



Research Article

Analysis of Genetic Parameters and Agro-Morphological Characterization in Maize (*Zea mays* L.) Inbred Lines

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Abstract | Genetic analysis and agro-morphological characterization of maize (*Zea mays* L.) inbred lines enables increased genetic gain in the maize breeding program. Twenty-four maize inbred lines were evaluated in a alpha lattice design with three replications in 2022 to study genetic parameters and agro-morphological characters of inbred lines at Rampur, Chitwan, Nepal. The number of ears per plant had the highest genotypic coefficient of variation (28.16%) and phenotypic coefficient of variation (34.49%). Thousand-kernel weight had the highest broad-sense heritability (87%). The maximum distance (706.40) between cluster centroids in the cluster analysis showed that clusters 2 and 5 had the greatest genetic dissimilarity. Inbred lines RML-76 produced the highest yield (2262.2 kg/ha) followed by RML-17 (2069.94 kg/ha) and CML-581 (1957.91 kg/ha). The grain yield had positive correlation with the number of kernel rows per ear ($r = 0.89$, $p < 0.01$), the length of the ear ($r = 0.85$, $p < 0.01$) and the number of kernels per kernel row ($r = 0.84$, $p < 0.01$). The results suggest that maize productivity can be increased by utilizing genetic variability.

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Introduction

Maize (*Zea mays* L.) is a widely produced crop for fodder, food, and fuel (Muitire *et al.*, 2021). Maize is the second most important cereal crop in Nepal. The area under maize cultivation is 1,000,000 ha with production of 3,000,000 mt in Nepal (FAOSTAT, 2022). The maize sub-sector contributes 3.15% to the national gross domestic

product (GDP) and 9.5% to the agricultural GDP of Nepal, while it has 24.97% contribution to total cereal production and covers 27% of the area used for food crops (MoALD, 2022). The productivity of the maize crop is lower (3.06 t/ha) than that of China (6.29 t/ha), Bangladesh (8.57 t/ha), USA (11.11 t/ha), India (3.20 t/ha), and the world average (5.87 t/ha) for 2021 (FAOSTAT, 2022). The low yield of crops is associated with the low adaptation of hybrid

maize varieties.

A wise selection of good inbred lines is essential for the development of high-yielding hybrids. Developing good inbred lines requires an in-depth understanding of variability. Crop plant development is more influenced by the genetic variability of the available germplasm. Genetic diversity is measured by phenotypic traits and molecular markers (Govindaraj *et al.*, 2015). The ability of any inbred maize line to successfully combine with other inbred lines to produce superior F_1 offspring is ultimately what determines its value in heterosis breeding (Arifin *et al.*, 2018). The heterogeneity in genes across maize genotypes significantly affects grain yield. The interactions between the environment and genetics impact grain yield. The hybrid performance is determined by the degree of genetic diversity among two parental inbred lines. The higher heterosis is shown by more diverse parents and vice versa (Dermail *et al.*, 2018). Inbred parental lines with high genetic variability are needed in maize breeding efforts in order to develop a distinct, adaptable hybrid with higher levels of heterosis expression. Therefore, a hybrid breeding effort requires an understanding of genetic variability. In heterosis breeding, it aids in choosing the best parental inbred lines.

Multivariate analysis is the most widely utilized technique for assessing genetic variability and investigating genetic relationships and variation patterns among genotypes. The most popular multivariate analysis tools are principal component analysis (PCA) and cluster analysis (CA) (Khalid *et al.*, 2020). PCA is used to assess the correlations between various combinations of test plant traits (Walubita *et al.*, 2022). It helps to clarify, condense, and forecast correlations among various variables (Wang *et al.*, 2022). The analysis used was for determining secondary traits for rice under salinity stress (Anshori *et al.*, 2019), maize's tolerance to drought stress (Fadhli *et al.*, 2020), and the genotype selection of popcorn (Leite *et al.*, 2022). The multivariate analysis of various agro-morphological traits in maize was applied in other study (Barth *et al.*, 2022). A powerful statistical method for assessing genetic diversity is cluster analysis with Euclidean distance (Vus *et al.*, 2020). Breeders and geneticists use cluster analysis to group maize accessions and find subsets of accessions. Cluster analysis is a technique that helps to identify the homogenous variables at various levels of similarity

in the form of a dendrogram (Oyekunle *et al.*, 2015). In order to make selection more successful, genotypes are grouped according to how complicated they are with regard to traits (Bista *et al.*, 2022).

The extent to which genotypic variability may be employed by selection is determined by heritability and the genetic progress of the specific trait. Genetic parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and heritability estimates can be used to formulate breeding strategies for high-yielding crops and to gain a better understanding of genetic advance (GA) (Singh and Kumar, 2017; Saha *et al.*, 2019). The PCV and GCV thus provide information on the level of variability in a population, whereas heritability depicts the component of a character that is passed down to future generations (Girma *et al.*, 2018; Pal *et al.*, 2016). A statistical measure used to quantify how closely two variables are associated with each other is the correlation coefficient. This provides information on the degree of relationship as well as the degree to which traits are interdependent (Shrestha *et al.*, 2023). Plant breeders use heritability analysis to aid in genotype selection from genetic diversity (Terfa and Gurmu, 2020). When breeders are knowledgeable about heritability, genetic advance, and the influence of environment on the appearance of desired traits, they may develop more suitable cultivars more quickly (Al-Daej, 2022). In order to generate relevant data, plant breeding programs have used heritability estimation (Merrick *et al.*, 2022; Egeland, 2023). Consequently, heritability estimates for yield component traits may be utilized as a starting point for further research when choosing genotypes for the required traits. A breeder will be able to ascertain how much the environment affects yield by evaluating genotypic and phenotypic differences in yield and yield components of many crop genotypes (Schmidt *et al.*, 2019).

By understanding the correlation between yield and its attributing traits, breeders can improve the effectiveness of selection. The correlation coefficient establishes the relationship between two traits of plants (Kinfe and Tsehaye, 2015; Rocha *et al.*, 2019). Breeders can utilize correlation analysis to identify important relationships between morphological traits and integrate them with genetic variable analysis (Roy *et al.*, 2018; Wang *et al.*, 2020). Determining the strength of the relationship is aided by the correlation coefficient. The values of the coefficient of correlation

between traits are important in the selection process. A useful tool for illustrating the magnitude and direction of selection is the correlation coefficient, which summarizes the relationship between traits (Aman *et al.*, 2020).

In Nepal, the research findings on agro-morphological characterization and genetic analysis of maize inbred lines are very limited. Therefore, this study was carried out to analyze the level of genetic diversity and agro-morphological variability among the inbred lines of maize. This study measures the genetic advance, heritability, variability, and correlation of the growth, yield, and yield-related traits among the maize inbred lines. The findings of this study help to identify the better parental lines that are required for hybridization. The findings from this study will be helpful in developing novel hybrids for Terai and inner Terai regions of Nepal.

