



Essential Oil Components, Metabolite Profiles, and Idioblast Cell Densities in Galangal (*Kaempferia galanga* L.) at Different Agroecology

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ABSTRACT

Galangal is widely cultivated for the multifunctional plant. This study analyzed the essential oil chemical components, metabolite profiles, and idioblast cell densities in seven galangal accession grown at two different altitudes (locations A and B). The galangal accessions included: Purbalingga, Cilacap, Purworejo, Karanganyar, Pacitan, Madiun, and Galesia 2 which was used as the control. The results showed that the highest essential oil content was obtained from MAD (3.22%) at location A. The highest levels of ethyl-p-methoxycinnamate (EPMC) were obtained from PBG (74.8%) at location B and PCT (71.6%) at location A. The metabolite profiles of the galangal rhizomes were divided into two clusters based on the metabolite content. The first cluster had one accession: PCT from location A with genkwanin as the metabolite marker. The second cluster consisted of CLP from location A and PBG, MAD, and GAL2 from location B. The highest density of idioblast cells was found in PCT (90.5 cells/mm²) at location A and PBG accessions (77.1 cells/mm²) at location B. The PBG and PCT accessions can be recommended as a candidate of superior varieties based on their high EPMC content.

INTRODUCTION

The Zingiberaceae family is a group of plants that are widely used as raw materials for traditional medicines, herbs and spices, food coloring, cloth, antimicrobials, insecticides, and in the food industry (de Souza Tavares et al., 2013; Gowda, Kress, & Htun, 2012; Harit, Barapatre, Prajapati, Aadil, & Senapati, 2013; Jan, Rabbani, & Shinwari, 2012; Tripathi, Chawla, Upadhyay, & Trivedi, 2013; Velayudhan, Dikshit, & Abdul Nizar, 2012). One potential medicinal plant from this family that has been widely cultivated is galangal (*Kaempferia galanga* L.). As a medicinal plant, galangal contributes 6.33% of biopharmaceutical plants in Indonesia (Salim & Munadi, 2017), and has been used in herbal medicine with proven efficacy and safety based on clinical trials equivalent to modern medicine trials (Pujiasmanto, 2009).

Galangal productivity in Indonesia in 2017 was 1.77 kg/m² and decreased in 2018 and 2019 by 1.46 kg/m² and 1.45 kg/m², respectively (BPS, 2019). Galangal productivity in the three main producing center provinces in West Java, Central Java, and East Java in 2019 only reached 4.26 kg/m², which was still lower than ginger and turmeric at 5.77 kg/m² and 6.82 kg/m², respectively. Domestic galangal production has not currently met the market demand in Indonesia for the drug and cosmetic industry, which has caused the product to be imported from China, Malaysia, Thailand, India, Vietnam, Pakistan, and Myanmar, even though the imported quality does not necessarily meet the standards for large industrial production in Indonesia (DINPERINDAG JATENG, 2014; Putri et al., 2014). The increasing market demand for galangal products, coupled with the plant's ability to grow over a wide area encourages farmers to cultivate this plant in new

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potential areas.

The selection of new cultivation areas must consider the ever-changing agroecological conditions of each location, to produce the desired yield and quality of medicinal plants (Purwadi, 2011). Rostiana, Rosita, & Rahardjo (2009) stated that galangal optimally grows at an altitude of 50–600 m asl with the range daily temperature of 25–30°C, 2500–4000 mm/year rainfall, and full sunlight or 25–30% shading up to the plant reached 6 months old. Galangal prefers soil with good drainage, clay to sandy loam texture, and soil pH ranges 5.5–6.5. Further information regarding the suitability of different agroecological conditions is needed to support the expansion of galangal production. For example, altitude differences alter environmental conditions, such as sunlight intensity, air temperature, wind, and rainfall (Unal, Guvensen, Dereboyulu, & Ozturk, 2013).

The essential oil and ethyl-p-methoxycinnamate (EPMC) contents are quality parameters that can be measured by analyzing galangal rhizomes, and vary widely in various environments. The EPMC is a galangal identity compound derived from cinnamic acid derivatives that contributes to the distinctive taste and plant aroma (Tripathi, Chawla, Upadhyay, & Trivedi, 2013). The chemical contents and essential oil components of galangal vary depending on the genotype and planting location. This indicates an interaction between genetic traits and a growing environment is expressed by the chemical components and active ingredients. EPMC levels have been measured at 2.27–17.92% at optimally harvested rhizomes, after 10–12 months of planting (Rostiana & Subaryanti, 2010). The chemical components of other galangal essential oils are pentadecane, 1,8-cineole, 3-carene, borneole, camphene, kaempferol, kaempferid, cinnamaldehyde, p-methoxycinnamic acid, pinene, thymol, methyl cinnamate, and ethyl cinnamate (Munda, Saikia, & Lal, 2018; Srivastava et al., 2019; Yang et al., 2018).

Cultivar selection is expected to produce potential genotype that has wider adaptation to suboptimal conditions (Cramer, Urano, Delrot, Pezzotti, & Shinozaki, 2011; Ncube, Finnie, & Van Staden, 2012; Pavarini, Pavarini, Niehues, & Lopes, 2012; Ramakrishna & Ravishankar, 2011). Anggraito et al. (2018) has stated that a genotype when grown in different environments will produce different secondary metabolite compounds.

