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GENETIC VARIABILITY STUDIES OF INDONESIAN GARLIC (*ALLIUM SATIVUM* L.) ACCESSIONS BASED ON MORPHOLOGICAL TRAITS

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SUMMARY

Garlic (*Allium sativum* L.) is one of the relevant strategic vegetable commodities in the world. Since garlic is a widely known sterile crop, developing new cultivars has relied mainly on clonal selection. Determining genetic variability among the local accessions of garlic is a vital step in a garlic breeding program. In the presented study, 14 local garlic accessions incurred evaluation for their genetic variability based on morphological traits and SSR markers. The results showed that local accessions displayed high genetic variability based on the morphological and molecular characteristics. Principle component analysis (PCA) indicated that 75.26% of total variation came from four PCs mainly determined by the traits, viz., plant height, number of leaves, leaf length, leaf width, degree of leaf waxiness, intensity of anthocyanin coloration at the base of the pseudo-stem, bulb diameter, leaf density, cross-section shape of leaf, and the shape of the basal plate. Molecular analysis based on 10 SSR markers revealed that high allelic variation (2-12 alleles) was evident among garlic accessions

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with an average number of 6.4 alleles, and the average polymorphism information content (PIC) value was 0.67 (0.32–0.88). Nine out of 10 SSR markers showed a PIC value >0.5, indicating that these markers were more informative for the genetic variability analysis of the garlic. The phylogenetic analysis also signified that the 14 garlic accessions could become two main groups with a 0.58 similarity coefficient. These results could benefit further selection and assist Indonesia's future garlic breeding program.

Keywords: local accessions, genetic variability, molecular markers, homonym, clustergram

Key findings: We used morphological traits and molecular markers (SSR) to reveal that Indonesian garlic accessions collected from different geographical regions had high genetic variability. This finding would substantially contribute to the groundwork for forthcoming garlic breeding programs.

INTRODUCTION

Garlic is one of the prominent species of the genus *Allium* consumed worldwide as spices and traditional medicine. Garlic has eight pairs of chromosomes (2n = 16) (Konvicka & Levan, 1972) and is believed to have originated from Central Asia (Vavilov, 1951). Its production is by asexual propagation through cloves (Ben-Michael *et al.,* 2018; Winiarczyk *et al.,* 2018). It also has well-known antioxidant properties (Shang *et al.*, 2019) and cooking flavors (Abe *et al.*, 2020).

Genotypic and phenotypic assessment is essential for improvement through plant breeding and conservation strategies (Chen *et al.*, 2021). Since garlic rarely produces true seeds, achieving cultivar development is mainly through clonal selection (Shiga *et al.*, 2015; Dhall, 2016; Karklelienė *et al.,* 2018). However, in garlic, the phenotypic plasticity and epigenetics have complicated clones' identification because some of the phenotypes gain more effects from environmental factors (Sánchez-Virosta *et al*., 2021; Aswani *et al.,* 2023).

Molecular markers can reveal numerous genetic variations among the genotypes at the DNA level, providing a more accurate, reliable, and efficient explanation for germplasm characterization without influences from environmental factors. Therefore, the garlic germplasm characterization should continue using phenotypic and molecular approaches for more accurate and reliable results. Specifically, simple sequence repeat (SSR) markers have been a standard tool in genetic variability studies and are often applied in plant breeding (Sharma *et al.,* 2022; de-Oliveira *et al.,* 2023), including garlic, due to their informativeness and stability (da Cunha *et al.,* 2014; Poljuha *et al.,* 2021; Li *et al.,* 2022).

From 1980 until now, Indonesia has released nine garlic varieties, of which production has not been able to meet domestic demand. Hence, the dependency of Indonesia on imported garlic has been increasing for decades (Purba *et al.*, 2022). Despite the urgency for developing superior varieties, very limited exploration and characterization of Indonesia's local garlic accessions transpired (Hardiyanto *et al.*, 2007; Wahyuni *et al.,* 2023). Moreover, most previous studies discussed their agronomical aspects, such as, adaptation (Kurniaty *et al.*, 2022; Saidah *et al.*, 2022), cultivation techniques (Aswani *et al.*, 2022; Dianawati *et al.*, 2022), temperature, and photoperiod effect on their vegetative growth (Azmi *et al.*, 2022; Kendarini *et al.*, 2022). The genetic information on the available Indonesian local garlic germplasm is still scarce, yet it is necessary for breeding new varieties. The pertinent study assessed the genetic variability among various local accessions of garlic (*A. sativum* L.) collected from diverse regions of Indonesia based on morphological characteristics and SSR markers.

MATERIALS AND METHODS

Plant material and experimental design

Bulbs from 14 garlic accessions collected from various regions in Indonesia served as plant material in this study (Table 1, Figure 1). The experiment began in July to November 2021, with climate conditions tending to be wet due to the rainy season. The trial plots created followed a randomized complete block design (RCBD) with a single factor, i.e., garlic accession and three replications. As many as 20 cloves of each garlic accession incurred planting at the Margahayu Experimental Field, Lembang, West Java, Indonesia, with an altitude of 1250 masl (6°48'06.4"S 107°38'56.1"E).

The research adopted the Innovative Technology method for garlic cultivation (Basuki *et al.*, 2019). Creating planting beds had dimensions of 100 cm in width, 30 cm in height, and 50 cm between beds. The recommended doses of 2 t ha^{-1} of dolomite, 15 t ha^{-1} of chicken manure, and 375 kg ha^{-1} of phosphate fertilizer (SP36) proceeded with application to the top of the bed. Cloves' planting followed the planting space of 12.5 cm × 12.5 cm, with organic mulch (rice straw) as a cover. On the 21st, 35th, 49th, and 63rd days after planting (DAP), nitrogen (ZA, 286 kg ha^{-1}) and potassium (KCl, 50 kg ha^{-1}) fertilizers spreading onto each planting row ensued.

