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Tissue Culture and Photoperiod for Enhancing Secondary Metabolite on Jasmine

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ABSTRACT

Jasmine is a commercially ornamental flower crop cultivated plant that is highly valued for producing jasmine essential oil as a secondary metabolite. Essential oil is extracted from jasmine, which is in great demand in the perfumery, flavor, and food additives industry. Plant tissue culture technologies could be authorized by regular sterile conditions from explants for the way for secondary metabolite multiplication. This study aims to investigate the effects of callus formation and photoperiod on the quality, quantity, and secondary metabolites of jasmine through tissue culture. The method used in this study was a completely randomized factorial design with callus formation and photoperiod. The result showed that the callus formation and photoperiod did not influence the callus quality obtained from profiling. Callus quality observation revealed the best result on a three-month-old callus and 12 hours of the photoperiod (1.86). Furthermore, the secondary metabolite of the callus highlighted that a one-month-old callus and 6 hours of the photoperiod produce the highest Jasmone level of 0.17%.

Keywords: Callus*; Jasminum officinale*; Ornamental plant; Photoperiod; Plant growth regulator

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INTRODUCTION

Jasmine (*Jasminum officinale* L.) is a native plant of the Indonesian archipelago (Heyne, 1987), which belongs to the olive family (Oleaceae). The existence of local culture makes jasmine exhibit different names in various Indonesian native languages, such as malate (Madurese), mlati (Javanese), manduru (Manado Malay), mayora (Timor), selupan (Malay), mundu (Bima), melur (Batak Karo), elung (Bugis), and malati (Sundanese). Approximately 200 recorded species belong to the genera Jasminum, but in 1988, 300 species were reported (Jones and Gray 1988), and the number of cultivated species was 47. In Indonesia, Central Java, East Java, Lampung, and North Sumatra produce the significant commodity of jasmine. However, as an ornamental plant, the harvested area of jasmine decreased by 36.59 percent in 2018 (the Indonesian Central Bureau of Statistics 2019).

The main part of jasmine used as an essential oil is flowers; besides, almost all parts have a commodity value (Yulia et al. 2012). The chemical compounds in jasmine flowers and leaves create a sweet, spicy, and cool taste. At the same time, the roots exhibit spicy, sweet, and somewhat toxic (Hariana 2013). Phytochemical screening by Rastogi et al. (1990) reported the presence of indole, eugenol, linalool, and other active compounds in jasmine flowers. The highest component of jasmine flower oil is indole. Indole is an alkaloid compound that demonstrates anti-cancer activity (Siswandono and Soekardjo 2008). The existence of various compounds makes jasmine oil highly used by industry as a raw material for perfume, soap, cosmetics, food production, and pharmaceutical industries (Hidayat et al. 2015).

Secondary metabolites can be obtained by direct extraction from plant organs. However, Fejér et al.(2018) stated that intensive use of plants can reduce genetic variance. Furthermore, the use of intact plants requires plants that are cultivated on a large scale, and there are substantial costs for the extraction, isolation, and purification processes (Ningsih 2014). In vitro culture (including cell and tissue culture) for secondary metabolite production has been released from various commercial pharmaceutical and bioreactor plants (Marchev et al. 2014). The advantages of tissue culture in the production of secondary metabolites compared to intact plants are the absence of climatic limitations, the ability to be done on a small area of land, and the ability to produce bioactive compounds under controlled conditions (Collin and Edwards 1998).

Previous studies show that shoot culture can be used to produce secondary metabolites such as menthofuran

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in *Mentha piperita* L. Fejér et al. (2018) and rosmarinic acid in *Salvia virgata* (Dowom et al. 2017). The callus culture of *Naringi crenulata* was able to produce 1,3,4,5-tetrahydroxy cyclohexane carboxylic acid (Singh et al. 2018). Furthermore, the production of secondary metabolites from plant cell culture also demonstrates various advantages, including manipulating bioactive compound biosynthesis in sterile and controlled cultures (Docimo et al. 2015; Dias et al. 2016). In addition, in vitro techniques have a high potential to produce secondary metabolites without being affected by the harvest season and minimize cross-contamination compared with in vivo cultures (Murch and Saxena 2006; Fazili et al. 2022).

Nugroho et al. (2007) reported that the analysis of the secondary metabolite chromatogram profile of ginger showed 83% of the compounds contained in the Curcuma callus were the same as those found in the parental plant (Nugroho et al. 2007). A similar result showed that in vitro-grown gotu kola (*Centella asiatica*) produced more varied secondary metabolites compared to the field or in vivo gotu kola (Diniz et al. 2023). Therefore, this study aims to investigate the effects of callus formation and photoperiod on the quality, quantity, and secondary metabolites of jasmine through tissue culture.

