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Research Article

Differential response of selected bread wheat (*Triticum aestivum* L.) genotypes for salt tolerance by using multiple parameters

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Abstract

The aim of the present study was to identify new sources of salt tolerance on the basis of relative performance of multiple parameters with respect to salt tolerance indices at 10.0 dSm^{-1} in wheat. Ten bread wheat genotypes were evaluated in pots following completely randomised design for salinity tolerance (EC_{iw}=10.0 dSm^{-1}) imposed 21 days after sowing (S1) and at the time of sowing (S2). In the present study Kharchia65, UP1109 and K9423 were found to be the most tolerant while HD2009 and AKAW4627 were salt sensitive among studied genotypes according to cluster analysis based on relative salt tolerance indices of multiple parameters. The differences among genotypes of bread wheat reflected important genetic variability under salinity, which can be further explored and used for the wheat breeding programs. Cluster analysis with multiple agronomic parameters simultaneously can facilitate rankings of salt tolerance of wheat genotypes.

Keywords: Salinity, bread wheat, salt tolerance indices, sodium, potassium

1. Introduction

Salinity is one of the major factors reducing plant growth and productivity worldwide, and affects about 7% of world's total land area (Flowers *et al.*, 1997). Percentage of cultivated land affected by salt is even greater with 23% of the cultivated land being saline and 20% of the irrigated land suffering from secondary salinization. Furthermore, there is also a dangerous trend of a 10% per year increase in the saline area throughout the world (Ponnamieruma, 1984). In India, about 6.73 million hectare land area is salt affected and out of which 3.77 and 2.96 million hectares are under sodicity and salinity, respectively (Mondal *et al.*, 2010). Wheat is the second most important crop after rice in India and occupies approximately 28.5 million hectare area. According to some estimates by FAO (2006, 2007) and Rosegrant *et al.* (2001), the global wheat production must increase by at least 1.6 percent annually to meet a projected wheat demand of 760 million tons by 2020. In order to achieve this goal it is not only important to increase genetic yield potential of varieties but also incorporate biotic and abiotic stress tolerance genes.

Improving wheat productivity will be essential to meet the growing demand for food under shrinking cultivable land area. It is imperative in this context to look for tools not only to increase the crop productivity but also ensure protection against loss of potential productivity due to environmental vagaries (Kumar *et al.*, 2012). Improving salt tolerance of wheat genotypes has been hampered by a number of factors, such as the lack of effective evaluation methods for salt tolerance to screen the genotypes in breeding programs, low selection efficiency using overall agronomic parameters and a complex phenomenon involving morphological, physiological and biochemical parameters among genotypes (Zeng *et al.*, 2002). Compared with conventional techniques that score and rank salt tolerance genotypes based on single parameter, some success has already been realized by using multiple agronomic parameters simultaneously at different growth stages (Shannon, 1997; Zeng *et al.*, 2002). An appropriate statistical method is needed to analyse multiple agronomic parameters simultaneously to facilitate ranking of genotypes for salt tolerance (Zeng *et al.*, 2002). Cluster analysis is commonly used multivariate statistical technique suggested for comparisons of genotypes

(Jollife *et al.*, 1989). However, multivariate analysis in the screening of genotypes for salt tolerance has been applied only in potato (Khrais *et al.*, 1988) and rice (Zeng *et al.*, 2002). The objective of the present study was to identify new sources of salt tolerance on the basis of relative performance of multiple parameters with respect to salt tolerance indices at ECiw=10.0 dS m^{-1} in bread wheat.

2. Materials and methods

Pot experiment was laid out by planting ten bread wheat genotypes (Kharchia65, UP1109, K9423, PBW373, PBW343, HUW468, K9162, PBW154, AKAW4627 and HD2009), obtained from gene pool of ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal, and Sardar Vallabhbhai Patel University of Agriculture and Technology (SVPUA&T), Meerut, India. The experiment was laid out at the experimental farm, Department of Agriculture Biotechnology, SVPUA&T, Meerut, during November, 2012-13 and 2013-14 and the experimental soil was sandy loam with initial pH 7.2 and ECe 1.13 dSm^{-1}. To create the irrigation water of desired salinity level (EC_{iw}=10.0 dSm^{-1}), required quantity of NaCl, Na_2SO_4 and $CaCl_2$ (4.091g/l: 0.71g/l: 1.1g/l) were thoroughly mixed with irrigating water (7:1:2 ratio) to the pots. The pot experiment was performed in complete randomized design (CRD) with three replications. Two levels of soil salinity i.e, control (normal irrigation water) and saline (pre sowing with normal water and saline irrigation after 21 days of sowing) were maintained and applied.

