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Original Article

Shading effects on Tacca (*Tacca leontopetaloides* L. Kuntze) agronomy and metabolites: Implications for cultivation strategies

Wardah Wardah[1], Ridwan Ridwan[2*], Marwan Setiawan[1], Riska Ayu Purnamasari[1], Asep Sadili[1], Siti Sundari[1], Emma Sri Kuncari[1], Dini Ariani[3], Parwa Oryzanti[1], Gusmaini[2], Dibyo Pranowo[2], Handi Supriadi[2], Tintin Febrianti[4]
[1] Research Center for Ecology and Ethnobiology, National Research and Innovation Agency, Indonesia
[2] Research Center for Estate Crops, National Research and Innovation Agency, Indonesia
[3] Research Center for Food Technology and Processing, National Research and Innovation Agency, Indonesia
[4] Faculty of Agriculture, Garut University, Indonesia

**Corresponding author's email:* ridw009@brin.go.id
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Abstract

Tacca (*Tacca leontopetaloides* (L.) Kuntze) is a tuberous plant commonly growing in coastal areas, both in open and shaded areas. This study aimed to identify the agronomical characteristics and metabolite content of Tacca growing under various levels of natural shading. The research was conducted in the southern coastal area of Garut Regency (West of Java-Indonesia) by observing the plant growth and production, and metabolite content of Tacca tubers under conditions of natural full sunlight (0% shade/FL), moderate shade (40% shade/MS), and heavy shade (75% shades/HS) in three different locations as replications. Three mature plants (±7 months old) in each shade level and replication were randomly selected as observed samples. The result presented that Tacca's growth increased with the increasing shading levels. Nonetheless, tuber production showed the highest value under MS, which increased by 76.64% and 35.20% compared to FL and HS, respectively. Fourteen metabolites were detected in Tacca tuber grown in MS and FL, while only eight metabolites were detected in HS. The highest concentration of the metabolites obtained in MS were 8 metabolites (57%) and followed by FL and HS with 3 (21%) and 1 metabolite (7%), respectively. Moderately shaded areas have great potential to be used as cultivation land to produce high-quality Tacca tubers.

Keywords: Coastal area, Light intensity, Metabolites, Shading

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Introduction

Tacca (*Tacca lentopetaloides* (L.) Kuntze) is a tuberous herbaceous plant belonging to the Taccaceae family and commonly grows wild on the coastal area. In Indonesia, this plant has been reported to grow in many coastal areas, such as Krakatau Islands, Java, Karimunjawa, Madura, and Kangean Islands (Susiarti et al., 2012; Syarif et al., 2014; Wardah et al., 2017). One area that has been using this tuber for generations is the southern coastal area of Garut Regency, particularly in the districts of Pamengpeuk, Cikelet, and Caringin (Wardah et al., 2017)

This plant tuber is primarily used as a carbohydrates source to substitute rice and wheat flour due to its high carbohydrate content (Vu et al., 2017; Ridwan et al., 2020) with moderate glycemic index, so it is safe for consumption by people with diabetes (Nurhayati et al., 2022). In addition, this tuber also contains protein, fiber, fat, and various types of minerals such as calcium (Ca), natrium (Na), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn), copper (Co), and phosphorus (P). As a natural ingredient, this tuber is also reported to contain various types of compounds, including tannins, saponins, alkaloids, and flavonoids which are known to be beneficial for health (Basit et al., 2023; Ogbonna et al., 2017; Rachmawati et al., 2022). This indicates that this tuber can be developed as a food ingredient, healthy food, and even a functional food. In Indonesia, although some people have utilized it, Tacca plant has not been cultivated intensely. To meet their needs, the people take from nature directly without any cultivation and preservation efforts. This practice can lead to a massive decrease in the Tacca population, and in the long term, it can cause the extinction of this plant. The longevity and actual low economic value of this plant seem to be the main reason that it cannot compete with other commercial crops such as paddy, maize, shallots, and chilies for planting in productive lands. Therefore, unproductive land, such as land under vegetation along the coast, can be an alternative to cultivating this plant.