Metabolomics aims to identify and quantify metabolite compounds in an organism under certain conditions (Arbona, Manzi, de Ollas, & Gómez-Cadenas, 2013; Kusano et al., 2015). The metabolomic approach has been widely used as a tool for studying organisms subjected to biotic and abiotic stress and offers a more comprehensive analysis of metabolite profiles when plant metabolic conditions change (Widodo et al., 2009). Various strategies are used to detect and measure the presence of metabolite compounds, including liquid chromatography-mass spectrometry (LC-MS). Metabolite profile analysis through LC-MS is carried out in the liquid phase due to its wide detection ability (Obata & Fernie, 2012), and its ability to detect secondary metabolite groups in plants (Sangwan et al., 2015). For example, the results of LC-MS analysis on *Curcuma aeruginosa* rhizomes detected 175 compounds, which were dominated by the sesquiterpenes group and were divided into two clusters based on their antioxidant activity (Septaningsih, Darusman, Afendi, & Heryanto, 2018). Growth stage, environment, plant material, and post-harvest processes are the main factors that influence the biosynthesis and composition of metabolites in rhizomes (Yudthavorasit, Wongravee, & Leepipatpiboon, 2014).

Secondary metabolite compounds, such as essential oils, resins, mucilages, and tannins, are stored in idioblast cells (Victório, Kuster, & Lage, 2011), which are contained in a tissue that is different in shape, size, content, and function from the surrounding cells (Nugroho, 2018). Oil-filled idioblasts in the rhizome of *Zingiber officinale* are located in pith tissue (Liu, Specht, Zhao, & Liao, 2020). Oil cell-containing idioblasts have also been reported in the rhizome of *Alpinia zerumbet* (Victório, Kuster, & Lage, 2011). The number of oil cells increases with plant age, as seen in the adult rhizomes of *Zingiber officinale* (10–12 MAP), which had more essential oils than the younger plants did (Mu, Liu, Kuang, Zou, & Liao, 2015). Similar results were also reported by Liu, Specht, Zhao, & Liao (2020), that the amount of starch decreased in mature rhizomes, while the number of oil cells increased.

This study aimed to assess and analyze the chemical components of galangal rhizome essential oils, obtain metabolite profiles from selected galangal accessions, and analyze the density of idioblast cells of seven accessions of galangal. The selection was based on the most promising

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accession in producing EPMC constituents when planted under different agroecological conditions. The results are expected to open up opportunities for further researches related to secondary metabolites in galangal rhizomes, especially PBG and PCT accessions as candidates for superior varieties.

MATERIALS AND METHODS

Time and Locations

The planting materials were gathered from Central Java and East Java from December 2016 to April 2017. The experiment was carried out in two different sites, i.e. at the altitude of 214 m a.s.l. (location A), and at 780 m a.s.l. (location B) from June 2017 until May 2018. Climatic conditions and soil fertility of both locations were presented in Table 1 and Table 2. The isolation of essential oils was carried out at the testing laboratory of the Indonesian Spice and Medicinal Crops Research Institute (ISMCR), Cimanggu, Bogor, Indonesia. Chemical components of galangal rhizome essential oils were determined using gas chromatography-mass spectroscopy (GC-MS) and analyzed at the DKI Jakarta Regional Health Laboratory, Rawasari, Central Jakarta, Indonesia. Metabolite compound profiles were analyzed using liquid chromatography-mass spectroscopy (LC-MS) at the Advanced Laboratory of IPB University. Idioblast cell density was analyzed at the Laboratory of Ecology and Plant Resources, Department of Biology, Faculty of Mathematics and Natural Science, IPB University.

Plant Materials and Instruments

Six accessions of galangal: Purbalingga (PBG), Cilacap (CLP), Purworejo (PWJ), Karanganyar (KRA), Pacitan (PCT), Madiun (MAD), and one control variety of Galesia 2 (GAL 2) were studied. The origin of galangal accessions used is listed in Table 3. GC-MS (Agilent Technologies 7890-5975, Germany), LC-MS (Thermo Scientific, Germany), and a light microscope (Olympus CX23, Japan) with a digital camera (OptiLab, Mikonos) were used for analysis.

Table 1. The average climatic conditions at the two locations at 214 and 780 m a.s.l. from June 2017 to May 2018

Location	Altitude (m a.s.l.)	Light intensity (%)	Temperature (°C)	Relative humidity (%)	Rainfall (mm/year)	The amount of rain (day/year)
A	214	21-64	27-28	76-86	3605	243
B	780	26-70	21-23	79-93	3276	252

Remarks: Source = The Climatology Station at Darmaga Bogor; Value indicates lowest-highest.

Experimental Design

This study was designed in a split-plot randomized block experiment with 3 replications. The growing locations in different altitudes (214 and 780 m a.s.l.) served as the main plot, and galangal accessions were designated as the subplot. The number of experimental units was 54 plots and the size of each plot was 2.4 x 1.2 m. The plant density on each plot was 60 plants with a planting distance of 15 x 20 cm.

