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Research Article

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Quantity essential oil from rose callus leaf (*Rosa hybrid* L. variety *Hybride tea purple*): Results of light elicitation

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ABSTRACT

Essential oil from rose petals roses are very small, the average ranged from 0.01 to 10.0%. Local Americana beauty red roses and roses Holand from Cipanas contains oil rose about 0.08 to 0.14%, with citronellol 27.23 % and geraniol 16.18%, in East Java (Batu) content of local Rosa hybrida varieties Hybride Tea purple containing β-citronellol 21.07 % and Geraniol 0.18 % In Indonesia, produce rose essential oil is low, compared rose essential oil of Kashmir and Bulgarian. The essential rose oil research try to improve the callus of rose leaf with elicitation abiotic. This Research of essential oil in rose leaf callus induced by elicitor Long exposures (0 hours / day, 12 hours / day and 24 hours / day) and light intensity (1200 Lux and 2400 Lux). Formation of the results obtained Geraniol need light. The content is highest Citronellol in the dark treatment

Keywords: abiotic elicitor, callus, citronellol and geraniol

INTRODUCTION

Rose essential oil is a vegetable oil that produces a distinctive fragrant. In Indonesia, grown roses from the Netherlands, the varieties of *Hybride tea* [4]. Extraction of essential rose oil has been done through the process of distillation from rose petals. The essential rose oil made up of 75 % is composed dominant about *geraniol* and *citronellol* then 25 % *rose champor*, the containing compounds *nerol, linalool, phenyl ethyl alcohol, farnesol, stearoptene, α-pinene , β-pinene, α-terpinene, limonene, p-cymene, Camphene, β-caryophyllene, neral, citronellyl acetate, geranyl acetate, neryl acetate, eugenol, methyl eugenol, rose oxide, α-damascenone, β-damascenone, benzaldehyde, benzyl alcohol, rhodinyl acetate* and *phenyl ethyl formate* [14].

The local red roses from Cipanas West Java contains *geraniol* 27.23 % and *citronellol* 16.18 % [14]. Preliminary research on the oil content of rose petals local varieties of *tea Hybride* contained in three locations Batu East Java with 5 varieties: *Hybride white tea, Hybride yellow tea, Hybride rose tea, Hybride red tea* and *Hybride purple tea*. Only one location in the village Sidorame with *purple tea* varieties Hybride containing *citronellol* and *geraniol*. Ingredients obtained: *citronellol* (21.73%) and *geraniol* (0.18%) [7]. The problem is there is oil content rose in Indonesia is very low when compared to the industry standard rose essential oil in Turkey. The content of *Rosa damascena* Mill *citronellol* was 35.1%. and 17.9% *geraniol* [2][12].

In an effort to improve the content of the research conducted through tissue culture techniques and elicitation techniques. Increased content of volatile oil carried through the elicitation technique using light. Elicitation is a method to induce phytoalexin formation, secondary metabolites that have been or other secondary metabolites that normally do not accumulate in plants [5]. Elicitation is the process of adding elicitor with the aim to induce and enhance the formation of secondary metabolites [6]. culture usually requires long irradiation or irradiation ranges from 10-24 hours long. Long exposures are optimal is 16 hours [10].

EXPERIMENTAL SECTION

This study was conducted in labobatorium Biotechnology Faculty of Pharmacy, Airlangga University.

Leaf explants of shoots roses varieties of *Hybride tea purple* measuring 2 cm and the leaves were taken to the three that are still colored red, grown on Murashige and Skoog medium with the addition of plant growth regulator 0.1 mg/L Napthalene Acetic Acid and 3 mg/L Benzyl Amino Purin [11].

Callus two months old in subculture to elicitation using factorial completely randomized design with two factors. The first factor while irradiation with 3 treatments as follows: 0 hours/day (total darkness), 12 hours/day and 24 hours/day (total light). The second factor light intensity with 2 treatments as follows: 1200 Lux and 2400 Lux.

