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Morphological characterization of maize (*Zea mays* L.) genotypes for drought tolerance

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ABSTRACT

Drought stress poses a major challenge to global maize (*Zea mays* L.) production, significantly affecting food security. This study aimed to identify drought-tolerant maize genotypes by evaluating their morphological characteristics under controlled and field conditions. Morphological traits, such as shoot and root length in the seedling stage and plant height, canopy temperature, ear length, and kernel weight at vegetative and reproductive stages, were analyzed under drought stress conditions induced by PEG and reduced field capacity. Significant genetic variability was observed among the genotypes for most traits, with phenotypic coefficient of variation (PCV) consistently higher than genotypic coefficient of variation (GCV). Heritability estimates ranged from 37% to 99.82%, with traits such as plant height, kernel weight per ear, ear weight, hundred-seed weight, and yield per pot demonstrating both high heritability and genetic advance. Yield showed positive correlations with plant height, ear length, ear diameter, kernel number per row, and kernel weight per ear, while negatively correlating with days to ear moisture loss. Principal Component Analysis (PCA) revealed that the first five components explained 83.46% of the total variation, with yield-related traits contributing most to the variation. Cluster analysis revealed eight distinct groups at the seedling stage and six at the vegetative and reproductive stages, with genotypes in Clusters 5 and 6 exhibiting superior drought tolerance, particularly in traits like root number, kernel number per ear, and kernel weight per ear. This study identified BHM-7, BHM-14, and BHM-15 as genotypes with superior drought tolerance at the reproductive stage, while Black, Violet, and White Vutta showed resilience at the seedling stage, exhibiting greater plant vigor under drought stress. Recognizing these genotypes as a significant step toward breeding drought-resistant maize varieties, contributing to food security and sustainable agriculture.

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INTRODUCTION

Maize, also known as corn, is a versatile and staple crop that plays a crucial role in global agriculture and cuisine. Originating in Central America over 9,000 years ago, maize has transcended its origins to become a fundamental food source and raw material in many parts of the world. As of 2022, global maize production reached approximately 1.17 billion tons, cultivated over around 202 million hectares (Ahmad *et al.*, 2024). From 2009 to 2022, maize production in Bangladesh increased from 1.23 million tons to 4.31 million tons, underscoring its growing importance as a staple crop (BBS, 2022). However, the crop is often subjected to water scarcity, which can severely impede its growth and productivity. Field experiment data published between 1980 and 2015 showed that drought (approximately 40% water loss) reduced maize yields by 39.3% (Daryanto *et al.*, 2016). So, the study of morphological characterization of maize genotypes for drought tolerance is of paramount importance in the realm of genetics and plant breeding, as it seeks to elucidate the intricate relationships between genotype, phenotype, and environmental factors, particularly in the context of abiotic stressors such as drought. Consequently, the identification and characterization of maize genotypes that exhibit superior drought tolerance is a pressing concern for researchers and breeders alike, as it holds the potential to mitigate the adverse effects of water scarcity on crop yield and ensure food security for burgeoning populations. In this regard, the morphological characterization of maize genotypes serves as a crucial first step in the process of developing drought-tolerant cultivars, as it enables the identification of key morphological traits that are associated with enhanced drought tolerance and can thus be targeted for improvement through breeding efforts (Gazal *et al.*, 2018).

Most of the global maize cultivation occurs in rainfed environments, making it particularly susceptible to drought compared to other cereal crops (Hall *et al.*, 1981). Drought triggers cellular adaptive responses, including stomatal closure, stress-protective metabolite synthesis, and enhanced antioxidant defenses (Gupta *et al.*, 2020; Choudhary *et al.*, 2021). Drought during the reproductive phase severely impacts crop yield, with traits like Anthesis-Silking Interval (ASI) and kernel number per row (KNR) being important for breeding drought-resistant varieties (Monneveux *et al.*, 2008; Jia *et al.*, 2020).

Approximately 67% of maize production in developing nations comes from low-income countries, where moisture stress is a major constraint. Drought during flowering can delay silk emergence and cause embryo abortion, leading to yield losses estimated at 24 million metric tons annually, or about 17% of normal production (Moss & Downey, 1971; Aslam *et al.*, 2013; Singh *et al.*, 2020).

Addressing the impact of drought on maize is crucial for sustaining productivity and enhancing food security. Research should focus on not only final yield but also underlying traits like ASI and KNR. Morphological traits, such as plant height and ear characteristics, are essential for drought resistance. Understanding the genetic basis of these traits can help identify candidate genes and optimize breeding strategies (Al-Naggar *et al.*, 2020; Maqbool *et al.*, 2021).

Despite advances in maize genetics and breeding, a significant gap remains in the morphological characterization of genotypes for drought tolerance. Most research has focused on identifying quantitative trait loci (QTL) and candidate genes, neglecting the comprehensive analysis of morphological traits and their role in drought resistance. Additionally, complex interactions among genotype, phenotype, and environment, including epistatic and pleiotropic effects, are largely unexplored. Addressing these gaps will enhance our understanding of the genetic and morphological basis of drought tolerance, leading to more effective breeding strategies (Kondwakwenda *et al.*, 2021). This study aims to evaluate the performance of fifteen maize genotypes under control and PEG-induced drought stress, analyze genotypic variability and relationships among yield and its attributing traits under stress, and identify the best-performing genotypes based on performance during the seedling and reproductive stages under stress conditions.

