EVALUATION OF WHEAT GENOTYPES FOR SALINITY TOLERANCE USING PHYSIOLOGICAL INDICES AS SCREENING TOOL

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Abstract

Salinity is a major threat to world food security, to ensure future food needs of an increasing world population, development of salt tolerant crop varieties are necessary. Effective screening techniques for salinity tolerance would be beneficial in developing high yielding and salt tolerant wheat varieties. In the present study, an attempt for rapid screening of wheat genotypes for salt tolerance was made. Twenty wheat genotypes were evaluated for salinity tolerance under laboratory/green-house conditions using different physiological indices like germination stress tolerance index (GSI), shoot length stress tolerance index (SLSI), root length stress tolerance index (RLSI) , shoot dry biomass stress tolerance index (SDSI). The data was pooled together to different multivariate techniques including correlation and cluster analysis to assess the diversity for salt tolerance in wheat genotypes. Highly significant and positive correlations were found between GSI, SDWSI and RDWSI. Cluster analysis classified 20 genotypes into three divergent groups. The members of first cluster (Abadgharr, Bhakkar-2000, Chakwal-86, Kiran-95, LU-26-S, Margalla-99, Marvi Pak-81, Sarsabaz) exhibited adequate degree of salt tolerance on the basis of various physiological stress tolerance indices, whereas, cluster-2 included genotypes (Bhattai, Pasban-90, Shafaq-2006, Soghat-90) with medium level of salt tolerance and cluster-3 consisted of wheat genotypes (Inqilab-91, Iqbal-2000, Kohistan-97, PARI-73, Punjab-90, Sehar-2006 and Uqab-6) with lower level of salt tolerance and did not perform upto the mark. On the basis of results and scores obtained, indicated that physiological indices can be used as a selection tool for salinity tolerance in wheat.

Key words: Phsiological indices, Screening techniques, Cluster analysis, Salinity and wheat.

Introduction

Salinity is a serious problem of soil degradation, limiting agricultural productivity in many regions of the world (Rengasamy, 2006). Approximately 800 million hectares of land in the world is affected by soil salinization. Seed germination and seedling growth are major problem to establish a vigorous crop stand, so these traits can be utilized to screen plant germplasm for salt tolerance (Ashraf *et al.*, 2007). Salinity mainly causes hyper-osmotic stress and hyper-ionic toxic effects, which leads to germination inhibition and seedling growth (Hasegawa *et al.*, 2000). Salt resistance is an inherent trait of plants to withstand the adverse effects in the leaves or root zone (Odjegba & Chukwunwike, 2012).

Wheat is grown throughout the world to fulfill ever increasing population and it is the staple food of people of Pakistan. Pakistan is the 4th biggest producer in Asia and ranks 11th in the world in wheat production (Saeed *et al.*, 2012). In wheat, the energy source for early seedlings is endosperm which is more vulnerable due to delicate radicles (Lin *et al.*, 2012). Screening of wheat germplasm is important to determine genetic potential for salt tolerance which strengthens the breeding for high yielding and salt tolerant wheat varieties. Breeders seek to produce and identify genotypes that are tolerant to osmotic and ionic stress (Janmohammadi *et al.*, 2008). The variation among the genotypes for the physiological indices at germination and early seedling has been analyzed in many crop plants (Hakim *et al.*, 2010; Kausar *et al.*, 2012). This helps in identifying the tolerant varieties, which can be further studied and economically exploited to cultivate them on salt-affected lands. The objective of present study was to evaluate 20 wheat genotypes under NaCl stress and to assess the variability in their genotypic response to salinity. Our study is an attempt to compare the usefulness of several stress indices for identification of genotypes with better performance at different levels of salt stress.

Materials and Methods

Experimental conditions: The experiment was conducted under lab conditions using 0, 50, 100 and 150 m*M* NaCl levels to determine the salt tolerance potential of 20 wheat genotypes using physiological indices as screening tool. The seeds were obtained from Plant Breeding and Genetics Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan and Wheat Research Institute, AARI (Ayub Agricultural Research Institute), Faisalabad, Pakistan. The experiment was conducted at Plant Stress Physiology Lab, NIAB, Faisalabad, Pakistan. The seeds were surface sterilized with 10% sodium hypochlorite solution for five minutes and washed three times with distilled water. Ten seeds of each genotype were grown in Petri-plates containing filter papers moistened with above-mentioned solutions of salinity and placed in a Growth Chamber (Sanyo-Gallenkamp, UK) running at $28\pm2^{o}C$ and 10 h photoperiod with 80 μM S^{-1} m^{-2} light intensity was maintained. Germination was recorded when the radical was of 5 mm in length.

Physiological indices: To calculate the germination stress tolerance index (GSI), promptness index (PI) was estimated using following formulae (Ashraf *et al.,* 2008):

$$PI = nd_1 (1.00) + nd_2 (0.75) + nd_3 (0.50) + nd_4 (0.25)$$

where: nd_1, nd_2, nd_3 and nd_4 showed the number of seeds germinated on the 1st, 2nd, 3[rd and] 4th day, respectively. A germination stress tolerance index (GSI) was calculated in terms of percentage as follows:

PHSI = (Plant height of stressed plants / Plant height of control plants) x100
RLSI = (Root length of stressed plants / Root length of control plants) x100
SFSI = Shoot fresh weight of stressed plants / Shoot fresh weight of control plants) x 100
RFSI = (Root fresh weight of stressed plants / Root fresh weight of control plants) x 100
SDSI = (Shoot dry weight of stressed plants / Shoot dry weight of control plants) x 100
RDSI = (Root dry weight of stressed plants / Root dry weight of control plants) x 100

Statistical analysis: The data thus obtained were subjected to analysis of variance (Steel *et al.*, 1997). The data were analyzed using ANOVA and subsequent comparison of means was performed using the Duncan's Test at 5% probability. The cluster analysis and coefficient of variation analysis was performed using the MStatC and Minitab-6. The layout used was completely randomized design with three replications.

