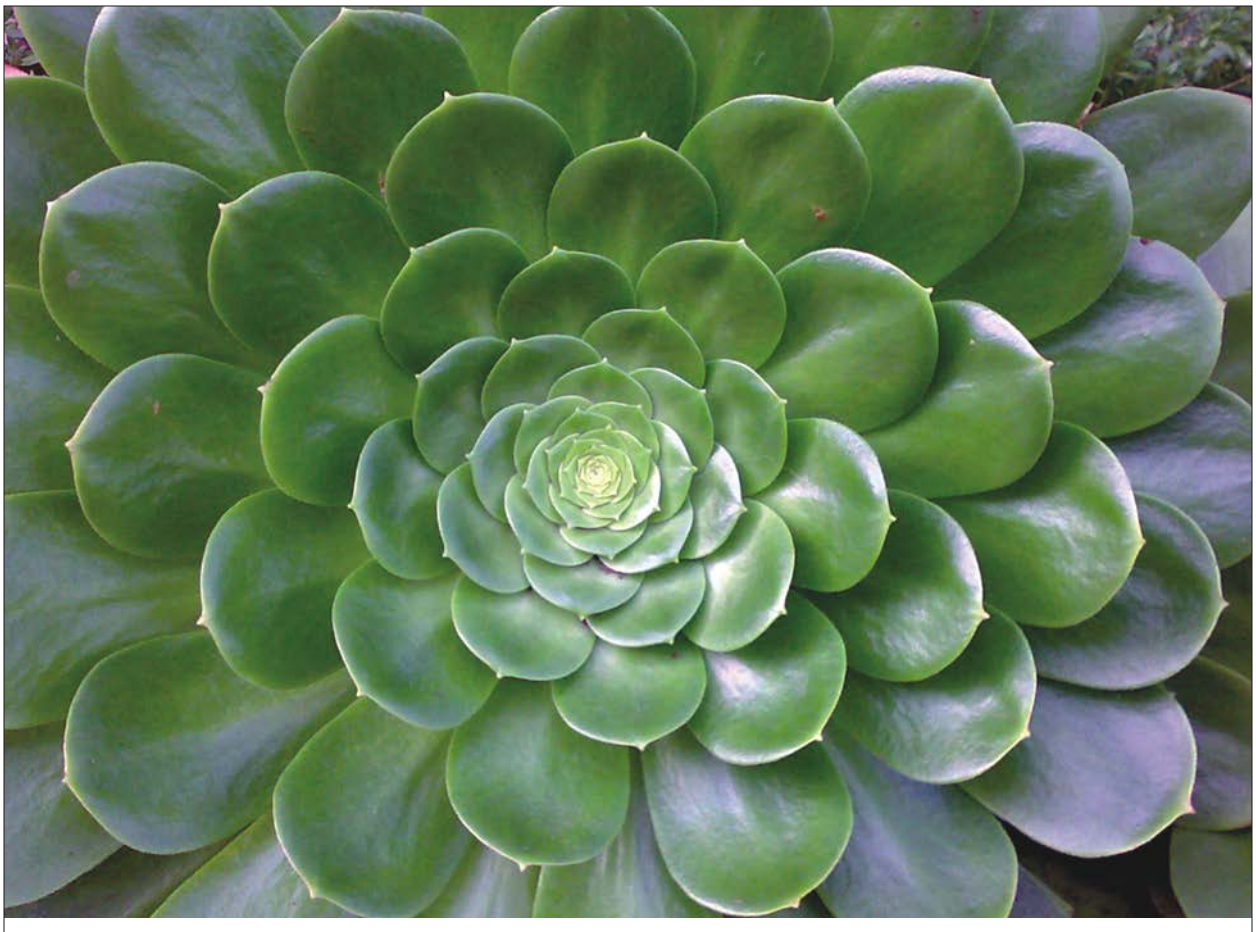

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Research Article
Phenotypic Diversity, Heritability and Genetic Advance Among Quality Protein Maize (*Zea mays* L.) Inbred Lines Under Low Soil Nitrogen Environment

[1,2]Bedasa M. Dosho, [2]B.E. Ifie, [2]I.K. Asante, [2]E.Y Danquah and [1]Habtamu Zeleke

[1]School of Plant Sciences, Haramaya University, Dire Dawa, Ethiopia
[2]West Africa Centre for Crop Improvement, University of Ghana, College of Basic and Applied Sciences, Legon, Accra, Ghana

Abstract

Background and Objective: Phenotypic diversity, heritability and genetic advance are the key factors in the maize breeding program. This study was conducted to assess genetic variability, heritability and genetic advance among quality protein maize inbred lines under a low soil nitrogen environment. **Materials and Methods:** One hundred and sixty-six maize inbred lines including five checks were evaluated under low soil nitrogen (30 kg ha^{-1}) environment. **Results:** The results indicated the existence of genetic variability among inbred lines under low N. Grain yield (28%), ear height (23%), kernel row number per ear (20%) and stay green characteristics (24%) were shown a low heritability estimate, whereas ear diameter (65%) was shown a high heritability estimate. Moderate heritability estimates were observed for day to anthesis (48%), days to silking (46%), plant height (50%), days to harvesting (53%) and 1000 kernel weight (41%) and ear length (41%). All agronomic characters showed a low genetic advance of the mean (GAM) except grain yield (31.11%) which showed greater GAM and stay green characteristics (12.78%) which showed moderate GAM. Principal component analysis was extracted from the first five PCs which accounted for 73.12% of the total cumulative variations existing among the considered maize inbred lines. Based on their Euclidean distance, the inbred lines were also grouped into four clusters. **Conclusion:** Based on the data analysis results, genetic variability was pointed out among maize inbred lines with regards to low soil nitrogen.

Key words: Cluster analysis, genetic advance, genetic variability, heritability, low soil nitrogen, principal components

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Corresponding Author: Bedasa M. Dosho, School of Plant Sciences, Haramaya University, Dire Dawa, Ethiopia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maize (*Zea mays* L., 2n = 2x = 20) is one of the three most important food crops and is widely grown in the world together with rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). The majority of developing countries people depend on maize as a source of calories, proteins, vitamins and minerals[1,2]. In more than 25 developing countries particularly in Africa, Latin America and Asia, maize is accounted for 15-56% of the total daily calories of poor people and is used to combat food insecurity, hunger and malnutrition[3]. As estimated by FAO food balance sheets, one-fifth of total daily calories and 17-60% of total daily protein of people in 12 African countries were supplied from maize[4].

Maize yield is dependent on the biosynthesis efficiency and storage of starch in the maize kernel. The kernel contains 70-75% starch which accounts for 65-75% of kernel dry weight, 8-10% protein, 4-5% oil and 14% other constituents (fibre)[5]. There are two classes of maize kernels, in which the majority of biochemical components exist (i.e., endosperm and germ (embryo)). The endosperm and the germ account for 80 and 10% of the kernel dry weight, respectively and also the endosperm account for 90% of the kernel starch. While the germ accounts for 30% oil and 18% protein. The endosperm and the germ contain most of the protein in the mature maize kernel, with the difference in both quantity and quality. Protein in the mature maize kernel germ is superior in both quantity and quality to the protein in the endosperm[4-6].

About 3% of the kernel nitrogen is covered by albumins which are soluble in water, 3% by globulins which are soluble in salt and 60% by prolamins which are soluble in alcohol with or without added reducing agents and 34% glutelins which are soluble in dilute alkali[3]. The prolamins account for the majority of the kernel proteins which are collectively known as the zeins. The zeins contain a high amount of amino acids like glutamine, proline, leucine and alanine, which are relatively poor in protein quality. The zeins are classified into alpha (α- 22 and 19 kD), beta (β-14 kD), gamma (γ-27 and 16 kD) and delta (δ-(10 kD) based on their primary gene and amino acid sequences. The α-zeins account for about 80% of the total zeins in the kernel, which are deficient in essential amino acids lysine and tryptophan[7,8].

In countries where maize is consumed as the primary or sole protein source, malnutrition is common due to the lack of essential amino acids lysine and tryptophan in maize. To overcome this problem, in the 1960s and 1970s Purdue University scientists identified natural mutant genes conferring high lysine and tryptophan content in normal maize, opaque-2 (o2)[9] and floury-2 (fl2)[10]. For the development of higher lysine and tryptophan content in maize through conventional methods of plant genetic manipulation, the o2 gene was identified as more suitable for genetic manipulation in plant breeding programs than other mutant genes[4]. The o2 gene in maize encodes a transcriptional activator that controls the expression of various genes during kernel development, particularly some of the most abundant endosperm storage protein genes. According to Vivek *et al.*[11], homozygous recessive opaque-2(o2o2) has substantially higher lysine and tryptophan contents than either heterozygous or homozygous dominant for the opaque-2 locus.

