

Research Article

Screening of maize (*Zea mays* L.) hybrids based on drought tolerance under hydroponic conditions

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Abstract

Maize (*Zea mays* L.) is an important cereal adversely affected by drought stress. Screening the germplasm of maize crop is prerequisite to categorize the genotypes based on drought tolerance and sensitivity. With the objective to sort out the contrasting genotypes, eight maize varieties (V_1 =FH-988, V_2 =20R52, V_3 =FH-1046, V_4 =Rustam-1, V_5 =FH-949, V_6 =Pioneer30Y87, V_7 =FH-1137, and V_8 =Rustam-12) were grown under well-watered (WW= 0 MPa) and water stressed (DD = -0.60MPa) conditions. Drought was estimated by applying PEG-8000. Plants were harvested four weeks after transplanting and the evaluation was done on the basis of various morphological (root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, root shoot ratio, number of leaves) and biochemical parameters (potassium and chlorophyll contents in leaves). Relative leaf water contents and membrane stability index were also measured. The data collected was analyzed statistically at 1% probability level and Least Significant Difference Test (LSD) was applied to separate the significant treatment means. The results showed that the water stress adversely affected on the morphological parameters. The maize hybrid FH-988 had significantly higher ($p<0.01$) morphological and biochemical parameters and found more drought tolerant. While maize hybrid FH-1137 had lower these parameters as compared to all other maize hybrids and was considered as drought sensitive genotype. Study findings showed that the screening of drought tolerant genotypes could be a better source to mitigate the drought stress impacts on maize in drought prone regions.

Keywords: Drought tolerant; Hydroponic; Maize; Water stress

Introduction

Maize (*Zea mays* L.) is a multipurpose crop and is used as food, feed, fodder, fuel and important industrial products like corn syrup, starch, lactic acid, dextrose gluten, grain cake corn, flakes, jellies, corn oil, acetone and alcohol [1]. It is considered as the king of

grain crops among leading cereals and ranked third after wheat and rice [2]. Maize share to the value added in agriculture is 2.2 % and to GDP 0.4 %. The cultivated area has increased to 1144 thousand hectares under maize crop during 2015-16 which as of 1142 thousand hectares showing an increase of 0.2 % over

last year's area. During 2015-16 maize crop production reached at 4.920 million tonnes, over the last year's production of 4.937 million tonnes showing a decrease of 0.3 % with an average of 4301 kg ha⁻¹ (Govt. Pakistan, 2015-16). Owing to its major contribution in GDP, so need more attention to protect maize plants from adverse effects of drought stress by selection of suitable varieties.

Drought is a meteorological term that means minimum rainfall occurred in areas over an extended period and has been provoked by climate change brought about by global warming through the burning of fossil fuels, deforestation, and lack of reforestation and greenhouse gas emissions, [3]. Drought is viewed as the insufficiency of water availability, including precipitation in agriculture. Water is becoming an important resource and that directly effect on farming [4]. Numerous studies revealed that 50% reduction in yield of major crop plants is caused by drought [5] which is a common characteristic of less or unpunctual rainfall regions [6] Water is the essential constituent of actively growing plants ranging from 70-90% of fresh biomass [7].

Water scarcity is the chief abiotic factor reducing maize growth and development around the world. Water stress affects plant structure, growth, development, as well as physiological, morphological functions and i.e. is the results decreased in yield [8]. Water shortage causes denaturing of proteins, production of reactive oxygen species (ROS) and decrease in photosynthetic activity [9].

Drought tolerance refers to the capability of a plant to survive water deficit with low tissue water potential and to produce product with least thrashing of produce in a water deficient environment relative to a water check free environment [10]. Understanding of physiological behavior of plants under drought conditions may result in estimating varieties of crops [11]. As many plant species

under drought condition the variation in different metabolic activities, reduction of relative water content in plants and decrease in plant vigor [12, 13] was observed. Maize seedling feedback varies its growth rate at different field capacities of soil and accumulates a variety of compatible solutes such as proline, glycine betaine, mannitol, and an adaptive mechanism of tolerance to drought [14].