MATERIALS AND METHODS

Plant Materials and Experimentation

The experiment was conducted in two different conditions. The first one was conducted at the seedling stage in the growth chamber using PEG as a stress inducer. The second one was done in the field using different field capacity at the vegetative and reproductive stages. The investigation involved 15 maize genotypes, including those released by the Bangladesh Agricultural Research Institute (BARI), local landraces and half-sib populations. List of maize genotypes used to successfully conclude the trial (Table 1).

Table 1: List of maize genotypes and sources used in the experiment

S. No.	Pedigree	Description
1	BHM-7	Hybrid
2	BHM-15	Hybrid
3	Mohor	Inbred parent
4	BHM-12	Hybrid
5	Black	Open-pollinated population from hill tract
6	BM-1	Inbred parent
7	Bornali	Inbred parent
8	BHM-14	Hybrid
9	MA-13	Inbred parent
10	Purple	Open-pollinated population from hill tract
11	BHM-13	Hybrid
12	BHM5	Hybrid 7
13	BARI Mishti	Inbred parent
14	White Vutta	Open-pollinated population from hill tract
15	MA-12	Inbred parent

Seedlings Treatment

The seedling experiment was executed at the Experimental Growth Chamber, a control condition and two treatments were used. 10% PEG was added to Treatment 1 starting on Day 1 and Treatment 2 starting on Day 7. In CRD (Completely Randomized Design), 15 genotypes and 3 interventions were arranged in 15 Petri dishes in 3 replications. The data was collected after 10th and 17th days after setting up the experiment. The data of total length, shoot length, root number, maximum root length, minimum root length and moisture loss (%) were collected.

Plant Treatment in the Field

This experimental trial was executed at the Experimental Field Laboratory. The trial was done in the same-sized pot containing 20 kg of soil. Those pots were organized in the greenhouse with three replications. Drought treatment was applied to the corn plants at the growth stages for 40 days. There was no drought stress applied to the corn plants before and after the treatment period, which indicated that the plants were re-watered after the 40 days of drought treatment. The data collection was carried out during planting until harvesting at 128 days after sowing (DAS).

Statistical Analysis

Analysis of variances of the data was carried out according to standard texts and subjects (Snedecor & Cochram, 1967; Clark, 1973). SAS software version 9.3 (SAS, 2010) was used to carry out the ANOVA. Correlation coefficient was estimated by using Minitab. Genotypic and phenotypic variances were estimated according to the formula given by Johnson *et al.* (1955). Heritability in the broad sense (h^2_b) was calculated using the formulas proposed by Johnson *et al.* (1955) and Hanson *et al.* (1956). In order to calculate the coefficient of variation for both the genotype and the phenotype, Burton (1952) and Singh and Chowdhury (1985) methods were utilized. The genetic advance was estimated using the formulas provided by Johnson *et al.* (1955) and Allard (1960). Genetic advance in percentage of mean was estimated using the formula devised by Comstock and Robinson (1952). The phenotypic correlations were calculated using the formula proposed by Miller *et al.* (1958). MINITAB 19 was used to perform Principal Component Analysis (PCA) using Holland's (2008) procedure. The standardized mean values were used to construct a two-way hierarchical clustering heatmap using Minitab and RStudio 4.3.1.

RESULTS

The results of analysis of variance showed highly significant (0.1% level) variation among the genotypes for most of the studied traits (namely germination rate, survival rate, total length, shoot length, maximum root length, minimum root length considering experiment 1 whereas plant height, ear length, ear girth, kernel length, 100 seed Weight, yield per plot, yield per ha for experiment 2 (Tables 2, 3 & 4).

Estimation of Genetic Parameters for Morphological Characters

For plant height, phenotypic variance (108.40) slightly surpassed genotypic variance (103.10), suggesting environmental effects. The coefficients of variation were 14.33% (phenotypic) and 13.97% (genotypic). High heritability (95.11%) and significant genetic advance (20.40, 21.81% of mean) were noted for plant height in Table 5, consistent with previous studies (Shahzad *et al.*, 2023; Ilyas *et al.*, 2019). Canopy temperature

Table 2: Analysis of variance (mean square) for different characters of 15 maize genotypes at seedling stage

SV	DF	GR	SR	TL	SL	RN	MXRL	MNRL	RFW	SFW	RDW	SDW
Gen.	14	0.11357***	0.20448***	21.650***	25.26***	10.146***	67.04***	3.2431***	0.019695***	0.022041***	0.000576***	0.000377***
Tre.	2	3.17363***	1.81067***	541.648***	1282.20***	145.489***	2072.29***	14.5647***	0.287053***	0.573642***	0.001979***	0.005066***
Rep.	2	0.00719	0.00867	0.012	0.01	0.956	0.02	0.0574	0.000001	0.000000	0.000000	0.000001
Gen.* Tre.	28	0.09792***	0.08114***	28.596***	14.10***	7.925***	51.92***	3.0824***	0.013097***	0.008651***	0.000370***	0.000151***
Err.	88	0.00317	0.00314	0.004	0.01	0.342	0.01	0.0241	0.000000	0.000000	0.000000	0.000000