Results

The salt stress significantly reduced seed germination in all wheat genotypes (Table 1) which resulted significant (p≤0.05) variations in GSI. All the salinity treatments differed significantly, GSI gradually decreased with the increase in salinity and it was 89.94, 77.06 and 60.10% under 50, 100 and 150 m*M* NaCl, respectively (Table 1). Wheat genotypes, Abadgharr (100 %), Kohistan-97 (100%) and Bhattai (98.72%) exhibited maximum and PARI-73 minimum GSI (73.99%) values at 50 m*M* NaCl salinity level. At 100 and 150 m*M* NaCl salinity levels Abadgharr showed 100 and 92.79% GSI, respectively while it was the least in PARI-73 (63.97 and 37.12%, respectively) under these levels. The overall genotypic means and ranking for GSI indicated that Abadgharr was at first, Chakwal-86 at second and LU-26S at third position while PARI-73 was at 20th position.

Salinity inhibited shoot length of all wheat genotypes which influenced the SLSI and variations among all the salinity treatments were significant for this index (Table 2), which was significantly decreased (71.43, 54.62 and 38.95 %, respectively at 50, 100 and 150 m*M* NaCl) with the increase in salinity levels. Under 50 m*M* NaCl treatment, all wheat genotypes responded differently, however the highest SLSI (93.25%) was exhibited by Pasban-90 closely followed by Pak-81(88.79%), minimum SLSI was observed in PARI-73 (41.52%). At 100 m*M* NaCl salinity level, Chakwal 86 (72.96%), Margalla-99 (72.77%) maintained the highest SLSI closely followed by Abadgharr (69.41%) while minimum was again in PARI-73. Under the highest salinity level (150 m*M* NaCl), the genotype Abadgharr was successful in maintaining the highest (60.38%) SLSI and the least values were demonstrated by PARI-73 (14.92 %) and Iqbal-2000 (19.32 %). On the basis of genotypic mean, Abadgharr ranked at

first, Bhattai at second and Soghat-90 at third position while PARI-73 at 20th positions.

Root length stress tolerance index (RLSI) of all wheat genotypes was significantly affected by salinity stress (Table 3); it reduced with increase in salinity stress (73.49, 60.76 and 42.92% under 50, 100 and 150 m*M* NaCl, respectively). Variations among all salinity levels were significant for RLSI. At 50 m*M* NaCl, the minimum value (59.15%) for RLSI was examined in case of Bhakkar-2000 while maximum was exhibited by Abadgharr (88.34%) closely followed by and Pasban-90 (84.73%). Under 100 m*M* NaCl stress, the highest RLSI (76.28%) was exhibited by Kohistan-97 closely followed by Abadgharr (68.53%) and the lowest value (41.06%) was recorded for Bhakkar-2000 closely followed by Marvi (42.17%). Bhattai showed the highest RLSI (59.91) under 150 m*M* NaCl while the minimum was exhibited by Chakwal-86 (20.97%) closely followed by Iqbal-2000(23.99%). On the basis of genotypic means, Bhattai and Abadgharr were ranked as 1st and 2nd and Marvi 20th.

Shoot and root fresh biomass stress tolerance index (SFSI, RFSI) were significantly influenced by salinity (Tables 4 and 5), both were gradually decreased with increase in salinity levels (SFSI= 65.72, 50.20 and 34.22%; RFSI= 69.58, 54.80 and 36.62% under 50, 100 and 150 m*M* NaCl levels, respectively). Under 50 m*M* NaCl salinity level, Bhattai (86.1%) and Abadgharr (84.80%) maintained the highest SFSI and Chakwal-86 (87.78%) and Abadgharr (85.57%) possessed the maximum RFSI, while minimum SFSI was in Kiran-95 (42.22%) and Kohistan-97 (42.87%) and the lowest values of RFSI were obtained for Punjab-90 (37.06%) and Iqbal-2000 (44.73%). Kohistan-97 and PARI-73 exhibited poor performance for SFSI (19.43 and 22.59%, respectively) and for RLSI, Punjab-90 and Uqab-6 (30.91 and 30.97%, respectively) at 100m*M* NaCl level. At the highest salinity level, maximum SFSI was recorded for Sarsabz (55.13%) and Abadgharr (50.89%) while RFSI was the highest in Bhattai (55.27%), Abadgharr (45.13%) and Kohistan-97 (45.06%). Under the highest salinity level (150 m*M* NaCl), wheat genotypes Pasban-90(10.17%) and Maragalla-99 (17.29%) showed the poor performance for SFSI and Uqab-6 (19.67%) for RFSI. Overall genotypic means indicated that Abadgharr and Bhattai scored maximum points for SFSI and RFSI and ranked as 1st and 2nd while PARI-73 was at 20th position, for both of these indices.

