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Physiological Markers for Screening Sorghum (*Sorghum bicolor*) Germplasm under Water Stress Condition

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ABSTRACT

Seed of eighty accessions collected from the National Agricultural Research Council, Islamabad, Pakistan were subjected to drought stress for ten days in greenhouse. Leaf stomatal conductance, osmotic potential, water potential, turgor pressure, shoot length and root length were measured to select physiological markers for drought tolerance and evaluate the potential of different sorghum accessions for drought tolerance. The accessions differed significantly in all physiological parameters. In most accessions, reductions were observed under water stress for all traits. In contrast, significant increases in measurements of root length were also observed under water stress conditions in some accessions. Among proportional contribution of osmotic potential, water potential, turgor pressure, root length, shoot length and stomatal conductance towards drought tolerance: the osmotic potential was the highest contributing among all parameters for drought tolerance. So, it might be used as selection trait for drought tolerance. Among 80 sorghum accessions, only five accessions (80265, 80114, SS-95-4, SS-97-7 & 80377) were most water stress tolerant. © 2010 Friends Science Publishers

Key Words: Sorghum; Seedling growth; Drought stress; Leaf water relations

INTRODUCTION

Drought tolerance is a complex trait. The genetic and physiological mechanisms governing its expression are poorly understood (Boyer, 1982; Ejeta *et al.,* 2007) yet, few drought tolerance determinants have been identified (Araus *et al*., 2002; Bruce *et al*., 2002). Understanding these mechanisms and breeding for drought-tolerant crops has been one of the major goals of plant breeders.

The crop plants are usually under stress at one growth stage or another. The plant species able to withstand such stresses have great economic importance. Drought stress is the major limitation to the production of crop plants in the rain fed areas (Nikus *et al*., 2004). The detrimental effects of drought stress on plants are a consequence of osmotic strain on the cytoplasm. In many plants, drought stress decreases stomatal conductance and transpiration (Earl, 2002; Ribas-Carbo *et al*., 2005). Under drought conditions, stomatal closure helps to maintain higher leaf water potential and thereby leaf water content (Nakayama *et al.,* 2007). Drought often induces changes in plant water relation parameters such as turgor pressure, osmotic pressure and water potential (Basra *et al.,* 1999).

In Pakistan, the demand for animal products is increasing in view of the ever-rising population that expected to reach 2.17 million by 2020 (FAO, 2005). Such demand calls for continuous supply of fodder through out the year. Sorghum is the most capable crop in meeting the demand for large quantities of high-quality green fodders, especially in the drier parts of the world. Nonetheless, its genetic potential for drought tolerance has to be exploited to the maximum possible to secure the increasing need for food.

To improve our present knowledge about drought tolerance we need to investigate attributes like stomatal conductance, leaf water potential, osmotic potential, turgor pressure and shoot and root length. Studying the impact of such parameters will assist in identifying some selection criteria that might prove useful in developing drought tolerant genotypes. The objectives of the present study were to investigate the performance of different sorghum accessions under water stress condition and to identify the physiological markers attributable to drought tolerance.

MATERIALS AND METHODS

Eighty sorghum accessions collected from National Agricultural Research Council, Islamabad, Pakistan were arranged in a completely randomized design with three replications and two factors representing stressed and non-moisture stressed (control) conditions. Ten seeds of each accession were sown at a depth of 1 cm in earthen pots each

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filled with 10 kg of sand and supplemented with Hoagland solution (20 mL per pot) as nutritive media (Hoagland & Arnon, 1950). The accessions under control were irrigated daily (total 7 irrigations) with 50 mL of distilled water per pot, whereas the stressed conditions were simulated by withholding irrigation from sowing. After ten days from sowing, three plants were used each accession in each replication to measure stomatal conductance (gs), osmotic potential (Ψπ), water potential (Ψw), turgor pressure (Ψp), shoot length (SL) and root length (RL).

The (gs) were for three randomly selected flag leaves of intact plants from each genotype were measured between 10-11 h with the help of a porometer. Water potential (Ψw) was measured in units of pressure using pressure chamber; three fully expanded flag leaves were sampled per replicate from each treatment between 1100 and 1300 h. This pressure is balance pressure and is equal in magnitude but opposite in sign, to the negative pressure that existed in the xylem column before the plant tissue was excised. Plant leaves from each replication were washed in distilled water, blot dried with tissue paper and transferred to eppendorf tubes in deep freezer. The frozen samples were thawed, crushed with glass rod and the sap was centrifuged out at 1100 rpm. Osmotic pressure was measured with micro osmometer by calibrating the equipment in m.osmolkg^{-1} of water. The pressure was converted into potential by putting a negative sign as prefix to the values (Basra *et al.,* 1999). The concentration unit's milli osmole kg^{-1} of water was converted into pressure units, MPa using Vent Hoff relationship at 20^{o}C (Nobel, 1983).

$$\Psi\pi = -\,miRT$$

Where *m* is the concentration in molarity of the solute, *i* is the Van't Hoff factor, the ionization constant of the solute (1 for glucose, 2 for NaCl, etc.) *R* is the ideal gas constant, and *T* is the absolute temperature.

