Research Article

Biosynthesis of secondary metabolites (gingerol, shogaol, and zingerone) from callus of three ginger varieties

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ABSTRACT

Objective: The purposes of this research are as follows: to investigate the quantity of callus of three varieties of ginger; to investigate the quality of callus of the three varieties of ginger; and to evaluate the ginger oil derived from callus of the three varieties of ginger. **Methods:** The research used a complete randomized design on three varieties of ginger (*Gajah, Emprit,* and *Merah*) with 10 repetitions and four test samples. If analysis of variance revealed significant differences, analysis continued by least significant difference at 5%. This research used MS (*Murashige Skoog)* medium. After 3 months of incubation, analysis of the contents of gingerol, shogaol, and zingerone in the ginger callus was carried out. **Results:** The greatest quantity of callus was produced by *Gajah* ginger. *Emprit* and *Merah* gingers tend to have a compact callus, and *Gajah* ginger callus tends to be friable. **Conclusion:** The best composition of secondary metabolites was found in *Emprit* ginger which contained 1.181% gingerol, 0.118% shogaol, and 0.098% zingerone.

KEY WORDS: Biosynthesis, Ginger callus, Ginger variety, Rhizome explant, Secondary metabolite

INTRODUCTION

Ginger (*Zingiber officinale* R.) is one of the spices that has long been cultivated in Indonesia and has high economic value as a spice, essential oil, aromatic scent, and medicinal herb.[1] Ginger is one of the important export commodities and ingredients of traditional medicine as well as of phytopharmacy that has been commonly used in the herbal medicine industry in Indonesia.[2] Ginger oil contains gingerol, shogaol, and zingerone. These compounds have pharmacological and physiological effects such as antioxidant, anti-inflammatory, analgesic, anticarcinogenic, nontoxic, and mutagenic effects.[3]

The conventional process for extracting ginger oil is not efficient; it requires a large quantity of rhizomes and solvents, as well as vast agricultural fields and a lot of time.[4] Rhizome production is highly influenced by climate and also influenced by the age of *Merah* ginger.[5]

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One alternative to fulfill the needs of phytopharmacy for medicinal herbs and spices is by developing tissue culture techniques through metabolite biosynthesis processes.[6] The production of secondary metabolites through plant tissue culture processes is a more effective way to improve these contents than conventional methods.[7]

Some of the advantages of using plant tissue culture techniques for the production of secondary metabolites include: not depending on environmental factors such as climate, pest, geographic, and seasonal constraints; the production system can be regulated, where production is carried out when needed and in the desired amount; quality and production results are more consistent and reduce land use.[8]

To produce secondary metabolite compounds in ginger, a profiling process was done to investigate the content of gingerol, shogaol, and zingerone in three varieties of ginger that was grown on Murashige and Skoog medium.[9] The purposes of this research are: to investigate the quantity and quality of callus of the three varieties of ginger and to obtain callus from the three varieties of ginger that can produce the best composition of ginger oil.[10]

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METHOD OF RESEARCH

Time and Location of Research

The research was done in the Laboratory of Tissue Cultures in the Department of Agriculture of Wijaya Kusuma University, Surabaya. This research was carried out from early January 2018 to December 2018.

Experimental Design

The method used a complete randomized design on three varieties of ginger (*Gajah, Emprit,* and *Merah*); within each repetition, there were 10 test units with four test samples. The designations were: V1= *Gajah,* V2= *Emprit,* and V3= *Merah*. After incubation of callus on MS medium for 3 months, profiling was done to determine the composition of gingerol, shogaol, and zingerone in the callus.

Media and Planting

The MS medium used was a medium package created by the Bunga Harapan florist shop that produces various tissue culture media according to the researchers' orders. This medium was not made in the research laboratory. In this study, the researchers modified it by only adding 5 ppm NAA and 3.5 ppm BAP.

