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Variability of Agronomic and Metabolomic Characteristics of Nine Accessions of Cardamom (*Amomum compactum*) From Central Java, Indonesia

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Abstract

Amomum compactum (*Javanese cardamom*) is valued for its unique flavor and aroma, commonly used as a culinary spice or medicinal ingredient. The numerous accessions of cardamom in Indonesia present a significant potential for developing this spice. However, the specific potential of each accession remains to be discovered. This study records the variation of agronomical traits and metabolomic profiles of nine cardamom accessions from Central Java, Indonesia. Multivariate analysis using a heatmap on agronomical traits indicated significant variation among accessions, with clustering based on growth locations. Genetic parameter analysis revealed high phenotypic variability, but varying broad-sense heritability among traits, suggesting the influence of both genotypic and environmental factors. Metabolomic analysis using GC-MS reveals the presence of specific compounds in certain accessions, such as beta-panasinsene in the Kulonprogo Putih accession, 1-docosene and alpha-terpinene in the Banyumas Putih accession, and 9-tricosene in the Banyumas Hybrid, which can serve as markers for these accessions.

Keywords: essential oil, exploration, Java local accessionsplant breeding, traits

Introduction

The Javanese cardamom (*Amomum compactum*) and True cardamom (*Elettaria cardamomum*) are two cardamoms cultivated in Indonesia. *Amomum compactum* is a prominent spice and medicinal plant, with Indonesia's export volume reaching $16.48 million (BPS, 2018). West Java and Central Java provinces are Indonesia's top *producers of A. compactum*, with outputs of 89,000 tons and 26,500 tons, respectively (BPS, 2021). *A. compactum* contains essential oils frequently used for treating various diseases (Ashrafi et al., 2020). In 2018, the demand for *A. compactum* for herbal medicine consumption in simplicia form reached 1,409 tons, and in herb form, 9,862 tons (Aditya and Dona, 2020). *A. compactum* also offers health benefits, including free radical scavenging, antimicrobial, cytotoxic, and anti-inflammatory activities (Li et al., 2021). Research by Arista et al. (2023) indicates that Javanese cardamom contains variable patterns of monoterpenes, sesquiterpenes, diterpenes, and other compounds, with 1,8-Cineol being the main compound of the essential oil studied. Eucalyptol (1,8-cineole), an essential oil from *A. compactum*, is potentially a therapeutic agent for SARS-CoV-2 (Sabulal and Baby, 2021). This data highlights the significance of essential oils in A. compactum for the healthcare industry and their increasing demand. However, more specific data are needed on the essential oil content or other secondary metabolites in locally cultivated *A. compactum* accessions. Comprehensive metabolomic profiling of each individual provides valuable insights into the biological functions of cells, tissues, or organisms in response to genetic, physiological, or environmental stimuli, aiding in the evaluation of phenotype and the determination of mechanisms (Li et al., 2021).

Amomum compactum belongs to the Zingiberaceae family, is a leafy herb with a robust rhizome, and can reach a height of 180 cm (Das et al., 2018). *Amomum compactum* is commonly known as the "Queen of Spices" due to its unique flavor and aroma, frequently used as a culinary spice and for medicinal purposes (Alagupalamuthirsolai et al., 2020). According to Droop and Newman (2014), there are 24 species of Javanese cardamom distributed in Indonesia, seven of which are endemic to Sumatra, while the

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others are found in Southeast Asia. *A. compactum* flowers are white with green fruit capsules when unripe and turn orange-brown when ripe, irregularly polygonal in shape, with a diameter of 4 mm, and white arils (Das et al., 2018). The long cultivation history and rapid seed production capability result in high morphological variation in this species, making it challenging to classify and differentiate *A. compactum* types (Droop et al., 2013). The lack of morphological character descriptions can be a significant obstacle in enhancing the value of *A. compactum*. Research by Windarsih et al. (2021) demonstrates that the morphological traits of vegetative and generative organs can be used to describe and classify plants within the Zingiberaceae family, enabling farmers to identify economically valuable Zingiberaceae groups. Research on *A. compactum* in Indonesia is still limited. The potential of *A. compactum* accessions for development has yet to be fully understood, necessitating a study to examine the morphological and metabolomic aspects of local accessions. This study aims to investigate the correlation between morphological traits and metabolomic profiles in nine *A. compactum* accessions from Central Java, to accurately assess the potential of local *A. compactum* accessions in terms of genetics, cultivation techniques, and the development of new superior varieties.