Morphological characterization

The morphological characterization referred to the descriptors for *Allium* sp. developed by the International Plant Genetic Resources Institute (IPGRI) and the International Union for the Protection of New Varieties of Plants (UPOV) (Table 2). Five randomly selected plants of each accession gained characterization for eight qualitative and quantitative traits. Observing the density of leaves (LD), foliage attitude (FL), foliage color (FC), and the cross-section shape of the leaf (CSL) happened 60 days after planting (DAP), with plant height (PL), the number of leaves (NL), leaf length (LL), leaf width (LW), and pseudo-stem diameter (PSD) scrutiny was at 90 DAP. Leaf length assessment ensued by measuring the nearest part from the pseudo-stem to the leaf tip of the longest leaf. Meanwhile, the leaf width observation began measuring the widest part of the longest leaf. Probing the weight of the dry bulb (WDB), bulb diameter (BD), bulb height (BH), bulb shape (BS), and the shape of the basal plate (SBP) occurred after the curing process or one month after harvest (90–120 DAP). Bulbs' weight from sample plants used a digital scale for WDB. Using the digital caliper helped measure BD from the broadest part of the bulb, while the BH measurement started from the basal part to the tip of the bulb (the nearest part to the base of the pseudo-stem).

Table 1. Fourteen Indonesian garlic accessions used in the study.

No.	Accessions	Location of collection
1	Bengkulu	Bengkulu Regency, Bengkulu
2	Maja	Majalengka Regency, West Java
3	Merek	Kabanjahe, Karo Regency, North Sumatera
4	Doulu	Kabanjahe, Karo Regency, North Sumatera
5	Tes	Lembang, West Java
6	Lumbu Putih Cipanas	Cianjur Regency, West Java
7	Lumbu Putih Tegal	Tegal Regency,Central Java
8	Lumbu Kuning Tegal	Tegal Regency, Central Java
9	Palu	Palu Regency, Central Sulawesi
10	Lumbu Kuning Temanggung	Temanggung Regency, Central Java
11	Lumbu Hijau Karanganyar	Karanganyar Regency, Central Java
12	Lumbu hitam	Ciwidey, Bandung Regency, West Java
13	Jawa	Karanganyar Regency, Central Java
14	Eban NTT	North Central Timor Regency, East Nusa Tenggara

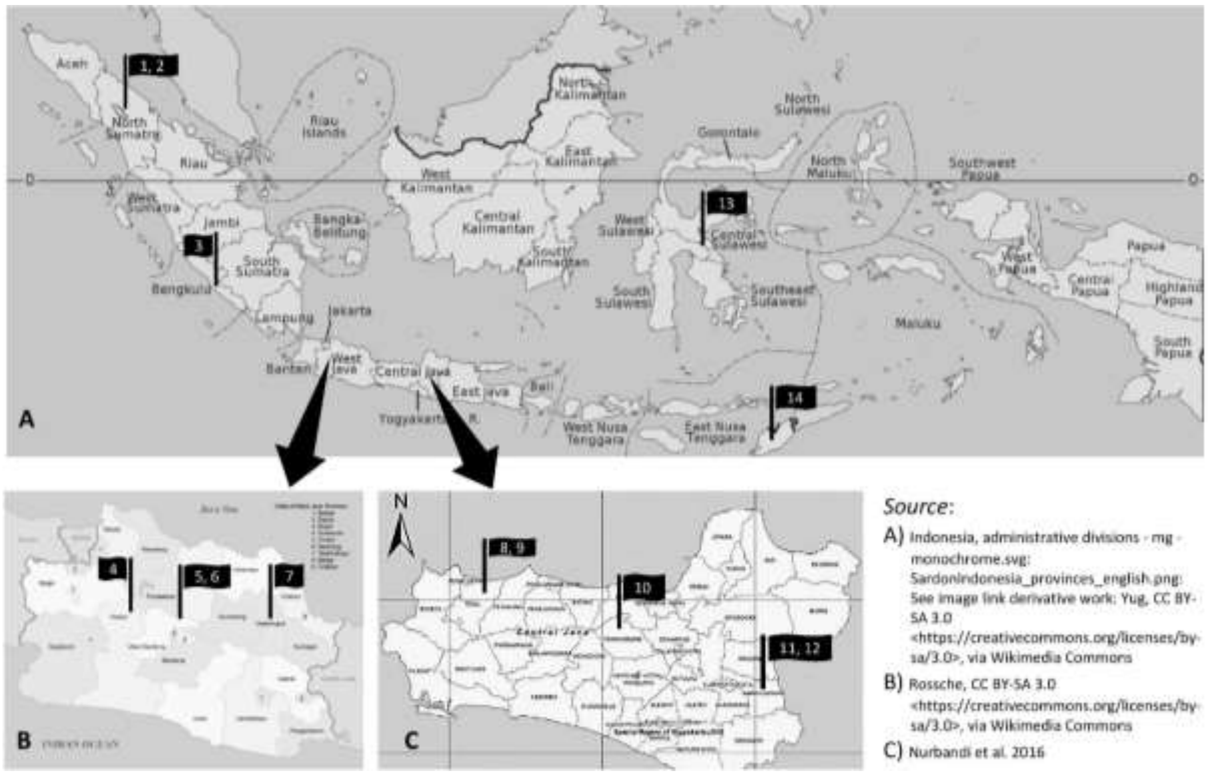

Figure 1. Indonesia map showing locations of 14 collected garlic accessions.

Table 2. Scores of qualitative characteristics.

Qualitative characteristics	Score
Density of leaves (LD)	3 = low; 5 = medium; 7 = high
Foliage attitude (FA)	3 = prostrate; 5 = intermediate; 7 = erect
Degree of leaf waxiness (LW.1)	3 = weak; 5 = medium; 7 = strong
Foliage color (FC)	1 = light green; 2 = yellow-green; 3 = green; 4 = grey-green; 5 = dark green; 6 = bluish green; 7 = purplish-green; 8 = moderate yellowish green; 9 = moderate olive green; 10 = moderate yellow-green; 11 = greyish olive green
Cross-section of leaf (CSL)	1 = circular; 2 = semi-circular; 3 = square; 4 = pentagonal; 5 = V-shape; 6 = flat; 7 = triangular; 8 = concave; 9 = very concave
Intensity of anthocyanin coloration at the base of pseudo-stem (APD)	1 = weak; 2 = medium; 3 = strong
Bulb shape (BS)	1 = circular, basal plate prominent; 2 = heart-shaped, basal plate retracted; 3 = broadly ovate, basal plate even
Shape of basal plate (SBP)	1 = prominent; 2 = retracted; 3 = even