There have been various methods adopted to increase the drought resistance of maize by entering genes for drought resistance into modified genotypes having screens of international collection, conventional methods of breeding and crosses with maize cultivars, complete field experiments of particular genotypes, conducting screening programs to select the drought tolerant varieties have been recognized and well documented in maize crop varieties [15].

Drought tolerance of plants is research issue in the world and also in Pakistan. The major emphasis of plants breeder and researcher is to get optimal yield under drought stress. Yield losses of maize in water deficit areas are 40-70% [16] which is a huge loss.

Keeping in view the above facts, the trial was conducted in hydroponics having particular objectives; i) to identify the maize genotypes (drought sensitive and drought tolerant) based on growth and ionic estimation ii) to evaluate the effects of drought on maize performance.

Materials and methods

The current experiment was conducted under hydroponic conditions at Ghazi University, Dera Ghazi Khan, Pakistan (121 m above sea level, 30.050° latitude North and 70.633° longitude East) 2016. The seeds of 8 maize hybrids including FH-988, 20R52, FH-1046, Rustam-1, FH-949, Pioneer30Y87, FH-1137, and Rustam-12 were acquired from Seed Company Jullundur Private Limited (JPL) and Maize & Millet Research Institute (MMRI), Yousafwala Sahiwal. The

experiment was laid out in Completely Randomized Design (CRD) with three replications during Kharif season. The plastic containers were used with capacity of 3.5 L water. The crop nursery was sown in iron trays in pure sand and was transplanted after 8 days. Polystyrene sheet was used for supporting the plants in solution culture. Size of polystyrene sheet was according to the size of pot. Holes were made on polystyrene sheet at distance. One plant was transplanted at each hole. Plastic pots were filled with Hoagland's solution.

Four days after transplanting, plants were subjected to 0 and -0.60 MPa (Drought) without and with PEG-8000 (-0.60 MPa, 15%) in Hoagland's nutrient solution, respectively. pH (6.0 - 6.5) was maintained by adding H₂SO₄ or NaOH daily basis. After 7 days interval solution was changed in each treatment. Fresh air was supplied in with the help of aeration pump. All the plants from each pot were harvested four weeks after transplanting, then each plant was washed with tap water and then with distilled water. The data for the following attributes were recorded.

1). Root length (cm) and shoot length was measured with the help of meter rod. 2). Root fresh weight and shoot fresh weight (g plant⁻¹): Roots were cut on the base of the shoot, and then root fresh weight (g) was taken; furthermore shoots were separated from roots to calculate shoot fresh weight (g). 3). Root dry weight (g plant⁻¹): Root material was placed in an oven at 70°C for 72 hours to get constant dry weight then root dry weight (g) was calculated. 4). Shoot dry weight (g plant⁻¹): Shoot material was placed in an oven at 70°C for 72 hours to get constant dry weight then root dry weight (g) was calculated.

5). Root: Shoot: Root/shoot ratio was calculated using formula as proposed by Root/shoot ratio = Root length / shoot length [17], 8). Number of leaves: Total number of leaves was calculated, and average was taken

for statistical analysis. 6). Membrane Stability Index (MSI) %: Leaf Membrane stability index (MSI) was determined according to the method of [18] as modified by [20]. Leaf samples (0.1 g) were placed in 10 ml of double-distilled water in two sets. One set was kept at 40 °C for 30 min and its conductivity recorded (C₁) using a conductivity meter. The second set was kept in a boiling water bath (100 °C) for 15 min, and its conductivity was also recorded (C₂). The MSI was calculated as
MSI = 1 - (C₁/C₂) x 100