Extraction of essential oils from callus elicitation results done by weighing 100 g of callus wind dried for 24 hours, then soaked with n-Hexan with a ratio of 1 : 3 for 24 hours. Strain and cool evaporated [1]

Sample extraction yield with 5 mL n-Hexan, divortex for 3 minutes, centrifuged at 2.500 rpm for 5 minutes. Supernatant (hexan phase) was taken and injected for analysis Gas chromatography analysis - MS spectrophotometer

RESULTS AND DISCUSSION

Callus and Color Quality

In Table 1. the quality obtained the friable callus with brown translucent color on dark treatment, the quality of compact and friable callus with green translucent color on irradiation treatment 12 hours/day and the quality of compact callus with green color on treatment irradiation 24 hours/day.

Irradiation on the growth of plant organs in vitro requires full light, while the splitting process does not require light [13]. The light effect on the formation of clorofil [8]. This is consistent with the results of the study showed that callus formed on treatment while the dark is brown translucent color that gets the green light. According Widyastuti green callus can improve growth, enhance the cell's ability to bind CO2 for photosynthesis purposes optimal light intensity in 1000 to 5000 lux more than it will inhibit the growth of.callus.

Table 1. Observations Color and Quality Callus Generated After Elicitation Light Treatment

Treatment (weeks)		Color of Callus	Quality of Callus
L1	4	Brown translucent	Friable
	6	Brown translucent	Friable
	8	Brown translucent	Friable
L2I1	4	Green translucent	Compact and Friable
	6	Green translucent	Compact and Friable
	8	Green translucent	Compact and Friable
L2I2	4	Green translucent	Compact and Friable
	6	Green translucent	Compact and Friable
	8	Green translucent	Compact and Friable
L3I1	4	Green	Compact
	6	Green	Compact
	8	Green	Compact
L3I2	4	Green	Compact
	6	Green	Compact
	8	Green	Compact

Weight Callus

In Table 2. the tendency of dark treatment resulted in the lightest callus. While the provision of various length and intensity of light affects callus heavier. The more the intensity and duration of light irradiation heavier callus formed. The low light intensity effect on carbohydrate production decline resulting in lower weight of the plant [3]. Effect of light intensity deals with the process of photosynthesis. In this process of photosynthesis light energy needed to form carbohydrates [15]. The greater the amount of energy the optimal formation of the large amount of light energy received depends on the intensity of light. The higher the light intensity the greater the energy, the more carbohydrates are formed.

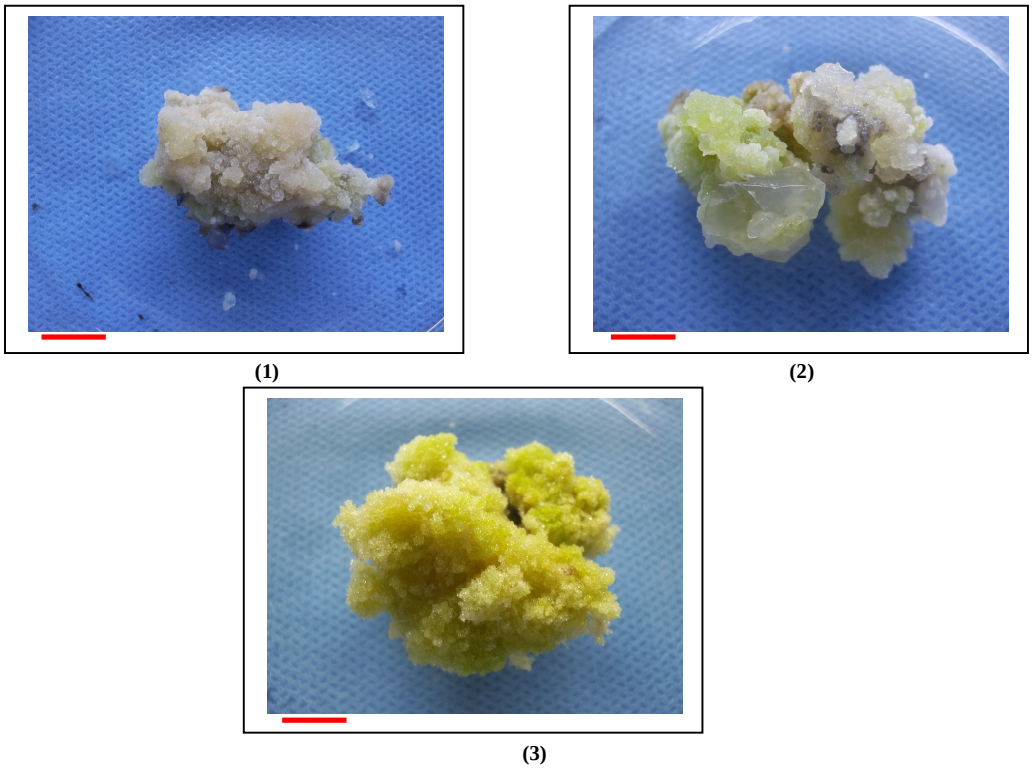
Table 2. Observations Wet weight Shaped Rose Leaves Callus After elicitation Light Treatment