The nutritive value of milk protein is considered to be higher than that of normal maize protein. However, milk is a protein source that very few people can afford it. Maize homozygous for the o2 mutant has a quality value equivalent to 90% of that of milk, but it is associated with different undesirable characteristics like reduction in grain yield, ear rots and weevils[4,11]. By combining the genetic systems of the gene mutant opaque-2 (o2) and modified o2 endosperm gene, CIMMYT scientists developed hard endosperm maize with high lysine and tryptophan content, called quality protein maize (QPM)[12]. Quality protein maize (QPM) is described as a range of maize cultivars with twice the content of limiting amino acids lysine and tryptophan and 30% less leucine as compared to normal maize[4,13].

In crop production, nitrogen is a major factor that can be supplied through organic matter or biological nitrogen fixation and chemical synthesis. In maize growth and development, low nitrogen supply causes a reduction in seed production, leaf chlorosis and reduced root branching[14]. Due to the high cost of chemical nitrogen fertilizer developing countries' farmers have not been applying it to their farms which led to yield loss. In addition to yield loss, 70% of the chemical nitrogen fertilizer applied to maize is lost due to leaching, denitrification and surface runoff that affect climate change[15,16]. To solve food and nutritional security in developing countries, the development of genotypes that yield better under limited N supply by improving nitrogen-use efficiency (NUE) of maize is the most important strategy with today's climate change[17].

Nitrogen-use efficiency (NUE) for maize is defined as the capacity of the crop to produce biomass and grain yield per unit of available N in the soil and it is the product of N-uptake efficiency and N-utilization efficiency[18]. Nitrogen can be up-taken by a plant in the form of nitrate and ammonium and their N-uptake efficiency depends on the characteristics of root morphology and physiology with the activity of nitrate and ammonium transporters[18]. The NUE is a highly complex, polygenically inherited trait, with significant genetic variance

which is widely found in various germplasm[17,19]. Therefore, developing genotypes with better yield under low N is the strategy behind the maize breeding program. Thus, this study was initiated to determine genetic variability among QPM inbred lines under low soil nitrogen and cluster the inbred lines into relatively homogenous groups.

MATERIALS AND METHODS

Plant materials, study site and experimental design: One hundred and sixty-six including five checks were used. The study was conducted at Haramaya University (Ethiopia) research site (rare) in 2018-2019, a mid-altitude agro-ecological region located at 8.37°N latitude and 42.02°E longitudes with an elevation of 2,050 m.a.s.l with a maximum and minimum temperature of 24.4 and 9.8°C, respectively. The study area receives maximum rainfall from July to September with an average annual of 820 mm. The soil of the area is characterized by sandy clay.

Depletion of soil nitrogen: Depletion of nitrogen from the experimental site was done by growing a high population density of maize plants without fertilizer application and removing all biomass after harvest from the field. Before planting under a low N environment, the soil chemical property of the site was determined at Haramaya University soil laboratory. Soil analysis results indicated that the total nitrogen content at Rare was 0.08%, which is considered low soil nitrogen at the site. Due to a limited number of seeds for each inbred line an augmented design was used to evaluate the inbred line's performance under low N environments (30 kg ha^{-1}). Two seeds per hill were planted on a one-row length of 5.1 m at a 30 cm distance between plants and 75 cm between rows. The plants were thinned to one plant per hill to give 44,444 plant populations per hector. The 30 kg N ha^{-1} nitrogen fertilizer was applied in the form of UREA creating a low soil N environment. This is because the resource-poor farmers in Sub-Saharan African countries apply 20-30 kg ha^{-1} fertilizer below recommended rate due to high cost and low access to commercial fertilizer for their crops. In addition to nitrogen fertilizer, the recommended rate of 100 kg P_2O_5 ha^{-1} in the form of tricalcium phosphate was applied.

Collection of agronomic characters: Based on the International Board for Plant Genetic Resources (IBPGR) descriptor list[20] phenotypic characters were for grain yield (t ha^{-1}) (GY), as shelled grain weight at harvest adjusted to 12.5% moisture content, days to 50% anthesis (days) (DTA): Number of days from planting to when pollens have shed 50% of the plants, days to 50% silking (days) (DTS): Number of days from planting to when silks have emerged 50% of the plants, Anthesis-silking interval (days) (ASI): The difference between days to 50% silking and 50% anthesis, plant aspect (1-5) (PASP): Plant aspect was recorded on a scale of 1-5 based on general plant type (plant and ear height), uniformity of plants, disease and insect damage and lodging, where 1 good plant type and 5 poor plant type, ear aspect (1-5) (EASP): Ear aspect could be recorded based on a scale of 1-5, where 1 means good ears (consider ear size, husk cover, ear rot, total numbers of ear harvested per plot, ear damage caused by insects or/and diseases and other acceptable characters), while 5 means poor ear with undesirable characters, number of ears per plant (count) (NEPP): It counted as a number of ears with at least one fully developed grain divided by the number of harvested plants, plant height (cm) (PLH): From the ground level to the base of the first tassel branch, after the milk stage, ear height (cm) (ERH): From ground level to the node bearing the uppermost ear. After the milk stage, ear length (cm) (ERL): The length of the uppermost ears of sampled plants in cm after de-husking, ear diameter (cm) (ERD): The diameter of the sample plant in cm after de-husking in the middle of the cob and stay-green characteristics: Stay green scored four weeks after silking on a scale of 1-9 based on the % of dead leaf area below the ear (where, stay green score: 1 = 0-10% dead leaf area, 2 = 10-20% dead leaf area, 3 = 20-30% dead leaf area, 4 = 30-40% dead leaf area, 5 = 40-50% dead leaf area, 6 = 50-60% dead leaf area, 7 = 60-70% dead leaf area, 8 = 70-80% dead leaf area, 9 = 80-90% dead leaf area Banzinger *et al.*[18].

Data analyses

Analysis of variance: Statistical data analyses were performed on the mean of five sample plants used for the collection of measured traits and on a per plot basis for the scored traits. All statistical data analyses were done using R software version 4.1.0. Analysis of variance for a single environment in which checks and newlines (non-replicated lines) were considered as fixed effects, hierarchical ANOVA can be estimated using DAU. Test() function in the agricolae package for R software. During the analysis of variance for augmented experimental design, check lines that were replicated in the experiment were used for estimating error variance in the linear model in Table 1. Statistical analysis for the linear model procedure was performed to fit ANOVA of a single environment in which checks and non-replicated lines considered as fixed effects is as follows.

The linear model for a single environment is as follows:

$$Y_{ij} = \mu+\beta_i+C_j+\tau_{k(i)}+e_{ij}$$

Where:
Y_{ij} = Response variable
μ = General mean
β_i = Random effect of the i^{th} blocks
C_j = Fixed effects of j^{th} checks
$\tau_{k(l)}$ = Fixed effects of $k(i)^{th}$ new inbred lines in blocks
e_{ijk} = Random errors

Broad-sense heritability (h^2) for inbred lines over environments was calculated from components of variance as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\frac{\sigma_{gxy}^2}{2}\right) + \sigma_e^2}$$

Where:
h^2 = Broad-sense heritability
σ_g^2 = Genetic variance of inbred lines
σ_{gxy}^2 = Genetic variance of inbred lines by year interactions
σ_e^2 = Error variance

The heritability can be categorized as low heritability (0-30 %), moderate heritability (30-60%) and high heritability (60% and above)[21].

Genetic advance: Measures genetic gain under selection, which was computed from "selection intensity of 2.06 when 5% of the population is selected" a phenotypic standard deviation of the character in the population and heritability in the broad sense of the trait as follows:

$$GA = k\times\sigma^2p\times h^2_B$$

$$GAM = \frac{GA}{x}\times 100$$

Where:
GA = Genetic advance
GAM = Genetic advance as percent of mean
k = Standardized selection differential at 5% selection intensity (2.063)
σ^2p = Phenotypic standard deviation of the character in the population
h^2 = Heritability in a broad sense of the trait
x = Mean for the trait

The observed genetic advance as percent of mean values was classified as low (less than 10%), moderate (10-20%) and high (greater than 20%).

