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Maize hybrids' genetic variability based on qualitative and quantitative traits

Abstract. Genetic variability was a prerequisite to doing a plant breeding program. A broad genetic variability allows plant breeders to select a desired genotype. This research aims to assess the maize hybrid's genetic variability based on qualitative and quantitative traits. This research was conducted in the Bone district, south Sulawesi, from November 2022 to March 2023. Fifteen maize hybrids were arranged in a randomized complete block design with three replications. The variables observed are qualitative and quantitative traits. Principal component (PCA) and cluster analyses assessed the genetic variability. The result indicated that based on a loading factor greater than 0.70, the qualitative traits such as intensity of green color, anthocyanin coloration of brace roots, length of lateral branch, intensity anthocyanin coloration of silk, and degree of zigzag displayed high variability. quantitative like days to anthesis, days to silk, leaf length, 1000 seeds weight, yield, ear diameter, number of row seeds per ear, ear height, ear length, and number of seeds per row also exhibit high variability. Cluster analysis shows a broad genetic variability on qualitative and quantitative traits demonstrated by Euclidean levels 6.68-10.93 and 3.43-5.08, respectively, and generated the dendrogram that divides genotypes into four main clusters for qualitative and five for quantitative traits.

Keywords: Genetic variability · Maize hybrid · Qualitative traits · Quantitative traits

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Introduction

Maize is one of the world's most essential crops. Maize kernels contain water (10.49±0.01%), ash (1.45±0.01%), protein (11.78±0.05%), fat (5.59±0.22%), crude fiber (6.84±0.07%), total carbohydrates (70.69±0.21%) and energy of (380.19±1.56 kcal/100g) based on dry weight (Murningsih et al., 2019; Rouf Shah et al., 2016). In addition to its use as human foodstuffs, livestock feed, chemical goods, and biofuels, maize is a source of life and prosperity for people in several countries. It is expected that, in terms of production and trade, maize—currently the most-produced cereal—will surpass all other crops in the next ten years. The developing world's need for maize will double (Erenstein et al., 2022). Hence, maize yield must be enhanced to satisfy these demands.

The enhancement of maize yield can be achieved by developing a novel high-yield maize cultivar. Novel high-yield maize cultivar development relies on genetic variability (Kotschi & Horneburg, 2018; Mengistu et al., 2020) Genetic variability is population variation among members (Litrico & Violle, 2015) When genetic variability is broad, plant breeders can combine the desired traits to develop novel varieties (Ahmar et al., 2020; Swarup et al., 2021). A comprehensive understanding of the germplasm's genetic variability is needed to recombinate traits correctly.

Maize is a plant with board variability. Genetic variability can be estimated using qualitative and quantitative traits (Alemu et al., 2020). Qualitative traits in maize are typically controlled by one or a few genes and exhibit discrete variations. These traits are often used to identify and classify maize varieties and hybrids. Qualitative traits provide easy markers for initial selection, while quantitative traits are complex and influenced by multiple genes, requiring advanced breeding techniques to achieve desired improvements. The range of trait variability in plant genetic materials is an excellent resource for plant breeders to develop and improve new varieties with desired traits (Darrudi et al., 2018). Because of this, breeding programs need to look at how different quantitative and qualitative traits are in genetic resources (Bhadmus et al., 2022; Bhandari et al., 2017; Wang et al., 2023).This information helps plant breeders develop optimal breeding strategies for breeding populations.

Principal component analysis (PCA) and clustering analysis are commonly used to measure genetic variability. PCA reduces data dimensionality by transforming it into principal components, effectively estimating population variability based on traits. Studies on upland rice (Tuhina-Khatun et al., 2015), wild cassava (Karuniawan et al., 2017), sunflower (Dudhe et al., 2020), and alfalfa (Sayed et al., 2022) have utilized PCA to determine genetic variability. In maize, PCA was used to identify key traits for breeding drought-resistant varieties (Esen et al., 2022) and identified the heritability and genetic variability traits like grain yield, kernels per ear, ear diameter, and thousand kernel weight (Matin et al., 2022; Rai et al., 2021; Yadesa et al., 2022). PCA with SNP data was used by Ayesiga et al., (2023) to identify genetically of distinct maize inbred lines. Clustering analysis groups genotypes based on similarities, visualizing these relationships in dendrograms for easier understanding. This research aims to assess genetic variability in maize hybrids using qualitative and quantitative traits, providing insights for developing maize breeding programs.