10). Leaf potassium contents (m mol g⁻¹Dw): Plant samples digestion was done according to [21]. Took one g dried ground sample from the leaf of each maize hybrid in the separate digestion tubes. Ten ml of di-acid (6.67 ml HNO₃ and 3.33 ml of HClO₄) was added in each digestion tube and placed these tubes for overnight at room temperature so that the process of digestion could be accelerated. These tubes were stirred for the complete dissolution of plant parts. The digestion tubes were heated over the stove on low heat and fumes were produced. The tubes were heated until the material of the tubes became colorless. Then these tubes were removed from the stove and cooled them. A small amount of distilled water was added in each colorless digested material so that the process of filtration could be done. After the completion of the filtration process, the volume of the extract was made up to 100 ml in volumetric flasks separately for each sample. This filtrated extract was used for the determination of potassium contents in leaf by using flame photometer (Sherwood Flame photometer, Model-410; Sherwood Scientific, Ltd, Cambridge UK). 11). Leaf chlorophyll contents (SPAD Value): Chlorophyll contents of the leaves were determined by using SPAD instrument (model SPAD-502; Minolta Corp., Ramsey, N.J.). 12). Relative water contents (RWC) %: Relative water contents were determined

according to [20]. For the determination of RWC, the 2nd leaf from the shoot of maize plant was removed with a sharp razor blade. Its fresh weight (fresh mass, FW) was determined immediately. For the determination of turgid weight (TW) leaves were put in the distilled water inside the closed plastic bags. The leaves were allowed for imbibitions for overnight (24 hours) by placing plastic bags under dim light (around 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in the laboratory under the naturally fluctuating temperature. After the completion of imbibitions, the leaf samples were again weighed, and turgid weight (TW) was recorded. After recording the turgid weight, the leaf samples were placed at 70 °C in an oven for 72 hours. After this, oven-dry weight (DW) of leaf samples was determined. All the measurements were made on an analytical scale, with the precision of 0.0001 g. RWC was calculated by using the values of FW, TW, and DW by the given equation.

$$\text{RWC (\%)} = \frac{[(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})] * 100}{1}$$

Statistical analysis: The collected data were analyzed statistically by using Fisher's

analysis of variance (ANOVA) technique. Least Significant Difference (LSD) test was applied ($p \leq 0.01$) to compare significant treatments means using Statistics version 8.1 and according to [22].

Results

The analyzed data for root and shoot length of eight maize hybrids showed that imposing of water stress caused a significant reduction in root and shoot length of all maize hybrids (Table 1). However, considerable variations were observed among the maize hybrids when grown under normal and PEG-induced water stress. Cultivars FH-1046 and FH-988 produced maximum root and shoot length than the other cultivars under well-watered conditions, whereas under water stress conditions FH-988 followed by FH-1046 were the maximum root, shoot length producers. In contrast, cultivar FH-1137 was the minimum root and shoot length under both normal and water stress conditions. Similarly, Rustam-1 followed by FH-949, Pioneer 30Y87 and Rustam-12 were intermediate in root and shoot length among all cultivars under both normal and water stress conditions.

Table 1. Effect of water deficit stress (DD) on root length, shoot length, root fresh weight, and shoot fresh weight of eight maize hybrids

Maize Hybrids	Root length (cm)		Shoot length (cm)		Root fresh weight (g)		Shoot fresh weight (g)	
	WW	DD	WW	DD	WW	DD	WW	DD
FH-988	41.33 A	36.66 BCD	90.00 A	72.00 F	25.09 B	20.46 GH	63.33 A	47.00F G
20R52	39.33 AB	33.33 EF	85.33 B	67.33 G	24.48 BC	18.60 IJ	60.33 B	43.66 H
FH-1046	42.00 A	35.33 CDE	92.00 A	69.66 FG	26.63 A	19.61 HI	64.66 A	44.66 GH
Rustam-1	37.33 BC	31.66 FG	84.33 BC	66.33 G	23.63 CD	17.72 JK	57.33 C	40.66 I
FH-949	34.66 CDE	30.00 GH	81.66 BCD	61.66 H	22.78 DE	16.90 KL	54.33 D	40.00 I
Pioneer 30Y87	34.00 DEF	28.00 HI	79.00 DE	55.66 IJ	21.74 EF	15.38 M	51.66 DE	35.66 J
FH-1137	27.66 HI	25.66 I	77.00 E	52.00 J	19.83 H	13.25 N	45.00 GH	32.33 K
Rustam-12	34.00 DEF	28.66 H	80.66 CDE	58.00 HI	21.41 FG	16.00 LM	49.33 EF	38.33 IJ

