

# Relationship analysis and genetic diversity of tea *Camellia sinensis* germplasm from illegitimate seeds based on morphological characters

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**Abstract.** Maxiselly Y, Bakti C, Murgayanti, Ernah, Wahyudin AA, Prayoga MK, Karuniawan A. 2024. Relationship analysis and genetic diversity of tea *Camellia sinensis* germplasm from illegitimate seeds based on morphological characters. *Biodiversitas* 25: 3486-3495. Tea (*Camellia sinensis* (L.) O. Kuntze) is a plant with self-incompatible traits and requires significant efforts to assemble superior clones. The generation of the superior clone is from seed-derived genetic material that demands comprehensive information on population variance, genetic diversity, and the level of relationship among accessions. Therefore, this research aimed to determine population variances, genetic diversity, and relationships of tea germplasm accessions based on morphological characteristics. The experiment was conducted from August 2023 to January 2024 at the Indonesian Research Institute Tea and Cinchona, Mekarsari Village, Pasir Jambu District, Bandung Regency, West Java, Indonesia. An experimental method, "no layout design," was used to identify morphological traits of 36 characters in 50 selected accessions from the population. Observational data were analyzed to determine variance values, principal component analysis (PCA), heatmap correlation, and cluster analysis using XLSTAT software. The results showed a wide variation of 50% among the 36 characters, with genetic diversity in the 2 Principal Components (PC) at 38.99%. The cluster analysis of accessions had an Euclidean distance range, measuring a measure of the straight line distance between two points of 2.23 to 12.86. This variation was caused by a high correlation of some characteristics, such as leaf length, width, and leaf area, as the potential to develop the new tea clone.

**Keywords:** Accession, cluster analysis, euclidean distance, principal component analysis, variance

## INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) is among the most important plantation commodities in Indonesia. Moreover, in 2020, the country produced 138 thousand tons of tea, reaching 8th among tea-producing countries worldwide (Muflihah et al. 2023). This crop is a beverage that has flavor profiles, stimulating effects, and health benefits such as helping manage low blood pressure, lowering the risk of obesity, and preventing cancer and diabetes (Wang et al. 2022; Mastur et al. 2023). Despite this significant production, the development of breeding tea continues to advance to support sustainable plantations. Generally, the conventional breeding process takes more than 10 years to assemble new superior clones (Bharti and Chimata 2019). This process starts with exploring and identifying germplasm, which functions as a genetic bank for the subsequent assembly of superior clones (Sriyadi 2012). Tea germplasm has been categorized into seedling germplasm, seed production, and clonal plantation, as well as plant introduction (Wibowo and Martono 2022). Seedling germplasm includes plants with unclear origin (illegitimate) and known pedigree (Ma and Chen 2018),

which is currently identified as a monoclonal plantation. Clonal plantations have similar genetic constitutions, leading to narrow genetic diversity that can cause endangered potency for future breeding. Additionally, it has a risk of overpopulation pests and climate change, which causes reduced productivity. The reliance on monoclonal tea plant materials in a singular location poses a substantial risk, particularly in the event of pest or disease outbreaks and unforeseen environmental changes (Pokharel et al. 2023). Developing a population with wide genetic diversity, including individual and population buffering, is essential to mitigate this risk. Individual buffering is the genotype traits of each plant, while population buffering is the interaction genotype traits of one population in the same environment (Bocci et al. 2020). The tea plant is self-incompatible, and long-term allogamy makes it exceedingly diverse, resulting in a broad genetic variety (Xia et al. 2020). A tea seed comprises an embryo and many extraembryonic layers that serve as a coating. It usually results from fusing the nuclei of male and female gametes (Patel et al. 2018). The tea germplasm of illegitimate seed plantations has been recorded in Indonesia. Plant material of illegitimate seed from

*Camellia sinensis* var. *assamica* was used for the F1 clonal to recommend plant material for the heritage clone in Indonesia's tea plantation (Sriyadi 2015). Creating new varieties of tea from illegitimate seeds is an attempt to expand the range of new traits in tea plants.

Predictor characters are used to assess the potential of germplasm as parents in designing breeding programs. It's the breeders who, by analyzing selected parents' diversity and genetic relationships, can predict the success of a breeding method. Research to determine the level of genetic diversity of tea plants is conducted by observing morphological characters. Moreover, estimating characters based on morphology traits to analyze genetic diversity is a general standard in germplasm evaluation (Lee et al. 2019). The method can use leaf criteria, canopy architecture, and the biological structure of flowers (Zakir 2019), with greater genetic diversity in leaf-related traits, such as shape and sides.

Genetic diversity and relationships can be analyzed using population variance, Principal Component Analysis (PCA), and cluster analysis. Statistical methods based on the population variance of tea plants have been reported (Khomaeni and Sriyadi 2011). PCA and cluster analysis examine the similarities and dissimilarities between genotypes to explore plant characteristics (Jarwar et al. 2019). Correlation between the characters is also crucial to report as a relation involving diversity patterns' distribution (Thammanu et al. 2021). The traits correlation is capably illustrated by heatmap analysis when using multiple variables (Haarman et al. 2015). Based on these facts, this research aims to determine the extent of genetic variability among the tea gene pool in Indonesia following different analysis approaches on morphological characters.

