

Multi-trait Selection for Excess Moisture Tolerance in Maize at Seedling Stage

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Introduction

Maize is an important and widely cultivated crop for food, feed, forage and fuel across tropical and temperate regions of the world (Xu, *et al.*, 2009). In Bangladesh, the maize crop grown during the *Kharif* I (spring) and *Kharif* II (summer-rainy) season occasionally faces extreme climatic conditions and abiotic pressure limiting crop growth, and eventually yield potential. Among the abiotic stresses, excessive soil moisture (EM), caused by flooding, water-logging or a high water table, is one of the most important constraints for maize production and productivity in Bangladesh. EM causes major changes in physical and chemical properties in rhizosphere (Zaidi *et al.*, 2003). Gaseous diffusion rates in flooded soil is about 100 times lower than air (Kennedy *et al.*, 1992), and respiration of plant roots, soil micro-flora and fauna leads rapid exhaustion of soil oxygen, and thereby causes hypoxia/anoxia condition. There is no proper ventilation system in maize plants for gaseous exchange between aboveground plant parts and inundated roots. Therefore, plant roots suffer with extreme oxygen stress, hypoxia followed by anoxia, whenever it faces prolonged (>3 days) EM (Zaidi *et al.*, 2003). Considering these facts, the present study was undertaken to evaluate the degree of EM tolerance in maize inbred lines at the seedling stage.

Materials and methods

The experiment was conducted at the Plant Breeding Farm, Gazipur, Bangladesh, on 210 maize inbred lines. Screening was done using the cup method (Zaidi *et al.*, 2004); the cup size was 500 ml (12 cm height, 10 cm diameter). The inbred lines were exposed to EM stress after germination. Each plastic tray (40cm x 28cm x 14cm) contained six cups. Four cups remained under normal moisture (NM) condition outside the tray (as check). Different morphological parameters were recorded during and after the moisture stress treatment.

For individual and combined analyses, a linear model using the alpha-lattice design (Messmer, *et al.*, 2011) with three replications was used. Statistical analyses were performed using R-statistics software Version 3.0.2 for Windows (R Development Core Team, 2013). For each trait, components of phenotypic variance were estimated from analysis of variance

(ANOVA) using restricted maximum likelihood method. The linear mixed effect “lmer” command in the lme4 package was used to estimate the variance components. The best linear, unbiased predictors (BLUPs) were estimated for each line and for each trait using the same models used to estimate variance components. The random effect “ranef ” command in the lme4 package, was used to estimate BLUPs for all terms in the model. The genotypic, phenotypic correlation and broad-sense heritability were estimated using agricolae R-package. The relative trait value for each trait was calculated by dividing trait value under the EM treatment with the corresponding trait value under the NM treatment. By using the relative trait value, a G×T biplot was constructed from a two-way matrix of eight traits and 210 genotypes by STAR software. The first two PCs were plotted. Genotypes were also plotted according to scores on each PC, with traits plotted on the basis of the eigenvectors on each PC.

The relative trait values were standardized and subjected to PCA using SAS. The PCs whose eigenvalues were larger than one were retained (Bayuelo-Jiménez, *et al.*, 2011) and used to calculate EM tolerance indicator (EMTI_all) using the formula:

$$EMTI = \sum_{i=1}^n PC_i * CR_i$$

where n is the number of PCs whose eigenvalues were larger than 1 (Bayuelo-Jiménez, *et al.*, 2011), and CR (contribution rate) was the rate for trait variation. Based on EMTI_{all} at 5 percent selection intensity, we selected at least 14 most tolerant and 14 most susceptible lines. The inbred lines showing better trait performance under EM were called as EM responders, while those with opposite performance were called as non-responders, following Bayuelo-Jiménez *et al.* (2011). In this study, the trait performance under the NM conditions was defined as NMPI (NM performance indicator), which was calculated as follows: the standardized trait values for each trait were subjected to PC analysis and the PCs whose eigenvalues were larger than 1 were used to calculate NMPI following formula:

$$NMPI = \sum_{i=1}^n PC_i * CR_i;$$

where n is the number of PCs whose eigen values were larger than 1, and CR was the rate for trait variation. The tested accessions could be classified

into four groups based on the two criteria, P-response and EM tolerance, which can be called as tolerant and responsive group (TR), sensitive and responsive group (SR), tolerant and non-responsive group (TNR), and sensitive and non-responsive group (SNR), respectively. From the extreme lines of each of the four groups, the top 5 percent were selected based on NMPI and EMTI_all-values