MATERIALS AND METHODS

Study area

Previous studies show that shoot culture can be used to produce secondary metabolites such as menthofuran in *Mentha piperita* L. Fejér et al. (2018) and rosmarinic acid in Salvia virgata (Dowom et al. 2017). The callus culture of *Naringi crenulata* was able to produce 1,3,4,5-tetrahydroxy cyclohexane carboxylic acid (Singh et al. 2018). Furthermore, the production of secondary metabolites from plant cell culture also demonstrates various advantages, including manipulating bioactive compound biosynthesis in sterile and controlled cultures (Docimo et al. 2015; Dias et al. 2016). In addition, in vitro techniques have a high potential for production. This research was conducted at the Faculty of Agriculture, Wijaya Kusuma University Surabaya, East Java, in August 2017. The experimental method used a completely randomized factorial design with two factors, which are the following: Factor I (Callus Formations) consists of 3 levels: U1, one-month age; U2, two-month age; U3, three-month age. The second factor (photoperiods) consists of 5 levels: P1, 0 hours of light period; P2, 6 hours of light period; P3, 12 hours of light period; P4, 18 hours of light period); and P5, 24 hours of light period. The treatment was carried out on young leaf callus of Jasminum officinale, which was expected to affect the quality of the callus formed and could be friable quality (crumb callus) and compact quality (dense) (Junairiah et al. 2017; Zhou et al. 2018).

Explanting and planting

J. *officinale* young leaf extract was sterilized with 20% Clorox plus one drop of Tween for 5 minutes followed by 10% Clorox and then one drop of Tween was added for 10 minutes. Finally, 5% Clorox and one drop of Tween were added for 20 minutes. It was rinsed with sterile water, and young leaves of Jasminum officinale were cut to a size of approximately 1 x 1 cm and planted in a culture tube containing 40 mL of modified Murashige and Skoog (MS) medium Table 1. Callus can grow a month after planting, and the conditions mentioned above have been carried out with various photoperiods.

Table 1. Murashige and Skoog Medium with modifications from (Taji et al. 2002)

Macro Nutrient	Amount ($mg.L^{-1}$)	Micronutrient	Amount ($mg.L^{-1}$)
NH_4NO_3	400	Na_2 – EDTA	32.8
KH_2PO_4	170	$FeSO_4.7H_2O$	27.6
$Ca(NO_3)_2.4H_2O$	556	$MnSO_4.4H_2O$	16.9
$MgSO_4.7H_2O$	370	$ZnSO_4.4H_2O$	8.60
K_2SO_4	990	H_2BO_3	6.20
$CaCl_2$	96	KI	0.83
Vitamin and Amino Acid	**Amount ($mg.L^{-1}$)**	$NaMoO_4.2H_2O$	0.25
Thiamine	1	Na_2 – EDTA	32.8
Nicotinic acid	0.5	$CuSO_4.5H_2O$	0.025
Pyridoxine HCl	0.5	$CoCl_2.6H_2O$	0.025
Glycine	2		
Mio – Inositol	100		
Sucrose	30.000		
Agar (Bacto)	7.000		

Data observation

Observations made in this study include morphological analysis and secondary metabolite analysis. Morphological analyses were observed at intervals of a week, while observations of secondary metabolic content were observed at the end of the observation, around the age of callus 4 months after planting.

Morphological analysis

Analysis of callus morphology includes (a) analysis of callus quantity and (b) callus quality. According to Wattimena (1988), the callus morphology analysis method measures the quality and quantity of callus. The callus quantity was observed by measuring the cohesiveness and weakness of the callus using a loop needle. If the loop needle is inserted into the callus and the callus breaks, the quality of the callus is friable (crumbs), whereas if the callus is not broken, the callus is compact. Meanwhile, to observe the callus quantity, scoring was carried out as follows: Score 1. The size of the callus formed is the same as the explant size, Score 2. The callus formed is twice as big as the explant and Score 3: the callus formed is twice as large as the explant.

Secondary metabolite analysis

Analysis of secondary metabolite was done through *extraction* method using maceration and gas chromatography analysis: Mass Spectrophotometry**.**

The extraction method uses maceration. *J. officinale* callus that were incubated in the laboratory for 2 months were dried. According to the treatment given, the number of the *J. officinale* callus sample was 15. The sample is macerated, soaked in 200 mL of hexane for 24 hours, and then evaporated at room temperature. Soaking for 24 hours can produce jasmine oil (a liquid that floats). After that, 5 mL of jasmine oil is pipetted for observation.