Tacca is reported to be able to grow both in the shade and in locations that are exposed to direct sunlight (Wawo et al., 2015; Ogbonna et al., 2017). Plant growth and production were generally reported to be affected by the environment, including shade or light intensity. The growth and production of seasonal crops such as lettuce (Kosma et al., 2013), rice (Ginting et al., 2015), maize (Suryanto et al., 2014), barley (Ridwan et al., 2018), and soybeans (Pratiwi and Artawi, 2018) were reported to decrease with increasing shade. Perennial crops such as Rosewood (*Dalbergia cochinchinensis* Pierre) and Teak (*Tectona grandis* L.f.) were also reported to show growth in the same pattern (Leksungnoen et al., 2021). Even so, some plants grow and produce optimally in low light or shaded conditions, such as *Curcuma alismatifolia* Gagnep., and *Kaempferia parviflora* Wall. ex Baker, and *Tacca integrifolia* Ker Gawl. (Abdullah et al., 2017), *Colocasia esculenta* (L.) Schott (Gondim et al., 2018), and *Citrus hystrix* DC. (Budiarto et al., 2019). The Metabolite profile of plants also has been reported to change due to distinct light intensity (Reshef et al., 2017; Abreu et al., 2019). However, until now, there is no available data yet to describe the growth and production of Tacca plants in quantity and quality in such conditions. However, this information is essential to ensure that this plant can be cultivated well under shaded conditions. Therefore, this study aimed to identify the agronomic characteristics of this plant and its tuber metabolite content under various levels of natural shading.

Material and Methods

Research location and plant observation samples

The research was conducted in the southern coastal area of Garut Regency, particularly in growing areas of Tacca in Sayang Heulang, Cigadog, and Rancabuaya. Three different natural shading levels, namely full sunlight (FL), moderate shades (MS), and heavy shades (HS) were used in this study. The observation plots were made with a size of 9 m^2 for each shading level with three replications. In each plot, three mature plants (±7 months old) were randomly selected as observation samples. Finally, there were 9 observation plots and 27 plant samples in total.

Climatic and edaphic observations

Light intensity, temperature, and humidity were observed directly at each observation plot at mid-day (12.00 local time) on clear days using a lux meter (Sandryadi et al., 2022), thermohygrometer (Junior et al., 2020), respectively. Soil moisture was observed using the gravimetric method (Dobriyal et al., 2012) at the Plant Physiology Laboratory, KST Soekarno, National Research and Innovation Agency of

Indonesia. The soil samples were taken from 5 random points at each location at 20 cm depth.

Agronomic and physiological observations
Growth parameters observed included plant height, leaf number, leaf petiole length and diameter, leaf length and width, and flower stalk diameter. Plant height was measured at the flower stalk, and observed from the ground surface to the top of the flower. The leaf petiole and flower stalk diameter were observed at a height of 5 cm from the soil surface. The leaf length was measured from the base to the tip of the leaf, while the leaf width was measured at the widest part of the leaf. The physiological parameter observed was the chlorophyll content using the chlorophyll meter apparatus (Konica Minolta SPAD-502Plus-Japan) at 5 points of each leaf and then averaged. Jiang et al. (2017) reported that SPAD value and total chlorophyll content were significantly correlated in tomato leaves (r=0.869), while Villa e Vila et al. (2022) estimating chlorophyll content using SPAD-502 Plus was also directly correlated with the absolutes content in okra leaves. The observed tuber production included dimensions (cm^3, length x width x height) and fresh weight. The tubers were stored in the freezer at -34˚C for further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis
Frozen peeled Tacca tubers were extracted using absolute ethanol at 1:10 (w/v) at 25˚C for five days. The extract was then evaporated at 45˚C for 1 hour. Analysis of tuber metabolites was carried out using GC-MS (Gas chromatography-mass spectrometry) by following the method used by Turhadi et al. (2019). Briefly, 5 µL of the extract was injected into a GC-MS device (Agilent Technologies 7890, Palo Alto, CA, USA) connected to a Mass Selective Detector and a Chemstation Data System (Model 5975C inert MSD with Triple-Axis Detector, Agilent Technologies, Palo Alto, CA, USA). The column used was the capillary column HP Ultra 2 (0.11 µm). The analysis was set as injection temperature (250°C), ion source temperature (230°C), interface temperature (280°C), and quadrupole temperature (140°C). The carrier gas used was helium, with a 1.2 ml/minute flow rate. The range of the detected mass spectrum was 40-650 m/z. Metabolite was then identified based on the Wiley W8N08.L database.

