International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 3, p. 344-351, 2020

INNSPUB

RESEARCH PAPER OPEN ACCESS

Screening at an early growth stage in maize (*Zea mays* L.) for detection of drought tolerant genotypes

Usman Saleem[1*], Naeem Akhtar[1], Muhammad Afzal[1], Salman Saleem[2], Muhammad Azmat[3], Shadab Shaukat[1]

[1] *College of Agriculture, University of Sargodha, Sargodha, Pakistan*

[2] *MSM, National Agriculture Research Centre, Islamabad, Pakistan*

[3] *University of Agriculture, Faisalabad, Pakistan*

Key words: Maize, Correlation, moisture, heritability, correlation.

http://dx.doi.org/10.12692/ijb/16.3.344-351 Article published on March 29, 2020

Abstract

Maize is the largest produced grain on earth and plays significant role in food security. The study was aimed to explore the genetic variation and the degree to which seedling characters of maize are associated with each other under normal and water deficit environment. Fifty maize genotypes were sown in sand culture in Completely Randomized Design under factorial arrangement with three repeats under different water regimes. Pooled analysis of variance (two way) showed that all sources of variations were statistically significant except interaction of inbred lines × treatments for leaf relative water content (LRWC). Inbred line YP-12 exhibited maximum values for LRWC (68 %), Root length (34.23 cm), FRTWT (12.36 g), DRTWT (5.68 g) and root to shoot ratio (3.39) under water deficit regime. Maximum genetic advance (9.81) and heritability (93%) was recorded for dry root weight (DRTWT) under water deficit environment. Strong significant positive association was detected between fresh shoot weight (FRTWT), Leaf relative water content (LRWC) and fresh shoot weight (FSTWT) under water deficit environment. The YP-12 genotype considered as tolerant inbred line/genotype and could be used in maize developing program to tailor drought tolerant maize hybrids.

* **Corresponding Author:** Usman Saleem ✉ usman.saleem@uos.edu.pk

Introduction

Maize is the largest produced grain on earth and plays significant role in food security. Large range of products made from maize share considerable portion in global economy (Haq *et al.,* 2015). The increasing demand of maize and maize products can only be fulfilled by increasing the per unit area yield of crop. However, the effect of yield limiting factors are increasing drastically with every passing day and drought stress is the single major factor restraining high grain yield for maize than any other factor globally (Nisar *et al*., 2016).

Optimum moisture availability is necessary for plants to carry out all the growth and physiological processes (Saleem *et al.,* 2016a) and sub-optimal moisture availability results in decreased nutrient uptake, cell size, turgor potential, plant growth and ultimately yield losses (Saleem *et al*., 2016b). At cellular level the reduced water availability increases the production of ROS (reactive oxygen species) which react to the cell organelles, resulting in cell death (Cruz de Carvalho, 2008).

Plant roots are the primary organ which respond to the water shortage in soil, resultantly the root growth is increased to extract more water and shoot growth is inhibited (Sharp, 2002) and the genotypes which develop better root mass are able to extract additional water from soil (Rosas 2005). Stomatal closure is one of the first reaction of maize plant to counter water stress by minimizing losses through transpiration, likewise the RWC (relative water content) is also reduced during stress and genotypes which maintain good RWC under stress are considered as drought tolerant (Ahmad *et al.,* (2016).

The genotypes showing good tolerance to drought stress can be used directly or as source material for incorporating tolerance for drought in breeding material. The response of plant to water stress is almost same at all the growth stage but at seedling and later stages the plants are more sensitive (Saleem *et al.,* 2011). Screening at early growth stage provides an opportunity of pre-selection of genotypes/inbred lines before their evaluation in field for water limited regime (Rekha *et al.,* 2011). The correlation analysis is very useful to understand the nature of association in the traits and systematic selection based on correlation can yield better genetic gains in short span of time. The existing study was formulated to explore the variation present in the available genotypes/germplasm and the degree to which seedling parameters of maize are linked with each other under different water regimes.

