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KORELASI KARAKTER PERTUMBUHAN DAN HUBUNGAN GENETIK AKSESI *Cinchona Ledgeriana* MOENS ASAL 4 TETUA SETELAH PEMBERIAN KOMPOS LIMBAH KOPI DAN PUPUK HAYATI

GROWTH TRAITS CORRELATION AND GENETIC RELATIONSHIP OF Cinchona Ledgeriana MOENS ACCESSIONS DERIVED FROM 4 PARENTAL LINES AFTER COFFEE WASTE COMPOST AND BIOFERTILIZER UTILIZATION

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ABSTRAK

Kina (*Cinchona ledgeriana* Moens) merupakan tanaman perkebunan ekonomis tinggi yang memiliki manfaat sebagai obat bahan alam. Produktivitas kina dapat ditingkatkan melalui budidaya plasma nutfah potensial dan pemeliharaan dengan menggunakan pupuk hayati dan organik. Penelitian ini bertujuan untuk mempelajari hubungan genetik aksesi plasma nutfah kina dan korelasi antar sifat morfologi yang diberikan aplikasi pupuk hayati dan organik. Studi ini dilaksanakan di Balai Penelitian Teh dan Kina Gambung, Pasir Jambu, Bandung, Jawa Barat dari bulan November 2023 hingga Januari 2024. Sebanyak dua belas aksesi dipilih dari populasi dan dievaluasi berdasarkan enam karakteristik morfologi pertumbuhan. Analisis yang digunakan adalah analisis korelasi Pearson, principal component analysis (PCA), dan clustering untuk mengetahui korelasi antar karakter pertumbuhan dan hubungan antar plasma nutfah. Hasil penelitian diperoleh beberapa karakter pertumbuhan memiliki korelasi yang tinggi (>0,5) yaitu tinggi tanaman dengan diameter batang, diameter batang dengan jumlah daun, tinggi tanaman dengan jumlah daun, dan tinggi tanaman dengan tebal kulit batang. Populasi tanaman kina menunjukkan keragaman genetik yang cukup tinggi teridentifikasi melalui ciri morfologi, dengan total nilai PC1 dan PC2 mencapai 73,36%. Analisis klaster menghasilkan empat kelompok dari 12 aksesi, di mana kelompok 1 mencakup 50% dari keseluruhan sampel. Pemberian kompos limbah kopi dan biofertilizer berpengaruh terhadap karakter batang kina, meskipun genotipe memiliki respon yang beragam terhadap pemberian kompos limbah kopi dan biofertilizer terhadap sifat pertumbuhannya.

ABSTRACT

Cinchona (Cinchona ledgeriana Moens) is a plantation crop with high economic value with benefits as a natural medicine. Cinchona productivity can be enhanced by cultivating the potential germplasm and maintenance using bio and organic fertilizers. This research aims to study the genetic relationships and correlations among cinchona germplasm accessions' morphological characteristics under bio- and organic-fertilizer treatments. From November 2023 to January 2024, the research was conducted at the tea and cinchona research center in Gambung is situated in the Pasir Jambu District of Bandung Regency, West Java. The research evaluated 12 accessions, which were selected from the population, by examining six of their morphological growth characteristics. The analysis uses Pearson's correlation, principal component analysis (PCA), and Clustering to determine the correlation between growth characters and the relationships among germplasm. The results obtained some growth characters have a high correlation (>0.5), namely plant height with stem diameter, stem diameter with leaf number, plant height with leaf number, and plant height with bark thickness. The population of cinchona plants exhibits wide genetic diversity based on morphological characteristics. The data analysis revealed that the first two principal components explained 73.36% of the variation. Using cluster analysis, the 12 accessions were categorized into four major groups, and Group 1 was the most prevalent, accounting for 50% of the 12 accessions. The coffee waste compost and biofertilizer influence cinchona stem characters, even though the genotypes have various responses to coffee waste compost and biofertilizer applications for their growth traits.