Results

Descriptive statistics and ANOVA revealed a wide range of variability in the germplasm (Table 1). The heritability (h^2) ranged from 0.59 to 0.93. Genetic variance was found low to moderate (0.15–0.42) for most traits (Table 2). The relatively low genetic variances may be a reflection of the fact that the germplasm panel consisted of breeding lines that experienced strong selection. In addition, low genetic variance may reflect complex genetic inheritance and/or a strong environmental influence on trait expression. All of the traits fitted within the normal distribution in both EM and NM conditions, except some traits showed skewness (Figure 1). BLUPs revealed strong positive correlation between BLUPs and means, which is shrinkage of the BLUPs toward the mean of population (Figure 2). Shoot length (SL) was significantly correlated with other root traits, indicating that direct selection based on SL might be sufficient to improve other traits (Table 3). The first two principal components explained about 84.55% of the total variation among lines for the seven seedling traits. The relative magnitudes of eigenvectors for the first principal component was 61.43%, explained mostly by root length, shoot length, root dry weight, shoot dry weight, root shoot ratio and total dry weight measurements. The genotype \times traits biplot showed superior genotypes with a relatively greater expression of combinations of favorable traits

(Figure 3). The results suggest that MRL, MSL, SDW and RDW could be helpful for identifying the superior genotypes in elite germplasm. Based on both selection traits LPTI_all and NPPI at 5% of threshold level, we selected seven, three, six and three number of inbred lines from the groups of TR, TNR, SR, and SNR, respectively (Figure 4). These selected lines could be of potential use for improvement of EM tolerance in maize.

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Table 1. Descriptive statistics of measured traits under EM and NM treatments.

Descriptive Statistics	Traits measured															
	MSL		MRL		SFW		RFW		SDW		RDW		RSR		TDM	
	NM	EM	NM	EM	NM	EM	NM	EM	NM	EM	NM	EM	NM	EM	NM	EM
Mean	59.5	35.9	38.6	45.4	10.0	3.11	3.10	2.22	0.72	0.35	0.20	0.22	0.76	1.21	0.94	0.55
Sd	11.3	6.08	8.54	10.2	3.90	1.13	1.39	0.86	0.28	0.11	0.10	0.07	0.18	0.29	0.36	0.17
Variance	129.	36.9	72.9	105.	15.1	1.27	1.92	0.74	0.08	0.01	0.01	0.00	0.03	0.08	0.13	0.03
Kurtosis	0.20	-0.1	-0.1	-0.1	0.39	0.15	0.99	0.15	0.75	1.08	1.34	1.31	0.98	0.81	0.94	0.58
Skewness	0.28	-0.1	0.22	0.07	0.39	0.32	0.79	0.44	0.67	0.39	0.93	0.69	0.54	0.40	0.72	0.28
Minimum	23.2	18.1	18.0	6.21	1.33	0.21	0.14	0.14	0.13	0.03	0.02	0.03	0.27	0.20	0.17	0.07
Maximum	93.2	55.3	68.7	78.8	26.2	7.41	9.11	5.47	1.85	0.88	0.67	0.54	1.64	2.55	2.45	1.38
CV (%)	20.7	15.8	21.2	22.5	38.8	36.3	44.8	38.7	38.8	31.4	50.0	31.8	23.6	23.9	38.3	30.9
h^2	0.67	0.59	0.75	0.69	0.72	0.78	0.89	0.76	0.79	0.82	0.81	0.87	0.67	0.70	0.93	0.80

MSL=maximum shoot length, MRL= maximum root length, SFW=shoot fresh weight, RFW=root fresh weight, SDW=shoot dry weight, RDW=root dry weight, RSR=root shoot ratio, TDM=total dry matter, NM=normal phosphorus, EM= low-phosphorus, Sd= standard deviation, CV= coefficient of variation, h^2 =Heritability

Table 2. Estimates of variance components (proportion of the total).