## MATERIALS AND METHODS

### Study area

The research was carried out using the experimental method "no layout design (*rancangan tanpa tata ruang*)". It was conducted from August 2023 to January 2024 at the Indonesia Research Institute Tea and Cinchona (IRITC), Mekarsari Village, Pasir Jambu District, Bandung Regency, West Java Province, Indonesia. The site is located at an elevation of 1,250 masl, with climate type B based Schmidt Ferguson, 2,960 mm.year<sup>-1</sup> rainfall, and andisol soil with a pH of around 4.5 up to 5.5.

### Material

A total of 50 accessions were collected from the germplasm of the tea plantation, each representing an illegitimate seed planted by R.E Kerhoven in 1907, estimated 116 years old. These accessions were obtained from tea plantations that had previously experienced pruning two years ago. Meanwhile, the tools that were used included labels, calipers with an accuracy of 0.1 mm,

meters, an RHS Color Chart, a screw micrometer with an accuracy of 0.01 mm, a magnifying glass, a ruler, and a protractor.

## Procedures

### Selected accessions

The basis for consideration for the selected accessions was tea plants from seed propagation of the experimental field. Therefore, to facilitate observation and recording, the experimental field was plotted into a plot covering a one-hectare. After creating a plot, the plant population was calculated, and 50 accessions were selected located in the A2 block of the IRITC field using the criteria of tea plants with healthy conditions, namely damaged tea bushes. Subsequently, labeling was carried out using a code label, describing a population plot and number of accessions (A2.001-A2.097).

### Collecting data

A total of 36 traits were examined based on the morphological data to investigate the morphological diversity, as shown in Table 1. Moreover, these traits were selected by a modified descriptor by the International Plant Genetic Resources Institute (IPGRI) and the International Union for the Protection of New Varieties of Plants (UPOV) (IPGRI 1997; UPOV 2020). The leaf-related traits such as leaf shape, leaf upper surface, leaf thickness, leaf apex, leaf apex habit, leaf base shape, leaf margin, leaf area, length of leaf, the width of leaf, leaf venation, leaf vestiture surface, leaf pubescence, leaf waxiness, leaf blade attitude, and leaf cross section were identified using five most fully expanded mature and intact leaves collected from each accession. Meanwhile, the flower-related traits such as length of pedicel, diameter of flower, inner petal color, outer side sepal pubescence, style length, style splitting, position to stamens, ovary pubescence, ovary pubescence density included the completely developed open flowers stage, as shown in the research procedures of morphological characterization were presented in Table 1.

### Maintenance of tea plants

Maintenance was regularly conducted, including fertilization, weeding, and pest and disease control. Fertilization occurs when rainfall ranges from 60-100 mm within 10 days. Weed control is carried out by manual weeding and herbicides; control weeds with herbicides that contain glyphosate at a dose of 2 L.hectare<sup>-1</sup>. Pest and disease control use a mixture of the active ingredients chlorpyrifos and cypermethrin at a dose of 125 mL.150 L<sup>-1</sup> per a hectare. Pesticide application is carried out 10 days before plucking and after plucking. These activities were adjusted according to the procedures and schedules of the experimental field to obtain morphological characters of the selected accessions.

