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Physiological, Biochemical and Morphological Study in Wheat (*Triticum aestivum* L.) RILs Population for Salinity Tolerance

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Abstract

Soil salinity is among the major abiotic stresses affecting crop yield. Phenotypic and biochemical studies are needed for screening of salt tolerant genotypes that could be used in breeding programme. The present study was conducted to ascertain the morphological or agronomical traits contributing for yield and to identify salt tolerant RILs. A mapping population of 94 recombinant inbred lines (RILs) (F8) developed from cross between wheat genotype Kharchia 65 and HD 2009 were sown under stressed and non-stressed condition in microplots. STI was calculated to normalize the data. Salinity tolerance value was calculated through PCA and STI values. Based on the salinity tolerance value the RILs were grouped into three groups tolerant, moderately tolerant and susceptible. PCA indicated that the first three components accounted for 63.35% of the total variations. Tiller number showed significant positive correlation with number of earheads, plant height, length of earheads, spike number, K^+ concentration and 1000 grain weight. Proline is an important biochemical parameter and its accumulation under salt stress condition is related with salinity tolerance and ultimately to grain yield. The study concluded that PCA based grouping is an effective tool to identify tolerant genotypes and that tiller number is a good parameter for evaluating salinity tolerance at early growth stages in plants.

Keywords: STI, PCA, salinity tolerance, wheat (*Triticum aestivum* L.)

1. Introduction

Wheat is one of the most important world's food crops which contribute substantially in food and nutritional security. However, production and productivity of wheat is affected by several abiotic constraints including high temperature, low temperature, drought and salinity. Salinity stress is among them the major abiotic stress affecting 7% of world land area (Flower et al., 1997). In India, an area of about 7 mha is already under salinity and 3.6 mha under sodicity problem and still larger area is coming under potential salinity problem due to injudicious use of water under canal irrigation system (Hollington, 1998). There are two ways to deal with this problem: one is to reclaim soil by the application of chemicals and the other is to develop varieties which can withstand salinity. However the technologies to reclaim land and maintain salt balance is expensive and even in many situations soil amendments based reclamation are either not possible or economically viable. Therefore, development of salt tolerant cultivars is a viable proposition. Conventional and artificial breeding approaches are used to develop varieties tolerant to salinity stress. Development of salt tolerant varieties needs morphological and physiological studies of the genotypes conducted under controlled and stressed conditions in field, to identify salt tolerant genotypes and use them in breeding programs.

A number of methods have been developed to identify salt tolerant genotypes of wheat on yield basis. Tolerance index was proposed by Rosielle and Hamblin (1981), Yield index by Gavuzzi et al. (1997) and Yield stability index was introduced by Bauslama and Schapaugh (1984). As salinity stress is a complex inherited trait the use of simply inherited traits associated with yield are more useful than selection based on grain yield or biomass production under abiotic stress (Ashraf, 2004). Evaluation of genotypes using multiple agronomic traits is recommended as most of them are significantly correlated with each other (Zeng et al., 2002).

Principal component analysis (PCA) is a multivariate analysis method that finds correlation between a large set of variables in terms of few independent factors (Beheshtizadeh et al., 2013). In salinity studies this statistical

tool is used to know how much a physiological or morphological trait is contributing to the yield variable. PCA has been used in various studies to identify variability, a particular trait or component is contributing towards yield (Gholizadeh et al., 2014; Mohamed, 2013; Choukan, 2011). Based on components derived from PCA biplot can be developed to show the genotype x enviornment interaction. In biplot the first two component of PCA are used to produce a two dimensional diagram to demonstrate genotype to environment variation. In the milieu, the present study is an attempt to identify the salt tolerant RILs and ascertain the morphological or agronomical traits contributing for yield trait.

2. Material and Methods

2.1 Plant Material

A mapping population of 94 recombinant inbred lines (RILs) (F8) developed from cross between wheat genotype Kh 65 (salt tolerant) and HD 2009 (salt sensitive) were taken and seeds of the RILs were sown under normal and alkaline soil (pH 9.2) in microplot (12 m × 6 m) adopting the completely randomized design. The pH of the soil was maintained at pH 9.2 by regularly sampling of soil till maturity. Varieties Kh 65, HD 2009, HD 2967 and KRL1-4 were used as checks after every 20 RILs. Sowing in microplots was done in three rows with a row-to-row distance of 1.5 m and in each row, 10-15 plants of each RIL were sown with a spacing of 3 cm. Five plants of each RIL were randomly selected from both the control and treated microplots for evaluation of morphological, agronomical and physiological traits from germination to maturity stage. Germination percentage, tiller number at 45 and 65 days, chlorophyll content, days to heading, days to anthesis, plant height, number of earheads, number of spike, length of spike and 1000 grain weight were recorded for statistical analysis. Due to presence of homogenous error and non-significant year X genotype effect pooled analysis of data was done.