Materials and Methods

Research Site. This research was conducted between November 2022 and March 2023 in the Bone district of South Sulawesi. The location is situated at a latitude of 5.06607°S and a longitude of 120.2120°E. This site is dryland with Latosol soil at an altitude of 80 m above sea level and has a D2 climate type according to Oldeman & Frere, (1982)

Plant Materials. Fifteen hybrids were used in this research. Thirteen conventional crosses maize, including TH 1- TH 13, and two commercial hybrids that have high yields and are widely adopted, NK 6172 and P 32, are used as check varieties (Table 1).

Research Methods. This study used a Randomized Complete Block Design (RCBD) with three replications. Each plot had four rows, each 5 meters long. The spacing between rows was 0.70 m, and the spacing between individual maize plants within a row was 0.20 m. Initially, two seeds were sown per hill, but after two weeks, seedlings were thinned into one per hill to maintain a plant density of 71, 428 plants per hectare, allowing only one plant per stand to grow. Standard agricultural practices were applied for field maintenance, following recommended guidelines.

Table 1. The maize material used in this experiment.

No	Code	Origin	
1	TH 1	LG 1 X G 222	Tested variety
2	TH 2	MP 2 X LG 1	Tested variety
3	TH 3	LN 64 X LG 1	Tested variety
4	TH 4	LN 86 X G 222	Tested variety
5	TH 5	B 992 X MP 2	Tested variety
6	TH 6	SB 35 X MP 4	Tested variety
7	TH 7	RE 71 X G 222	Tested variety
8	TH 8	IM 33 X RS 261	Tested variety
9	TH 9	HF 22 X G 222	Tested variety
10	TH 10	G 222 X MP 2	Tested variety
11	TH 11	MT 20 X CL 14	Tested variety
12	TH 12	LN 18 X HF 22	Tested variety
13	TH 13	RS 122 X G 222	Tested variety
14	NK 6172	NK 6172	Check Variety (commercial hybrid)
15	P 32	P 32	Check Variety (commercial hybrid)

Data Collections. The observed variables were divided into two types: qualitative and quantitative traits. The qualitative traits consisted of various traits, such intensity of green color (IGC), undulation of margin of blade (UMB), attitude of blade (AB), degree of zigzag (DZ), anthocyanin coloration of brace roots (ACBR), anthocyanin coloration of internodes (ACI), anthocyanin coloration of silks (ACSi), intensity anthocyanin coloration of silk (IACS), anthocyanin coloration of sheath (ACSh), anthocyanin coloration at base of glume (ACBG), anthocyanin coloration of glume excluding base (ACGEB), anthocyanin coloration of fresh anthers (ACFA), density of spikelet (DS), shape of tassel (ST), angle between main axis and lateral branches (ABMALB), number of primary lateral branches (NPLB), attitude of lateral branches (ALB), length of main axis above lowest lateral branch (LMAALLB), length of main axis above highest lateral branch (LMAAHLB), length of lateral branch (LLB), and shape of stem (SS). The quantitative traits included days to flowering, growth traits, yield components, and yield. Days to flowering consist of days to anthesis (DA) and days to silk (DS). Meanwhile, growth traits comprise plant height (PH), ear height (EH), ear diameter (SD), leaf length (LL), leaf width (LW), leaf angle (LA),. The yield component contains moisture content (MC), ear length (EL), ear diameter (ED), number of row seeds per ear (NRE), number of seeds per row (NSR), 1000 seeds weight (W 1000), shelling percentage (SP), fresh ear weight (FEW), yield (Y).