Variance components for genotypic and phenotypic effects were estimated from the mean squares and their expectations such as:

$$\sigma^2g = \frac{\sigma^2t - \sigma^2e}{Block}$$

$\sigma^2p = \sigma^2g+\sigma^2e$ (for the single environment)

$\sigma^2p = \sigma^2g+ \sigma^2_{gxe} +\sigma^2e$ (for over environments)

Where:
σ^2g = Genotypic variance
σ^2t = Mean squares of treatments
σ^2e = Mean squares of error
σ^2p = Phenotypic variance

Genetic and phenotypic coefficients of variation were also estimated from genotypic and phenotypic variances with their overall mean as follows:

$$GCV = \frac{\sqrt{\sigma^2g}}{x}\times 100$$

$$PCV = \frac{\sqrt{\sigma^2p}}{x}\times 100$$

Where:
GCV = Genetic coefficient of variation
PCV = Phenotypic coefficient of variation
x = Over all means of a trait

Principal component analysis: To avoid differences in scales, the data were standardized (0,1) using a scale. Unit = TRUE of R version 4.1.0 as mean to zero and standard deviation one. The principal component analysis (PCA) was computed using princomp() and the amount of variance retained by each principal component was done by getting Eigenvalue () to identify useful characters which are more important to explain much of the variability by identifying characters that load the most in explaining the observed variation. fviz_eig () was used in determining the number of PCA by visualizing the screen plot. Screen-plot is used to determine the cut point for the number of PC. From the output of PCA, get_pca_var() was used to provide coordinates, a correlation between variables

Table 1: ANOVA for mean squares and their expectancies for augmented design for a single environment

Source of variation	Df	MS	Expected MS
Total	n-1	-	$\sigma^2_e+\sigma^2_b+\sigma^2_t$
Blocks (b)	b-1	Msb	-
Treatments (t)	t-1	Mst	$\sigma^2_e+\sigma^2_t$
Checks (c)	c-1	Msc	$\sigma^2_e+\sigma^2_c$
Augmented (inbred lines (l))	t-1	Msl	$\sigma^2_e+\sigma^2_t$
Checks vs. augmented (cvs.l)	1	Mslvs.c	$\sigma^2_e+\sigma^2_l+\sigma^2_c$
Check+check vs. augmented (c+cvs.l)	1+(l-1)	Msc+cvs.l	$\sigma^2_e+\sigma^2_{c+c}+\sigma^2_l$
Error	(b-1) (c-1)	Mse	σ^2_e

df: Degrees of freedom and MS: Mean squares

and axes, squared cosine and contributions variables in the PCA. The quality of representation of the variables on the factor map for PCA was determined by square cosine(cos2). Square cosines of the variables on the selected dimensions were visualized using the corrplot() function and bar plot of variables cos2 using the fviz_cos2(). Variable correlation bi-plot based on the contribution of variables to the first two principal components were visualized using fviz_pca_var(). The most significantly associated variables with the first selected principal components were tested using dimdesc() function. Individual matrices containing all the results including coordinates, a correlation between individuals and axes, squared cosine and contributions were provided by get_pca_ind() and visualization of the individuals on the factor map based on their contribution and cos2 were provided by using fviz_pca_ind(). Add Ellipses = TRUE using fviz_pca_biplot() was used to group individuals based on their origin on the first two principal components as ellipses. A three-dimensional bi-plot was constructed by using scatterplot3d().

Cluster analysis: Before performing cluster analysis for all 166 maize inbred lines, the dissimilarity matrix between observations using the function dist() and pre-cluster visualization using the fviz_dist(dist()) were computed. Cluster analysis was made using the hierarchical cluster analysis. After pre-cluster visualization, all 166 maize inbred lines were grouped into respective classes using function HCPC() with nb. clust = -1,0 or any positive integer in R based on the Euclidean distance matrix. The results of the cluster analysis were presented in the form of a dendrogram to depict the degree of similarity and inter-relationships among characters. Visualization of the dendrogram was performed by using function fviz_dend() of R. Individual visualization on the first two PCAs (factor map) was performed using R base function fviz_cluster() and plot() for combining the hierarchical clustering and the factorial map. The variable that described the most in each cluster were also computed.

RESULTS

Analysis of variance and variance components: Analysis of Variance (ANOVA) showed significant ($p<0.05$) differences among treatments, control, augmented, control vs. augmented and control+control vs. augmented for most of the agronomic characters except anthesis silking interval in Table 2. Highly significant ($p<0.01$) differences were observed among augmented (new inbred lines) inbred lines for all characters. As indicated in Table 2, grain yield ranged from 0.5-4.3 t ha^{-1} with a mean of 2.34 t ha^{-1}. Days to anthesis ranged from 69-103 days with a mean of 85.83 days. Days to silking ranged from 72-106 with a mean of 89 days. Plant height ranged from 60-199 cm with 103 cm. Stay green characteristics ranged from 2-9 with a mean of 4.66. This indicates that there is genetic variability among inbred lines for the environment under which they are evaluated.

Phenotypic diversity: The proportions of phenotypic variances of inbred lines under a low N environment for all agronomic characters were greater than both genetic and environmental variances, while the proportion of environmental variances was greater than genetic variances for all agronomic characters in Table 3. The levels of variability apparent among the lines were assessed based on the genetic and phenotypic coefficient of variation which had the potential for genetic change due to the genetic makeup of the lines and the environment where the lines have grown. The genetic coefficient of variation was ranged from 1.03% for days to harvesting to 27.31% for grain yield, while the phenotypic coefficient of variation ranges from 1.41% for days to harvesting to 63.7% for grain yield (Table 3). This indicates that those traits that had higher phenotypic coefficient variation were also showed a higher genotypic coefficient of variation. For all the agronomic characters the GCV values were lower than PCV values, showing that the characters were more influenced by environments than their genetic makeup. Grain yield had higher GCV and PCV values of 27.31 and 51.96%, respectively, whereas days to harvesting had low GCV

Table 2: Mean square for quality protein maize inbred lines evaluated under a low N environment

		Mean square														
Sources of variation	DF	GYD	DTA	DTS	ASI	PLH	ERH	NEPP	DTH	TKW	EASP	ERL	ERD	KRNPE	NKPR	NKPE	SG
Block(adj.)	10	0.369	9.844*	11.760*	0.330	130.8**	63.401*	0.048	2.444	602.9	0.104	1.456	0.017	0.879	14.482	4912.4*	0.564
Treatment(adj.)	170	1.224**	45.446**	47.229**	0.501	462.47**	109.57**	0.231**	39.638 **	4064.9**	0.233	6.963**	0.559**	2.757**	23.904**	7496.2**	5.563**
Control	4	1.141**	204.545**	232.727**	0.384	1401.2**	2.13	0.035	144.327**	722.9	0.384	3.141**	0.639**	3.783**	10.695	8702.4**	11.136**
Augmented	165	1.280**	42.177**	43.76**	0.540*	486.5**	130.274**	0.229**	40.143 **	4402.6**	0.262*	7.81**	0.568**	2.797**	25.435**	8007.6**	5.308**
Control vs. augmented	1	5.334**	69.395**	76.574**	0.001	3575.4**	154.482*	2.572**	115.244**	2272.6*	0.749*	0.302	1.647**	10.529**	67.18**	214.7**	47.579**
Control+control vs. augmented	166	1.226**	41.613**	42.759**	0.504	439.85**	112.16**	0.236**	37.116 **	4145.5**	0.229	7.055**	0.557**	2.732**	24.223**	7467.1**	5.429**
Error	40	1.07	4.107	4.633	0.34	38.42	25.68	0.09	2.93	474.2	0.15	0.81	0.026	0.72	6.99	1907.2	1.25
Mean		2.34	85.83	89.02	3.07	103.51	36.25	1.55	177.00	253.77	2.25	13.14	3.89	12.57	27.89	352.78	4.66
Maximum		4.83	103	106	4	199	100	4	187	426.4	4	36.7	11.96	16.4	42.60	664.56	9
Minimum		0.5	69	72	2	60	13	0.38	168	79	1.5	7.4	2.7	9.2	16.40	166.00	2
SE		0.44	2.02	2.15	0.58	6.19	5.06	0.30	1.71	21.72	0.39	0.90	0.16	0.85	2.64	43.67	1.11
CV (%)		19	2.40	2.40	19	6	14	19.6	1	8.6	17.3	6.9	4.2	6.8	9.50	12.40	24

*Significant at p<0.05 probability level, **Significant at p<0.01 probability level, SE: Standard error, CV (%): Coefficient of variance, GYD: Grain yield, DTA: Days to anthesis, DTS: Days to silking, ASI: Anthesis silking interval, PLH: Plant height, ERH: Ear height, NEPP: Number of ear per plant, DTH: Days to harvest, TKW: Thousand kernels weight, EASP: Ear aspect, ERL: Ear length, ERD: Ear diameter, KRNPE: Kernels row number per ear, NKPR: Number of kernels per row, NKPE: Number of kernels per ear and SG: Stay green

and PCV values of 1.03 and 1.41, respectively. The coefficient of variation, particularly the genetic coefficient of variation determines the reliability of the lines for use in a breeding program in which high proportions of GCV to PCV value are more preferred in breeding work. Except stay green (13.44%) and grain yield (27.31%), all agronomic characters had a low genetic coefficient of variation.