Material and methods

The study consisted of fifty inbred lines assembled from different indigenous sources (Maize and Millet Research Institute, Yousafwala, Sahiwal, Maize Research Station, Faisalabad and Aliakbar Seeds (Pvt.) Ltd.) was conducted in the glass house of College of Agriculture, University of Sargodha during February 2017. Seeds of inbred lines were planted in polyethene bags (18 × 10 cm) filled with equal quantity of sand in Completely Randomized Design under factorial arrangement with three repeats under water deficit and normal conditions. Seeds were placed at a uniform depth of 2.5 cm. Temperature and relative humidity during the experimental period ranged from 19.7 °C to 24.6 °C and 51.8 % to 65.5 % respectively. Ten seedling of each genotype were maintained in each repeat under both moisture treatments i.e normal and water deficit conditions. After one week of sowing 100 ml Hogland solution was applied to both the treatments, however after two weeks of sowing distilled water was used to irrigate the normal set only.

Seedlings were uprooted after the completion of third week by applying water to avoid any injury to the seedlings. Seedlings were carefully washed with distilled water after uprooting to eliminate any sand particles in the root zone. Data was recorded on Shoot length (STL), root length (RTL), fresh shoot weight (FSTWT), fresh root weight (FRTWT), dry shoot weight (DSTWT), dry root weight (DRTWT), root to shoot ratio (RTSTR) and leaf relative water content (LRWC). Leaf relative water content (LRWC) was calculated by the method proposed by Matta and

Lamaina, 2001.

$$\text{Leaf relative water contents (LRWC)} = \frac{(\text{Fresh weight}) - (\text{Dry weight})}{(\text{Turgid weight}) - (\text{Dry weight})} \times 100$$

Leaf samples were kept in water for 24 hrs to record turgid weight and dry weight was recorded after placing the leaf samples at 65 °C for 48 hrs.

Statistical studies

Data collected for each parameter were analysed for analysis of variance using SAS software version 9.2 (SAS, 2008). Broad sense heritability (h^2) for the parameter under studied were concluded by the formula defined by Allard (1960).

$h^2_{bs} = (\sigma^2_g) / (\sigma^2_p) \times 100$

h^2_{bs} represents the heritability broad sense whereas σ^2_p and σ^2_g represent phenotypic and genotypic variances respectively. Genetic advance (GA) was calculated as formulated by Johnson *et al.*, 1995.

$$Genetic\ advance\ (GA) = K(\sigma p)\ h$$

Where, K represents the selection intensity at 5% i.e 2.06 and σ_p and h represent phenotypic standard deviation of the parameter and broad sense heritability respectively. Phenotypic and genotypic correlation was determined as described by Kown and Torrie, 1964.

Result and discussion

Analysis of variance

Results of variance analysis (one way) exhibited in Table 1 indicated that the inbred lines/genotypes significantly differ from each other under normal and water limited regimes. Similarly pooled analysis of variance (two way) showed that all sources of variations were statistically significant except interaction of inbred lines × treatments for leaf relative water content (LRWC) (Table 1).

Table 1. Mean squares of the seedling traits under normal and water limited regimes.

	LRWC	RL	SL	FRTWT	FSTWT	DRTWT	DSTWT	RTSTR
Analysis of Variance (One Way)								
Normal condition								
Genotypes	0.11**	65.89**	39.34**	25.54**	61.68**	9.083**	1.39**	1.568**
Error	0.02	10.101	6.121	0.054	0.724	0.023	0.199	0.04
Water Deficit Condition								
Genotypes	0.09**	42.86**	19.71**	21.2**	32.54**	4.768**	0.984**	1.589**
Error	0.01	3.304	1.403	0.6	0.739	0.041	0.061	0.024
Analysis of Variance (Two way)								
Treatments	0.395**	995.15**	1565.04**	233.41**	632.28**	258.18**	211.39**	1.216**
Inbred lines	0.036**	50.45**	75.41**	14.38**	28.49**	17.7**	11.54**	2.34**
Treat.× Inbred	0.002	22.19**	38.19**	1.42**	4.39**	1.35**	1.11**	0.325**
Error	0.004	3.98	5.24	0.021	0.342	0.028	0.11	0.021

** = significant at probability level of 0.01, LRWC= Leaf relative water content, RL= Root length, SL= Shoot length, FRTWT= Fresh root weight, FSTWT= Fresh shoot weight, DRTWT, Dry root weight, DSTWT= Dry shoot weight, RTSTR= Root to shoot ratio.

Non-significant interaction of inbred lines × treatments for LRWC was also stated by Haq *et al.*, 2015. Presence of genetic variability in the germplasm to breed water stress plant was also supported by wang *et al.*, 2011.