Statistical analysis
Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was performed at α=0.05 using SPSS for Windows version 21 (SPSS Inc., Chicago, IL, USA). Heatmap clustering analyses were conducted online using Metaboanalyst 5.0 (https://www.metaboanalyst.ca/).

Results and Discussion

Research sites conditions
Light intensity of mid-day reaching the surface of the plants in moderate (MS) and heavy shade (HS) were 60% and 25%, respectively, compared with full sunlight (FL) (Table 1). It means that approximately 40% and 75% of the light was blocked by the canopy of vegetation around the growing area of the Tacca plants. The distinct shade levels affected the air temperature, humidity, and soil moisture content. Air temperature decreased by approximately 17% and 26% in MS and HS, respectively, compared with FL, while air humidity and soil moisture content increased by approximately 114% and 145%, and 65.70% and 154.33%, respectively (Table 1). Based on the data, this study determined 0% shade as FL, 40% shade as MS, and 75% as HS.

Table-1. Light intensity, air temperature, air relative humidity, and soil moisture content of research location.

Shading Level	Light Intensity (Lux)	Air Temperature (˚C)	Air Relative Humidity (%)	Soil Moisture Content (%)
Full Sunlight	130420	38.9	29	14.15
Moderate Shade	78566	32.2	62	23.44
Heavy Shade	32402	28.9	71	35.98

Agronomical and physiological data

Light is one of the main ecological factors affecting plant growth and development. Every plant requires different lighting conditions to optimally grow and produce, including Tacca plants. The growth of Tacca was significantly affected by different light intensities or shading, which was indicated by several observed growth variables. In this study, the vegetative growth of Tacca linearly increased with increasing shade, which began under moderate shade (MS) and increased more under heavier shade (HS). Among variables, leaf number, and flower stalk diameter were the only variables not significantly affected by shading. Plant height, leaf petiole length, leaf petiole diameter, leaf length, and leaf width significantly increased in MS and HS by approximately 38% and 60%, 103% and 106%, 66% and 64%, 43% and 85%, and 116% and 172%, respectively compared within FL. The chlorophyll content of the leaves indicated by SPAD value also showed a higher value under shade conditions by approximately 24% and 31% in MS and HS, respectively, than in FL (Fig. 1).