Materials and Methods

Plant materials

For this study, 24 inbred lines of maize were grown in single location. The names and pedigree information of the inbred lines is given in Table 1. The official source of these inbred lines is NMRP (National Maize Research

Program), Rampur, Chitwan, Nepal. The inbred lines used in experiment are of S7 stage of selfing.

Experimental site

The experiment was carried out in the research field of the National Maize Research Program, Rampur, Chitwan, Nepal during spring to summer season (March to July) of 2022. The experimental site is situated at 228 meters above sea level with 27°40' North latitude and 84°19' East longitude. Before the experiment, the initial soil samples were taken from 0–15 and 15–30 cm depth, and their properties were analyzed at the laboratory of the National Soil Science Research Centre (NSSRC), Khumaltar, Lalitpur, Nepal. The soil of the research plot was sandy loam with an average pH of 6.36. The average values of soil properties showed that there were 2.81% organic matter, 0.12% total nitrogen, 49.55 mg/kg available phosphorous, 82.19 mg/kg available potassium, 69.70% sand, 13.40% silt, and 16.90% clay (Table 2). The climatic data is given and the source of this climatic data is NMRP, Rampur, Chitwan, Nepal (Figure 1). During the experimental period, climatic data were collected on a monthly average. The range of the temperature was between 35.21 °C in July (maximum temperature) and 15.78 °C in March (minimum temperature).

Table 1: List of maize inbred lines used in the experiment.

S. No.	Inbred lines	Pedigree/source population	SN	Inbred lines	Pedigree/source population
1	CML-581	CML161/CML451	13	RL-290	20-29-S-29S11-3-1-3-1-1-2-BB
2	RML-17	(P24F26*27F1)-4-1-B-1-1-BB-f	14	RL-292	20-29-S-29S11-2-1-1-1-2-1-BB
3	RML-76	CLRCYE00-7	15	RL-293	20-29-S-29S12-4-2-1-2-1-1-BB
4	RML-96	AG27	16	RL-294	20-29-S-29S11-2-1-1-1-2-2-BB
5	CAL-14137	WLCY2-7-1-2-1-5-B-2-2-2-1-B*9-#-B2-BH	17	RL-84	Upahar-10-3-1-2-1
6	RL-143	Upahar-54-3-4-3-1	18	RML-122	CML-Q-494-W
7	RL-208	Arun-2-S1-10-1-4-2-1-4-1-2-2-BB	19	RML-134	CML-Q-493-Y
8	RL-254	BIO981-5-6-1-2-1-1-1-1-1-BB	20	RML-145	HY18R-Y75-7
9	RL-257	HPY-S1-7-5-2-2-1-1-2-BB	21	RML-191	CAL1465
10	RL-273	POP-446-8-4-1-2-1-2-1-BB	22	RML-32	CA00320
11	RL-278	POP-446-9-3-3-1-1-1-1-BB	23	RML-46	P31C4S5B-39-#-#-1-B-B
12	RL-279	Gaurav-132-3-3-1-2-BB	24	RML-64	Pob24C5HC34-2-3-B-f-2#-BBB-f

Table 2: Details of soil properties of experimental field.

S. No	Soil depth (cm)	pH	OM %	Total N %	Available P ₂ O ₅ (mg/ kg)	Available K ₂ O (mg/ kg)	Sand %	Silt %	Clay %
1	0-15	6.52	3.07	0.13	67.29	76.16	70.70	15.40	13.90
2	15-30	6.20	2.55	0.11	31.8	88.22	68.70	11.40	19.90
	Mean	6.36	2.81	0.12	49.55	82.19	69.70	13.40	16.90

There was no rainfall in March, The highest total rainfall (649.90 mm) in June and April having the lowest rainfall (71.80 mm). Similarly, April had the lowest relative humidity (77.64%), while June had the highest relative humidity (88.57%) (Figure 1).

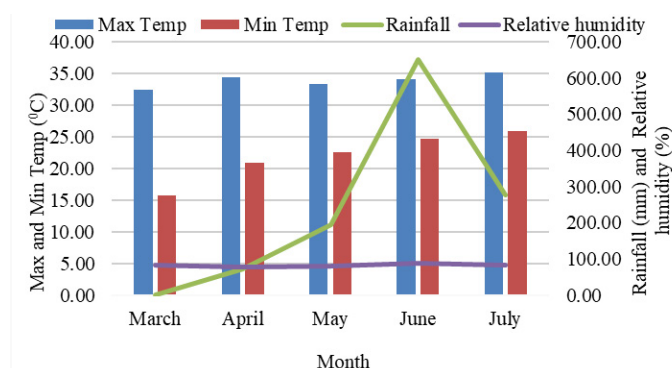


Figure 1: Climate data of the experimental location.

Experimental design and cultural practices

The maize inbred lines were evaluated in an alpha lattice design with three replications. The row to row spacing was 75 cm, and the plant to plant spacing was 25 cm in each individual plot. The plot size was four rows, each 3.5 m long. In the experimental field, well-decomposed farmyard manure (FYM) at the rate of 6 t/ha was applied one week before sowing. The chemical fertilizer at the rate of 180:60:40 kg N:P₂O₅:K₂O [nitrogen (N), phosphorus (P), and potassium (K)] per ha was applied through urea, di-ammonium phosphate (DAP), and muriate of potash (MOP). A full dose of P₂O₅, a half dose of N, and

a full dose of K₂O fertilizer were applied at the time of land preparation. After seed sowing, the remaining half of the N dose was divided into two doses: The first half dose was applied 45 days after sowing, and the second half dose was applied 90 days after sowing. During the maize growing period, two manual weeding and hoeing operations were carried out. Weeds were hoed twice at 18 and 36 days after sowing. Fungicides were not applied in the experimental field. The experiment was conducted under natural conditions and no artificial inoculation was provided for screening against disease and pests. Knee-high stage, tasseling stage, and milking stage were the three critical growth stages of maize at which furrow irrigation was applied.

Data collection

Five plants were chosen at random from each experimental plot, and data (phenological and morphological data) were collected from them (Table 3).

Morphological traits were observed at the flowering stage.

The leaf area (LA) was determined using below formula (Radford, 1967).

$$LA = K (L \times W) \dots (1)$$

Where LA = leaf area (cm²), K = constant (0.75), L = leaf length (cm), and W = maximum leaf width (cm).

Table 3: List of phenological and morphological traits measured.

