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SCREENING OF DIFFERENT ACCESSIONS OF THREE POTENTIAL GRASS SPECIES FROM CHOLISTAN DESERT FOR SALT TOLERANCE

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Abstract

Experiments for evaluation of 22 accessions of three grass species (*Panicum antidotale, Cenchrus ciliaris and Sporobolus iocladus)* from Cholistan for salinity tolerance were conducted in a growth chamber under controlled environmental conditions at NIAB, Faisalabad, Pakistan. Germination stress tolerance index (GSTI), plant height and dry matter stress tolerance indices (PHSTI, DMSTI), cell membrane stability (% injury) and relative saturation deficits (RSD) of the germinating seeds were measured in all accessions under 0 or 1.5 % NaCl level. The results indicated that the accessions with high GSTI, cell membrane stability (less % injury), PHSTI, DMSTI and low RSD were more salt tolerant than the others thus seem promising for getting good productivity in salt-affected areas. These protocols are low cost, rapid and reliable for screening the germplasm of grasses against salinity.

Keywords: germination stress tolerance index, cell membrane stability, salt tolerance

Introduction

Among the arid regions of Pakistan, Cholistan desert is a very important segment that covers a large area (85000 km^2) in the Bahawalpur Division of Punjab, Pakistan. This region demands an immediate attention of plant scientists and Government funding agencies for rehabilitation. The desert temperature shoots up to 52°C during summer and average rainfall is 120 to 200 mm, which aggravate the problems of aridity (Naeem *et al.,* 2000). Cholistan vegetation is facing severe problem of drought, salinity and over-grazing by livestock. But shortage in fodder/ grasses for grazing occurs during summer in Cholistan and other parts of the country. So the present situation demands biological endeavors to focus on plantation of salt and drought tolerant plants so as to overcome the problems of both salinization and drought. Grasses are the promising plants, if grown, may minimize the loss caused by the above-mentioned effects. So, the present work was undertaken to screen different available accessions of three elite Cholistan grass species for salt and drought tolerance with a view that tolerant ones could be recommended for cultivation in other parts of Pakistan hit with salinity and drought but having less harsh climate than that in Cholistan.

The screening criteria for salt tolerance so far are based on field performance of different plant species under adverse environments for high plant productivity (Ashraf *et al.,* 1996), but it requires full season field data. It is not always an efficient approach, especially in saline locations because salinity in fields is in patches (Ashraf *et al.,* 2005).

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Alternative methods under laboratory or greenhouse conditions using seedlings as test and other available low cost rapid and reliable techniques can be used. An evidence has been presented that okra *(Hibiscus esculentus*) genotypes with high emergence under high salinity in laboratory showed better emergence and survival in salt-affected fields than those genotypes that do not emerge under laboratory conditions (Ashraf *et al.*, 2005). Several other physiological characteristics have been reported as being reliable indicators for the selection of germplasm possessing high degree of salinity tolerance. These characteristics include seed germination and seedling growth under saline conditions (Ashraf *et al.*, 1992; 2002), the degree of electrolyte leakage (cell membrane stability, CMS) from salinity-damaged leaf cells and the water relations of plants (Ashraf *et al.*, 2005). The success of these approaches requires evidence that the salt tolerance of accessions/genotypes tested under laboratory and greenhouse conditions also reflects this character under field conditions (Sammons *et al.*, 1978). It has been found that significant correlations exist between field and laboratory data (Ashraf *et al.*, 1996; 1999; 2005). However, the objectives of this study were: (1) to evaluate the genetic variability of salinity tolerance among 22 accessions of three elite Cholistan grasses and (2) to determine whether the parameters mentioned above are effective criteria for screening salt tolerant accessions.

Materials and Methods

Germination: Twenty two accessions of three grasses from Cholistan desert of Pakistan were chosen for this study (Table 1). Fifty seeds of each accession were germinated in 0.0 or 1.5 % NaCl solution (EC 23.5 dS m^{-1}). Seeds were sown in plastic bowls (dia. 14 cm x depth 8 cm) containing washed river sand. The conductivity of the sand was estimated by EC meter which was 20 µS cm^{-1}. Salinity was created with analar grade NaCl and each treatment had three replications. The experiment was repeated three times. The bowls were placed in a controlled growth chamber at 28±2°C. Germination was recorded when the radicle reached 5 mm in length. The results were expressed in terms of a promptness index (PI) following George, (1967):

$$\text{Pl} = \text{nd2}(1.00) + \text{nd4}(0.75) + \text{nd6}(0.50) + \text{nd8}(0.25)$$