Materials and Methods

Morphological Characterization of Central Java Cardamom Accessions

The research was conducted at cardamom cultivation locations in several districts, including Banyumas, Kulon Progo, Magelang, and Purworejo. Forty clumps were observed per accession in each farmer's field. The study was conducted in August 2023. The observed plants were local farmer-cultivated cardamom accessions. There was a total of nine accessions across all locations, coded based on the accession's origin with the first letter and local names: BH (Banyumas Hybrid), BM (Banyumas Merah), BP (Banyumas Putih), KM (Kulonprogo Merah), KP (Kulonprogo Putih), MM (Magelang Merah), PB (Purworejo Hybrid), PM (Purworejo Merah), and PP (Purworejo Putih). The age of the clump is greater than 10 years. Quantitative measurements were conducted on (1) stem height (SH): measuring the height of the two tallest stems in one clump, measured from the ground surface to the tip of the leaves, (2) stem diameter (SD): the diameter of the two tallest stems in one clump, measured 15 cm from the ground surface, (3) the first leaf position (FLP): the distance from the ground surface to the first leaf, (4) leaf length (LL): the length of the leaf from the leaf end, measured from the base to the tip of the leaves, (5) leaf width (LW): the width of the leaf at its widest point, (6) Inter-stem distance (ISD): the distance between three stems in one clump (stem a to stem b, stem b to stem c, stem c to stem a), (7) number of fruits per stalk (NFS), (8) Number of stems per clump (NSC): the number of stems in one clump, (9) weight of 100 fresh fruits (W100F), (10) weight of 100 dry seeds (W100D), and (11) number of seeds per fruit (NSF). Forty plants of each accession were measured, using the method described by Droop and Newman (2014) and the Descriptors for Cardamom by the International Plant Genetic Resources Institute in 1994.

GC-MS Metabolomic Analysis

Bioactive compounds were identified using untargeted metabolomic analysis, aiming to detect potential bioactive compounds in cardamom plant accessions through GC-MS in this study. Samples consisting of harvested seeds from nine cardamom plant accessions were utilized.

The extraction process began with finely grinding each sample, weighing approximately 1 g. A portion of 100 µL of the sample was mixed with approximately 900 µL of methanol. This mixture was vortexed to ensure thorough homogenization. Subsequently, the vortexed samples were injected into the GC-MS instrument for further analysis. The extraction method follows the protocol described by Arista et al. (2023).
Metabolic compound analysis was performed using an Agilent Technologies 7890 Gas Chromatograph, equipped with an Autosampler and a 5975 Mass Selective Detector, controlled by the ChemStation data system. The electron impact ionization mode was employed with an electron energy of 70 eV. The chromatographic column used was an HP Ultra 2 Capillary Column, 30 m long, with an internal diameter of 0.20 mm and a film thickness of 0.11 µm. The initial oven temperature was set at 80°C without a hold, then ramped at 3°C per minute to 150°C and held for 1 minute. This was followed by a ramp at 20°C per minute to 280°C and a hold for 26 minutes. The injection port temperature was set at 250°C, the ion source temperature at 230°C, the interface temperature at 280°C, and the quadrupole temperature at 140°C. Helium was used as the carrier gas at a constant flow rate of 1.2 mL per min. The injection volume was 5 µL with an 8:1 split mode.

Data Analysis

Observation data were automatically standardized using the Unit Variance (UV) scale with Minitab

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22.0 software, and the standardized data were then analyzed using the multivariate statistical method of Principal Component Analysis (PCA) Biplot. Multivariate analysis was also performed with a heatmap dendrogram using a correlation matrix to identify and understand the relationships between variables. Genetic parameter analysis on morphological characteristics was conducted to determine the phenotypic coefficient of variation using the Sivasubramanian and Menon (1973) calculation:
Phenotypic coefficient of variation (PCV) = ( ) × 100
where:

$$\text{Phenotypic coefficient of variation (PCV)} = \left(\frac{\sigma p}{\mu}\right) \times 100$$

σ_p = Phenotypic standard deviation.
μ = Phenotypic mean.

Broad-sense heritability values for morphological characteristics were calculated using the Stansfield (1991) calculation:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

where:
H^2 = Broad-sense Heritability
= Genotypic variance
= Total phenotypic variance

The metabolomic data obtained from the GC-MS analysis were analyzed using multivariate statistical analysis. The study was performed using R software with the pHeatmap package.

