Kristanto, 2006), to improve the content produced through tissue culture techniques and biosynthesis. Sources of carbohydrates that are often used in tissue culture medium are sucrose, glucose and fructose. Suryaningsih *et al.* (2013) inducing citronellol in calluses induced from the leaf of *Jasminum sambac* L. included 20 % fructose in the Murashige and Skoog medium they used. This present study also used precursor compounds (sucrose, glucose and fructose) in the tissue culture medium to increase the formation of compounds of benzyl acetate and jasmone in *Jasminun sambac* L. calluses

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MATERIALS AND METHODS

Plant Materials . *Jasminum sambac* L. leaf explants, with shoots 2 cm in length and three leaves were grown on Murashige and Skoog (MS) medium modified with kinetin 1 mg/l and 0.1 mg/l 2,4 diclorophenoxy acetic acid (Kong, *et al.*, 2012).

Experimental Design. The study was conducted at the Tissue Culture Laboratory of Agriculture, Wijaya Kusuma Surabaya University between April and August 2014.

This research was carried out using a completely randomized design with 2 factors: 2 types of explant, young leaf and callus; and 3 types of media modified with carbohydrate, namely MS + 20 % fructose, MS + 20 % glucose and MS + 20 % sucrose.

Leaf and callus explants of jasmine with shoots, grown on Murashige and Skoog were provided with 20 % precursor medium and incubated for 3 months.

Surface sterilization. Shoots were sterilized with 20, 10 and 5 % (v/v) hypochloride for 5, 10 and 20 minutes, then washed with sterile water. After that explants were cut and cultured on modified Murashige and Skoog medium.

Callus Induction. Calluses formed after three weeks incubation on modified Murashige and skoog medium with addition of 20 % carbohydrate.

Secondary Metabolite Analysis. Spectrophotometry was used to detect jasmine oil (benzyle acetat and jasmone) from calluses at 8 and 12 weeks old.

RESULTS AND DISCUSSION

The quality obtained in the friable calluses maintained on modified MS + 20 % sucrose with callus explant treatment, the quality of compact and friable calluses on modified MS + 20 % glucose with callus or leaf explant treatment, and the quality of compact callus on modified MS + 20 % fructose with callus or leaf explant treatment, and modified MS + 20 % sucrose with leaf explant treatment is shown in Figure 1. Results were in accordance with the work of Prakoeswa *et al.* (2006) where the quality of calluses depended on the conditions that influenced the growth of the explant source. The formation of quality calluses is indicated by the direction in growth: friable callus growth leads to callus embryogenesis, but compact callus growth leads to organogenesis (Prakoeswa *et al.*, 2009).

In Table 1 the quantity of calluses formed under different carbohydrate and explant treatment are shown. The treatments with callus explants maintained on MS + 20 % glucose and MS + 20 % sucrose produced the highest callus growth compared to the other treatments.

Taji *et al.* (2002) showed that callus growth is influenced by planting materials, properties of tissue, explant age, the composition of medium, source of carbohydrate and species of plant. Nurchayati and Aphiah (2010) reported that sucrose is needed to induce the formation of a callus. Quantity of propagation is indicated by the number of calluses formed from the cell division, and in general, the growth and development of a callus is influenced by elements in the medium. Explants of different species need different compositions of medium; specific nutrients are needed for growth. Success in plant tissue culture techniques is highly dependent on the medium used, which should contain macro nutrients, micro nutrients, vitamins and a carbon replacement source (Rahmawati, 2006).

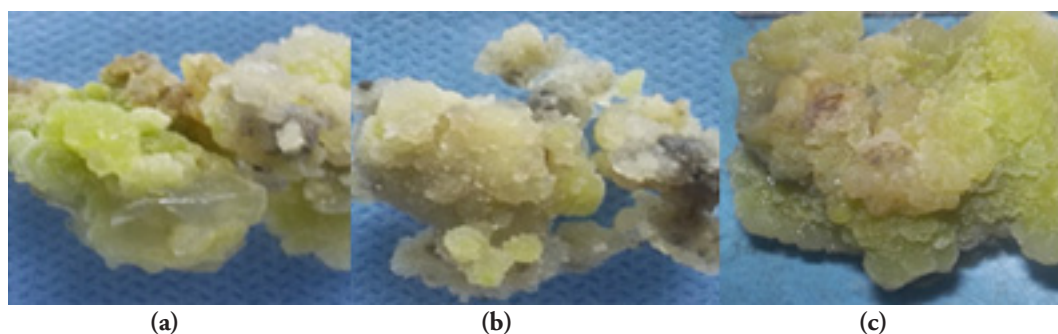


Figure 1. Photographs of calluses produced on different media, illustrating how quality was judged.

(a) Appearance of compact and friable callus on MS + 20 % glucose. (b) Appearance of compact callus on MS + 20 % Fructose. (c) Appearance of friable callus on MS + 20 % sucrose.

Table 1. The quality and quantity of calluses formed with different carbohydrate and explant sources.

Treatment	Quality of calluses	Quantity of calluses/explant
Young leaf explants with MS + 20 % fructose	Compact	1 – 2
Young leaf explants with MS + 20 % glucose	Compact and friable	> 2
Young leaf explants with MS + 20 % sucrose	Compact	1 – 2
Callus explants with MS + 20 % fructose	Compact	1 – 2
Callus explants with MS + 20 % glucose	Compact and friable	> 2
Callus explants with MS + 20 % sucrose	Friable	> 2

Table 2 shows the quantity of benzyl acetate and jasmine produced with different carbohydrate and explant treatments. The highest content of benzyl acetate and jasmine was found in callus explants grown on MS + 20% fructose.