Data analysis

Data computation for RCBD's analysis of variance continued to the LSD test whenever significant differences emerged. The statistical parameters, including mean, range, standard deviation (SD), standard error (SE), and coefficient of variations (CV) for each quantitative trait's estimation used Microsoft Excel from Windows per the following formula:

$$\sigma = \frac{\sqrt{\sum(x_i - \mu)^2}}{N} \quad (1)$$

$$S.E. = \frac{\sigma}{\sqrt{n}} \times 100 \quad (2)$$

$$C.V. = \sigma/\mu \quad (3)$$

Where:

σ = population standard deviation

N = the size of the population

x_i = each value from the population

μ = the population mean

Pearson's correlation coefficient measured the strength of a linear association between two variables, where the value r = 1 means a perfect positive correlation and the value r = -1 means a perfect negative correlation. ANOVA, *Pearson*'s correlation, and clustergram ran computations using software Rv.4.2.0 (https://www.r-project.org/).

Molecular analysis

Genomic DNA extraction from young leaves of the garlic's different accessions used a modified CTAB protocol (Doyle and Doyle, 1987). The quantity of DNA stock solution determination utilized a NanoDrop™2000 spectrophotometer (Thermo Scientific, USA), while for the quality, the study performed the method on 1% agarose gel with 1× Tris-acetate-EDTA (TAE) buffer at 90 volts for 30 min. The genomic DNA of the different garlic accessions bore PCR amplification using 10 SSR markers collected from some references as primers. PCR profiling setup continued with an initial denaturation temperature at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, and the final extension at 60 °C for 15 min. The PCR product separation proceeded on 6% vertical polyacrylamide gel electrophoresis in a tank containing 1× Tris borate EDTA (TBE) buffer at 80 V for 1.5 h.

The number of alleles detected, chief allele frequency, gene diversity, heterozygosity, and polymorphism information content (PIC) of each SSR marker reached identification using PowerMarker V3.25. Generating a similarity data matrix file for clustering analysis by NTedit version 1.04 occurred. Additionally, similarity coefficients based on Simple Matching (SM) coefficients estimation used the SIMQUAL module in NTSYS-pc v. 2.10e software. Phylogenetic dendrogram construction engaged the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) through Sequential Agglomerative Hierarchical Non-overlapping (SAHN).

RESULTS

Morphological traits variability

The descriptive statistics showed that the parameters, i.e., plant height, number of leaves, leaf length, bulb diameter, and bulb height, had a lower coefficient of variance (<10%), and the traits leaf width, pseudo-stem diameter, and weight of dry bulb had a higher coefficient of variance (>10%) (Table 3). The garlic accessions evaluated in this study also revealed significant differences for all the quantitative morphological traits (Table 4).

The results indicated that the garlic accession Lumbu Hitam displayed the tallest plant on average (54.13 cm), while landrace Lokal Jawa displayed the shortest plants (40.87 cm). The accession Lumbu Hitam had the longest (49.39 cm) and the widest leaves (2.11 cm), whereas the shortest (29.53 cm) and narrowest leaves (0.87 cm) resulted in the garlic accession Lokal Jawa. The broader pseudo-stem diameter was visible in the accession Lumbu Hitam (9.89 mm), while the smallest was in the Eban NTT (5.59 mm). Garlic landrace Lumbu Hitam had the highest dry bulb weight (18.34 g), whereas Bengkulu displayed the lowest value for the said trait (9.19 g). The accession Lumbu Kuning Tegal produced the largest bulb diameter (35.55 mm), while the landrace Doulu produced the tiniest bulb diameter (22.00 mm). Furthermore, the maximum values for the bulb height were evident in accession Maja (26.70 mm) and the smallest value of bulb height in the garlic landrace Doulu (18.05 mm).

Table 3. Descriptive statistics for quantitative traits of 14 garlic accessions.

Quantitative characteristics	Means	Range	S.D.	S.E. (±)	C.V. (%)
Plant height	47.86	40.87-55.13	4.54	1.21	8.27
Number of leaves	6.88	5.87-7.73	0.58	0.15	7.31
Leaf length	39.04	29.56-49.39	4.67	1.25	4.23
Leaf width	1.46	0.87-2.11	0.31	0.08	10.18
Pseudo-stem diameter	7.10	5.59-9.89	1.28	0.34	12.11
Weight of dry bulb	13.29	9.19-18.34	2.42	0.65	14.80
Bulb diameter	29.51	22.22-35.55	4.23	1.13	8.54
Bulb height	22.89	18.05-26.70	2.56	0.68	7.89

SD = Standard deviation; SE = Standard error; CV = Coefficient of variation.

Table 4. Means of quantitative traits of garlic accessions.