1. INTRODUCTION

The cinchona plant (*Cinchona ledgeriana* Moens) originated in South America, including Peru, Bolivia, Ecuador, and Colombia, and was introduced to Europe in 1640 (Wiranova *et al*., 2025). It has been cultivated in various tropical countries, including Indonesia, since the Dutch brought it to Java Island (Kacprzak, 2013). The cinchona plant is an industrial plant with alkaloids in its stem bark. It has substantial value and is used in the pharmaceutical, food, and beverage industries (tonic water) (Maxiselly *et al.,* 2021). Cinchona plant (*Cinchona ledgeriana*) has high economic value due to its alkaloid compounds, such as cinchona and quinine, which function as antimalarials (Ruiz-Mesia *et al.,* 2005). This plant has long been cultivated in Indonesia, especially in highland areas, because it requires a low temperature with sufficient rainfall.

Production and quality of cinchona plants still face various challenges, including optimizing growth and yield quality through proper fertilization (Maxiselly *et al.,* 2020). Plant maintenance techniques will support results on plant quantity and quality. The maintenance of cinchona plants includes proper fertilization using combinations of organic and inorganic fertilizers, which can be recommended as a technique for cultivating cinchona plants in the immature phase to accelerate the growth of cinchona stem diameter as one of the quality criteria for cinchona stems (Maxiselly *et al.,* 2017). Maxiselly *et al.,* (2022) reported that applying vermicompost as one of the organic fertilizers in immature cinchona plants is able to increase leaf number and stem diameter.

Other organic fertilizers, such as coffee waste compost and biofertilizers, positively impact plant growth and help maintain environmental sustainability. Coffee waste, rich in nitrogen, phosphorus, and potassium, can enhance soil fertility and provide the nutrients plants need (Alexander *et al.,* 2008). Two types of waste are generated during coffee production: solid waste from stripping coffee skins in dry processing and liquid waste from washing and peeling coffee beans in wet processing (Czekata *et al.,* 2023). A combination of organic fertilizer from coffee waste and a biofertilizer was applied to improve all measured growth characteristics, including plant height, stem diameter, number of primary branches, primary branch length, leaf area, and chlorophyll index (Maxiselly *et al.,* 2023). On the other hand, biofertilizers contain microorganisms that can enhance nutrient availability around the plant roots, strengthen the root system, and increase the plant's resistance to diseases (Bonita *et al.,* 2024). With the combination of these two types of fertilizers, there is expected to be a significant improvement in the growth and quality of *Cinchona ledgeriana* Moens yields.

In addition to growth factor studies, genetic analyses of the plant's germplasm are needed. Information on genetic relationships based on growth traits can support the effective utilization of plant genetic resources and improve breeding sustainability (Kumar *et al*., 2026). Genetic diversity and relationships can be assessed using population variance, Principal Component Analysis (PCA), and cluster analysis. These methods, particularly PCA and cluster analysis, help reveal the degree of similarity or difference among genotypes to characterize plant traits (Maxiselly *et al.,* 2024a). Research on the genetic relationship of plant genetic resources can provide insights into genetic diversity and plant responses to fertilization treatments, ultimately contributing to optimizing plant breeding programs (Ng & Tan, 2015). Through this approach, a correlation between growth and the genetic relationship of the *Cinchona ledgeriana* germplasm, treated with coffee waste compost and biofertilizer, is expected to be obtained. This research aims to identify the correlation between growth and the genetic relationship of the *Cinchona ledgeriana* germplasm applied with coffee waste compost and biofertilizer. The results of this research are expected to serve as a basis for developing effective and environmentally friendly fertilization strategies to enhance the productivity of cinchona plants in Indonesia.

2. MATERIALS AND METHODS

2.1 Plant Materials

The planting materials used were genotypes from 4 parental lines of a 2-year-old cinchona germplasm collection (Cib, Ckb, QRC, Afrika) species *Cinchona ledgeriana*, which originated from seed, with 12 accessions in total. The accessions were evaluated for growth after the application of organic fertilizer made from coffee waste an organic fertilizer made from coffee waste.