S.	Trait	Method of measurement
Phenological traits		
1	Days to 50% anthesis (days)	The number of days from planting until 50% of the plants in each net plot had anthesis.
2	Days to 50% silking (days)	The number of days from planting until 50% of the plants reached the silk emergence stage
Morphological traits		
3	Plant height (cm)	The average of height from the ground level to the base of the tassel
4	Ear height (cm)	From ground level to the upper ear node
5	Stem diameter (mm)	Measured (using a Vernier caliper) stem diameter just below upper ear
6	Number of tassel branches	The number of primary and secondary tassel branches was counted for five selected plants
7	Number of leaves	The number of leaves above and below the ears were recorded for five selected plants
8	Leaf length and leaf width (cm)	Length and maximum width of fully expanded leaves were measured
Yield components		
9	Number of ears per plant	Counting all the ears on the five randomly selected plants
10	Ear diameter (cm)	Measured (using a Vernier caliper) as an average of the same five ears used for ear length estimation
11	Ear length (cm)	Measured as an average of the same five ears
12	Number of kernel rows per ear	Counted using the same five ears used for ear length estimation
13	Number of kernels per kernel row	Counted using the same five ears used for ear length estimation

Thousand kernel weight (g) was estimated randomly from grains of the same five ears after shelling, adjusted to 12.5% grain moisture.

Grain yield (kg/ha): It was calculated using the formula provided (Equation 2) based on fresh ear weight at 12.5% moisture content.

$$\text{Grain yield } \left(\frac{\text{kg}}{\text{ha}} \right) = \frac{\text{F.W.} \left(\frac{\text{kg}}{\text{plot}} \right) \times (100 - \text{HMP}) \times S \times 10000}{(100 - \text{DMP}) \times \text{NPA}} \dots (2)$$

Where; FW = the fresh weight of ears per plot (kg) at harvest; HMP= moisture percentage of grain at harvest; DMP= desired moisture percentage, i.e., 12.5%; NPA= the area of the net harvest plot, m²; S= shelling coefficient, i.e., 0.8

Disease and insect scoring

Southern leaf blight (SLB) was observed in the field. The scoring of disease was done on overall observation basis but not on individual plant basis. Disease was scored using a 0–5 scale (CIMMYT, 2004). Where, 0 (Immune) = No visible lesion. 1.0 (Resistance)= Plants with one or two to few scattered lesions on lower leaves covering up to 10% of leaf area.

2.0 (Moderately resistant)= Moderate number of lesions on leaves covering 11–25% leaf area.

3.0 (Moderately susceptible)= Abundant lesions on lower leaves, few on other leaves covering 26–50% leaf area.

4.0 (Susceptible)= Lesions abundant on lower and mid leaves, extending to upper leaves covering 51–75% leaf area.

5.0 (Highly susceptible)= Lesions abundant on almost all leaves, plant prematurely dried with 76–100% leaf area covered.

The fall army worm and stem borer were observed in a maize field. The severity of insect pest infestation was scored on a visual observation of the foliar damage attributed to each pest using a 1–5 scale (Tefera *et al.*, 2011).

Where;

1.0 (Highly resistant) = No visible leaf feeding damage

2.0 (Resistant) = Few pin holes on older leaves, several shot-holes injuries on a few leaves.

3.0 (Moderately resistant) = Several shot-hole injuries common on several leaves or small lesions, elongated lesions (> 2 cm long) on a few leaves.

4.0 (Susceptible) = Elongated lesions on several leaves,

several leaves with elongated lesions or tattering
5.0 (Highly susceptible) = Most leaves with elongated lesions or severe tattering, plant dying as a result of foliar damage.

Statistical analysis

The experimental data were processed using MS Excel 2019, and the data on ANOVA, heritability were analyzed using R-4.4.0 software. The data were analyzed using alpha lattice design with one-way ANOVA. The least significant difference (LSD) was used to compare the treatment means at the 5% level of significance (Gomez and Gomez, 1984). The Pearson correlation coefficient of growth, yield, and its attributing traits was worked out using R studio software by the Metan package. Principal component analysis and cluster analysis was carried out using Minitab 21.1.0.

The phenotypic coefficients of variation (Equation 3), genotypic coefficients of variation (Equation 4) (Singh and Chaudhary, 1985), heritability (in the broad sense) (Equation 5) (Johnson *et al.*, 1955) were estimated for evaluated traits.

$$PCV (\%) = \frac{\sqrt{\delta^2 p}}{\bar{x}} \times 100 \dots (3)$$

$$GCV (\%) = \frac{\sqrt{\delta^2 g}}{\bar{x}} \times 100 \dots (4)$$

Where, $\delta^2 g$ = genotypic variance; $\delta^2 p$ = phenotypic variance; $\bar{x}$ = sample mean.

$$\text{Heritability } (h^2) = \frac{d^2 g}{d^2 p} \dots (5)$$

The correlation between traits in this study was assessed using Pearson's correlation coefficient approach (Equation 6).

$$r = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2} \sqrt{\sum_i (y_i - \bar{y})^2}} \dots (6)$$

Where; r = Pearson correlation coefficient; x_i = x variable; y_i = y variable; Σ = Mean of y variable.

Results and Discussion

Growth, yield and yield attributing traits

The maize inbred lines showed significant differences for plant height, ear height, number of leaves above the ear, total number of leaves, silking days, anthesis

days, number of primary tassel branches, number of secondary tassel branches, number of ears per plant, number of kernel rows per ear, number of kernels per kernel row, thousand grain weight, grain yield, and insect score (Tables 4, 5, 6). The minimum coefficient of variation (1.14%) was found in silking days (Table 5). The inbred RML-76 produced the highest grain yield (2262.22 kg/ha), followed by RML-17 (2069.94 kg/ha) and CML-581 (1957.91 kg/ha), respectively (Table 6).

Maize inbred lines serve as parental lines for hybrid development. The development of inbred lines is one of the important tasks of maize breeders. The appropriate genotype selection for the parents and the performance of their cross-combinations are key factors

in hybridization success (Khan *et al.*, 2022). A significant degree of genetic variability allows plant breeders to combine outstanding characteristics according to their preferences (Priyanto *et al.*, 2023). Utilizing the variability found in inbred lines efficiently and effectively is essential for the development of high-yielding and superior hybrids. In our study, significant differences for plant height, ear height, number of leaves above the ear, total number of leaves, number of primary tassel branches, number of secondary tassel branches, number of kernel rows per ear, number of kernels per kernel row, thousand grain weight, grain yield, and insect score were found among the maize inbred lines (Tables 4, 5, 6). The results of the present study are consistent with those of Yadesa *et al.* (2022) and Jilo *et al.* (2018) and who found a wide range of

Table 4: Mean values for growth traits of 24 maize inbred lines.

