



RESEARCH ARTICLE

Screening and Inheritance Pattern Studies of Maize Seedlings under Normal and Water Stress Conditions

Anwar ul Haq^{1*}, Muhammad Hammad Nadeem Tahir¹, Muhammad Ahsan¹, Rashid Ahmad² and Hafiz Muhammad Akram³

¹Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, Pakistan

²Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

³Agronomic Research Institute, AARI, Faisalabad, Pakistan

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ABSTRACT

An experiment was conducted during 2011-12 at Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan to investigate the inheritance pattern of seedling traits under drought condition. Fifty maize inbred lines collected from different sources were screened at seedling stage for water stress tolerance in glasshouse. Data were collected for fresh root and shoot length, fresh root and shoot weight, dry root and shoot weight, root /shoot ratio and relative water content and analysed statistically. All seedling traits were significantly higher ($P \leq 0.05$) for drought tolerant genotype (Y-26) than drought sensitive genotypes. One tolerant genotype Y-26 and one susceptible (Y-113) inbred lines were selected on the basis of dry root weight, root/shoot ratio, relative water content and used to develop six generations (P_1 , P_2 , F_1 , F_2 , BC_1 and BC_2). The generations were evaluated in glasshouse under normal and water stress conditions. Additive and dominance genetic effects were prominent for all seedling traits under normal condition except fresh and dry root weight which had additive genetic effects. Similarly additive and dominance gene action was involved for all seedling traits under water stress condition except fresh root length and fresh and dry root weight, which were under additive genetic control.

*Corresponding Author:

anwarulhaq312@gmail.com

INTRODUCTION

Maize is one of the oldest domesticated as well as cultivated monoecious food crops of Maydae tribe. Among leading cereal crops it ranks first in the world and third in Pakistan followed by wheat and rice (Anonymous 2013a, b). In Pakistan, currently it is grown on an area of 1.085 million hectares with annual production of 4.631 million tons and average yield of 4268 kg ha⁻¹ (Anonymous 2013b). Being a C₄ plant, maize gives higher yield among cereal crops, though it produces highest yield under abundant water and high soil fertility, yet it is least tolerant to stresses (Ludlow and Muchow, 1990). It shares an important position in global economy and trade, while ever increasing demand of maize in comparison with wheat and rice has made it distinct food and feed crop.

Water stress is among the most important abiotic factors which can limit crop production (Rao and Singh, 2006). Incidence of water stress at seedling

stage may lead to higher dry root weights, longer roots and higher root/shoot ratio (Takele, 2000; Kashiwagi et al., 2004). Drought-adapted plants are often characterized by deep and vigorous root system (Dhanda et al., 2004; Khan et al., 2004). Roots are less sensitive than shoots to growth retardation under drought stress. Therefore root/ shoot ratio of plants increases when water availability is limiting (Wu and Cosgrove, 2000). Root/shoot ratio is a useful and reliable selection parameter for development of drought tolerant cultivars (Zekri, 1991; Tavakol and Pakniyat, 2007). The genetic variability plays a leading role in any breeding programme (Tengan et al., 2012) and the success of any crop improvement programme does not depend only on the amount of genetic variability present in the population, but also on the extent to which it is heritable (Ahmad et al., 2011; Wang et al., 2011).

Setty (1975) and Wang et al. (2000) observed that additive genetic effects were significant for fresh root

and shoot weight, dry root and shoot weight in maize. Akbar et al. (2008) reported additive and dominance genetic effects for fresh root and shoot length, fresh shoot and root weight, while root/shoot ratio was found under additive genetic control. Root/shoot ratio in maize was under additive genetic control (Rao and Singh, 2004). Non additive genetic control was involved in the expression of root length, fresh root and shoot weight and dry shoot weight in maize (Zia and Chaudhary, 1980; Rahman et al., 1994). Partial dominance type of gene action was involved for the inheritance of dry root weight (Zia and Chaudhary, 1980) and dominance type of genetic effects were present for this trait in maize (Rahman et al., 1994).