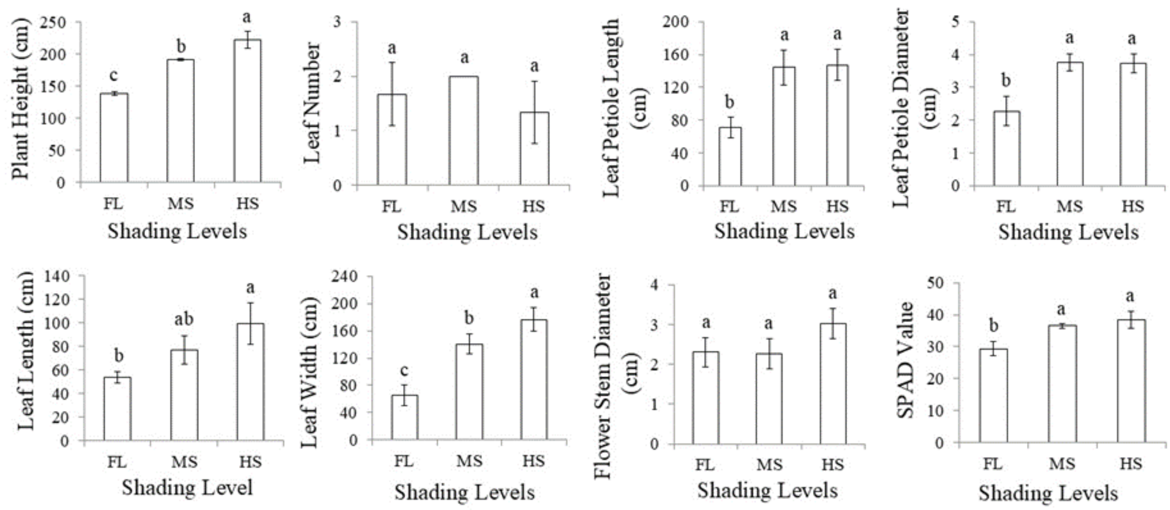

Figure-1. Growth and SPAD value of Tacca (*Tacca leontopetaloides* (L.) Kuntze) grown under several shading levels (0%, 40%, 75%) in nature. Bar was a standard deviation from 3 replications. The letters indicated the significance level according to ANOVA and DMRT at p≤ 0.05.

The data indicate that shaded growing environmental conditions are the preferred environment for these plants to support their vegetative growth. Another plant of the same genus, namely *Tacca integrifolia* Ker. Gawl. was reported to have optimal growth in shaded conditions as well, as indicated by chlorophyll content, photosynthetic rate, plant height, leaf size, and leaf petioles, which were much higher in shaded conditions than in full sunlight conditions (Abdullah et al., 2017). It seems to be caused by the genetic factor of the plant, which is not strong with direct sunlight exposure. Under full sunlight conditions, the light received by the Tacca plant was more than its optimal needs. It was reinforced by the leaf area, which was smaller in full sunlight than in shade conditions. Gondim et al. (2018) reported that taro plants, which prefer shade conditions exposed to direct sunlight, minimize transpiration due to excess light by reducing their leaf area. Excess light can induce the formation of excess reactive oxygen species (ROS), which can degrade cell membranes and their organelles inside, including photosynthetic apparatus such as chlorophyll (Taiz et al., 2015). This causes the photosynthesis of this plant to decrease and ultimately reduces its growth. Under shaded conditions, this plant gets optimal lighting levels, which makes the photosynthetic process well undergo, and finally causes the plants to optimally grow.

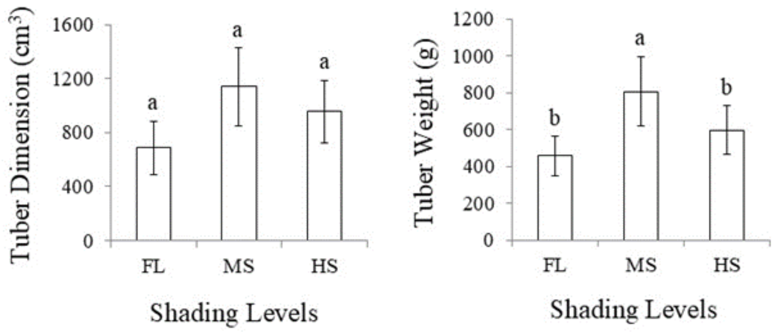

Figure-2. Tuber production of Tacca (*Tacca leontopetaloides* (L.) Kuntze) grown under several shading levels (FL, MS, HS) in nature. Bar was a standard deviation from 3 replications. The letter indicated the significance level according to ANOVA and DMRT at p≤ 0.05.

Figure-3. Tacca (*Tacca leontopetaloides* (L.) Kuntze) tuber production under several shading levels. Full sunlight (a), moderate shade (b), heavy shade (c).

The three-dimensional size of Tacca tubers showed no significant differences in the three levels of shade, but there was a tendency to increase in shaded conditions (MS and HS). However, tuber weights as the main indicator of Tacca tuber production were significantly affected by the different levels of shade. Tacca tuber showed optimal production under moderate shade (MS) levels, approximately 77% and 31% higher than Tacca tuber under FL and HS, respectively (Fig. 2 and 3). It means that moderate shade conditions have the potential to be used in the Tacca cultivation program to optimize tuber production. Higher tuber production under a moderate shade has also been reported in *Curcuma longa* L. (Srikrishnah and Sutharsan, 2015) and *Solanum tuberosum* L. (Hamdani et al., 2018; Schulz et al., 2019).