The qualitative trait and days to flowering were observed 55 days after planting (DAP). The growth traits were observed at 85 DAP in ten plant samples. Harvest was done at the two middle rows of plants at 105 DAP, where a black layer at the base of kernel seeds appeared, indicating that the seeds were physiologically mature. Ears were collected from two middle rows of each plot. The yield component was observed at ten ear samples. The yield (Y) was corrected to 15% moisture and converted to t/ha. A digital scale was utilized to weigh each genotype yield.

Data Analysis. The genetic variability was investigated using a multivariate analysis method with Principal Component Analysis, and the level of similarity between varieties was measured with cluster analysis. The data was processed using Euclidean distance and the standardized Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and shown as a dendrogram. A variable with a loading factor greater than 0.7 is identified as a variable that contributes to diversity (Jollife & Cadima, 2016). This analysis used Numerical Taxonomy and Multivariate System (NTSYS) software version 2.02 (Rohlf, 2000) and IBM SPSS Statistic version 23 (IBM, 2015).

Results and Discussion

The genetic variability of qualitative and quantitative traits in this study was determined using the PCA method. PCA is a multivariate technique that reduces the dimensionality of original variables that are highly correlated into mutually independent variables called Principal Components (PC). This method's maximum

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number of principal components is equal to the number of original variables(Agustina & Waluyo, 2017). Each PC represents the percentage of variance a given variable contributes to the overall variance (Aristya et al., 2017). The eigenvalue indicates the magnitude of the contribution of PC to the total variance. The first PC (PC1) has the highest eigenvalue and the most significant proportion of variance, while the subsequent PCs have lower eigenvalues than PC1 and explain the remaining variance (Pachauri et al., 2017).

Many approaches to determining the number of PCs are necessary to explain the minimum total variability. This study determines the criteria for PCs with eigenvalues higher than one (Woolford, 2015). A loading factor expresses the correlation between the measured variables and the PC. The higher the loading factor's value, the closer the variable correlation is to the PC (Hefny et al., 2017). Variables that significantly contribute to variability are identified by a loading factor value exceeding 0.70 (Kaiser, 1974).

Genetic variability based on the qualitative trait. The PCA identified six principal components with eigenvalues greater than one based on qualitative traits. These components collectively explain 83.07% of the total variability (Table 2). The identified PCA could explain almost all the variability in qualitative traits Only 16.93% of the variability in qualitative traits is not explained by these principal components. Huqe et al (2021) reported that the total diversity represented by a PC with an eigenvalue of more than one is sufficient to describe diversity in a population.

PC 1 provides 23.70% of the total variability and has an eigenvalue of 4.98. This PC is composed of the intensity of green colour, anthocyanin coloration of brace roots, and length of lateral branch with loading factor values 0.74, 0.80 and 0.74 respectively. The second PC (PC2) had an eigenvalue of 3.68, explaining 17.53% of the variability. It was strongly associated with one trait: the intensity of anthocyanin coloration of silk (loading factor= -0.72). The eigenvalue and contribution to the total variation of PC3 are 3.11 and 14.79. The length of main axis above highest lateral branch with a loading factor -0.82 was a variable associated with PC 3. The degree of zigzag(loading fac tor=-0, 79) affects the variation at PC 4, which is 12.93%. The fifth PC (PC5) and

Table 2. Eigenvalues, variation explained (%), cumulative variance (%), and loading factor of qualitative trait.