Note: The Values with a similar lowercase letter uppercase letter within the column of water deficit stress, root length, shoot length, root fresh weight, and shoot fresh weight of eight maize hybrids are not significantly different ($p < 0.05$). Water stress reduced root and shoot fresh weight of all cultivars as compared to normal conditions. However, cultivars FH-988, FH-1046 and 20R52 greater root and shoot fresh weight than that of all other cultivars. The maximum reduction in fresh biomass was

observed in cultivar FH-1137 due to drought stress; however, under water stress and well-watered conditions cultivars Rustam-1 followed by FH-949, Rustam-12 and Pioneer30Y87 were recorded as intermediate root and shoot fresh biomass. The data regarding mean root dry weight and shoot dry weight of different maize hybrids under control, and -0.6 MPa of PEG-induced stress are depicted in (Table 2). Plant dry biomass was severely affected due to drought stress; dry weights of plants were significantly decreased. The dry weight of root and shoot of maize hybrids under well-watered and drought (Table 2) showed that minimum reduction in root and shoot dry weight was exhibited by maize hybrids FH-988, FH-1046 and 20R52. However, minimum dry biomass was recorded in FH-1137 under both water levels, while all other cultivars were intermediate.

Plants when face water stress, root to shoot ratio is increased. Root proliferation is an important parameter to assess drought tolerance in different genotypes as tolerant genotypes under drought stress have more root penetration to explore water from more depth as compared to sensitive ones. More shoot length was recorded under condition as compared to water stress. In (Table 2) indicated that drought caused significant reduction in root: shoot and number of leaves while cultivars differed considerably in root: shoot and number of leaves. The maximum root: shoot and number of leaves were recorded in CVS. FH-988, FH-1046 and 20R52 while minimum in FH-1137 and Rustam-1, FH-949, Rustam-12, and Pioneer 30Y87 were intermediate under both normal and water stress conditions.

Table 2. Effect of water deficit stress (DD) on root dry weight, shoot dry weight, and root: shoot, number of leaves of eight maize hybrids

Maize Hybrid	Root dry weight (g)		Shoot dry weight (g)		Root: Shoot		Number of leaves	
	WW	DD	WW	DD	WW	DD	WW	DD
FH-988	3.33 AB	2.88 DE	7.37 AB	5.01 DE	0.45 BCD	0.50 A	10.33 A	9.33 ABC
20R52	3.20 BC	2.46 FG	6.71 BC	4.51 EFGH	0.45 BCD	0.49 AB	9.66 AB	8.33 BCDE
FH-1046	3.50 A	2.68 EF	7.57 A	4.80 DEF	0.45 BCD	0.50 A	10.66 A	9.00 ABCD
Rustam-1	3.13 BC	2.33 GH	6.19 C	4.31 FGHI	0.43 CD	0.48 ABC	9.00 ABCD	7.66 CDEF
FH-949	3.03 CD	2.19 HI	5.25 D	3.87 HIJ	0.41 D	0.48 ABC	9.00 ABCD	7.33 DEF
Pioneer 30Y87	3.00 CD	1.8 IJ	4.69 DEFG	3.54 JK	0.42 D	0.49 AB	8.33 BCDE	6.33 FG
FH-1137	2.11 HI	1.45 K	4.06 GHIJ	3.10 K	0.35 E	0.47 ABC	7.00 EFG	5.33 G
Rustam-12	2.85 DE	1.97 IJ	4.60 DEFG	3.76 IJK	0.41 D	0.48 ABC	8.33 BCDE	6.66 EFG

Note: The Values with a similar lowercase letter and uppercase letter within the column of water deficit stress, on root dry weight, shoot dry weight, and root: shoot, number of leaves of eight maize hybrids are not significantly different ($p < 0.05$).