2.1. Phenotypic observation: The morpho-physiological and biochemical observations of all the genotypes were recorded at the time of pre-maturity. The investigated traits were plant height (PH), number of tillers $plant^{-1}$ (NT), number of productive tillers $plant^{-1}$ (NPT), spike length (SL), spikelet $spike^{-1}$ (SN), average biomass $plant^{-1}$ (AB), test weight (TW), grain yield $plant^{-1}$ (GY), leaf area (LA), relative water content (RWC), potassium (K^+) and sodium (Na^+) were measured using standard protocols. RWC was determined for all genotypes following the procedure of Turner (1981).

All the recorded data were subjected to statistical analysis using (SPSS 19.0) software.

2.2. Ranking of genotypes for salt tolerance: All the data were converted to salt tolerance indices before cluster analysis following the method of Zeng *et al.* (2002) to allow comparisons among genotypes for salt tolerance by using multiple agronomic parameters. A salt tolerance index was defined as the observation at salinity divided by the average of the controls. Cluster group ranking numbers can be assigned to cluster groups based on cluster means, and were used to score genotypes. Cluster analysis was followed as per the methods described by Jollife *et al.* (1989). Cluster group rankings were obtained based on Ward's minimum variance cluster analysis of the averages of the salt tolerance indices for five parameters of morphological characters (plant height, number of tillers $plant^{-1}$, number of productive tillers $plant^{-1}$, spike length, and spikelet $spike^{-1}$), three parameters of yield attributes characters (average biomass $plant^{-1}$, test weight and average yield $plant^{-1}$) and four parameters of physio-biochemical characters (SPAD value, leaf area, and relative water contents and K/Na ratio). The distance between two clusters was calculated as the ANOVA sum of squares between the two clusters in all the parameters analyzed. The clusters were merged in each generation to minimize the within-cluster sum of squares. The procedures are described in the SPSS User's Guide (SPSS, version 19.0). The cluster groups were identified in Dendrogram (Fig. not shown). The number of cluster groups was determined by calculating the pseudo t^2 which reached a local maximum. The cluster group rankings were obtained from the averages of means over multiple parameters in each cluster group, i.e., cluster mean, in order from highest to lowest averages. A sum was obtained by adding the numbers of cluster group ranking at each salt level in each genotype. The genotypes were finally ranked based on the sums in order that those with the smallest sums were ranked as the most tolerant and those with the largest sums were ranked as the least tolerant in terms of relative salt tolerance.

3. Results and discussion

In the present investigation, plant height, tillers $plant^{-1}$, productive tillers $plant^{-1}$, spike length, spikelet $spike^{-1}$, all these studied characters, were decreased with increasing salinity. However, the relative salt tolerance indices (RSTI) in terms of these parameters varied among genotypes (Table 1). The RSTI of plant height ranged from 0.60 to 0.97. Tiller $plant^{-1}$ is most salinity sensitive trait in wheat (El-Hendawy *et al.*, 2005). Thus to increase yield under stress condition it is necessary to maintain high plant density. Tiller formation included tiller number and tiller biomass. Salinity reduces tiller number by delaying and reducing tiller emergence at the vegetative stage. After tiller emergence, growth of tillers at all stages is inhibited by salinity due to its damage on the essential metabolic reaction in plants, resulting in low tiller biomass and small tiller size (Maas and Poss, 1989).