The values determined for water potential and osmotic potential were used in calculating turgor pressure of flag leaves as per the following equation given by Kramer (1983):

$$\Psi w = \Psi\pi + \Psi p$$

The plants were carefully uprooted and the lengths of shoots and roots were measured in centimeters using a ruler.

The recorded data were subjected to analysis of variance (Steel *et al.,* 1997). The multivariate scoring index (Principle component analysis) was employed to find out the best performing accessions under water stress using the data recorded for the six physiological parameters.

RESULTS

Of the eighty accessions grown, only fifty survived under water stress conditions and were subjected to further evaluation. As indicated by the data shown by Table II, the measurements of gs, Ψw,Ψπ, Ψp, SL and RL were decreased under water stress compared to control conditions. The response of different accessions was different under water stress. The highest respective decrease in measurement was shown by the accessions: 80174 (63.9%), 80210 (33.6%), 80091 (7.8%), 80205 (83.9%), 80174 (46.6%) and 1728 (35.6%) in stomatal conductance, water potential, osmotic potential, turgor pressure, shoot length and root length. In contrast, some significant increase in measurements for root length was also observed. Accessions that showed increased measurements for root length were: SS-95-I, 80107, 80234, 80011, 80236, 80114, 80364, SS-98-3, SS-97-9, 80203, 80210, 80377, 80204, 80154, F9706, SS-97-6, 80319 and 80080.

The analysis of variance (Table I) indicated that the accessions differed significantly (P=0.01-0.05) in all traits. Moisture condition also differed significantly for all the characters except for Ψp. Interaction of accessions with moisture condition was significant for stomatal conductance, shoot length and root length; suggesting differential performance of accessions for these parameters under different moisture conditions.

Multivariate scoring of principle components analysis (Fig. 1) was carried out using replicated data of gs, Ψw,Ψπ, Ψp, SL and RL. A review of the figure showed that sorghum accessions 80265, 80114, SS-95-4, SS-97-7 and 80377 depicted the highest multivariate scores; whereas the accessions 80199, 80365, 80369, 80374 and 80381 depicted the lowest scores. The 5 accessions (from upper side of the graph) were screened as most drought tolerant.

Based on the results obtained from principle component analysis, the proportional contributions in drought tolerance for osmotic potential, water potential, turgor pressure, root length, shoot length and stomatal conductance were: 20.8 %, 23.5 %, 16.5%, 15.9%, 13.3% and 10%, respectively (Fig. 2), thus the osmotic potential was the highest contributing among all parameters for drought tolerance and it could, therefore be used as selection trait for identifying drought tolerant genotypes.

DISCUSSION

Daily or seasonal water stress to which a plant is subjected induces a range of plant responses (Pessarakli, 1999). Perhaps the most critical plant response under drought conditions is stomatal regulation of water loss. The classical control system involves stomatal closure as a result of water loss of guard cell turgor at lower leaf water potentials (Paleg & Aspinall, 1981). Stomata have a high capacity of response to changes in the plant water status and they close as leaf water potential decreases. They are most sensitive to changes in atmospheric humidity (Lange *et al.,* 1971; Sandford & Jarvis, 1986). There was a notable fall in gs, in plants subjected to water stress at seedling stage (Table I). This fall in gs is associated with lower leaf water potential. Decrease in leaf water potential was associated with reduced water supply from the soil to the roots and

Table I: Leaf water relations under stressed and non-stressed (control) conditions in sorghum

