Annals of Bangladesh Agriculture
Journal homepage: bsmrau.edu.bd/aba

ORIGINAL ARTICLES

Genetic diversity in maize at germination and seedling stages under simulated drought conditions

Md. Abdul Mannan[1*] **and Ferdousi Begum**[2]

[1] Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh
[2] Plant Breeding Division, Bangladesh Agricultural Research Institute, Gazipur 1701, Bangladesh

ARTICLE INFO

Keywords:
Drought tolerance, genotypes, germination, maize, variability.

Received : 05 November 2023
Revised : 12 December 2023
Accepted : 27 December 2023
Published : 30 December 2023

Citation:
Mannan, M. A. and F. Begum. 2023. Genetic diversity in maize at germination and seedling stages under simulated drought conditions. *Ann. Bangladesh Agric.* 27(2):19-30.

ABSTRACT

Ninety seven maize (*Zea mays*) genotypes were evaluated for germination and early seedling characters under PEG6000 (8%) induced drought stress to identify drought tolerant genotypes. Descriptive statistics for percentage of seed germination and abnormal seedling, shoot length, root length, and shoot dry weight, root dry weight, seedling length, seedling dry weight, and vigor index revealed significant variation among the genotypes studied. The percentage of germination and abnormal seedlings varied greatly among the genotypes. The first and second principal components (PC) of PCA results accounted for 73.15 and 26.85% of the total variation of maize genotypes, respectively. The first PC variation consisted primarily of relative seedling shoot dry weight and relative seedling root dry weight. Cluster analysis grouped the genotypes into four clusters. Cluster III genotypes performed better in terms of relative shoot dry weight, relative root dry weight, and number of abnormal seedlings, indicating drought tolerance. Very poorly performance was shown by genotypes in cluster IV, whereas moderately performance was shown by genotypes in cluster II and I. The clusters differed significantly from one another, according to D^2 and multivariate analysis. Thirty six genotypes viz. BD-814, BD-810, BD-812, BD-821, BD-815, BD-10015, BD-10238, CML-552, BD-822, BD-826, CML-341, CML-555, CML-591, CML-523, CML-564, BD-817, BD-10018, BD-10240, CML-577, CML-342, CML-556, BD-837, CML-544, BD-827, BD-806, BD-835, BD-840, BD-842, BD-10010, CML-562, BD-10239, BD-10016, CML-593, CML-568 and BD-10234 demonstrated relatively drought tolerance during the germination and early seedling stages might be used in future breeding program.

Introduction

Occasionally, plants are subjected to shifting unfavorable biotic and/or abiotic circumstances, which might endanger their existence and prevent them from reaching their full potential (Suzuki *et al*., 2014). When available water does not keep up with the constant transpirational loss of water by plants, drought stress is imposed on the plants (Farooq *et al*., 2009). Drought is one of the major abiotic factors limiting crop productivity globally. Global climate change is predicted to result in losses in crop yield (Webber *et al*., 2018) because it

---

[*] **Corresponding Author:** Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh. Email: mannanagr@bsmrau.edu.bd

https://doi.org/10.3329/aba.v27i2.72515

would exacerbate drought stress, which will lower the yield of highly valued crops and significantly affect the ecological fitness of crops planted. By developing crop varieties tolerance to drought using available germplasms, crop yield can be sustained for future generations (Osborn and Ferguson, 2019). Plant materials that will be valuable to both current and future generations of people are known as plant genetic resources (PGR). The PGRs have long been acknowledged as essential sources of genotypic diversity needed for the development of future crop varieties (Hammer, 1998).

In terms of cereal crop production worldwide, maize (*Zea mays* L.) is rated third behind rice and wheat (Cooper *et al*., 2014). It is extensively cultivated across the globe in many agro-ecological conditions. Due to the growing worldwide human population, there is an increasing need for maize cultivation as a source of food, fodder, oil, and biofuel. On the other hand, the global yield loss of maize annually as a result of drought is estimated to be roughly 15% (Edmeades, 2018; Adewale *et al*., 2018). Between 2011 and 2020, maize production of Bangladesh increased significantly from 1,954 to 4,700,000 tons, increasing at an annual rate, peaking at 17.14% in 2019 and then falling to 14.63% in 2020 (USDA, 2020). Khandoker *et al*. (2018) found that maize production was decreased by 22.4% in drought prone areas than normal environment and loss of yield (70%), loss of plant growth (55%) and problem in flowering stage (43%) were reported to be the major effects of drought in Bangladesh. Under climate change, major areas that produce maize would get warmer, dryer and more vulnerable to severe droughts. These changes could have alarming effects on maize production under hotter climate scenarios, while the exact effects will depend on the location and alter rainfall patterns (Edmeades, 2013). In this situation, cultivating drought-tolerant varieties of maize is necessary to counteract anticipated production declines and maintain productivity in susceptible areas (Tesfaye *et al*., 2018).