2.2 Experimental Design

From November 2023 to January 2024, the research was carried out at the Tea and Cinchona Research Center, located in Gambung, Pasir Jambu District, West Java, at approximately 1,300 meters above sea level. The design was carried out using "no layout design or called *rancangan tanpa tata ruang*", the design is suitable for limited material and high variation based on the originating material (Maxiselly *et al*., 2024a). The observations were made using a descriptive method by observing the morphological characteristics of the cinchona plant. All of the accessions were already applied by the recommended dosage of coffee waste compost (3 kg per plant) and biofertilizer (10 mL per plant) (Maxiselly *et al*., 2024b). The coffee waste compost was applied around the plants, whereas the biofertilizer was sprayed onto the leaves and watered into the soil.

2.3 Data Collection

The observed morphological attributes of the cinchona plant included stem diameter, leaf chlorophyll index, number of leaves, plant height, leaf area, and bark thickness. Plant height was measured from the stem base to the growing point using a measuring tape. The stem diameter is measured with a caliper at a height of 3 cm above the ground surface. The number of leaves is counted manually using a hand counter on all fully opened leaves per plant. Leaf area is measured on fully opened leaves by taking a photo and calculating the area in ImageJ. Using a Digital Chlorophyll Meter, chlorophyll content is determined by clamping the device onto a plant's leaves and reading the resulting Chlorophyll Content Index (CCI) value from its screen. Measurements were taken on three leaves to achieve a single cumulative chlorophyll value. The thickness of the bark was measured by scraping a small amount of bark using a cutter and then measuring it with a caliper.

2.4 Data Analysis

Data analysis of all accessions was performed using the average of the sample, Pearson correlation, Principal Component Analysis (PCA), and cluster analysis methods with the XLSTAT 2024.4.1 software.

3. RESULTS AND DISCUSSION

3.1 Growth traits correlation of *Cinchona ledgeriana* Germplasm

The correlation between the growth traits of *Cinchona ledgeriana* germplasm is shown in (Figure 1) and Table 1. Figure 1 presents a correlation map among growth traits of *C. ledgeriana* germplasm, which is explained in more detail in Table 1 below. The results of the Pearson Correlation test on all morphological growth traits of the *Cinchona ledgeriana* germplasm, including plant height, stem diameter, leaf area, leaf number, leaf chlorophyll index, and bark thickness, showed positive correlations between plant height and leaf number, plant height and bark thickness, and stem diameter and leaf number. The analysis results do not show any correlation between plant height and leaf area and leaf chlorophyll index, stem diameter and leaf area and leaf chlorophyll index and bark thickness, leaf area and leaf number and leaf chlorophyll index and bark thickness, leaf number and leaf chlorophyll index and bark thickness, and leaf area index and bark thickness.

In Table 1, plant height does not correlate with leaf area and leaf chlorophyll index but positively correlates with stem diameter, leaf number, and bark thickness; The correlation coefficients were r = 0.893, r = 0.637, and r = 0.627. The correlations are classified as very strong, strong, and firm. The diameter of the stem is not correlated with leaf area, leaf chlorophyll index, and bark thickness but is very strongly correlated with the number of leaves; the positive correlation criterion for these two parameters is (r=0.881).

According to Maxiselly *et al.,* (2022) organic fertilizer will improve cinchona growth characteristics, including stem diameter. One of the most crucial traits of cinchona is stem development, which is related to quinine salt composition. Based on Maxiselly *et al.* (2023b), a combination of coffee waste and biofertilizer, effective for boosting coffee plant growth, is hypothesized to affect the cinchona plant. Balancing fertilizer application will support cinchona development, specifically the growth trait (Maxiselly *et al.,* 2017).

Table 1. Pearson Correlation Test Among Growth Characters of Cinchona Accessions.