To develop new lines, three things are essential to play with, which are the variation for the trait, heritability and mode of inheritance of the trait. A plant breeder always requires controlled genetic variation that enables him to evolve drought tolerant maize lines (Thirunanai et al., 2000). Existing variability plays an important role in the success of selection which depends upon the gene action that controls the trait under improvement (Tengan et al., 2012). The present study was devised to investigate the genetic variability and study the inheritance pattern of seedling traits in maize germplasm under normal and water stress conditions.

MATERIALS AND METHODS

The present study was conducted during 2011-12 in the glasshouse of Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. The mean temperature and relative humidity in the glasshouse ranged between 25.7 and 34.4°C and 60.3 - 79.3%, respectively. Fifty maize inbred lines were collected from Maize and Millet Research Institute (MMRI), Yousafwala, Maize Research Station, Ayub Agricultural Research Institute (AARI), Faisalabad, and Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The inbred lines were sown in the polythene bags measuring 18 × 9 cm filled with pre-irrigated washed river sand at a uniform depth of 3 cm. The experiment was laid out in a completely randomized factorial design with three replications under both normal and water stress conditions. Ten seedlings of each inbred line per replication were grown. After seven days of sowing, 150 ml of Hoagland solution was applied to both normal and water stress treatments, whereas 15 days after sowing, 150 ml of Hoagland solution was applied to normal set only.

After 21 days of sowing, water was applied to both sets of experiments prior to uprooting. The uprooted seedlings were washed with tap water carefully so that root and shoot are not damaged. The data were recorded for various seedling traits. Fresh root and

shoot lengths were measured in centimetre (cm), fresh and dry root and shoot weights were measured in gram (g) using electronic balance (CHYO-MJ- 500). Root/shoot ratio was calculated using formula as proposed by Nour et al. (1978).

Root/shoot ratio = Dry root weight / dry shoot weight
Relative water content was determined during stress period according to Mata and Lamattina (2001). When water stress appeared, leaf samples were detached from selected plants and placed in polythene bags, fresh leaf weight was recorded by using electronic balance in the laboratory. Turgid leaf weight was recorded after keeping the leaf samples in water overnight. Dry leaf weight was calculated after oven drying the leaf samples at 70°C for 24 hours. Relative water content was determined by using the formula given below

$$\text{RWC \%} = [\text{Fresh Weight} - \text{Dry Weight}] / [\text{Turgid Weight} - \text{Dry Weight}] \times 100$$

One tolerant (Y-26) and one susceptible (Y-113) inbred lines were selected on the basis of dry root weight, root/shoot ratio and relative water content under water deficit conditions. During spring 2011, the selected lines were sown in the field and the female parent was hand emasculated and pollinated to produce sufficient seed of F₁ generation. During the next season, F₁ along with parents were grown in the field and F₁ was selfed to get F₂ generation. F₁ was also crossed with P₁ and P₂ to develop BC₁ and BC₂ respectively. Six generations were sown in glasshouse following the aforesaid procedure for screening of inbred lines. The experiment was laid out in a completely randomized factorial design with three replications and two water treatments. Each replication consisted of 10 seedlings of P₁, P₂ and F₁, 20 seedlings of back crosses and 50 seedlings of F₂ generation under both conditions. The data on seedling traits were recorded and subjected to analysis of variance (Steel et al., 1997) to determine the variability among the entries. Generation means analysis was performed according to Mather and Jinks (1982) to study the inheritance pattern of seedling traits in maize. Standard error (SE) of generation means was computed by performing a nested analysis of variance (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Pooled analysis of variance (Table 1) indicated that treatments, inbred lines and treatment × inbred lines interactions were significant (P≤0.01) for all traits except treatment × inbred line interaction for relative water content. Analysis of variance pertaining to the seedling parameters evaluated under normal and water stress condition is presented in Table 2. Significant variation among the inbred lines was found for all the parameters under both conditions, which showed sufficient genetic variability in the available maize

Table 1: Mean square values from pooled analysis of variance for seedling traits of maize inbred lines under normal and water stress condition