The explants used were the rhizomes of ginger plants from the start of planting until they grow rhizomes at around 12–14 months. After that time, at around 2 months, rhizomes were taken as planting material. The rhizomes used were sliced or cut near the buds. Explants were sterilized with 0.1% HgCl for 1 min, and 20% Clorox was added with 1 drop of Tween for 5 min; the process continued with 10% Clorox + 1 drop of Tween for 10 min and 5% Clorox + 1 drop of Tween for 20 min, then rhizomes were rinsed 3 times with sterilized water. Afterward, the explants were cut to approximately 0.5 cm^2 and were planted in culture tubes in the prepared medium.

Data Observation

Observations were done every week by visually observing the growth of callus:

- Quantity of Callus

Visually observed once a week by scoring of:

- 1 = score when there is no callus on explants measuring 0.5 cm^2
- 2 = score when the explant has started to grow callus that is smaller than the explant
- 3 = score when the explant grows callus which is around 1–2 times the size of the explant
- 4 = score when the explant has grown callus more than twice the size of the explant

- Quality of Callus

Visually observed every week by scoring of:

- 1 = no callus
- 1–2= compact callus
- 2–3= friable callus.

A callus with a score between 1 and 2 is a compact callus, which is dense and slow growing and which contains more secondary metabolites. A callus with a score between 2 and 3 is callus that is friable, tends to grow faster and is better used for vegetative propagation.[11]

Composition of Secondary Metabolites

Extraction of gingerol, shogaol, and zingerone from ginger callus was carried out by the Industrial Research and Consultation Agency (Badan Penelitian dan Konsultasi Industri [BPKI]) of the Research Laboratory and Research and Consultation Center, Jalan Ketintang Baru XVII No. 14, Surabaya, East Java. Compounds were extracted and visually observed using a spectrophotometer.

Gingerol analysis

Callus was weighed and dried until the maximum moisture content was 10%. The callus was distilled, the steam flowing through the cooler so that the vapor melted; this vapor was stored in a separating flask. The distillation was carried out until the aroma of ginger oil was disappear. The obtained distillate was put into a separator flask in which gingerol was obtained.

Shogaol analysis

Dried callus was put into a separating flask with alcohol and benzene solvents (1:50) for 24 h at a temperature of 40–60ºC. Liquid was separated from callus residue with Whatman 40 filter paper so that a clear filtrate was obtained. The absorbance value of filtrate was measured at a wavelength of 212 nm; shogaol was measured using a calibration curve.

Zingerone analysis

To analyze zingerone, the same stages were used as for shogaol analysis, except that the solvent was benzene, ether, and ethanol (1:1:1), and the wavelength was 240.5 nm.

Data Analysis

Univariate data for callus quantity and quality were analyzed using one-way ANOVA with SPSS 18; if significant differences were found, analysis continued by least significant difference test at 5%.

RESULTS OF RESEARCH

Quantity of Callus

In Table 1, the results show that there was a significant difference in the growth of callus from the 5th week. It seems that callus growth on *Gajah* ginger is faster than growth on *Emprit a*nd *Merah* ginger.

Quality of Callus

Table 2 shows that at week 7 there was no significant difference in quality, even though it appeared that

Table 1: Quantity of ginger callus for Gajah, Emprit and Merah varieties.

Treatment	Average/week after planting											
	1	2	3	4	5	6	7	8	9	10	11	12
Gajah	1	1	1	1	1.78[a]	1.80[a]	1.80[a]	1.95[a]	1.95[a]	2.53[a]	2.53[a]	2.81[a]
Emprit	1	1	1	1	1.46[b]	1.46[b]	1.46[b]	1.46[b]	1.46[b]	1.60[b]	1.60[b]	1.64[b]
Merah	1	1	1	1	1.40[b]	1.42[b]	1.45[b]	1.45[b]	1.49[c]	1.57[c]	1.61[b]	1.60[b]
LSD 5%	NS	NS	NS	NS	0.09	0.10	0.11	0.11	0.11	0.12	0.12	0.15

Average values followed by the same letter in the same column indicate significant indifference based on LSD 5% test. NS=non-significant.