Accessions	gs(cm/s)		Ψw (MPa)		Ψπ (MPa)		Ψp (MPa)		SL (cm)		RL (cm)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
80162	13.58	6.86	-4.07	-4.13	-4.82	-4.84	0.71	0.55	15.17	14.12	6.22	5.86
SS-98-5	7.97	3.66	-3.57	-3.64	-3.71	-3.94	0.30	0.15	11.10	10.77	7.67	5.16
BR-123	6.52	3.36	-3.10	-3.16	-3.75	-3.84	0.68	0.65	12.98	10.08	13.17	10.07
80205	21.37	13.32	-4.15	-4.17	-4.17	-4.27	0.10	0.02	12.73	10.59	6.53	6.22
80091	7.63	4.86	-3.33	-3.59	-3.56	-3.86	0.27	0.23	11.59	10.73	7.07	6.51
SS-95-4	11.88	7.70	-4.87	-5.02	-5.37	-5.64	0.61	0.49	12.54	11.27	3.47	4.11
80199	12.01	5.92	-4.03	-4.09	-4.51	-4.60	0.52	0.48	14.40	12.78	7.43	6.53
80361	8.48	7.74	-3.55	-3.64	-3.85	-3.97	0.33	0.30	11.72	10.11	6.84	6.83
80363	7.01	6.27	-3.21	-3.28	-3.43	-3.52	0.24	0.23	16.27	14.03	5.55	5.51
80107	9.18	8.15	-4.09	-4.15	-4.18	-4.27	0.12	0.09	11.75	11.22	5.23	5.73
80353	10.25	5.91	-4.42	-4.85	-4.86	-4.97	0.12	0.44	7.38	6.88	6.49	6.35
80056	13.53	11.17	-4.14	-4.16	-4.58	-4.74	0.58	0.44	14.30	11.39	6.60	5.90
80077	11.65	8.69	-4.84	-4.85	-5.15	-5.17	0.32	0.30	13.20	11.95	5.58	5.15
80074	6.86	4.44	-3.16	-3.22	-3.39	-3.44	0.22	0.23	11.44	10.29	4.47	4.32
80234	4.96	3.23	-3.22	-3.27	-4.50	-4.87	1.60	1.29	12.76	10.86	5.55	5.46
80373	9.84	8.23	-4.53	-4.55	-4.76	-4.77	0.21	0.22	12.33	10.75	5.52	5.56
80381	9.28	7.84	-3.80	-3.83	-4.34	-4.41	0.57	0.54	16.88	13.25	5.24	4.25
80174	14.33	5.17	-3.93	-3.96	-4.39	-4.45	0.49	0.46	16.79	8.97	4.80	3.58
80308	6.75	5.85	-3.06	-3.15	-3.57	-3.75	0.59	0.51	11.93	9.14	4.06	3.76
FC 26 II	19.63	11.24	-3.48	-3.55	-3.59	-3.64	0.09	0.11	15.68	15.33	6.97	6.57
80011	10.75	5.65	-4.41	-4.52	-4.86	-5.08	0.56	0.44	12.50	12.18	6.68	7.14
80236	13.94	11.26	-3.68	-3.74	-4.05	-4.14	0.40	0.37	12.18	11.92	4.95	5.72
80114	10.64	8.95	-3.58	-3.61	-4.31	-4.67	1.06	0.73	14.38	14.33	3.43	3.52
80364	15.02	13.27	-2.23	-2.33	-3.14	-3.23	0.90	0.88	10.47	10.07	7.15	7.42
SS-98-3	10.47	9.94	-2.77	-2.96	-4.39	-4.54	1.58	1.55	11.29	9.80	3.18	3.55
SS-97-9	8.14	5.19	-2.06	-2.24	-4.18	-4.25	2.01	1.98	11.95	11.27	3.24	3.40
80265	12.86	6.67	-2.26	-2.46	-4.47	-4.69	2.24	2.21	11.22	9.79	5.23	4.87
80203	12.03	10.20	-2.95	-3.29	-4.73	-4.84	1.55	1.51	5.88	5.82	5.12	5.44
80210	9.97	8.34	-1.67	-2.52	-4.06	-4.15	1.63	1.56	6.83	5.59	3.10	3.76
80377	14.42	14.06	-2.32	-2.70	-4.33	-4.40	1.70	1.65	11.87	10.49	4.75	4.89
80204	11.35	8.23	-5.13	-5.27	-5.25	-5.40	0.13	0.12	8.36	7.37	3.78	3.84
80154	13.22	10.28	-3.45	-3.53	-4.85	-4.97	1.44	1.40	15.49	13.99	5.25	5.47
80269	13.46	11.00	-3.36	-3.46	-3.41	-3.49	0.04	0.03	10.68	9.59	3.89	3.03
80374	17.90	15.57	-5.14	-5.28	-5.83	-5.94	0.66	0.65	17.45	14.36	9.22	7.47
80214	28.25	13.38	-4.17	-4.30	-4.49	-4.59	0.29	0.25	10.05	9.31	3.41	2.82
80369	14.55	11.64	-4.77	-4.79	-5.47	-5.56	0.77	0.70	11.07	10.12	5.59	4.29
1632	13.94	12.26	-4.14	-4.20	-4.92	-5.00	0.80	0.78	12.66	11.06	8.00	6.26
1728	33.35	22.05	-3.47	-3.44	-4.38	-4.50	1.07	0.91	13.85	12.28	4.98	3.20
F9706	12.97	9.15	-3.76	-3.84	-4.32	-4.34	0.50	0.30	11.53	11.11	4.15	4.16
SS-97-7	11.78	8.58	-3.37	-3.44	-4.46	-4.54	1.10	1.09	6.68	6.26	7.14	7.17
80319	11.66	9.75	-3.17	-3.29	-3.71	-3.70	0.40	0.31	19.08	17.03	5.07	5.25
80159	17.76	14.42	-3.34	-3.54	-4.11	-4.15	0.61	0.59	10.81	10.69	5.34	5.33
80158	42.94	23.15	-3.41	-3.62	-4.84	-4.87	1.25	1.12	9.16	8.86	5.83	4.15
80175	9.75	4.46	-3.87	-3.99	-4.39	-4.45	0.47	0.41	10.60	9.73	5.58	4.77
80365	9.68	9.76	-4.12	-4.16	-4.34	-4.33	0.18	0.15	8.95	8.37	3.56	2.97
80376	12.52	10.81	-4.11	-4.21	-4.30	-4.35	0.14	0.11	11.24	10.57	5.89	4.81
80136	15.79	13.29	-3.26	-3.38	-3.48	-3.55	0.17	0.15	12.08	11.25	9.31	8.47
80283	10.35	5.95	-3.56	-3.68	-4.73	-4.77	1.10	1.09	9.68	9.17	11.94	10.16
80121	6.45	3.42	-3.76	-3.84	-3.94	-3.97	0.13	0.11	10.86	10.31	3.16	2.20
80080	28.91	14.35	-3.27	-3.37	-3.95	-4.06	0.69	0.68	11.27	11.24	6.37	6.50