Mulabagal and Tsay (2004) showed that an increase in secondary metabolite production can be induced through provision of an efficient precursor compound as a source of carbohydrate. According to Nurchayati and Aphiah (2010), the concentration of the additional carbohydrate absorbed by the callus cells for the formation of secondary metabolites varies. This is because sugar is used as an energy source, and the carbon source affects the signals that in turn affect gene expression in the formation of secondary metabolites.

Fructose is the base material of respiratory metabolism since it metabolises so fast that it can be used in the process of glycolysis. It can also accelerate the formation of cell membranes, amino acids and cell wall components including lignin during the growth of calluses (Ribkahwati *et al.*, 2010).

Table 2. Benzyl acetate and jasmine content analysis.

Treatment	8 week old calluses (%)		12 weeks old calluses (%)	
	<i>Benzyl Acetat</i>	<i>Jasmone</i>	<i>Benzyl Acetat</i>	<i>Jasmone</i>
Young leaf explants with MS + 20 % fructose	1.19 ab	1.10 a	1.20 b	1.12 ab
Young leaf explants with MS + 20 % glucose	1.02 c	1.08 b	1.01 d	1.10 b
Young leaf explants with MS + 20% sucrose	1.15 b	0.96 b	1.17 c	0.99 b
Callus explants with MS + 20 % fructose	1.22 a	1.12 a	1.27 a	1.15 a
Callus explants with MS + 20 % glucose	1.15 b	0.96 b	1.16 c	0.99 b
Callus explants with MS + 20 % sucrose	1.19 ab	1.10 a	1.21 b	1.12 ab
LSD 5 %	S	S	S	S
Values proceeded by different letters within a column are statistically significantly different from each other. S: Significant				

CONCLUSION

The results of the observations that have been obtained in this study can be summarized as follows. The quality of friable callus on MS + 20 % sucrose with callus explant, the quality of compact and friable calluses on MS + 20 % glucose with callus or leaf explant, and the quality of compact callus on MS + 20 % fructose with callus or leaf explant and MS + 20 % Sucrose with leaf explant have all been explored and characterised. The highest quantity of calluses were grown on MS + 20 % glucose and MS + 20 % sucrose, and the highest amount of benzyl acetate and jasmine was produced by callus explants grown on MS + 20% fructose.

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REFERENCES

- Davallo, B.L., Kazemitabar, S.K., Gholipour, A. and Ghanbari, S. 2014. Callus Induction on *Jasminum sambac* L. by 2,4 – Dichlorophenoxy acetic acid Hormone. *International Journal of Biosciences* 5(2): 114-118.
- Kong, U.W., Wongsawad, P. and Buddharak, P. 2012. Shoot Bud and Young Leaf Induction of *Jasminum* spp in *In Vitro* Culture. *International Journal of Applied Agricultural Research* 7(1): 17.
- Kristanto, H. 2006. Kajian Penambahan Fruktosa pada Multiplikasi *Rosa* sp secara *In Vitro*. Laporan Penelitian Lab. KJ. Faperta UWKS Surabaya.
- Mulabagal, V. and Tsay H.S. 2004. Plant Cell Cultures – An Alternative and Efficient Source for the Production of Biologically Important Secondary Metabolites. *International Journal of Applied Science and Engineering* 2(1): 29–48.
- Nurchayati, Y. and Afiah, F. 2010. Kandungan Asam Askorbat Pada Kultur Rosela (*Hibiscus sabdariffa* L.) dengan Variasi Konsentrasi Sukrosa dalam Media MS. *Majalah Obat Tradisional* 15(2): 71–74.
- Prakoeswa, S.A., Ribkahwati and Suryaningsih, D.R. 2010. *Teknik Kultur Jaringan Tanaman Implementasi Beserta Aplikasi, dan Hasil Penelitian*. Dian Prima Lestari.
- Rahmawati. 2006. Pengaruh Jenis Gula terhadap Akumulasi Isoflavon pada Kalus Bengkoang (*Pochyrhizus esus* L) Univ. Brawijaya Malang.
- Ramawat, K.G. 2008. *Plant Biotechnology*. S. Chand & Company LTD. New Delhi. 93 – 134.
- Ribkahwati, Arijanti, Susilo and Dwie Retna. 2010. *Teknik Elisitasi Dengan Elisitor Biotik secara in vitro*. Irvi Jaya.
- Rukmana, R. 2004. *Budidaya Bunga Matahari*. Semarang. Aneka Ilmu.
- Satuhu, S. 2004. *Melati Penanganan Segar dan Pembuatan Minyak Bunga Melati*. Jakarta. Penebar Swadaya.
- Suryaningsih, D.R., Prakoeswa, S.A. and Ribkahwati. 2013. Biosintesis Benzyl Acetat dan Jasmone dengan 3 Macam Prekursor Karbohidrat. Laporan Penelitian DIKTI.
- Taji, A., Kumar, P. and Lakshmanan, P. 2002. *In Vitro* Plant Breeding. *Food Products Press* 15: 44.