Accessions	PH	NL	LL	LW	PD	WDB	BD	BH
Bengkulu	53.13^{a}	6.92^{abcd}	38.89^{de}	1.49^{bcd}	6.72^{cde}	9.19^{d}	25.76^{ef}	20.68^{de}
Maja	44.07^{def}	6.73^{bcde}	42.22^{bc}	1.22^{de}	5.61^{e}	15.68^{ab}	32.29^{abcd}	26.70^{a}
Merek	42.67^{ef}	5.87^{e}	39.22^{cde}	1.26^{d}	6.94^{cde}	10.35^{d}	30.25^{bcde}	23.52^{abcd}
Doulu	42.47^{ef}	6.00^{de}	34.22^{f}	1.41^{cd}	6.27^{de}	15.43^{abc}	22.20^{f}	18.05^{e}
Tes	47.56^{bcdef}	6.73^{bcde}	38.81^{de}	1.56^{bc}	8.56^{ab}	14.53^{bc}	34.15^{abc}	23.12^{bcd}
Lumbu Putih Cipanas	53.32^{abc}	7.73^{a}	41.08^{bcd}	1.59^{bc}	6.51^{cde}	11.61^{cd}	26.02^{ef}	20.28^{de}
Lumbu Putih Tegal	47.40^{bcdef}	7.42^{ab}	34.33^{f}	0.99^{ef}	5.84^{e}	11.70^{cd}	26.61^{ef}	21.17^{cde}
Lumbu Kuning Tegal	46.00^{cdef}	6.40^{cde}	38.33^{de}	1.56^{bc}	7.99^{bc}	11.91^{bcd}	35.55^{a}	23.34^{abcd}
Palu	48.67^{abcde}	7.13^{abc}	40.00^{cde}	1.54^{bc}	6.59^{cde}	12.60^{bcd}	27.35^{e}	21.62^{cd}
Lumbu Kuning Temanggung	50.55^{abcd}	6.88^{abcd}	43.56^{b}	1.71^{b}	8.68^{ab}	12.36^{bcd}	34.69^{ab}	25.30^{ab}
Lumbu Hijau Karanganyar	51.22^{abcd}	7.45^{ab}	39.83^{cde}	1.72^{b}	7.54^{bcd}	15.22^{abc}	34.99^{a}	26.30^{ab}
Lumbu Hitam	54.13^{ab}	7.73^{a}	49.39^{a}	2.11^{a}	9.89^{a}	18.34^{a}	29.59^{cde}	24.50^{abc}
Lokal Jawa	40.87^{f}	6.53^{bcde}	29.53^{g}	0.87^{f}	6.66^{cde}	14.15^{bc}	27.76^{de}	25.19^{ab}
Eban NTT	46.00^{cdef}	6.73^{bcde}	37.00^{ef}	1.37^{cd}	5.59^{e}	12.92^{bcd}	25.86^{ef}	20.69^{de}

Means followed by the same letters are not significantly different according to LSD test with α = 5%. PH=plant height (cm); NL=number of leaves; LL=leaf length (cm); LW=leaf width (cm); PD=pseudo-stem diameter (mm); WDB=weight of dry bulb (g); BD=bulb diameter (mm); BH=bulb height (mm).

In this study, the garlic accessions displayed low leaf density and erect to intermediate foliage attitude (Table 5). For leaf waxiness, the varied degrees ranged from low to medium. There was also quite a variation in leaf colors within the green spectrum. Among 14 garlic accessions, the landrace Lumbu Kuning Tegal exhibited flat leaves. Most garlic accessions exhibited broadly ovate basal plate shapes, with uniform shapes, except for the accession Eban NTT, which had circular and prominent basal plate shapes.

Pearson's correlation analysis showed that bulb diameter has a positive correlation with bulb height (0.78) and pseudo-stem diameter (0.56) (Figure 2). However, the pseudo-stem diameter is positively associated with leaf length (0.60) and leaf width (0.74). Plant height also showed a positive link with the leaf length (0.64), leaf width (0.71), and number of leaves (0.77). Furthermore, the leaf length also revealed a strong positive correlation with the leaf width (0.83).

Among the garlic accessions, the four principal components (PCs) explained 75.26% of total morphological trait variations (Table 6). The first PC explaining 30.76% of the total variation has plant height, the number of leaves, leaf length, leaf width, degree of leaf waxiness, and intensity of anthocyanin coloration at the base of pseudo-stem determining it. Meanwhile, the second PC, implying 24.66% of the total variation, has main determinants from the density of leaves,

Table 5. Qualitative traits of garlic accessions.

Accessions	LD	FA	LW.1	FC	CSL	APD	BS	SBP
Bengkulu	Low	Erect	Medium	Moderate yellow green	Strongly concave	Medium	Broadly ovate	Even
Maja	Low	Erect	Medium	Moderate yellowish green	Slightly concave	Medium	Broadly ovate	Even
Merek	High	Intermediate	Low	Moderate yellow green	Slightly concave	Weak	Broadly ovate	Prominent
Doulu	Low	Intermediate	Low	Moderate olive green	Slightly concave	Weak	Broadly ovate	Prominent
Tes	Medium	Prostate	Low	Moderate yellowish green	Slightly concave	Medium	Broadly ovate	Prominent
Lumbu Putih Cipanas	Low	Erect	Medium	Moderate yellow green	Strongly concave	Medium	Broadly ovate	Even
Lumbu Putih Tegal	Low	Erect	Low	Moderate olive green	Strongly concave	Medium	Broadly ovate	Prominent
Lumbu Kuning Tegal	Medium	Intermediate	Low	Moderate yellow green	Flat	Weak	Broadly ovate	even
Palu	Low	Erect	Medium	Moderate olive green	Strongly concave	Medium	Broadly ovate	even
Lumbu Kuning Temanggung	Medium	Intermediate	Medium	Moderate olive green	Slightly concave	Weak	Broadly ovate	Prominent
Lumbu Hijau Karanganyar	High	Intermediate	Medium	Moderate yellowish green	Slightly concave	Medium	Broadly ovate	even
Lumbu Hitam	High	Erect	High	Greyish olive green	Slightly concave	Strong	Broadly ovate	Prominent
Lokal Jawa	High	Erect	Medium	Moderate olive green	Slightly concave	Weak	Broadly ovate	Prominent
Eban NTT	Low	Erect	Medium	Moderate olive green	Strongly concave	Medium	Circular	Prominent

LD=density of leaves; FA=foliage attitude; LW.1=leaf waxiness; FC=foliage color; CSL=cross-section shape of leaf; APD=Intensity of anthocyanin at the base of pseudo-stem; BS=bulb shape; SBP=shape of basal plate.

Table 6. PCA of morphological traits of garlic accessions.