Results and Discussion

Agronomic Traits and Environmental Conditions

The cardamom plantations are located in Banyumas, Kulon Progo, Magelang, and Purworejo, covering 70, 1, 4.5, and 0.5 hectares, respectively. The cultivated cardamom accessions are diverse. The commonly encountered accessions in these plantations include local red, local white, and hybrid accessions. However, only local red accessions were found in Magelang, and only two accessions, local red and local white, were found in Kulonprogo. Plantation owners tend to replace less productive accessions with more productive ones, so in some locations, such as Magelang and Kulonprogo, only one or two local accessions are found. According to Droop and Newman (2014), there are 24 types of cardamom distributed in Java and Sumatra. Each type has unique morphological traits, both quantitatively and qualitatively.

The topography of the four plantation locations is hilly, with similar elevations ranging from 400 to 600 meters above sea level (masl). This elevation meets the growth requirements for cardamom plants, which vary between 300-800 masl (Dirjenhorti, 2019). According to Zakir (2019), the appropriate elevation for cardamom cultivation is 600-1200 masl. Besides elevation, other factors such as temperature and air humidity need to be considered. The average temperature in the studied plantations ranges from 23 °C to 27°C, aligning with the growth requirements of cardamom, which is 20-30°C. The suitable humidity range for cardamom growth is 40-75% (Dirjenhorti, 2019), but the average air humidity at the four research locations is higher than the optimal range, averaging between 81% and 84% (Table 1).

Table 2 shows the mean and standard deviation of 11 growth parameters of cardamom from different regions. The average and relative deviation percentages assess variation within and between accessions. The results indicate that the tallest plant height was found in the accession from Kulon Progo, with an average of 243.83 cm and a low relative deviation percentage of 3.72%, indicating high consistency. Conversely, Banyumas Putih had an average plant height of 175.36 cm with a higher relative deviation percentage (22.27%), indicating more significant variability (Table 2). Shades with light intensities of 100%, 75%, 50%, and 25% significantly affected cardamom plant height (Rini et al., 2022).

In contrast, Lamxay and Newman (2012) reported that some cardamom types exhibit distinct plant heights, such as *Amomum muricarpum*, which reaches a height of 2-2.5 m, and Amomum tenellum, which typically grows to around 1 m. Variation among accessions in the Purworejo region was also significant, with parameters such as leaf length and width showing substantial differences between accessions, including Purworejo Merah and Purworejo Putih. The results differ from those of Droop and Newman (2014), which described Javanese cardamom leaves (*Amomum compactum*) as lanceolate with a base tapering to a sharp point, measuring 41-42 cm in length and 10.5-17 cm in width.

The highest weight of 100 fruits was also found in the Kulonprogo Putih, with an average of 123.44 g and a relative standard deviation percentage of 4.62%, indicating good stability. In contrast, the Banyumas Hybrid had a high fresh fruit weight of 113.09 g per 100 fresh fruits, with a higher variability, as indicated by a coefficient of variation of 37.31%.

Comparisons between accessions revealed significant variation in the parameter of fresh fruit weight, with

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Table 1. Environmental conditions at different cardamom locations

Origin	Land area (ha)	Accession	Elevation (masl)	Temperature (°C)	Relative humidity (%)	Shade trees
Banyumas	70	Banyumas Merah Banyumas Putih	500-600	27.1	82	Damar, pines
Kulonprogo	1	Kulonprogo Merah Kulonprogo Merah	500-600	23.1	84	Bamboo
Magelang	4.5	Magelang Merah Magelang Putih	400-500	26.1	81	Teaks, Albizia
Purworejo	0.5	Purworejo Merah Purworejo Putih	500-600	23.1	84	Albizia

Table 2. Stem height, stem diameter, leaf position, and leaf size of different cardamom accessions

Accession	Stem height (cm)		Stem diameter (cm)		1st leaf position (cm)		Leaf length (cm)		Leaf width (cm)	
	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%
Banyumas Merah	187.19	7.76	1.36	7.83	77.53	21.01	35.70	11.33	8.56	18.24
Banyumas Putih	175.36	22.27	1.31	9.55	77.40	25.92	38.05	14.92	8.22	10.35
Banyumas hibrida	209.43	14.56	1.43	8.97	83.18	24.04	41.21	11.79	9.16	12.75
Kulonprogo Merah	203.57	13.11	1.39	10.27	88.20	29.87	37.67	13.59	9.28	9.58
Kulonprogo Putih	243.83	3.72	1.40	1.80	55.57	5.02	34.08	4.89	8.52	8.51
Magelang Merah	174.87	21.62	1.33	12.84	80.80	25.83	34.03	22.60	8.65	10.86
Magelang Putih	202.64	16.34	2.52	32.86	69.58	18.47	35.05	40.44	5.13	52.05
Purworejo Merah	186.20	21.06	2.89	41.16	64.78	25.06	24.02	27.29	5.64	48.83
Purworejo hibrida	188.73	19.22	1.72	27.26	69.40	32.57	33.46	31.15	4.94	62.60
Average	196.87	15.52	1.70	16.95	74.05	23.09	34.81	19.78	7.57	25.97