During the early crop establishment phase, maize seedlings are more sensitive to drought stress than during flowering and the longer anthesis-silk interval. Seedlings are particularly vulnerable to drought stress because they require less water than later vegetative and reproductive stages (Maiti *et al*., 1996; Cao and Wj, 2004). Depending on the inherent characteristics of the seed and the surrounding environmental parameters like moisture and temperature, maize seedlings emerge 4–9 days after germination. From the moment of emergence (VE) to the 5-leaf (V5) stage, the maize seedling stage begins (Bell, 2017). At this point, the plants are extremely vulnerable to environmental stressors like drought, and any severe stress at this point will cause the crop to fail completely (Fakorede, 2008; Bashir *et al*., 2016). Under stress situations, selection indices based on early seedling characteristics may enhance grain yield selection efficiency in addition to grain yield factors (Abdel-Ghani *et al*., 2015).

Some researchers have employed measures of drought tolerance to identify potential genotypes that are tolerant to drought, based on characteristics of early seedlings under control conditions and drought stress (Avramova *et al*., 2016; Meeks *et al*., 2013; Liu *et al*., 2013; Akinwale *et al*., 2017). Furthermore, maize drought tolerance has recently been assessed using drought sensitivity indices, which are based on how seedling traits respond to stress compared to the control (Grzesiak *et al*., 2012; Ahmed *et al*., 2019). Principal component analysis, biplot and clustering techniques are also being utilised more frequently to compare drought tolerance of maize (Arisandy *et al*., 2017; Hefny *et al*., 2017; Badr and Brüggmann, 2020). In seed germination studies, high molecular weight polyethylene glycol (PEG) solutions are commonly used to mimic osmotic stress effects in a Petri dish (*in vitro*) for plants, ensuring a consistent water potential throughout the experiment (Sidari *et al*., 2008; Dodd and Donovan, 1999).The rationale of this study is to determine whether a core collection of maize germplasm from the Bangladesh Agricultural Research Institute, Bangladesh and the CIMMYT, Mexico is drought-tolerant. To find potential drought-tolerant accessions, we modified the use of variation in germination and seedling attributes and their correlations. These methods are practical and efficient. This method avoids the tedious and time-consuming selection that must be done in the field to identify prospective high grain yielding genotypes (Adewale *et al*., 2018; Abdel-Ghani *et al*., 2013). In order to identify genotype groups tolerant to drought stress, the main objective of the present study was to examine the effects of osmotic stress generated by PEG6000 on early seedling features and germination characteristics of maize.

Materials and Methods

The study was performed in the month of November, 2020 in Seed Science Laboratory of Bangabandhu sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, following completely randomized design (CRD). Seeds of 97 maize (*Zea mays*) genotypes were collected from CIMMYT and Plant Genetic Resource Centre, Bangladesh Agricultural Research Institute (BARI) listed in Table 1. Seeds of every accession were split into two groups of fifty, one for use as a control and the other for treating drought stress by subjecting them to 8% concentrations of PEG6000.

Table 1. List of genotypes and their sources which are used in the experiment

SL. No.	Name of genotype	Source	SL. No.	Name of genotype	Source	SL. No.	Name of genotype	Source
1	BD-798	BARI	34	BD-834	CIMMYT	67	CML-577	BARI
2	BD-799	BARI	35	BD-835	CIMMYT	68	CML-562	CIMMYT
3	BD-800	BARI	36	BD-836	CIMMYT	69	CML-563	CIMMYT
4	BD-801	BARI	37	BD-837	CIMMYT	70	CML-564	CIMMYT
5	BD-802	BARI	38	BD-838	CIMMYT	71	CML-579	CIMMYT
6	BD-803	BARI	39	BD-839	CIMMYT	72	CML-580	CIMMYT
7	BD-804	BARI	40	BD-840	CIMMYT	73	CML-588	CIMMYT
8	BD-805	BARI	41	BD-841	CIMMYT	74	CML-339	CIMMYT
9	BD-806	BARI	42	BD-842	CIMMYT	75	CML-340	CIMMYT
10	BD-807	BARI	43	BD-843	CIMMYT	76	CML-341	CIMMYT
11	BD-808	BARI	44	BD-844	CIMMYT	77	CML-342	CIMMYT
12	BD-810	BARI	45	BD-845	CIMMYT	78	CML-543	CIMMYT
13	BD-811	BARI	46	BD-846	CIMMYT	79	CML-544	CIMMYT
14	BD-812	BARI	47	BD-847	CIMMYT	80	CML-440	CIMMYT
15	BD-813	BARI	48	BD-848	CIMMYT	81	CML-444	CIMMYT
16	BD-814	BARI	49	BD-10005	CIMMYT	82	CML-589	CIMMYT
17	BD-815	BARI	50	BD-10006	CIMMYT	83	CML-590	CIMMYT
18	BD-817	BARI	51	BD-10010	CIMMYT	84	CML-591	CIMMYT
19	BD-819	BARI	52	BD-10015	CIMMYT	85	CML-592	CIMMYT
20	BD-820	BARI	53	BD-10016	CIMMYT	86	CML-593	CIMMYT
21	BD-821	BARI	54	BD-10018	CIMMYT	87	CML-600	CIMMYT
22	BD-822	BARI	55	BD-10233	CIMMYT	88	CML-601	CIMMYT
23	BD-823	BARI	56	BD-10234	CIMMYT	89	CML-549	CIMMYT
24	BD-824	BARI	57	BD-10235	CIMMYT	90	CML-587	CIMMYT
25	BD-825	BARI	58	BD-10236	CIMMYT	91	CML-522	CIMMYT
26	BD-826	BARI	59	BD-10237	CIMMYT	92	CML-523	CIMMYT
27	BD-827	BARI	60	BD-10238	CIMMYT	93	CML-550	CIMMYT
28	BD-828	BARI	61	BD-10239	CIMMYT	94	CML-552	CIMMYT
29	BD-829	BARI	62	BD-10240	CIMMYT	95	CML-555	CIMMYT
30	BD-830	BARI	63	BD-10241	CIMMYT	96	CML-556	CIMMYT
31	BD-831	BARI	64	BD-10242	CIMMYT	97	CML-568	CIMMYT
32	BD-832	CIMMYT	65	CML-570	CIMMYT			
33	BD-833	CIMMYT	66	CML-576	CIMMYT			