Inbred lines	PH (cm)	EH (cm)	SDia (mm)	NLAE (No.)	NLBE (No.)	TNL (No.)	LL (cm)	LB (cm)
CML-581	183.15	91.56	12.85	6.57	7.55	14.34	66.82	7.26
CAL-14137	176.61	85.72	13.17	5.1	7.15	12.27	66.96	7.39
RL-143	136.56	77.22	13.22	5.08	7.37	12.67	74.34	8.41
RL-208	134.85	78.47	13.54	4.4	6.92	11.24	67.01	7.38
RL-254	157.25	86.31	14.29	6.57	7.78	14.73	73.02	8.64
RL-257	139.05	75.88	13.46	5.01	7.06	12.04	65.13	7.96
RL-273	128.4	62.53	13.55	5.71	7.28	13.07	64.25	8.49
RL-278	143.33	64.54	13.28	5.54	7.73	13.7	73.71	7.85
RL-279	139.36	70.79	13.44	5.27	6.97	12.12	61.77	7.3
RL-290	137.11	70.21	13.13	5.44	6.47	11.4	73.14	7.94
RL-292	154.22	92.98	12.88	7.27	7.19	14.34	62.1	7.82
RL-293	146.67	68.79	13.46	6.41	7.28	13.7	68.67	8.32
RL-294	129.41	55.94	14.13	6.33	6.83	12.83	65.56	7.56
RL-84	154.37	75.05	12.73	6.06	7.46	13.7	70.34	7.27
RML-122	147.92	74.8	13.71	5.27	6.52	11.32	71.88	7.59
RML-134	148.54	64.62	13.57	5.19	7.55	13.07	72.27	7.59
RML-145	174.9	83.56	12.8	5.71	7.1	12.75	66.12	7.8
RML-17	160.67	86.48	13.27	5.71	7.24	12.99	68.19	7.33
RML-191	156.35	80.47	13.3	6.06	6.92	12.75	66.78	7.64
RML-32	129.87	61.7	13.78	5.62	7.15	12.75	66.99	8.42
RML-46	151.65	76.72	14.11	5.27	6.7	11.64	71.94	8.2
RML-64	148.46	77.22	13.99	6.24	7.37	13.7	73.3	8.75
RML-76	154.29	74.38	13.95	6.85	7.55	14.58	71.94	7.28
RML-96	143.56	70.88	12.97	5.62	7.28	12.99	69.21	7.82
Grand mean	149.02	75.28	13.44	5.76	7.18	12.95	68.81	7.83
SEM	9.6	4.58	0.93	0.29	0.56	0.6	4.16	0.52
LSD (0.05)	23.79	13.04	1.77	0.79	1.17	1.59	9.05	1.09
CV (%)	8.38	8.78	9.99	6.66	11.25	6.37	6.94	7.24
P value	0	0	0.27	0	0.16	0	0.02	0.02

PH, Plant height (cm); EH, Ear height (cm); SDia, Stem diameter (mm); NLAE, Number of leaves above ear; NLBE, Number of leaves below ear; TNL, Total number of leaves; LL, Leaf length (cm); LB, Leaf width (cm).

Table 5: Mean values for growth and phonological traits of 24 maize inbred lines.

Inbred lines	LA (cm ²)	SD (day)	AD (day)	NPTB (No.)	NSTB (No.)	TL (cm)	TNTB (No.)
CML-581	366.81	62.15	60.17	9.06	1.06	26.01	11.47
CAL-14137	373.14	62.54	61.47	10.53	3.78	31.07	18.79
RL-143	466.94	62.54	61.47	14.75	1.06	27.62	15.75
RL-208	376.77	60.97	58.88	16.2	1.16	29.55	14.57
RL-254	467.69	63.32	62.33	7.78	2.62	31.33	13.45
RL-257	392.86	62.54	60.17	9.34	1.07	26.76	12.64
RL-273	408.04	60.58	58.45	16.22	1	27.64	17.24
RL-278	434.56	62.54	61.47	14.57	1.07	28.39	15.13
RL-279	349.41	62.54	61.47	15.49	2.36	26.96	18.04
RL-290	435.23	63.72	62.76	10.81	1.24	32.09	13.76
RL-292	367.24	63.32	62.33	20.81	2.25	27.26	16.62
RL-293	427.62	64.11	63.19	10.26	1.72	24.66	16.18
RL-294	377.75	63.32	62.33	12.28	1	26.61	14.07
RL-84	384.47	64.11	62.33	8.7	1.75	32.28	12.09
RML-122	410.93	62.54	61.47	6.13	1.07	27.69	10.72
RML-134	413.43	62.93	60.17	14.75	2.91	28.67	19.35
RML-145	392.13	62.54	61.47	8.88	1.07	27.39	11.59
RML-17	380.91	63.32	61.47	12.46	1.9	28.81	15
RML-191	387.34	61.76	60.17	9.61	1.07	27.18	10.66
RML-32	420.66	62.54	60.6	13.84	1.7	27.65	15.25
RML-46	440.52	61.76	60.6	11.45	3.04	28.98	16.68
RML-64	476.14	63.32	62.33	13.65	1.75	28.8	15.25
RML-76	393.82	62.54	61.47	9.8	1.24	29	14.07
RML-96	409.25	62.93	61.47	10.9	2.62	28.34	13.95
Grand Mean	406.4	62.69	61.25	12.01	1.73	28.36	14.68
SEM	47.99	0.53	0.52	1.05	0.26	1.38	2.44
LSD (0.05)	92.93	1.37	1.43	3.08	0.77	3.62	5.78
CV (%)	12.6	1.14	1.16	12.38	20.43	6.63	23.16
P value	0.04	0	0	0	0	0.01	0.02

LA, Leaf area (cm²); SD, Silking day; AD, Anthesis day; NPTB, Number of primary tassel branch; NSTB, Number of secondary tassel branch; TL, Tassel length (cm); TNTB, Total number of tassel branches. SEM, Standard error of the mean; CV, Coefficient of variation; LSD, Least significance difference.

variability in maize traits, including grain yield, row numbers per ear, number of kernels per row, ear length, ear diameter, and thousand-kernel weight. In the maize genotypes, [Mukri et al. \(2022\)](#) and [Al-Naggar et al. \(2022\)](#) found that there was significant genetic variability for yield and attributes that contributed to yield.

Correlation analysis

Correlation analysis (Pearson's correlation coefficient) between yield and yield attributing traits ([Figure 2](#)) showed that grain yield was significantly and positively correlated with the number of kernel rows per ear ($r = 0.89, p < 0.01$), number of kernels per kernel

row ($r = 0.84, p < 0.01$), ear length ($r = 0.85, p < 0.01$), and Thousand grain weight ($r = 0.79, p < 0.01$). However, the insect score ($r = -0.66, p < 0.01$) showed a significant negative correlation with yield. The grain yield and disease score were negatively correlated ($r = -0.33, p > 0.05$) ([Figure 2](#)).