ECe >7.5 dSm^{-1} in soil water could eradicate most of the secondary tillers and greatly reduce formation of tertiary and lateral tillers. The yield potential of wheat is greatly dependent on the number of tillers $plant^{-1}$ that is affected in the early life cycle. Number of tillers regulates grain yield by its prime influence on the number of spikes in wheat (Simons and Hunt, 1983). The RSTI of tillers $plant^{-1}$ ranged from 0.40 to 0.91. The RSTI of productive tillers $plant^{-1}$ ranged from 0.36 to 0.91. The RSTI of spike length ranged from 0.81 to 0.97. The RSTI of spikelet $spike^{-1}$ ranged from 0.76 to 0.96. The salt tolerance indexes (STI)

of all morphological character studied was ranged from 0.60 to 0.94. Genotypes were divided into three and two cluster groups (Fig. not shown) by simultaneous analysis on salt tolerance indexes based on five parameters of morphological characters using Ward's minimum-variance cluster analysis. Plant biomass decreased with increase in salinity levels. The RSTI of biomass plant^{-1} ranged from 0.47 to 0.90. The RSTI of 1000 grain weight ranged from 0.57 to 0.91. Salinity significantly reduced the grain yield and the effect increased with salinity level. The RSTI of grain yield plant^{-1} ranged from 0.03 to 0.86 (Table 1). The salt tolerance indexes (STI) of all yield components studied was ranged from 0.39 to 0.89. Genotypes were divided into five and three cluster groups (Fig. not shown) by simultaneous analysis on salt tolerance indexes based on three parameters of yield related characters using Ward's minimum-variance cluster analysis. Salt stress causes inhibition of growth and development, reduction in photosynthesis, respiration, and protein synthesis and disturbs nucleic acid metabolism reported by Boyer (1965) and Levine *et al.* (1990). Decrease in uptake of K, Mg^{2+}, Ca^{2+}, and thereby decrease in growth at higher sodium concentration have also been reported by Poonia *et al.* (1972).

Salinity stress at different phenological stages inhibits photosynthetic activities of the plant because it had a direct inhibitory effect on the Calvin cycle enzymes (Ottender and Oquist, 1991). The RSTI of chlorophyll content (SPAD) ranged from 0.69 to 0.98. The RSTI of leaf area ranged from 0.50 to 0.97. The RSTI of relative water

Table 1. Relative salt tolerance indices (RSTI) of different traits studied in selected wheat genotypes under salinity (EC_{iw}=10.0 dSm^{-1}) in 2012-13 and 2013-14.

Genotypes	T	PH	NT	NPT	SL	NS	STI	AB	TW	GY	STI	SPAD	LA	RWC	K / Na	STI
Kharchia 65	S1	0.93	0.91	0.91	0.97	0.96	0.94	0.90	0.91	0.86	0.89	0.94	0.97	0.97	0.25	0.78
	S2	0.89	0.80	0.81	0.93	0.87	0.86	0.78	0.86	0.05	0.56	0.88	0.94	0.90	0.11	0.71
HD 2009	S1	0.89	0.66	0.75	0.90	0.87	0.81	0.66	0.82	0.54	0.67	0.83	0.81	0.90	0.21	0.69
	S2	0.75	0.56	0.52	0.85	0.76	0.69	0.47	0.66	0.03	0.39	0.69	0.69	0.60	0.07	0.51
PBW 343	S1	0.94	0.84	0.80	0.93	0.92	0.89	0.69	0.85	0.70	0.75	0.92	0.86	0.95	0.41	0.78
	S2	0.86	0.59	0.51	0.85	0.87	0.74	0.54	0.77	0.04	0.45	0.91	0.63	0.84	0.27	0.66
AKAW 4627	S1	0.97	0.48	0.49	0.89	0.91	0.75	0.88	0.86	0.54	0.76	0.89	0.77	0.96	0.10	0.68
	S2	0.60	0.40	0.39	0.82	0.81	0.60	0.65	0.61	0.04	0.43	0.76	0.50	0.93	0.07	0.56
K 9423	S1	0.97	0.71	0.83	0.85	0.95	0.86	0.89	0.89	0.85	0.88	0.98	0.94	0.94	0.25	0.78
	S2	0.87	0.48	0.50	0.81	0.88	0.71	0.71	0.75	0.03	0.50	0.95	0.82	0.85	0.18	0.70
PBW 373	S1	0.90	0.78	0.66	0.92	0.95	0.84	0.90	0.80	0.69	0.80	0.98	0.93	0.91	0.18	0.75
	S2	0.86	0.55	0.44	0.89	0.92	0.73	0.72	0.57	0.04	0.45	0.91	0.81	0.58	0.10	0.60
HUW 468	S1	0.88	0.83	0.82	0.96	0.96	0.89	0.87	0.84	0.51	0.74	0.91	0.88	0.93	0.22	0.73
	S2	0.86	0.63	0.54	0.83	0.92	0.75	0.73	0.60	0.04	0.46	0.88	0.77	0.50	0.12	0.57
K9162	S1	0.93	0.87	0.89	0.91	0.92	0.90	0.73	0.81	0.79	0.78	0.87	0.89	0.74	0.22	0.68
	S2	0.91	0.82	0.70	0.85	0.92	0.84	0.57	0.63	0.05	0.41	0.83	0.78	0.44	0.12	0.55
PBW 154	S1	0.97	0.90	0.75	0.91	0.92	0.89	0.77	0.85	0.55	0.72	0.95	0.93	0.64	0.40	0.73
	S2	0.94	0.44	0.36	0.83	0.86	0.68	0.66	0.70	0.03	0.47	0.89	0.50	0.53	0.16	0.52
UP 1109	S1	0.90	0.83	0.82	0.93	0.90	0.88	0.79	0.87	0.80	0.82	0.97	0.90	0.92	0.53	0.83
	S2	0.83	0.75	0.53	0.90	0.86	0.77	0.65	0.68	0.05	0.46	0.87	0.71	0.86	0.18	0.66