Table 1. Germination stress tolerance index (GSI) of various wheat genotypes.

| Genotypes | Salinity treatments (NaCl in mM) | | | | Ranking |
| | 50 | 100 | 150 | Means | |
	GSI (%)				
Abadgharr	100.00	100.00	92.79	97.59 a	1
Bhakkar-2000	82.82	77.77	72.72	77.77 fg	8
Bhattai	98.72	85.00	66.00	83.24 cde	5
Chakwal-86	96.39	89.18	82.88	89.48 b	2
Inqilab-91	92.79	85.58	56.75	78.38 efg	7
Iqbal-2000	89.33	79.08	43.98	70.79 hij	14
Kiran-95	89.76	66.25	51.40	69.13 ij	16
Kohistan-97	100.00	60.56	61.23	73.93 ghi	13
LU-26-S	92.79	90.09	75.68	86.19 bc	3
Margalla-99	86.48	78.37	63.06	75.97 fgh	11
Marvi	96.05	88.25	69.69	84.66 bcd	4
Pak-81	89.19	76.58	57.25	74.34 fghi	12
PARI-73	73.99	63.97	37.12	58.36 kl	20
Pasban-90	90.00	58.56	40.18	62.91 kl	19
Punjab-90	96.39	73.11	38.14	69.21 ij	15
Sarsabaz	86.48	83.78	68.53	79.60 def	6
Sehar-2006	91.95	77.04	60.99	76.66 fg	10
Shafaq-2006	80.18	65.64	43.49	63.11 kl	18
Soghat-90	78.83	60.62	57.24	65.56 jk	17
Uqab -6	86.59	81.66	62.78	77.01 fg	9
Mean	89.94a	77.06b	60.10 c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 2. Shoot length stress tolerance index (SLSI) of various wheat genotypes.

| Genotypes | Salinity treatments (NaCl in mM) | | | | Ranking |
| | 50 | 100 | 150 | Means | |
	SLSI (%)				
Abadgharr	82.66	69.41	60.38	70.82 a	1
Bhakkar-2000	80.33	52.19	51.62	61.38 bc	7
Bhattai	83.44	63.76	47.87	65.02 b	2
Chakwal-86	81.19	72.96	32.31	62.15 bc	4
Inqilab-91	42.76	26.49	26.11	31.79 k	19
Iqbal-2000	71.13	39.76	19.32	43.40 i	16
Kiran-95	51.63	47.97	39.11	46.24 h	15
Kohistan-97	75.23	47.45	24.61	49.10 fg	13
LU-26-S	79.64	56.02	49.63	61.76 bc	6
Margalla-99	80.15	72.77	32.56	61.83 bc	5
Marvi	75.31	47.74	32.57	51.87 f	12
Pak-81	88.79	61.48	25.07	58.45 d	9
PARI-73	41.52	24.47	14.92	26.97 l	20
Pasban-90	93.25	50.83	22.09	55.39 e	11
Punjab-90	78.08	61.82	27.46	55.79 e	10
Sarsabaz	83.23	60.57	39.16	60.99 bc	8
Sehar-2006	62.39	42.07	22.15	42.20 i	17
Shafaq-2006	68.1	55.09	23.92	49.04 fg	14
Soghat-90	76.72	64.25	50.77	64.00 b	3
Uqab -6	54.61	29.96	26.37	36.98 j	18
Mean	71.43 a	54.62 b	38.95 c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 3. Root length stress tolerance index (RLSI) of various wheat genotypes.

Genotypes	Salinity treatments (NaCl in mM)				Ranking
	50	100	150	Means	
Abadgharr	88.34	68.53	49.74	68.87 a	2
Bhakkar-2000	59.15	41.06	39.23	46.48 f	18
Bhattai	80.84	66.51	59.91	69.09 a	1
Chakwal-86	62.08	48.94	20.97	44.00 fg	19
Inqilab-91	75.6	57.75	46.08	59.81cd	9
Iqbal-2000	79.84	54.13	23.99	52.65 e	15
Kiran-95	67.67	42.26	32.32	47.42 f	16
Kohistan-97	81.98	76.28	29.15	62.47 bc	6
LU-26-S	77.84	59.65	47.38	61.62 bc	7
Margalla-99	63.59	48.94	28.56	47.03 f	17
Marvi	61.79	42.17	24.35	42.77 fg	20
Pak-81	75.43	53.25	30.74	53.14 e	14
PARI-73	74.91	67.39	27.13	56.48 d	12
Pasban-90	84.73	54.42	39.39	59.51cd	10
Punjab-90	80.92	70.51	43.56	65.00 b	4
Sarsabaz	78.97	59.87	43.64	60.83 bc	8
Sehar-2006	76.16	67.32	46.51	63.33 bc	5
Shafaq-2006	74.91	67.39	27.13	56.48 d	12
Soghat-90	75.94	66.83	55.24	66.00 b	3
Uqab -6	72.68	62.82	36.31	57.27 d	11
Mean	73.49 a	60.76 b	42.92 c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 4. Shoot fresh biomass stress tolerance index (SFSI) of various wheat genotypes.