Traits	Genotype	Treatment	Genotype × Treatment	Residual
Maximum shoot length (cm)	0.19	0.59	0.12	0.1
Maximum root length (cm)	0.42	0.12	0.30	0.16
Shoot fresh weight (g)	0.15	0.55	0.19	0.11
Root fresh weight (g)	0.27	0.29	0.23	0.21
Shoot dry weight (g)	0.17	0.39	0.26	0.18
Root dry weight (g)	0.26	0.13	0.43	0.18
Root:shoot ratio	0.16	0.49	0.19	0.16
Total dry matter (g)	0.22	0.38	0.24	0.16

Table 3. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlations under EM conditions

	MSL	MRL	SFW	RFW	SDW	RDW	RSR	TDM
MSL		0.28*	0.76 **	0.79**	0.65**	0.51**	-0.52 **	0.71**
MRL	0.30*		0.41 **	0.59**	0.43**	0.44**	0.76**	0.52**
SFW	0.73**	0.40**		0.73**	0.87**	0.66**	-0.11	0.83**
RFW	0.72**	0.57**	0.71**		0.76**	0.73**	0.23	0.87**
SDW	0.77**	0.41**	0.82**	0.74**		0.71**	-0.02	0.97**
RDW	0.75**	0.43**	0.61**	0.73**	0.71**		0.07	0.89**
RSR	-0.51**	0.75**	-0.11	0.20	-0.04 ^s	0.06		0.11
TDM	0.53**	0.55**	0.80**	0.73**	0.91**	0.89**	0.10	

MSL=maximum shoot length, MRL= maximum root length, SFW=shoot fresh weight, RFW=root fresh weight, SDW=shoot dry weight, RDW=root dry weight, RSR=root: shoot ratio, TDM=total dry matter, NP=normal phosphorus, LP= low-phosphorus, *, ** indicate significance at 5% and 1% level, respectively