Better growth of Tacca under shaded conditions allegedly positively affects tuber production. Tubers are a strong sink of photosynthate and act as a storage organ for food reserves (Chen and Setter, 2003). Moderate shade is an optimal light intensity for this plant so that the photosynthetic process runs optimally, leading to optimal vegetative growth and tubers production. In heavy shade, the light received by the Tacca plant is probably not enough to make the photosynthetic process usually run, even though its vegetative growth has been increased as a mechanism

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to increase the surface area for capturing sunlight. This causes the production of Tacca tubers not to reach the optimal point even though it tends to be higher than in full sunlight conditions.

Metabolite content

The metabolite content of Tacca tuber also altered with differences in light intensity. In this study, the number of metabolites detected under higher light exposure conditions (MS and FL) was almost two times more than under heavily shaded conditions (HS). The GC-MS chromatogram showed that there were 14 prominent peaks both in FL and MS, but only 8 in HS (Fig. 4), which illustrated the number of metabolites detected at each shading level. Eight metabolites were detected in all shading levels, namely linoleic acid, mandenol, Z, Z-3,13-Octadecadien-1-ol, octadecanal, Icosyl 2-ethylbutanoate, oleic acid, linoleic acid chloride, and DL- α-Tocopherol, while six metabolites were detected in FL and MS but were not detected in HS, namely palmitic acid, Ethyl cyclohexanepropionate, (Z)-9,17-Octadecadienal, 11-Dodecenyl trifluoroacetate, trimethylammonium acetate, and carbonic acid, 2-dimethylamino ethyl neopentyl ester (Table 2).

The concentration based on the peak areas of the metabolites differed among the three shading levels. Linoleic acid concentration significantly increased only in the heavy shading level (HS). The concentration of DL-α-Tocopherol, octadecanal, oleic acid, and linoleic acid chloride significantly decreased in HS, but statistically had the same concentration in FL and MS. Trimethylammonio acetate and carbonic acid 2-dimethylaminoethyl neopentyl ester significantly decreased in MS compared to FL and were not detected in HS. Palmitic acid, ethyl cyclohexanepropionate, (Z)-9,17-Octadecadienal, and 11-Dodecen-1-ol trifluoroacetate were detected both in FL and MS with statistically comparable concentrations but were not detected in HS (Table 2). The heatmap clustering analysis based on the tuber metabolite concentration divided the Tacca plants grown under FL, MS, and HS conditions into two groups. The Tacca grown under HS were grouped alone, while the Tacca grown under FL and MS were grouped into one other group (Fig. 5). FL and MS grouped into the same group because they tended to have the same pattern, while HS grouped alone because it had a much different pattern from FL and MS. In general, the concentrations of metabolites detected at the three shading levels were lower in HS compared to FL and MS. Moderate shade condition (MS) showed the highest number of the highest concentration of metabolites with 8 metabolites.

High light intensity causes the temperature of the surrounding environment to increase, which causes higher evaporation compared to environments with low light intensity. This was evident by lower soil moisture in high-light intensity areas (Table 1). High evaporation causes lower water availability, so water absorption by plants declines. This was exacerbated by the soil type in this area, which has a sandy soil texture, so the water storage capacity is low. This condition causes drought and heat stress, which forces this plant to respond physiologically. Generally, plants will respond to drought and heat stress by accumulating certain compounds to make osmotic adjustments to increase their water use efficiency and to scavenge Reactive Oxygen Species (ROS) (Ridwan et al., 2023). Six metabolites were not detected in heavy shade but were detected in moderate shade and full sunlight conditions and appear to be synthesized for such functions.