Table 2: Quality of ginger callus for Gajah, Emprit and Merah varieties.

Treatment	Average/week after planting											
	1	2	3	4	5	6	7	8	9	10	11	12
Gajah	1	1	1	1	1.48	1.53	1.69	1.75	1.75[a]	2.22[a]	2.65[a]	2.88[a]
Emprit	1	1	1	1	1.52	1.55	1.64	1.72	1.72[b]	1.72[b]	1.74[b]	1.74[b]
Merah	1	1	1	1	1.45	1.48	1.50	1.48	1.48[c]	1.59	1.63[c]	1.63[c]
LSD 5%	NS	NS	NS	NS	NS	NS	NS	NS	0.18	0.21	0.15	0.14

Average values followed by the same letter in the same column indicate significant indifference based on LSD 5% test. NS=non-significant; LSD=least significant difference (method of Ronald Fisher).

Gajah and *Merah* ginger quality was highest and lowest, respectively. Due to the values were not significantly different, there is no statistic letter notation. In the 12th week, *Gajah* ginger callus tended toward friable callus while the *Emprit* and *Merah* ginger callus tended toward compact callus.

Composition of Secondary Metabolites

Profiles of gingerol, shogaol, and zingerone in the three varieties of *Gajah, Emprit,* and *Merah* gingers are shown in Tables 3-5.

From the results of profiling, the composition of the secondary metabolites of *Emprit* ginger was the best, containing 1.181% gingerol, 0.118% shogaol, and 0.098% zingerone. In comparison, the composition of secondary metabolites in the *Gajah* variety was 0.150% gingerol, 0.130% shogaol, and 0.056% zingerone, and in the *Merah* variety was 0.170% gingerol, 0.096% shogaol, and 0.047% zingerone.

Table 3: Gajah ginger profile

Code	Gingerol %	Shogaol %	Zingerone %
V1U5S1	0.150	0.130	0.056
V1U9S2	0.136	0.127	0.050
V1U4S3	0.128	0.120	0.054
V1U10S4	0.132	0.125	0.050
V1U2S1	0.126	0.126	0.051
V1U8S3	0.145	0.125	0.050
V1U6S2	0.118	0.128	0.052
V1U7S4	0.127	0.126	0.050
V1U1S2	0.143	0.125	0.056
V1U3S1	0.116	0.120	0.055

Table 4: Emprit ginger profile

Code	Gingerol %	Shogaol %	Zingerone %
V2U8S1	0.181	0.118	0.098
V2U5S2	0.176	0.110	0.080
V2U7S3	0.780	0.105	0.076
V2U10S4	1.742	0.112	0.082
V2U9S4	1.800	0.106	0.086
V2U1S3	1.765	0.110	0.085
V2U3S2	1.702	0.115	0.080
V2U6S1	1.748	0.108	0.078
V2U2S2	1.752	0.112	0.075
V2U4S4	1.738	0.106	0.070

Table 5: Merah ginger profile

Code	Gingerol %	Shogaol %	Zingerone %
V3U10S1	0.170	0.096	0.047
V3U7S3	0.164	0.085	0.038
V3U3S4	0.160	0.091	0.040
V3U9S2	0.162	0.088	0.035
V3U4S1	0.165	0.090	0.030
V3U5S2	0.163	0.086	0.040
V3U8S4	0.168	0.078	0.036
V3U2S3	0.165	0.081	0.038
V3U6S1	0.166	0.075	0.040
V3U1S4	0.160	0.080	0.035

DISCUSSION

Quantity of Callus

Table 1 shows that callus growth started in observation week 5; there were significant differences in the quantity of callus grown. Growth on *Gajah* ginger was faster than that on *Emprit* and *Merah* ginger. Explants as a plant commodity and their compatibility with the medium are factors that support the growth of callus.