BARI-Bangladesh Agricultural Research Institute, Gazipur, Bangladesh, CIMMYT, Mexico

The International Seed Testing Association (ISTA, 2017) protocol was followed in the germination of the control and drought treatment seeds. The seeds for each treatment were separated into three sets of sixteen to seventeen seeds each of which was considered as three replications, and each set of seeds were allowed to germinate on filter paper in Petri dishes in a germinator maintained at 25°C, 75% humidity, and total darkness that had been moistened with either 8% PEG6000 or distilled water.

After nine days of seed sowing, the 8% PEG treatment and the control showed germination. Seeds with radicles measuring at least 3 mm were considered to have germinated. A number of abnormal seedlings (AS), which did not develop into healthy seedlings and included a small number of albinos, were noted for every set of seeds. Five seedlings on the tenth day were chosen for each replicate of the 8% PEG6000 treatment and the control group. The parameters measured were: the number of abnormal seedlings, the length of the shoots and roots, the shoot and root dry weight, the length and dry weight of the seedlings, and the vigor index. Rather than using absolute values, relative values of seed and seedling traits were used to make comparisons more meaningful in terms of identifying drought stress tolerance genotypes or groups of genotypes. Relative performance for each variable was calculated as the variable measured in PEG6000-induced drought-treated seeds divided by the variable measured under controlled conditions.

Data on nine relative seed and seedling traits for each of the 97 genotypes were subjected to multivariate analysis using principal component analysis and discriminant functional analysis. To select genotypes with diverse characters associated with different quantitative traits, a multivariate analysis was performed with the SPSS program, version 16 according to the procedure described by Rojas *et al*. (2000). Deviations of orthogonal variables using principal component analysis (PCA) and genotyping into homogeneous groups using the non-hierarchical K-means technique of cluster analysis were performed.

Results and Discussion

Variability in quantitative characters

The nine plant characteristics of the 97 maize genotypes subjected to PEG6000 induced drought stress at the germination and early seedling shown in Table 2, revealed that there were wide ranges of variations among those characters, but variations narrowed down in drought-treated plants. Seed germination rate varied from 68.00 to 96.00% with a mean of 84.97% in control condition, and 0.00 and 92% with a mean of 58.24% in drought-treated seeds. The reduction of germination percentage due to water deficit stress was almost 27%. Drought generally reduced the germination capacity of seeds. In comparison to the control conditions, the current study's results demonstrated that osmotic stress had a negative impact on seed germination performances. According to Yang *et al*. (2018), a high seed nutritional imbalance, harmful ions, and a decreased soluble osmotic potential were linked to the decrease in germination when using PEG. It was observed that the control treatment had the highest germination percentage, indicating that germination was proceeding well due to the water content. The study discovered that the responses of various genotypes to osmotic stress varied. Stress conditions cause seeds to lose some of their capacity to absorb and utilize water, which makes their tolerance mechanisms less effective in promoting healthy growth (Islam *et al*., 2018).

The relative performance of the genotypes showed a wide range of variations in all the seedling characters assessed (Table 2). PEG6000 induced drought stress increased the abnormal seedling in the tested genotypes. In drought condition abnormal seedling was 41.46%, but only 15.04% under control. Our result was supported by Tarawneh *et al*. (2019), who observed the percentage of abnormal seedlings increased due to the delayed appearance of radicles and plumules in wheat seeds when exposed to PEG and the reduced growth of seedlings under this treatment. It is widely accepted that the first effect of the moisture shortage brought on by drought is lower germination, which hinders early crop establishment and causes poor plant stand during the early seedling phase (Abdel-Ghani *et al*., 2015). Water scarcity has an important effect on the shoot and root length of maize plants. The interaction between genotype and environment determines the phenotypic response of any trait. Minimum seedling shoot length was 1.30 cm and maximum was 25.73 cm with an average of 9.66 cm in controlled conditions but in drought conditions seedling shoot length was observed 0.0-21.02 cm with an averageof 4.85 cm. The root length was 1.76-34.20 cm with an average of 14.34 cm but in drought conditions seedling root length was 0.00-21.77 cm with an average of 6.93 cm. Several studies (Ahmad *et al*., 2013; Faisal *et al*., 2017) examined the reduction in shoot length under drought conditions as well; their conclusions agreed with those of the present study. Past research has indicated that root length may be a significant characteristic for drought stress tolerance (Leishman and Westoby, 1994). However, other genotypes were categorized as drought-susceptible because of their poor performance. The genotypes that displayed the maximum mean values of root length in comparison to other tested genotypes were identified as drought-tolerant. Selection for maximum root length was suggested to be a good indicator of future improvements in drought tolerance in wheat breeding (Dhanda *et al*., 2004).

