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ASSESSMENT OF WATER STRESS TOLERANCE IN DIFFERENT MAIZE ACCESSIONS AT GERMINATION AND EARLY GROWTH STAGE

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Abstract

A pot experiment was conducted to screen 60 maize accessions for water stress tolerance. In preliminary experiment, 60 maize accessions were grown at four moisture levels (100%, 80%, 60% and 40% of field capacity) and evaluated on the basis of survival rate. About 10 maize accessions namely NC-9, M-14, B-42, NC-3, N18, W187R, NC-42, NC-8 and A50-2 with highest survival rate at low moisture level (40% of FC) were selected as water stress tolerant and other 10 maize accessions namely T-7, WFTMS, B-34, NC-7, N48-1, OH54-3A, T-5, UMZ, NC-4 and USSR with lowest survival rate at mild stress (moisture level 80% of FC) were selected as susceptible to water stress. In the 2nd experiment, these selected maize accessions were further evaluated on the basis of survival rate, relative cell membrane injury (RCI %age), and stomatal conductance. Broad sense heritability for these traits was also estimated and it was found that RCI% could be used as main selection criterion for drought tolerance in maize. Furthermore, on the basis of this selection criterion, NC-9 was found as highly water stress tolerant, while T-7 recognized as drought susceptible.
Keywords: drought, selection criteria, screening and selection, stress tolerance

Introduction

Of various abiotic stresses, water stress is one of major environmental constraints that limit crop productivity world wide (Araus *et al*., 2002; Boyer, 1982). Pakistan also faces serious problems of shortage of water due to low and irregular rain fall, (less than 100 mm) which resulted in heavy crop losses (Govt. of Pakistan, 2003). Furthermore, in view of various climatic change models scientists suggested that in many regions of world, crop losses due to increasing aridity will further increase in future (Athar & Ashraf, 2005). Thus, the threat and effects of shortage of water on crop productivity are becoming more alarming. Therefore, different effective measures should be adopted to reduce crop losses. It has been estimated that it is very necessary to improve crop productivity by 20% in the developed countries and by 60% in the developing countries (Owen, 2001). Screening and selection of plants of different crops with considerable water stress tolerance has been considered an economic and efficient means of utilizing drought-prone areas when combined with appropriate management practices to reduce water loss (Rehman *et al*., 2005). Several physiological characteristics have been reported as being reliable indicators for the selection of genotypes/cultivars for drought tolerance (Ashraf *et al*. 1999), e.g., photochemical activity of photosystem II (PS-II) calculated as F_v/F_m and chlorophyll in canola (Kauser *et al*., 2006), and cell membrane stability (CMS) (Dedio, 1975). Thus, improved water stress tolerance is one of major objectives of plant breeding programs for crops grown in dry areas.

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Maize is efficient user of moisture for dry matter production and requires 500-800 mm of water during life cycle of 80-110 days (Critchley & Klaus, 1991). At the time of tesseling maize requires 135 mm / month and this may increase up to 195 mm / month during hot windy conditions (Jamieson *et al*., 1995). Keeping in view all above information, present study was conducted to screen maize accession for water stress tolerance. In addition, genotypic variation was also assessed for some physiological traits that could be used as selection criteria for future breeding program.

Materials and Methods

The seed of 60 maize accessions was collected from different research stations of Pakistan viz National Agricultural Research Centre (NARC), Islamabad, Maize and Millet Research Institute (MMRI), Sahiwal, and University of Agriculture Faisalabad. The collected germplasm was multiplied in the fields of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad for this study. Polythene bags (18 × 9 cm) were filed with sandy loam soil (pH 7.8 and EC 1.7 dS/m) and 30 seedlings of each accession were established in each treatment in two factor factorial completely randomized design. Plants were subjected to different moisture levels (40, 60, 80 and 100% of field capacity) by controlling water application three weeks after sowing. Moisture levels were maintained on alternate days by irrigating the polythene bags with tap water (EC 0.927 dS/m). At 50% mortality, half strength Hoagland's nutrient solution was applied to all the experimental units to recover the surviving seedlings. After 24 hours survived seedlings were counted and survival rate of the each accession was estimated using following formula;

$$\text{Survival Rate (\%)} = (\text{Number of survived seedlings} / \text{Total number of seedlings}) \times 100$$

The accessions selected in preliminary experiment were reassessed for drought tolerance on the basis of survival rate, relative cell injury percentage (RCI%) and stomatal conductance under four moisture levels. Forty earthen pots (11" × 12") filled with soil (pH 7.8 and EC 1.7 dS/m) of each accession were arranged following two factor factorial completely randomized design and two seeds of each accession were sown 3 cm deep in each experimental unit. Nine seedlings of each were tagged randomly at 20 days of age. The survival rate of 20 selected maize accessions was estimated following the same procedure explained above.