Genotypes	Salinity treatments (NaCl in mM)				Ranking
	50	100	150	Means	
Abadgharr	84.8	62.74	50.89	66.14 a	1
Bhakkar-2000	55.58	41.19	45.48	47.42 de	13
Bhattai	86.1	66.38	45.35	65.94 a	2
Chakwal-86	80.61	58.65	28.15	55.80 bc	5
Inqilab-91	48.41	41.45	34.43	41.43 f	15
Iqbal-2000	78.95	50.01	23.28	50.75 cd	8
Kiran-95	42.22	29.79	23.67	31.89 h	18
Kohistan-97	42.87	19.43	3.53	21.94 i	20
LU-26-S	73.24	62.78	35.11	57.04 b	4
Margalla-99	60.25	37.99	17.29	38.51g	16
Marvi	72.74	54.53	31.18	52.82 bc	7
Pak-81	73.66	45.12	29.81	49.53 cd	10
PARI-73	47.87	22.59	5.14	25.20 i	19
Pasban-90	74.06	60.08	10.17	48.10 de	12
Punjab-90	72.35	59.89	19.03	50.42 cd	9
Sarsabaz	75.02	62.51	55.13	64.22 a	3
Sehar-2006	71.23	52.81	23.95	49.33 cd	11
Shafaq-2006	62.63	50.87	26.28	46.59 de	14
Soghat-90	76.58	44.14	38.56	53.09 bc	6
Uqab -6	50.94	31.18	22.21	34.78 h	17
Mean	65.72 a	50.20 b	34.22 c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 5. Root fresh biomass stress tolerance index (RFSI) of various wheat genotypes.

Genotypes	Salinity treatments (NaCl in mM)				Ranking
	50	100	150	Means	
Abadgharr	85.57	69.69	45.13	66.80 a	2
Bhakkar-2000	59.06	54.39	34.17	49.21 de	14
Bhattai	83.85	65.43	55.27	68.18 a	1
Chakwal-86	87.78	68.71	39.82	65.44 a	3
Inqilab-91	71.27	60.18	37.16	56.20 bc	8
Iqbal-2000	44.73	41.38	14.99	33.70 gh	19
Kiran-95	72.11	59.48	40.39	57.33 bc	6
Kohistan-97	64.39	42.89	45.06	50.78 cd	13
LU-26-S	75.45	61.66	17.21	51.44 cd	12
Margalla-99	80.74	62.65	41.49	61.63 ab	5
Marvi	81.42	51.98	35.19	56.20 bc	8
Pak-81	84.41	55.22	25.77	55.13 bc	10
PARI-73	57.03	36.33	20.39	37.92 g	16
Pasban-90	80.93	66.67	22.41	56.67 bc	7
Punjab-90	37.06	30.97	23.49	30.51 h	20
Sarsabaz	84.69	66.05	42.27	64.34 ab	4
Sehar-2006	62.51	38.53	4.63	35.22 g	17
Shafaq-2006	81.26	47.75	30.38	53.13 cd	11
Soghat-90	65.29	39.99	24.14	43.14 f	15
Uqab -6	51.66	30.91	19.67	34.08 g	18
Mean	69.58 a	54.80 b	36.62 c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 6. Shoot dry biomass stress tolerance index (SDSI) of various wheat Genotypes.

Genotypes	Salinity treatments (NaCl in mM)				Ranking
	50	100	150	Means	
Abadgharr	89.64	78.06	71.44	79.71 a	1
Bhakkar-2000	63.61	40.38	19.17	41.05 g	16
Bhattai	84.74	49.02	35.24	56.33 cd	12
Chakwal-86	84.74	70.76	43.03	66.18 b	3
Inqilab-91	71.41	53.76	21.2	48.79 f	14
Iqbal-2000	81.61	68.37	32.89	60.96 bc	7
Kiran-95	59.75	32.39	20.37	37.50 h	18
Kohistan-97	82.52	64.08	33.02	59.87 bc	9
LU-26-S	79.73	60.64	45.05	61.81 bc	6
Margalla-99	77.61	55.93	39.46	57.67 cd	10
Marvi	78.85	65.31	43.29	62.48 bc	5
Pak-81	84.12	76.88	38.63	66.54 b	2
PARI-73	48.92	40.62	17.59	35.71 h	20
Pasban-90	68.52	27.41	14.63	36.85 h	19
Punjab-90	78.58	68.2	22.73	56.50 cd	11
Sarsabaz	78.99	69.38	48.42	65.60 b	4
Sehar-2006	91.76	53.22	34.7	59.89 bc	8
Shafaq-2006	63.43	42.82	30.08	45.44 f	15
Soghat-90	72.77	52.58	27.06	50.80 e	13
Uqab -6	52.89	38.23	25.11	38.74 h	17
Mean	73.53 a	57.53 b	38.72 c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Salinity stress significantly (p≤0.05) influenced shoot and root dry biomass stress tolerance indices (SDSI and RDSI, respectively). Significant differences in SDSI and RDSI were exhibited by all the treatments (Tables 6 and 7), both traits were decreased by the increase in salinity levels (SDSI, 73.53, 57.53 and 38.72%; RDSI, 66.63, 51.42 and 36.50 % under 50, 100, and 150 m*M* NaCl salinity, respectively). At 50 m*M* NaCl level, maximum SDSI was recorded for Sehar (91.76%) followed by Abadgharr (89.64%), Chakwal-86 and Bhattai (84.74%), Pak-81 (84.12%) while the lowest value for SDSI (48.92%) was noted for PARI-73. Under this treatment the highest values for RDSI were recorded for LU-26S (87.28%) and Abadgharr (87.07%) and the lowest for Pasban-90 (45.22%) closely followed by PARI-73 (51.36). Under 100 m*M* NaCl salinity level, the highest SDSI was estimated for Abadgharr (78.06%) closely followed by Pak-81 (76.88%) and it was the lowest in Pasban-90 (27.41%) which was statistically at par with Kiran-95 (32.39). Similarly, wheat genotype Abadgharr also maintained the highest RDSI (79.26) and the lowest was in Kohistan-97 (24.5) under 100 m*M* NaCl salinity levels. At 150 m*M* NaCl salinity level, maximum values for SDSI and RDSI were noted for Abadgharr while the minimum were in Pasban-90 and Sehar-2006. On the basis of overall means genotypes Abadgharr and Bhattai ranked first, second and PARI-73 on 20th positions.

