Study of Genetic Diversity and Relationship of 100 Cardamom (Elettaria cardamomum) Lines Based on Morphological Characters

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Abstract. Cardamom (Elettaria cardamomum) is belong to family Zingiberaceae which the fruit is not rhizome. This plant is phenomenal in spice as called “queen of spices”. Cardamom is not only used as a food additive in cooking but also is used in traditional medicine. In Indonesia, cardamom has two types, namely Java cardamom (Amomum compactum) and small or true cardamom (Elettaria cardamomum). This study aims to determine genetic diversity and relationship between 100 cardamom lines based on their morphological characteristics. This experiment was carried out conducted at the Manoko Research Station from January 2019 to December 2019. The seeds used are 100 lines from cross-pollination of cardamom parents. Seeds are planted in a 30 cm x 30 polybag filled with soil and manure. Morphological traits parameters observed divided into two categories are a quantitative and qualitative character. Morphological quantitative traits include plant height, number of tillers, number of leaves, stem diameter, leaf length, leaf width, and leaf thickness. Whereas qualitative morphological characteristics are leaf color, leaf shape, leaf base shape, and leaf tip shape. The value of genetic diversity, which a big show by the morphology characteristics is the number of tillers, number of leaves, stem diameter, and leaf thickness where shows the coefficient variety value more than 25%. Cluster analysis created three major groups.

Keywords: Breeding, spices, morphological, cluster analysis, superiority

1. Introduction
Cardamom is known as “Queen of spices” belongs to the monocotyledonous family Zingiberaceae, besides that cardamom is indigenous to South India and Sri Lanka, and is believed the plant has been originated in the moist evergreen rainforests of the Western Ghats of South India [1]. Cardamom can grow in mid-elevation until high elevation about the altitude of 600-1300 m asl. Based on plant botany, cardamom is divided into 3 genera, namely Elettaria, Amomum, and Aframomum. However, in the world of international trade, cardamom divided into three types, namely green cardamom, black cardamom, and Madagascar cardamom [2].

The basic chromosome number of cardamom is x = 12 and 2n = 48 which indicates a balanced tetraploid nature [3]. Cardamom has bisexual flowers, is self-compatible, but crosses occur frequently. The blooming period of cardamom flowers occurs for 8-15 hours per day. The peak flowering is spread over a period of six months from May to October [4]. The essential oil content in cardamom is Alpha pinene, sabinene, beta pinene, cineole [5], and 1.8 Cineole [6]. Cardamom propagation generally using seed and suckers, its showing considerable variation in cultivation [7]. Cardamom is used as an additive flavor, perfumery, and traditional medicine.
There are two types of cardamom that grow in Indonesia, namely Java cardamom (*Amomum cardamomum / compactum*) and green cardamom (*Elettaria cardamomum*). Java cardamom is native plant from Indonesia and grows wild in the forests on the Java Island. Currently, Java cardamom is widely cultivated in various regions in Indonesia, such as Central Java, East Java, West Java and West Sumatra, which are center production area of *Amomum compactum* [8]. Green cardamom is also called small cardamom, true cardamom, and green cardamom which grows in India, Guatemala, Sri Lanka, Nepal, Indonesia, Costa Rica, Mexico and Tanzania [3]. Green cardamom grows in the moderate to highlands between 600 m above sea level - 1400 m above sea level.

The genetic diversity and heritability of a plant is an important component in plant breeding program. Plant genetic diversity is enhanced through introduction, conventional (crossover), and biotechnology (mutation, transgenic, gene transfer). The genetic diversity determines the effectiveness of selection; therefore, selection will be effective if the diversity in the high population [9]. Genetic diversity can be observed in intra-species, inter-species, segregated populations or mutant plant populations [10]. Heritability is a genetic parameter used to measure the ability of genotypes in plant populations to inherit their characteristics [11]. Estimation of heritability value aims to determine the plants trait influenced by genetic or environmental factors. In addition, the heritability value is to facilitate the selection of plants in the population. This study aims to determine the diversity of the first generations from cardamom parents cross.

2. Material and method
2.1. Plant material
The material used in this study is 100 cardamom lines produced from the crossing of cardamom parents naturally. The research was conducted from January to December 2019 at the Manoko Research Station, Indonesian Spice and Medicinal Research Institute, Lembang, West Java, Indonesia (1,500 m asl).

2.2. Observation on morphological traits
The study was conducted with a descriptive method which observed the morphological character of plants. Morphological characters of plants observed are quantitative data. The quantitative plant morphological characters observed were plant height, number of tillers, number of leaves, stem diameter, leaf length, leaf width, and leaf thickness. The Leaf observations were taken from the third leaves of cardamom plants. Observations of plant morphology characters follow the guidelines for plant morphology descriptors [12].