Heritability estimates and genetic advance: The estimated heritability for agronomic characters ranged from 0.04 (4%) for the anthesis-silking interval to 0.65 (65%) for ear diameter. Grain yield (28%), anthesis-silking interval (4%), ear height (23%), number of ears per plant (12%), ear aspect (5%), kernel row number per ear (20%), number of kernels per row (18%), number of kerns per ear (21%) and stay green characteristics (24%) were showed low heritability estimate, whereas ear diameter (65%) was showed high heritability estimate. Moderate heritability estimates were observed for day to anthesis (48%), days to silking (46%), plant height (50%), days to harvesting (53%), 1000 kernel weight (41%) and ear length (41%) (Table 3). In our study, the GAM for agronomic characters under consideration ranged from 1.55% for days to harvesting to 31.11% for grain yield. All agronomic characters were showed low GAM except grain yield (31.11%) which showed greater GAM and stay green characteristics (12.78%) which showed moderate GAM (Table 3). With interrelationships between genetic advance as percentage of mean for the concerned agronomic characters, grain yield showed high GAM (31.11%) but low broad-sense heritability (28%), whereas ear diameter showed high broad-sense heritability (65%) but low GAM (9.37%).

Relative importance and quality of variables in explaining variations: Before computing principal component and cluster analyses, the most correlated variables within the data were standardized (0,1) using the scale in R as mean to zero and standard deviation one. Highly correlated variables in the data indicate that there is a redundancy in the data in which the principal component analysis can be used to reduce the variables into a smaller number of new variables that explain most of the variances in the variables. Principal component analysis computed for maize inbred lines evaluated under low N environments at Raare has extracted the first five PCs by using normalized variables which accounted for 73.12% of the cumulative variations existing among the considered maize inbred lines under low N environments in Table 4. Out of total cumulative variations, about 31.05% of the total variation among inbred lines was explained by the first PC, which was

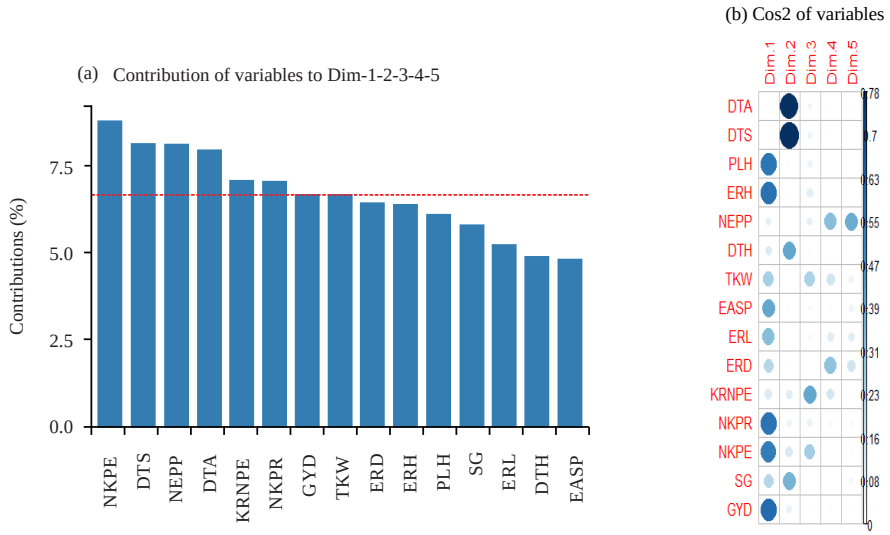

Fig. 1(a-b): Contribution of the variables to principal components and square cosine (cos2) of variables on the principal components, (a) Contribution of the variables to the first five principal components and (b) Square cosine (cos2) of the variables on the first five principal components

GYD: Grain yield, DTA: Days to anthesis, DTS: Days to silking, ASI: Anthesis silking interval, PLH: Plant height, ERH: Ear height, NEPP: Number of ears per plant, DTH: Days to harvest, TKW: Thousand kernels weight, EASP: Ear aspect, ERL: Ear length, ERD: Ear diameter, KRNPE: Kernels row number per ear, NKPR: Number of kernels per row, NKPE: Number of kernels per ear and SG: Stay green

Table 3: Estimate of genetic, phenotypic and environmental variances, genetic advance and heritability with standard error for QPM inbred lines under a low N soil environment

					Coefficient of variation				
Traits	σ^2_g	σ^2_p	σ^2_e	Mean	GCV (%)	PCV (%)	GA	GAM	h^2_B±SE
Grain yield	0.49	1.48	0.20	2.25	27.31	51.96	0.70	31.11	0.28±0.44
Days to anthesis	3.76	7.87	4.11	86.15	2.26	3.27	2.77	3.22	0.48±2.02
Days to silking	3.87	8.51	4.63	89.36	2.21	3.28	2.76	3.09	0.46±2.15
Anthesis-silking interval	0.01	0.35	0.34	3.08	3.94	19.40	0.05	1.62	0.04±0.58
Plant height	38.55	76.97	38.42	101.20	6.00	8.48	9.04	8.93	0.50±6.19
Ear height	7.63	33.31	25.68	35.77	7.62	15.92	2.73	7.63	0.23±5.06
Number of ears per plant	0.01	0.10	0.09	1.49	7.30	20.69	0.08	5.37	0.12±0.30
Days to harvesting	3.34	6.27	2.93	176.58	1.03	1.41	2.73	1.55	0.53±1.71
Thousand kernels weight	326.43	800.63	474.20	255.62	7.12	11.15	23.90	9.35	0.41±21.72
Ear aspect	0.01	0.16	0.15	2.22	3.86	17.64	0.04	1.80	0.05±0.39
Ear length	0.56	1.37	0.81	13.16	5.69	8.91	0.99	7.52	0.41±0.90
Ear diameter	0.05	0.07	0.03	3.95	5.66	7.01	0.37	9.37	0.65±0.16
Kernel row number per ear	0.19	0.91	0.72	12.70	3.42	7.57	0.39	3.07	0.20±0.85
Number of kernels per row	1.54	8.53	6.99	27.58	4.45	10.47	1.08	3.92	0.18±2.64
Number of kernels per ear	508.09	2415.29	1907.20	352.21	6.39	13.93	21.26	6.04	0.21±43.67
Stay green	0.39	1.64	1.25	4.93	13.44	27.50	0.63	12.78	0.24±1.11

σ^2_g: Genotypic variance, σ^2_p: Phenotypic variance, σ^2_e: Environmental variance, GCV: Genetic coefficient of variation, PCV: Phenotypic coefficient of variation, GA: Genetic advance, GAM: Genetic advance as percent of mean, h^2_B: Broad sense heritability and SE: Standard error

highly associated with high grain yield, tall plant and ear height, high thousand-grain weight, long ear length, high number of kernels per row and ear. While the second PC accounted for about 18.64%, which was associated with more contribution of days to anthesis and silking and days to harvesting. Kernel row number per ear and number of kernels per ear showed more contribution in creating variation among inbred lines in the third PC, which accounted for about 9.70% of the total cumulative variations. The fourth and fifth PCs accounted for about 7.80 and 6.00% of the total cumulative variation existing among inbred lines, which are characterized by good ear aspect and big ear diameter. The quality of variables in explaining variations among inbred lines under a low soil nitrogen environment was detected in Fig. 1. The