The correlation analysis indicated significant and positive correlations between GSI and SFSI, RFSI, SDSI and RDSI; same was the case with SLSI and SFSI, RFSI, SDSI and RDSI. Significant and positive correlations were also obtained between RLSI and SFSI, RFSI, SDSI and RDSI, and relationships between SFSI and RFSI, SDSI and RDSI were also positive (Table 8). The data indicated that the genotypes with high GSI, SLSI, SFSI, RFSI, SDSI and RDSI were tolerant to salt stress.

The cluster analysis based on complete linkage correlation coefficient distance was performed in the present study which split the twenty wheat genotypes into three clusters (Fig. 1). Clustering of salt tolerant (cluster 1) i.e. Abadgharr, Bhakkar-2000, Chakwal-86, Kiran-95, LU-26-S, Margalla-99, Marvi, Pak-81 and Sarsabaz; medium salt tolerant (cluster 2) and susceptible (cluster 3) genotypes proposed the use of members of these clusters as parents to build up populations for selection of transgressive segregants against salt stress in subsequent generations and for the development of quantitative trait loci (QTLs) related with salt tolerance. The results suggested that utilization of genetic variability for various morphophysiological markers contributing towards salt tolerance available in wheat would be important for cultivar development with considerable salt tolerance at early and terminal growth stages.

Discussion

It is an established fact that tolerance at adult stage is reflected by the tolerance at seedling stage of plant. This fact has been exploited with success in maize (Khan *et al.,* 2003a), sorghum (Kausar *et al.,* 2012) wheat (Ali *et al.,* 2002; Khan *et al.,* 2003 b), soybean (Kamal *et al.,* 2003), cotton (Azhar and Ahmad, 2000). In maize, the variation

at seedling stage affects the yield potential at maturity (Akram *et al.,* 2010). A major objective of wheat breeders is to develop salt tolerant varieties, however, the genotypes showing clear differences to environmental stresses and adequate screening techniques are limited. Results of present study indicate that stress tolerance indices could explain some of the mechanisms indicating tolerance to salinity.

From the data regarding physiological indices like, GSI, SLSI, RLSI and DMSI it is evident that they can be used to screen the wheat germplasm for salt tolerance. The genotype Abadgharr was the highest scorer for the physiological indices followed by LU-26-S, Chakwal-86, Marvi, Sarsabaz, Pak-81, Bhakkar-2000, Kiran-95, and Margalla-99, which clustered them in cluster 1 in dendogram (Fig. 1) categorized as tolerant one. While wheat genotypes PARI-73, Inqilab-91, Uqab-6, Kohistan-97, Iqbal-2000, Sehar-2006, and Punjab-90 maintained scores below average and clustered them in the 3rd cluster (Fig. 1) and categorized as sensitive or non-tolerant ones. These findings are in accordance with the results of Kausar *et al.* (2012) and Ashraf *et al.* (2008). So, the screened wheat genotypes have a genetic potential for salt tolerance and Abadgharr can directly be cultivated on salt-affected soils having salinity below 15mM NaCl.

Salinity causes a decrease in radicle and plumule growth, which increased with the increase in salinity levels. Presence of salinity in the growth medium reduced the absorption of water due to decrease in osmotic potential of the medium which adversely affects cell division and differentiation (Ashraf *et al.,* 2005). The growth of plumule, radical, biomass and consequently all physiological indices are negatively affected by salinity (Kausar *et al.,* 2012), which may be due to ionic toxicity, disturbance in nutrients uptake (Akhtar *et al.,* 2012) osmotic effects of salinity (Iqbal *et al.,* 1998; Ashraf *et al.,* 2005) and water absorption (Ashraf and Sarwar, 2002), resulting in reduction in biosynthesis of enzymes, plant hormones necessary for seedling/plant growth (Bor *et al.,* 2003). Rejili *et al.* (2010) observed a decrease in *Oued dkouk* population due to reduction in germination percentage that resulted due to osmotic effect of salts present in the growth medium. The salinity stress imposed at germination stage, damages cell membranes of the seedling due to which cell membrane permeability increases, resulting the replacement of Ca^{2+} with Na^+ and leakage of K^+ (Takel, 2000). All these activities disturb the plant osmotic adjustment (Ashraf *et al.,* 2002).

An analysis of correlations between the various stress tolerance parameters revealed some important associations among the germination, shoot and root length and biomass/growth related characters. Therefore significant and positive correlations were recorded among GSI, SFSI, RFSI, SDSI and RDSI, which clearly indicated that these physiological indices can be utilized to screen the germplasm for salt tolerance. Riga and Vartanian (1999), Khan *et al*. (2007), Khan *et al*. (2010), and Kausar *et al.* (2012) found a positive correlation between GSI and stress tolerance in tobacco, wheat, Brassica and sorghum and concluded that GSI reflects the stress tolerance

potential of plant and is good screening tool to for salinity tolerance. The reproducibility and consistency of the genotypic differences determined by above mentioned physiological indices, plus the positive correlation between GSI, SFSI, SDWSI, suggest that physiological indices could be a reliable and efficient method for assessing salt tolerance in wheat germplasm. The information regarding significant correlation among the characters is important for initiation of any breeding program because it provides a chance for selection of desirable genotypes with desirable traits simultaneously (Ali *et al.*, 2009).