Effect of drought on membrane stability index (MSI) and leaf potassium contents (K^+) of eight maize hybrids are listed in Table 3. Compared to control, PEG addition caused a significant reduction in MSI and leaf K^+ substances in all maize hybrids at -0.6 MPa. The higher decrease in MSI and leaf K^+ contents was observed in maize hybrid FH-1137 followed by maize hybrid Pioneer

30Y87 while the minimum reduction was observed in maize hybrid FH-988 followed by maize hybrid FH-1046 at water stress. All other maize hybrids showed a reduction in between these maize hybrids.

Application of PEG-8000 (0.6 MPa, 15%) resulted in a significant decrease in SPAD chlorophyll value and relative water contents (Table 3). Chlorophyll contents were

significantly reduced in all maize hybrids with maximum reduction in chlorophyll contents was observed in maize hybrid FH-1137 while the minimum reduction was noted in maize hybrid FH-988 under drought. The highest reduction in relative water contents was observed in maize hybrid FH-

1137, and lowest reduction in leaf relative water contents was found in maize hybrid FH-988. While the maize hybrids 20R52 followed by Rustam-1, FH-949, Rustam-12 and Pioneer 30Y87 showed intermediate performance under both well-watered and drought.

Table 3. Effect of water deficit stress (DD) on membrane stability index, leaf potassium contents, leaf chlorophyll contents, relative water contents of eight maize hybrids

Maize Hybrid	Membrane stability index %		Leaf potassium contents (m mol g ⁻¹ dwt ⁻¹)		Leaf chlorophyll contents (SPAD Value)		Relative water contents (RWC) %	
	WW	DD	WW	DD	WW	DD	WW	DD
FH-988	83.50 A	78.66 E	174.67 A	123.00 F	38.33 A	32.86 C	89.43 B	82.33 GH
20R52	82.33 B	76.33 G	169.00 B	118.33 G	38.00 A	30.60 DE	88.20 BC	80.13 IJ
FH-1046	84.50 A	77.66 EF	177.35 A	121.67 FG	39.00 A	32.16 C	91.16 A	81.66 HI
Rustam-1	81.33 BC	74.33 H	162.33 C	112.33 H	35.00 B	30.36 DE	86.66 CD	79.33 JK
FH-949	80.66 CD	72.66 I	155.33 D	106.33 I	33.10 C	29.43 EF	85.66 DE	78.33 KL
Pioneer 30Y87	79.83 D	70.33 J	154.67 D	99.00 JK	32.36 C	27.50 G	84.66 EF	76.00 M
FH-1137	76.66 FG	69.00 K	144.00 E	96.67 K	30.40 DE	25.26 H	82.33 GH	72.00 N
Rustam-12	78.66 E	71.66 I	152.33 D	102.67 IJ	31.76 CD	28.56 FG	83.33 FG	77.66 L

Note: The Values with a similar lowercase letter and uppercase letter within the column of water deficit stress, on membrane stability index, leaf potassium contents, leaf chlorophyll contents, with relative water contents of eight maize hybrids and soil layers are not significantly different ($p < 0.05$).

Discussion

It is well clear that selection of appropriate plants from small or large germplasm collection particularly morphological and biochemical traits is a viable method that promotes the crop improvement for drought stress tolerance [23-25]. A simple method of screening maize genotypes against drought tolerance using potential morphological (root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, , number of leaves) and biochemical (potassium and chlorophyll contents in leaves, leaf relative water contents and membrane stability index) selection criteria were tested in the present study. The different sets of maize cultivars were tested in the present study that showed wide variations for drought stress tolerance at early growth stages. For example, drought stress conditions genotype, FH-988 proved to be most tolerant of drought, while FH-1137 the

most sensitive being the least production of all parameters.