SOV	Treatments	Inbred lines	Treatment × Inbred	Error
FRL(cm)	1196.48**	79.33**	18.09**	0.75**
FSL (cm)	4478.45**	38.76**	18.45**	1.006**
FSW (g)	2480.44**	73.78**	8.91**	0.401**
FRW (g)	943.95**	40.80**	2.75**	0.39**
DSW (g)	64.48**	1.81**	0.432**	0.055**
DRW (g)	226.06**	13.90**	1.08**	0.034**
R/S Ratio	1.176**	2.11**	0.441**	0.016**
RWC	0.427**	0.027**	0.001	0.003

** = Highly significant at 0.01 probability; **FSL**=Fresh shoot length; **FRL**=Fresh root length; **FSW**=Fresh shoot weight; **FRW**=Fresh root weight; **DSW**= Dry shoot weight; **DRW**= Dry root weight; **R/S Ratio**= Root: shoot ratio; **RWC**= Relative water content

Table 2: Mean square values and coefficients of variation for seedling traits in maize under normal and water stress condition

Trait	Level	Mean Square (df 49)	CV%
FRL (cm)	Normal	40.350**	3.41
	Water Stress	18.810**	4.26
FSL (cm)	Normal	66.560**	2.86
	Water Stress	41.930**	3.47
FSW(g)	Normal	62.740**	4.59
	Water Stress	31.600**	6.47
FRW (g)	Normal	27.570**	7.10
	Water Stress	19.060**	11.02
DSW(g)	Normal	1.470**	8.03
	Water Stress	0.874**	11.18
DRW(g)	Normal	10.060**	5.34
	Water Stress	4.652**	6.84
R/S Ratio	Normal	1.470**	9.01
	Water Stress	1.627**	9.84
RWC	Normal	0.014**	9.53
	Water Stress	0.015**	6.70

** = highly significant (P<0.01); **FSL**= Fresh shoot length; **FRL**= Fresh root length; **FSW**=Fresh shoot weight; **FRW**= Fresh root weight; **DSW**= Dry shoot weight; **DRW**= Dry root weight; **R/S Ratio**= Root: shoot ratio; **RWC**= Relative water content

Table 3: Range of different maize seedlings traits under normal and water stress condition

Traits	Normal	Water stress
FRL(cm)	19.0 – 34.95	15.85 – 36.03
FSL (cm)	18.97 – 36.0	16.23 – 26.73
FSW (g)	7.06 – 24.84	5.10 – 15.95
FRW (g)	5.83 – 19.48	4.01 – 12.73
DSW (g)	1.86 – 4.78	1.21 – 3.27
DRW (g)	2.09 – 8.70	1.09 – 7.35
R/S Ratio	0.62 – 3.04	0.55 – 3.43
RWC	0.47 – 0.76	0.41 – 0.70

FSL= Fresh shoot length; **FRL**= Fresh root length; **FSW**= Fresh shoot weight; **FRW**= Fresh root weight; **DSW**= Dry shoot weight; **DRW**= Dry root weight; **R/S Ratio**= Root: shoot ratio; **RWC**= Relative water content

germplasm that could be manipulated for further improvement in maize breeding for water stress tolerance (Mehdi and Ahsan, 2000; Dhanda et al., 2004; Wang et al., 2011; Qayyum et al., 2012).