The composition of the medium also affects growth, along with the research treatments. Therefore, in this research, callus quantities were determined by the treatment of explants of ginger varieties. There were more calluses on the *Gajah* ginger variety than on the *Emprit* and *Merah* ginger varieties. Furthermore, it obtained that the growth of callus on *Emprit* ginger was slower because the energy for its growth was used to form secondary metabolites, as the content of these secondary metabolites was highest.[11] Arijanti *et al.*

stated that a plant medium should contain all nutrients needed to ensure the growth of explants.[12]

Quality of Callus

The quality of callus was determined by the characteristics of callus produced by the explants. According to Wattimena, callus can be divided into two types: compact callus and friable callus.[13] Compact callus is compact and indestructible if pinned by a needle, while friable callus tends to grow into new individuals.[14] In this research, the callus of *Gajah* ginger was friable, while the callus of *Emprit* and *Merah* gingers was compact. The growth was different due to the specific responses to the media and treatments.[12]

In Table 2, it can be seen that by week 9 there was a significant difference in the quality parameters of the calluses. In week 12, *Gajah* ginger callus tended to be friable, while *Emprit* and *Merah* ginger callus was compact. Based on callus quality, it was found that the *Emprit* variety has a compact size that produces more secondary metabolites than the more friable *Gajah*. This is in accordance with Rahmawati who examined yam bean (*Pachyrhizus* sp.) and also found that compact callus has more secondary metabolites than friable callus.[11]

Content of Secondary Metabolites

Tables 3-5 show the secondary metabolite composition of the ginger callus (gingerol, shogaol and zingerone), analyzed by the BPKI of the *Laboratorium Penelitian dan Konsultasi Penelitian*, Surabaya, Jawa Timur. The results show that *Emprit* ginger had the best composition of gingerol, shogaol, and zingerone (only the zingerone level was slightly below that of *Gajah* ginger).

Secondary metabolites can be formed by a biosynthesis process of the plant's cells. Vickery and Vickery showed a biosynthesis map of carbohydrates that produce secondary metabolites of terpenoid ginger oil.[15] The production of these secondary metabolites by developing plant tissue cultures is very much affected by some factors, such as:

a. Genetic factors, and
b. Environmental factors outside the culture itself.[16]

These factors vary depending on the type of culture used and can be manipulated to produce the desired compounds in large quantities. The expression of secondary metabolite compounds depends on the stages of development of the organism that produces them. Cell differentiation determines the synthesis of the compounds.[13]

The composition of the medium also plays a factor in determining the success of the tissue culture technique. Differences in medium composition can produce different growth and development of explants *in vitro*.[17] Zenk *et al*. conducted a study on *Morinda citrifolia* plants to produce the secondary metabolite anthraquinone.[18] They used a treatment with genes from this plant and succeeded in increasing anthraquinone production by 10 times.

Ernawati *et al*. conducted research on the plant *Polygonum tinctorium*; transgenic treatment in these plants can produce the secondary metabolite anthocyanin and successfully increase secondary metabolites by 8 times.[19] From the two results above, it can be seen that genetic factors influence the production of secondary metabolites. In *in vitro* cultures, the production of secondary metabolite compounds is also associated with cell differentiation or the tissues cultured. In conclusion, to gain secondary metabolite compounds in large quantities using the tissue culture technique, the explants planted should be directed to form callus.[20]

CONCLUSION

The conclusion of this research is as follows:

- The quantity of callus was best on *Gajah* ginger.
- Callus on *Gajah* ginger tended to be friable, while *Emprit* and *Merah* ginger callus tended to be compact.
- The gingerol, shogaol, and zingerone contents were best on *Emprit* ginger.
- It is recommended to conduct further research into stimulating an increase in the composition of ginger oil, for example, by the addition of biotic and abiotic elicitors.

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