Characteristics	PC-1	PC-2	PC-3	PC-4
Plant height	0.80	-0.31	-0.28	-0.24
Number of leaves	0.76	-0.39	0.13	0.04
Leaf length	0.85	0.11	-0.13	-0.16
Leaf width	0.81	0.18	-0.36	-0.18
Pseudo-stem diameter	0.65	0.62	-0.30	0.08
Weight of dry bulb	0.39	0.31	0.32	0.49
Bulb diameter	0.31	0.71	0.33	-0.44
Bulb height	0.36	0.58	0.64	-0.04
Leaf density	0.26	0.72	0.09	0.30
Foliage attitude	0.35	-0.63	0.38	0.11
Leaf waxiness	0.76	-0.25	0.24	0.22
Foliage color	0.33	-0.19	-0.68	0.14
Cross-section shape of leaf	0.06	-0.83	0.04	0.26
Intensity of anthocyanin coloration at the base of pseudo-stem	0.74	-0.45	0.16	0.15
Bulb shape	0.12	0.43	-0.20	0.02
Shape of basal plate	-0.16	0.45	-0.26	0.72
Eigenvalue	4.92	3.94	1.80	1.37
Variance (%)	30.76	24.66	11.25	8.57
Cumulative of variance (%)	30.76	55.42	66.68	75.26

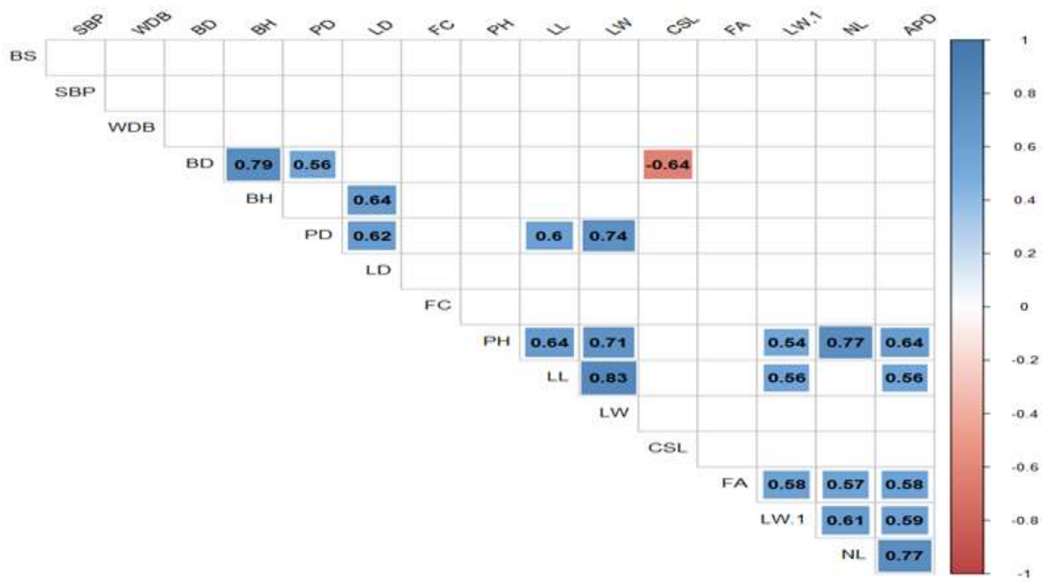

Figure 2. *Pearson's* significant correlation analysis ($P < 0.05$) among quantitative and qualitative morphological traits within the garlic accessions. PH=plant height (cm); NL=number of leaves; LL=leaf length (cm); LW=leaf width (cm); PD=pseudo-stem diameter (mm); WDB=weight of dry bulb (g); BD=bulb diameter (mm); BH=bulb height (mm); LD=density of leaves; FA=foliage attitude; LW.1=leaf waxiness; FC=foliage color; CSL=cross-section shape of leaf; APD=Intensity of anthocyanin at the base of pseudo-stem; BS=bulb shape; SBP=shape of basal plate.

foliage attitude, cross-section shape of leaf, and pseudo-stem diameter. The third PC explaining 11.25% of total variations was due to bulb height and foliage color. The fourth PC, determined by the weight of the dry bulb and bulb diameter, described 8.57% of the total variation.

Biplots showed that the garlic accessions were scattered and clustered in all four quadrants as per the chief contributing variables of the given PC (Figure 3A, 3B). For example, in the upper right quadrant, the garlic accession Lumbu Hitam was separated far from other accessions, i.e., Lumbu Hijau Karanganyar, Lumbu Kuning Temanggung, which had clustering around the long vectors of pseudo-stem diameter, bulb height, bulb diameter, and leaves' density (Figure 3A). The accession Lumbu Hitam had a significantly higher value for these traits than the other garlic accessions. Meanwhile, in Figure 3B, it was also noticeable that the garlic accession Doulu became separated in the lower right quadrant and far from the bulb height vector and other accessions since it had the lowest value for this trait.

The first group of heatmaps only consisted of the garlic accession Lumbu Hitam with the darkest red colors, indicating the superiority of this accession for most characteristics among all the accessions (Figure 4). The second group consisted of the accessions Merek, Lumbu Kuning Tegal, Maja, Lumbu Hijau Karanganyar, Tes, and Lumbu Kuning Temanggung. The third group comprised the garlic accessions, *viz*., Eban NTT, Lumbu Putih Tegal, Palu, Bengkulu, and Lumbu Putih Cipanas. It also enunciated that the Eban NTT was the only accession with a different bulb shape, a circular bulb marked with the darkest blue color. The fourth group included the garlic landraces Doulu and Jawa, marked with weaker intensity colors than the rest of the accessions. These garlic accessions had lower values for most attributes except leaves' density in the Lokal Jawa (dark orange).