Trait and Component	Principal Component					
	1	2	3	4	5	6
Intensity of green colour	**0.74**	0.38	0.29	0.32	-0.25	-0.01
Undulation of margin of blade	0.32	0.30	-0.52	-0.32	0.24	0.49
Attitude of blade	-0.32	-0.54	-0.19	0.51	0.17	0.37
Degree of zigzag	0.01	0.24	-0.17	**-0.79**	-0.05	0.16
Anthocyanin coloration of brace roots.	**0.80**	0.12	0.14	0.33	-0.06	0.10
Anthocyanin coloration of internodes	0.54	0.57	0.46	0.25	-0.01	0.15
Anthocyanin coloration of silks	-0.52	0.66	-0.17	-0.16	-0.31	-0.20
Intensity anthocyanin coloration of silk	0.15	**-0.72**	0.21	0.34	0.33	0.23
Anthocyanin coloration of sheath	0.35	0.39	0.69	-0.01	-0.23	0.02
Anthocyanin coloration at base of glume	0.11	0.43	-0.17	-0.07	0.69	-0.27
Anthocyanin coloration of glume excluding base	-0.15	-0.41	0.62	0.05	0.26	-0.45
Anthocyanin coloration of fresh anthers	0.64	0.30	0.10	-0.41	0.42	-0.15
Density of spikelet	-0.64	0.39	0.27	-0.11	0.43	0.10
Shape of tassel	-0.35	0.63	-0.03	0.42	-0.18	-0.02
Angle between main axis and lateral branches	-0.68	0.33	0.36	0.24	-0.02	0.15
Number of primary lateral branches	-0.63	0.44	0.17	-0.11	0.39	0.25
Attitude of lateral branches	-0.55	0.43	0.12	0.55	-0.02	0.16
Length of main axis above lowest lateral branch	-0.18	0.15	-0.64	0.54	0.16	-0.27
Length of main axis above highest lateral branch	0.14	0.24	**-0.82**	0.35	-0.04	-0.22
Length of lateral branch	**0.74**	0.24	-0.35	0.23	0.06	0.18
Shape of stem	0.37	0.22	0.17	0.34	0.58	-0.08
Eigen Values	4.98	3.68	3.11	2.71	1.86	1.11
Percent of Variance (%)	23.70	17.53	14.79	12.93	8.85	5.27
Cumulative percentage (%)	23.70	41.23	56.02	68.95	77.80	83.07

the sixth PC explained the variability 8.85% and 5.27% respectively. No variable has an absolute loading factor value of more than 0.70 in PC5 and PC6. The variables at PC5 and PC6 account for a negligible portion of the variance, indicating these variables do not contribute to the variability in these PCs.

Of the four PCs that show traits that contributed to each PC, only PC1 shows positive values, while the other is negative. this means that traits that contributed to PC1 (the intensity of green colour, anthocyanin coloration of brace roots, and length of lateral branch) show the maximum contribution to the variability in PC1. Meanwhile, traits the intensity of anthocyanin coloration of silk, the length of main axis above highest lateral branch, and degree of zigzag contributed to PC2, PC3, and PC even though they have contributed to variability, their contribution is not optimal. Traits with positive loading factors contributed the most to variability, whereas the negative traits contributed but not optimally (Arif et al., 2023; Saleem et al., 2023).

This study only shows the PCA biplot PC 1 vs. PC 2. Previous research has revealed that PC 1 and 2 have the highest variation and are considered enough to represent all variations (Lee et al., 2020; Ravikumar & Somashekar, 2017). The distribution of genotypes across the vectors in the biplot diagram results in the formation of distinct genotype groups (Khan et al., 2022; Leite et al., 2018). Figure 1 shows the biplot PC1 vs. PC2 for qualitative traits. Han et al (2019) say that the point position in the biplot represented the degree of similarity. The lines that connect the traits to the biplot origin are called trait vectors. The angle between two trait vectors describes their correlation (Maulana et al., 2023). A closer angle shows a closer correlation. Two traits that show an acute angle mean have a positive correlation, an absolute angle has a negative correlation, and a right angle is not correlated.

Figure 1 shows that based on the angle to the UMB trait, the ASCh trait has a strong positive correlation. The AB trait shows a weak negative correlation, and the ABMALB trait does not correlate. The ASCh trait exhibits a higher degree of similarity to the UMB trait than the ACI trait, even though their angles to the UMB trait are nearly identical. This similarity between the two traits is influenced by both the length of their vectors and the cosine of the angle between them. Notably, the ACI trait vector is longer than the ACSh trait vector despite having nearly the same angle.