Gas chromatography analysis - mass spectrophotometry (GC-MS). Following Vandana et al. (2018), the preparation of the sample that was analyzed using GC-MS was carried out by dissolving a small amount of callus dry extracts into 5 mL n-hexane and homogenizing for 3 minutes (Vandana et al. 2018). Next, it was centrifuged at 2,500 rpm for 5 minutes, and the supernatant (n-hexane phase) was filtered and injected into the GC – MS chromatography column. Analysis was performed by two replications.

RESULTS AND DISCUSSION

Quality of callus

The analysis of variance regarding the effect of callus formation and photoperiod on callus quality did not show any significant interactions. Although it is not statistically different, the quality values show that the longer the callus life and the longer the light exposure time resulted in a greater quality of the callus produced. The mean results of observations of the quality of callus formed at 4 to 10 weeks after planting are shown in Table 2. These results indicate that the treatment carried out in this study did not exhibit a significant effect on the callus quality of jasmine plants.

The quality of callus growth can be seen from the callus's texture, color, and weight (Ariati et al. 2012). According to the result, the callus texture from the treatment tends to be compact. A compact callus texture is considered good because it can accumulate more secondary metabolites in plants (Indah and Ermavitalini 2013). The compact texture of callus generally has small cell sizes with dense cytoplasm, large nuclei, a lot of carbohydrate content, and nodule-like structures (Adri 2012). Nodules are pro-embryonic masses and can be used as inoculums for induction as somatic embryos. Many factors influenced the formation of callus textures, including the type of plant used, the nutrient composition of the medium, growth regulators, and environmental conditions (Hariyati et al. 2016).

The quantity of callus

The results of the variance analysis showed that the callus formation 4–5 weeks after planting and 7–9 weeks after planting did not show any interaction with callus quantity. The significant interaction in each treatment occurred at the age of 6 weeks after planting (Table 3). Based on the result, three months of age of callus formation showed different mean values for each treatment. The best average yield, as indicated by the three-month-old callus with photoperiod for 12 hours of the light period, is 1.86. However, the lowest yield was a one-month-old callus without light exposure, with a value of 1.32.

Table 2. The mean results of callus quality observations formed in various treatments (callus formation and photoperiod treatment from 4–10 weeks after planting

Treatments		Age (Week After Planting)						
Callus Formation (month)	**Photoperiod (hour)**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	**0**	1.20	1.30	1.40	1.60	1.80	2.10	2.30
1	**6**	1.20	1.30	1.40	1.60	1.80	2.10	2.30
1	**12**	1.20	1.30	1.40	1.60	1.80	2.10	2.30
1	**18**	1.20	1.30	1.40	1.60	1.80	2.10	2.30
1	**24**	1.20	1.30	1.50	1.60	1.80	2.10	2.30
2	**0**	1.20	1.40	1.50	1.80	2.00	2.40	2.60
2	**6**	1.20	1.40	1.56	1.90	2.13	2.40	2.60
2	**12**	1.20	1.46	1.56	1.90	2.13	2.40	2.60
2	**18**	1.20	1.46	1.52	1.90	2.13	2.40	2.60
2	**24**	1.20	1.46	1.70	1.90	2.40	2.40	2.60
3	**0**	1.20	1.60	1.70	2.10	2.40	2.60	2.80
3	**6**	1.20	1.60	1.70	2.10	2.40	2.60	2.80
3	**12**	1.20	1.60	1.70	2.10	2.40	2.60	2.80
3	**18**	1.20	1.60	1.70	2.10	2.40	2.60	2.80
3	**24**	1.20	1.60	1.70	2.10	2.40	2.60	2.80
Least Significant Difference (5%)		NS	NS	0.04	NS	NS	NS	NS

Remark: NS= non-significant

Table 3. The mean results of the observation of the callus quantity formed in various treatments