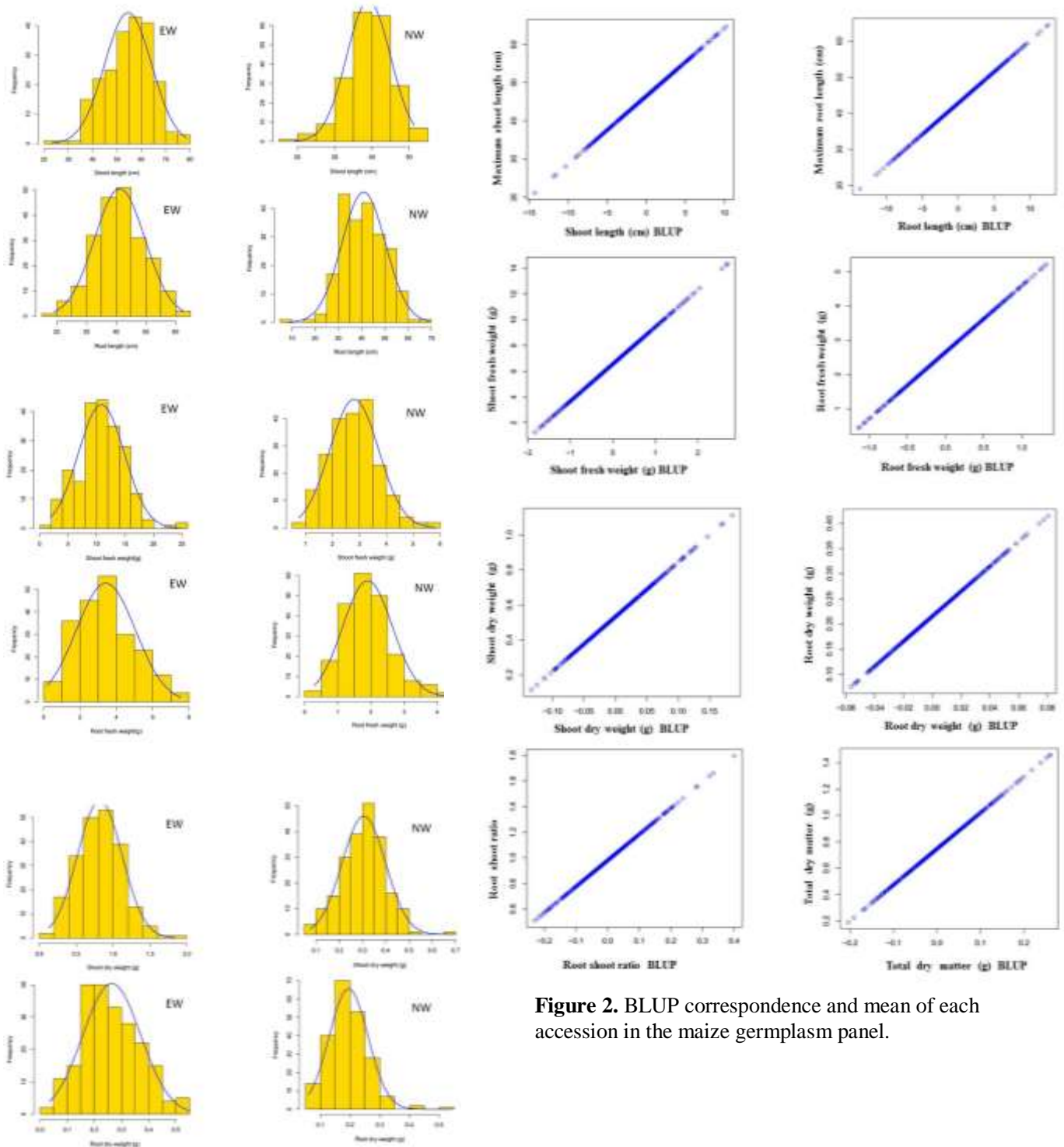


Figure 1. Frequency distribution for traits measured under EM and NM conditions.