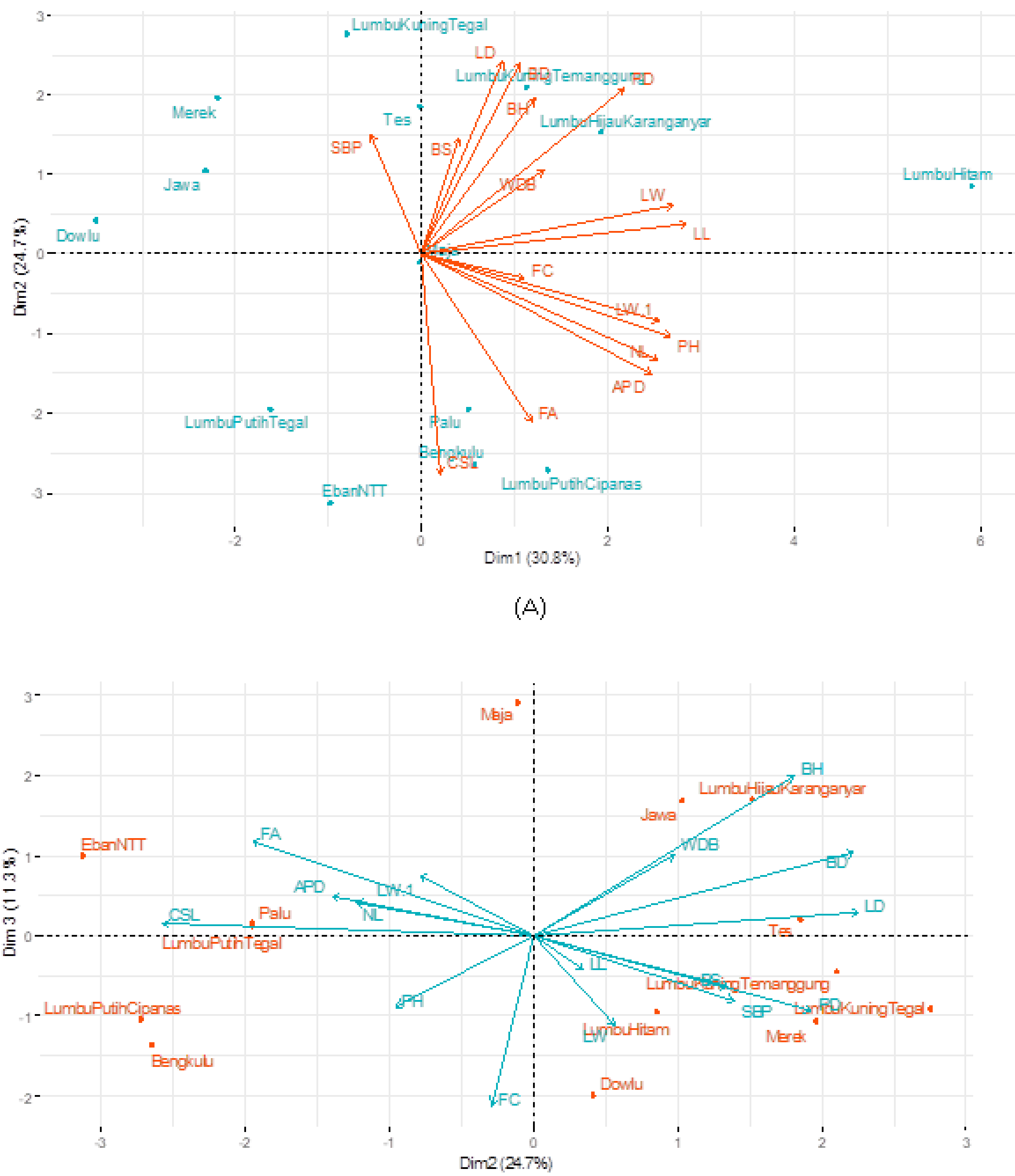

Figure 3. Biplot of garlic morphological traits data: the first and second PCs (A), the second and third PCs (B). PH=plant height (cm); NL=number of leaves; LL=leaf length (cm); LW=leaf width (cm); PD=pseudo-stem diameter (mm); WDB=weight of dry bulb (g); BD=bulb diameter (mm); BH=bulb height (mm); LD=density of leaves; FA=foliage attitude; LW.1=leaf waxiness; FC=foliage color; CSL=cross-section shape of leaf; APD=Intensity of anthocyanin at the base of pseudo-stem; BS=bulb shape; SBP=shape of basal plate.

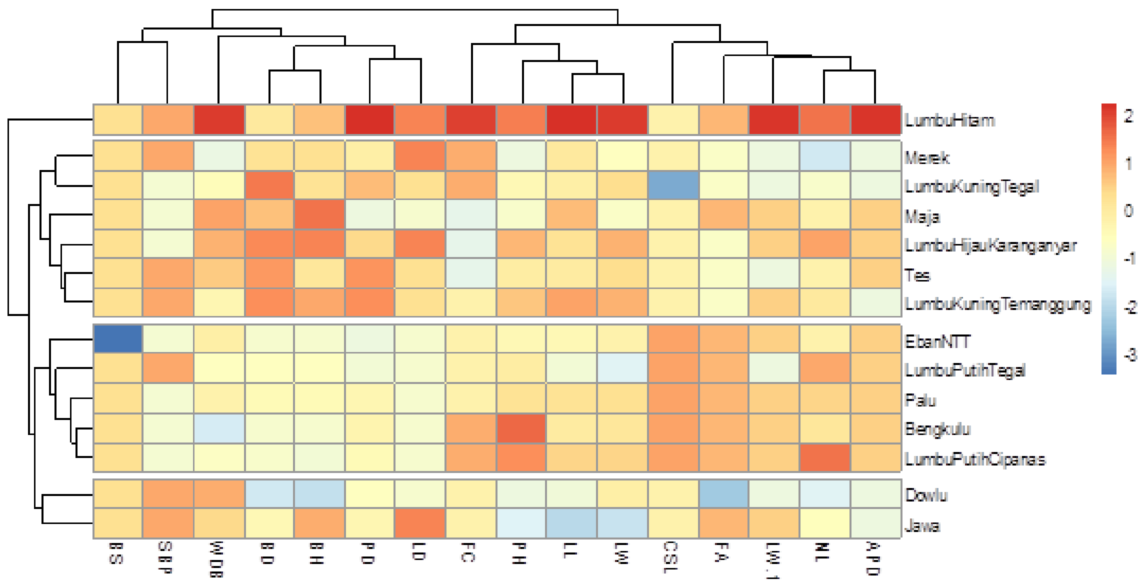

Figure 4. Heatmap from clustergram analysis of 14 garlic accessions and 16 morphological traits. PH=plant height (cm); NL=number of leaves; LL=leaf length (cm); LW=leaf width (cm); PD=pseudo-stem diameter (mm); WDB=weight of dry bulb (g); BD=bulb diameter (mm); BH=bulb height (mm); LD=density of leaves; FA=foliage attitude; LW.1=leaf waxiness; FC=foliage color; CSL=cross-section shape of leaf; APD=Intensity of anthocyanin at the base of pseudo-stem; BS=bulb shape; SBP=shape of basal plate.