Fresh root length ranged from 19.0–34.95 cm under normal condition, while under water stress it ranged from 15.85–36.03 cm. The only root trait which acts positively under water stress conditions is fresh root length because photosynthetic assimilates are diverted more towards root than shoot in such situation, therefore root length increases while other traits under water stress decrease for tolerant genotypes. For susceptible genotypes all seedling traits showed decreasing trend under water stress. The results are in accordance with Khan et al. (2004). Fresh shoot length ranged from 18.97–36.0 cm under normal condition, while under water stress it ranges from 16.23–26.73cm. The results are in agreement with Ahsan et al. (2011). Similarly fresh shoot weight ranged between 7.06 g and 24.84 g under normal condition, whereas a range of 5.10–15.95 g was observed under water stress. Ahsan et al. (2011) reported a range of 3.55-12.55 g for fresh shoot weight. The slight difference in range may be due to different genetic material and environmental conditions. A range of 5.83-19.48g was noted for Fresh Root Weight (FRW) under normal condition, while it ranged from 4.01–12.73 g under water stress. Ahsan et al. (2011) and Qayyum et al. (2012) observed the range of 0.83-17.01g under water stress for FRW and the results are in accordance with our results. The range of 1.86–4.78 g was exhibited for dry shoot weight (DSW) under normal condition; however this range was 1.21–3.27 g under water stress. Yagmur and Kaydan (2008) also confirmed the same pattern with a range of 0.83-3.21. Dry root weight ranges from 2.09 –8.78 g under normal condition, whereas its range was 1.09 – 7.35g under water stress. Root/ shoot ratio ranged between 0.62–3.04 under normal condition; however it changed to 0.55–3.43 under water stress. The roots are less sensitive than shoots and direction of dry matter accumulation is away from the shoot to the root, so root/shoo ratio increase for water stress tolerant lines. Aslam and Tahir (2003) and Chohan (2012) reported a range of 1.33 - 6.11 for root/ shoot ratio under water stress. The reported ranges are different due to variability in genetic material and water stress level Wu and Cosgrove (2000) and Thomas and Howarth (2000) confirmed that root/shoot ratio is significantly affected by water stress and decrease in water supply increases root/shoot ratio. High root/shoot ratio under water stress may be due to redirection of growth and dry matter accumulation away from the shoot to the root, which is in accordance with our results. The ranges of 0.47–0.76 and 0.41–0.70 were observed for RWC under normal and water stress conditions respectively. Aslam and Tahir (2003) also observed the same range. All the

Table 4: Maize Inbred Lines for maximum and minimum seedling traits with mean values under normal and water stress condition

Traits	Normal		Water stress	
	Maximum	Minimum	Maximum	Minimum
FRL(cm)	OH54-34(34.95)	B34-2B(19.0)	Y-26(36.03)	Y-113(15.85)
FSL (cm)	Y-15(36.0)	W64SP(18.97)	W-10(26.73)	W64SP16.23)
FSW (g)	A-239(24.84)	OH-8(7.06)	Y-54(15.95)	Q-67(5.10)
FRW (g)	A-239(19.48)	OH-28(5.83)	Y-26(12.73)	Y-113(4.01)
DSW (g)	OH-41(4.78)	OH54-34(1.86)	Y-54(3.27)	Q-67(1.21)
DRW (g)	Y-52(8.78)	Q-67(2.09)	Y-26(7.35)	Y-113(1.09)
R/S Ratio	Y-52(3.04)	OH33-1(0.62)	Y-26(3.43)	Y-113(0.55)
RWC	Y-26(0.76)	Y-113(0.47)	Y-26(0.70)	Y-113(0.41)

FSL= Fresh shoot length; **FRL**= Fresh root length; **FSW**= Fresh shoot weight; **FRW**= Fresh root weight; **DSW**= Dry shoot weight; **DRW**= Dry root weight; **R/S Ratio**= Root: shoot ratio; **RWC**= Relative water content

Table 5: Estimates of the best fit model for generation means of seedling parameters (\pm standard error) by weighted least squares analysis under normal and water stress conditions

Traits	Genetic effects					
	m	[d]	[h]	[i]	[j]	[l]
	Normal					
FRL	28.85 \pm 0.48	1.601 \pm 0.15	4.314 \pm 0.61	-1.844 \pm 0.51	-	-
FSL	28.79 \pm 0.59	-1.213 \pm 0.16	3.323 \pm 0.72	-2.410 \pm 0.58	-	-
FSW	11.26 \pm 0.11	-1.131 \pm 0.11	4.294 \pm 0.20	-	-	-
FRW	11.63 \pm 0.22	1.790 \pm 0.12	-	-1.361 \pm 0.26	1.370 \pm 0.37	2.835 \pm 0.31
DSW	3.20 \pm 0.07	-0.698 \pm 0.07	1.819 \pm 0.12	-	-	-
DRW	6.66 \pm 0.13	1.67 \pm 0.07	-	-1.51 \pm 0.15	-	2.03 \pm 0.21
R/S Ratio	1.89 \pm 0.05	1.007 \pm 0.06	0.910 \pm 0.27	-	-	-0.162 \pm 0.07
	Drought condition					
FRL	25.90 \pm 0.27	2.69 \pm 0.14	-	-3.86 \pm 0.32	-	2.00 \pm 0.40
FSL	20.57 \pm 0.15	-1.220 \pm 0.15	4.597 \pm 0.25	-	-	-
FSW	9.31 \pm 0.39	-0.795 \pm 0.12	3.197 \pm 0.55	-1.075 \pm 0.41	-0.657 \pm 0.32	-
FRW	10.03 \pm 0.19	2.156 \pm 0.11	-	-2.318 \pm 0.23	-0.842 \pm 0.31	1.333 \pm 0.29
DSW	4.31 \pm 0.46	-	-4.302 \pm 1.16	-2.259 \pm 0.45	-	3.492 \pm 0.74
DRW	4.93 \pm 0.13	1.54 \pm 0.08	-	-1.56 \pm 0.17	-	1.75 \pm 0.23
R/S Ratio	1.79 \pm 0.06	0.835 \pm 0.06	0.916 \pm 0.27	-	0.360 \pm 0.15	-0.764 \pm 0.27