Where nd2, nd4, nd6 and nd8 = number of seeds germinated on the 2nd , 4th , 6th and 8th day respectively. A germination stress tolerance index (GSTI) was expressed in percentage and was calculated as follows:

$$\text{GSTI} = (\text{PI of stressed seeds} / \text{PI of control seeds}) \times 100$$

Seedling growth: Seeds of the 22 accessions were sown under conditions similar to those used for the germination test. Salinity treatments (0 and 1.5 % NaCl) were created with three replications. The experiment was repeated three times. The plastic bowls were placed in a growth chamber at 30/25°C day/night temperature with 10 h photoperiod (4000 Lux) fluorescent lights installed in the chamber. The seedlings were harvested after 14 days and their shoot and root lengths were recorded. The plants were then dried at 70 °C for 48 h in an oven. Stress tolerance indices, namely plant height stress tolerance index (PHSTI) and dry matter stress tolerance index (DMSTI) were calculated as follows:

PHSTI = (Plant height of stressed plants/ Plant height of control plants) x 100

DMSTI = (Dry weight of stressed plant /dry weight of control plant) x 100

Cell membrane stability (CMS): Cell membrane stability was determined as described by Ashraf *et al.* (2005) while screening salt tolerant germplasm of okra. In the present experiments, plants of different accessions were grown as described above. When plants were of 30 days old, one g leaf discs cut from the flag leaf (0.5 cm each) and placed in a 50 ml test tube after washing three times with deionized water. The leaf discs were submerged in 30 ml of 1.5 % NaCl solution (T_I) or in deionized water as a control (C_1) and both were left for 24 h at 25°C. The leaf discs were then washed quickly with deionized water and allowed to remain in 30 ml deionized water for another 24 h at 25°C. The electrical conductivity (EC) of the extract was then measured with a conductivity meter (WTW, Germany). The leaf discs, still in the same solution, were then killed by autoclaving for 20 minutes and EC was again determined at 25 °C (T_2, C_2). Cell membrane stability as percent injury was calculated as follows:

$$\text{Percent injury} = [1-\{(1-T_1/T_2)/\ (1-C_1/C_2)\}] \times 100$$

Water relations: For water relation measurements, the above accessions were grown in a similar way using same treatments as described above. Six weeks after sowing, second fully expanded leaf was collected from each accession in the morning and weighed immediately. They were then placed in a test tube containing 10 ml of distilled water and left overnight at room temperature. The leaves were then taken out, water was removed from the surface and the leaves were weighed again to obtain saturated weight (turgid weight). After drying at 70 °C in an oven for 48 h, their dry weight determined. The relative saturation deficit (RSD) was calculated as follows.

RSD= (Saturated weight - Fresh weight/ Saturated weight) x 100

The correlations among all the above-mentioned parameters were calculated.

Results

Germination stress tolerance index (GSTI) differed significantly in different accessions of all three grasses examined. Maximum value for germination stress tolerance index (93) was observed in accession LS3/6 of *Sporobolus iocladus* followed by accessions MW1/1 and BJ1/2 of *Panicum antidotale* having GSTI value 92 (Table 2). The lowest value for GSTI (40) was observed in accession KS1/4 of *Cenchrus ciliaris*. Similarly, plant height stress tolerance index (PHSTI) also varied significantly among all the accessions. However, the highest PHSTI value (93.13) was observed in LS3/6 (*S. iocladus*) followed by BJ1/2 and MW1/1 (*P. antidotale*) having values 90.63 and 88.41, respectively (Table 3). Minimum value (47.26) for PHSTI was recorded in accession KS1/4 (*C. ciliaris*). Accession LS3/6 (*S. iocladus*) also maintained the highest dry matter stress tolerance index (86.23) closely followed by MW1/1 (81.48) and BJ1/2 (79.68) of *P. antidotale* (Table 4). The poorest performance for DMSTI was noted in accession KS1/4 (*C. ciliaris*). The values of DMSTI for other accessions ranged between 45.24 and

78.27 (Table 4). Based on these three indices, LS3/6 (*S. iocladus*) was ranked as the top and KS1/4 (*C. ciliaris*) occupied the bottom place in ranking.

Table 1. Accessions of three grasses of Cholistan desert used in the study.