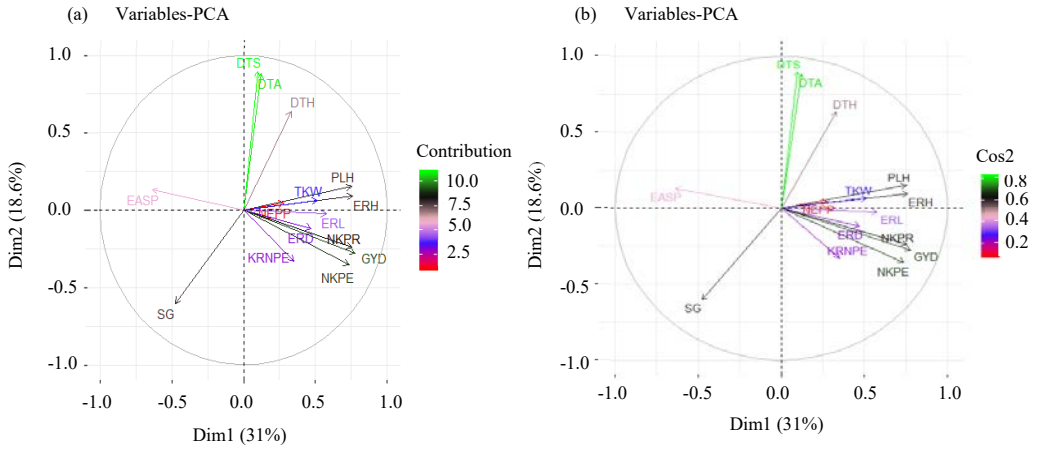

Fig. 2(a-b): Variable's correlation plot based on the contributions and quality of variables to the first two principal components, (a) Variable's correlation plot based on the contributions and (b) Variable's correlation plot based on the quality

DTA: Days to anthesis, DTS: Days to silking, ASI: Anthesis silking interval, PLH: Plant height, ERH: Ear height, NEPP: Number of ear per plant, DTH: Days to harvest, TKW: Thousand kernels weight, EASP: Ear aspect, ERL: Ear length, ERD: Ear diameter, KRNPE: Kernels row number per ear, NKPR: Number of kernels per row, NKPE: Number of kernels per ear, SG: Stay green and GYD: Grain yield

Table 4: Eigenvectors, eigenvalue, proportion of variance and cumulative proportion of agronomic traits of QPM inbred lines for the first five principal components evaluated under a low N soil environment

	Eigenvectors				
Agronomic characters	PC-1	PC-2	PC-3	PC-4	PC-5
Grain yield	0.779	-0.277	-0.111	-0.135	0.127
Days to anthesis	0.124	0.884	0.225	0.097	-0.112
Days to silking	0.097	0.898	0.241	0.113	-0.082
Plant height	0.754	0.153	-0.262	-0.068	-0.023
Ear height	0.763	0.094	-0.316	-0.071	-0.065
Number of ears per plant	0.280	0.045	0.294	-0.578	0.624
Days to harvest	0.331	0.636	0.130	0.012	-0.057
Thousand grain weight	0.515	0.065	-0.496	0.392	0.244
Ear aspect	-0.635	0.131	0.168	-0.128	0.249
Ear length	0.576	-0.024	-0.187	-0.328	-0.313
Ear diameter	0.471	-0.117	0.014	0.564	0.388
Kernel row number per ear	0.350	-0.329	0.632	0.380	-0.009
Number of kernels per row	0.757	-0.241	0.279	-0.193	-0.165
Number of kernels per ear	0.736	-0.356	0.521	0.046	-0.144
Stay green characteristics	-0.475	-0.603	0.008	0.075	-0.203
Eigen value	4.66	2.78	1.45	1.17	0.89
Proportion of variance (%)	31.05	18.64	9.70	7.80	6.00
Cumulative proportion (%)	31.05	49.69	59.36	67.16	73.12

number of kernels per ear, days to silking, the number of ears per plant, days to anthesis, kernel row number per ear and the number of kernels per row were the variables more contributed to the creation of variation among inbred lines in the first five PCs in Fig. 1a. High quality of trait contribution in explaining variances was observed by plant height, ear height, number of kernels per row, number of kernels per ear and grain yield in the first PC, days to anthesis and days to silking in the second PC, kernel row number per ear in the third PC, number of ear per plant and ear diameter in the fourth PC and number ear per plant in the fifth PC in Fig.1b.

Variable's correlation plot in the first two principal components: The majority of the variances were accounted for by PC1 and PC2, which accounted for about 49.69% of the cumulative variances in the measured variables of QPM inbred lines evaluated under low soil nitrogen (Table 4). variables grouped under cluster-1 were considered as those variables explained more variances in the first PC, except for the number of ears per plant and clusters 2 and 3 were considered as those variables explained more variances in the second PC except ear aspect in Fig. 2a. High qualities of variables contribution were observed by grain yield, plant height, ear

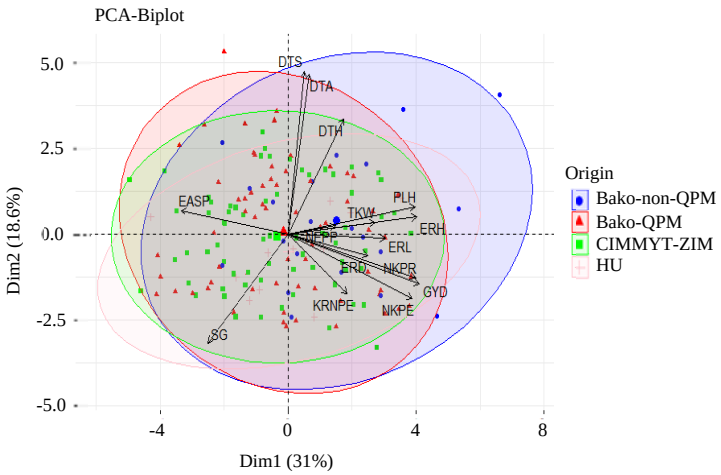

Fig. 3: Ellipse PCA bi-plot for the contribution of inbred lines and variables based on inbred lines' collection source in explaining variation in the first two principal components for 166 QPM and non-QPM maize inbred lines evaluated under a low N environment

height and the number of kernels per year in the first PC and days to silking, anthesis and harvesting and stay green in the second PC in Fig. 2b. Figure 2 indicated that those variables grouped under cluster-1 in PC1 were positively correlated and grouped as well as in cluster-2 but variables grouped under cluster-3 were negatively correlated in PC1.

Contributions and associations of variables in the first five principal components: The most significantly associated variables in the first five principal components are presented in Table 5, indicating that except for days to anthesis and silking all variables showed greater contributions in creating variations among the inbred lines. Most variables significantly contributed to variations among the inbred lines with a positive significant association with each other except ear aspect (-0.635**) and stay green (-0.476**) which was a negatively significant association with other variables in the first PC. Positive significant contribution and association were observed in the second PC by days anthesis (0.884**) and silking (0.898**), plant height (0.152**) and days to harvest (0.637**) and negative significant contributions and association by kernel row number per ear (-0.329**), number of kernels per row (-0.241**), number of kernels per ear (-0.356**), stay green (-0.603**) and grain yield (-0.277**). Positive significant contributions and associations were observed in the third PC by days to anthesis (0.224**) and silking(0.242**), number of ear per plant (0.294**), ear aspect (0.168**), kernels row number per ear (0.632**), number of kernels per row (0.279**) and number of kernels per ear (0.521**) but negative significant contributions and associations by plant height (-0.262**), ear height (-0.316**), thousand kernel weight (-0.496**) and ear length (-0.187**). Thousand kernels weight (0.392**), ear diameter (0.565**) and kernel row number per ear (0.380**) were observed as positive significant contributions and association, but negative significant contributions and association among the number of ears per plant (-0.578**), ear length (-0.328**) and the number of kernels per row (-0.193**) in the fourth PC and also, high and positive significant contribution and association among the number of ear per plant (0.624**), thousand kernel weight (0.244**), ear aspect (0.249**) and ear diameter (0.388**) and negative significant contribution and associations among the number of kernels per row (-0.165**), stay green and grain yield (-0.203**) in Table 5. Ellipse PCA bi-plot constructed for the contribution of inbred lines and variables based on their collection sources in explaining variation in the first two principal components for 166 QPM and non-QPM maize inbred lines evaluated under a low N environment are delineated in Fig. 3. Based on the ellipse PCA bi-plot constructed, more variations were observed among maize inbred lines due to inbred lines collected from Bako-non-QPM, due to more variability in days to anthesis and silking. Except for days to anthesis and silking, all variables were contributed from small to medium in the creation of variability among all maize inbred lines collected from all sources. The distribution of inbred lines was also constructed on the first three coordinates through a three-dimensional scatter plot indicating variability among inbred lines in Fig. 4.