gs = stomatal conductance, Ψw = water potential, Ψπ = osmotic potential, Ψp = Turgor pressure, SL = shoot length, RL = root length, cm/s = Centimeter per second, MPa = Mega Pascal, cm = centimeter

ultimately to the leaves (Wood & Goldsbrough, 1997; Mastrorilli *et al.,* 1999; Pleijel *et al.,* 2000). Differences in stomatal responses to water stress help determine the relative ability of genotypes to cope with drought conditions.

Leaf water potential, osmotic potential and turgor pressure are significantly decreased by water stress as indicated from Table I (Wood & Goldsbrough, 1997; Mastrorilli *et al.,* 1999; Pleijel *et al.,* 2000). And this could be caused by accumulation of osmotica (Na^+, K^+, Cl^-, sugar, amino acids & proline etc.) at cellular level (Nepomuceno *et al.,* 1998) so that turgor and turgor dependent processes may be maintained at a significant lower water availability (Kramer, 1983).

Water stress also reduced both shoot length and root length as clear from Table I (Luis *et al*., 1999; Meo, 2000). Because plant growth is the result of cell division and enlargement, water stress directly reduces growth by deceasing CO_2 assimilation and reducing cell division and elongation (Kramer, 1983). But drought stress effected more

Table II: Mean squares from analysis of variance for different seedling traits of sorghum accessions grown under stressed and no-stressed moisture conditions

Source of variations	df	Seedling traits †					
		gs	Ψw	Ψπ	Ψp	SL	RL
Accessions (A)	49	180.99**	2.09*	2.09*	1.97*	36.37**	20.50**
Moisture (T)	1	1234.36**	1.13*	0.85*	0.02^{NS}	78.88**	16.32**
A x T	49	23.65**	0.03^{NS}	0.01^{NS}	0.04^{NS}	3.39**	1.05*
Error	200	0.59	0.10	0.06	0.14	0.99	0.28

†: gs = stomatal conductance, Ψw = water potential, Ψπ = osmotic potential, Ψp = Turgor pressure, SL = shoot length, RL = root length
* Level of Significance at 0.01
** Level of Significance at 0.05

Fig. 1: Multivariate scores over osmotic potential, water potential, turgor pressure, root length, shoot length and stomatal conductance under control and water stress