Research Implementation

The planting materials were shiny skin, slightly hard meat textures, healthy rhizomes, and free from pest and disease and physical defects. The planting materials should also have 3–9 g in weight, 2–3 shoots with a height less than 1 cm. Galangal seedlings were planted at the depth of 7 cm with the shoots facing upward. Manure (20 t/ha), urea (250 kg/ha), SP 36 (200 kg/ha), and KCl (200 kg/ha) or the equivalent of 8.64 kg/plot of manure, 90 g/plot urea, 70 g/plot SP 36, and 70 g/plot KCl were applied to the soil. Galangal cultivation techniques were following Rostiana, Rosita, & Rahardjo (2009). Harvesting was carried out 12 months after planting.

Essential oil isolation was carried out based on Bhuiyan, Begum, & Anwar (2013) using the hydrodistillation method. Essential oil components were analyzed using GC-MS, and metabolite profiles were analyzed using LC-MS (Han, Lee, Kim, & Lee, 2015). Idioblast cell density analysis was performed based on methods published by Subaryanti (2005). The fresh rhizome was sliced using a razor blade, then immersed in 5% NaOH for 48 hours. After rinsing with distilled water, the rhizome slices were stained with 0.03% Sudan IV, then 1–2 drops of glycerin were given. The density of the idioblast cells was calculated using a light microscope. The calculation was carried out in four fields of view with three replications. The formula for the number of idioblast cells obtained was divided by the field of view (cells/mm²).

Table 2. The soil fertility condition at two locations

Location	Soil texture (%)			C/N ratio	pH	CEC (MEK/g)
	Sand	Dust	Clay			
A	9.7	10.3	80.1	6.8	5.0	18.8
B	9.9	28.7	61.4	6.0	5.4	19.7

Table 3. Accessions of galangal used in this study based on their altitudes

No.	Accessions	Village	District	Regency	State	Altitudes (m asl)
1	PBG	Bedagas	Pengadegan	Purbalingga	Central Java	100
2	CLP	Tayem	Karangpucung	Cilacap	Central Java	100
3	PWJ	Kaliboto	Bener	Purworejo	Central Java	300
4	KRA	Sambirejo	Jumantono	Karanganyar	Central Java	300
5	PCT	Ngunut	Bandar	Pacitan	East Java	600
6	MAD	Doho	Dolopo	Madiun	East Java	600
7	GAL 2	Balittro	Balittro	Balittro	West Java	-

Data Analysis

The data were analyzed by analysis of variance at 95% confidence level using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The mean comparison was performed using Duncan's Multiple Range Test at $\alpha = 5\%$ (Mattjik & Sumertajaya, 2006). The essential oil components of the GC-MS chromatogram were identified and compared with spectroscopic data available in the Wiley W9N11.L database. Identification of metabolites from the LC-MS chromatogram was carried out by matching the m/z value in the mass array table with the accurate mass of metabolites from the Zingiberaceae family in the Natural Products Dictionary (dnp.chemnetbase.com). The identification phase of LC-MS was based on an online database and a database that is independently compiled. The online database for metabolite compounds in the Zingiberaceae family refers to the Kyoto Encyclopedia of Genes and Genomes Pathway Database (<http://www.genome.jp/kegg/pathway.html>), Pubchem (<https://PubChem.ncbi.nlm.nih.gov/>), and The Human Metabolome Database (HMDB) (<http://www.hmdb.ca/>). The independently compiled database refers to metabolite compounds found in the Zingiberaceae family through LC-MS detection which have been reported in several scientific publications and have been summarized in this study (Asamenew *et al.*, 2019; Han, Lee, Kim, & Lee, 2015). Multivariate analysis was performed using the MetaboAnalyst 4.0 program (<http://www.metaboanalyst.ca>) (Xia & Wishart, 2016).

RESULTS AND DISCUSSION

General Conditions of the Planting Locations

Based on the combined environmental condition data from June 2017–May 2018, the air temperature at location A ranged from 27–28°C, relative humidity was 76–86%, light intensity was 21–64%, total rainfall was 3605 mm/year, and the number of rainy days per year was 243. At location B, the air temperature was 21–23°C, relative humidity was 79–93%, light intensity was 26–70%, total rainfall was 3276 mm/year, and the number of rainy days per year was 252 (Table 1).

In general, the agro-climatic conditions of the planting sites at the two locations were quite good. Air temperature and light intensity influenced the growth and development of galangal accessions. The air temperature at location B was lower than the air temperature at location A, but the light intensity at location B was higher than the light intensity at location A, which was not optimal. Soil conditions influenced rhizome yield and the number of rhizomes. The results of soil analysis were listed in Table 2. The organic carbon content at location A was classified as mid-level but was low at location B. The total N and C/N ratios at both locations were low. The CEC value was moderate and the soil texture was classified as clay and dusty. According to Rostiana, Rosita, & Rahardjo (2009), high yield galangal production requires special soil requirements: textured clay to sandy loam, land slope < 3%, with latosol, regosol soil types, associations between latosol-andosol, regosol-latosol, and regosol-lithosol, and soil pH

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5.5–6.5. The land must also be free from disease, especially bacterial wilt. Soil conditions at the two research locations were good enough to support the growth of galangal accessions.