In this study, there was a significant and positive correlation between grain yield and ear length, thousand grain weight, number of kernel rows per ear, and number of kernels per kernel row ([Figure 2](#)). Similar results have been obtained by [Sesay et al. \(2017\)](#) for the number of kernel rows per ear and ear length. According to [Fan et al. \(2008\)](#), the number

of ears per plant, ear length, ear diameter, number of kernels per row, and thousand-kernel weight have a significant correlation with grain yield. [Hemavathy \(2020\)](#) observed that the number of kernel rows per ear was significantly and positively correlated with the yield of maize. The correlation between yield and disease as well as yield and insect scores was negative ([Figure 2](#)). The positive correlation between traits suggests that improving any trait may have a positive effect on desirable traits since a correlation between traits is created by the existence of connected genes and the occurrence of an interallelic effect between genes ([Cömertpay et al., 2012](#)). Analysis of correlation coefficients is a popular method for determining

the degree and direction of relationships among individuals, particularly those that have to do with productivity. The correlation coefficient, in accordance with [Sadeghi \(2022\)](#) aids in identifying how closely two variables are related. This is very beneficial for evaluating and developing selection criteria for the required traits. Trait correlation is essential when utilizing secondary trait selection to identify superior genotypes with high yields ([Amegbor et al., 2022](#)). [Akfindarwan et al. \(2023\)](#) used correlation coefficient analysis to identify the selection criteria for S2 maize, whereas [Epule et al. \(2022\)](#) used it to determine the yield and harvest area of maize.

Table 6: Mean values for yield and yield attributing traits of 24 maize inbred lines.

Inbred lines	NEPP (No.)	ED (mm)	EL (mm)	NKRPE (No.)	NKPKR (No.)	TGW (g)	GY (kg/ha)	DS (0-5)	IS (1-5)
CML-581	1.84	33.66	137.08	16.38	20.76	266.23	1957.91	1.24	1.25
CAL-14137	1.05	31.48	128.44	11.92	17.15	242.24	1558.35	1.21	2.36
RL-143	1.84	33.49	128.4	15.93	21.16	266.67	1920.29	1.39	1.62
RL-208	1.05	31.62	124.9	12.14	18.15	219.12	1599.93	1.24	1.99
RL-254	1.04	32.53	124.88	11.92	19.56	239.19	1590.67	1.48	2.73
RL-257	1.44	33.24	135.81	14.87	19.34	247.04	1786.37	1.24	1.99
RL-273	1.05	31	122.34	10.63	17.43	173.75	1453.73	1.57	1.62
RL-278	1.05	31.74	126.59	11.23	19.88	218.25	1453.63	1.48	1.99
RL-279	1.05	31.29	122.1	11.08	17.64	185.53	1350.87	1.21	2.73
RL-290	1.05	32.26	127.7	12.45	18.61	241.37	1590.18	1.24	2.36
RL-292	1.04	30.4	126.3	11.16	16.2	238.75	1506.9	1.39	1.99
RL-293	1.05	32.52	125.09	12.29	19.11	244.86	1582.09	1.24	1.99
RL-294	1.84	33.58	132.75	15.1	20.54	247.04	1852.16	1.21	1.62
RL-84	1.05	32.21	124.92	11.99	18.23	231.33	1562.62	1.48	2.73
RML-122	1.05	30.96	125.74	11.69	19	213.45	1597.89	1.39	2.73
RML-134	1.05	32.09	128.49	11.84	19.66	234.39	1722.63	1.3	1.25
RML-145	1.05	32.34	122.7	12.29	19.66	238.75	1601.64	1.21	1.99
RML-17	1.45	33.48	131.37	16.46	21.75	272.78	2069.94	1.21	1.25
RML-191	1.05	32.87	130.45	11.99	20.28	233.95	1733.62	1.24	1.62
RML-32	1.05	33.04	126.74	13.2	20.77	254.02	1801.52	1.3	1.99
RML-46	1.05	31.53	126.91	12.52	17.25	234.39	1593.28	1.39	1.99
RML-64	1.84	32.66	127.54	12.67	19.99	238.75	1799.17	1.39	1.99
RML-76	1.45	34.84	138.2	16.38	23.57	272.34	2262.22	1.21	1.25
RML-96	1.05	32.72	129.52	11.46	20.15	234.82	1946.39	1.57	1.99
Grand Mean	1.23	32.4	128.12	12.9	19.41	237.04	1703.92	1.33	1.96
SEM	0.18	1.32	6.31	1.21	1.17	9.87	164.43	0.26	0.32
LSD(0.05)	0.49	2.97	13.41	3.21	2.95	27.57	412.32	0.49	0.86
CV(%)	20.41	5.48	5.92	13.22	6.99	5.76	11.35	28.28	23.61
P value	0	0.07	0.08	0	0	0	0	0.28	0

NEPP, Number of ears per plant; ED, Ear diameter (mm); EL, Ear length (mm); NKRPE, Number of kernel rows per ear; NKPKR, Number of kernels per kernel row; TGW, Thousand grain weight (g); GY, Grain yield (kg/ha); DS, Disease score (0-5); IS, Insect score (1-5). SEM, Standard error of the mean; CV, Coefficient of variation; LSD, Least significance difference.

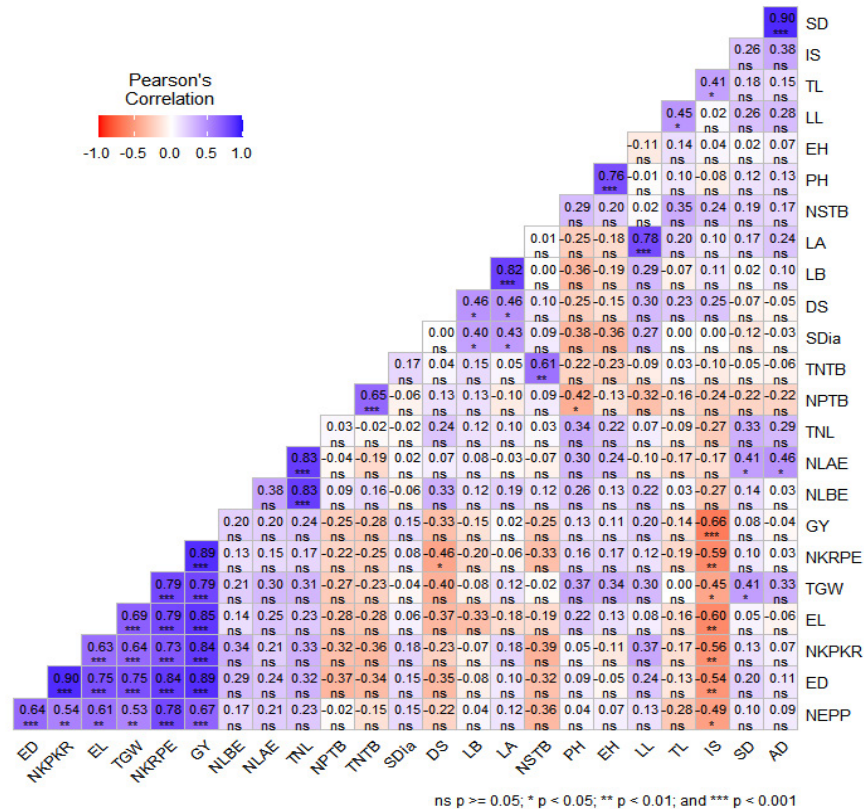


Figure 2: Pearson's correlation coefficient among growth, yield and its attributing traits of 24 maize inbred lines. PH, Plant height (cm); EH, Ear height (cm); TNL, Total number of leaves; LA, Leaf area; SD, Silking day; AD, Anthesis day; TL, Tassel length (cm); NTB, Number of tassel branches; NEPP, Number of ears per plant; ED, Ear diameter (mm); EL, Ear length (mm); NKRPE, Number of kernel rows per ear; NKPKR, Number of kernels per kernel row; TGW, Thousand grain weight (g); GY, Grain yield (kg/ha); DS, Disease score (0-5); IS, Insect score (1-5).