Variables	Plant height (cm)	Stem Diameter (cm)	Leaf Area (cm^2)	Leaf Number	Leaf Chlorophyll Index	Bark Thickness (mm)
Plant height (cm)	**1**	**0.893**	0.343	**0.637**	0.431	**0.627**
Stem Diameter (cm)	**0.893**	**1**	0.159	**0.881**	0.396	0.474
Leaf Area (cm^2)	0.343	0.159	**1**	-0.186	0.127	-0.090
Leaf Number	**0.637**	**0.881**	0.186	**1**	0.227	0.303
Leaf Chlorophyll index	0.431	0.396	0.127	0.227	**1**	0.433
Bark Thickness (mm)	**0.627**	0.474	0.090	0.303	0.433	**1**

Note: Values shown in bold are significantly different from 0, based on an alpha level of 0.05. This is consistent with the Sig. value < 0.05, indicating that the parameters are said to be correlated; conversely, if the Sig. Value> 0.05, the parameters are said to be uncorrelated. Positive correlation with a strong degree of relationship (0.60-0.799). The positive correlation with the degree of relationship is powerful (0.80–1.000) [27].

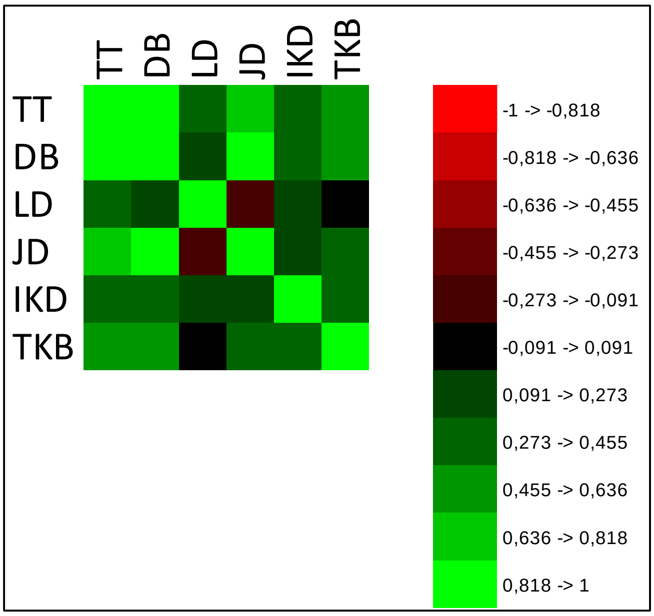

Figure 1. Correlation Map Between Growth and Genetic Relationship of *Cinchona ledgeriana* Accesions*, Code characters:* TT: Plant height; DB: Stem diameter; LD: Leaf area; JD: Number of leaf; IKD: Leaf chlorophyll index; TKB: Bark thickness.

3.2 Relationship of Accession Based on Cluster Analysis

Cluster analysis is used to determine genetic relationships based on morphological similarity among plants. Cluster analysis is illustrated as a dendrogram containing similarity or dissimilarity coefficient values that indicate the degree of similarity among plants (Dencic *et al.,* 2016). Coefficients with a value of <60% have a distant relationship, while coefficients with a value of >60% have a close relationship. Therefore, accessions with lower similarity values have greater potential for use in breeding programs (Karamang *et al.,* 2023).

The relationship of accession can be determined through cluster analysis. Cluster analysis is used to clarify the grouping of closely related accessions based on the distance between accessions. Figure 2 shows whether there is a sibling relationship or not, even if they originate from the same parent. It is evident from Figure 2 that there are four distinct groups, namely C1, C2, C3, and C4. Afrika parental group has a morphological appearance similar to some parts of Cib 5 and Ckb 3, while the other accessions exhibit different characteristics. The results of the AHC analysis are identical to the results of the PCA analysis.

Cluster analysis is increasingly utilized as a genetic approach to assess the relationships of genetic materials according to the traits being examined (Ene *et al.,* 2022). Through cluster analysis, 12 accession samples were obtained and divided into four major groups. Accessions within the same group indicate a close relationship (Prayoga *et al.,* 2017). The results of cluster analysis grouped the 12 tested accessions into two major clusters, with a genetic similarity value of 25% (Soegianto *et al.,* 2011). Group 1 dominates the sample population group, accounting for 50% of the 12 accessions. Each group consists of the following: Group 1 consists of 6 accessions, Group 2 consists of 2 accessions, Group 3 consists of 3 accessions, and Group 4 consists of 1 accession. The members of the accessions can be seen in Table 2.