**Table 1.** Morphological characterization recorded for the current study

Characters	Criteria
<b>Growth-plant-related traits</b>	
Tree Habit (TH)	[1] = Shrub, [2] = Semi-arbour, [3] = arbour
Growth Habit (GH)	[1] = Fastigiate, [2] = Upright, [3] = Upright to spreading, [4] = Spreading
Plant Height (PH)	Measured from ground level up to unplucked plants in cm units.
Number of Nodes (NN)	Recorded on shoots lateral branch at tea plucking point.
<b>Leaf-related traits</b>	
Pigment young leaf at productivity season (PigL)	Identification of purple pigment in young leaf and petiole. [0] = Absent, [1] = Present
Immature Leaf Color (ILC)	Observation of the color of the unfurled leaf with RHS color chart. [1] = Yellow, [2] = Dark Green, [99] = Other
Mature Leaf Color (MLC)	Observation on the third leaf below the apical bud. RHS color codes are given below: [1] = Light Green (green group 138 A), [2] = Green (green group 138 B), [3] = Greyed-green (greyed-green group 191 A), [4] = Grayed-yellow (greyed-yellow group 160 A), [5] = Yellow-green (yellow-green group 147 B), [99] = Other
Leaf Shape (LS)	Observation on the fifth leaf below bud of a flush growth. [1] = Ovate, [2] = Oblong, [3] = Elliptic, [4] = Lanceolate
Leaf Upper Surface (LUS)	Observation on the fifth leaf below bud of a flush growth. [1] = Smooth, [2] = Rugose
Leaf Thickness (LT)	Observation on the fifth leaf and using a screw micrometer with an accuracy of 0.01 mm. Average score of five leaves.
Leaf Apex (LA)	Observation on the fifth leaf below bud of a flush growth. [1] = Acute, [2] = Blunt (Obtuse), [3] = Attenuate
Leaf Apex Habit (LAH)	[1] = Downturned (recurved), [2] = Straight
Leaf Base Shape (LBS)	Observation on the fifth leaf below bud of a flush growth. [1] = Attenuate (acute), [2] = Rounded, [3] = Blunt (obtuse)
Leaf Margin (LM)	Observation on the fifth leaf below bud of a flush growth. [1] = Entire, [2] = Wavy, [3] = Serrulate, [4] = Biserrate, [5] = Denticulate
Leaf area (L.cm <sup>2</sup> )	Observation on the fifth leaf below the bud of a flush growth with ImageJ software, use units cm <sup>2</sup> , and then average that.
Length of Leaf (LL)	Five leaves Observation use units cm. Observation on the fifth leaf below bud of a flush growth.
Width of Leaf (WL)	Five leaves were observed at maximum breadth, using units cm. The fifth leaf was observed below the bud of a flush growth.
Leaf Venation (LV)	[1] = Indistinct, [2] = Distinct with bulation
Leaf Vestiture Surface (LVS)	Observation on the lower surface. [1] = Glabrous, [2] = Appressed, [3] = Pubescent, [4] = Villous
Leaf Pubescence (LP)	Observation on the first leaf abaxial side with lup. [3] = Sparse, [5] = medium, [7] = Dense
Leaf Waxiness (LW)	[0] = Absent, [1] = Present
Density of Bud (DoB)	[1] = Sparse ( $\leq 4$ ), [2] = Intermediate (5 - 9), [3] = Dense ( $\geq 10$ )
Leaf Blade Attitude (LBA)	[1] = Erect ( $<35^\circ$ ), [2] = Semi-Erect ( $35^\circ - 75^\circ$ ), [3] = Horizontal ( $76^\circ - 90^\circ$ ), [4] = Drooping ( $>90^\circ$ )
Petiole Color (PC)	RHS code color below: [1] = Green (green group 139 A, 133 A, 137 C); [2] = Yellow-green (yellow-green group 144 A to 147 A); [3] = Green with greyed-purple color (green group 133A, greyed-purple group 186 B); [99] = Other
Young Shoot Color (YSC)	Observation on the bud. [1] = Green, [2] = Bronze, [3] = Red, [99] = Other
Young Shoot Pubescence (YSP)	Observation on the density pubescence of bud with lup. [3] = Sparse, [7] = Dense
Leaf: Cross Section (LCS)	Sample mature leaf below bud of a flush growth. [1] = Folded upward, [2] = Flat, [3] = Recurved
<b>Flower-related traits</b>	
Flower: Length of Pedicel (FLP)	Observation on the stage showed completely developed open flowers; the average of ten pedicels was measured in unit mm with an accuracy of 0.1 mm.
Flower: Diameter (FD)	Compare at least 10 flowers. [3] = small, [5] = medium, [7] = large
Flower: Inner Petal Color (IPC)	Observation on the stage completely developed open flowers. [1] = White; [2] = Greenish; [3] = pink
Sepal: outer side Pubescence (SoP)	Observation on the stage completely developed open flowers. [0] = Absent, [1] = Present
Flower: Length Style (FLS)	Observation on the stage completely developed open flowers and measured in units mm with an accuracy of 0.1 mm.
Style: Splitting (SS)	Observation on the stage completely developed open flowers. [1] = low/Geniculate, [2] = medium/ascending, [3] = high/terminal
Stigma: Position about stamens (SP)	Observation on the stage completely developed open flowers. [1] = far below, [3] = same level, [5] = far above
Ovary: Pubescence (OP)	Observation on the stage completely developed open flowers. [0] = Absent, [1] = Present
Ovary: Density of Pubescence (ODP)	Observation on the stage completely developed open flowers. [3] = sparse, [5] = medium, [7] = dense

## Data analysis

The data obtained from morphological characters were tested statistically using Microsoft Excel 2016 to obtain the minimum value (min), maximum value (max), mean, standard deviation (STDEV), and variance of the population ( $\sigma^2$ ), which indicated data diversity. The variance of the population was a measure of variability with two-digit numbers multiplied by the root of the standard deviation of each character. Saadah et al. (2023) from Anderson and Bancroft (1952) suggested a variance value equal to or greater than two multiplied by the standard deviation of variance ( $\sigma^2 \geq 2 \cdot \text{STDEV } \sigma^2$ ), indicating state-wide variability; the opposite will be stated as narrow variability.

The level of genetic diversity and the relationship between accessions based on morphological characters was analyzed using XLSTAT 2016. Moreover, XLSTAT was also applied to show the contribution of characters to population diversity by PCA, visualized into a biplot graph. Hierarchical cluster agglomerative analysis was used to determine genetic diversity based on the dissimilarity matrix of Euclidean distance and Ward's Linkage modeling. This research used Pearson's matrix data obtained from PCA to determine the correlation between characters, which was interpreted into a correlation heatmap. The visualization software used for this method was R Program version 4.3.3 with R packages ggcorrplot.