Notes: RSD is the relative standard deviation in percentage.

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the highest values observed in the Banyumas Hybrid and Kulonprogo Putih. However, the latter had a lower relative deviation percentage, indicating better stability than Banyumas hybrid. Hasnah et al. (2023) demonstrated the impact of environmental variables, including daily temperature, humidity, light intensity, pH, and soil nutrient content, on the weight of fresh cardamom fruit.

Overall averages indicated that the fresh fruit weight of 100 fruits had the highest value (96.19 g) with a coefficient of variation of 16.26%. At the same time, other parameters, such as plant height and first leaf position, showed more moderate variation, with relative deviation percentages of 15.52% and 23.09%, respectively. Generally, the first leaf position in all *Amomum* species is 0.5-1 m above the ground; however, some species have their first leaf position more than 1 m above the ground (Droop and Newman, 2014). This data indicates significant morphological variation among accessions in different regions, which can be utilized to develop superior plant varieties.

The heatmap analysis in Figure 1 illustrates the relationship between various agronomic parameters in the nine observed accessions. Red colors indicate higher-than-average parameter values, while blue colors indicate lower values. Kulonprogo Putih and Purworejo Putih stand out with several parameters, indicating high plant height, stem diameter, and fruit weight. Conversely, accessions like Bayumas Putih and Banyumas Merah tend to have average or lower parameter values. The dendrograms on both axes indicate that parameters and accessions are grouped based on similar traits. The morphological similarities shown in the heatmap indicate that accessions from the same region tend to exhibit high levels of similarity. For instance, accessions from Banyumas (Merah, Putih, and Hybrid) and accessions from Purworejo exhibit similar morphological patterns. These patterns suggest significant variation among accessions from different regions, which can be optimized for breeding and increasing plant productivity.

The Principal Component Analysis (PCA) illustrated in Figure 2 displays the distribution of accessions based on the first (First Component) and second (Second Component) principal components. The scree plot reveals that the first two components account for most data variability, with significant eigenvalues exceeding 75%, before sharply declining. Accessions are grouped into clusters in the score plot based on their principal component values. Kulonprogo Putih and Purwokerto Putih are in the negative quadrant of the first principal component, indicating significant differences from accessions Banyumas Putih and Banyumas Merah, which are in the positive quadrant. This suggests that these two groups have distinct traits.