Table 7: Estimation of PCV, GCV, heritability, genetic advance and GAM for growth, yield and its attributing traits of 24 maize inbred lines.

Traits	Genotype variance (Vg)	Phenotypic variance (Vp)	GCV (%)	PCV (%)	Heritability
Plant height (cm)	272.84	428.79	11.08	13.9	0.78
Ear height (cm)	109.89	153.58	13.93	16.46	0.83
Total number of leaves	1.31	1.99	8.84	10.89	0.79
Leaf length (cm)	22.54	45.37	6.90	9.79	0.66
Leaf width (cm)	0.35	0.67	7.56	10.45	0.68
Leaf area (cm ²)	2083.37	4706.25	11.23	16.88	0.61
Silking days	0.93	1.44	1.54	1.91	0.78
Anthesis days	1.57	2.08	2.05	2.35	0.86
Tassel length (cm)	4.89	8.43	7.8	10.24	0.73
Total number of tassel branches	9.45	21	20.94	31.22	0.62
Number of ears per plant	0.12	0.18	28.16	34.49	0.79
Ear diameter (mm)	2.02	5.17	4.39	7.02	0.56
Ear length (mm)	37.83	95.44	4.8	7.63	0.57
Number of kernel rows per ear	4.54	7.45	16.52	21.16	0.76
Number of kernels per kernel row	3.68	5.52	9.88	12.1	0.80
Thousand grain weight (g)	638.51	825.15	10.66	12.12	0.87
Grain yield (kg/ha)	64670.31	102094.36	14.92	18.75	0.78
Disease score (0-5)	0.04	0.18	15.04	31.9	0.37
Insect score (1-5)	0.3	0.51	27.95	36.44	0.74

Table 8: Cluster means of 24 agro-morphological traits of 24 maize genotypes.

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Centroid
Plant height (cm)	154.86	150.61	139.36	142.7	154.29	149.02
Ear height (cm)	82.78	76.64	70.79	68.47	74.38	75.28
Stem diameter (mm)	13.2	13.39	13.44	13.62	13.95	13.44
Number of leaves above ear	5.59	5.73	5.27	5.84	6.85	5.76
Number of leaves below ear	7.30	7.13	6.97	7.18	7.55	7.18
Total number of leaves (No.)	13.01	12.82	12.12	13.01	14.58	12.95
Leaf length (cm)	68.62	69.1	61.77	69.02	71.94	68.81
Leaf width (cm)	7.74	7.89	7.3	7.96	7.28	7.83
Leaf area (cm ²)	401.88	409.86	349.41	414.1	393.82	406.4
Silking days (d)	62.64	62.67	62.54	62.8	62.54	62.69
Anthesis days (d)	60.82	61.4	61.47	61.18	61.47	61.25
Number of primary tassel branch	11.4	11.86	15.49	12.51	9.8	12.01
Number of secondary tassel branch	1.27	1.81	2.36	1.84	1.24	1.73
Tassel length (cm)	27.3	29.03	26.96	27.88	29	28.36
Number of tassel branch	13.72	14.74	18.04	14.76	14.07	14.68
Number of ears per plant	1.64	1.05	1.05	1.31	1.45	1.23
Ear diameter (mm)	33.47	31.72	31.29	32.83	34.84	32.4
Ear length (mm)	133.17	125.54	122.1	129.25	138.2	128.12
Number of kernel rows per ear	15.91	11.85	11.08	12.71	16.38	12.9
Number of kernels per kernel row	20.75	18.35	17.64	20.23	23.57	19.41
Thousand grain weight (g)	263.18	227.95	185.53	240.49	272.34	237.04
Grain yield (kg/ha)	1973.63	1557.58	1350.87	1782.58	2262.22	1703.92
Disease Score (0-5)	1.27	1.36	1.21	1.34	1.21	1.33
Insect score (1-5)	1.53	2.21	2.73	1.74	1.25	1.96

Phenotypic and genotypic coefficients of variation and Heritability

The maximum value of GCV was observed in the number of ears per plant (28.16%). The minimum value of GCV was observed in silking days (1.54%). Similarly, the maximum value of PCV was found in insect score (36.44%). The minimum value of PCV was found in silking days (1.91%) (Table 7).

The maximum values of broad-sense heritability were found in thousand grain weight (87%) followed by anthesis days (86%), respectively. The minimum value of heritability was found in the disease score (37%) (Table 7).

For all of the traits, the phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) (Table 7). Similar results were obtained by Iseghohi *et al.* (2023). The disease score had a higher phenotypic variance than genotypic variance, indicating that the environment had an important effect on this trait. GCV indicates

a large level of genetic variation, but only heritability estimates and genetic gain may be used to calculate the amount of heritable variation (Rao and Rao, 2015). Plant breeders can select genotypes from genetic diversity with the use of heritability estimation. In our study, high heritability was found for plant height, ear height, total number of leaves, anthesis day, silking day, number of ears per plant, grain yield, thousand grain weight, number of kernel rows per ear, and number of kernels per kernel row, while low heritability was found for disease score (Table 7). Plant breeders can choose the direction of selection using high heritability values (Bartaula *et al.*, 2019), which will be used as a guide in selection (Tessema *et al.*, 2022). The low heritability values of observed traits suggest that environmental influences on their traits (Dochtermann *et al.*, 2019; Mustakim *et al.*, 2019).

Cluster analysis

Based on growth and yield traits, the 24 maize inbred lines have been grouped into five clusters (Table 9,

Table 9: Grouping of 24 maize inbred lines by Euclidean average linkage method.

Cluster 1	Cluster 2	Cluster3	Cluster 4	Cluster 5
CML581, RL-257, RL-143, RML-17	CAL-14137, RL-84, RL-208, , RML-122, RML-145, RL-254, RL-290, RL-293, RML-46, RL-273, RL-278, RL-292	RL-279	RL-294, RML-134, RML-191, RML-32, RML-96, RML-64	RML-76

Figure 3). The largest cluster overall was cluster 2 with 12 inbred lines, followed by cluster 4 with 6 inbred lines, cluster 1 with 4 inbred lines, cluster 3 with 1 inbred line and Cluster 5 with 1 inbred line (Table 9).