Different researchers have used cluster analysis to group different wheat genotypes based on various characteristics and found similarities of wheat genotypes within a group (Nookra & Khaliq, 2007). The genotypes closest to each other are grouped into one cluster. The cluster-I comprised of nine out of twenty genotypes, all nine performed significantly better than others for all the tested indices and categorized as salt tolerant ones. Cluster-II comprised of four genotypes having similarities between most of the traits studied and were medium in performance thus considered as medium tolerant. While cluster-III comprised of 7 genotypes shows less similarity with other genotypes for the characters under study and performed not upto the mark therefore, categorized as sensitive or non tolerant ones. Literature emphasizes on the use of cluster analysis to screen the crop germplasm for stress tolerance (Vahdati *et al.*, 2009; Farshadfar & Elyasi, 2012; Noorifarjam *et al.*, 2013). Selected genotypes could be used in further breeding programmes for salt tolerance.

Table 7. Root dry biomass stress tolerance index (RDSI) of various wheat genotypes.

Genotypes	Salinity treatments (NaCl in mM)				Ranking
	50	100	150	Means	
Abadgharr	87.07	79.26	66.04	77.46 a	1
Bhakkar-2000	48.02	41.98	27.13	39.04 g	15
Bhattai	71.52	59.85	23.27	51.55 de	8
Chakwal-86	72.58	76.92	27.83	59.11 c	5
Inqilab-91	65.42	40.51	38.88	48.27 f	11
Iqbal-2000	77.26	61.89	41.72	60.29 c	4
Kiran-95	51.36	41.72	19.69	37.59 g	16
Kohistan-97	52.36	24.5	19.92	32.26 h	20
LU-26-S	87.28	57.25	50.22	64.92 b	2
Margalla-99	66.37	53.9	23.49	47.92 f	12
Marvi	70.66	53.59	29.6	51.28 de	9
Pak-81	86.65	48.57	31.47	55.56 d	6
PARI-73	51.36	31.91	19.5	34.26 h	19
Pasban-90	45.22	39.1	24	36.11gh	18
Punjab-90	74.79	47.81	39.69	54.10 d	7
Sarsabaz	80.93	54.84	50.49	62.09 b	3
Sehar-2006	84.11	58.67	10.63	51.14 de	10
Shafaq-2006	54.58	41.3	23.6	39.83 g	14
Soghat-90	66.13	33.62	24.98	41.58 g	13
Uqab -6	55.51	32.69	24.43	37.54 g	17
Mean	66.63 a	51.42 b	36.50 c		

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 8. Correlation among different screening techniques.

Techniques	GSI	SLSI	RLSI	SFWSI	RFWSI	SDWSI	RDWSI
GSI							
SLSI	0.316N.S						
RLSI	-0.119N.S	0.233NS					
SFWSI	0.512*	0.655**	0.261NS				
RFWSI	0.460*	0.546*	-0.068NS	0.494*			
SDWSI	0.678**	0.455*	0.098NS	0.552*	0.283NS		
RDWSI	0.710**	0.378NS	0.093NS	0.752**	0.347NS	0.842**	

** = Significant (p<0.01); GSI = Germination stress tolerance index; SLSI = Shoot length stress tolerance index; RLSI = Root length stress tolerance index; SFWSI = Shoot fresh weight stress tolerance index; RFWSI =Root fresh weight stress tolerance index; SDWSI = Shoot dry weight stress tolerance index; RDWSI = Root dry weight stress tolerance index

Dendrogram with Complete Linkage and Absolute Correlation Coefficient Distance
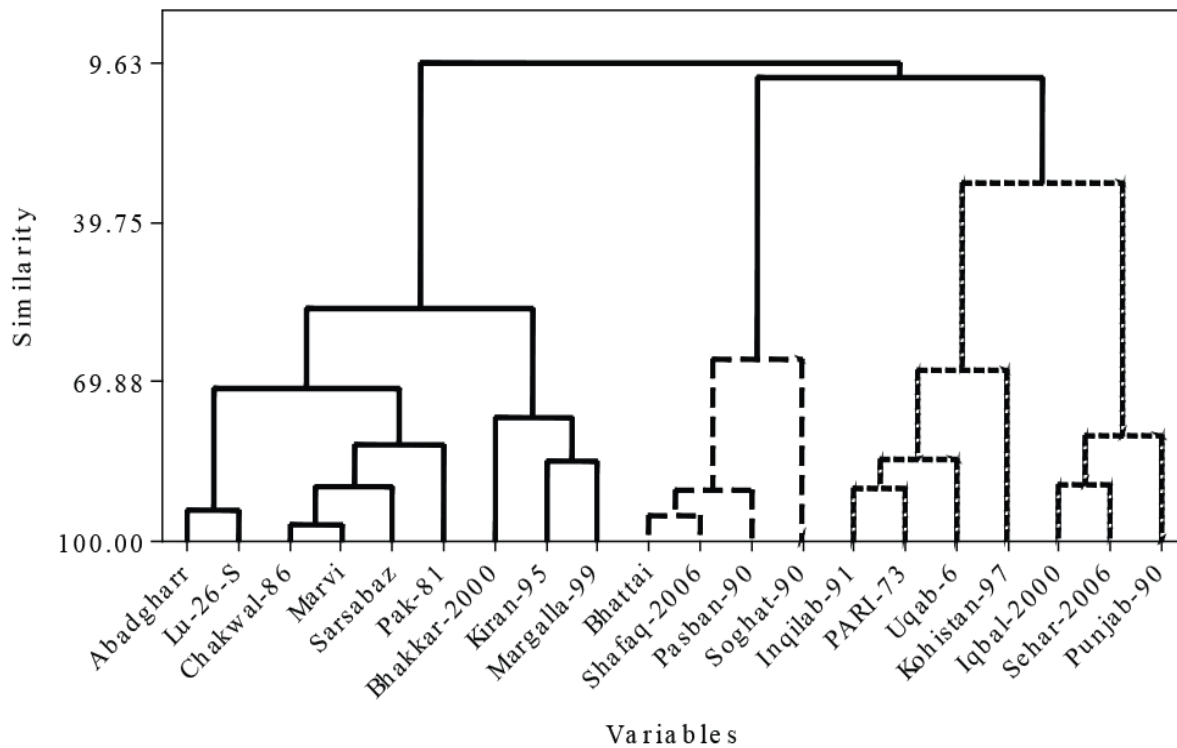