All parameters were significant at 5 % probability level of significance; **FRL**= Fresh root length; **FSL**= Fresh shoot length; **FSW**= Fresh shoot weight; **FRW**= Fresh root weight; **DSW**= Dry shoot weight; **DRW**= Dry root weight; **R/S Ratio**= Root: shoot ratio; d= Additive genetic effects h= Dominance genetic effects i= additive \times additive interaction j= additive \times dominance interaction l= dominance \times dominance interaction

seedling traits decreased under water stress except root/shoot ratio in maize (Aslam and Tahir, 2003; Khan et al., 2004; Chohan, 2012). Dry root weight, root/shoot ratio and relative water content are suitable criterion for selection of drought resistant genotypes at seedling stage (Colom and Vazzana, 2003; Tavakol and Pakniyat, 2007). Per cent relative water content (RWC %) decreases with the decrease in osmotic potential and increases in drought stress. The inbred line Y-26 gave maximum values for FRW, DRW, R/S ratio and RWC under water stress, proving it tolerant, while inbred line Y-113 gave minimum values for FRW, DRW, R/S ratio and RWC under water stress confirming its susceptibility.

Inheritance studies

The results pertaining to the gene action of seedling traits under normal and drought conditions are presented in Table 5.

1. Fresh root length

The model with four parameters [mdhi] was found best fitted to the data for fresh root length under normal condition suggesting the presence of both additive and dominance genetic effects along with the additive \times additive interaction, whereas the four parameters model [mdil] was best fit to the data showing the presence of additive genetic effects for inheritance of fresh root length along with the additive \times additive and dominance \times dominance interaction under water stress condition. The results are in line with Ekanayake et al. (1985), Ana et al. (1997) and Akbar et al. (2008). Positive additive effects indicated that it may be used to fix the increase for fresh root length under both conditions. Positive dominance effects under normal condition indicated that increase may be used for the development of hybrids under normal and water stress condition.

2. Fresh shoot length

For fresh shoot length the four parameters model [mdhi] was best fitted to the data showing the presence of both additive and dominance gene action along with the additive \times additive interaction under normal irrigation, whereas under water stress condition three parameters model [mdh] was best fit, suggesting the presence of both additive and dominance gene action with no interaction. Fresh shoot length exhibited the importance of additive and dominance genetic effects in maize (Akbar et al., 2008) and rice (Ekanayake et al., 1985), while Zia and Chaudhary (1980) and Rahman et al. (1994) reported non additive type of genetic effects. The variation in genetic effects may be due to use of different genetic material. Positive dominance effects with no interaction suggested that it may be used to increase fresh shoot length for hybrid development in maize under both conditions.

3. Fresh shoot weight

The three parameters model [mdh] was best fitted to the data for fresh shoot weight under normal condition depicting the presence of both additive and dominance gene action. The five parameters model [mdhij] was best fitted to the data indicating the presence of both additive and dominance gene action along with the additive \times additive and additive \times dominance interaction under water stress condition. The results are in comparison with Akbar et al. (2008) in maize and Ekanayake et al. (1985) in rice, Negative additive effects were observed, which indicated that decrease may be fixed but cannot be useful to decrease fresh shoot weight under normal condition. Similarly positive dominance effects indicated that increase may be used for hybrid development under normal condition, but under water stress condition, positive dominance effects and negative additive \times dominance interaction indicated complex interaction, so further selection is recommended till later generations to select desirable combination for hybrid development in maize under water stress condition.