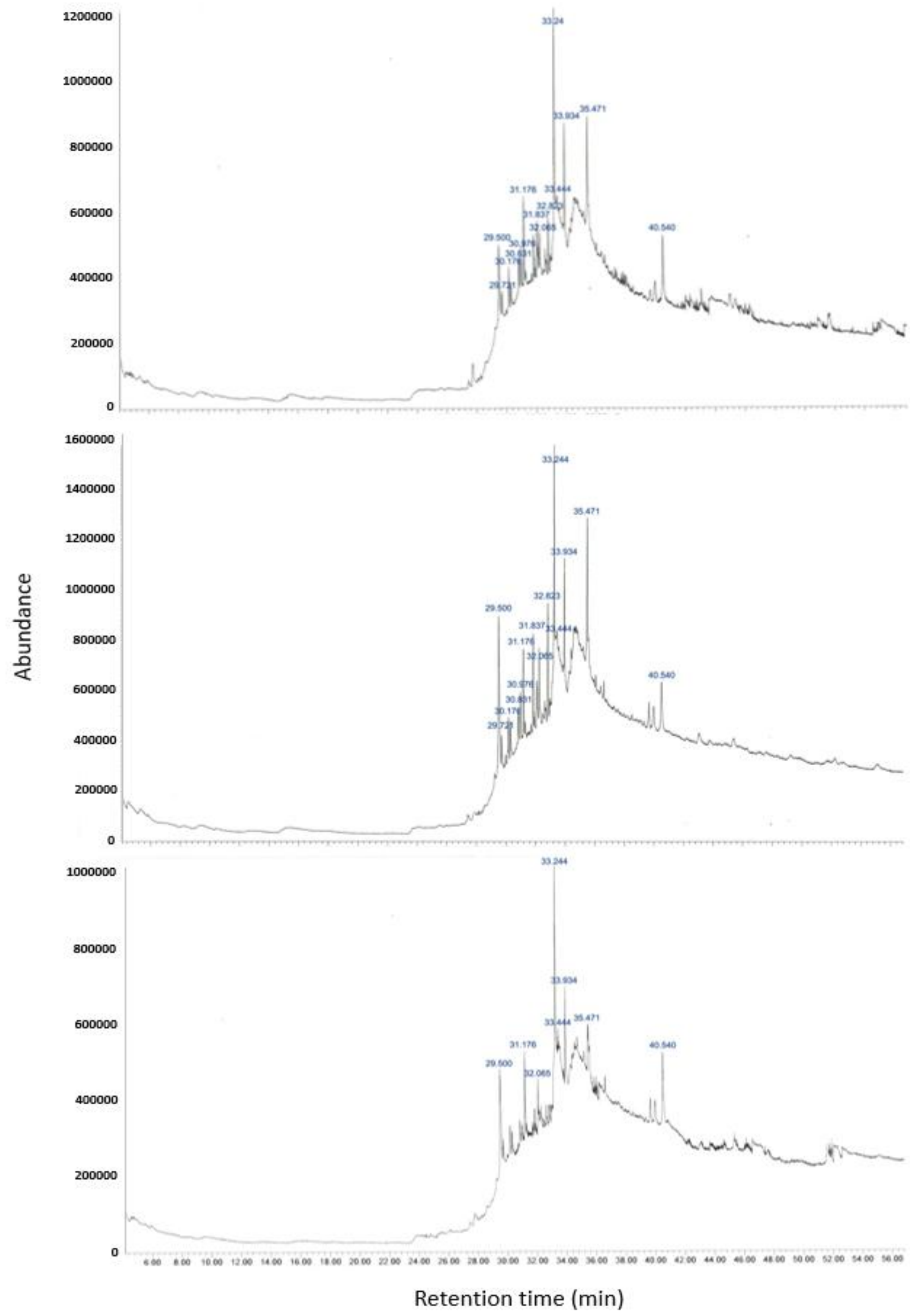

Figure-4. The chromatogram of GC-MS analysis of Tacca (*Tacca leontopetaloides* (L.) Kuntze) tuber several shading levels. Full sunlight (a), moderate shade (b), heavy shade (c).

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Table-2. Metabolites detected in Tacca (*Tacca leontopetaloides* (L.) Kuntze) tuber in full sunlight (FL), moderate shade (MS), and heavy shade (HS) conditions by GC-MS method.