Fig. 1. Dandogram from cluster analysis for salt tolerance in different wheat genotypes based on physiological indices: a screening tool. Clusters detail; Cluster: 1 Abadgharr, LU-26-S, Chakwal-86, Marvi, Sarsabaz, Pak-81, Bhakkar-2000, Kiran-95, and Margalla-99; Cluster 2: Bhattai, Shafaq-2006, Pasban-90, and Soghat-90; Cluster 3: PARI-73, Inqilab-91, Uqab-6, Kohistan-97, Iqbal-2000, Sehar-2006, and Punjab-90

Conclusion

The findings of this study clearly indicated that physiological indices can be utilized to screen the wheat germplasm for salt tolerance. Positive and significant correlations among different indices and cluster analysis also proved that wheat genotypes screened on the basis of physiological indices are salt tolerant. Tolerant genotypes can directly be recommended for cultivation on salt-affected soils or can be used to develop high yielding salt tolerant wheat cultivars.

References

Akram, M., M.Y. Ashraf, R. Ahmad, E.A. Waraich, J. Iqbal and M. Mohsan. 2010. Screening for salt tolerance in maize (*Zea mays* L.) hybrids at an early seedling stage. *Pak. J. Bot.*, 42(1): 141-154.

Akhtar, M., F. Hussain, M.Y. Ashraf, T.M. Qureshi, J. Akhter and A.R. Awan. 2012. Influence of salinity on nitrogen transformations in soil. *Comm. Soil Sci. Plant Analy.*, 43: 1674-1683.

Ali, M.A., N.N. Nawab, A. Abbas, M. Zulkiffal and M. Sajjad .2009 .Evaluation of selection criteria in *Cicer arietinum* L. using correlation coefficients and path analysis. *Australian J Crop Sci.*, 3: 65-70.

Ali, Z., A.S. Khan and M.A. Asad. 2002. Salt tolerance in bread wheat: genetic variation and genetic variation and heritability for growth and ion relation. *Asia. J. Plant Sci.*, 1: 420-422.

Ashraf, M.Y. and G. Sarwar. 2002. Salt tolerance potential in some members of Brassicaceae. Plysiological studies on water relations and mineral contents. In: *Prospects for Saline Agriculture* (Eds.): Ahmad, R. and K.A. Malik. Kluwer Academic Publishers Springer, the Netherlands, pp. 237-245.

Ashraf, M.Y., K. Akhtar, G. Sarwar and M. Ashraf. 2002. Evaluation of arid and semi-arid ecotypes of guar (*Cyamopsis tetragonoloba* L.) for salinity (NaCl) tolerance. *Journal of Arid Environments*, 52: 437-482.

Ashraf, M.Y., A.H. Khan, A.R. Azmi and S.S.M. Naqvi. 1999. Comparison of screening techniques used in breeding for drought tolerance in wheat. In: *Proc. New Genetical Approaches to crop improvement II*. Ed. Naqvi, S.S.M. 513-525.

Ashraf, M., S. Rahmatullah, M.A. Kanwal, A. Sarwar Tahir and L. Ali. 2007. Differential salt tolerance of sugarcane genotypes. *Pak. J. Agri. Sci.*, 44(1): 50-57.

Ashraf, M.Y., F. Hussain, J. Akhtar, A. Gul, M. Ross and G. Ebert. 2008. Effect of different sources and rates of nitrogen and supra optimal level of potassium fertilization on growth, yield and nutrient. *Pak. J. Bot.*, 40(4): 1521-1531.

Azhar, F.M. and R. Ahmad. 2000. Variation and heritability of salinity tolerance in upland cotton at early stage of plant development. *Pak. J. Bio. Sci.*, 3: 1991-1993.

Bor, M, F. Ozdemir and I. Turkan. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet (*Beta maritima* L.). *Plant Science*, 164(1): 77-84.

Bybordi, A. and J. Tabatabaei .2009. Effect of salinity stress on germination and seedling properties in canola cultivars (*Brassica napus* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 37: 71-76.

Chauhan, B.S. and D.E. Johnson. 2009. Ecological studies on *Cyperus deformis*, *Cyperus iria* and *Fimbristylis miliacea* three troublesome annual sedge weeds of rice. *Ann. App. Biol.*, 155: 103-112.