Here, SV=Sources of variance, Gen.=Genotypes, Rep.=Replication, Err.=Error, GR=Germination Rate (%), SR=Survival Rate (%), TL=Total Length (cm), SL=Shoot Length (cm), RN=Root Number, MXRL=Maximum Root Length (cm), MNRL=Minimum Root Length (cm), RFW=Root Fresh Weight (g), SFW=Shoot Fresh Weight (g), RDW=Root Dry Weight (g), SDW=Shoot Dry Weight (g), PH=Plant Height (inch), CT=Canopy Temperature (°C), IL=Internode Length (cm), TLL=Tassel Leaf Length (cm), TLP=Total Leaf Plant^{-1}, CN=Cob No., EL=Ear Length (cm), ED=Ear Diameter (cm), KRE=Kernel Rows Ear^{1}, KNR=Kernel Numbers Row^{-1}, KNE=Kernel Number Ear^{1}, EML=Ear Moisture Loss (%), KWE=Kernel Weight Ear^{1} (g), EW=Ear Weight (g), HSW=100 seed Weight (g), TDW=Total Dry Weight (g), CN=Cob Number, YP=Yield Pot^{-1}. *, ** & *** indicates significant at 5%, 1% and 0.1% level of probability respectively

Table 3: Analysis of variance (mean square) for different characters of 15 maize genotypes at vegetative stage

SV	DF	PH	CT	IL	NBT	TLL	TLW	TLC	CLC	TLP	CN
Gen.	14	314.6***	0.6033***	2.3327***	37.02***	171.12***	2.4893***	128.52***	97.18***	3.2349***	0.52063***
Tre.	1	22816.5***	14.0028***	98.5960***	1416.10***	6690.84***	75.6250***	1327.87***	1137.07***	80.2778***	0.17778
Rep.	2	4.0	0.2858**	0.0001	0.14	0.56	0.0333	0.57	0.71	0.1444	0.01111
Gen.* Tre.	14	59.6***	0.5273***	1.2022***	25.39***	232.66***	3.3155***	52.98***	74.10***	1.3968***	0.36825**
Err.	58	5.3	0.0446	0.0374	3.71	4.57	0.3839	2.54	1.09	0.3169	0.11456

Here, SV=Sources of variance, Gen.=Genotypes, Rep.=Replication, Err.=Error, GR=Germination Rate (%), SR=Survival Rate (%), TL=Total Length (cm), SL=Shoot Length (cm), RN=Root Number, MXRL=Maximum Root Length (cm), MNRL=Minimum Root Length (cm), RFW=Root Fresh Weight (g), SFW=Shoot Fresh Weight (g), RDW=Root Dry Weight (g), SDW=Shoot Dry Weight (g), PH=Plant Height (inch), CT=Canopy Temperature (°C), IL=Internode Length (cm), TLL=Tassel Leaf Length (cm), TLP=Total Leaf Plant^{-1}, CN=Cob No., EL=Ear Length (cm), ED=Ear Diameter (cm), KRE=Kernel Rows Ear^{1}, KNR=Kernel Numbers Row^{-1}, KNE=Kernel Number Ear^{1}, EML=Ear Moisture Loss (%), KWE=Kernel Weight Ear^{1} (g), EW=Ear Weight (g), HSW=100 seed Weight (g), TDW=Total Dry Weight (g), CN=Cob Number, YP=Yield Pot^{-1}. *, ** & *** indicates significant at 5%, 1% and 0.1% level of probability respectively

Table 4: Analysis of variance (mean square) for different characters of 15 maize genotypes at reproductive stage

SV	DF	EL	ED	KRE	KNR	KNE	EML	KWE	EW	HSW	TDW	YP
Gen.	14	27.594***	0.6206**	10.4873***	75.80***	8425**	444.871***	488.2***	2110***	86.56***	1824***	2238***
Tre.	1	261.803***	6.7788***	20.5444**	1013.38***	496696***	424.079***	62832.0***	246281***	165.38**	289567***	124546***
Rep.	2	0.711	0.1041	0.8444	48.13	4043	9.346	0.8	1	13.51	34	713
Gen.* Tre.	14	27.583***	0.4247*	14.7825***	92.83***	11137**	469.836***	315.2***	801***	125.38***	753***	1910***
Err.	58	0.332	0.2247	2.9709	16.43	3640	6.618	0.3	2	24.13	12	485

Here, SV=Sources of variance, Gen.=Genotypes, Rep.=Replication, Err.=Error, GR=Germination Rate (%), SR=Survival Rate (%), TL=Total Length (cm), SL=Shoot Length (cm), RN=Root Number, MXRL=Maximum Root Length (cm), MNRL=Minimum Root Length (cm), RFW=Root Fresh Weight (g), SFW=Shoot Fresh Weight (g), RDW=Root Dry Weight (g), SDW=Shoot Dry Weight (g), PH=Plant Height (inch), CT=Canopy Temperature (°C), IL=Internode Length (cm), TLL=Tassel Leaf Length (cm), TLP=Total Leaf Plant^{-1}, CN=Cob No., EL=Ear Length (cm), ED=Ear Diameter (cm), KRE=Kernel Rows Ear^{-1}, KNR=Kernel Numbers Row^{-1}, KNE=Kernel Number Ear^{-1}, EML=Ear Moisture Loss (%), KWE=Kernel Weight Ear^{-1} (g), EW=Ear Weight (g), HSW=100 seed Weight (g), TDW=Total Dry Weight (g), CN=Cob Number, YP=Yield Pot^{-1}. *, ** & *** indicates significant at 5%, 1% and 0.1% level of probability respectively