## RESULTS AND DISCUSSION

### Variance of population

Variability is an important benchmark in developing genotypes and can be obtained from the variance value of each character in the population, compared with twice the standard deviations. The population variance for each observed character of all accessions is shown in Table 2. The results showed that the variance among the 36 morphological characters of tea germplasm observed was a wide variation on 18 characters, which is 50% of the characters observed. Environmental effects, such as uncontrolled conditions in the field, different adaptive abilities in similar environments, and variation between accessions, could cause wide phenotypic variation. Genetic diversity is not only caused by the genetic constitution of plants but is also influenced by adaptation to their environment (Walter et al. 2024). Wide variations could also indicate the effectiveness of selection in plant breeding activities (Ahmad et al. 2021). The broad variation of morphological characters on tea indicated that the characters of both distanced parental cultivars have been segregating in hybrid progenies (Thuvaraki et al. 2017). The wider the variation in a population, the more diverse the character traits used to control genetics in the population. Selection is made on characters with wide variations, while those with narrow variations are avoided. The level of genetic variation in tea plants is also influenced by the treatment given by humans, such as selecting traits and developing them according to industry needs (Jiang et al. 2023). The broad variability in morphological characters in the tea population from seed

propagation was found according to some previous research (Khomaeni and Sriyadi 2011). For example, it was obtained that the yield characters per bush and the number of young shoots as characters varied widely for parent plant selection, obtaining 8 accessions among 105 in one population. Therefore, the characters with wide variations in this research could be used as considerations for further selection. Harvest-related characteristics are a crucial aspect of tea plant diversity. These harvest-related characteristics include shoot number, leaf area, and dry weight. Tea plants' wide range of characteristics provides a foundation for developing new superior varieties (Kottawa-Arachchi et al. 2017). Another crucial variable is leaf pubescence; based on Thuvaraki et al. (2017), a high correlation was found between the ordered arrangement of leaf pubescence trait and tea quality, implying that pubescence is a determinant in tea quality and hence important in quality selection.

### Principal component analysis

PCA simplifies and transforms correlated variables, enabling the identification of diversity within a dataset, which is interpreted into Principal Components (PC). Table 3 shows the PCA results of 50 *Camellia sinensis* genotypes based on morphological characters from seed propagation. The analysis obtained 5 PCs with a total variation contribution of 56.87% and an eigenvalue ranging from 2.015 to 9.428. The PC values shown from F1 to F5 have a value of  $>1$ , which means that this value greatly influences the grouping of genotypes used (Maxiselly et al. 2023). Recent research on PCA by Lin et al. (2024) reported that the accumulation of 5 PCs accounted for 55.54% of the 33 traits identified among morphology and biochemical traits in the local population of tea in Shiqian, Guizhou province, China.

PC interpretation is done by considering factor loading, the correlation value between the original data characters, and PC (Zhao et al. 2021). The factor interpretation process is carried out by observing the significant factor loading of a character among the factors, as shown in Table 4. In this research, the value of factor loading, which was considered more than 0.5, was identified as a trait with a significant and positive correlation with diversity in each PC (Prayoga et al. 2022). In F 1, flower-related traits were obtained, such as length of pedicel, diameter of flower, inner petal color, outer side pubescence in sepal, length style, splitting of style, position about stamens, the existence of pubescence and density of pubescence in ovary, while F 2 showed growth and tree habit, as well as leaf area, length, and width. Positive and significant characters were also obtained at plant height and number of nodes on the F 3, which showed growth plant-related traits. Although this research did not identify significant factor loading on F 4, F 5 showed only one significant character: leaf petiole.

The biplot constructed by the first two components is shown in Figure 1, with the cumulative of both axes accounting for 38.99% of the total variation. Specifically, F 1 and F 2 contribute 26.19% and 12.80% of the total phenotypic variations, respectively, showing homogeneous diversity. A comparison of narrow diversity for

morphological characters in tea plantations has been previously reported (Kamau et al. 2020; Khiavi et al. 2021; Dargah et al. 2023). Based on the results, the total of two PCs is much more limited than the component in this research. Kamau et al. (2020) stated that the % cumulative variation in the two PCs was 34.5%. Dargah et al. (2023) reported variations in F 1 and F 2, respectively, of 17.7% and 13.5%, while Khiavi et al. (2021) found F 1 of 16.96% and F 2 of 16.18%. Khiavi et al. (2021) also stated that the contribution of PC based on molecular markers only reached 76.61% for 10 PC. Characters related to leaf morphology were reported to be among the vegetative characters. Similarly, this research found another component besides flower morphology as reproductive characteristics, which were identified as traits of F 1.

Dargah et al. (2023) stated that the number of differences in the germplasm examined was acceptable due to tea propagation in Iran originating from open-pollinated seeds. However, the origin of the available germplasm in this research was from the seeds of old tea plants with unknown parents (illegitimate seeds). The biplot graph also shows the distribution of accessions in the four quadrants, each expressing accession groups and influential characters. The distribution of accessions is dominated by quadrant I (Positive-Positive), followed by quadrant IV (Positive-Negative), then quadrant II (Negative-Positive), and quadrant III (Negative-Negative). Additionally, there are 20 accessions in Quadrant I, 9 in Quadrant II, 4 in Quadrant III, and 17 in Quadrant IV.