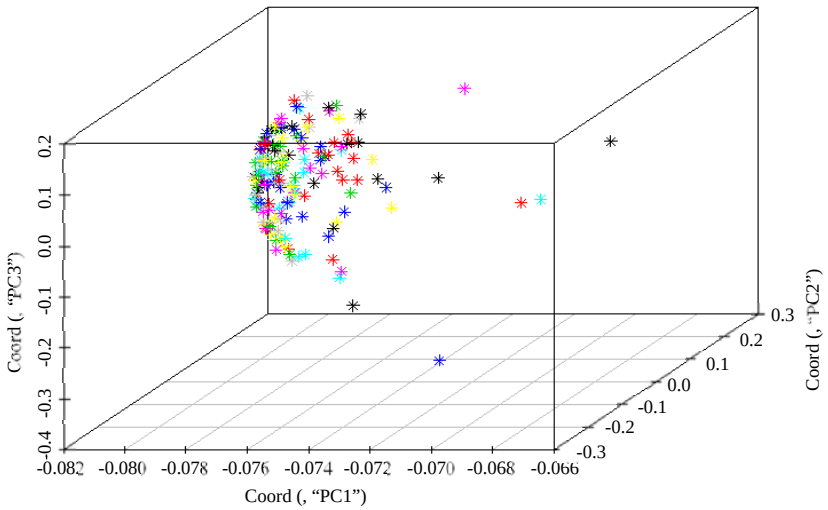

Fig. 4: Distribution of inbred lines according to (three-dimensional scatter plots) to the first three coordinates evaluated under a low N soil environment

Table 5: Most significantly associated variables in the first five principal components

	Correlation				
Variables	PC-1	PC-2	PC-3	PC-4	PC-5
Grain yield	0.779**	-0.277**	-	-	-0.313**
Days to anthesis	-	0.884**	0.224**	-	-
Days to silking	-	0.898**	0.242**	-	-
Plant height	0.754**	0.152**	-0.262**	-	-
Ear height	0.763**	-	-0.316**	-	-
Number of ears per plant	0.280**	-	0.294**	-0.578**	0.624**
Days to harvest	0.331**	0.637**	-	-	-
Thousand kernels weight	0.515**	-	-0.496**	0.392**	0.244**
Ear aspect	-0.635**	-	0.168**	-	0.249**
Ear length	0.576**	-	-0.187**	-0.328**	-
Ear diameter	0.472**	-	-	0.565**	0.388**
Kernel row number per ear	0.350**	-0.329**	0.632**	0.380**	-
Number of kernels per row	0.757**	-0.241**	0.279**	-0.193**	-0.165**
Number of kernels per ear	0.736**	-0.356**	0.521**	-	-
Stay green characteristics	-0.476**	-0.603**	-	-	-0.203**

Variables were highly significantly correlated to each other in the specific principal component

Grouping of inbred lines into their respective classes: After pre-cluster visualization of inbred lines based on their Euclidean distance, the inbred lines were grouped into four clusters in which the number of inbred lines per cluster varied from 50 in cluster-3 to 32 in cluster-4 in Table 6 and also cluster dendrogram was constructed to group inbred lines into their respective clusters under hierarchical clustering based on principal component (HCPC) method using ward-clustering analysis in Fig. 5. Hierarchical clustering on the factor-map is also described for the inbred lines based on the first two PCs using a three-dimensional plot in Fig. 6. Thirty-seven inbred lines were grouped under cluster-1 which accounted for about 22.89% of the total maize inbred lines included. Cluster-1 was characterized by low in days to silking (85.53 days) and anthesis (82.37 days) with the short plant (83.47cm) and ear height (27.43). A low number of kernels per ear (24.17 number) and low grain yield (1.34 t ha^{-1}) were considered as the characteristics of cluster-2, which included 46 inbred lines in the cluster and accounted for about 27.71% of the total inbred lines included for this study. Cluster-3 which included 50 inbred lines and accounted for about 30.12% of the total inbred lines, was characterized by the high number of kernels per ear (399.68 number) and grain yield (2.25 t ha^{-1}) and cluster-4 is characterized by high days to silking (93.53 days) and anthesis (90.13 days) with tall plant height (120.47 cm), long ear height (15.06 cm) and high grain yield (3.10 t ha^{-1}). Thirty-two inbred lines were grouped under cluster-4 which accounted for about 19.28% of the total inbred lines included in the study. All individuals in all clusters had high coordinates on axes one and two.

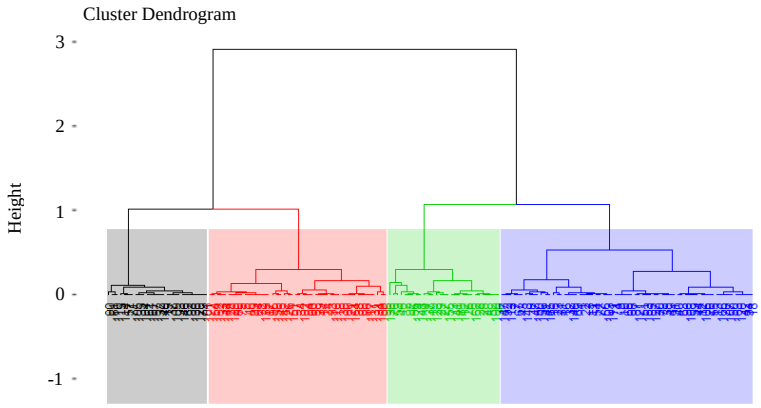

Fig. 5: Cluster dendrogram for inbred lines based on the agronomic characters under low soil nitrogen

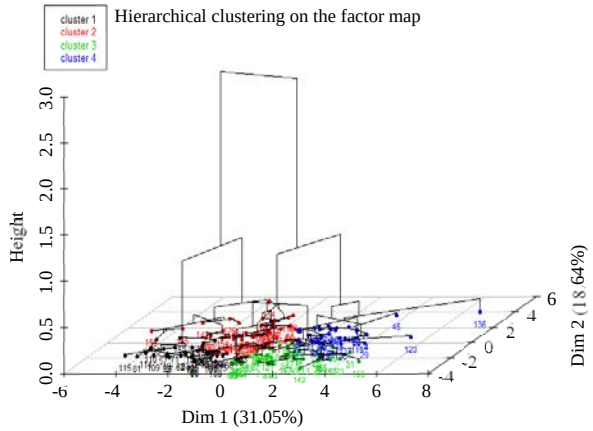

Fig. 6: Three-dimensional plot combining the hierarchical clustering and the factor map on the first two principal components

Table 6: Variables and principal components describe the most in each cluster

	Cluster-1		Cluster-2		Cluster-3		Cluster-4	
Variables	Mean in category	Overall mean	Mean in category	Overall mean	Mean in category	Overall mean	Mean in category	Overall mean
Days to anthesis	82.37	86.15	90.17	86.15	82.78	86.15	90.13	86.15
Days to silking	85.53	89.36	93.70	89.36	85.62	89.36	93.53	89.36
Plant height	83.47	101.20	94.12	101.20	108.86	101.20	120.47	101.20
Ear height	27.43	35.77	31.06	35.77	39.82	35.76	46.09	35.77
Number of ears per plant	-	-	-	-	-	-	1.71	1.49
Days to harvest	171.03	176.58	179.43	176.58	-	-	180.81	176.58
Thousand kernels weight	208.92	255.62	-	-	282.87	255.62	280.73	255.62
Ear aspect	2.58	2.23	2.45	2.22	1.93	2.22	1.92	2.22
Ear length	11.42	13.16	12.28	13.16	14.07	13.16	15.06	13.16
Ear diameter	3.68	3.95	3.72	3.95	4.19	3.95	4.21	3.95
Kernel row number per ear	12.18	12.70	12.11	12.70	13.54	12.70	-	-
Number of kernels per row	24.83	27.58	24.17	27.58	30.57	27.58	31.06	27.58
Number of kernels per ear	302.54	352.21	292.17	352.21	414.83	352.21	399.68	352.21
Stay green characteristics	6.79	4.93	-	-	-	-	2.59	4.93
Grain yield	1.64	2.25	1.34	2.25	3.02	2.25	3.10	2.25
PC1	-2.28	-1.38	-1.25	-1.39	1.35	-1.39	2.40	-1.39
PC2	-1.19	-3.28	1.61	-3.28	-1.38	-3.28	1.25	-3.28
Number of inbred lines in each cluster	38		46		50		32	
Percentage of inbred lines out of population	22.89		27.71		30.12		19.28	