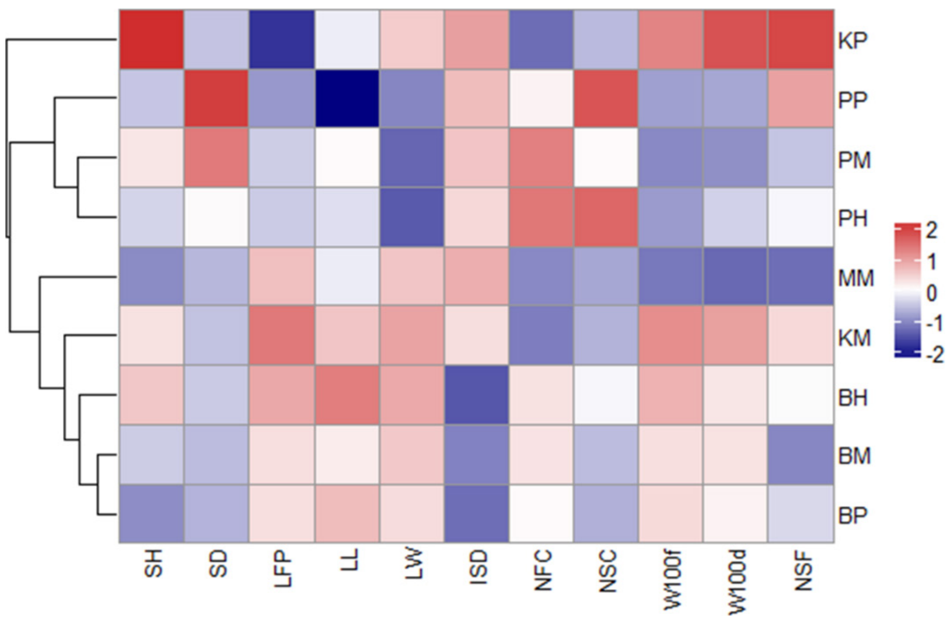

Figure 1. Cluster analysis based on agronomic traits of nine cardamom accessions from Central Java, Indonesia. SH = stem height, SD = stem diameter, FLP = first leaf Position, LL = leaf length, LW = leaf width, ISD = inter-stem distance, NFS =nNumber of fruits per Stalk, NSC = number of stems per clump, W100f = weight of 100 fresh fruits, W100d = weight of 100 dry seeds, and NFS = number of seeds per Fruit. BH=Banyumas Hybrid, BM= Banyumas Merah, BP = Banyumas Putih, KM= Kulonprogo Merah, KP = Kulonprogo Putih, MM= Magelang Merah, PB= Purworejo Hibrida, PM=Purworejo Merah, and PP=Purworejo Putih.

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The loading plot indicates the contribution of agronomic variables to the principal components. For instance, leaf length (PD) and the weight of 100 fresh fruits (B100b) have a substantial positive contribution to the first principal component, while stem diameter (DB) and leaf width (LD) show smaller contributions. Combining the loading and score plots, the biplot provides a comprehensive view of how each accession correlates with agronomic variables. Kulonprogo Putih and Purworejo Putih are far from the center (Figure 2). In contrast, accessions near the center, such as Banyumas Putih and Banyumas Merah, show more uniform and less extreme values for these variables. Overall, this PCA analysis highlights significant variation among accessions, which can be utilized for breeding programs. Specific dominant traits in certain accessions offer opportunities to select and develop superior varieties with desired characteristics.

Broad-sense heritability is the ratio of genotypic variance (including additive, dominant, and epistatic effects) to phenotypic variance (Majhi, 2019). The calculation of the phenotypic variance coefficient in Table 4 indicates a broad phenotypic variance coefficient except for stem height. The broad-sense heritability estimates range from low to moderate to high values. Low heritability values suggest that environmental influences predominantly affect phenotypic variance in traits such as first leaf height and the number of seeds per fruit. High heritability values indicate that genetic influences predominantly affect traits such as stem diameter, the number of fruits per cluster, and the number of stems per clump. Traits with high heritability can be used as selection criteria to identify accessions that are suited to specific breeding objectives (Firmansah et al., 2024). Traits such as stem height, leaf length, leaf width, inter-stem distance, the weight of 100 fresh fruits, and the weight of 100 dry seeds show moderate heritability values, indicating that both genotype and environment significantly influence phenotypic variation.

Compound Identification

The heatmap analysis presented in Figure 3 illustrates the relative distribution of various chemical compounds among different accessions from several regions. This heatmap illustrates significant variations in chemical composition among accessions, represented by a gradient of colors ranging from red