Table 5: Genetic Parameter of different characters for 15 maize genotypes

Traits	GV	PV	Heritability (%)	GCV (%)	PCV (%)	GA	GA (%)
PH (cm)	103.10	108.40	95.11	13.97	14.33	20.40	28.07
CT (℃)	0.19	0.23	80.68	1.24	1.38	0.80	2.29
IL (cm)	0.77	0.80	95.34	19.96	20.44	1.76	40.15
TLL	55.52	60.09	92.39	19.99	20.80	14.75	39.59
TLC	41.99	44.53	94.30	13.19	13.58	12.96	26.39
TLP	0.97	1.29	75.43	7.26	8.36	1.76	12.98
CN	0.14	0.25	54.16	28.06	38.13	0.56	42.54
EL (cm)	9.09	9.42	96.48	20.38	20.74	6.10	41.23
ED (cm)	0.13	0.36	37.00	10.20	16.77	0.46	12.78
KRE	2.51	5.48	45.75	12.71	18.79	2.21	17.71
KNR	19.79	36.22	54.64	20.92	28.30	6.77	31.85
KNE	1595.00	5235.00	30.47	18.42	33.37	45.41	20.95
KWE	162.63	162.93	99.82	27.55	27.58	26.25	56.70
EW (g)	702.67	704.67	99.72	29.19	29.24	54.53	60.05
HSW (g)	20.81	44.94	46.31	26.49	38.92	6.39	37.13
TDW (g)	604.00	616.00	98.05	21.09	21.30	50.13	43.02
YP (g)	584.33	1069.33	54.64	39.31	53.18	36.81	59.87

Here, PH=Plant Height (inch), CT=Canopy temperature (°C), IL=Internode Length (cm), TLL=Tassel Leaf Length (cm), TLC=Tassel Leaf Chlorophyll, TLP=Total Leaf Plant^{-1}, CN=Cob No, EL=Ear Length (cm), EG=Ear Diameter (cm), KRE=Kernel Rows Ear^{-1}, KNR=Kernel Numbers Row^{-1}, KNE=Kernel Number Ear^{-1}, EML=Ear Moisture Loss (%), KWC=Kernel Weight Ear^{-1} (g), EW=Ear Weight (g), HSW=100 seed Weight (g), TDW=Total Dry Weight (g), YP=Yield Plant^{-1} (g)

and internode length show high heritability (80.68% and 95.34%, respectively) but low genetic advance. Tassel leaf length exhibited environmental influence, with genotypic and phenotypic coefficients of variation at 20.80% and 19.99%. Root lodging demonstrated moderate genetic advance (14.75%) and high heritability (92.39%) with a genetic advance percentage of mean at 39.59%. Tassel leaf chlorophyll content shows a moderate genetic progress and high heritability (94.30%) but total leaf plant^{-1} exhibits a low level of genetic advancement and a very high heritability of 75.43%.

In comparison to genotypic variance (0.14), phenotypic variance for cob number (0.25) was larger. Table 5 revealed that it had very low genetic advance (0.56) and strong genetic advance in percentage of mean (42.54%), with moderate heritability values (54.16). Phenotypic variance for ear length and width was higher than genotypic variance, with ear length showing high heritability (96.48%) and ear diameter showing moderate heritability. Kernel rows per ear, kernel number per row, and kernel number per ear exhibited higher phenotypic variance and moderate heritability.

Kernel weight per ear and ear weight indicated strong genetic influence, characterized by high heritability and significant variation. In contrast, 100-seed weight had moderate heritability (46.31%) and low genetic advance, as noted by Ilyas *et al.* (2019).

For yield per pot, phenotypic variance (1069.33) was higher than genotypic variance (584.33). The phenotypic coefficient of variation (53.18%) exceeded the genotypic coefficient (39.31%). Yield per plot showed moderate heritability (54.64%) with high genetic advance (36.81) and a very high genetic advance percentage (59.87%), despite Saif-ul-Malook *et al.* (2014) reporting high heritability.

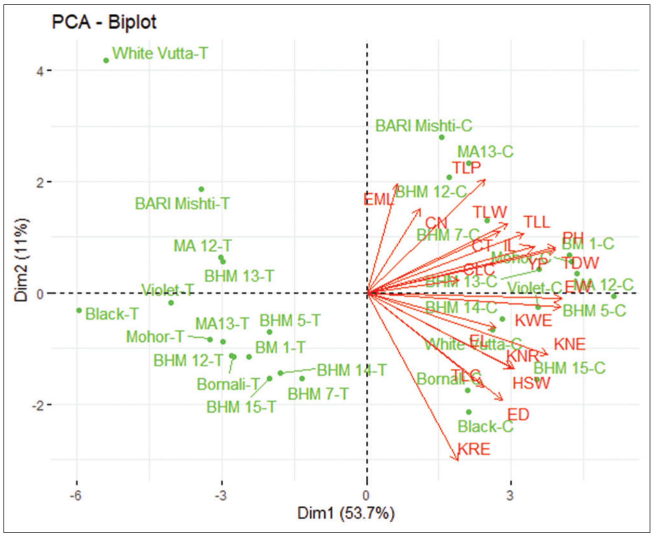

Figure 1: Biplot for important characters of four maize genotypes

Estimation of Correlation Coefficients

Correlation coefficients were calculated to assess relationships between traits. Notably, grain yield per plant positively correlated with plant height (0.755***), tassel leaf length (0.659***), tassel leaf chlorophyll (0.462***), total leaves per plant (0.674***), cob number (0.501**), kernel weight per ear (0.845***), and total dry weight (0.782***) (Table 6). The strongest association was between grain yield per plant and kernel weight per ear. Yield per plant had a negligible negative correlation with ear moisture loss (-0.026). The highest correlation under drought stress was between total dry weight and kernel weight per ear (0.919***). Zarei *et al.* (2012) reported similar positive correlations among traits like plant height and ear length with yield per plant. Bello *et al.* (2010) found significant correlations of grain yield with plant height and ear weight. Salami *et al.* (2007) noted a significant correlation between plant height and yield per plant, while Olakojo and Olayoye (2011) reported a non-significant correlation between plant height and ear height, contrasting with current findings.