Relative cell injury percentage (RCI %): RCI % was estimated following the procedure proposed by Sullivan (1972). Two leaf discs (10 mm of diameter), one from each side of midrib, from one leaf of each tagged seedling were washed and grouped in to two sets on the basis of samples i.e. side of midrib, one disc from each side. On set was treated with 50°C (treatment) for one hour and second with 25 °C temperature (control) for one hour in water bath (MEMMERT-WB1, Germany) with attached shaker (MEMMERT-SV1422, Germany). 10 ml of de-ionized water added in each vial and placed at 10 °C for 24 hours. Vials were placed on mechanical shaker (EYELA-MMS, RIKAKIKAI CO., LTD.) to mix the contents at room temperature. EC of sap in vials was measured. Then these vials were autoclaved at 121 °C with 15 psi for 10 min. to release all the electrolytes from the cells. Vials were allowed to cool at room temperature and EC was measured. RCI % was calculated by following formula (Sullivan, 1972).

$$RCI\ \% = 1-[\{1-(T1/T2)\} / \{1-(C1 / C2)\}] \times 100$$

Where,

T1 = EC of sap of treated discs (50 °C) before autoclaving
T2 = EC of sap of treated discs (50°C) after autoclaving
C1 = EC of sap of treated discs (25°C) before autoclaving
C2 = EC of sap of treated discs (25°C) after autoclaving

Stomatal conductance: Data for stomatal conductance were recorded using diffusion porometer (AP4, Delta-T Devices, Cambridge, UK) at 1.00 and 2.00 pm (Kirda *et al.*, 2005) from one leaf of nine selected seedlings in each treatment one weak after imposition of water stress. To further elucidate these findings, all accessions were grouped in to four categories i.e. "A" (fully turgid and green), "B" (not fully turgid and green), "C" (wilted and light green) and "D" (wilted, rolled and light green).

Statistical analysis: For assessment of variability the data was analyzed using analysis of variance following Steel & Torrie (1980).

Results and Discussion

ANOVA and mean data for survival rate of 60 maize accessions at varying levels of moisture stress showed that increasing level of water deficit caused a significant reduction in survival of all maize accessions examine in the present study (Table 1; 2). However, highest water stress-induced reduction in survival rate of all maize accession was observed at the lowest level of moisture regime. Maize accessions were also differed significantly for survival rate at varying levels of moisture stress. However, accessions NC-9, M-14, B-42, NC-3, N-14, W187R, T-4, NC-42 and NC-8 showed more than 66% survival rate at the lowest moisture level. Furthermore, these accessions were excelled other at other moisture regimes. However, T-7, WFTMS, B-34, NC-7, N48-1, OH54-3A, T-5, UMZ, NC-4 and USSR showed less than 21% survival rate at 40% of FC and less than 41% survival rate at 80% of FC. Similar results of water stress induced reduction in survival rate were observed in a number of crop plants (Liptay *et al*. 1998). Since tolerance to water stress is a genetically controlled, variation in survival rate at varying levels of water stress seems to be controlled by a number of physiological and biochemical processes. Thus, selected maize accessions of highly water stress tolerant and sensitive were re-evaluated for drought tolerance using some physiological selection criteria.

Imposition of simulated water stress reduced the survival rate and stomatal conductance, while it caused a significant increase in relative cell injury percentage in both water stress tolerant and water stress sensitive accessions. However, adverse effects of water stress was less in selected 10 water stress tolerant maize accessions compared with those of water stress sensitive accessions. As different crop species have varying responses to water stress (Ashraf & Mehmood, 1990) one finds a spectrum of responses within same species and may be classified as tolerant or sensitive to water stress. However, water stress tolerant plants have ability to maintain water and ion homeostatic conditions by lowering water loss through transpiration, and/or accumulation of organic compatible solutes. In view of water conservation strategy in plants to acclimatize water stress, leaf stomatal conductance has long been considered a promising selection criterion for drought resistance although some contrasting reports have been