Compounds	**RT**	**Formula**	**Concentration** (%)		
			FL	**MS**	**HS**
Linoleic acid	29.500	$C_{18}H_{32}O_2$	12.37[b]	13.21[b]	21.68[a]
Palmitic acid	29.721	$CH_3(CH_2)_{14}COOH$	2.64[a]	3.05[a]	0.00[b]
Ethyl cyclohexanepropionate	30.176	$C_{11}H_{20}O_2$	3.42[a]	3.50[a]	0.00[b]
9,17-Octadecadienal, (Z)-	30.831	$C_{18}H_{32}O$	3.84[a]	4.34[a]	0.00[b]
11-Dodecen-1-ol trifluoroacetate	30.976	$C_{14}H_{32}F_3O_2$	2.50[a]	2.71[a]	0.00[b]
Linoleic acid ethyl ester	31.176	$C_{20}H_{36}O_2$	5.57[a]	7.17[a]	5.6[a]
Trimethylammonio acetate	31.837	$C_5H_{13}NO_2$	4.72[a]	2.60[b]	0.00[c]
3,13-Octadecadien-1-ol	32.065	$C_{18}H_{34}O$	1.74[b]	2.53[a]	1.93a[b]
Carbonic acid, 2-dimethylaminoethyl neopentyl ester	32.823	$C_{10}H_{21}O_3$	6.04[a]	3.62[b]	0.00[c]
Octadecanal	33.244	$C_{18}H_{36}O$	18.52[a]	19.82[a]	13.75[b]
2-Ethylbutyric acid, eicosyl ester	33.444	$C_{26}H_{52}O_2$	5.05[a]	4.33[a]	4.01[a]
Oleic Acid	33.934	$C_{18}H_{34}O_2$	6.76[a]	6.77[a]	2.47[b]
Octadeca-9,12-dienoyl chloride	35.471	$C_{18}H_{31}ClO$	7.11[ab]	8.29[a]	4.41[b]
Alpha-Tocopherol	40.519	$C_{29}H_{50}O_2$	9.38[a]	11.41[a]	4.46[b]

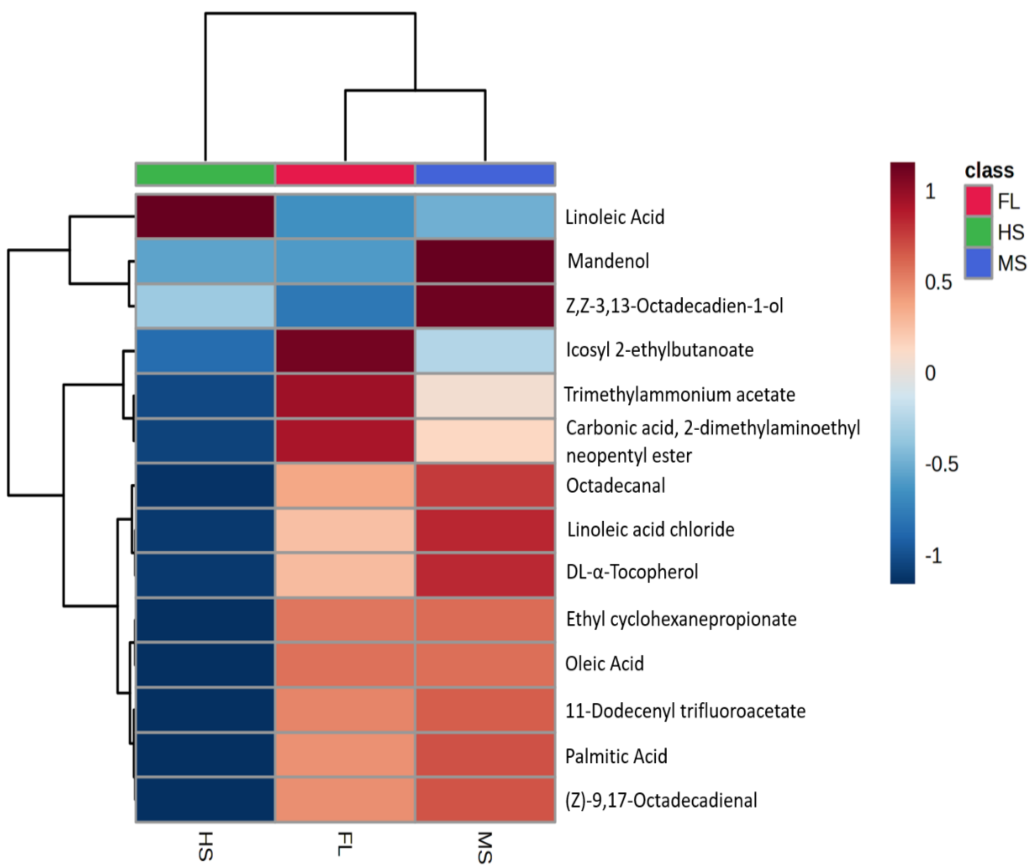

Figure-5. Heatmap clustering analysis of Tacca plant grown under full sunlight (FL), moderate shade (MS), and heavy shade (HS) according to the tuber metabolites content.