Genotypic variations based on SSR markers

In the relevant study, the SSR markers showed a polymorphism for all the garlic accessions. Fifty-three amplicons incurred generation from 10 SSR markers (Table 7). The total number of alleles produced varied from two to 12, with an average of 6.40 per locus. The chief allele frequencies ranged from 0.17 to 0.71 for each locus, with an average of 0.41. Gene diversity ranged from 0.40 to 0.89, with an average of 0.71, and the heterozygosity ranged from zero to one, with an average of 0.67. The PIC value represents the relative informativeness of each SSR marker, calculated with an average of 0.67. The highest PIC value of 0.88 was evident for the SSR marker ASA10. Genetic similarity coefficients among the garlic accessions are available in Table 8. In this study, among the 14 garlic accessions, the calculated coefficient of similarity values ranged from 0.48 to 0.97 (Table 8). The highest genetic similarity coefficient (0.48) was notable between the garlic accessions Eban NTT and Lumbu Hitam and between Eban NTT and Lokal Jawa. The minimum genetic similarity coefficient (0.97) was prominent between the two accessions, i.e., Lumbu Putih Tegal and Lumbu Putih Cipanas.

Based on Nei's coefficient, the phylogenetic tree revealed that 14 garlic accessions could continue to classify into two prime clusters with a 0.58 genetic similarity coefficient (Figure 5). The first cluster consisted of six garlic accessions, and the second cluster consisted of eight. The first cluster could proceed further division into two subclusters: the IA subcluster, comprising five accessions (Bengkulu, Maja, Lumbu Putih Cipanas, Lumbu Putih Tegal, and Palu), and the IB subcluster, comprising only one accession (Eban NTT). The second cluster comprised the remaining eight garlic accessions (Merek, Lumbu Hijau Karanganyar, Tes, Lumbu Kuning Temanggung, Doulu, Lumbu Kuning Tegal, Lumbu Hitam, and Jawa).

Table 7. Details of SSR markers used in this study.

Markers	Sequence	MAF	NA	GD	H	PIC
AS440	F: AATGTGGTTTTGGGTTTAATGG R: TGGAGCATCAAATATAACGACC	0.39	6	0.71	1.00	0.67
AS589	F: AACAGTCGAAAGCGTGGATTG R: TACGGCTTGCTACCAAAGAC	0.35	7	0.78	0.35	0.75
ASA10	F: GGGGGTTACCTGAACCTGTTA R: GGGGGTTACCTGAACCTGTTA	0.17	12	0.89	1.00	0.88
AS11065	F: TCTTTGCATCTCTGTCTTGCAT R: GAAGGCACGATTACATTTCTCG	0.39	6	0.72	0.57	0.68
ASA24	F: TTGTTGTGCCGAGTTCCATA R: CAGCAATTTACCAAAGCCAAG	0.28	5	0.76	1.00	0.72
AS1722	F: AGCTGAGGTCTCAAAACCAAA R: ATGTTCTCTTGATTTGCCGC	0.71	2	0.40	0.00	0.32
AS739	F: AACAGGGATCTTTGCTTCAGC R: GATCTGTTGTGGTTGGATGTTC	0.46	6	0.71	0.71	0.68
AS987	F: GTACCAACTCTTTCCTAACGC R: TCCAATAGTTGTGATGACAGG	0.50	6	0.63	0.14	0.57
AS6580	F: AACTGGATCAGCCGGTACTC R: AACTGGATCAGCCGGTACTC	0.46	8	0.73	1.00	0.71
ASA06	F: GGTGCCGGAGTACTACGAGG R: GGACATCTTTCCATTCATCCTGC	0.35	6	0.76	1.00	0.73
Mean		0.41	6.4	0.71	0.67	0.67
Total			53			

Primers were referred to MAF = major allele frequency; NA = number of alleles; GD = genetic diversity; H = heterozygosity; PIC = polymorphism information content.

Table 8. Genetic similarity coefficient matrix of 14 garlic accessions based on 10 SSR markers calculated using SIMQUAL module in NTSYS-pc v. 2.10e software.

	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0,82	1,00											
3	0,63	0,58	1,00										
4	0,60	0,58	0,68	1,00									
5	0,55	0,56	0,82	0,73	1,00								
6	0,68	0,63	0,63	0,50	0,61	1,00							
7	0,71	0,66	0,66	0,50	0,58	0,97	1,00						
8	0,53	0,55	0,61	0,77	0,63	0,53	0,53	1,00					
9	0,71	0,69	0,66	0,56	0,58	0,81	0,84	0,53	1,00				
10	0,56	0,61	0,74	0,74	0,82	0,63	0,63	0,68	0,66	1,00			
11	0,68	0,66	0,89	0,69	0,81	0,61	0,65	0,56	0,68	0,73	1,00		
12	0,63	0,71	0,68	0,77	0,73	0,53	0,56	0,84	0,63	0,77	0,73	1,00	
13	0,60	0,65	0,61	0,74	0,63	0,53	0,56	0,74	0,60	0,68	0,66	0,84	1,00
14	0,60	0,61	0,55	0,45	0,63	0,66	0,63	0,42	0,63	0,58	0,53	0,48	0,48

Note: The numbers stand for the garlic accession numbers, and their complete names are represented in Table 1.