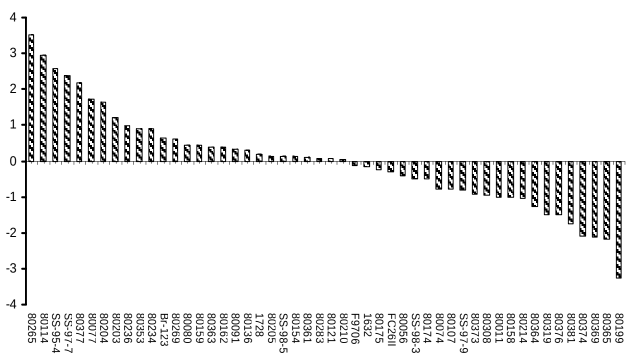
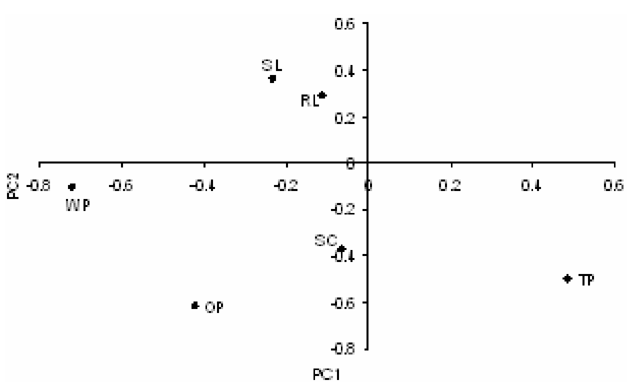


Fig. 2: Principle component analysis performed for osmotic potential (Ψπ), water potential (Ψw), turgor pressure (Ψp), root length (RL), shoot length (SL) and stomatal conductance (SC) of 50 accessions of sorghum



shoot growth than root growth and in certain cases root growth increased (Salih *et al.,* 1999; Younis *et al.,* 2000).

However as was observed in this study, root grew at a comparatively faster rate. This might be due to the reason that the minimum turgor pressure required for root growth was not much affected by water stress hence; root growth was not terminated, but ensued at slow rate. On the other hand, Hsiao and Xu (2000) reported that the growth region of roots is hydraulically isolated from the vascular system. This isolation protects the roots from low Ψw in the mature xylem and facilitates the continued growth into new moist soil volume. Any loss in turgor pressure as a consequence of the imbalance in the plant water content could result in reduced growth (shoot & root length) and even in the total absence of growth under dry environmental conditions. Never the less, the relationship between turgor loss and cell enlargement is unclear Takele (2000). Dhanda *et al.* (2004) and Kashiwagi *et al*. (2004) concluded that these seedling traits can be reliably utilized for screening of water stress tolerant genotypes in various crops.

Under water deficiency growth is readily inhibited and growth of roots if favored over that of leaves. For roots, when water potential (Ψw) is suddenly reduced, osmotic adjustment occurs rapidly to allow partial turgor recovery and reestablishment of Ψw gradient for water uptake. These adjustments permit roots to resume growth under low Ψw. In contrast, in leaves under reductions in Ψw of similar magnitude, osmotic adjustment occurs slowly, leading to marked growth inhibition (Hsiao & Xu, 2000).

Presence of substantial amount of variability in the available germplasm is prerequisite for triumphant breeding programme (Ali *et al*., 2008). The genetic material was significantly differed for all the physiological traits under study indicated greater genetic variability among the accessions (Table II). Significant differences were also found for treatments for stomatal conductance, leaf water potential, osmotic potential, turgor pressure, shoot length and root length suggesting differences in performance of accessions under different moisture conditions. Dhanda *et al.* (2004), Khan *et al.* (2004) and Hajime (1999), also observed significant differences for various seedling traits contributing to drought in wheat, maize and sorghum, respectively.

Multivariate analysis handles simultaneously a number of variables of common effects whereby similar data patterns being summarized, noise removed and the internal or some times hidden structures of the data being elucidated. Principal component analysis (PCA) is the most frequently used multivariate method. The 10 accessions (five each from upper & lower side of the graph) represent two distinct patterns or groups with differing responses to water stress could be advanced for further testing to drought tolerance. The results exhibited that accessions 80265, 80114, SS-95-4, SS-97-7 and 80377 are water stress tolerant among the accessions studied based on the seedling traits and can be further exploited in hybridization programme. Principle component analysis revealed that higher root length, shoot length with lower leaf water potential, osmotic potential and turgor pressure under water stress could be utilized as selection criteria for drought tolerance in sorghum at seedling stage. The most drought tolerant and susceptible genotypes might be used further in hybridization programme to create maximum genetic variability.

CONCLUSION

Generally reduction was observed in all the physiological traits in most of the accessions of sorghum under water stress. The sorghum accessions 80265, 80114, SS-95-4, SS-97-7 and 80377 were most drought tolerant, while the accessions 80199, 80365, 80369, 80374 and 80381 were most drought susceptible. These accessions might be utilized in hybridization programme to create maximum genetic variability. Osmotic potential might be used as selection trait for drought tolerance as it contributes maximum towards water stress among all the drought parameters.

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