The Content and Yield of Essential Oils of Galangal

One indicator of the quality of galangal rhizomes is the high content of essential oils. Essential oils (volatile oil) are a complex mixture of volatile and non-volatile organic compounds from plants that have a distinctive smell and taste (Sirousmehr, Arbabi, & Asgharipour, 2014; Tisserand & Young, 2014). Essential oils are also known as etheric oils (aetheric oils), and aromatic oils. Essential oils are a large group of vegetable oils that are viscous at room temperature, volatile, and give a distinctive aroma. Essential oils are generally colorless to pale yellow (Moghaddam & Mehdizadeh, 2017), and are widely used in health products, cosmetics, and perfume, as well as in the pharmaceutical and the food and beverage industry (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). Essential oils also provide physiological and ecological functions for plants, including protection against predators and pests, attracting pollinators, strengthening the immune system, and being used for the interaction and communication between organisms and their environment (Bouwmeester, Schuurink, Bleeker, & Schiestl, 2019; Meena, Jangra, Wadhwa, Monika, & Wati, 2017).

The content and yield of essential oils were significantly influenced ($p < 0.05$) by the interaction between location and galangal accessions (Table 4). The essential oil content from location A was 0.98–3.22% and 1.33–2.80% at location B. The yield of essential oil at location A was 0.50–2.67% and 1.25–5.12% at location B. The highest essential oil content was obtained at both locations A and location B from MAD (3.22%) and PBG (2.80%), respectively. The highest yield was obtained from PBG (5.12%) at location B. MAD accession in location A is different from other accessions, except with GAL 2, meanwhile, PBG accession in location B is different from KRA accession. The essential oil contents that met the quality standards of SNI and MMI included MAD and GAL 2 from location A, and PBG, CLP, PCT, and MAD from location B. The high content and yield of essential oils of galangal rhizome in the two planting locations varied greatly but met the Indonesian National Standard, $> 2\%$ (BSN, 2005),

and the Indonesian Materia Medika standard of 2.4–3.9% (DEPKES RI, 1977) was MAD and GAL 2 at location A and PBG, CLP, PCT, and MAD at location B. Our findings are consistent with those of Armando (2009), who found that the yield of essential oils from galangal rhizomes was 2.8–5.8%. Karami, Khoshbakht, Esmaeili, & Maggi (2020) reported that the quantity and quality of *Oliveria decumbens* essential oils are affected by environmental factors such as altitude, temperature, latitude, and soil properties such as soil acidity and sand content.

The high essential oil content (3.22%) of the MAD accession at location A is predicted to be related to nutrients, particularly the organic carbon content of the soil since the organic carbon content at location A was higher than at location B (2.74 and 1.76%, respectively) (Table 2). Yulipriyanto (2010) reported that the availability of organic carbon in the soil increases microorganism populations since microorganisms thrive when organic matter is available as a food source. Soils richer in organic matter and minerals produced higher essential oil yields (Fernández-Sestelo & Carrillo, 2020). Soil microelements are very important for the optimal sustainability of enzymatic processes (Purwoko, 2007). The availability of nutrients, especially organic carbon content, will also increase the essential oil levels as secondary metabolites (Widyastuti & Sugiarso, 2003). Suryawati & Murniyanto (2011) reported that the organic carbon content increases the essential oil content in the ginger rhizome (*Zingiber officinale*). In addition to nutrients (organic carbon), adequate rainfall (130–526 mm/month) at location A was also responsible for the high levels of essential oils in the MAD accessions. A greater amount of annual rainfall produced a higher essential oil yield and of better quality (Fernández-Sestelo & Carrillo, 2020). Water available in the soil is absorbed by the roots through xylem vessels, then is transported to the stems and leaves. In the leaves, water is used to synthesize organic compounds such as carbohydrates, fats, proteins, and other organic materials (Lakitan, 2018; Taiz & Zeiger, 2010).

Several other factors also affect the levels of the essential oils, such as soil fertility, genetic variation, growing environment, and geographic conditions (Aragaw, Alamerew, Michael, & Tesfaye, 2011; Bermawie, Syahid, Ajijah, Purwiyanti, & Martono, 2013; da Costa *et al.*, 2014; Kavitha & Menon, 2013; Preetha, Hemanthakumar, & Krishnan, 2016; Rostiana & Subaryanti, 2010).

Table 4. The essential oil content and yield of galangal accessions at different locations

Location	Accessions	Essential oils (%)	Yield (%)
A (214 m a.s.l.)	PBG	1.40 c-f	1.77 b-d
	CLP	0.98 f	0.53 e
	PWJ	1.06 ef	0.65 e
	KRA	1.17 d-f	0.50 e
	PCT	1.01 f	2.38 bc
	MAD	3.22 a	2.67 b
	GAL 2	2.31 a-e	2.51 bc
	Means	1.59	1.57
B (780 m a.s.l.)	PBG	2.80 ab	5.12 a
	CLP	2.22 a-f	2.16 b-d
	PWJ	1.76 b-f	1.71 cd
	KRA	1.33 c-f	1.25 de
	PCT	2.53 a-c	2.67 b
	MAD	2.37 a-d	2.35 bc
	GAL 2	1.66 b-f	2.11 b-d
	Means	2.09	2.48

Remarks: Numbers followed by the same letters on the same column are not significantly different based on DMRT 5%