Principle Component Analysis

From the principal component analysis, it was observed that the first five principal components (PC) describe most of the variation (83.46%) and they have eigenvalue greater than 0.9. (Table 7) The first principal component PC1 accounted for 53.721%, which is the highest of the total variability. The most significant characteristics in this component were kernel weight ear^{-1} (0.30), ear weight (0.30), total dry weight (0.29), plant height (0.29), and yield pot^{-1} (0.29) for their high co-efficient. The second component PC2, which explained 10.969% of the variation, had positive contributions from kernel row ear^{-1} (0.49), ear diameter (0.31) and kernel numbers row^{-1} (0.22) (Table 7). The third principal component (PC3) explained 8.076% variation (Table 7) was governed by traits like

Table 6: Phenotypic correlation coefficients between yield and yield attributing traits for drought tolerance

	YP	PH	CT	IL	TLL	TLC	TLP	CN	EL	ED	KRE	KNR	KNE	EML	KWE	EW	HSW	TDW
YP	1.00	0.755***	0.517***	0.635***	0.659***	0.462***	0.674***	0.501***	0.404***	0.350***	0.221*	0.393***	0.637***	-0.026	0.845***	0.645***	0.142	0.782***
PH		1.00	0.601***	0.856***	0.747***	0.398***	0.700***	0.128	0.676***	0.414***	0.209*	0.540***	0.668***	0.236*	0.864***	0.814***	0.229*	0.903***
CT			1.00	0.435***	0.528***	0.377***	0.473***	0.127	0.396***	0.231*	0.15	0.317**	0.457***	0.102	0.533***	0.622***	-0.041	0.611***
IL				1.00	0.716***	0.471***	0.562***	0.007	0.455***	0.296**	0.17	0.428***	0.530***	0.238*	0.789***	0.756***	0.309**	0.826***
TLL					1.00	0.367***	0.509***	0.154	0.451***	0.312**	0.189	0.403***	0.511***	0.161	0.723***	0.681***	0.105	0.675***
TLC						1.00	0.303**	-0.082	0.149	0.358***	0.316**	0.335***	0.458***	0.284**	0.626***	0.590***	-0.014	0.487***
TLP							1.00	0.258	0.530***	0.307**	0.17	0.431***	0.577***	0.019	0.699***	0.587***	0.18	0.753***
CN								1.00	-0.1	-0.167	-0.270**	-0.224*	-0.056	-0.236*	0.06	0.023	-0.071	0.074
EL									1.00	0.336***	0.365***	0.597***	0.562***	0.025	0.516***	0.509***	0.092	0.557***
ED										1.00	0.675***	0.608***	0.782***	0.077	0.535***	0.506***	0.381	0.464***
KRE											1.00	0.519***	0.640***	-0.173	0.367***	0.286**	0.167	0.263*
KNR												1.00	0.745***	0.133	0.565***	0.544***	0.277**	0.553***
KNE													1.00	0.03	0.790***	0.668***	0.316**	0.737***
EML														1.00	0.107	0.151	0.289**	0.244*
KWE															1.00	0.811***	0.273**	0.919***
EW																1.00	0.261**	0.825***
HSW																	1.00	0.293**
TDW																		1.00

*, ** & *** indicates significant at 5%, 1% and 0.1% level of probability respectively. Here, PH=Plant Height (inch), CT=Canopy temperature (°C), IL=Internode Length (cm), TLL=Tassel Leaf Length (cm), TLC=Tassel Leaf Chlorophyll, TLP=Total Leaf Plant^{-1}, CN=Cob No, EL=Ear Length (cm), EG=Ear Diameter (cm), KRE=Kernel Rows Ear^{-1}, KNR=Kernel Numbers Row^{-1}, KNE=Kernel Number Ear^{-1}, EML=Ear Moisture Loss (%), KWC=Kernel Wt. Ear^{-1} (g), EW=Ear Wt.(g), HSW=100 seed weight (g), TDW=Total Dry Wt. (g), YP=Yield Plant^{-1} (g)

Table 7: Eigen value, % Variance and cumulative (%) total variance of the principal components

Variables	PC1	PC2	PC3	PC4	PC4	PC5
PH	0.29	-0.13	0.07	-0.10	-0.10	0.06
CT	0.20	-0.18	-0.09	0.20	0.20	-0.01
IL	0.25	-0.13	0.01	-0.13	-0.13	-0.27
TL	0.24	-0.17	-0.06	-0.22	-0.22	-0.12
TLC	0.18	0.28	-0.35	0.10	0.10	-0.44
TLP	0.18	-0.33	0.20	0.27	0.27	0.34
CN	0.08	-0.25	-0.42	-0.25	-0.25	0.58
EL	0.20	0.10	0.26	-0.35	-0.35	0.19
ED	0.21	0.31	0.11	0.24	0.24	0.20
KRE	0.14	0.49	0.14	0.04	0.04	0.14
KNR	0.22	0.22	0.33	-0.05	-0.05	0.06
KNE	0.28	0.18	0.05	0.10	0.10	0.18
EML	0.05	-0.32	0.54	0.30	0.30	-0.18
KWE	0.30	0.04	-0.09	0.06	0.06	-0.01
EW	0.30	0.02	-0.10	0.02	0.02	-0.04
HSW	0.23	0.22	-0.05	-0.18	-0.18	0.01
TDW	0.29	-0.13	-0.03	0.03	0.03	-0.05
YP	0.29	-0.13	-0.03	0.03	0.03	-0.05
Eigenvalue	10.744	2.194	1.615	1.169	1.169	0.969
% Variance	53.721	10.969	8.076	5.846	5.846	4.846
% Cumulative Variance	53.721	64.69	72.765	78.611	78.611	83.457