Average shoot dry weight was 0.04 mg per seedling in controlled conditions but in drought conditions it was 0.02 mg. In controlled conditions average root dry weight per seedling was 0.02 mg but in drought conditions it was 0.01 mg. Minimum seedling length was 3.06 cm and it was maximum 57.62 cm with an average 24.0 cm in controlled condition but in drought conditions average seedling length was 11.77 cm. In controlled conditions average seedling dry weight was 0.06 mg per plant and it was 0.03 mg under drought conditions. In controlled conditions average leading to a considerable reduction in shoot and root length, fresh and dry weights. All of the aforementioned parameters decreased when the PEG6000-induced drought was imposed in this study (Table 2). Many researchers agreed that decrease in seed germination and seedling growth under water stress is a common response (Kosar *et al*., 2018). Reduced germination, cell division, and elongation are the results of severe osmotic pressures causing a water deficit, which first reduces the capacity of seeds to absorb water, activates hydrolytic enzymes, and interupt the nutrients to the embryo (Fahad *et al*., 2017).

Table 2. Range and mean for quantitative characteristics of 97 maize genotypes subjected to drought stress

Variables	Control[1]		Drought[2]	
	Range	Mean[3]	Range	Mean[3]
Germination (%)	68.00-96.00	84.97±5.93	0.00-92.00	58.24±23.80
Abnormal seedling (%)	4.00-32.00	15.04±5.93	8.00-100.00	41.46±23.80
Shoot length (cm plant^{-1})	1.30-25.73	9.66±7.13	0.00-21.02	4.85±3.18
Root length (cm plant^{-1})	1.76-34.20	14.34±9.52	0.00-21.77	6.93±3.88
Shoot dry weight (mg plant^{-1})	0.00-0.10	0.04±-.02	0.00-0.14	0.02±0.02
Root dry weight(mg plant^{-1})	0.00-0.07	0.02±0.02	0.00-0.03	0.01±0.01
Seedling length (cm plant^{-1})	3.06-57.62	24.00±15.60	0.00-30.04	11.77±6.14
Seedling dry weight (mg plant^{-1})	0.00-0.14	0.06±0.03	0.00-0.14	0.03±0.02
Vigor index	0.00-12.56	5.53±2.64	0.00-5.23	2.11±1.16

[1]Germination at controlled condition, [2] Germination at drought conditions (8% PEG 6000), [3]Mean±standard deviation

Principal component analysis

By using principal component analysis (PCA), the genotypic variance in the level of drought tolerance in 97 maize genotypes was explicated succinctly and clearly. The contribution of each principal component (PC) to the overall variance is estimated using the Eigen values corresponding to each PC. While the coefficients of the latent vector linked to a certain PC show how much each original value contributed to the PC. The first PC (PC 1) accounted for about 73.15% of the total seedling contributed greatly for this variation (Table 3 & 4). Therefore, the seed and seedling traits that separate genotypes along with the PC 2 is number of abnormal seedling.rincipal component (PC) analysis is a multivariate statistical analysis, which is used to examine and deconstruct big and complicated data sets. Plant scientists have employed principal component analysis to ascertain grouping and diversity in wheat seedling traits (Wang *et al*., 2001; Sisodia and Rai, 2017). El-Deeb and Mohamed (1999) showed that this approach reduces the number of associated variables from a bigger one to smaller ones.

Table 3. Initial Eigen values and per cent of variation in nine plant characters of 97 maize genotypes subjected to drought

Function	Eigenvalue	% of variance	Cumulative %	Canonical correlation
1	8.66[a]	73.15	73.15	0.910
2	3.15[a]	26.85	100	0.869

First 2 canonical discriminant functions were used in the analysis variation and the associated traits explained by this component were shoot dry weight and root dry weight. In contrast germination had the negative impact. Seedling traits that separate genotypes along the PC 1 axis is seedling shoot dry weight. The PC 2 accounted for about 26.85% of the total variation of the genotypes and abnormal

Table 4. Latent vectors associated with the first two principal components

Relative plant characters	Principal components	
	1	2
Germination	-0.00130	-0.01822
Abnormal seedling	0.00069	-0.00510
Shoot length	0.02631	0.01764
Root length	0.02487	0.00751
Shoot dry weight	0.04552	0.01323
Root dry weight	0.03009	0.01608
Seedling length	0. 01903	0.419040
Seedling dry weight	0.02974	-0.00612
Vigor index	0.00499	0.00229

Cluster analysis

The 97 genotypes were grouped into four clusters based on variation using K-means non-hierarchical cluster analysis (Table 5). cluster III had the most genotypes (36), followed by cluster II (25), I (22), and IV (14). Cluster III had the best relative performance in terms of germination%, shoot length, root length, shoot dry weight, root dry weight, seedling length, seedling dry weight, and vigor index, but the worst in terms of abnormal seedling percent. All of these characteristics were lowest in Cluster IV, but abnormal seedling was highest. In clusters I and II, the mean values of the study characters were moderate. The clustering pattern of 97 maize genotypes revealed that genotypes in cluster III performed better in all parameters tested. Cluster IV performed the worst out of the four clusters. Cluster I performed significantly worse than cluster II. Table 6 shows the members of each cluster's group. According to earlier research by Iqbal *et al*. (2018), the cluster analysis divided the genotypes of rice into various clusters, guaranteeing that there was high to moderate variability among the genotypes. Four clusters were proposed in this study, with thirty six genotypes included in cluster III. This 36 genotypes combination exhibited the highest values for nearly all germination and early seedling characteristics (Table 5 & 6).