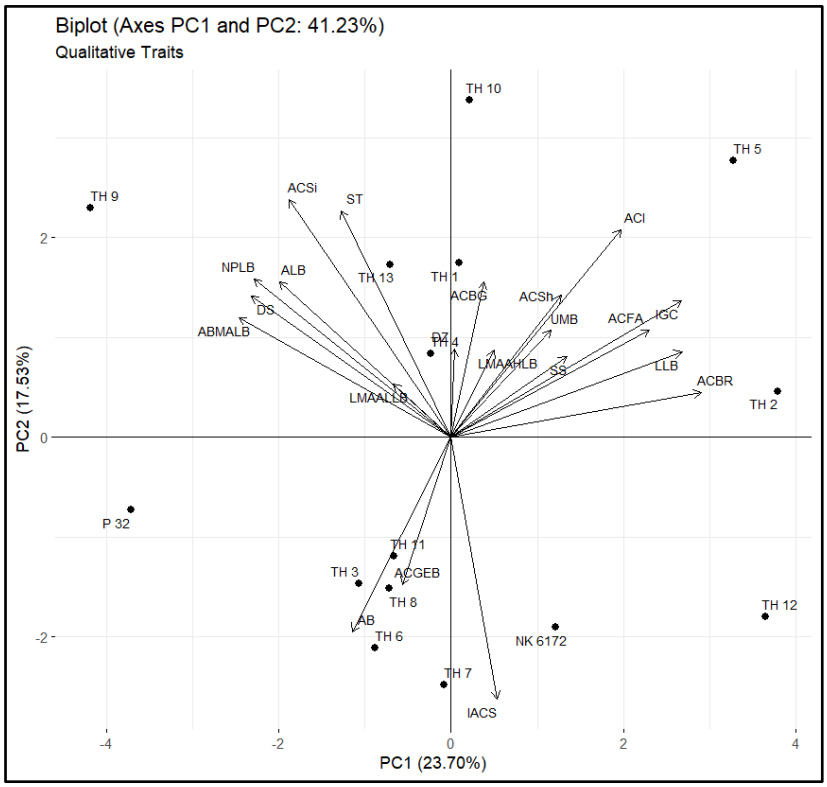

Figure 1. Biplot PCA (PC1 vs PC2) for qualitative traits.

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Genetic variability based on the quantitative trait. The PCA generates the quantitative traits into five PCs with eigenvalues between 1.22 and 5.47 Rojas-Valverde et al. (2020), state that eigenvalues quantify the effectiveness of a factor in capturing the maximum variance from each analyzed variable. The generated PCs represent 84.46% of the total variation. In the other research, Prayudha et al., (2019) generated five PCs for morphology traits and four for agronomy traits in purple-fleshed sweet potato clones. These PCs account for 89.42% and 84.79% of the explained variation, respectively.

In this research on quantitative traits, PC1 covers 32.19% of the variability, influenced by traits such as days to anthesis, days to silk, leaf length, 1000 seed weight, and yield, with loading factor values of 0.90, 0.90, 0.77, -0.77, and -0.77, respectively. PC2, which explains 20.39% of the variation, is impacted by the number of row seeds per ear (loading factor = 0.99) and ear diameter (loading factor = 0.77). Ear height, with a loading factor of 0.75, influences PC3, covering 13.03% of the variability. PC4 explains 13.03% of the variability, driven by the trait ear length (loading factor = 0.81). Lastly, the number of seeds per row, with a loading factor of 0.71, affects PC5, contributing 7.16% to the total variation (Table 3). Although 1000 seed weight and yield affect variability, their contribution is not as high as that of the other traits. Moreover, the 1000 seed weight and yield have negative loading factors, while the others have positive ones. This information aligns with the findings of Gewers et al., (2021) that even though the negative loading factor shows a high contribution, the statistically significant number was fewer than the positive one.

Figure 1 shows the PCA biplot PC 1 vs. PC 2 for quantitative traits, and objectively presents the correlations between these traits. Consistent with the qualitative trait pattern. The Y vector exhibits a positive correlation with both FEW and W 1000, as evidenced by its acute angle to these vectors. In the meantime, the NSR and LA vectors and the Y vector form a straight line. With a cosine value of -1, the straight line shows that Y strongly correlates negatively with NSR and LA. On the other hand, SP and MC form a right angle with the Y vector. Thus, there is no correlation between these traits and Y.MC from a right angle with the Y vector. Thus, there is no correlation between these traits and Y.z

Table 3. Eigenvalues, Variation Explained (%), Cumulative Variance (%), and Loading Factor of Quantitative Trait.