Farshadfar, E. and P. Elyasi. 2012. Screening quantitative indicators of drought tolerance in bread wheat (*Triticum aestivum* L.) landraces. *European J. Experimental Biology*, 2: 577-584.

Ghazizade, M., P. Golkar and F. Salehinejad. 2012. Effect of salinity stress on germination and seedling characters in Safflower (*Carthamus tinctorius* L.) genotypes. *Annals of Biological Research,* 3(1): 114-118.

Hakim, A.M., A.S. Juraimi, M. Begum, M. M. Hanafi1, M.R. Ismail and A. Selamat. 2010. Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.).*Afr. J. Biotechno.*, .9(13): 1911-1918.

Hasegawa, P.M., A.R. Bressan, K.J. Zhu and J.H. Bohnert. 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 51: 463-499.

Janmohammadi, M., P. Dezfuli Moradi and F. Sharifzadeh. 2008. Seed invigorations techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. *Gen. Appl. Plant Physiol.,* 34(3-4): 215-226.

Kamal, A., M.S. Qureshi, M.Y. Ashraf and M. Hussain. 2003. Salinity induced changes in growth and some physio-chemical aspects of two soybean (*Glycine max* L.) genotypes. *Pak. J. Bot.,* 35: 93-97.

Kausar, A., M.Y. Ashraf, I. Ali, M. Niaz and Q. Abbass. 2012. Evaluation of sorghum varieties/lines for salt tolerance using physiological indices as screening tool. *Pak. J. Bot.*, 44(1): 47-52.

Khan, A.A., S.A. Rao and T.M. McNilly. 2003a. Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). *Euphytica*, 131: 81-89.

Khan, A.S., M.A. Asad and Z. Ali. 2003b. Assessment of genetic variability for NaCl tolerance in wheat. *Pak. J. Agri. Sci.,* 40: 33-36.

Khan, M.A., S. Yasmin, R. Ansari, M.U. Shirazi and M.Y. Ashraf. 2007. Screening for salt tolerance in wheat genotypes at an early seedling stage. *Pak. J. Bot.*, 39: 2501-2509.

Khan, M.A., M.Y. Ashraf, S.M. Mujtaba, M.U. Shirazi, M.A. Khan, A. Shereen, S. Mumtaz, M.A. Siddiqui and G.M. Kaleri. 2010. Evaluation of high yielding canola type *Brassica* genotypes/ mutants for drought tolerance using physiological indices as screening tool. *Pak. J. Bot.*, 42: 3807-3816.

Lin, J., X. Li, Z. Zhang, X. Yu, Z. Gao,Y. Wang, J. Wang, Z. Li and C. Mu. 2012. Salinity-alkalinity tolerance in wheat: Seed germination, early seedling growth, ion relations and solute accumulation. *Afr. J. Agric. Res.*, 7(3): 467-474.

Murillo-Amador, B., R. Lopez-Aguilar, J. Larrinaga-Mayoral Kaya and H.A. Flores. 2002. Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. *J. Agron. Crop Sci.*, 188: 233-247.

Nookra, R.I. and I. Khaliq. 2007. An efficient technique for screening wheat (*Triticum aestivum* L.) germplasm for drought tolerance. *Pak. J. Bot.,* 39(5): 1539-1546.

Noorifarjam, S., E. Farshadfar and M. Saeidi. 2013. Evaluation of drought tolerant genotypes in bread wheat using yield based screening techniques. *European J. Experimental Biology*, 3: 138-143.

Odjegba, J.V. and C.I. Chukwunwike. 2012. Physiological responses of *Amaranthus hybrids* L. under salinity stress. *Indian J. Innovations Dev*., 1(10): 742-748.

Redmann, R.E. and M. Belyk. 1994. Growth of transgenic and standard canola (*Brassica napus* L.) varieties in response to soil salinity. *Can. J. Plant Sci.*, 74(4): 797-799.

Rejili, M., M.A. Vadel, A. Guetet, M. Mahdhi, B. Lachiheb, A. Ferchichi and M. Mars. 2010. Influence of temperature and salinity on the germination of *Lotus creticus* (L.) from the arid land of Tunisia. *Afr. J. Eco*., 48: 329-337.

Rengasamy. P. 2006. World salinization with emphasis on Australia. *J. Exp. Bot*., 57(5): 1017-1023.

Riga, P. and N. Vartanian. 1999. Sequential expression of adaptive mechanism is responsible for drought resistance in tobacco. *Aust. J. Plant Physiol*., 26:211-220.

Saeed, B., Gul, H. Shah, P. Khan, A. Anwar, S. Azra and S. Ali. 2012. Yield of wheat varieties under solid and skip row geometries . *ARPN Journal of Agricultural and Biological Science*, 7(8): 591-594.

Steel, R.G.D., J.H. Torrie and D.A. Deekey. 1997. Principles and procedures of statistics: *A Biometrical Approach*. 3rd ed. McGraw Hill Book Co. Inc. New York, 400-428.

Takel, A. 2000. Seedling emergence and growth of sorghum genotypes under variable soil moisture deficit. *Agron. J*., 48: 95-102.

Vahdati, K., N. Lotfi, B. Kholdebarin, D. Hassani, R. Amiri, M.R. Mozaffari and C. Leslie. 2009. Screening for drought-tolerant genotypes of persian walnuts (*Juglans regia* L.) during seed germination. *Hortscience*, 44: 1815-1819.