Table 5. Comparison profile of the four groups of maize genotypes classified by K-means clustering (Cluster figures are means of relative values for the genotypes in each cluster)

Relative plant characters	Cluster			
	I	II	III	IV
Germination	66.1	68.7	74.6	43.0
Abnormal seedling	316.9	272.3	212.0	1250.0
Shoot length	57.2	77.1	81.4	44.1
Root length	54.6	65.7	78.4	29.4
Shoot dry weight	53.8	60.8	68.4	47.3
Root dry weight	59.4	60.0	66.1	38.2
Seedling length	111.8	142.8	160.1	73.5
Seedling dry weight	113.2	120.8	134.5	85.5
Vigor index	29.6	39.8	82.7	16.8

Table 6. Group members of 97 maize genotypes classified into 4 groups by K-means clustering

Cluster	Name of genotypes / Accession	Member
I	BD-831, BD-808, BD-843, CML-592, BD-824, CML-601, CML-576, CML-440, CML-579, BD-10006, CML-590, BD-798, BD-830, BD-839, BD-844, BD-803, CML-600, BD-804, BD-802, BD-10005, BD-825.	22
II	CML-588, BD-829, BD-10237, CML-589, CML-340, CML-587, BD-813, BD-820, CML-339, BD-845, BD-799, BD-805, BD-823, BD-832, BD-834, CML-549, BD-10236, CML-570, CML-563, CML-543, BD-10235, BD-819, BD-828, BD-10233, BD-10242, CML-550.	25
III	BD-814, BD-810, BD-812, BD-821, BD-811, BD-815, BD-10015, BD-10238, CML-552, BD-822, BD-826, CML-341, CML-555, CML-591, CML-523, CML-564, BD-817, BD-10018, BD-10240, CML-577, CML-342, CML-556, BD-837, CML-544, BD-827, BD-806, BD-835, BD-840, BD-842, BD-10010, CML-562, BD-10239, BD-10016, CML-593, CML-568, BD-10234.	36
IV	BD-800, BD-838, BD-841, BD-801, BD-10241, CML-522, BD-833, BD-836, BD-846, BD-847, BD-848, BD-807, CML-580, CML-563, CML-444.	14

Discriminant function analysis

The step-wise discriminant function analysis (DFA) was used to identify the set of discriminatory functions that contributed to the classification of 97 maize genotypes into four distinct clusters (Table 6). DFA is especially helpful in defining genotype groups as prior classification criteria. Furthermore, it generates a graphical output that shows the existence of groups (Singh et al., 1991). According to the chi-square test, discriminant functions 1 and 2 were significant at a probability level of 0.00 (Table 7). Seedling dry weight and seedling length were two seedling characteristics that distinguished groups along the first canonical variable (Table 8). It was assumed that seedling dry weight was the most important factor in explaining the greatest variation by stepwise DFA. In function 2, the percentage of abnormal seedlings was the most important factor in explaining the maximum variance. The discriminant function technique helps the researcher with indirect selection for higher potential genotypes by requiring the establishment of selection criteria based on a multiplicity of crop parameters. Within plant breeding, a linear combination of yield-related traits is referred to as the selection index. These results are consistent with Muhammad et al. (2014), who found that cane productivity and stalk crop are significant characteristics to take into account when trying to increase sugar yield.

Table 7. Chi-square test of 3 discriminatory functions contributed in grouping of 97 maize genotypes into 4 clusters

Test of Function(s)	Walk's Lambda	Chi-square	df	Sig.
1 through 3	0.042	425.90	27	0.000

Table 8. Correlation matrix between discriminating variables and standardized canonical discriminant function of 97 maize genotypes as classification criteria

Variables evaluated	Canonical discriminant function	
	1	2
Relative abnormal seedling	0.062	0.516*
Relative shoot length	0.263	0.172
Relative root length	0.241	0.151
Relative shoot dry weight	0.596*	0.023
Relative root dry weight	0.337*	0.008
Relative seedling length	0.124	0.019
Relative seedling dry weight	0.147	0.012
Relative vigor index	0.192	0.229

Mahlonobis distance

The Mahoronobis distance (D2) between groups (clusters) computed by DFA showed that the four clusters were statistically different from each other with a probability of 0.01 (Table 9). Clusters IV and III were the most divergent with a distance matrix of 17.76 units. Cluster III grouped genotypes that performed well for most characters. In contrast, genotypes grouped into cluster IV performed very poorly. The most similar clusters were II and III (9.51 units) and Clusters I and II (11.23 units). A similar D2 analysis has been used by several authors to identify distances between clusters (Bansal *et al.*, 1999; Hedge and Patil, 2000; Jha *et al.*, 1999; Mass and Hoffman, 1977; Pradhan and Roy, 1990; Mannan *et al*., 2010; Mannan and Mamun, 2018).