The control variety, GAL 2, also had high essential oil levels at location A (2.31%). GAL 2 is a superior variety that is highly responsive and more stable in a variety of growing environments. GAL 2 also adapts specifically to growing environments with an altitude of 350–650 m asl, and its essential oil content usually reaches 2.06–6.64% (Rostiana & Effendi, 2007; Rostiana, Haryudin, & Rosita, 2006). The high essential oil content from the PBG accession at location B is thought to be related to genetic factors, light intensity, and air temperature. The light intensity at location B was 26–70% higher than at location A, which was 21–64% (Table 1). Light as an energy source activates the photosynthetic process; the photosynthate produced from the photosynthetic process is then translocated to the rhizome as a storage organ (sink) (Buntoro, Rogomulyo, & Trisnowati, 2014). Photosynthate in the form of starch is not always used as energy for growth (primary metabolite), but can also be converted into other secondary metabolites, such as essential oils (Widiyanto & Siarudin, 2013). Thus, the light intensity can increase essential oil levels (Rehman, Hanif, Mushtaq, Mochona, & Qi, 2016), especially since galangal requires 25–30% full light intensity or shade until the plants are 6

months old (Rostiana, Rosita, & Rahardjo, 2009). In turmeric plants (*Curcuma domestica*), 70% light intensity produced the highest amount of essential oil contents (4.77%) (Syahid, Syukur, Kristina, & Pitono, 2012). Since the PBG accessions have been cultivated for long periods in their place of origin (Purbalingga) at an altitude of 100 m asl, it is thought to have been able to adapt to the test location altitude of 780 m asl and temperature of 21–23°C. Fernández-Sestelo & Carrillo (2020) reported that higher altitudes favored obtaining higher essential oil quality.

The optimal altitude for galangal growth is 50–600 m asl, at a temperature of 25–30°C (Rostiana, Rosita, & Rahardjo, 2009). Long light exposure accompanied by low air temperatures results in maximum photosynthate production in the form of carbohydrates (Yaqoob & Nawchoo, 2017), which are used as a substrate for the formation of essential oils through glycolysis. Glycolysis produces pyruvic acid, which undergoes various reactions to become geranyl pyrophosphate, a precursor compound of essential oils (Croteau, Kutchan, & Lewis, 2000). In *Porcelia macrocarpa*, maximum essential oil levels were obtained at low temperatures (da Silva *et al.*, 2013).

Table 5. The components of essential oil of galangal rhizome at different altitudes from GC-MS analysis in percent area

No.	Compounds	The essential oil content of accessions in locations																	
		PBG		CLP		PWJ		KRA		PCT		MAD		GAL 2					
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B		
1	EPMC	27.97	74.77	59.90	30.89	13.28	43.72	12.51	54.01	71.62	48.36	28.84	39.76	38.46	43.11				
2	<i>Ethyl cinnamate</i>	-	5.39	-	-	8.51	9.71	8.90	4.95	5.17	9.40	9.15	-	8.16	-				
3	<i>3-carene</i>	3.83	-	-	2.36	-	-	-	9.13	-	-	3.40	-	7.95	1.13				
4	<i>4-tetradecene</i>	-	-	-	-	-	1.82	-	1.65	-	-	-	-	-	2.19				
5	<i>1,8-cineole</i>	1.22	-	-	-	-	-	-	-	-	-	1.40	-	1.33	-				
6	<i>Naphthalene</i>	1.51	-	-	-	-	-	-	-	-	-	-	-	-	-				
7	<i>Cinnamic acid</i>	-	-	-	-	-	-	-	-	-	-	-	12.33	-	11.68				
8	<i>Endo-borneole</i>	-	-	-	-	-	-	1.68	1.04	-	-	-	-	-	-				
9	<i>Trans-geraniol</i>	-	-	-	-	-	-	2.54	-	-	-	-	-	-	-				
10	<i>2-propenoic acid</i>	9.02	-	7.73	12.99	-	-	-	-	-	-	-	-	-	-				
11	<i>Pentadecane</i>	17.54	8.10	8.80	17.44	31.66	17.62	26.49	6.79	26.49	-	36.98	16.93	13.74	17.43				
12	<i>8-heptadecane</i>	-	-	-	-	1.72	-	-	-	-	-	1.79	-	1.23	-				
13	<i>Patchouli alcohol</i>	-	-	1.87	-	-	-	-	-	-	-	2.99	-	1.47	-				
14	<i>γ-muurolene</i>	-	-	-	-	-	-	4.30	1.79	4.30	-	1.19	-	-	-				
15	<i>Germacrene-B</i>	-	-	-	-	-	-	1.23	-	1.23	-	-	-	-	-				
16	<i>Caryophyllene</i>	-	-	-	-	-	2.66	-	-	-	-	2.05	-	-	-				
17	<i>α-amorphene</i>	-	-	-	-	-	-	-	-	-	-	-	-	1.20	-				
	Means	10.18	29.42	19.56	15.92	13.79	15.11	8.24	11.34	21.76	28.88	9.75	23.0	9.19	15.11				