T- Treatments; PH-Plant height; NT- Number of tillers plant^{-1}; NPT- Number of productive tillers plant^{-1}; SL- Spike length; SN- Spikelet number spike^{-1}; AB-Average biomass plant^{-1} TW-Test weight; GY-Average grain yield; SPAD- Chlorophyll content; LA- Leaf area; RWC-Relative water content; K/Na ratio; STI-Salt tolerance index S1- Salinity (EC_{iw}=10.0 dSm^{-1}) imposed on 21 days after sowing (Salinity level1) S2- Salinity (EC_{iw}=10.0 dSm^{-1}) imposed at the time of sowing (Salinity level2)

content ranged from 0.44 to 0.97 (Table 1). El-Hendawy (2009) reported that upper two leaves on main stem can be used as selection criteria. Seed weight, grains plant^{-1} and fertile spikes were found poor selection Criteria in controlled conditions but these traits were efficient under saline field conditions. Concentration of potassium was found to be poor criteria while K^+/Na^+ discrimination was found useful in controlled and field conditions. The RSTI of K/Na ranged from 0.07 to 0.53. The salt tolerance indexes (STI) of four parameters of physio-biochemical characters studied was ranged from 0.51 to 0.83 (Table 1). Genotypes were divided into five cluster groups (Fig. not shown) by simultaneous analysis on salt tolerance indexes based on four parameters of physio-biochemical characters using Ward's minimum-variance cluster analysis. In the analysis of the relationships between seed yield plant^{-1} and the other parameters, productive tiller plant^{-1} (0.776**) and spikelet spike^{-1} (0.652*) contributed the most variation to seed yield plant^{-1} when data from all genotypes were combined (Table 3).

In this study, relative salt tolerance index (RSTI) and salt tolerance index (STI) among wheat genotypes was evaluated using cluster analysis. As earlier pointed out by Khrais *et al.* (1988), Zeng *et al.* (2002) and Hendway *et al.* (2005 and 2009) all the data studied were converted to relative salt tolerance indices before further analysis to allow comparisons among genotypes for salt tolerance. The genotypes were finally ranked based on the sums, such that those with the smallest and largest sums were ranked respectively as the most and least tolerant genotypes in terms of relative salt tolerance (Table 2). Advantages of using a multivariate analysis in the evaluation of salt tolerance are that it allows: (1) a simultaneous analysis of multiple parameters to increase the accuracy of the genotype ranking; (2) the ranking of genotypes even when plants are evaluated at different salt levels and salt tolerance varies with salinity levels, especially when the salt tolerance indices are averaged across salt levels; and (3) a more convenient and accurate estimation of salt tolerance among genotypes by simply adding the numbers in cluster group ranking at different salt levels.