Accession No.	**Botanical name of**	**Common name of**	**Collection site**
MW1/1	*Panicum antidotale*	Murrate	Masu Wala Toba
BJ 1/2			Bijnot Fort
RD 1/1			Roda Wala Toba
RD 1/2			Roda Wala Toba
KH 1/6			Khokhran Wali
KWT 1/2			Khiwtal Wala
SH 2/1			Shheedan Wala Toba
SH 2/2			Shheedan Wala Toba
KS 1/1			Shheedan Wala Toba
Local-2	*Cenchrus ciliaris*	Dhaman grass	Cholistan Institute of Desert Studies
Local-4			- do -
Local-8			- do -
Local-10			- do -
Local-14			- do -
Local-15			- do -
Local-16			- do -
Local-17			- do -
Australian			- do -
KS 1/2			- do -
KS 1/3			- do -
KS 1/4			- do -
LS3/6	*Sporobolus iocladus*	Sporobolus	Lalsohanra whispering Hill

Cell membrane stability (percent injury) showed that the results obtained on the basis of this index revealed that accessions LS3/6 of *S. iocladus* and MW1/1 and BJ1/2 of *P. antidotale* showed the least injury (%) or the highest cell membrane stability (CMS) (28.73, 29.14 and 29.03 respectively as compared to the other accessions (Table 5). The highest percent injury or the lowest CMS values i.e. >60 % were recorded for accessions Australian (60.47), KS1/3 (61.23) and KS1/4 (63.87) of *Cenchrus ciliarus*.

Salinity adversely affected the relative saturation deficit that was reduced in all the accessions (Table 6). Maximum values for relative saturation deficit (RSD) i.e. 30.78 and 30.99 were recorded in accessions KS1/3 and KS1/4 of *C. ciliaris* respectively, while accession BJ1/2 of *P. antidotale* showed the lowest value (9.47) for RSD closely followed by LS3/6 (10.63).

Highly significant and positive correlations were recorded among germination, plant height and dry matter stress tolerance indices but all these parameters had negative and significant relationships with percent injury and relative saturation deficit (Table 7). The data indicated that the accessions with high GSTI, PHSTI, DMSTI, and less percent injury and RSD were tolerant to salinity.

Table 2. Germination stress tolerance index (GSTI) of 22 accessions of three Cholistan grasses.

Grass species	Acc. name	Germination stress tolerance index (%)	Ranking
Sporobolus iocladus	LS 3/6	93	1
Panicum antidotale	MW 1/1	92	2
Panicum antidotale	BJ 1/2	92	2
Cenchrus ciliaris	KS 1/2	87	4
Panicum antidotale	RD 1/1	85	5
Panicum antidotale	KS 1/1	82	6
Panicum antidotale	RD 1/2	80	7
Cenchrus ciliaris	Local-2	76	8
Cenchrus ciliaris	Local-4	73	9
Cenchrus ciliaris	Local-8	71	10
Cenchrus ciliaris	Local-10	70	11
Cenchrus ciliaris	Local-14	67	12
Cenchrus ciliaris	Local-16	66	13
Cenchrus ciliaris	Local-15	64	14
Panicum antidotale	KH 1/6	63	15
Panicum antidotale	KWT 1/2	54	16
Cenchrus ciliaris	Local-17	53	17
Panicum antidotale	SH 2/1	48	18
Panicum antidotale	SH 2/2	47	19
Cenchrus ciliaris	KS 1/2	45	20
Cenchrus ciliaris	Australian	43	21
Cenchrus ciliaris	KS 1/4	40	22

Table 3. Plant height stress tolerance index (PHSTI) of 22 accessions of three Cholistan grasses.

Grass species	Acc. name	Plant height stress tolerance index (%)	Ranking
Sporobolus iocladus	LS 3/6	93.13	1
Panicum antidotale	BJ 1/2	90.63	2
Panicum antidotale	MW 1/1	88.41	3
Cenchrus ciliaris	RD 1/1	83.74	4
Panicum antidotale	KS 1/1	80.74	5
Panicum antidotale	RD 1/2	80.68	6
Cenchrus ciliaris	KS 1/2	76.78	7
Cenchrus ciliaris	Local-16	75.38	8
Cenchrus ciliaris	Local-10	74.41	9
Cenchrus ciliaris	Local-15	72.38	10
Cenchrus ciliaris	Local-14	70.41	11
Cenchrus ciliaris	Local-2	67.32	12
Cenchrus ciliaris	Local-8	65.11	13
Cenchrus ciliaris	Local-4	63.88	14
Panicum antidotale	KH 1/6	63.20	15
Panicum antidotale	KWT 1/2	60.13	16
Panicum antidotale	SH 1/2	57.48	17
Panicum antidotale	SH 2/2	53.27	18
Cenchrus ciliaris	Local-17	50.48	19
Cenchrus ciliaris	Australian	48.93	20
Cenchrus ciliaris	KS 1/3	48.16	21
Cenchrus ciliaris	KS 1/4	47.26	22

Table 4. Dry weight stress tolerance index of 22 accessions of three Cholistan grasses.