**Table 2.** The variance of the population of characters

Variable	Min	Max	Mean	STDEV	$\sigma^2$	2*STDEV	Note
GH	2.000	4.000	3.400	0.756	1.739	1.512	Wide
TH	1.000	2.000	1.800	0.404	1.271	0.808	Wide
PigL	0.000	1.000	0.060	0.240	0.980	0.480	Wide
ILC	1.000	2.000	1.160	0.370	1.217	0.741	Wide
MLC	1.000	99.000	76.040	41.284	12.851	82.568	Narrow
LS	1.000	4.000	2.460	1.182	2.174	2.363	Narrow
LUS	1.000	2.000	1.640	0.485	1.393	0.970	Wide
LA	1.000	3.000	2.340	0.872	1.867	1.743	Wide
LAH	1.000	2.000	1.560	0.501	1.416	1.003	Wide
LBS	1.000	2.000	1.140	0.351	1.184	0.701	Wide
LM	3.000	4.000	3.020	0.141	0.752	0.283	Wide
LV	1.000	2.000	1.440	0.501	1.416	1.003	Wide
LVS	1.000	2.000	1.820	0.388	1.246	0.776	Wide
LBA	1.000	4.000	1.660	0.717	1.694	1.435	Wide
LW	0.000	1.000	0.360	0.485	1.393	0.970	Wide
PC	1.000	99.000	9.600	26.633	10.321	53.266	Narrow
DoB	1.000	3.000	2.500	0.707	1.682	1.414	Wide
YSC	1.000	3.000	1.100	0.364	1.207	0.728	Wide
YSP	3.000	7.000	5.160	2.014	2.838	4.028	Narrow
LP	3.000	7.000	4.120	1.154	2.149	2.308	Narrow
LCS	1.000	3.000	1.560	0.760	1.744	1.520	Wide
PH	74.000	114.000	91.260	9.337	6.111	18.674	Narrow
NN	2.000	8.000	5.560	1.473	2.427	2.946	Narrow
LL	5.920	14.820	9.886	1.979	2.813	3.958	Narrow
WL	2.740	6.240	4.039	0.745	1.726	1.490	Wide
L.cm2	11.868	63.039	27.723	10.836	6.584	21.672	Narrow
LT	0.234	0.762	0.287	0.070	0.530	0.141	Wide
FD	3.000	99.000	74.240	42.199	12.992	84.397	Narrow
IPC	1.000	99.000	73.560	43.355	13.169	86.710	Narrow
SoP	0.000	99.000	73.260	43.866	13.246	87.731	Narrow
SS	1.000	99.000	73.920	42.743	13.076	85.485	Narrow
SP	1.000	99.000	73.920	42.747	13.076	85.494	Narrow
OP	0.000	99.000	73.500	43.457	13.184	86.914	Narrow
ODP	1.000	99.000	74.080	42.477	13.035	84.954	Narrow
FLP	2.560	99.000	74.309	42.082	12.974	84.164	Narrow
FLS	2.050	99.000	74.338	42.032	12.966	84.064	Narrow

Note: Min: Minimum; Max: Maximum; STDEV: Standard deviation;  $\sigma^2$ : Variance. Code characters: TH: Tree Habit; GH: Growth Habit; PH: Plant Height; NN: Number of Nodes; PigL: Pigment young leaf at productivity season; ILC: Immature Leaf Color; MLC: Mature Leaf Color; LS: Leaf Shape; LUS: Leaf Upper Surface; LT: Leaf Thickness; LA: Leaf Apex; LAH: Leaf Apex Habit; LBS: Leaf Base Shape; LM: Leaf Margin; L.cm<sup>2</sup>: Leaf area; LL: Length of Leaf; WL: Width of Leaf; LV: Leaf Venation; LVS: Leaf Vestiture Surface; LP: Leaf Pubescence; LW: Leaf Waxiness; DoB: Density of Bud; LBA: Leaf Blade Attitude; PC: Petiole Color; YSC: Young Shoot Color; YSP: Young Shoot Pubescence; LCS: Leaf Cross Section; FLP: Flower: Length of Pedicel; Flower: FD: Diameter; Flower: IPC: Inner Petal Color; Sepal: SoP: outer side Pubescence (SoP); Flower: FLS: Length Style; Style: SS: Splitting; Stigma: SP: Position in relation to stamens; Ovary: OP: Pubescence; Ovary: ODP: Density of Pubescence

**Table 3.** Eigenvalue, variability, and cumulative of 5 PCs

	F1	F2	F3	F4	F5
Eigenvalue	9.4282	4.6067	2.3923	2.0318	2.0148
Variability (%)	26.1895	12.7965	6.6453	5.6438	5.5965
Cumulative %	26.1895	38.9860	45.6314	51.2752	56.8717