4. Fresh root weight

For fresh root weight the five parameters model [mdijl] was found best fitted to the data exhibiting predominance of additive gene action along with the additive \times additive, additive \times dominance and dominance \times dominance interaction under both conditions. Additive genetic effects were more important than dominant genetic effects under normal and water stress condition (Setty, 1975; Wang et al., 2000), while additive and dominance genetic effects were reported by Akbar et al. (2008). The variation in results may be due to difference in genetic material and environmental conditions. Positive additive effects indicated that increase may be fixed for fresh root weight both under normal and water stress conditions. Positive dominance \times dominance interaction suggested

that increase in fresh shoot weight may be used for hybrid development under both conditions.

5. Dry shoot weight

For dry shoot weight the three parameters model [mdh] was best fitted to the data showing the presence of both additive and dominance gene action with no interaction for this trait under normal condition, while under drought condition four parameters model [mhil] was best fitted to the data exhibiting existence of dominance gene action for the inheritance of the trait along with the additive \times additive and dominance \times dominance interaction. Additive and dominant genetic effects contributed equally to the expression of dry shoot weight in rice (Ekanayake et al., 1985). The results are different from Setty (1975), who reported additive type of gene action. Variation in results may be due to different genetic material. Positive dominance effects indicated that increase in dry shoot weight may be used for hybrid development under normal condition. Whereas negative dominance effects along with positive additive \times dominance interaction under water stress condition indicated ambiguous results, therefore it is suggested that further progeny selection may be continued till further generations.

6. Dry root weight

Four parameters model [mdil] was best fitted to the data for dry root weight suggesting the existence of additive genetic effects along with the additive \times additive and dominance \times dominance interaction under both normal and water stress conditions. Additive genetic effects were crucial for dry root weight under normal and water stress condition. Additive genetic effects were also reported for dry root weight in maize by Setty (1975) and Akbar et al. (2008). Dominant genetic effects for dry root weight were claimed by Rahman et al. (1994). Positive additive effects indicated that it may be used to fix the increase for dry root weight both under normal and water stress condition but cannot be useful at this stage because negative additive \times additive interaction indicated that both effects are in opposite direction, so further progeny selection is recommended. Positive dominance \times dominance interaction suggested that increase may be used for hybrid development in maize under both conditions.

7. Root/ shoot ratio

For root: shoot ratio the four parameters model [mdhl] was best fitted to the data showing the presence of both additive and dominance genetic effects for the inheritance of the trait under normal condition along with dominance \times dominance interaction, whereas five parameters model [mdhjl] was found best fitted to the data depicting the presence of both additive and dominance gene action along with the additive \times dominance and dominance \times dominance interaction under water stress condition. Rao and Singh, (2004) and Akbar et al. (2008) reported additive genetic effects for

root/shoot ratio in maize. The experiment was conducted under different environmental conditions. Additive and dominant genetic effects were important equally to the expression of root/shoot ratio in rice (Ekanayake et al., 1985), which is according to our results. Positive additive effects indicated that increase for root/shoot ratio may be fixed for synthetic development in maize under normal condition, whereas under water stress condition, positive additive effects along with positive additive \times dominance interaction indicated that situation is unclear, which suggest further progeny selection in succeeding generations.

Conclusion

Significant genetic variability was present among the inbred lines indicating that these traits may be further improved through selection. Dominance genetic effects with no interaction under normal conditions suggest that increase for FRL, FSL, FSW and DSW may be used for hybrid development, whereas increase for FRL and FSL may be used for hybrid development due to presence of positive dominance \times dominance interaction and positive genetic effects respectively under water stress. The inbred lines Y-26 and Y-52 were comparatively tolerant under water stress, while inbred lines Y-113 and Y-126 were comparatively more susceptible to water stress. The results indicate that the lines Y-26 and Y-52 are the best options for utilization in future breeding programme for water stress tolerance, while the lines Y-113 and Y-126 being susceptible may not be utilized for drought tolerance breeding.

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