Table 2. Ranking of genotypes (GR) for their relative salt tolerance index (RSTI) in cluster analysis

Genotypes	GR based on MC[a]	GR based on YC[b]	GR based on PYC[c]	Sum	Finale GR[d]	TD[e]
Kharchia65	1	1	1	3	1	MT
UP 1109	1	1	2	4	2	T
K 9423	2	1	2	5	3	T
PBW 373	2	2	2	6	4	MDT
PBW 343	1	5	1	7	5	MDT
HUW 468	1	5	3	9	6	MDS
K9162	1	3	5	9	6	MDS
PBW 154	1	6	3	10	7	MDS
AKAW 4627	3	4	4	11	8	S
HD 2009	2	7	4	13	9	MS

MC[a]- Morphological characters (PH-Plant height; NT- Number of tillers plant^{-1}; NPT- Number of productive tillers plant^{-1}; SL- Spike length; SN- Spikelet number spike^{-1}) YC[b]- Yield components (AB-Average biomass plant^{-1} TW-Test weight; GY-grain yield), PYC[c]- Physio-biochemical characters (SPAD- Chlorophyll content; LA- Leaf area; RWC-Relative water content; K/Na ratio), [d]Genotypes were finally ranked based on the sums with the smallest sum being the most relatively tolerant, TD[e]- Tolerance degree; MT- Most toerant; T- Tolerant; MDT; Moderate tolerant; MDS- Moderate susceptible; MS- Most susceptible; S- Susceptible

Table 3. Relationships between grain yield and others parameters (RSTI) studied in this investigation under salinity.

			PH	NT	NPT	SL	NS	AB	TW	SPAD	LA	RWC	K/Na
Relationship to yield plant^{-1}	Correlation (r)	2012-13	0.183	0.395	0.776**	-0.027	-0.036	0.013	0.374	0.460	0.587	0.024	0.389
		2013-14	0.244	0.124	0.125	0.157	0.652*	0.440	0.186	0.359	0.377	0.443	-0.058

**, * Correlation is significant at the 0.01 and 0.05 level.

In the present investigation, cluster group rankings were obtained based on ward's minimum variance cluster analysis of the averages of the salt tolerance indices for five parameters of morphological characters (plant height, tillers plant^{-1}, productive tillers plant^{-1}, spike length, spikelet spike^{-1}), three parameters of yield attributes characters (average biomass plant^{-1}, test weight, average grain yield plant^{-1}) and four parameters of physio-biochemical characters (SPAD value, leaf area, relative water contents and K/Na). At heading salinity suppresses reproductive development, spikelet formation and ultimately spikelet number (Mans and Rawson, 2004). Due to their response to salinity and significant positive correlation with yield these two traits could be used to evaluate wheat genotypes under saline field conditions. Decline in grains spike^{-1} was mainly due to decline in spikelet spike^{-1} as revealed by positive correlation between them. The 1000 grain weight was less affected as compare to the other yield components because it was determined at maturity which is the least salt sensitive stage in wheat (Frank *et al.*, 1997).

In the present study, wide genotypic differences were observed for relative salt tolerance in terms of spikelet spike^{-1} and productive tiller plant^{-1}. Spikelet spike^{-1} and productive tiller plant^{-1} contributed most of the variations to seed yield under salinity among parameters investigated when data were averaged across all genotypes. Genotypic differences were also identified in the other studied traits, but these characters were not strictly correlated with relative salt tolerance based on seed yield among genotypes. In conclusion, cluster analysis (according to ward minimum variance) based on relative salt tolerance indices of studied traits were performed, Kharchia65, UP1109 and K9423 were found the most tolerant while HD2009 and AKAW4627 were found most susceptible among studied genotypes. Our result supported by the previous finding of Zeng *et al.* (2002), El-Hendawy *et al.* (2005 and 2009), Shahzad *et al.* (2012) and Ahmad *et al.* (2013). The wide range of relative salt tolerance indices for different traits indicates that genotypes had broad genetic base for these traits. These genotypes can be utilized in breeding programs for further improvement and development of salt tolerant varieties of wheat for the salinity affected areas. In a breeding program where a large number of genotypes have to be evaluated, relative salt tolerance indices can be computed for different agronomic parameters. Cluster analysis can be used to facilitate the ranking of the genotypes for salt tolerance.

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