The highest values of plant height (154.86 cm) and ear height (82.78 cm) were recorded among the inbred lines of cluster 1 (Table 8). The highest value of tassel length (29.03 cm) and disease score (1.36) were found in cluster 2. Cluster 5 had the highest values of total number of leaves (14.58), anthesis days (61.47), ear diameter (34.84 mm), ear length (138.20 mm), number of kernel rows per ear (16.38), number of kernels per kernel row (23.57), thousand grain weight (272.34 g), and grain yield (2262.22 kg/ha) (Table 8).

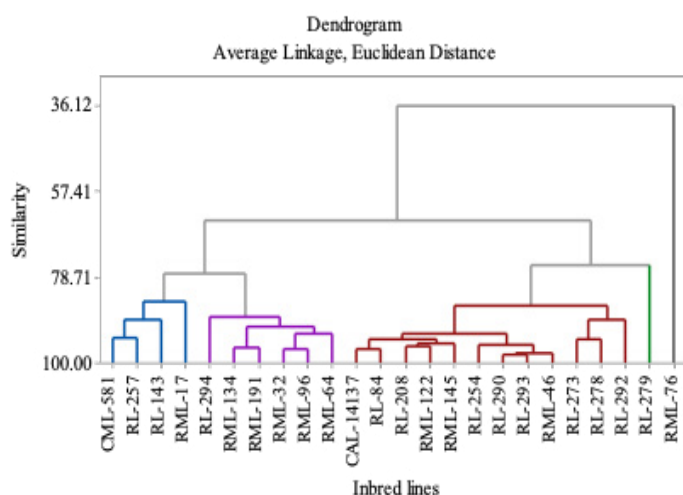


Figure 3: Cluster analysis of 24 maize inbred lines evaluated for agro-morphological traits.

The distance between cluster centroids ranged from 193.77 to 916.96 (Table 10). The minimum distance (193.77) between cluster centroids was found between cluster 1 and cluster 4, indicating genetic similarity between the inbred lines in respective clusters, and the maximum distance (916.96) was found between cluster 3 and cluster 5, indicating genetic dissimilarity between the inbred lines in respective clusters. The maximum distance from the centroid (120.40) was shown by cluster 2, and the minimum distance from the centroid (0.00) by cluster 3 and cluster 5 (Table 11).

Table 10: Inter and intra cluster distances between clusters among 24 maize inbred lines.

Clusters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	0	417.80	630.28	193.77	289.07
Cluster 2		0	220.09	225.73	706.40
Cluster 3			0	440.15	916.96
Cluster 4				0	481.44
Cluster 5					0

Table 11: Different statistics of Euclidean distance and cluster analysis of 24 maize inbred lines.

Clusters	No. of observations	Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
Cluster 1	4	21205	68.16	99.33
Cluster 2	12	52968.8	60.82	120.40
Cluster 3	1	0	0	0
Cluster 4	6	18977.2	50.72	81.08
Cluster 5	1	0	0	0

Cluster analysis has been shown to be helpful in classifying genotypes under different conditions and in assisting in the selection of the best parents for hybrid breeding programs (Al-Naggar *et al.*, 2020; Bhatti *et al.*, 2020). In order to increase the success of selection, the fundamental objective of genotype clustering is to categorize genotypes according to how complex they are with respect to attributes (Bista *et al.*, 2022). The mean values of agro-morphological traits present in five clusters, which indicate their broad genetic bases, are given (Table 8). These findings were similar to those found by Wendwessen (2023). Five clusters of maize inbred lines were made in our study based on agro-morphological traits (Table 9), demonstrating that the evaluated inbred lines had enough genetic diversity. These results are similar to those reported by previous researchers (Anusha *et al.*, 2022; Matin *et al.*, 2017; Mounika *et al.*, 2018; Hannok *et al.*, 2023). Clusters 3 and 5 had the highest intra-cluster distance, showing genetic dissimilarity between the inbred lines in those clusters (Table 10). The genotypes that were grouped together in those clusters had the

highest intra-cluster distance, which suggests that there was substantial genetic variation between them. So, there is a large chance that genotypes inside these clusters will exchange genes. Cluster 2 had the highest value of maximum distance from the centroid (Table 11). These results are supported by the results of Varaprasad and Shivani (2017).

Principal component analysis

The eigenvalue, proportion of variance, and cumulative proportion are given (Table 12). The first seven principal components were found eigen value more than 1 and all together 84.6 % of variability was explained. The first principal component was explained by 28.7 % of variability and 14.7 % of variability was explained by second principal component and 13.0 % of variability was explained by third principal component (Table 12). The total variance given by

the first principal component was mainly associated with insect score, disease score, number of tassel branches, number of primary tassel branches, number of secondary tassel branches, tassel length, and leaf width. The total variance provided by the second principal component was mainly associated with the total number of leaves, anthesis days, silking days, number of leaves above the ear, leaf area, leaf length, insect score, tassel length, number of secondary tassel branches, leaf width, disease score, number of leaves below the ear, thousand grain weight and stem diameter (Table 12).

Figure 4 displays the distribution of maize inbred lines for the first two principal components. Those inbred lines which are distant from the other inbred lines indicates their usefulness in breeding program.

Table 12: Eigen analysis and principal component coefficients based on correlation matrix of 24 variables measured in 24 maize inbred lines

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Proportion of Variance	0.287	0.147	0.130	0.100	0.071	0.068	0.044	0.039
Cumulative Proportion	0.287	0.434	0.564	0.664	0.734	0.803	0.846	0.885
Eigen Values	6.888	3.518	3.124	2.398	1.698	1.636	1.049	0.926
Plant height (cm)	-0.112	-0.016	-0.442	0.010	0.109	-0.272	0.219	-0.085
Ear height (cm)	-0.070	-0.031	-0.390	0.029	0.090	-0.274	0.475	0.170
Stem diameter (mm)	-0.021	0.149	0.323	-0.043	0.137	0.107	0.236	-0.647
Number of leaves above ear	-0.150	0.186	-0.217	0.280	-0.314	0.148	0.094	-0.248
Number of leaves below ear	-0.120	0.232	-0.073	0.396	0.036	-0.252	-0.279	-0.028
Total number of leaves	-0.162	0.251	-0.175	0.407	-0.169	-0.063	-0.109	-0.163
Leaf length (cm)	-0.083	0.362	0.140	-0.233	0.181	-0.216	-0.120	0.156
Leaf width (cm)	0.066	0.326	0.273	0.095	-0.100	-0.024	0.460	0.074
Leaf area (cm ²)	-0.006	0.422	0.265	-0.085	0.045	-0.129	0.239	0.156
Silking days (d)	-0.087	0.303	-0.235	-0.115	-0.032	0.441	-0.166	0.146
Anthesis days (d)	-0.056	0.327	-0.229	-0.142	-0.084	0.450	0.006	0.104
Number of primary tassel branch	0.127	-0.084	0.134	0.435	0.141	0.203	-0.006	0.385
Number of secondary tassel branch	0.123	0.138	-0.210	0.090	0.542	0.061	0.059	-0.263
Tassel length (cm)	0.077	0.202	-0.124	-0.241	0.320	-0.212	-0.299	0.061
Number of tassel branch	0.144	0.036	0.090	0.328	0.496	0.250	-0.030	0.016
Number of ears per plant	-0.279	-0.011	0.134	0.064	-0.013	0.100	0.222	0.230
Ear diameter (mm)	-0.352	0.022	0.100	-0.062	0.011	0.022	-0.133	-0.078
Ear length (mm)	-0.322	-0.118	0.007	-0.012	0.102	-0.017	-0.053	-0.121
No. of kernel rows per ear (No.)	-0.345	-0.095	0.064	-0.044	0.104	0.053	0.086	0.119
No. of kernels per kernel row (No.)	-0.327	0.049	0.148	-0.044	-0.040	-0.058	-0.223	-0.072
Thousand grain weight (g)	-0.323	0.076	-0.097	-0.068	0.193	0.078	0.126	0.178
Grain yield (kg/ha)	-0.353	-0.049	0.096	-0.021	0.119	-0.033	-0.018	-0.017
Disease score (0-5)	0.146	0.287	0.098	0.156	-0.168	-0.326	-0.157	0.114
Insect score (1-5)	0.239	0.167	-0.162	-0.297	-0.124	0.076	0.025	-0.101