Mean performance of inbred lines

Maximum leaf relative water contents (LRWC) of 75% and 68% were recorded in inbred line YP-12 and minimum of 52% and 39% in inbred line US-17 for normal and water limited regimes (Table 2).

Table 2. Minimum and maximum mean values of different traits under normal and water limited regimes.

	Normal		Water Deficit	
Parameters	Minimum	Maximum	Minimum	Maximum
LRWC	0.52 (US-17)	0.75 (YP-12)	0.39 (US-17)	0.68 (YP-12)
RL	20.55 (US-25)	37.41 (YP-10)	16.13 (US-17)	34.23 (YP-12)
SL	19.42 (YP-13)	35.21 (US-15)	17.81 (YP-13)	24.32 (US-25)
FRTWT	6.01 (US-7)	18.23 (YP-2)	4.35 (US-17)	12.36 (YP-12)
FSTWT	8.01 (US-29)	23.72 (YP-2)	6.01 (US-19)	14.87 (YP-9)
DRTWT	2.29 (US-17)	8.53 (YP-9)	1.18 (US-17)	5.68 (YP-12)
DSTWT	2.01 (US-24)	4.53 (US-29)	1.35 (US-19)	3.19 (YP-9)
RTSTR	0.84 (US-14)	2.99 (MS-8)	0.49 (US-17)	3.39 (YP-12)

LRWC= Leaf relative water content, RL= Root length, SL= Shoot length, FRTWT= Fresh root weight, FSTWT= Fresh shoot weight, DRTWT, Dry root weight, DSTWT= Dry shoot weight, RTSTR= Root to shoot ratio.

The presence of high LRWC under water stress regime is the indication of tolerance against water stress in crop plants. (Clarke and McCaig, 1982). Maximum shoot length (SL) of 35.21 cm was recorded for inbred US-15 under normal water regimes whereas US-25 gave 24.32 cm under water deficit conditions. However minimum shoot length (SL) of 19.42 cm and 17.81 cm was recorded in inbred lineYP-13 under normal and water deficit conditions. Reduction in shoot length (SL) under water deficit environment was also noticed by Rai (1984) and Ramdan *et al.,* 1985. Performance of maize inbred line YP-10 (37.41 cm) and YP-12 (34.23 cm) was maximum for root length (RL) whereas inbred line US-25 (20.55 cm) and US-17 (16.13 cm) gave lowest root length under normal and water deficit environments. Similar behaviour of inbred lines was reported by Mehdi *et al.,* 2001.

They also suggested that the use of root parameters for selection against drought stress can be helpful in selection of drought tolerant genotypes and these selected genotypes can be used in a breeding program to develop tolerant/resistant OPVs and hybrids for water deficient areas. Maximum fresh shoot weight (FSTWT) of 23.72 g and 14.87 g was recorded by inbred lines YP-2 and YP-9 whereas lowest values were recorded by US-29 (8.01 g) and US-19 (6.01 g) under normal and water deficit regimes. FSTWT was significantly affected by water stress conditions in most of the inbred lines, whereas higher values of FSTWT in YP-2 and YP-9 inbred lines might be due to accretion of inorganic and organic solutes (Ahsan *el al.,* 2011). Performance of YP-2 (18.23 g) and YP-12 (12.36 g) were maximum for fresh root weight (FRTWT) whereas US-7 (6.01 g) and US-17 (4.35 g) showed minimum performance under normal and limited water environments respectively. Aggarwal and Sinha, 1983 also reported similar results. Maximum dry shoot weight (DSTWT) of 4.53 g and 3.19 g were recorded for inbred line US-29 and YP-9 whereas minimum DSTWT of 2.01 g and 1.35 g was recorded for inbred lines US-24 and US-19. Inbred lines YP-9 and YP-12 produced maximum dry root weight (DRTWT) of 8.53 g and 5.68 g, whereas inbred line US-17 gave minimum values of 2.29 g and 1.19 g under irrigated and water stress environment respectively. Similar response of inbred lines was stated by Haq *et al.*, 2015. Root to shoot ratio (RTSTR) was also significantly affected under water deficit regime and increasing behaviour was observed in RTSTR under water limited condition (Thomas and Howarth, 2000). Stress tolerant plants/seedlings showed high RTSTR as compared to sensitive lines.