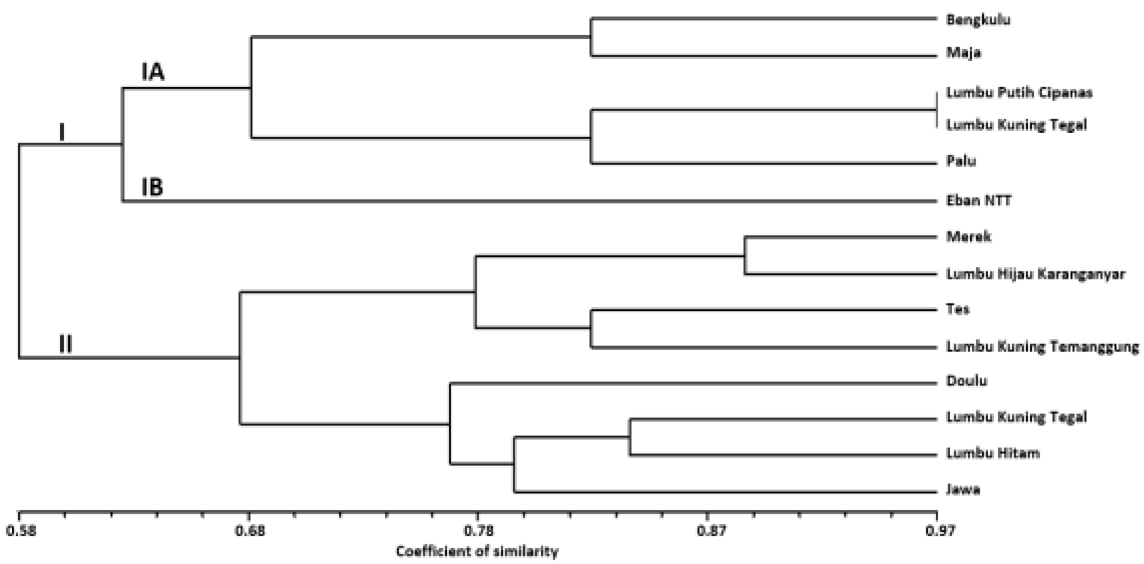

Figure 5. Dendrogram of 14 garlic accessions based on the UPGMA-SAHN.

DISCUSSION

The promising study showed high morphological variations among the garlic accessions, indicated by a wide range of plant height, number of leaves, leaf length, leaf width, pseudo-stem diameter, weight of dry bulb, bulb diameter, and bulb height. Previous studies have also shown that despite being vegetatively propagated, garlic has a wide range of variabilities in vegetative and bulb parameters (Kar *et al.,* 2013; Wang *et al.,* 2014; Hirata *et al.,* 2016; Tesfaye, 2021). Higher variability in any germplasm is necessary for a garlic breeding program since it indicates a higher possibility of developing a desirable variety. However, an accelerated selection still needs a correlation analysis to choose targeted characteristics among numerous traits.

Correlation analysis is beneficial in determining the target qualities for effective and efficient selection in accelerated breeding programs (Waldmann, 2019). The recent results also showed garlic accessions with greater bulb diameters can be a better selection than those with large pseudo-stem diameters. De-Oliveira *et al.* (2023) found that in garlic, the bulb size was one of the vital features in the selection program for high-yielding genotypes. A significant correlation between garlic's pseudo-stem diameter and bulb weight was also evident in other studies (Sandhu *et al*., 2015; Atif *et al.*, 2019; Kumari, 2021).

Morphological characterization supported with molecular analysis gave more reliable and consistent results in genetic diversity studies. This study had ten pairs of SSR markers generated, on average, 0.67 polymorphism. These values were similar and considered high according to the previous studies on garlic genotypes (Hoogerheide *et al.,* 2017; Kumar *et al.,* 2019; Mokate and Kesralikar, 2020; Mostafa *et al.,* 2020; Li *et al.,* 2022). The genetic diversity (GD) was 0.71, which was also higher than the recorded outcome in a previous garlic study (Jo *et al.*, 2012), which investigated 120 garlic (*A. sativum* L.) accessions by using seven SSR markers, and the obtained GD value was 0.59.

The garlic bulb seeds came from the different regions of Indonesia. Some accessions shared the same varietal name, and some had different local names. In the presented study, the garlic accessions sharing the same varietal names, yet collected from diverse provinces, such as, Lumbu Putih from

Tegal versus Lumbu Putih from Lembang, and Lumbu Hijau from Lembang versus Lumbu Hijau from Karanganyar can be distinguishable from each other using morphological traits and SSR markers (Figure 1). As suggested by previous research, the environmental conditions, such as, soil type, humidity, latitude, and altitude, can lead to garlic's wide range of genetic diversity (Tesfaye, 2021, 2022), even in narrow geographical areas (Poljuha *et al.*, 2021), or that even within an equal accession would have a lot of phenotypic variabilities (Khar *et al.*, 2008). It was also explainable that the long domestication in different environments or local adaptation may be due to mutations that have caused variations in some germplasm (Shaaf *et al.*, 2014; Kıraç and Dalda, 2022). These results also proved that the SSR markers employed can successfully distinguish the two phenotypically similar accessions at a molecular level. This phenomenon was also evident in previous research using molecular markers, identifying two different genotypes under the same name of cultivar (Ipek *et al.,* 2008, 2015).

Furthermore, both dendrograms based on morphological and molecular showed that the garlic accessions collected from varied islands, provinces, and regions received groupings in the same cluster (Figures 1, 4, and 5). For example, in the molecular dendrogram, the garlic accession Merek from North Sumatra (Sumatra Island) attained clustering with Lumbu Hijau Karanganyar from Central Java (Java Island). The accession, Doulu from Karo regency, North Sumatra, bore separation in considerable genetic dissimilarities (32%) with Merek, which was also from the same region. Other past studies on garlic also observed that the accessions were not clustered based on nor consistent with geographical regions (Poljuha *et al.,* 2021). Chen *et al.* (2021) stated that this phenomenon could be because of the long cultivation over decades, causing mutation and adaptation among the different garlic accessions and confusing the cultivar's identity among the different landraces.

During the four decades since the first cultivar, Lumbu Hijau's release in 1984, the track of seed exchange among farmers from different regions in Indonesia has unclear identification and recording. Also, similar characteristics could mislead the farming community and the breeders in field selection during seed bulb production. Hence, presumably, the seeds have been selected and domesticated for decades in each cultivated area without obvious information on the original seed sources of these garlic accessions.

CONCLUSIONS

Based on morphological traits and SSR markers, we found that high genetic variability existed among the 14 Indonesian local garlic accessions. Based on the results of PCA (eigenvalue) and clustergram, leaf width, leaf waxiness, the density of leaves, cross-section shape of leaves, pseudo-stem diameter, anthocyanin intensity at the base of pseudo-stem, and bulb diameter are the most distinctive characteristics. Also, accessions under the same name yet collected from diverse regions sustained separation into different groups, and some accessions from unlike regions attained clustering in the same group. An indication that this phenomenon is possibly due to the impurity of garlic seed bulbs produced by farmers. The individual *off-type* was often mistaken or missed at the *roguing* time and probably carried within seed exchanges and cultivated for many years. The other accessions with totally different names may have originated from mutation through long domestication or adaptation.

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