Grass species	Acc. name	Dry matter stress tolerance	Ranking
Sporobolus iocladus	LS 3/6	86.23	1
Panicum antidotale	MW 1/1	81.48	2
Panicum antidotale	BJ 1/2	79.68	3
Cenchrus ciliaris	RD 1/1	78.27	4
Panicum antidotale	KS 1/1	77.13	5
Panicum antidotale	RD 1/2	75.68	6
Cenchrus ciliaris	Local-8	73.27	7
Cenchrus ciliaris	KS 1/2	70.48	8
Cenchrus ciliaris	Local-16	70.10	9
Cenchrus ciliaris	Local-15	68.43	10
Cenchrus ciliaris	Local-4	66.73	11
Cenchrus ciliaris	Local-14	65.48	12
Cenchrus ciliaris	Local-2	64.23	13
Cenchrus ciliaris	Local-10	60.48	14
Panicum antidotale	KH 1/6	60.21	15
Panicum antidotale	SH 1/2	53.48	16
Panicum antidotale	KWT 1/2	50.71	17
Panicum antidotale	SH 2/2	50.21	18
Panicum antidotale	Local-17	48.41	19
Cenchrus ciliaris	KS 1/3	47.23	20
Cenchrus ciliaris	Australian	45.24	21
Cenchrus ciliaris	KS 1/4	44.49	22

Table 5. Cell membrane stability (percent injury) of 22 accessions of three Cholistan grasses.

Grass species	Acc. name	Percent injury by 1.5%	Ranking
Sporobolus iocladus	LS 3/6	28.73	1
Panicum antidotale	MW 1/1	29.14	2
Panicum antidotale	Bj 1/2	29.03	3
Cenchrus ciliaris	KS 1/1	30.71	4
Panicum antidotale	RD 1/2	31.28	5
Panicum antidotale	RD 1/1	33.48	6
Cenchrus ciliaris	Local-2	36.58	7
Cenchrus ciliaris	KS 1/2	38.71	8
Cenchrus ciliaris	Local-8	40.27	9
Cenchrus ciliaris	Local-16	41.48	10
Cenchrus ciliaris	Local-10	41.78	11
Cenchrus ciliaris	Local-15	41.97	12
Cenchrus ciliaris	Local-4	42.38	13
Cenchrus ciliaris	Local-14	42.73	14
Panicum antidotale	KH 1/6	42.87	15
Panicum antidotale	KWT 1/2	50.47	16
Panicum antidotale	SH 2/2	51.37	17
Panicum antidotale	SH 1/2	57.83	18
Panicum antidotale	Local-17	59.48	19
Cenchrus ciliaris	Australian	60.47	20
Cenchrus ciliaris	KS 1/3	61.23	21
Cenchrus ciliaris	KS 1/4	63.87	22

Table 6. Relative saturation deficit of (RSD) 22 accessions of three Cholistan grasses.

Grass species	Acc. name	Relative saturation	Ranking
Sporobolus iocladus	BJ 1/2	9.47	1
Panicum antidotale	LS 3/6	10.63	2
Panicum antidotale	MW 1/1	10.13	3
Cenchrus ciliaris	KS 1/1	11.48	4
Panicum antidotale	RD 1/2	11.23	5
Panicum antidotale	RD 1/1	13.78	6
Cenchrus ciliaris	Loca-2	14.42	7
Cenchrus ciliaris	KS 1/2	16.32	8
Cenchrus ciliaris	Local-16	17.42	9
Cenchrus ciliaris	Local-8	19.11	10
Cenchrus ciliaris	Local-15	20.28	11
Cenchrus ciliaris	Local-10	18.84	12
Cenchrus ciliaris	Local-14	17.63	13
Cenchrus ciliaris	KH 1/6	17.78	14
Panicum antidotale	Local-4	19.76	15
Panicum antidotale	SH 2/2	22.23	16
Panicum antidotale	KWT 1/2	25.48	17
Panicum antidotale	SH 1/2	26.47	18
Panicum antidotale	Local-17	27.4	19
Cenchrus ciliaris	Australian	29.44	20
Cenchrus ciliaris	KS 1/3	30.78	21
Cenchrus ciliaris	KS 1/4	30.99	22

Table 7. Correlation among different screening techniques.