Table 9. Pairwise Mahalanobis distance (D2) between 4 clusters of 97 maize genotypes

Cluster	1	2	3	4
1	-	-	-	-
2	11.23*	-	-	-
3	13.35*	9.51*	-	-
4	12.55*	13.13*	17.76*	-

* Distance differing from zero at a 95 % confidence interval.

Conclusions

Evaluation of germination and seedling root, shoot, and other characteristics was carried out in an osmotic stress simulation created by PEG6000 treatments, which served as a strong foundation for the drought tolerance of certain genotypes. The application of PEG6000 treatment resulted in a notable percentage of abnormal seedlings by dramatically reducing germination and delaying the early growth of seedlings. The study discriminated 36 genotypes viz. BD-814, BD-810, BD-812, BD-821, BD-815, BD-10015, BD-10238, CML-552, BD-822, BD-826, CML-341, CML-555, CML-591, CML-523, CML-564, BD-817, BD-10018, BD-10240, CML-577, CML-342, CML-556, BD-837, CML-544, BD-827, BD-806, BD-835, BD-840, BD-842, BD-10010, CML-562, BD-10239, BD-10016, CML-593, CML-568 and BD-10234 as drought tolerant, 14 genotypes were susceptible and rest 47 genotypes were moderaely drought tolerant among the genotypes studied. Therefore, the study made it possible to classify the tested genotypes of maize according to their level of tolerance and evaluate how well they tolerated the drought. In conclusion, screening for tolerant or sensitive maize germplasm quickly and affordably can be accomplished by utilising germination and early seedling traits.

Acknowledgements

The authors acknowledges the Research Management Wing (RMW), Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh for providing fund for conducting the research.

References

Abdel-Ghani, A. H., B. Kumar, J. Reyes-Matamoros, P. J. Gonzalez-Portilla, C. Jansen, J. P. San Martin, M. Lee and T. Lübberstedt. 2013. Genotypic variation and relationships between seedling and adult plant traits in maize (Zea mays L.) inbred lines grown under contrasting nitrogen levels. *Euphytica*. 189: 123–133. [CrossRef]

Abdel-Ghani, A. H., K. Neumann, C. Wabila, R. Sharma, S. Dhanagond, S. J. Owais, A. Börner, A. Graner and B. Kilian. 2015. Diversity of germination and seedling traits in a spring barley (Hordeum vulgare L.) collection under drought simulated conditions. Genet. Resour. Crop. Evol. 62: 275–292. [CrossRef]

Adewale,S. A., R. O. Akinwale, M. A. B. Fakorede and B. Badu-Apraku. 2018. Genetic analysis of drought-adaptive traits at seedling stage in early-maturing maize in bred lines and field performance under stress conditions. *Euphytica*. 214: 1–18. [Cross Ref]

Ahmad, M., G. Shabbir, N. Minhas and M. K. N. Shah. 2013. Identification of drought tolerant wheat genotypes based on seedling traits. *Sarhad J. Agric*. 29: 21–27.

Ahmed, H. G. M., M. Sajjad, M. Li, M. A. Azmat, M. Rizwan, R. H. Maqsood and S. H. Khan. 2019. Selection criteria for drought-tolerant bread wheat genotypes at seedling stage. *Sustainability*. 11: 2584. [CrossRef]

Akinwale, R. O., F. E. Awosanmi, O. O. Ogunniyi and A. O. Fadoju. 2017. Determinants of drought tolerance at seedling stage in early and extra-early maize hybrids. *Maydica*. (62) (9). : https://www.researchgate.net/publication/317218497:

Arisandy, P., W. B. Suwarno and M. Azrai. 2017. Evaluation of drought tolerance in maize hybrids using stress tolerance indices. *Int. J. Agron. Agric. Res*. 4: 46–54

Avramova, V., K. A. Nagel, E. H. D.Bustos, M. DuPlessis, F. Fiorani, T.S. Gerrit and A. Beemster. 2016. Screening for drought tolerance of maize hybrids by multi-scale analysis of root and shoot traits at the seedling stage. *J. Exp. Bot*. 67: 2453–2466. [Cross Ref]

Badr, A. and W. Brüggmann. 2020. Comparative analysis of drought stress response of maize genotypes using chlorophyll fluorescence measurements and leaf relative water content. *Photosynthetica*. 58: 638–645. [CrossRef]

Bansal, U. K., R. G. Saivi, N. S. Rani and A. Kaur. 1999. Genetic divergence in quality rice. *Oryza.* 36 (1): 20-23.

Bashir, N., S. Mahmood, Z. U. Zafar, S. Rasul, H. H. Manzoor and H. U. R. Athar. 2016. Is drought tolerance in maize (*Zea mays* L.) cultivars at the juvenile stage maintained at the reproductive stage? *Pak. J. Bot.* 48: 1385–1392.

Bell, J. 2017. Corn Growth Stages and Development; Texas A & M Agri Life Extension and Research Agronomist, Amarillo: Lubbock, TX, USA.

Cao, L. Z. X. and B. X. P. Wj. 2004. Discuss on evaluating method to drought-resistance of maize in seedling stage. *J. Maize Sci*. 12: 73–75.