RTSTR ranged from 0.84 (US-14) to 2.99 (MS-8) under normal irrigation condition as compared to 0.49 (US-17) to 0.68 (YP-12) in stress regimes. Increase in root to shoot ratio (RTSTR) was also reported by Wu and Cosgrove, 2000). This was the only parameter which raised under stress condition among all the other studied parameters. The inbred line YP-12 performed better for FRTWT, DRTWT and LRWC under limited water regimes and hence was considered as tolerant genotype and can be used in a breeding program to tailor maize hybrids for water limited environments. Scientists like Tavakol and Pakniyat, 2007 also reported that DRTWT, LRWC and RTSTR are suitable parameters to select tolerant genotypes at early growth stages. Lower values of FRTWT, LRWC and DRTWT produced by inbred line US-17 are evidence of its sensitivity for water stress.

Table 3. Heritability (h^2_{bs}) and genetic advance of the traits under normal and water limited regimes.

Parameters	LRWC	RL	SL	FRTWT	FSTWT	DRTWT	DSTWT	RTSTR
Normal condition								
Heritability (h^2_{bs}) %	60	65	64	94	92	94	66	91
Genetic Advance (GA)	7.03	3.15	4.50	10.78	4.13	11.30	7.61	8.09
Water Deficit Condition								
Heritability (h^2_{bs}) %	72	79	81	88	90	93	83	91
Genetic Advance (GA)	5.23	3.01	5.20	9.76	3.89	9.81	5.01	7.18

LRWC= Leaf relative water content, RL= Root length, SL= Shoot length, FRTWT= Fresh root weight, FSTWT= Fresh shoot weight, DRTWT, Dry root weight, DSTWT= Dry shoot weight, RTSTR= Root to shoot ratio.

Heritability, Genetic advance

The success of any breeding project largely dependents on heritability of the traits, higher the heritability the better will be the response to selection. The degree of heritability ultimately determines the improvement that can be attained through selection (Wang *et al.* 2011).

The heritability ranged from 60% (LRWC) to 94% (FRTWT and DRTWT) under normal treatment whereas 72% (LRWC) to 93% (DRTWT) under water deficit regime in the studied material and the heritability estimates were classified as suggested by Johnson *et al.,* 1995 i.e heritability less than 30 was graded as low, 30%-60% as moderate and more than 60% as high. Moderate to high heritability was observed for all the parameters under normal irrigation conditions whereas, whereas under water deficit regimes high heritability was verified for all the parameters as compared to normal irrigation conditions (Table 3). Maximum heritability of 94% was observed by DRTWT and FRTWT whereas minimum heritability was recorded for LRWC under both normal and water deficit environments. Presence of high heritability indicated that additive components are responsible for large proportion of the genetic variance and this information helps to guess the selection response in genetically mix population. Maximum genetic advance (GA) of 10.78 and 9.81 was observed for FRTWT whereas minimum was expressed by RL under both normal and water limited conditions. High heritability coupled with higher values of GA for FRTWT and DRTWT suggested that these parameters can be improved by simple selection (Najeeb *et al.,* 2009). High heritability with low GA for FSTWT indicated that the parameter is mostly under the influence of environment for its expression hence selection could be practiced at later stages in the breeding program.

Correlation analysis

The correlation analysis presented in Table 4 showed higher magnitude of genotypic correlations as compared to their respective phenotypic correlation coefficients indicating the dominant role of genetic factors instead of environmental factors. LRWC showed strong positive association with FRTWT and FSTWT under normal and water deficit environment. Similarly it also had significant association with RL, SL and dry root and shoot weight under water deficit condition. Significantly Positive association of root length (RL) was observed between SL, FRTWT and DSTWT under non stress environment whereas under water deficit regime RL showed significantly positive association with DSTWT and LRWC. The correlation pointed out that sufficient water supply helps in growth and development of roots to move deep into the soil under water shortage condition. Mirza, 1956 reported similar findings.

Shoot length (SL) had significant positive relationship with RL and RTSTR under normal and with LRWC, FRTWT, DRTWT, DSTWT and RTSTR under water limited conditions. Seedlings with more water contents will show more growth through cell division and enlargement which resulted in longer shoots. Length of root and shoot decreased under limited soil moisture regimes (Alkhafaf *et al.*, 1985). Association of fresh root weight (FRTWT) was positive with LRWC, RL, FSTWT and RTSTR under normal regimes whereas under water limited conditions it showed positive associations with LRWC, FRTWT and DSTWT. Seedlings having higher root weight definitely have better root system and will produce fresher shoot and root weight. It indicated that plants having tolerance against water stress at early growth stage will also represent tolerance at later stages of growth and development (Bocev, 1963).