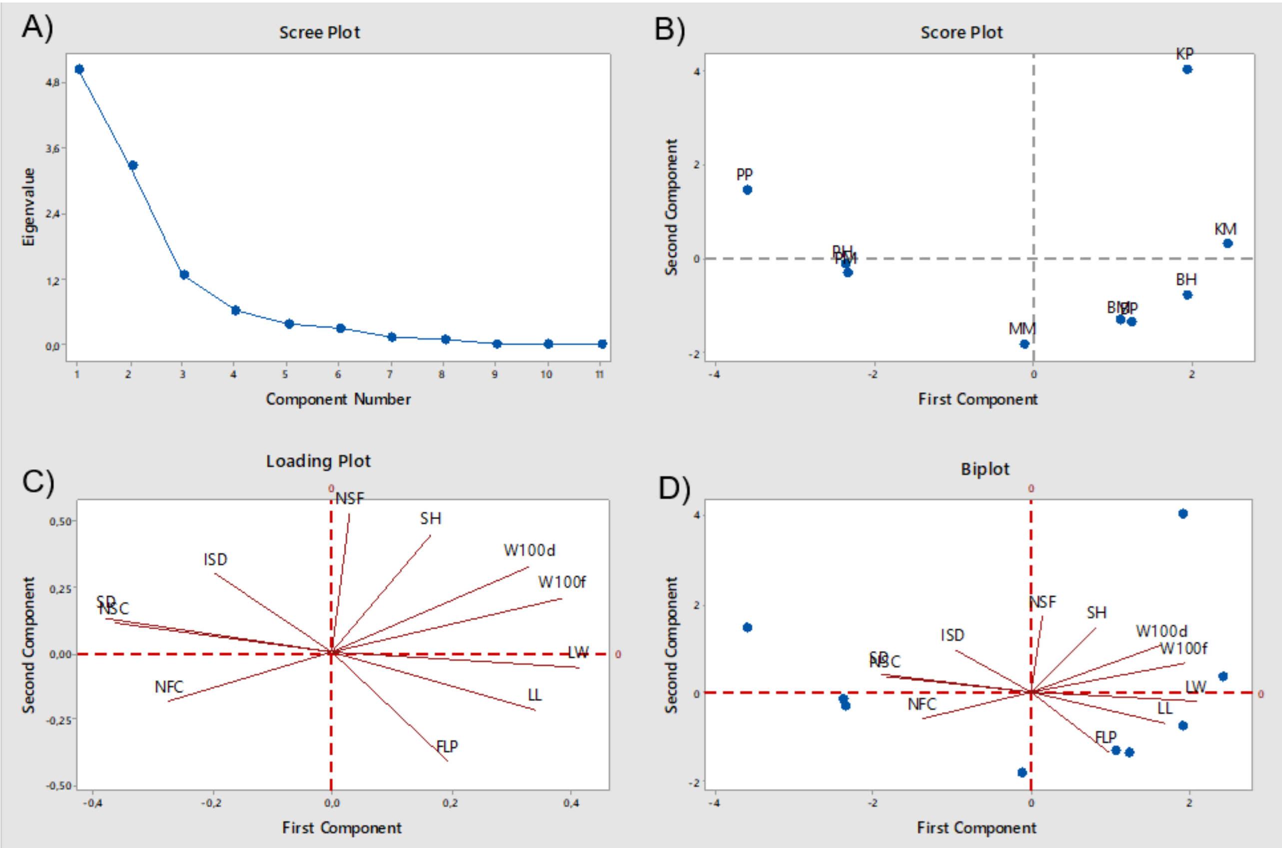

Figure 2. A) Scree plot (A), score plot (B), loading plot (C), and PCA biplot (D) of nine accessions of *A. compactum* based on agronomic traits. BH=Banyumas Hybrid, BM= Banyumas Merah, BP = Banyumas Putih, KM= Kulonprogo Merah, KP = Kulonprogo Putih, MM= Magelang Merah, PB= Purworejo Hibrida, PM=Purworejo Merah, and PP=Purworejo Putih.

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Table 3. Interstem distance, fruit number per stalk, stem number of clump, weight of fruits and seeds, and the number of seeds per fruit of cardamom accessions

Accession	Interstem distance (cm)		Fruit number per stalk		Stem number per clump		100-fruit weight (g)		100-dry seed weight (g)		Seed number per fruit	
	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%
Banyumas Merah	10.05	45.84	16.38	33.53	21.40	31.57	103.24	11.48	3.11	17.02	19.85	8.58
Banyumas Putih	9.35	37.98	14.95	35.17	19.73	41.23	104.41	19.89	2.97	31.32	21.88	22.69
Banyumas Hybrid	8.52	26.64	16.48	32.35	29.65	21.82	113.09	37.31	3.08	39.33	22.88	11.55
Kulonprogo Merah	15.71	27.89	7.90	17.70	20.10	28.00	121.22	3.19	3.69	17.52	23.97	13.26
Kulonprogo Putih	18.21	3.99	7.00	25.17	21.10	38.08	123.44	4.62	4.37	34.66	28.30	23.79
Magelang Merah	17.58	39.38	8.47	28.89	18.43	73.18	70.75	6.75	1.75	17.55	19.20	23.63
Purworejo Merah	16.69	22.41	22.50	8.24	31.53	40.60	73.94	26.84	2.04	48.66	21.40	17.32
Purworejo Putih	17.00	31.70	15.40	36.34	59.53	44.48	78.27	19.23	2.22	16.62	25.60	17.03
Purworejo Hybrid	15.90	36.28	23.00	6.23	56.50	34.03	77.32	17.02	2.53	29.97	22.65	16.23
Average	14.33	30.24	14.67	24.84	30.88	39.22	96.19	16.26	2.86	28.07	22.86	17.12

Notes: RSD is the relative standard deviation in percentage.