Trait and Component	Principal Component				
	1	2	3	4	5
Days to anthesis	**0.90**	-0.01	-0.07	0.28	-0.17
Days to silk	**0.90**	0.03	0.02	0.25	-0.13
Plant height	-0.36	0.07	0.51	0.50	-0.35
Ear height	-0.31	0.50	**0.75**	-0.06	0.00
Stem diameter	0.44	0.62	-0.44	0.17	0.03
Leaf length	**0.77**	0.03	0.05	0.26	-0.33
Leaf width	0.35	0.50	0.01	-0.48	0.29
Leaf angle	0.59	-0.20	-0.45	0.22	0.39
Moisture content	0.51	0.50	0.52	-0.12	-0.10
Ear length	0.18	-0.36	0.04	**0.81**	0.14
Ear diameter	-0.12	**0.90**	-0.26	0.12	0.05
Number of row seeds per ear	0.19	**0.77**	-0.40	0.11	-0.24
Number of seeds per row	0.29	-0.11	0.42	0.26	**0.71**
1000 seeds weight	**-0.77**	0.12	-0.47	0.01	0.06
Shelling percentage	-0.65	-0.48	-0.25	0.16	-0.25
Fresh ear weight	-0.57	0.61	0.10	0.45	0.16
Yield	**-0.77**	0.34	-0.07	0.47	0.13
Eigen Values	5.47	3.47	2.21	1.99	1.22
Percent of Variance (%)	32.19	20.39	13.03	11.70	7.16
Cumulative percentage (%)	32.19	52.58	65.60	77.30	84.46

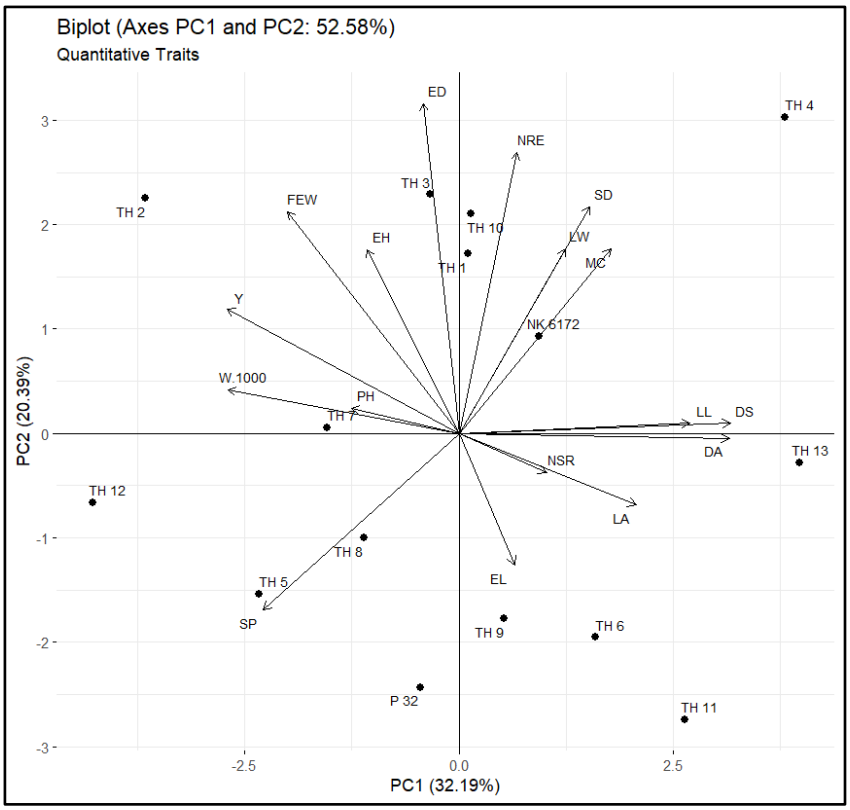

Figure 2. Biplot PCA (PC1 vs PC2) for quantitative traits.