Analysis of genetic diversity and relatedness among accessions can be a consideration for initial selection for clonal propagation. Genetic diversity dramatically influences the success of selection in plant breeding programs (Tiwari *et al.,* 2022). During crossing, it is not recommended to use accessions from the same group, as this can lead to high inbreeding. This will affect the traits of the offspring produced, as they have low variability during hybridization (Prayoga *et al.,* 2017). Information related to genetic relationships based on morphological characteristics needs to be accompanied by information based on molecular characteristics. Many plants that appear morphologically different still have genetic similarities within their genomes (Jarwah *et al.,* 2019). Morphological trait analysis can serve as a foundation and a basis for understanding other traits.

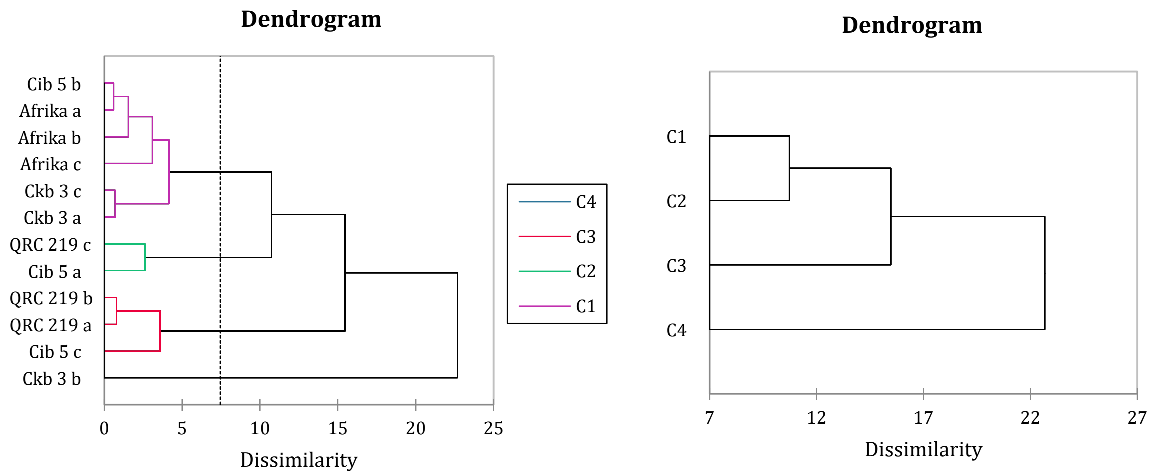

Figure 2. Cluster Analysis of 12 Cinchona Accessions by coffee compost and biofertilizer utilization.

Table 2. Members of Accession Groups Based on the Dendrogram

Cluster	1	2	3	4
Number	6	2	3	1
Percentage (%)	50%	16.7%	25%	8.3%
Accessions group	Afrika 1; Afrika 2; Afrika 3; Ckb 3 (a); Ckb 3 (c); Cib 5 (b).	Cib 5 (a); QRC 219 (c).	QRC 219 (a); QRC 219 (b); Cib 5 (c).	Ckb 3 (b)

3.3 Genetic Relationship by PCA and Clustering Analysis

Principal Component Analysis (PCA) is a multivariate analysis technique that helps pinpoint the most influential traits contributing to genetic diversity, allowing for more significant differences among the resulting groups (Meyer *et al.,* 2004). PCA used in exploration and characterization activities can show differences in characters and determine which character distributions influence the diversity of each accession. Table 3 shows the PCA results of 12 seed-origin genotypes of *Cinchona ledgeriana* based on morphological characters.