**Table 4.** Factor loading of 5 PCs

	F1	F2	F3	F4	F5
GH	-0.0379	<b>0.6307</b>	0.0737	-0.2786	0.3201
TH	0.0794	<b>0.6601</b>	0.2033	-0.1941	0.4376
PigL	0.1412	-0.6744	-0.0621	0.1871	0.0458
ILC	0.1563	-0.1347	0.4549	0.3848	0.0810
MLC	-0.0098	-0.1992	0.0051	0.2800	0.1685
LS	-0.2996	-0.3763	0.4300	-0.4275	0.0855
LUS	0.1542	0.0562	-0.5219	0.0471	0.0687
LA	0.2070	-0.1245	0.1541	0.3298	0.3509
LAH	0.0182	-0.1920	-0.3165	-0.0526	-0.4021
LBS	-0.0184	0.1456	-0.2100	0.0426	0.3247
LM	0.0895	0.0772	0.0836	-0.2743	-0.4221
LV	0.1766	0.0824	0.2913	0.0038	0.3923
LVS	-0.0519	0.2387	0.0368	-0.4051	0.3839
LBA	0.1437	0.2063	-0.3381	0.0709	-0.0428
LW	0.1883	-0.0127	-0.2358	0.1762	0.3392
PC	0.2047	-0.2511	-0.3267	0.3717	-0.2502
DoB	0.0440	-0.2888	0.3175	0.3382	-0.1274
YSC	0.0287	-0.6464	0.0161	0.1905	0.1364
YSP	-0.0071	-0.3551	-0.0709	0.2388	0.3166
LP	0.0236	-0.4161	-0.0690	0.3416	<b>0.5078</b>
LCS	0.1070	0.0720	-0.3129	-0.0582	0.0391
PH	0.0213	-0.1641	<b>0.6530</b>	0.0147	0.0843
NN	0.1157	0.0318	<b>0.5866</b>	0.2221	-0.4041
LL	0.2582	<b>0.8189</b>	0.0470	0.3675	-0.1226
WL	0.2035	<b>0.8251</b>	0.0845	0.4099	-0.0400
L.cm <sup>2</sup>	0.2410	<b>0.7948</b>	0.0921	0.4356	-0.1238
FD	<b>0.9934</b>	-0.0593	0.0111	-0.0838	-0.0015
IPC	<b>0.9935</b>	-0.0593	0.0118	-0.0826	-0.0006
SoP	<b>0.9935</b>	-0.0594	0.0118	-0.0820	-0.0002
SS	<b>0.9935</b>	-0.0595	0.0132	-0.0817	-0.0006
SP	<b>0.9932</b>	-0.0606	0.0123	-0.0821	-0.0020
OP	<b>0.9935</b>	-0.0598	0.0121	-0.0821	0.0002
ODP	<b>0.9932</b>	-0.0626	0.0172	-0.0792	-0.0007
LT	0.0527	0.1332	-0.3758	0.1117	0.1601
FLP	<b>0.9936</b>	-0.0584	0.0122	-0.0813	-0.0020
FLS	<b>0.9933</b>	-0.0603	0.0122	-0.0833	-0.0012

Note: Values in bold > 0.5 significantly correlate (Prayoga et al. 2022). Code characters: TH: Tree Habit; GH: Growth Habit; PH: Plant Height; NN: Number of Nodes; PigL: Pigment young leaf at productivity season; ILC: Immature Leaf Color; MLC: Mature Leaf Color; LS: Leaf Shape; LUS: Leaf Upper Surface; LT: Leaf Thickness; LA: Leaf Apex; LAH: Leaf Apex Habit; LBS: Leaf Base Shape; LM: Leaf Margin; L.cm<sup>2</sup>: Leaf area; LL: Length of Leaf; WL: Width of Leaf; LV: Leaf Venation; LVS: Leaf Vestiture Surface; LP: Leaf Pubescence; LW: Leaf Waxiness; DoB: Density of Bud; LBA: Leaf Blade Attitude; PC: Petiole Color; YSC: Young Shoot Color; YSP: Young Shoot Pubescence; LCS: Leaf Cross Section; FLP: Flower: Length of Pedicel; Flower: FD: Diameter; Flower: IPC: Inner Petal Color; Sepal: SoP: outer side Pubescence (SoP); Flower: FLS: Length Style; Style: SS: Splitting; Stigma: SP: Position in relation to stamens; Ovary: OP: Pubescence; Ovary: ODP: Density of Pubescence

In the biplot graph, the correlation between characters can be identified through the location between vectors. The longer vector line can be expressed as a character with the highest diversity and vice versa (Fitry et al. 2021). The characters that contribute to both PCs are related to flowering, followed by leaf area. Several vectors, including leaf length, width, and area, have almost the same length and are significantly close together in the same quadrant. Similarly, all flowering characters identified in the population have a harmonious correlation because of the same vector lines of identical length.