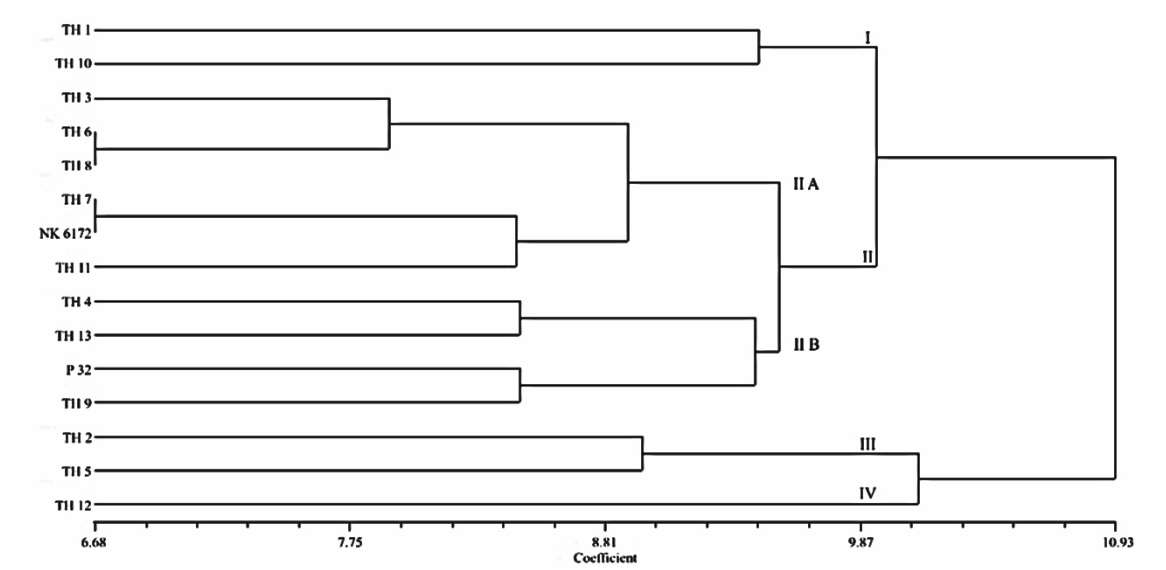

Figure 3. Dendrogram image based on the qualitative trait.

Genetic variability based on the cluster analysis. A cluster analysis based on qualitative and quantitative traits was used to assess the genetic diversity of maize varieties. Based on these traits, the cluster analysis shown as a dendrogram showed that each variety was not as similar as the others (Zhang et al., 2017). This can be seen from the Euclidean Distance in and Figure 4 of the dendrogram. Extensive relationships within genotypes are when the Euclidean distance between two genotypes is greater than 1, demonstrating the lack of a close relationship between the tested genotypes (Torres et al., 2019). The Euclidean value for the qualitative traits dendrogram ranges from 6.68 to 10.93; for quantitative traits, it ranges from 3.4 to 5.08.