Table 4. Coefficient of phenotypic variation and broad-sense heritability estimates for the cardamom morphological traits

Character	PCV (%)	PCV Criteria	H^2	H^2 Criteria
Stem height	18.67	Medium	0.26	Medium
Stem diameter	46.01	Large	0.53	High
Position of 1st leaf	27.75	Large	0.18	Low
Leaf length	25.74	Large	0.26	Medium
Leaf width	34.01	Large	0.44	Medium
Interstem distance	40.46	Large	0.40	Medium
Fruit number per cluster	46.09	Large	0.65	High
Seed number per cluster	67.45	Large	0.55	High
100-fruit weight	28.74	Large	0.49	Medium
100-seed dry weight	40.05	Large	0.41	Medium
Seed number per fruit	20.92	Large	0.27	Low

Notes: PCV = coefficient of phenotypic variation, H^2 = broad-sense heritability, CV criteria <10% = narrow, 10%-20% = medium, >20% = wide (Sivasubramanian and Menon, 1973). Heritability estimates criteria <0.2 = low, 0.2-0.5 = medium, >0.5 = high (Standsfield, 1991).

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(high value) to blue (low value). Dark red columns in the heatmap indicate that specific metabolites are exclusively found in one accession, making these compounds potential markers for that particular accession.

Accession Kulonprogo Putih's unique compound, beta-Panasinsene, is not found in other accessions. Beta-Panaxisene, a sesquiterpene, plays a role in defense mechanisms against fungal attacks (Le et al., 2019). Compounds such as 1-Docosene and alpha-terpinene are only found in accession Banyumas Putih. These compounds are involved in antimicrobial activity (Albratty et al., 2023), as well as anti-inflammatory and antioxidant functions (Natalia and Katarzyna, 2019). Accession Banyumas Hybrid has distinctive compounds, such as 9-tricosene and Nonacos-1-ene, whereas 1-nonadecene is only found in accession Banyumas Merah. These hydrocarbons are known for their biological activities, such as antibacterial, antifungal, and anticancer properties (Gajera et al., 2020).

Accession Purworejo Merah contains linalool, which is exclusive to this accession. Linalool is recognized for its antifungal properties; research by Xu et al. (2019) suggests that increased linalool activity can help inhibit fungal infections in strawberry plants. Compounds such as 9-Octadecenoic acid and Stigmasterol-3,6-dione are only found in Kulonprogo Merah; 9-Octadecenoic acid, a fatty acid derivative, offers numerous health benefits, including cholesterol reduction and applications in medications for cancer, cardiovascular diseases, and Alzheimer's (Ghavam et al., 2021).

Banyumas Putih stands out with high values of compounds such as 1-Tricosene and gamma-Sitosterol, represented by dark red colors. According to Gameil et al. (2019), 1-tricosene has potential as an anticancer compound. In plants, gamma-sitosterol plays a role in regulating growth and development, as well as aiding the plant's response to biotic and abiotic stress. This sterol enhances cell membrane strength, helping plants withstand pathogen attacks and unfavorable environmental conditions (Cabianca et al., 2021).

Kulonprogo Putih shows high values of alpha-pinene and Octacosanol, indicating a unique chemical composition compared to other accessions. Alpha-pinene is toxic to insect species such as *Sitophilus oryzae* (Sharma and Tiwari, 2021). Conversely, accessions such as Banyumas Hybrid