The primary metabolites detected in this study were the fatty acids group, most of which were poly and mono-unsaturated fatty acids. These metabolites are essential fatty acids and are known to have benefits for human health, such as protection from osteoarthritis, cancer, and autoimmune disorders (Kapoor et al., 2021; Odiase-Omoighe and Agoreyo, 2022). Linoleic acid, palmitic acid, and oleic acid are some kinds of fatty acids that have been reported to be affected by environmental stress. In this study, the linoleic acid concentration of Tacca tuber was higher in HS than in MS and FL. In contrast, palmitic acid and oleic acid were higher in MS and FL than in HS. These results are in agreement with Ullah et al. (2022), who reported that the palmitic acid and oleic acid content of wheat increased under water stress conditions, while linoleic acid and linolenic content decreased. He and Ding (2020) explained that the roles of fatty acids in stress defense can be through several ways, such as ingredients and modulators of cellular membranes in glycerolipids, a reserve of carbon and energy in triacylglycerol (TAG), stocks of extracellular barrier constituents, precursors of various bioactive molecules, and regulators of stress signaling.

One other important compound detected in Tacca tuber was α-tocopherol or vitamin E. In this study, vitamin E in the form of α-Tocopherol was significantly higher in the tuber of Tacca grown under FL and MS than in HS. The concentration of α-tocopherol in *Arabidopsis thaliana* (L.) Heynh was also reported to increase significantly after 3 days of high-light treatment (Ksas et al., 2015). Vitamin E is known to function in stress defense against biotic and abiotic stress, including high light and heat stress (Niu et al., 2022). Tocopherols can protect photosystem II of chloroplast from photooxidative due to their ability to reactive oxygen species quenching and protect the thylakoid membranes through the termination of lipid peroxidation chain-reactions (Spicher et al., 2017). The increase of tocopherol under high light and heat stress has been reported that caused by the activation of tocopherol cyclase (Tang et al., 2011). For human health purposes, vitamin E has an antioxidant activity which has beneficial for health, involves the prevention of oxidative stress, protection of the cell membrane, neuroprotective, wound healing, and prevention of some degenerative diseases, such as cardiovascular disease, cancer, and Alzheimer (Rizki et al., 2013; Ghosh et al., 2020).

Conclusion

The agronomical characteristics of Tacca plants were significantly affected by the distinct shading levels. The growth characteristics increased linearly with increasing shading levels, while tuber production reached an optimal point under moderate shade conditions (approximately 40% shade). The amount and concentration of Tacca tuber metabolites were higher under light exposure conditions both in full sunlight (FL) and moderate shade (MS) than in heavy shade conditions (HS), allegedly as a physiological defense mechanism. Fourteen metabolites were detected in Tacca tuber grown under FL and MS, but only eight metabolites were detected in HS. The highest concentration of the metabolites obtained in MS were 8 metabolites (57%), followed by FL and HS with 3 metabolites (21%) and 1 metabolite (7%), respectively. Based on the agronomical characteristics, Tacca plants grown under MS were closer properties to those grown in HS, but according to the metabolite content, they were closer to those grown in FL. Shaded areas, especially moderate shading level have a great potential to be used as cultivation land to produce high-quality Tacca tubers.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Contribution of Authors

Wardah W, Ridwan R & Setiawan M: Designed and conducted the research and interpreted the data.

Kuncari ES, Gusmaini, Pranowo D & Ariani D: Performed the chemical and statistical analysis.

Sadili A, Purnamasari RA, Sundari S, Oryzanti P, Supriadi H & Febrianti T: Visualized the data.

All authors contributed to writing, revising, and reviewing the paper. Each author has examined the final draft of the manuscript and consented to its publication.

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