Table 1. Mean survival rate (%) of 60 maize accessions grown in four moisture Levels

Accessions	40 % of FC	60 % of FC	80 % of FC	100 % of FC
T-7	0	10	20	100
WFTMS	0	13	23	100
B34	10	17	23	100
NC-7	11	20	33	100
N48-1	13	23	33	100
OH54-3A	17	23	37	100
T-5	20	27	37	100
UMZ	20	30	37	100
NC-4	20	30	40	100
USSR	20	30	40	100
A239	23	33	43	100
NC-13	23	33	43	100
B34-2B	27	37	43	100
T-1	27	37	47	100
OH8	27	37	47	100
36	30	40	47	100
PB7-1	30	40	47	100
JY1	30	40	50	100
20P2-1	30	40	50	100
52B4	33	43	50	100
WF-9	33	43	50	100
W82-3	33	43	53	100
T-6	33	43	53	100
Q88	33	43	53	100
A427-2	37	43	53	100
W64SP	37	43	57	100
A556	37	47	57	100
A545	37	47	57	100
PB77	40	47	57	100

Accessions	40 % of FC	60 % of FC	80 % of FC	100 % of FC
WM13RA	40	47	60	100
W64TMS	40	50	60	100
W10	40	53	60	100
OH41	43	53	60	100
AES204	43	53	63	100
NC-6	43	53	63	100
150P1	43	57	63	100
NC-41	47	57	64	100
USSR150	47	57	67	100
NC-5	47	57	67	100
NC-1	50	60	67	100
150PZ-1	50	60	67	100
Q66	50	63	70	100
L5-1	53	63	70	100
A509	53	63	70	100
53AP1	53	63	73	100
ANTIGUA-1	57	67	73	100
T-8	57	67	73	100
A521-1	60	67	73	100
A638	60	67	77	100
53P4	60	70	77	100
A50-2	63	73	77	100
NC-8	67	77	80	100
NC42	70	77	80	100
T4	73	80	83	100
W187R	73	80	87	100
N18	77	83	90	100
NC-3	80	87	90	100
B42	80	90	93	100
M14	83	90	93	100
NC-9	87	93	97	100
SE	1.56	1.52	1.49	

Table 2. Analysis of variance for survival rate (%) of 60 maize accessions grown in four moisture levels

Source of Variation	Degree of	Sum of	Mean Squares	F_{ratio}
Accessions (A)	59	149274	2530	58.57**
Moisture levels (M)	3	347575	115858	2682.25**
A × M	177	51517	291	6.74**
Error	480	20733		

* = *Significant at P ≤ 0.05;* ** = *Significant at P ≤ 0.01;* *NS = Non significant at P >0.05*

Table 3. Mean squares for survival rate and stomatal conductance of 20 maize accessions grown in four moisture levels, and RCI % in 100 % FC.

Source of Variation	Degree of	Survival rate	Stomatal	RCI (%)
Accessions (A)	19	4509.89**	20.50**	943.80** (19)
Moisture Levels (M)	3	34997.08**	317.10**	
A × M	57	535.39**	15.30**	
Error	160	89.58	1.10	4.5 (40)

Note: Figures in parenthesis show degree of freedom

Table 4. Mean stomatal conductance and leaf turgidity appearance of 20 maize accessions grown in four moisture levels.

	40 % of FC		60 % of FC		80 % of FC		100 % of FC	
Genotypes	Stomatal conductance ($mm\ s^{-1}$)	Appearance	Stomatal conductance ($mm\ s^{-1}$)	Appearance	Stomatal conductance ($mm\ s^{-1}$)	Appearance	Stomatal conductance ($mm\ s^{-1}$)	Appearance
W187R	42	D	43	C	44	B	46	A
NC-42	43	C	43	C	44	B	46	A
NC-9	40	A	42	A	43	A	43	A
M14	39	B	41	A	42	A	43	A
NC-4	42	D	43	C	44	B	45	A
T-7	44	D	45	C	45	C	46	A
B42	39	A	41	A	42	A	43	A
T-1	41	C	42	C	43	B	44	A
N18	41	C	42	C	43	B	44	A
B34	42	D	43	C	44	B	45	A
USSR	43	D	45	C	45	C	46	A
NC-7	42	C	42	C	44	B	44	A
UMZ	42	C	42	C	43	B	44	A
WFTMS	42	C	44	C	45	B	45	A
NC-8	44	C	44	C	45	B	46	A
A50-2	39	B	41	A	42	A	43	A
N48-1	41	D	42	C	43	B	44	A
NC-3	40	A	41	A	43	A	44	A
T-4	41	D	43	C	43	B	45	A
T-5	43	D	44	C	44	B	45	A
SE	0.42		0.52		0.37		0.40	