Remarks: - is not detected

Chemical Components in Galangal Essential Oil and EPMC Constituents

The chemical composition of essential oils varies greatly and can include aliphatic, aromatic, and terpenoid structures. Volatile components of essential oils are usually aromatic terpenes, aldehydes, ketones, phenols, volatile acids, and esters (Butnariu & Sarac, 2018). Several research results have indicated that galangal rhizomes contain significant EPMC aromatic compounds that can be fractionated from essential oils (Raina, Abraham, & Sivaraj, 2015; Rostiana & Subaryanti, 2010), as well as from the methanol extract of galangal rhizomes (Srivastava *et al.*, 2019; Umar *et al.*, 2012). Our findings confirm the results of these previous studies since EPMC was detected in all tested galangal accessions at both locations (Table 5). Chemical component differences in the galangal rhizomes planted at location A and B are thought to be due to differences in each accession's response to the environmental growing conditions since the chemical components are influenced by water availability and soil nutrients through a biosynthetic process related to enzyme activity (Bettaieb, Zakhama, Aidi Wannes, Kchouk, & Marzouk, 2009). Table 5 lists the 15 chemical components of galangal rhizome essential oil from location A, and the 10 compounds from location B. High levels of EPMC compounds were found at both location A (12.5–71.6%) and location B (30.9–74.8%). Accessions with the highest EPMC content from location A and location B were PCT (71.6%) and PBG (74.8%), respectively. Other components of galangal essential oils were 2-propenoic acid (7.73–12.99%), caryophyllene (2.05–2.66%), 3-carene (1.13–9.13%), α -muurolene (1.19–4.30%), patchouli alcohol (1.47–2.99%), and 1,8-cineole (1.22–1.40%). The chemical components found in galangal in China included EPMC, ethyl cinnamate, pinene, camphene, 3-carene, 1,8-cineole, borneol, encarvone, thymol, methyl cinnamate, cubenol, selinonol, bisabolol, and palmitic acid (Yang *et al.*, 2018). The essential oil components from galangal grown in India included EPMC, ethyl cinnamate, 1,8-cineole, 3-carene, dihydroterpineol, camphene, and α -terpineol (Srivastava *et al.*, 2019).

Different galangal varieties express different chemical properties, depending on the environment where they are grown. Different varieties have different production capacities and an optimal growing environment in which they should ideally

be grown (Rostiana, Haryudin, & Rosita, 2006). Chrysargyris, Mikallou, Petropoulos, & Tzortzakis (2020) reported that the effect of growing conditions defined by different environmental conditions—altitudes may significantly affect essential oil yield as well as the composition of the studied medicinal and aromatic plants (MAPs) in a species-dependent manner. These findings can be used to identify specific locations and ecosystems in which the cultivation of MAPs could be introduced for producing high added value products with improved quality and bioactive properties.

EPMC is recognized as a natural ester that is responsible for the aroma and pharmacological activities of the rhizome of galangal (Komala *et al.*, 2017; Umar *et al.*, 2012). For a long time, the rhizome of galangal has been used as a food flavoring and herbal drink in Indonesia. The herbal drink called 'beras kencur' which contains galangal as one of its ingredients is believed to be useful in increasing appetite and reducing stiffness and pain in the joints or muscles. EPMC is a monoterpene compound in the cinnamic acid group (Guzman, 2014). In plants, it plays an important physiological role in growth, development, reproduction, and disease resistance (Salvador *et al.*, 2013).

Metabolite Profile of Galangal by LC-MS

Five accessions were selected based on the abundance of EPMC content for further analysis using LC-MS. These accessions were CLP (59.9%) and PCT (71.6%) from location A, PBG (74.8%), MAD (39.8%), and GAL 2 (43.1%) from location B. The interaction of various metabolite compounds in various metabolic processes is necessary for the survival of an organism. These processes are particularly important in plants, as they are unable to move locations in times of biotic or abiotic stress. The production and concentration of metabolite compounds are determined by each plant's genetics and environmental conditions (Hardy & Hall, 2012; Weckwerth & Kahl, 2013). The metabolomic approach offers a comprehensive analysis of metabolite compound profiles under changing metabolic conditions and environmental conditions (Widodo *et al.*, 2009). Heatmap analysis combined with a hierarchical cluster analysis was performed on the five selected accessions. The results were divided into two clusters based on their metabolite content. Cluster one had one accession (PCT) with the genkwanin compound as a metabolite

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marker. Ten compounds were detected in cluster one: tectochrysin, gingerol, demethoxycurcumin, genkwanin, shogaol, phenylalanine, dihydroparadol, routine, galangin, and gingerone. Cluster two included four accessions (CLP, PBG, MAD, GAL 2), with eight compounds detected in CLP, 10 compounds in PBG, 2 compounds in MAD, and 6 compounds in GAL 2. The composition of compounds in cluster two included hydroxybenzoic

acid, tryptophan, coumaric acid, paradol, trimethoxyflavonol, curdione, gingerdiol, gingerol, bornyl acetate, caffeic acid, EPMC, retusine, gallic acid, curcumadiol, dihydroxyferulic acid, hexahydroxycurcumin, shogaol, kaempferol, nobiletin, furanodiene, galanganol, and oleamide. The marker compounds of cluster two were curdione in CLP; EPMC and turmerone in PBG; and galanganol and oleamide in GAL 2 (Fig. 1).