Table 4. Genotypic and phenotypic correlation associations between different traits under normal (upper diagonal) and water limited regimes (lower diagonal).

Parameters		LRWC	RL	SL	FRTWT	FSTWT	DRTWT	DSTWT	RTSTR
LRWC	G	1	0.323**	-0.136**	0.814**	0.335**	-0.076**	-0.391**	-0.234
	P		0.182	0.126	0.301	0.260	-0.376	-0.295	-0.217
RL	G	0.278**	1	0.689**	0.431**	0.347*	-0.279**	0.742**	0.338
	P	0.216*		0.272	0.225	0.328	-0.133	0.146	0.273
SL	G	0.726**	-0.571**	1	-0.354	-0.086	-0.618**	0.359	0.751**
	P	0.279*	0.174		0.218	-0.046	-0.306	0.067	0.683*
FRTWT	G	0.801**	-0.348**	0.898**	1	0.963**	0.339	-0.307	0.812**
	P	0.624**	-0.093	0.403*		0.253	0.099	-0.089	0.649*
FSTWT	G	0.983**	-0.973**	-0.119**	0.989**	1	0.431	-0.326	-0.209
	P	0.134	-0.131	0.098*	0.392**		0.297	-0.109	-0.195
DRTWT	G	0.478**	0.173	0.458**	-0.259**	-0.831**	1	-0.223	0.648**
	P	-0.137*	-0.125	0.247*	0.124	0.128		0.057	0.493*
DSTWT	G	0.468**	0.275**	0.683**	0.684**	-0.607**	-0.361**	1	0.824**
	P	-0.285*	0.158	-0.074*	0.078	0.097	-0.110		0.691*
RTSTR	G	0.371*	-0.357	0.416*	-0.509	-0.620*	-0.674*	-0.641*	1
	P	-0.215*	0.329	0.313	0.491*	-0.538*	0.553*	-0.498*	

G= Genotypic correlation, P= Phenotypic correlation, LRWC= Leaf relative water content, RL= Root length, SL= Shoot length, FRTWT= Fresh root weight, FSTWT= Fresh shoot weight, DRTWT, Dry root weight, DSTWT= Dry shoot weight, RTSTR= Root to shoot ratio.

Strong positive association of fresh shoot weight (FSTWT) was observed with LRWC, FRTWT and DSTWT and negative association with SL both under normal and water limited environments. Associations with RTSTR was found negative under both water treatments. Strong positive associations of FSTWT with FRTWT and LRWC indicating that selection with more LRWC could be helpful to improve FSTWT. Khan *et al.,* 2004 reported similar findings.

Correlation of dry root weight (DRTWT) was observed negative with LRWC, SL, and RL and strong positive association was found with RTSTR under normal environment. Dry shoot weight (DSTWT) had strong positive associations with RL and RTSTR under normal whereas under water deficit regimes it had significant positive associations with LRWC, RL, SL, and FRTWT. In current study positive associations of DSTWT with SL may be developed due to partial osmotic arrangement by the young seedlings, which assisted the plant to continue its growth.

Conclusion

Presence of significant genetic differences among the genotypes pointed out that these parameters may be enhanced through selection. It is accentuated from the study that selection standards for seedling parameter fluctuates with different levels of water. Therefore, it is suggested to perform early selection (at seedling stage) on the basis of dry root weight (DRTWT), leaf relative water content (LRWC) and root to shoot ratio (RTSTR) to accelerate the breeding program against water deficit environment. The YP-12 genotype considered as tolerant inbred line/genotype could be used in maize breeding program to tailor tolerant maize plants/hybrids for water limited areas whereas inbred lines US-17 and US-14 considered as sensitive to water stress and may not be used in breeding program particularly designed for water stress tolerance.

Acknowledgement

The author hereby acknowledged the services of Maize and Millets Research Institute, Yousafwala, Maize Research Station, Faisalabad and Aliakbar seeds (Pvt.) Ltd on account of providing breeding materials (inbred lines) and guidance throughout this and other part of author's Ph. D Research work.

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