A = *Fully turged and green in color (As in normal)* B = *Semi turged and green in color.*
C = *Semi wilted and light green in color.* D = *Wilted, rolled and light green in color.*

Table 5. Mean survival rate of 20 maize accessions grown in four moisture levels and mean RCI %

Accessions	Survival Rate (%)				RCI (%)
	40 % of FC	60 % of FC	80% of FC	100 % of FC	100 % of FC
T-1	0	7	43	100	70
T-7	7	13	33	100	83
N48-1	17	20	33	100	81
USSR	20	27	40	100	73
B34	20	27	40	100	75
WFTMS	23	30	40	100	87
T-5	27	37	43	100	69
NC-4	33	37	47	100	64
UMZ	33	37	47	100	61
NC-7	43	53	60	100	54
NC-8	47	57	67	100	52
NC-42	53	63	73	100	52
T-4	60	67	77	100	51
W187R	60	63	80	100	45
N18	70	80	87	100	45
NC3	77	83	90	100	41
B42	77	87	93	100	32
A50-2	80	87	93	100	37
M14	80	87	93	100	33
NC-9	83	90	97	100	30
SE	3.46	3.58	3.2	-	2.26

Table 6. Estimates of genotypic, phenotypic and environmental variances, and heritability in broad sense of 20 maize accessions.

Variances	Survival Rate (%) (SR)			Stomatal Conductance (mm s^{-1}) (SCD)				RCI (%)
	40 % of FC	60 % of FC	80 % of FC	40 % of FC	60 % of FC	80% of FC	100 % of FC	100 % of FC
Genotypic	715.03	745.22	526.22	4.72	18.00	2.32	3.87	301.61
Phenotypic	818.53	931.12	758.22	7.52	26.60	4.62	10.67	318.61
Environmental	103.50	185.90	232.0	2.80	8.60	2.30	6.80	17.00
$h^2_{B.S}$.	87.35	80.03	69.40	62.70	67.76	50.19	36.24	94.66

found in the literature (Ashraf & O'Leary, 1996; Sadaqat, 1999; Athar & Ashraf, 2005). Long ago, Spence *et al*. (1986) pointed out that stomata adapted to drought stress maintain stomatal opening at lower plant water potentials than non-adapted stomata. In the present study, water stress reduced the stomatal conductance of all accessions; however, water stress induced reduction in stomatal conductance was less in tolerant accessions and thus supported the view that stomatal conductance could be used as a selection criteria. Among water stress tolerant NC-9, M-14, B-42, NC-3 and A50-2 had lower stomatal conductance and maintained their turgidity. Similarly, among water stress sensitive cultivars N48-1, WFTMS, USSR, T-7 and B-34 showed high stomatal conductance and lost the turgidity. These results further emphasized that maintenance of plant water status in these maize accession was depend on regulation of stomatal conductance.

Drought susceptible accessions also showed a maximal relative cell membrane injury compared with those of drought tolerant accessions (Table 5). These results are similar to those of Tripathy *et al*. (2000) who considered RCI% as one of the major selection criterions of drought tolerance in cereals. Similarly, different researcher grouped the accessions of different crops with lesser RCI value as tolerant while accessions with higher RCI value were grouped as susceptible (Sullivan, 1972; Blum & Ebercon, 1981; Fokar *et al*., 1998). In addition, broad sense heritability of all the three traits was calculated and trait with high heritability was selected as selection criterion as suggested by Betran *et al*. (2003). Results for broad sense heritability depicted that with the in stress level there was a gradual increase in heritability of all these traits (Table 6). Fokar *et al*. (1998) reported high value of broad sense heritability for RCI% and suggested that screening and selection of individuals tolerant to water stress could be made using RCI%.

Overall, on the basis of RCI, and stomtal conductance accessions NC-9, M-14, B-42, A50-2 and NC-3 were selected as most drought tolerant and WFTMS, T-7, N48-1, B-34 and USSR were grouped as most drought susceptible. In conclusion, RCI% and stomatal conductance could be used as selection criteria for drought tolerance.

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