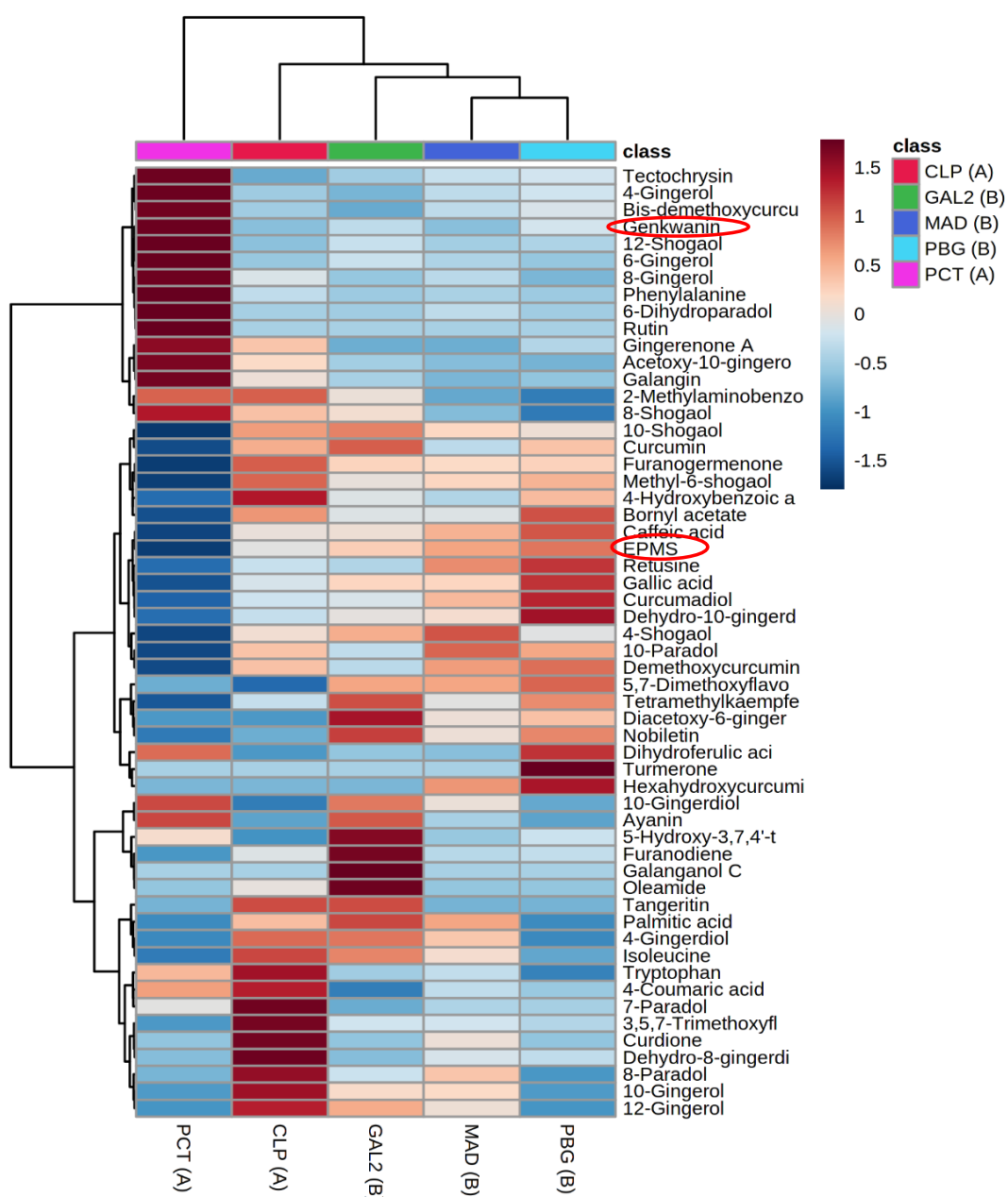


Fig. 1. The heatmap profile combined with hierarchical cluster analysis on selected galangal rhizome metabolite data at different altitudes from LC-MS analysis

Table 6. The density of idioblast cells in the cortex and pith of galangal accessions at different altitudes.

Locations	Accessions	Cortex (cells/mm ²)	Pith (cells/mm ²)
A (214 m a.s.l)	PBG	9.75 gh	77.8 b
	CLP	6.50 i	72.8 c
	PWJ	11.1 g	40.8 j
	KRA	10.5 gh	46.3 h
	PCT	9.33 h	90.5 a
	MAD	17.1 f	48.5 g
	GAL 2	3.83 j	39.6 jk
	Means	9.73	59.5
B (780 m a.s.l)	PBG	20.2 e	77.1 b
	CLP	29.6 d	59.3 f
	PWJ	31.9 c	45.3 h
	KRA	18.1 f	38.7 k
	PCT	68.3 a	66.3 d
	MAD	59.1 b	62.1 e
	GAL 2	17.7 f	42.8 i
	Means	34.9	55.9

Remarks: Numbers followed by the same letters on the same column are not significantly different based on DMRT 5%