DISCUSSION

Significant differences were observed among inbred lines for all agronomic characters except anthesis-silking interval under a low N environment indicating the high level of genetic variability among inbred lines. This variability among inbred lines is the most important in maize hybrid development programs through selection under a target environment. Different researchers have also reported genetic availability among maize inbred lines using morphological and molecular markers under different stress and non-stress environments[22,23]. Analysis of variances for inbred lines evaluated under a low N environment showed significant and highly significant differences for all traits which indicate high genetic variability among inbred lines due to differences in the genetic makeup of the inbred lines in response to low soil nitrogen. Under a low N environment, the performance of grain yield and other agronomic traits were significantly different among inbred lines because the inbred lines differ in genetic constituents and they were varied in response to varied N-environments. this finding is in agreement with other study[24] that reported on the agronomic performance of hybrids of white maize inbred lines under low-Nitrogen and managed environments. Estimates of variance components, coefficients of variability, heritability and genetic advance parameters are used to identify genetic variability among genotypes and determine genetic and environmental effects on various characters. Under a low N environment, a greater contribution of environmental variances than genetic variances was observed for all traits indicating environmental factors were more effects on the inbred lines' performances under a stress environment. High heritability for plant height (96.15%), ear diameter (72.49%) and ear length (65.83%) were reported[25] in maize inbred lines under an optimum environment.

The genetic coefficient of variation ranged from 1.03% for days to harvesting to 27.31% for grain yield, while the phenotypic coefficient of variation ranges from 1.41% for days to harvesting to 63.7% for grain yield. This indicates that those traits that had higher phenotypic coefficient variation also showed a higher genotypic coefficient of variation. For all the agronomic characters the GCV values were lower than PCV values, showing that the characters were more influenced by environments than their genetic makeup. According to another report[26], the traits had low (less than 10% phenotypic and genotypic coefficient of variations), moderate (10-20% phenotypic and genotypic coefficient of variations) and high (more than 20% phenotypic and genotypic coefficient of variations). Grain yield had higher GCV and PCV values of 27.31 and 51.96 %, respectively, whereas days to harvesting had low GCV and PCV values of 1.03 and 1.41, respectively. The coefficient of variation, particularly the genetic coefficient of variation determines the reliability of the lines for use in a breeding program in which high proportions of GCV to PCV value are more preferred in breeding work. Except stay green (13.44%) and grain yield (27.31%), all agronomic characters studied had a low genetic coefficient of variation. Other study[27-29] reported similar findings also provided the results of moderate PCV and GCV estimates for plant height and 100-grain weight. The presence of a significant degree of genetic diversity is indicated by GCV, but the amount of heritable variation can only be assessed using heritability estimates and genetic gain. Another study[30,31] also reported high GCV (25.9%) and PCV (26.91) for maize grain yield. The traits had low (less than 10% phenotypic and genotypic coefficient of variations), moderate (10-20% phenotypic and genotypic coefficient of variations) and high (more than 20% phenotypic and genotypic coefficient of variations).

Another study[32] stated that a trait controlled by additive gene action had high heritability and genetic advance, while those traits controlled by non-additive gene action had high heritability but low genetic advance. High estimates of heritability for ear diameter suggested that variations were passed down to offspring, implying that high-yielding varieties may be developed by selecting desirable inbred lines by crossing through indirect selection rather than direct selection. High heritability provides more options for selecting plant material with desired features under an optimum environment, which facilitates direct selection. Because of low heritability for grain yield and high for other secondary traits under stressful environments, indirect selection is considered in plant breeding programs. The findings on heritability estimates for all agronomic traits of maize inbred lines under a low soil nitrogen environment contradicted[33] because of the difference in growing environments. High heritability and high genetic progress are not always linked. As a result, high heritability does not imply a large genetic gain[34]. A heritability estimate in conjunction with genetic advances is proposed to anticipate the effectiveness of picking up superior genotypes. As indicated in the results, low estimates of heritability as well as GAM were found for the anthesis-silking interval, ear height, number of ears per plant, ear aspect, kernel row number per ear, number of kernels per row and number of kernels per ear. This is because the characters were more affected by the effects of the environment than the genetic makeup of the inbred lines, which indices that indirect selection is more important than direct selection. After all, the characters were governed by non-additive gene action. Wondimu *et al.*[35]

suggested that those characters had low heritability as well as low GAM, better management rather than selection to increase characters' performance.

In genetic diversity analysis, principal component analysis is used to substantiate the existence of morphological diversity among concerned genotypes by reducing the number of variables into a few correlated components that can explain much of the variability. For this study, the principal component analysis computed for maize inbred lines extracted the first five principal components (PCs), which accounted for 73.12% under a low N environment of the total cumulative variations existing among the inbred lines indicating different contributions of traits toward the genetic diversity encountered among the inbred lines. Days to anthesis, days to silking, grain yield and ear aspect were showed a greater contribution to the overall genetic variability among inbred lines under a low N environment[36] than 78.0% of the total variation in 32 white tropical mid-altitude QPM inbred lines using 17 morphological traits evaluated under optimum N environment explained by the first six PCs. Studies[37] also reported 75.1% of the total variation accounted for by the first five principal components in highland maize accessions of Ethiopia under a managed environment. In maize inbred lines adapted to highland agro-ecologies of Ethiopia 83% of the entire variation accounted for by the first nine PCs among 23 inbred lines[38].

In-Plant breeding program, clustering of genetic materials into homogenous groups is the most important aspect of selecting parental materials during hybrid development. For this study, maize inbred lines using agronomic data based on their estimates of genetic distance grouped into four homogenous clusters under a low N environment, indicating broad genetic diversity among inbred lines. The 23 maize inbred lines adapted to highland agro-ecologies of Ethiopia were grouped into five distinct clusters using morphological data[38]. The grouped highland maize accessions of Ethiopia into four homogenous clusters.

CONCLUSION

Under a low N environment, a greater contribution of environmental variances than genetic variances was observed for all traits. For all the agronomic characters tested the GCV values were lower than PCV values, showing that the characters were more influenced by environments than their genetic makeup. Grain yield (28%), anthesis-silking interval (4%), ear height (23%), number of ears per plant (12%), ear aspect (5%), kernel row number per ear (20%), number of kernels per row (18%), number of kerns per ear (21%) and stay green characteristics (24%) were showed low heritability estimate, whereas ear diameter (65%) was showed high heritability estimate. All agronomic characters showed low GAM except grain yield (31.11%) which showed greater GAM and stay green characteristics (12.78%) which showed moderate GAM. Principal component analysis computed for maize inbred lines evaluated under low N environments was extracted from the first five PCs by using normalized variables which accounted for 73.12% of the cumulative variations existing among the considered maize inbred lines. The number of kernels per ear, days to silking, number of ears per plant, days to anthesis, kernel row number per ear and number of kernels per row were the variables more contributed to the creation of variation among inbred lines in the first five PCs. The inbred lines were grouped into four clusters.

SIGNIFICANCE STATEMENT

This study discovered the genetic gain due to the level of soil nitrogen among maize inbred lines that can be beneficial for maize breeding programs. This study will help maize researchers in the development of high yielding maize hybrids under low soil nitrogen which will uncover the critical areas of African nutritional and food insecurity.

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REFERENCES

1. Badu-Apraku, B., B. Annor, M. Oyekunle, R.O. Akinwale and M.A.B. Fakorede *et al*., 2015. Grouping of early maturing quality protein maize inbreds based on SNP markers and combining ability under multiple environments. Field Crops Res., 183: 169-183.
2. Ranum, P., J.P. Pena-Rosas and M.N. Garcia-Casal, 2014. Global maize production, utilization and consumption. Ann. N.Y. Acad. Sci., 1312: 105-112.
3. Babu, R. and B.M. Prasanna, 2013. Molecular Breeding for Quality Protein Maize (QPM). In: Genomics of Plant Genetic Resources, Tuberosa, R., A. Graner and E. Frison (Eds.), Springer, Netherlands, ISBN: 978-94-007-7574-9, pp: 489-505.