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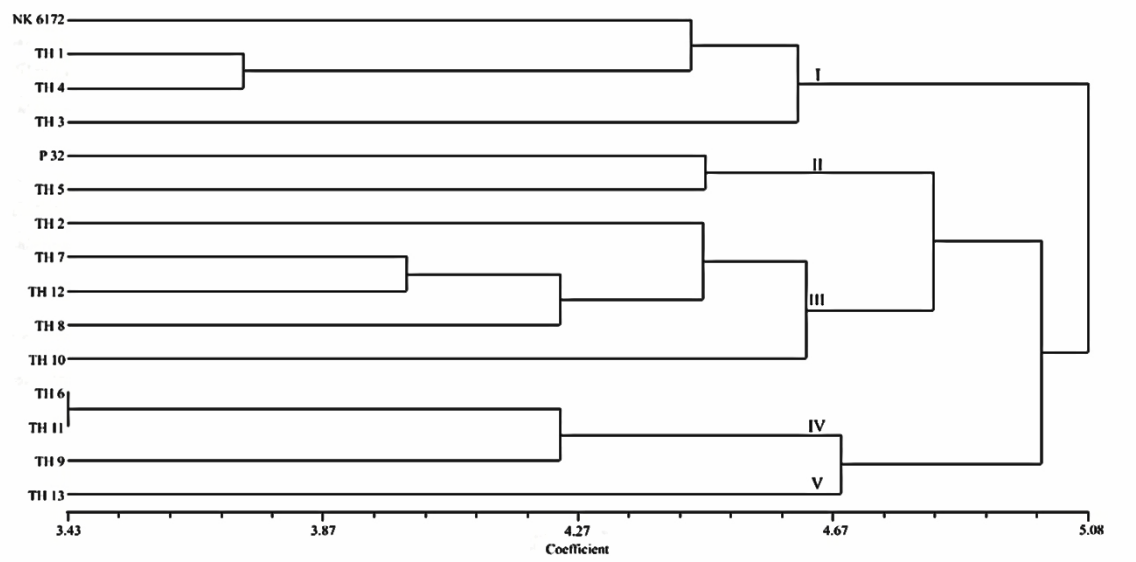

Figure 4. Dendrogram image based on quantitative trait

According to the Euclidean value of qualitative and quantitative traits, this study's maize varieties have a broad variability.

displays a dendrogram image based on qualitative trait. Four clusters of varieties of maize have been identified at Euclidean distance 9.87. Cluster I consists of TH 1 and TH 10. T Cluster II was divided into two subclusters. Five varieties constitute Cluster II A: TH 3, TH 6, TH 8, TH 7, and NK 6172. Furthermore, cluster II B involved TH 4, TH 13, P 32, and TH 9. Cluster III includes TH 2 and TH 5. The rest of the cluster (cluster IV) only contains one variety, i.e., TH 12.

Dendrograms based on quantitative traits appear in Figure 4. This dendrogram classified the maize variety into five clusters at Euclidean distance 4.67. NK 6172, TH 1, and TH 4 varieties constituted cluster I. Cluster II contains TH 3 and P 32. Cluster III is a cluster with the largest number of varieties (five varieties). The varieties are TH 2, TH 7, TH 12, TH 8, and TH 10. Cluster IV consisted of three varieties: TH 6, TH 11, and TH 9. There was one genotype, namely, TH 13, in cluster V.

In genetic variability, genetic distance plays an essential part in plant breeding. Besides genetic variation, genetic distance also plays a vital role in plant breeding. By studying genetic distance, plant breeders can identify sources of trait variation for plant breeding (Juma et al., 2021; Ustari et al., 2023). Genetic distance describes varieties' similarity degrees based on their traits. Two varieties with close genetic distance have a close relationship and high similarities, and vice versa. In dendrogram images, varieties with high similarity degrees are likely found in similar clusters (Metsalu & Vilo, 2015). Figure 1 shows that the highest level of similarity between varieties for qualitative traits was identified between TH 6 and TH 8 and between TH 7 and NK 6172. The varieties TH 6 and TH 11 have been identified as having the highest level of similarity in quantitative traits. The varieties TH 12 for qualitative qualities and TH 13 for quantitative traits exhibit the slightest similarity to the other varieties.

Conclusion

Based on loading factor values more than 0.70, the qualitative traits such as intensity of green colour, anthocyanin coloration of brace roots, length of lateral branch, intensity anthocyanin coloration of silk and degree of zigzag displayed high variability. Quantitative like days to anthesis, days to silk, leaf length, 1000 seeds weight, yield, ear diameter, number of row seeds per ear, ear height, ear length, and number of seeds per row also exhibit high variability. Cluster analysis shows a broad genetic variability on qualitative and quantitative traits demonstrated by Euclidean levels 6.68-10.93 and 3.43-5.08, respectively, and generated the dendrogram that divides genotypes into four main clusters for qualitative and five for quantitative traits.

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