This approach has been successful in uncovering the metabolic changes associated with various sample conditions in both plants and other organisms (Chiu *et al.*, 2016; Gupta & De, 2017; Olawode, Tandlich, & Cambray, 2018), and has also succeeded in identifying metabolite markers related to the environmental conditions of plants (Gupta & De, 2017). Plants can not move to avoid environmental changes, which can cause poor development and growth (Jorge *et al.*, 2016). The ability of plants to survive these conditions depends on their ability to adapt, which involves stress sensing, signal transduction, and the activation of several stress-related genes and metabolites. The metabolite compounds found in this study are thought to have a relationship with the environmental conditions at each location. Influenced by environmental factors, the respective group of secondary metabolites acts as a chemical interface between the plant and its environment. The chemical interaction between plants and their environment is mediated mainly by the biosynthesis of secondary metabolites, which exert their biological roles, as a plastic adaptive response to their environment. Such chemical interaction often includes variations in the production of plant metabolites (Gutbrodt, Dorn, Unsicker, & Mody,

2012; Pavarini, Pavarini, Niehues, & Lopes, 2012). Therefore, the study of these variations is very useful in the chemical characterization of plants of the same species which are collected from different regions and this is when the different geographical origin of plant material is taken into account (Vilela *et al.*, 2013). Some processes can be the main sources of variation in the levels of metabolites for individual plant species. These processes involve long term acclimation or local adaptation, seasonal differences related to phenology or environmental changes in the biotic and abiotic factors, geographical differences involving different populations (genetic differences within a plant species), or different environmental conditions of the growth location of the specific individuals, especially when they have genetic homogeneity (i.e. cultivars and/or clones) (Rahimmalek *et al.*, 2009).

Metabolomics is an interesting approach that has been used in the plant sciences, especially in ecological studies, in investigating the effects of environmental factors on plant metabolism. Metabolomic profile data have been utilized to compare different species from the same family, or individuals from populations from the same species growing under different environmental conditions, or changes in metabolite production of individuals within

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the same population at different seasons (Arbona, Manzi, de Ollas, & Gómez-Cadenas, 2013; Jones *et al.*, 2013). Due to the wide-distribution and great adaptive response capability of galangal to different environmental conditions, and its proficiency to yield potential therapeutic natural products, a specific and innovative metabolomics approach was used to obtain the respective chemical profiles of the samples and to correlate them with the environmental data (climate and soil) from distinct regions at different seasons (Raina, Abraham, & Sivaraj, 2015). The metabolomic approach is useful to understand how variations in plant metabolism can be a response to changes in the surrounding environmental conditions as reported by Sampaio, Edrada-Ebel, & Da Costa (2016) that the chemical features of *Tithonia diversifolia* in rationale with changes in environmental factors by taking into account the variations in metabolic profile perceived through the occurrence of major metabolites in various plant parts.

The Density of Idioblast Cell in Galangal Rhizome

The idioblast cell density was significantly ($p < 0.05$) influenced by the interaction between location and galangal accessions (Table 6). Idioblast density calculations were carried out in cells containing essential oils. The results of the idioblast density calculation showed that the distribution of idioblasts that contained oil cells was denser in the pith tissue than in the cortex tissue at both locations. Idioblasts that had the most oils were obtained from PCT (90.5 cells/mm²) at location A and PBG (77.1 cells/mm²) at location B. According to Nugroho (2018), idioblast cells are cells that are contained in a tissue that is different in shape, size, content, or function from the surrounding cells. Idioblasts in galangal plants contain oil cells and have a yellow to orange color, and they are denser in the pith tissue, which is thought to be related to the age of galangal at harvest (12 MAP). A higher number of idioblasts does not always lead to an increase in oil cells (de Souza, 2016). Instead, the number of oil cells increases with increasing plant age, causing the mature rhizomes to contain more essential oils than the young ones (Mu, Liu, Kuang, Zou, & Liao, 2015). Our results coincide with Liu, Specht, Zhao, & Liao (2020) that reported that *Zingiber officinale* rhizomes had the highest idioblast density in the pith. Oil cell-filled idioblasts were also detected in

the rhizome of *Alpinia zerumbet* (Victório, Kuster, & Lage, 2011). Apart from essential oils, idioblast cells can also store various other secondary metabolite compounds in large quantities, such as crystals, resins, mucilages, and tannins (Nugroho, 2018).

The high amount of EPMC levels found in these two accessions (PBG and PCT) indicate that they are capable of thriving in a large range of environmental conditions and can potentially overcome environmental stress conditions better. Furthermore, high levels of EPMC increase galangal's medicinal effectiveness and could help to alleviate the domestic galangal deficit in Indonesia. Thus, PBG and PCT are recommended as candidates for superior varieties. The suggestion that PBG and PCT optimally grow at an altitude of 200–800 m asl, temperature at 21–28°C, 3000–4000 mm/year rainfall, 75–95% humidity, and full sunlight or 21–70%. Soil conditions should allow for good drainage, with a clay texture to sandy loam, and soil pH 5–5.5. The other accessions that have the potential to be developed in the future are CLP (59.9%) and KRA (54.0%) based on the high EPMC content and MAD with the highest essential oil content (3.99%) compared to other accessions.

CONCLUSION

The components of galangal essential oil are EPMC, 2-propenoic acid, caryophyllene, 3-carene, α -muurolene, patchouli alcohol, and 1,8-cineole. The highest EPMC levels were found in PCT at location A and PBG at location B. The metabolite profile of cluster one included PCT from location A. Cluster two was CLP from location A, as well as PBG, MAD, and GAL 2 from location B. The highest density of idioblast cells at location A was found in PCT and PBG at location B.

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