Based on their morphological traits, a PCA was performed on 12 *Cinchona ledgeriana* accessions. The analysis produced two principal components (PCs) that collectively accounted for 73.36% of the total variation, with eigenvalues ranging from 1.186 to 3.216 (as shown in Table 3). The total variation value of 6 morphological characters in cinchona plants shows eigenvalues greater than one and a total variation value of 73.36% up to PC 2. PC 1 has a variation value of 53.60%, while PC 2 has a value of 19.77%. The PC values from PC1 to PC2 were greater than 1, indicating that these components significantly influenced the grouping of the accessions analyzed (Maxiselly *et al.,* 2023a). The closer an accession is to a specific character, the more valuable the character in that accession is compared to other accessions (Bonita *et al.,* 2024). Variables with an eigenvalue of more than 1 are retained, while values of less than 1 can be ignored (Munandari *et al.,* 2022).

Table 4 shows the grouping based on characteristics. Characters with bold numbers mean they influence the grouping. In PC1, the traits contributing to diversity included plant height, leaf number, stem diameter, leaf chlorophyll index, and bark thickness. The character that contributes to PC 2 is the leaf area. These five characters show significant differences between accessions. Although leaf chlorophyll index contributed to PC1, field measurements indicated relatively small differences among accessions.

The data show that each principal component has an eigenvalue > 1, but not all characters contribute to the principal components, as they are based on loading factor values. The loading factor is an index that reflects the degree of correlation between variables, observed factors, or artificial variables created in factor analysis (Santoso & Kusumo, 2016). The factor loading value that can be used as a reference for the significance of the variable relationship is ≤ -0.7 or ≥ 0.7 (Zhao *et al.,* 2021). According to Prayoga *et al.,* (2017) characters with high, positive factor loadings contribute most to the diversity of genetic sources.

As shown in Table 4, the Principal Components (PCs) are interpreted by examining their factor loadings, which indicate the correlation between the original data variables and each PC (Khan *et al.,* 2022). The results for PC1 and PC2 identified characters with factor loadings greater than 0.7, indicating a maximum positive correlation. The factor loadings for PC 1 that exceed 0.7 include plant height, stem diameter, and leaf count. On PC 2, there is a value greater than 0.7 for the leaf area character. If we compare the top characters, such as eigenvectors and factor loadings, they show similarities from PC 1 to PC 5. Researchers can use this option to determine the PC interpretation technique for identifying the contribution and correlation of distinguishing characters.

The biplot graph (Figure 3) shows that QRC 219 (b) and QRC 219 (a) have similar traits because they come from the same parental, but both accessions can also cluster with others, namely Cib 5 (c) from a different parental. This condition may occur because Cib 5 could have some similar morphological characters with QRC 219. However, there is one QRC 219, namely QRC 219 (c), that separates from the other QRCs and is positioned near Cib 5 (a) and Cib 5 (b). Although they come from the same parental, QRC 219, each accession has different morphological data.

All accessions of cinchona plants originating from Afrika parents, Afrika (a), Afrika (b), and Afrika (c), exhibit similar traits, placing them in the same quadrant and close positions. This suggests that the genetics inherited from Afrika parents are dominant despite similarities with Cib 5 (a), Cib 5 (b), and QRC 219 (c). Similarity between plants is marked by the position of the character points being close to the accession points. In this case, the characters are plant height, stem diameter, and bark thickness. The angles formed between vectors also help identify Vector orientation illustrates the correlation among characters. An acute angle between vectors (close to 0°) shows a strong positive correlation, while an obtuse angle indicates a strong negative one (Jarwar *et al.,* 2019).

Table 3. The Eigenvalue of Cinchona Accessions Diversity.

	PC1	PC2
Eigenvalue	3.216	1.186
Variability (%)	53.596	19.762
Cumulative (%)	53.596	73.358

Table 4. The Characters Influence Value on Grouping.

Traits	PC1	PC2
Plant height (PH)	**0.937**	0.197
Stem Diameter (SD)	**0.949**	-0.053
Leaf Area (LA)	0.153	**0.949**
Leaf Number (LN)	**0.786**	-0.426
Leaf Chlorophyll Index (LCI)	**0.575**	0.223
Bark Thickness (BT)	**0.682**	-0.106