4. Krivanek, A.F., H. De Groote, N.S. Gunaratna, A.O. Diallo and D. Friesen, 2007. Breeding and disseminating Quality Protein Maize (QPM) for Africa. Afr. J. Biotechnol., 6: 312-324.
5. Werle, A.J.K., F.R.A. Ferreira, R.J.B. Pinto, C.A. Mangolin, C.A. Scapim and L.S.A. Gonçalves, 2014. Diallel analysis of maize inbred lines for grain yield, oil and protein content. Crop Breed. Appl. Biotechnol., 14: 23-28.
6. Khan, N.U., M. Sheteiwy, N. Lihua, M.M.U. Khan and Z. Han, 2019. An update on the maize zein-gene family in the post-genomics era. Food Prod. Process. Nutr., Vol. 1. 10.1186/s43014-019-0012-5.
7. Boyer, C.D. and L.C. Hannah, 2000. Kernel Mutants of Corn. In: Specialty Corns, Hallauer, A.R. (Ed.), CRC Press, New York, ISBN: 9780849323775, Pages: 496.
8. Prasanna, B.M., S.K. Vasal, B. Kassahun and N.N. Singh, 2001. Quality protein maize. Curr. Sci. Assoc., 81: 1308-1319.
9. Wu, Y. and J. Messing, 2014. Proteome balancing of the maize seed for higher nutritional value. Front. Plant Sci., Vol. 5. 10.3389/fpls.2014.00240.
10. Wang, G., W. Qi, Q. Wu, D. Yao and J. Zhang *et al*., 2014. Identification and characterization of maize *floury4* as a novel semidominant opaque mutant that disrupts protein body assembly. Plant Physiol., 165: 582-594.
11. Vivek, B.S., A.F. Krivanek, N. Palacios-Rojas, S. Twumasi-Afriyie and A.O. Diallo, 2008. Breeding Quality Protein Maize: Protocols for Developing QPM Cultivars. CIMMYT, Mexico, ISBN: 978-970-648-160-3, Pages: 50.
12. Singh, R. and L. Ram, 2014. DNA aided introgression of opaque-2 allele for development of quality protein maize. Int. J. Sci. Res., 3: 3006-3011.
13. Xu, Y., R. Babu, D.J. Skinner, B.S. Vivek and H. Jonathan, 2009. Maize Mutant Opaque2 and the Improvement of Protein Quality Through Conventional and Molecular Approaches. In: Induced Plant Mutations in the Genomics Era, Shu, Q.Y. (Ed.), Joint FAO/IAEA Programme, Austria, ISBN: 978-92-5-106324-8, pp: 172-176.
14. Andrews, M., J.A. Raven and P.J. Lea, 2013. Do plants need nitrate? The mechanisms by which nitrogen form affects plants. Ann. Appl. Biol., 163: 174-199.
15. Erisman, J.W., J.N. Galloway, S. Seitzinger, A. Bleeker and N.B. Dise *et al*., 2013. Consequences of human modification of the global nitrogen cycle. Philos. Trans. Royal Soc. B: Biol. Sci., Vol. 368. 10.1098/rstb.2013.0116.
16. Cameron, K.C., H.J. Di and J.L. Moir, 2013. Nitrogen losses from the soil/plant system: A review. Ann. Appl. Biol., 162: 145-173.
17. Gelli, M., Y. Duo, A.R. Konda, C. Zhang, D. Holding and I. Dweikat, 2014. Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. BMC Genomics, Vol. 15. 10.1186/1471-2164-15-179.
18. Banziger, M., G.O. Edmeades, D.L. Beck and M.R. Bellon, 2000. Breeding for Drought and Nitrogen Stress Tolerance in Maize: From Theory to Practice. CIMMYT, Mexico, ISBN: 970-648-46-3, Pages: 68.
19. Simons, M., R. Saha, L. Guillard, G. Clement and P. Armengaud *et al*., 2014. Nitrogen-use efficiency in maize (*Zea mays* L.): from 'omics' studies to metabolic modelling. J. Exp. Bot., 65: 5657-5671.
20. Bioversity International, 2007. Developing Crop Descriptor Lists, Guidelines for developers-Bioversity Technical Bulletin No. 13. Bioversity International, Rome, Italy, ISBN: 9290437294, Pages: 71.
21. Aditya, J.P., P. Bhartiya and A. Bhartiya, 2011. Genetic variability, heritability and character association for yield and component characters in soybean (*G. max* (L.) Merrill). J. Cent. Eur. Agric., 12: 27-34.
22. Adu, G.B., B. Badu-Apraku, R. Akromah, A.L. Garcia-Oliveira, F.J. Awuku and M. Gedil, 2019. Genetic diversity and population structure of early-maturing tropical maize inbred lines using SNP markers. PLoS ONE, Vol. 14. 10.1371/journal.pone.0214810.
23. Azad, M.A.K., B.K. Biswas, N. Alam and S.S. Alam, 2012. Genetic diversity in maize (*Zea mays* L.) inbred lines. Agriculturists, 10: 64-70.
24. Ogunniyan, D.J., C.O. Alake and F.A. Anjorin and S.A. Makinde, 2018. Agronomic performance of hybrids of white low-nitrogen maize (*Zea mays* L.) inbred lines under managed nitrogen environments. Trop. Agric., 95: 18-30.
25. Hassan A.A., M. Abdikadir, M. Hasan, M.A.K. Azad and M. Hasanuzzaman, 2018. Genetic variability and diversity studies in maize *(Zea mays* L.) inbred lines. J. Agric. Vet. Sci., 11: 69-76.
26. Devi, K.R., B.S. Chandra, Y. Hari and V. Venkanna, 2018. Heterosis and inbreeding studies for agronomic and quality traits in rice *(Oryza sativa* L.). Agric. Sci. Dig., 38: 313-318.
27. Kandel, B.P., A. Poubel and S. Sharma *et al*., 2017. Variability study in yield attributing traits of early maize genotypes in the Western hill of Nepal. Nepal. J. Agri. Sci., 15: 13-18.
28. Sharma, B.K., S. Sharma, B.P. Kandel and J. Shrestha, 2018. Varietal evaluation of promising maize genotypes. Azarian J. Agric., 5: 120-124.
29. Nzuve, F., S. Githiri, D.M. Mukunya and J. Gethi, 2014. Genetic variability and correlation studies of grain yield and related agronomic traits in maize. J. Agric. Sci., 6: 166-176.
30. Ojwang, J.D., R. Nyankanga, N.V.P.R.G.G. Rao and J. Imungi, 2021. Evaluation of vegetable pigeonpea [*Cajanus cajan* (L.) Millsp] genotypes for yield stability. CABI Agric. Biosci., Vol. 2. 10.1186/s43170-021-00061-8.
31. Magar, B.T., S. Acharya, B. Gyawali, K. Timilsena, J. Upadhayaya and J. Shrestha, 2021. Genetic variability and trait association in maize (*Zea mays* L.) varieties for growth and yield traits. Heliyon, Vol. 7. 10.1016/j.heliyon.2021.e07939.

32. Krishna, D.M., D.M. Reddy, K.H.P. Reddy and P. Sudhakar, 2009. Character association and interrelationship of yield and quality attributes in rice (*Oryza sativa* L.). Andhra Agric. J., 56: 298-301.
33. Shengu, M.K., 2017. Path coefficient analysis of early maturing maize (*Zea mays*) inbred lines in central Rift Valley of Ethiopia. Plant, 5: 47-50.
34. Belay, N., 2018. Genetic variability, heritability, correlation and path coefficient analysis for grain yield and yield component in maize (*Zea mays* L.) hybrids. Adv. Crop Sci. Tech., Vol. 6. 10.4172/2329-8863.1000399.
35. Wondimu, T., A. Sentayehu, A. Amsalu and G. Weyessa, 2014. Variability and association of quantitative traits in anchote (*Coccinia abyssinica* (Lam.) Cogn.) in Ethiopia. Int. J. Plant Breed. Genet., 8: 1-12.
36. Wegary, D., B.S. Vivek and M.T. Labuschagne, 2014. Combining ability of certain agronomic traits in quality protein maize under stress and nonstress environments in Eastern and Southern Africa. Crop Sci., 54: 1004-1014.
37. Beyene, T., A.M. Botha and A.A. Myburg, 2005. Phenotypic diversity for morphological and agronomic traits in traditional Ethiopian highland maize accessions. South Afr. J. Plant Soil, 22: 100-105.
38. Terefe, W., A. Teklewold and K. Tesfaye, 2019. Characterization of selected maize inbred lines adapted to highland agro-ecologies of Ethiopia using morphological and molecular genetic distances. Adv. Crop. Sci. Technol., Vol. 7. 10.4172/2329-8863.1000421.