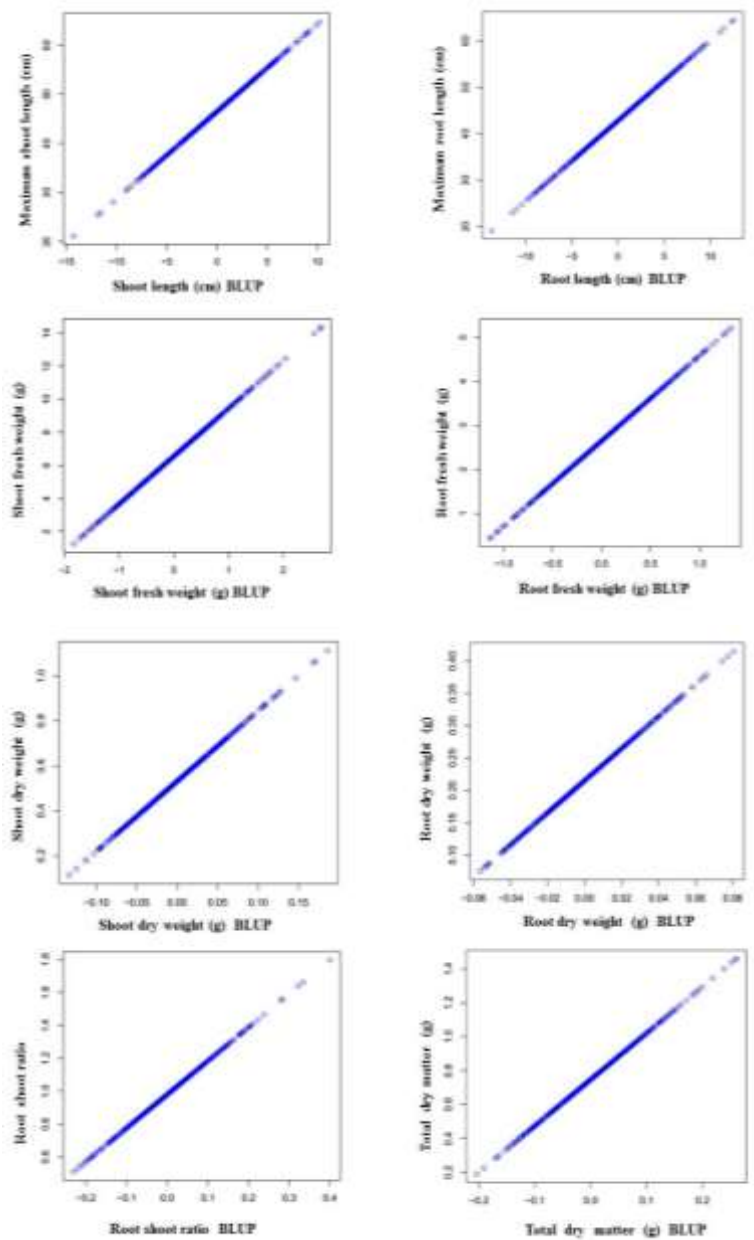


Figure 2. BLUP correspondence and mean of each accession in the maize germplasm panel.

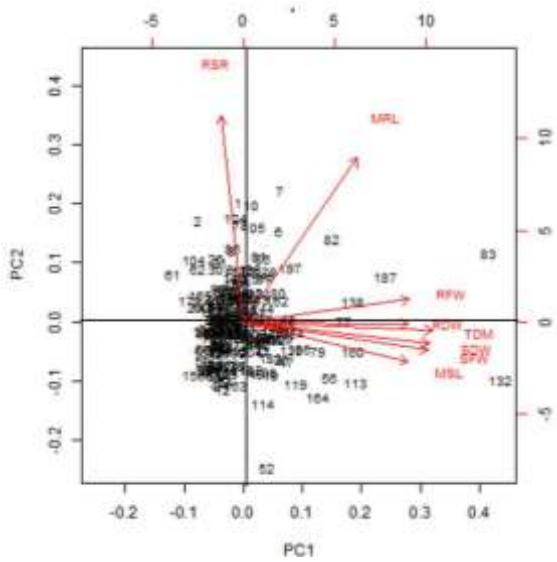


Figure 3. Genotype \times Trait biplot based on seedling traits of maize.

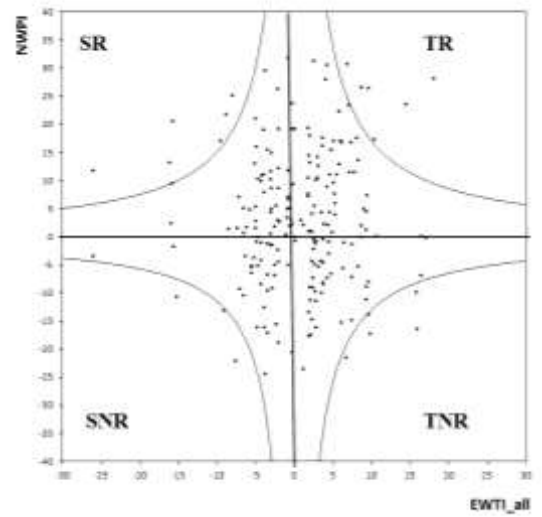


Figure 4. Classifying of the germplasm based on EM tolerance and NM responsiveness