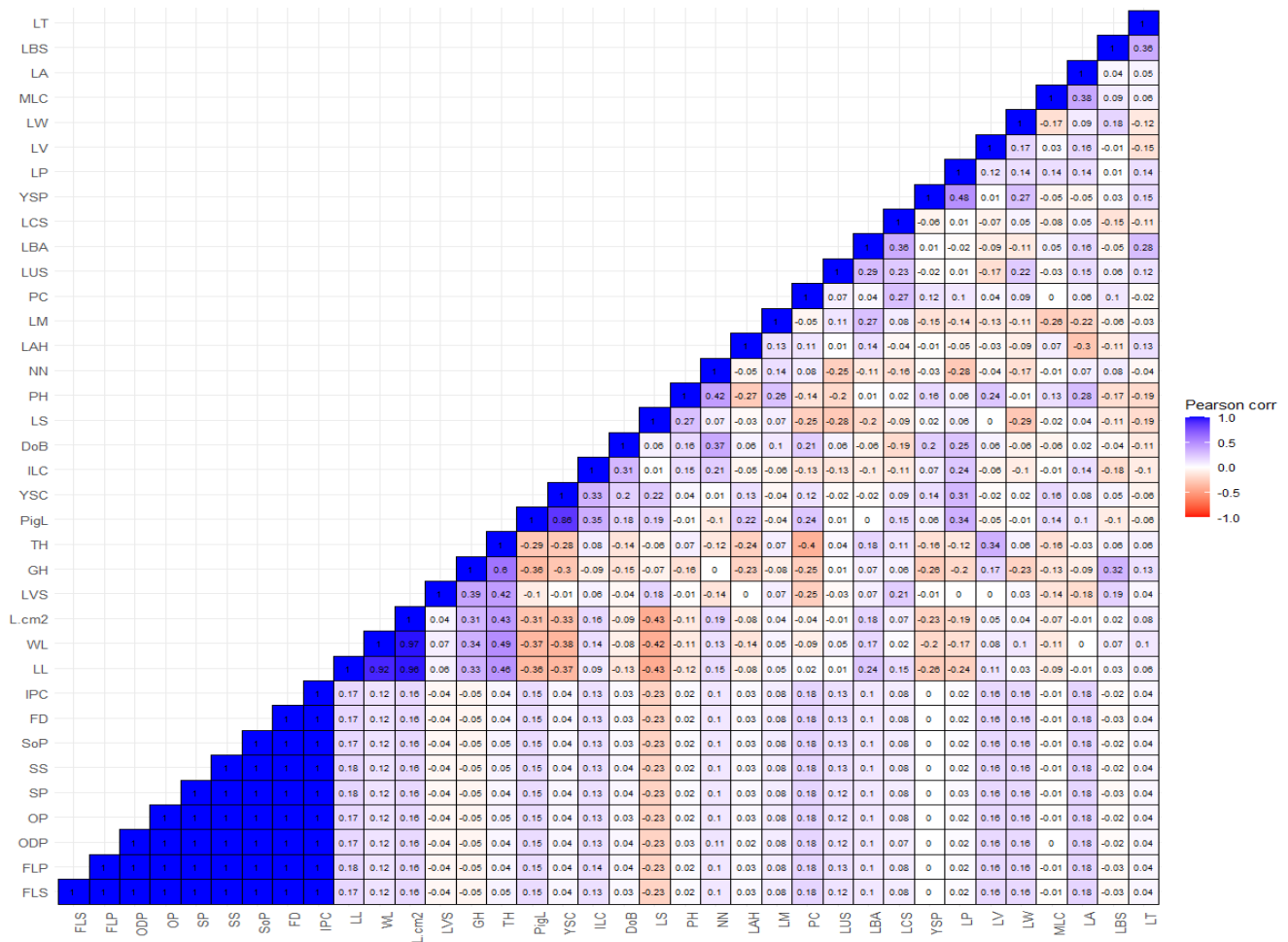
The position of the vectors and genotype points shows the magnitude of characters (Jarwar et al. 2019), as closer characters vector and accession show greater value. Accessions in quadrants I and IV show dominance by not being in the reproductive period, while quadrants II and III are in the flowering period. In Quadrant IV, 3 accessions had a greater distance than others in the same quadrant. This is differentiated from the direction of the leaf pigment character vector. Accessions A2.086 and A2.038 are adjacent to the leaf length, width, and leaf area vectors, having a larger leaf area than others.

The angles formed between vectors facilitate the identification of correlations between characters. Vectors that form an acute angle close to 0° indicate that the character has a strong and positive correlation, while an obtuse angle shows a strong and negative correlation (Khan et al. 2022). For example, the sharp angle indicates a positive correlation between the leaf pigment character vectors and shoot color. Between vectors, stem growth patterns and tree shape also correlate positively. The length, width, and area vectors are close to an angle of 0°, indicating a very strong positive correlation. However, the correlation between vectors is negative, approximately forming a 180° angle, which is found between shoot color and plant growth patterns.