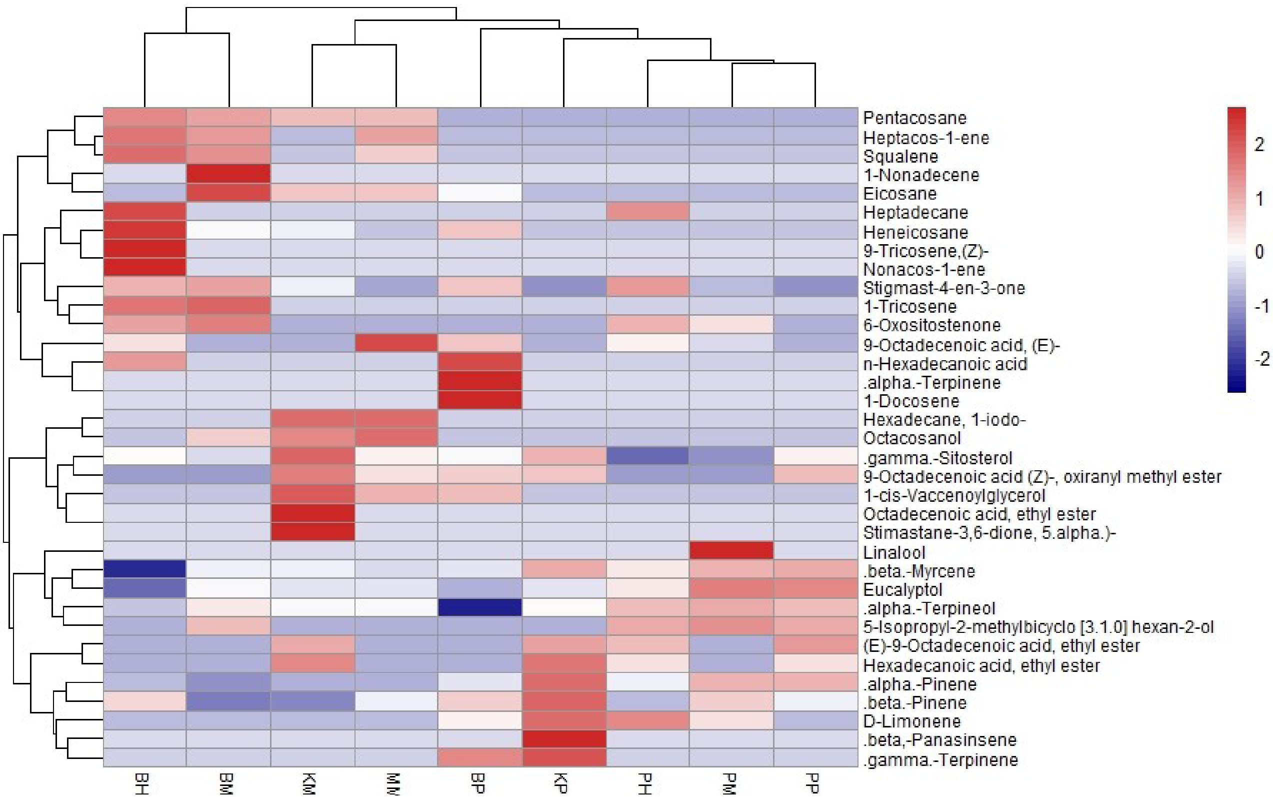

Figure 3. Heatmap cluster of nine accessions of *A. compactum* based on metabolite profiles. BH=Banyumas Hybrid, BM= Banyumas Merah, BP = Banyumas Putih, KM= Kulonprogo Merah, KP = Kulonprogo Putih, MM= Magelang Merah, PB= Purworejo Hibrida, PM=Purworejo Merah, and PP=Purworejo Putih.

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and Kulonprogo Merah tend to have lower values for specific compounds, as indicated by the blue colors, particularly for compounds like Eicosane and Heptadecane. Eicosane (alkane) has antioxidant, antimicrobial, and antifungal properties (Awan, 2023). Heptadecane is found in plants such as K. galanga and possesses antimicrobial properties that inhibit the growth of pathogens in rice plants, including *R. solanacearum* and *X. oryzae* (Suharti et al., 2023). This pattern indicates that Banyumas Hybrid and Kulonprogo Merah have lower concentrations of these compounds.

Clustering in the dendrogram shows that accessions with similar chemical profiles are grouped, such as Banyumas Hybrid and Banyumas Merah, indicating that these two accessions have more similar chemical profiles compared to others, like Kulonprogo Putih and Banyumas Putih. This variation is crucial in plant breeding, as certain chemical compounds may be associated with desirable agronomic traits, such as pest resistance or fruit quality.

Metabolite content analysis offers valuable insights into specific metabolites that can serve as markers for an accession. This information can be utilized in breeding programs to select accessions with desired chemical and morphological profiles.

Conclusions

The multivariate analysis using a heatmap for agronomic traits indicates significant variation among accessions from Central Java. However, it tends to exhibit clustering patterns based on the locations of growth. The results of the genetic parameter calculations align with the heatmap clustering pattern, demonstrating a high phenotypic coefficient of variation in morphological traits but differing heritability estimates among these morphological traits. This suggests that genotypic factors, environmental factors, or their combination influence phenotypic variability. Distinct compounds found in specific accessions can be used as markers for these accessions. For instance, beta-panasinsene in Kulonprogo Putih,1-docosene alpha-terpinene in Banyumas Putih, and 9-tricosene in Banyumas Hybrid can serve as unique identifiers for these accessions.

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