Here, PH=Plant Height (inch), CT=Canopy temperature (°C), IL=Internode Length (cm), TLL=Tassel Leaf Length (cm), TLC=Tassel Leaf Chlorophyll, TLP=Total Leaf Plant^{-1}, CN=Cob No, EL=Ear Length (cm), EG=Ear Diameter (cm), KRE=Kernel Rows Ear^{1}, KNR=Kernel Numbers Row^{-1}, KNE=Kernel Number Ear^{1}, EML=Ear Moisture Loss (%), KWC=Kernel Weight Ear^{1} (g), EW=Ear Weight (g), HSW=100 seed Weight (g), TDW=Total Dry Weight (g), YP=Yield Plant^{-1} (g)

ear moisture loss, kernel numbers row^{-1} and ear length. PC4 was dominated by a mixture of plant traits like ear diameter, ear moisture loss, total leaf plant^{-1} and canopy temperature (Table 7) and it explained 5.85% of data variation while PC5 (4.85% variance) was dominated by traits like cob number and total leaf plant^{-1}.

PCA Biplot

Principal Component Analysis (PCA)-biplot of 15 maize cultivars based on the variance in 15 morphological traits grown under control and drought conditions are shown in Figure 1. The first two components explained 53.7% and 11% of the variances, respectively. Arrows indicate the strength of the trait's influence on the first two PCs.

Cluster Analysis (Heatmap)

The hierarchical clustering heatmap of cultivars and traits along with the dendrogram for seedling stage is presented in Figure 2. A two-way cluster heatmap was created by splitting four groups at the variable level and eight clusters at the genotype level. The highly related traits such as SFW, SL and RFW were assembled in variable group-1; GR and SR in group-2; MXRL and TL in group 3 and MNRL in group 4.

At the seedling stage, genotypes in Cluster 5 showed the highest drought tolerance, followed by Cluster 7 and Cluster 8 in treatment 2 (Figure 2). Among the 9 traits measured, SFW and RFW had the lowest values, while the remaining 7 traits

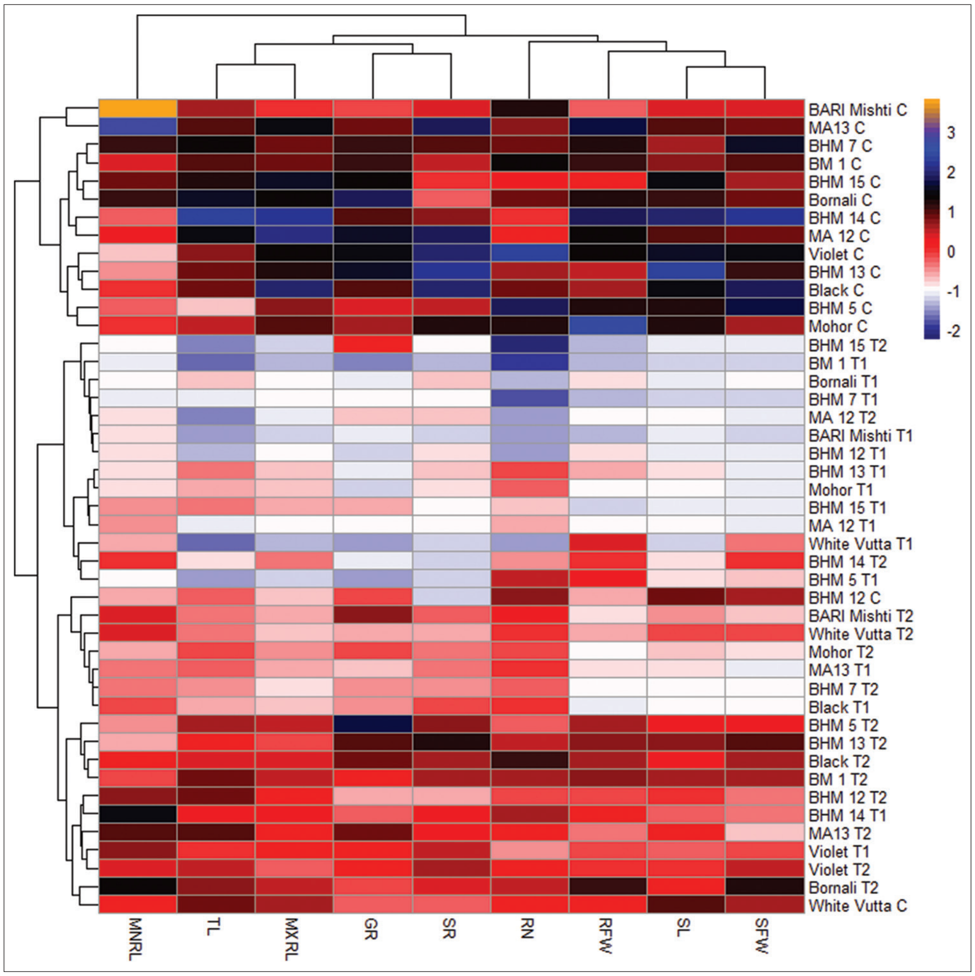

Figure 2: Cluster Heatmap Showing 15 Maize Genotypes Based on Different Traits at Seedling Stage. Here, GR=Germination Rate (%), SR=Survival Rate (%), TL=Total Length (cm), SL=Shoot Length (cm), RN=Root Number, MXRL=Maximum Root Length (cm), MNRL=Minimum Root Length (cm), RFW=Root Fresh Wt. (g), SFW=Shoot Fresh Wt. (g)

indicated greater drought tolerance. In treatment 2, RN and TL were highest in Cluster 7 and Cluster 6 compared to the control, followed by Cluster 5 in treatment 1. The heatmap highlights that cultivars in Cluster 7 and Cluster 8, such as BHM-5 T2, Black T2, and Violet T1 and T2, exhibited higher mean values than those in Cluster 3 and Cluster 4.