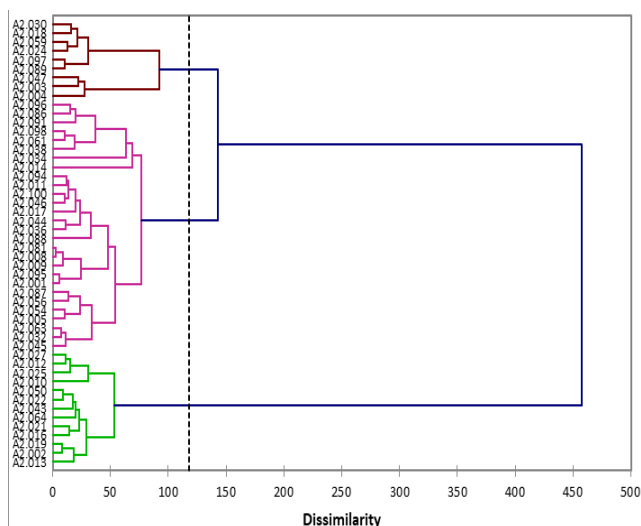
### Heatmap of correlation of morphological traits

The correlation matrix is used simultaneously for dependent factors between several characters. The results of this matrix contained the correlation coefficient between each and other variables (Gai et al. 2019). In this research, the correlation matrix based on Pearson's coefficient from PCA analysis was used, and the result was interpreted as a heatmap of the correlation cluster (Figure 2). Moreover, blue, white, and red indicate a positive, non-significant, and negative correlation. The heat map illustrates the influence of characters on variation between genotypes with differences in color intensity (Ismail et al. 2023). Hue shading indicates the relationship between traits, with darker intensity representing a greater correlation (Khan et al. 2022). A significant positive correlation was recorded between leaf pubescence and young shoot color ( $r = 0.31$ ), leaf pigment ( $r = 0.34$ ), and young shoot pubescence ( $r = 0.48$ ). In the growth-plant-related traits, the number of nodes identified a significant positive correlation with bud density ( $r = 0.37$ ) and plant height ( $r = 0.42$ ), and growth habit and tree habits showed a moderate positive correlation ( $r = 0.60$ ). A strong significance was found between leaf pigment and young shoot color ( $r = 0.86$ ).

**Figure 1.** Biplot graph on the PCA. Note: A2.001-A2.100 (tea germplasm that selected), Code characters: TH: Tree Habit; GH: Growth Habit; PH: Plant Height; NN: Number of Nodes; PigL: Pigment young leaf at productivity season; ILC: Immature Leaf Color; MLC: Mature Leaf Color; LS: Leaf Shape; LUS: Leaf Upper Surface; LT: Leaf Thickness; LA: Leaf Apex; LAH: Leaf Apex Habit; LBS: Leaf Base Shape; LM: Leaf Margin; L<sub>cm</sub><sup>2</sup>: Leaf area; LL: Length of Leaf; WL: Width of Leaf; LV: Leaf Venation; LVS: Leaf Vestiture Surface; LP: Leaf Pubescence; LW: Leaf Waxiness; DoB: Density of Bud; LBA: Leaf Blade Attitude; PC: Petiole Color; YSC: Young Shoot Color; YSP: Young Shoot Pubescence; LCS: Leaf Cross Section; FLP: Flower: Length of Pedicel; Flower: FD: Diameter; Flower: IPC: Inner Petal Color; Sepal: SoP: outer side Pubescence (SoP); Flower: FLS: Length Style; Style: SS: Splitting; Stigma: SP: Position in relation to stamens; Ovary: OP: Pubescence; Ovary: ODP: Density of Pubescence



**Figure 2.** Correlation heatmap showing characters' correlation with each other. Note: Coefficient -1 up to 1 indicated scores of pearson correlation. Code characters: TH: Tree Habit; GH: Growth Habit; PH: Plant Height; NN: Number of Nodes; PigL: Pigment young leaf at productivity season; ILC: Immature Leaf Color; MLC: Mature Leaf Color; LS: Leaf Shape; LUS: Leaf Upper Surface; LT: Leaf Thickness; LA: Leaf Apex; LAH: Leaf Apex Habit; LBS: Leaf Base Shape; LM: Leaf Margin; L.cm<sup>2</sup>: Leaf area; LL: Length of Leaf; WL: Width of Leaf; LV: Leaf Venation; LVS: Leaf Vestiture Surface; LP: Leaf Pubescence; LW: Leaf Waxiness; DoB: Density of Bud; LBA: Leaf Blade Attitude; PC: Petiole Color; YSC: Young Shoot Color; YSP: Young Shoot Pubescence; LCS: Leaf Cross Section; FLP: Flower: Length of Pedicel; Flower: FD: Diameter; Flower: IPC: Inner Petal Color; Sepal: SoP: outer side Pubescence (SoP); Flower: FLS: Length Style; Style: SS: Splitting; Stigma: SP: Position in relation to stamens; Ovary: OP: Pubescence; Ovary: ODP: Density of Pubescence



**Figure 3.** Grouped 50 accessions of cluster analysis

In conclusion, this research analyzed 50 accessions, with 50% of the observed characters showing high variability. Based on the results, PCA showed 38.99% variation cumulative to PC 2; a significant correlation was found in several characters, with the highly being flower-related traits, followed by leaf area-related. The cluster analysis of accessions produced three main clusters ranging from 2.23 to 12.86. This variation was caused by a high correlation of some characteristics, including leaf length, width, and area, which could potentially develop a new tea clone. Quantitative characteristics, such as the productivity potency of each accession and biochemical characteristics, can be studied to find more accuracy.



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