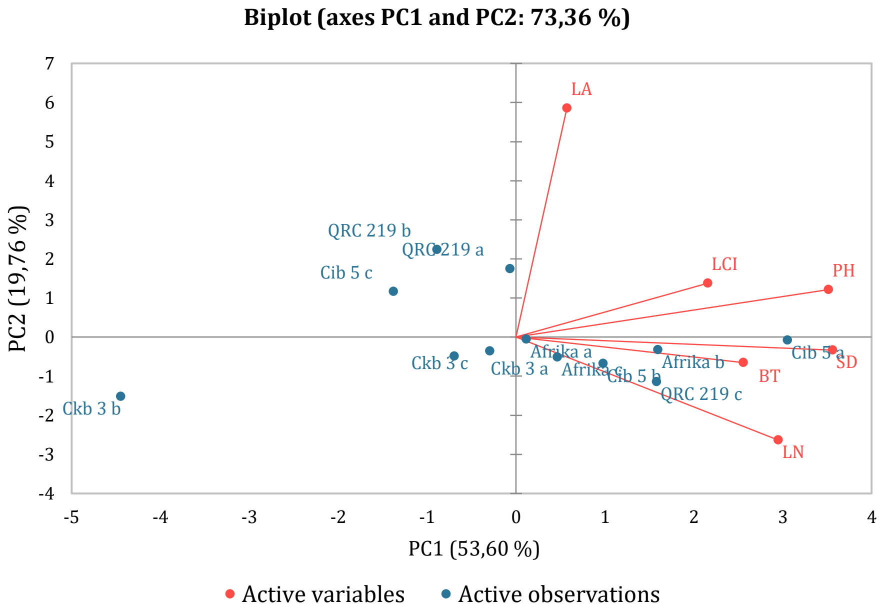

Figure 3. Biplot Graph of PC 1 and PC 2 of cinchona accession and growth traits.

Accession Cib 5 (a), Cib 5 (b), and Afrika are closely positioned, indicating they share similarities. The similarity lies in the stem diameter and bark thickness characteristics, unlike Ckb 3 (b), which is placed far apart, indicating a distant relationship. The position of the character vector and genotype point indicates the magnitude of the character in that genotype (Santoso & Kusumo, 2016). The distribution on the biplot can be said to cluster. That condition can occur due to differences in characteristics. Interpretation of factors is achieved by analyzing which characters display significant factor loadings among the identified factors (Maxiselly *et al.,* 2024). Based on previous reports, leaf morphology-related traits are included among vegetative characters, the characters related to leaf morphology are among the vegetative characters (Maxiselly *et al.,* 2024).

A character's contribution to clustering can be assessed from its loading value and the cumulative variance explained by the principal components. An eigenvalue can be said to influence clustering if it is greater than 1. Only PC1 and PC2 were used in this research because their contribution percentages were already higher than those of the others.

Each character's contribution to diversity may exist, but it does not show maximum contribution. Genetic variability underlies the genetic improvement of certain traits (Zhao *et al.,* 2021). With characters exhibiting variability in each component, which significantly contributes to accession total diversity and genetic distance between accessions, these accessions can be used as genetic improvement materials for cinchona in Indonesia. In addition, the selection of accessions can be adjusted according to the traits to be improved, while aligning with the diversity and average values of the characteristics (Cupic *et al.,* 2009).

The identical treatment will elicit different responses across the different accessions. In this case, applying coffee waste compost and biofertilizer has a varying effect for increasing cinchona growth. It illustrates that the diversity pattern of cinchona accessions shows wide variation in growth traits, while the fertilizer dosage is similar.

4. CONCLUSIONS

The twelve cinchona germplasm accessions treated with coffee waste compost and biofertilizer showed high variability in morphological traits, including stem diameter, leaf number, leaf area, leaf chlorophyll index, and bark thickness. The seed-origin cinchona plant population exhibits broad genetic diversity as indicated by morphological characteristics. This is indicated by the total PC value on PC 1 and PC 2 being 73.36%. Using cluster analysis, 12 accessions were identified and grouped into four major groups. Group 1 dominated the sample population group, accounting for 50% of the 12 accessions. The coffee waste compost and biofertilizer influence cinchona stem characters, even though the accessions have various responses to coffee waste compost and biofertilizer applications for their growth traits.

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