The hierarchical clustering heatmap of 15 maize cultivars and their associated traits for the reproductive stage is presented in Figure 3. The clustering resulted in six row-clusters for cultivars and three column clusters for their traits. Based on mean values, in treatment 1, cultivars in Cluster 6 exhibited greater drought tolerance, followed by Cluster-5, under stressed conditions in Figure 3. Among the 20 traits, the lowest values were observed for traits such as CN, EML, and HSW. In contrast, higher drought tolerance was indicated by greater mean values in the other 17 traits. The heatmap clearly illustrates those genotypes in Cluster 6 and Cluster 5, such as BHM 7, BHM 14, BHM 12, and BHM 15, had higher mean values than those in Cluster 4 (Figure 3), indicating that these cultivars exhibited greater drought tolerance compared to the cultivars in Cluster 4.

DISCUSSION

The ANOVA (Table 2) for different traits at the seedling stage revealed significant differences ($P<0.1\%$) among maize genotypes for most of the traits like GR, SR, SL, RN influenced by both genotype and drought stress. Similar variations were reported by Akinwale (2018) and Ahmad *et al.* (2004). Genotypes MA-12 and White Vutta displayed better drought resilience, maintaining higher emergence rates. Some genotypes showed reduced fresh shoot weight under drought, while others increased due to osmotic adjustments, highlighting the diverse effects of drought on maize seedlings. The (ANOVA) (Tables 3 & 4) at the vegetative and reproductive stages also revealed highly significant differences ($P<0.1\%$) for all measured traits, including PH, EL, ED, KRE, KNR, KNE, HSW, and YP. This aligns with the findings by Makumbi *et al.* (2015), who reported significant variability among 15 inbred lines in PH, EL, and YP. Similarly, Pandit *et al.* (2016) noted significant differences in PH, EL, and KNE. Shaw *et al.* (1988) also documented substantial variability in YP, PH, EL, and KNE. The observed variations are attributed to the high genetic variability among the genotypes under study, particularly

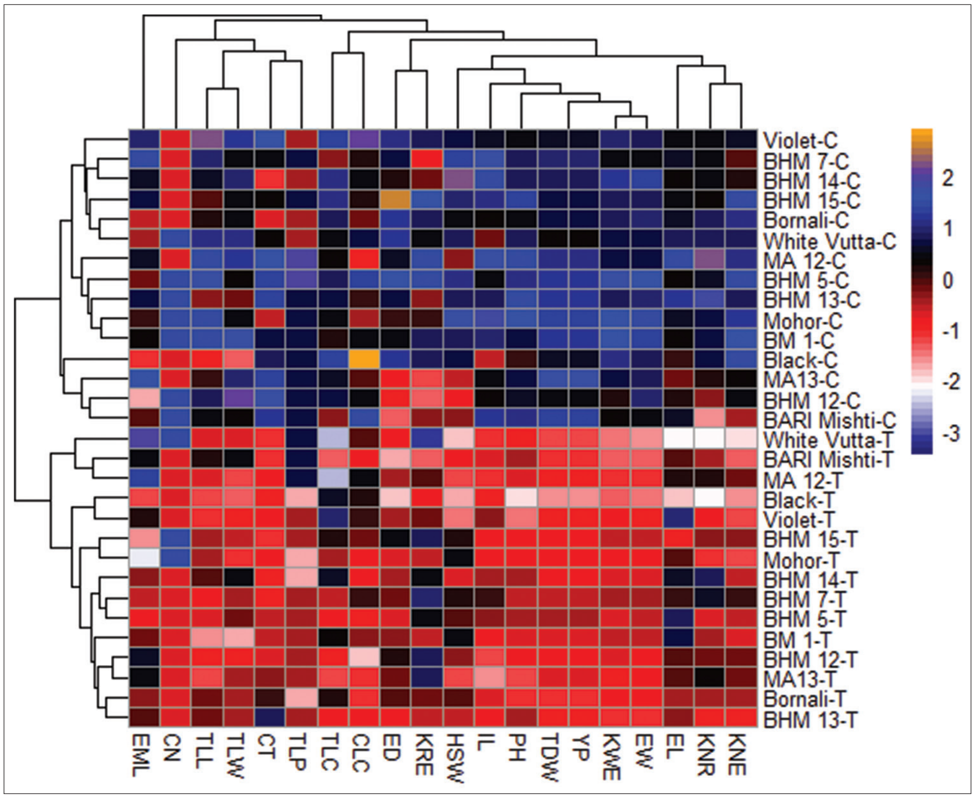

Figure 3: Cluster Heatmap Showing 15 Maize Genotypes Based on Different Traits at Reproductive Stage. Here, PH=Plant Height (inch), CT=Canopy temperature (℃), IL=Internode Length (cm), TLL=Tassel Leaf Length (cm), TLC=Tassel Leaf Chlorophyll, TLP=Total Leaf Plant^{-1}, CN=Cob No, EL= Ear Length (cm), EG=Ear Diameter (cm), KRE=Kernel Rows Ear^{-1}, KNR=Kernel Numbers Row^{-1}, KNE=Kernel Number Ear^{-1}, EML=Ear Moisture Loss (%), KWC=Kernel Wt. Ear^{-1} (g), EW=Ear Wt.(g), HSW=100 seed weight (g), TDW=Total Dry Wt. (g), YP=Yield Pot^{-1} (g)

in response to drought stress. Both genotype and treatment significantly influenced PH, EL, KRE, and YP, indicating a strong genetic divergence among the genotypes. These results justify further analysis to explore the underlying genetic factors contributing to drought resilience in maize.