Treatments		Age (Week After Planting)						
Callus Formation (month)	**Photoperiod (hour)**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	**0**	1.05	1.22	1.32f	1.46	1.68	1.90	2.11c
1	**6**	1.13	1.26	1.46d	1.61	1.86	2.13	2.43b
1	**12**	1.10	1.25	1.38c	1.57	1.78	2.04	2.33b
1	**18**	1.08	1.24	1.43d	1.66	1.82	2.11	2.40b
1	**24**	1.17	1.28	1.61c	1.70	2.00	2.40	2.76a
2	**0**	1.24	1.44	1.64c	1.73	1.90	2.15	2.52b
2	**6**	1.23	1.43	1.63c	1.72	1.83	2.06	2.30b
2	**12**	1.20	1.42	1.62c	1.70	1.84	2.08	2.33b
2	**18**	1.22	1.40	1.60c	1.71	1.73	2.03	2.24b
2	**24**	1.22	1.41	1.61c	1.82	1.82	2.05	2.28b
3	**0**	1.30	1.50	1.81b	2.00	2.15	2.48	2.81a
3	**6**	1.32	1.53	1.84ab	2.00	2,.21	2.43	2.81a
3	**12**	1.33	1.58	1.86a	2.02	2.26	2.53	2.86a
3	**18**	1.30	1.52	1.81b	2.00	2.14	2.42	2.83a
3	**24**	1.31	1.54	1.83ab	2.00	2.16	2.46	2.83a
Least Significant Difference (5%)		NS	NS	0.04	NS	NS	NS	0.04

Remark: NS= non-significant

Besides, other observations indicate that callus quantity exhibits a positive correlation with age after planting, which means that the longer the observation time, the greater the size of the callus produced in all treatments (Table 3), although the values vary. Increasing callus size indicated the presence of callus growth due to the treatment given. Growth is a permanent increase in the size of an organism or part of a plant in the form of an increase in the number and size of cells.

The result of this study is consistent with Salisbury and Ross (1995) statement that the ideal growth curve is to show the cumulative size as a function and time. Furthermore, the age of the callus affects the ability to grow the callus, which decreases, followed by physical changes in the callus. When the nutrient content in the culture medium is low, the cell regeneration process will slow down and affect the color and texture of the resulting callus. It is characterized by increasingly loose callus cells and a change in the color of the callus from yellowish white to brownish to brown (Lutfiah and Habibah 2022).

Secondary metabolite composition

The GC-MS results showed the existence of five secondary metabolites, which are the main secondary metabolites in jasmine oil. However, Hendaryono and Wijayani (1994) stated that callus culture usually produces more useful secondary metabolites, such as alkaloids. Various kinds of treatments showed that various variations exist regarding the content of the compounds obtained by each treatment with different peaks (Table 4).

Not all treatments show the existence of all secondary metabolites. Only the callus treatment aged two months without light exposure shows the value of the three secondary metabolites: *Linalyl acetate*, Linalool, and Jasmine. However, this study only focused on treatments that exhibited the highest levels of secondary metabolites. This one-month-old callus treatment was given light exposure for 6 hours per day with a value of 0.17%.

In addition, GC-MS results showed that several treatments exist that did not increase the levels of Jasmone or other secondary metabolites. It might be because the amount is too small due to callus formation and photoperiod. The environment influences callus growth and the production of secondary metabolites. Taji et al. (2002) used TL (Neon) lamps with intensities ranging from 600–1,000 Lux, and they stated that the growing environment could affect the production of secondary metabolites in tissue culture. Another factor influencing the result is the type of commodity and specific genetic traits. Also, light can influence plant development in vivo and in vitro. The culture condition is influenced by the duration of exposure, light color, and intensity. Next, light can affect the regulation of the production of primary and secondary metabolites (Siregar et al. 2010).

Table 4. GS–MS chromatography results for secondary metabolites of jasmine callus

Treatment		Secondary Metabolites (%)				
Callus Formation (month)	Photoperiod (hour)	Benzyl Acetate	Linalyl Acetate	Linalool	Jasmone	Peak
1	**0**	-	2.09	-	0.09	59
1	**6**	-	2.72	-	0.17	45
1	**12**	-	4.27	-	-	45
1	**18**	-	3.21	5.05	-	45
1	**24**	-	-	1.83	0.05	39
2	**0**	-	0.96	0.94	0.04	44
2	**6**	-	0.43	0.51	-	36
2	**12**	-	-	-	-	42
2	**18**	-	2.47	-	0.15	60
2	**24**	-	0.95	-	0.10	48
3	**0**	-	1.47	-	0.11	55
3	**6**	-	-	3.08	0.08	56
3	**12**	-	2.78	6.42	-	46
3	**18**	-	0.09	-	-	39
3	**24**	-	-	2.84	-	39

CONCLUSIONS AND SUGGESTIONS

This research showed that the quality of the callus obtained from profiling was not influenced by the age of the callus and the exposure duration. The results of callus quality observation showed that a three-month-old callus and 12 hours of photoperiod were the best results (1.86). Furthermore, the secondary metabolite of callus highlighted that a one-month-old callus and 6 hours of photoperiod produce the highest Jasmone level of 0.17%.

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