Treatment	Age of callus (days)							
	7	14	21	28	35	42	49	56
L1	0,942	1.038 ab	1.132 b	1.219 c	1.294 c	1.420 c	1.562 b	1.656 b
L2	0,84	0.964 b	1.175 b	1.432 a	1.719 a	2.087 a	2.310 a	2.416 a
L3	1,024	1.110 a	1.228 a	1.362 b	1.487 b	1.887 b	2.205 a	2.669 a
Duncan 5 %	TN	N	N	N	N	N	N	N

Profile Oil Rose

The results of the content analysis of secondary metabolites were performed using GCMS can be seen in table 3. Treatment of light capable of producing *geraniol* content, while in dark conditions *geraniol* not formed. The results of all treatments contained *citronellol* in any form treatment then *citronellol* a marker compound.

Light intensity affects the total energy received by jaingan or cells in tissue culture. Increasing the amount of chlorophyll formed by light followed by an increase in activity of the enzymes of photosynthesis [13]. In the production of secondary metabolites compounds with in vitro culture technique was also affected by light. Light affects the enzyme-inducing enzymes involved in the biosynthesis of secondary metabolite formation. Without light causes in vitro culture is not able to form some secondary metabolites.



(1) **(2)**

(3)

Figure (1) on the dark callus formed transparent with friabel quality (2) on the light intensity of 1200 lux callus formed transparent and green with friabel and compact quality (3) on the light intensity of 2400 lux callus formed green with compact quality. = 1 c

Table 3. Profiling Essential Oil Content in Callus Elicitation Results Light Rose Leaves

Ingredients Essential oil (%)	Lighting treatment (weeks)														
	L1			L2I1			L2I2			L3I1			L3I2		
	4	6	8	4	6	8	4	6	8	4	6	8	4	6	8
Benzaldehyde				0.363	0.403	0.326	2.241	0.551	0.361				0.367		
Benzyl Alcohol				2.010	5.754	4.680	3.484	1.992	3.405		2.980		2.142		
Phenethyl Alcohol				16.265	16.406	13.845	13.307	5.256	16.672	0.338		0.319			
Linalool										0.241					
β-Citronellol	36.629	31.025	31.623	31.624	18.248	22.054	29.542	21.077	30.664	7.209	6.067	6.361	5.848	5.583	5.872
Geraniol				2.375				2.185							
Geranyl acetate								1.859		2.488	1.998	2.132	2.610	3.407	2.228
Geraniol formate							0.520								
Geranyl benzoate							0.214		0.220	0.455					

CONCLUSION

The results of the observations that have been obtained in this study can be summarized as follows:
1. Quality of friable callus callus with translucent brown color on dark treatment. Quality compact and friable callus callus with green translucent color on irradiation treatment 12 hours/day. The quality of compact callus callus with green color on irradiation treatment 24 hours/day.
2. *Citonellol* as marker compounds.
3. Formation of Geraniol need light.
4. Highest Cironellol Content contained on the form of dark treatment on callus age of 2 weeks.
5. Network callus that forms on the dark loose produce callus, whereas the light treatment resulted in a denser network.

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