Cooper, M., C. Gho, R. Leafgren, T. Tang and C. Messina. 2014. Breeding drought-tolerant maize hybrids for the US corn-belt: Discovery to product. *J. Exp. Bot*. 65: 6191–6204. [CrossRef]

Desalegn, B., E. Kebede, H. Legesse and T. Fit. 2023. Sugarcane productivity and sugar yield improvement: Selecting variety, nitrogen fertilizer rate, and bioregulartor as a first-line treatment. *Heliyon*. 9(4): e15520.

Dhanda, S., G. Sethi and R. Behl. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agron. Crop Sci.* 190: 6–12. [CrossRef]

Dodd, G. L.and L. A. Donovan. 1999. Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. *Am. J. Bot*. PMID: 1044939486: 1146-1153.

Edmeades, G. O. 2018. A feature in james, drought tolerance in maize: An emerging reality. In Global Status of Commercialized Biotech/GM Crops. *Clive, J*., Ed.; ISAAA: Ithaca, NY, USA.

Edmeades, G.O. 2013. Progress in Achieving and Delivering Drought Tolerance in Maize an Update; ISAAA: Ithaca, NY, USA.

El-Deeb, A. and N. Mohamed. 1999. Factor and cluster analysis for some quantitative characters in sesame (*Sesamum indicum* L.). In Proceedings of the Annual Conference ISSR, Cairo University, Cairo, Egypt, 4–6 December. Volume 34.

Fahad, S. A., A. Bajwa, U. Nazir, S. A. Anjum, A. Farooq, A. Zohaib. 2017. Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Sci.* 8:1147. doi:10.3389/fpls.2017.01147.

Faisal, S., S. Mujtaba, M. Khan and W. Mahboob. 2017. Morpho-Physiological assessment of wheat (*Triticum aestivum* L.) genotypes for drought stress tolerance at seedling stage. *Pak. J. Bot*. 49: 445- 452.

Fakorede, M.A.B. and B. O. Opeke. 2008. Weather factors affecting the response of maize to planting dates in a tropical rainforest location. *Exp. Agric*. 21: 31–40. [CrossRef]

Farooq, M. A. Wahid, N. Kobayashi, D. Fujita and S. M. A. Basra. 2009. Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev*. 29: 185–212. [CrossRef]

Gola, A.Q. 2018. Effect of salt (NaCl) stress on germination and early seedling growth of muskmelon (*Cucumis melo* L.) Pure and Applied Biology 7(4): 273-282. doi:10.19045/bspab.2018.700218.

Grzesiak, S., M. T. Grzesiak, T. Hura, I. Marcinska, and A. Rzepka. 2012. Changes in root system structure, leaf matric potential and gas exchange of maize and triticale seedlings affected by soil compaction. *Environ. Exp. Bot*. 88, 2-10.

Hammer, K. 1998. Agrarbiodiversität und pflanzengenetische ressourcen. In Herausforderung und Lösungsansatz. Schriften zu Genetischen Ressourcen; Informationszentrum für Genetische Ressourcen (IGR) u. Zentralstelle für Agrardokumentation und Information (ZADI): Bonn, Germany, Volume 10, ISSN 0948-8332.

Hedge, S. G. and C. S. Patil. 2000. Genetic divergence in rainfed rice. *Karnataka J. Agril. Sci.* 13(3): 549-553.

Hefny, M., A. Ali, T. Byoumi, M. Al-Ashry and S. Okasha. 2017. Classification of genetic diversity for drought tolerance in maize genotypes through principal component analysis. *J. Agric. Sci. Belgrade.* 62: 213–227. [CrossRef]

Iqbal, T., I. Hussain, N. Ahmad, M. Nauman, M. Ali and S. Saeed. 2018. Genetic variability, correlation and cluster analysis in elite lines of rice. *J. of Sci. Agric.* 2:85-91. doi:10.25081/jsa.2018.v2.900

Islam, M. M., E. Kayesh, E. Zaman,T. A. Urmi and M. M. Haque. 2018. Evaluation of rice (*Oryza sativa* L.) genotypes for drought tolerance at germination and early seedling stage. *The Agriculturists.*16(1): 44-54.

ISTA, 2017. International Rules for Seed Testing; International Seed Testing Association: Bassersdorf, Switzerland.

Jha, S. K., I. P. Awasthi and D. M. Maurya. 1999. Genetic divergence in wild rice germplasm of eastern U. P. India. *Oryza*. 36 (20): 157-158.

Khandoker, S., M. A. Monayem Miah, M. A., Rashid, M. A., Khatun and M. Z. A. Talukder. 2018. Profitability of winter maize cultivation in drought prone areas of Bangladesh. *Bangladesh J. Agril. Res.* 43(2): 219-234.

Kosar, F. N., A. Akram, M. Ashraf, M. Sadiq and F. Al-Qurainy. 2018. Trehalose-induced improvement in growth, photosynthetic characteristics and levels of some key osmoprotectants in sunflower (*Helianthus* *annuus* L.) under drought stress. *Pak. J. of Bot.* 50(3):955-961.