Genetic analysis indicated significant variation among maize genotypes, particularly under drought stress. The phenotypic coefficient of variation (PCV) exceeded the genotypic coefficient of variation (GCV) for all traits, highlighting the environmental influence on trait expression, supported by Akanda *et al.* (1997). Traits such as YP, HSW, EW, CN and KWE exhibited substantial PCV and GCV, indicating high variability. In contrast, traits like CT, TL, ED, and KRE showed lower PCV and GCV, suggesting limited potential for improvement in drought tolerance.

Heritability is critical for selecting traits in drought-tolerance breeding programs (Farshadfar *et al.*, 2013). The study found that traits like PH, IL, KWE, EW, TDW, and EL had high heritability, indicating a reduced environmental impact on their expression. These traits are promising candidates for selection to enhance drought resilience in maize. Notably, KWE and EW demonstrated high heritability and significant genetic advancement, suggesting additive gene effects. Additionally, traits such as PH, EL, and YP also showed high genetic advancement as a percentage of the mean which suggests that these traits are subject to additive gene effects, presenting a favorable avenue for direct selection to enhance them (Wang *et al.*, 2018). The identification of important features for the selection and enhancement of maize genotypes for drought tolerance was made possible by the correlation coefficient analysis. This study evaluated the correlation coefficients between different variables, both genotypic and phenotypic, and showed how these traits interacted during a drought. PH, EW, KNE, TLL, KWE and TDW showed a strong positive and significant association with yield per pot, suggesting an additive genetic model with less environmental influence. These results are consistent with previous research by Bello *et al.* (2010), Zarei *et al.* (2012) and Mohanapriya *et al.* (2023) which also found a strong positive association between yield and similar features. In contrast, some traits, such as KRE, EML and HSW showed non-significant or negative correlations, as noted by Olakojo and Olayoye (2011). Those positive correlations, less affected by environmental changes, are crucial in selecting traits that perform consistently under drought stress.

Principal Component Analysis (PCA) effectively identifies key contributors to variance across traits, crucial for breeding programs (Akter *et al.*, 2009; Suryanarayana *et al.*, 2017). In this study, five principal components (PCs) captured the majority of variance, with 83.45% of total variability. This aligns with findings from Suryanarayana *et al.* (2017), where significant variance was also attributed to the first few PCs. The first few principal components (PC1 and PC2) explain most of the variance in the dataset and are influenced by critical yield-attributing traits such as PH, KWE, EW, and HSW. Across all PCs, hundred seed weight consistently displayed a positive value, warranting attention for yield enhancement. A similar sentiment was shared by Marker and Krupakar (2009). These traits could be key factors in improving maize yield under drought conditions. The PCA results suggest that focusing on these major contributors could lead to better selection criteria for drought-tolerant maize genotypes

Cluster analysis of 15 maize genotypes under drought stress revealed eight distinct clusters at the seedling stage and six at the vegetative and reproductive stages. In seedling stage, Cluster 5, Cluster 7, and Cluster 8 particularly including genotypes like BHM-5 T2, Black T2, and Violet T2, showed superior drought tolerance, exhibiting higher values in key traits like Root Number and Total Length, making them strong candidates for drought-tolerant breeding programs. In later stages, genotypes in Clusters 6 and 5 displayed strong drought tolerance, excelling in traits like Kernel Number per Ear, Kernel Weight per Ear, and Ear Length. Heatmap analysis also showed key correlations, with Plant Height, Internode Length, and Yield Pot-1 clustering together, indicating a strong link to drought resilience.

CONCLUSION

The analysis revealed significant genetic diversity among the genotypes, with minimal environmental influence, suggesting potential for effective selection. At seedling stage, Black Vutta, Violet Vutta, and White Vutta performed well and were grouped in Cluster 6. At the reproductive stage, BHM-7 excelled in yield-related traits under drought stress, while BHM-15 had the highest yield, and White Vutta was the most susceptible. Other notable genotypes were BHM-5 (tallest plants), BHM-14 (longest ears), and Mohor and Bornali (highest 100-seed weight). Heritability ranged from 30.47% to 99.82%, with yield traits showing the highest values, indicating strong genetic influence. Principal Component Analysis (PCA) identified five key components explaining 84.03% of the variation, with yield-related traits driving the first component. In conclusion, genotypes BHM-7, BHM-14, and BHM-15 show significant potential for breeding drought-tolerant maize. Crossbreeding these inbred lines could further enhance desirable traits, with ongoing multi-location trials aiming to provide drought-resilient varieties for farmers in dryland regions.

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AUTHORS' CONTRIBUTION

GHMS: Design, formulation, supervision of experiment, and writing of manuscript. FT, MSA: Conducting experiments, Data collection, Lab analysis, and writing of manuscript.

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