Techniques	GSTI	PHSTI	DMSTI	CMS	RSD
GSTI	-	-	-	-	-
PHSTI	0.993**	-	-	-	-
DMSTI	0.994**	0.992**	-	-	-
CMS	-0.987**	-0.973**	-0.974**	-	-
RSD	-0.988**	-0.966**	-0.976**	0.999	-
Yield (dry matter)	0.906**	0.884**	0.888**	-0.882**	-0.903**

** = Significant ($P \leq 0.01$); GSTI = Germination stress tolerance index; PHSTI = Plant height stress tolerance index; DMSTI = Dry matter stress tolerance index; CMS = Cell membrane stability (% injury by 1.5 % NaCl); RSD = Relative saturation deficit.

Discussions

According to the data presented in the present study, significant differences among accessions for germination, seedling growth, cell membrane stability and relative saturation deficit, plant height and dry matter stress tolerance indices were evident. However, not all the parameters studied appeared to be equally useful for screening of the accessions of these grasses accessions for sensitivity to salinity. All the screening techniques had significant correlations with yield (dry matter) except RSD indicating that they can be used to screen the germplasm of grasses.

Germination did not seem to reflect the stress tolerance response in grass germplasm as the accessions, KS1/1, MW1/1 of *Panicum antidotale* and Local-2 of *Cenchrus ciliarus* showed germination stress tolerance index in the range of 92-93 %. High germination stress tolerance index means that tolerant accessions would have high germination stress tolerance index (GSTI). However, the accessions, which exhibited higher values for GSTI, appeared to be medium in performance under saline conditions. They also could not compete with LS3/6 (*Sporobolus iocladus*) and other accessions of *Cenchrus ciliaris* and *Panicum antidotale* under stress conditions. In view of some earlier studies (Akram *et al.,* 1998; Ashraf *et al.,* 2005) the speed of germination and lengths of root, shoot and coleoptiles decreased under stress conditions. However, only on the basis of germination test, germplasm cannot be screened for salt tolerance. Similarly, Ashraf & Waheed (1992) based on their studies with chickpea also concluded that tolerance to drought or salt stress cannot be predicted from germination test alone. However, Ashraf *et al.* (1996; 1999) and Riga & Vertanian (1999) found a positive correlation between germination and stress tolerance in wheat and tobacco and concluded that germination could be useful in screening for stress tolerance (drought and salinity).

The reproducibility and consistency of the results determined for seedling growth of accessions of grasses for procedures used in the present studies plus the positive correlation between plant height, dry matter accumulation suggest that seedling growth could be a reliable and efficient method for screening grass germplasm for salt tolerance. The accessions having higher dry matter stress tolerance index (DMSTI) and plant height stress tolerance index (PHSTI) produced higher yield. These findings are in agreement with those of Ashraf *et al*. (2002; 2005).

The differences among grass accessions revealed that cell membrane stability (CMS, less percent injury) provide a positive correlation with relative saturation deficit (r = 0.999 at $P \leq 0.01$). The accessions, which showed higher membrane stability, had better performance in the subsequent stress studies. The accessions MW1/1, KS1/1 and LS3/6 having greater CMS are considered stress tolerant, which might have been due to high morpho-agronomical and biochemical changes and yield. These findings indicate that accessions can be screened on the basis of CMS. It is the most useful and efficient technique to screen the germplasm. Theoretically, it is also expected that membrane stability should play a role during abiotic stresses because it is influenced by osmotic adjustment which has important role in water balance and many enzymatic activities some of those involved in photosynthesis and respiration as they are membrane bound (Zidan *et al.,* 1992; Khan *et al.,* 1995).

The importance of plant water for the maintenance of turgidity is essential for the survival and growth of plants. Dehydration usually causes severe changes and disorganization of membranes and organelles, mechanical rupture of cell membranes and degradation of protoplasm (Shiferaw & Baker, 1999; Ashraf *et al.,* 1996). As is well evident that salt stress adversely affect plant growth due to its salt-induced osmotic stress and specific ion toxicity through Na^+ and Cl^- or other ions, so the accessions having smaller water deficit (relative saturation deficit) per unit area can combat the osmotic stress caused by salinity. The results of Yadav & Krishna (1987), Akbar *et al.* (1996) and Ashraf *et al*. (1999) also support the above findings.

From the results presented here, it can be concluded that the above screening methods are useful, reliable, cheaper and rapid to screen the germplasm consisting of large number of accessions or cultivars of grasses.

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