Leishman, M. R. and M. Westoby. 1994. The role of seed size in seedling establishment in dry soil conditions- Experimental evidence from semi-arid species. *J. Ecol.* 82: 249-258.[CrossRef]

Liu, M., M. Li, K. Liu, and N. Sui. 2013. Effects of drought stress on seed germination and seedling growth of different maize varieties. *J. Agric. Sci.* 7. [Cross Ref]

Maiti, R. K., L. E. Maiti, S. Maiti, A. M. Maiti, M. Maiti, and H. Maiti. 1996. Genotypic variability in maize cultivars (*Zea mays* L.) for resistance to drought and salinity at the seedling stage. *J. Plant Physiol*. 148: 741–744. [CrossRef]

Mannan, M. A. and M. A. Mamun. 2018. Selection of vegetable soybean suitable for Bangladesh through Multivariate analysis. *Ann. Bangladesh Agric..* 22(2): 51-57.

Mannan, M. A., M. A.Karim, Q. A. Khaliq, M. M. Haque, M. A. K.Mian and J. U. Ahmed. 2010. Assessment of genetic divergence in salt tolerance of soybean (*Glycine max* L.) genotypes. *J. Crop Sci. Biotech*., 13(1): 33-38. DOI: 10.1007/s12892-009-0091-y

Mass, E. V. and G. J. Hoffman. 1977. Crop salt tolerance-current assessment. J. Irrig. Drainage Div. ASCE 103 (IR2): 115-134.

Meeks, M., S. Murray,S. Hague and D. Hays. 2013. Measuring maize seedling drought response in search of tolerant germplasm.*Agronomy*. 3: 135–147. [Cross Ref]

Osborn, D. and J. N. Ferguson. 2019. Climate change and abiotic stress mechanisms in plants. *Emerg. Topics Life Sci*.3: 165–181.

Pradhan, K. and A. Roy. 1990. Genetic divergence in rice. *Oryza*, 27: 415-418.

Rojas, W. and P. B. Figueroa. 2000. Multivariate analysis of the genetic diversity of Bolivian quinua germplasm. *Plant Gen. Resources Newlt*., 122: 16-23.

Sidari, M., C. Mallamaci and A. Muscolo. 2008. Drought, salinity and heat differently affect seed germination of *Pinus pinea*. *J. For. Res.* 13: 326-330.

Sisodia, B. and V. Rai. 2017. An application of principal component analysis for pre-harvest forecast model for wheat crop based on biometrical characters. *Int. Res. J. Agric. Econ. Stat*. 8: 83–87.

Singh, A. K., H.T. Stalker and J. P. Moss 1991. 4-Cytogenetics and use of alien genetic variation in groundnut improvement. Developments in plant genetics and breeding, Volume 2, Part B, Pp. 65-77, ISSN 0168-7972, ISBN 9780444882608, https://doi.org/10.1016/B978-0-444-88260-8.50009-4.

Suzuki, N.; R. M. Rivero, V. Shulaev, E. Blumwald and R. Mittler. 2014. Abiotic and biotic stress combinations. *New Phytol.* 203: 32–43. [CrossRef] [PubMed]

Tarawneh, R. A., F. Szira, I. Monostori, A. Behrens, A. M. Alqudah, S. Thumm, U. Lohwasser, M. S. Röder, A. Börner and M. Nagel. 2019. Genetic analysis of drought response of wheat following either chemical desiccation or the use of a rain-out shelter. *J. Appl. Genet*. 60: 137–146. [CrossRef]

Tesfaye, K., G. Krusemanb, J. E. Cairnsc, M. Zaman-Allahc, D. Wegarya, P. H. Zaidid, K. J. Bootee, D. Rahutb and O. Erensteinb. 2018. Potential benefits of drought and heat tolerance for adapting maize to climate change in tropical environments. *Clim. Risk Manag*. 19: 106–119. [CrossRef]

USDA (United State Department of Agriculture), 2020. Production of maize in Bangladesh, 2011-2020". Data source: http://apps.fas.usda.gov/psdonline/app/index.html.

Wang, J., S. Li and K. E. Li. 2001. Effects of water limited deficit stress during different growth stages on leaf protective enzymes of winter wheat. *Acta Bot. Boreali Occident. Sin*. 21: 47–52

Webber, D., M. Babush, N. Schori-Eyal, A. Vazeou-Nieuwenhuis, M. Hettiarachchi, and J. J. Bélanger.2018. The Road to Extremism: Field and Experimental Evidence That Significance Loss-Induced Need for Closure Fosters Radicalization. *J. of Personality and Social Psychology*.114,270. https://doi.org/10.1037/pspi0000111

Yang, Z. Y., B. Z. Zhou,Y. Zhou, X. G. Ge, X.M. Wang and Y. H. Cao. 2018. Effects of Drought Stress Simulated by PEG on Seed Germination and Growth Physiological Characteristics of Phyllostachys edulis. *Forest Research* 31(6):47-54. doi:10.13275/j.cnki.lykxyj.2018.06.007.

Zheng, M., Y. Tao, S. Hussain, Q. Jiang, S. Peng, and J. Huang. 2016. Seed priming in dry direct-seeded rice: consequences for emergence, seedling growth and associated metabolic events under drought stress. *Plant Growth Regulation* 78(2): 167-178. doi:10.1007/s10725-015-0083-5.