2.3. Statistical analysis
The measurement of genetic diversity and correlation coefficient was based on mean values of lines using R software. The correlation coefficient for evaluate the relationships among the different variables in the experiment using R software. The coefficient of diversity is calculated by the following formula:

\[
CV = \frac{S}{\bar{x}}
\]

\[
S = \frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n - 1}
\]

Where: 
- \( S \) = Standard Deviation \\
- \( \bar{x} \) = means of observation value \\
- \( x_i \) = observation \( i = 1, 2, 3, 4, 5 \) \\
- \( n \) = total of sample observation

The value coefficient variance (CV) is used to estimate the level of diversity of the observed line characters, namely the CV value of 0% to 25% indicates low diversity, while the high diversity level is if the CV value is > 25%. Genetic diversity analysis among 100 cardamom lines was determined by cluster hierarchy analysis using R software.
3. Result and discussion

3.1. Genetic diversity

The results of the analysis showed a significant level of phenotypic variation between 100 lines of cardamom in morphological characters (Table 1). The highest mean value of the cardamom genotype for plant height characters has a value of 116.16 and leaf length characters that has a value of 37.01. The highest coefficient variety of genetics is the characteristics of number of tillers, number of leaves, stem diameter, and leaf thickness. The value of the coefficient variety of each character is 61.37%, 47.46%, 39.66%, and 37.55%. The largest variance is the plant height with a variance value of 667. The highest variance value shows the higher the fluctuation of data from one data to another.

| No | Characters               | Mean   | SD    | Minimum | Maximum | CV (%) | Variance |
|----|-------------------------|--------|-------|---------|---------|--------|----------|
| 1  | Plant Height (PH)       | 116.16 | 25.83 | 54      | 197     | 22.23  | 667      |
| 2  | Number of Tillers (NoT) | 2.33   | 1.43  | 0       | 7       | 61.37  | 2.04     |
| 3  | Number of Leaves (NoL)  | 11.21  | 5.32  | 2       | 38      | 47.46  | 28.3     |
| 4  | Stem Diameter (SD)      | 0.99   | 0.39  | 0.2     | 1.9     | 39.66  | 0.153    |
| 5  | Leaf Length (LL)        | 37.01  | 5.1   | 22      | 55      | 13.78  | 26       |
| 6  | Leaf Width (LW)         | 4.96   | 0.93  | 3       | 8.3     | 18.79  | 0.869    |
| 7  | Leaf Thickness (LT)     | 0.01   | 0.01  | 0.01    | 0.03    | 37.55  | 2.88E-05 |

* SD: Standard Deviation; CV: Coefficient of Variation

3.2. Traits correlations

Correlation analysis is to know the closeness of relationships between variables [13]. Correlation coefficients between morphological traits Rhizome given in table 2. Plant height and stem diameter trait has a positive correlation with all morphological traits. The results of Patin et al. (1998) research there is a significant and positive correlation between the yield/plants with the children/clumps on small cardamom [14]. Positive correlation is shown that the morphological traits of cardamom interact with each other. Negative correlation occurs between leaf width with number of tillers, and between leaf thickness with number of leaves. Negative correlation is shown no interaction traits with each other. Plant height trait has a strong significant correlation with leaf length, it is means plant height give effect with leaf length. Correlation between traits is so important because it make easy for the breeder to select important characters from the studied traits [15].

|     | PH     | NoT    | NoL    | SD     | LL     | LW     | LT     |
|-----|--------|--------|--------|--------|--------|--------|--------|
| PH  | —      | —      | —      | —      | —      | —      | —      |
| NoT | 0.247* | —      | —      | 0.368***| —      | —      | —      |
| NoL | 0.268***| 0.368***| —      | —      | —      | —      | —      |
| SD  | 0.297**| 0.122  | 0.08   | —      | —      | —      | —      |
| LL  | 0.675***| 0.119  | 0.237* | 0.31**| —      | —      | —      |
| LW  | 0.184  | -0.012 | 0.041  | 0.408***| 0.301**| —      | —      |
| LT  | 0.157  | 0.129  | -0.043 | 0.14  | 0.065  | 0.04   | —      |

Noted: * p < .05, ** p < .01, *** p < .001

PH: Plant Height; NoT = Number of Tiller; NoL = Number of Leaves; SD = Stem Diameter; LL = Leaf Length; LW = Leaf Width; Leaf Thickness
3.3 Cluster analysis
Cluster analysis to provide the closeness of a plant with other plants. Plants in the genus may not necessarily have the same proximity. Relationships between accessions can provide information about the characteristic traits of each group of accessions that are formed [16]. The result of eigenvalues shown three major group of 100 lines cardamom based on morphological (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Eigenvalue analysis for determines group of 100 cardamom based on morphological traits.

![Figure 2](image2.png)

**Figure 2.** Cluster analysis of 100 cardamom lines.

The eigenvalue results provide 3 groups whose results can provide strong support for group establishment in cluster analysis. Cluster analysis showed 100 cardamom lines divided into three major groups with dissimilarity distance 12 (Figure 2). The first generation of cardamom provided a good choice in the assembly of new varieties. The selection of parents should avoid the level of relationship that is too close because it increases the chance of gathering recessive genes and results in "deep cross depression" in the offspring [17]. The crosses between plants the farther of the relationship, the result will be less successful, but the possibility of obtaining superior genotypes was greater if the crosses were successful [18]. The more diverse the genetics, the more likely it is to obtain superior genotypes. Homozygosity occurs if the crosses between individuals who are genetically close or related to the same
kinship, on the other hand, heterozygosity occurs if crossing between individuals with a large genetic distance or distant kinship. Genetic diversity and superior in morphological characteristics provide breeders with opportunities or challenges to assemble new superior varieties.

4. Conclusion
In this study, based on the coefficient of variation value, the high level of accession diversity was indicated by morphological characteristics, namely the number of tillers (61.37%), number of leaves (47.46%), stem diameter (39.66%), and leaf thickness (37.55%). The results of the kinship analysis showed that the 100 cardamom lines were divided into three major groups.

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