Like other researchers, the findings of our study also showed that drought caused a reduction in fresh and dry weight of all maize hybrids [26, 27]. This reduction in maize fresh and dry weight was due to dehydration [28] because water stress causes denaturing of proteins, production of reactive oxygen species and decreased biomass of plants [10]. This study showed the decline of root and shoot length under drought stress conditions. Retardation in root and shoot length under low water availability was also reported by other workers in maize [29]. The root: shoot on length basis enhanced under drought as compared to well-watered treatment because the shoot is affected more from drought as compared to root. The increase in root: shoot under drought confirmed the previous results [15, 30]. The number of leaves was reduced under drought because drought stress leaf

initiation, suppress node formation and decrease axial growth rate for which the number of sheets becomes less [31]. Genetic variability for stress response could only be observed upon plant exposure to drought stress [32]. Thus, higher water stress tolerance in FH-988 might be due to the expression of water stress-responsive genes that can be translated into certain biochemical phenomena such as maintenance of relative water contents, potassium contents, osmotic adjustment, chlorophyll contents, membrane stability index [33, 34]. Using potassium contents as a selection criterion, it was promising to differentiate among the maize cultivars and optimistic correlation was found between this biochemical attribute and drought stress tolerance. Thus, potassium contents could be used as a proficient selection criterion for screening maize germplasm for drought stress tolerance. Drought tolerant maize hybrids (FH-988) uptake higher potassium contents under water stress conditions as compared to drought sensitive maize hybrid (FH-1137). Similarly, drought tolerant maize genotypes adapted to dry areas had higher potassium contents [35]. Such a positive relationship between potassium contents and drought stress tolerance was also observed in wheat genotypes [36]. Chlorophyll contents, relative water contents, and membrane stability index are very viable parameters that directly contribute to plant output [37]. In the present study, drought caused a significant reduction in Chlorophyll contents, relative water contents, and membrane stability index in all maize hybrids. The drought-tolerant varieties have higher chlorophyll contents, water contents, and membrane stability index [38]. The maize hybrid (FH-988) showed higher Chlorophyll contents, water contents and membrane stability index under drought stress conditions as compared to drought sensitive maize hybrid (FH-1137). The plants

that produced the highest chlorophyll content might have accumulated relatively more food reserves for the growth and development of more vigorous shoot and leaves. Higher relative water contents in water-stressed plants may be the result of lower rates of water loss due to stomatal closure and developed variety [39]. Cell membrane stability decreased by severe drought stress in other words in this condition destruction of cell wall increased [40].

Conclusion

It can be concluded from this study that the performance of the maize genotypes was different under drought stress. The present study revealed that at the seedling growth stage, shoot/root length, shoot/root fresh weight shoot/root dry weight, number of leaves, chlorophyll contents, membrane stability index, relative water contents and K^+ contents in the sheet could be useful parameters for screening maize genotypes against drought. Based on all above-recorded observations it was concluded that FH-988 is drought tolerant hybrid while FH-1137 is sensitive to induced drought under hydroponics. These results can be a good source for the plant breeders and plant physiologists engaged in the development of drought-tolerant maize genotypes. These drought tolerant genotypes could be exploited in the breeding program for the development of elite genotypes having high drought tolerance and have the potential to grow effectively on drought-affected regions. Further work is needed to evaluate the performance of this screened material in soil culture.

Authors' contributions

Conceived and designed the experiments: A Ali & M Tariq, Performed the experiments: M Maqbool & A Ali, Analyzed the data: M Rashid, SA Kalhoro & M Ahmed, Contributed materials/ analysis/ tools: FA Marri & KM Khan, Wrote the paper: A Ali & A Ahmed.

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