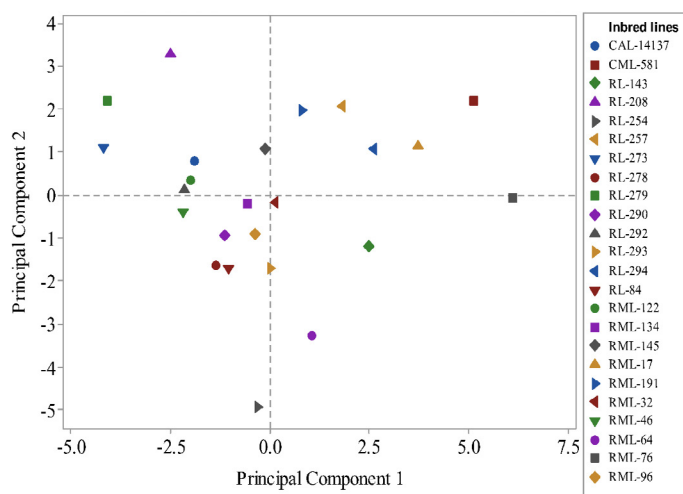


Figure 4: Distribution of maize inbred lines for the first two principal components.

The loading plot diagram (Figure 5) showed the distribution and the nature of diversity of traits along PC1 and PC2. The loading plot depicted that traits showed high degree of variation. The loading plot shows that the grain yield and yield attributing traits such as no. of kernel rows per ear, no. of kernels per kernel rows, ear length, ear diameter, thousand grain weight are further (Figure 5).

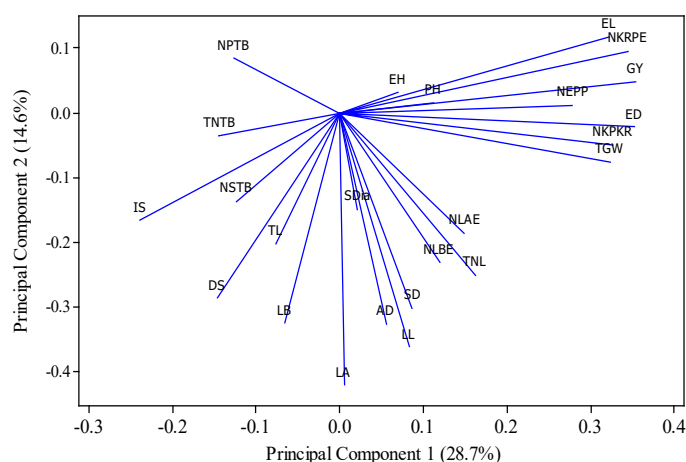


Figure 5: Loading plot of the studied 24 variables of 24 maize inbred lines.

Principal component analysis (PCA) is commonly used to make groups of genotypes and to minimize the number of variables. PCA aids in identifying the traits that can be utilized as indicators by explaining a large portion of the variation in the initial collection of variables. The selection of a cluster for the purpose of choosing parents for hybridization is well supported by traits that significantly contribute to divergence (Thakur *et al.*, 2017). In this study, 84.6% of the variance was explained by the first seven principal components (PC1 to PC7) with an Eigen value >1

(Table 12). In Baby corn, the first three principal components were found eigen value more than 1 and all together 87.49 % of variability was explained by Magudeeswari *et al.* (2019). Amegbor *et al.* (2022) found that the first four principal components (PCs) explained 96.4% of the variation of the agronomic traits along with PC1 accounting for 80.10% of the variation. Similarly, Langyan *et al.* (2022) reported that the first five PCs had eigen values >1, explaining more than 70% variance, of which the first three PCs contributed to 53% variance in maize.

The accuracy of the PCA can be determined by the magnitude of the eigen value. Kaviriri *et al.* (2023) found that an eigen value of 1 represents the practical limit of dimensionality in aggregating trait diversity.

Therefore, the eigenvalue can be an important statistic in the process of selection. The first PCs significantly contributed to overall variation when looking at a number of variables. Eigen values (in PCA) are of the utmost importance for numerical assessment to evaluate the variation induced by various variables on the dependent structure and their data matrix in graphical form (Singh *et al.*, 2020).

Conclusions and Recommendations

The number of leaves below ear, ear diameter, ear length, and disease score showed considerable variations among maize inbred lines. The values of PCV were higher than those of GCV for all evaluated traits, suggesting an environmental influence. The grain yield was positively correlated with days to 50% silking, plant height, ear diameter, and the number of kernel rows per ear, therefore, indirect selection for these traits may increase the grain yield. The cluster analysis revealed that the maximum distance between cluster centroids was found between cluster 3 and cluster 5, indicating genetic dissimilarity. The inbreds, namely RML-76, produced the highest grain yield, followed by RML-17, CML-581 and RML-96, respectively. These inbred lines would be suitable for higher grain yield and they can be utilized for developing high yielding hybrids.

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Novelty Statement

Maize is the second most important crop in Nepal. The hybrid seed was imported from India using a high amount of foreign exchange. Better parental lines, or inbred lines, are needed for maize hybridization programs, and this study sought to identify them.

Author's Contribution

Jiban Shrestha: Conceptualization, data curation, formal analysis, investigation, methodology, resources, writing original draft.

Surya Kant Ghimire, Krishna Hari Dhakal and Mahendra Prasad Tripathi: Conceptualization, supervision, validation, writing review and editing

Generative AI and AI-assisted technology statement

The authors have declared no Generative AI and AI-assisted technologies in the writing process

Conflict of interest

The authors have declared no conflict of interest.

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