2.2 Measurement of Na^+/K^+

For Na^+/K^+ measurement flag leaves samples were taken just after heading. The leaf samples were dried in oven for 48 hrs at 65 °C till they dry completely. Around 30 mg dried leaf samples were taken and digested in 0.5 ml of 0.5 N HNO_3 for 2 hrs at 80 °C (Munns et al., 2010). The digested samples were centrifuged and 100 µl of supernatant was diluted 100 times by making the volume 10 ml. Concentration of Na^+/K^+ was measured using flame photometer with standards in the range between 0.25 ppm to 20 ppm.

2.3 Measurement of Proline

Proline was extracted using ninhydrin reaction method (Cross et al., 2006). 50 mg of fresh sample of flag leaf was taken and added to 1 ml of 40:60 v/v ethanol and water mixture. The mixture was kept at 4 °C for overnight. After centrifugation of the mixture at 12,000 rpm for 10 min, 200 µl of the ethanolic extract was taken and to this added 300 µl of ninhydrin reaction (1% ninhydrin in 20:60 v/v ethanol and acetic acid mix). This mixture was incubated for 20 min at 90 °C in water bath. Absorbance of the chromophore was taken at 520 nm.

2.4 Statistical Analysis

2.4.1 Pearson's Correlation

Karl Pearson's correlation coefficient was estimated to find the linear association between various morphological, physiological and biochemical parameters using the following formula:

$$r = \frac{\sum XY - n\overline{X}\overline{Y}}{\sqrt{\sum X^2 - n\overline{X}^2}\sqrt{\sum Y^2 - n\overline{Y}^2}} \quad (1)$$

Where, r is the Pearson's correlation coefficient, X and Y are the variables for which the correlation has been estimated, and n is the number of observations.

2.4.2 Salt Tolerance Index and PCA Based Grouping

All the raw data were converted to relative values by calculating salt tolerance index (STI). The STI was calculated as follows: observation under salinity conditions divided by the mean of controls (Zeng et al., 2002). Principal component analysis was then employed on the normalized data (*i.e.* STI).

2.4.3 Principal Component Analysis and Biplot Analysis

Principal component analysis (PCA) is a popular statistical technique employed to convert a set of observations of possibly correlated variables into a set of principal components which are linearly uncorrelated using orthogonal transformation method. In other words, PCA constructs a linear combination of original variables which contain the same information but orthogonal to each other. By this process, the variables which contribute to variation in the original data have been taken into account. The model is defined in the following way (Stock

& Watson, 2002),

$$X_t = \Lambda_t F_t + e_t \quad (2)$$

Where X_t represents a N-dimensional vector of data, Λ_t is the rx1 common factor, F_t is the factor loading, and e_t is the associated idiosyncratic error-term of order Nx1. The model can be estimated through quasi-Maximum Likelihood Estimation (MLE) procedure.

The present study also used biplot analysis. Typically, a biplot is a scatter diagram having two components viz., PC1 and PC2 (derived from PCA) plotted on the axes.

After PCA, the salt tolerance value for each genotype was calculated using the following formula ((Ayyoob et al., 2013),

$$IGenotype = \frac{\sum_{i=1} XiWi}{\sum_i Wi} \quad (3)$$

Where, $I_{Genotype}$ is the index for each RIL/parent; X_i is the indicator variable for STI (i=1, 2, 3, ... 14); W_i is the weight of the variable = $\sum |Li| Ej$; L_{ij} is the factor loading of i^{th} variable on j^{th} factor; E_j is the eigen value of j^{th} factor (j = 1,2,3, ... Principal Components).

The genotypes were divided into three categories based on the salt tolerance value.

Salt tolerant = I_j > Mean +1/2 SD;

Moderately tolerant = Mean − 1/2 SD < I_j < Mean + 1/2 SD;

Susceptible = I_j < Mean − 1/2 SD.

3. Result

Four yield related parameters, tiller number, earhead number, number of spike and thousand grain weight were studied. Tiller number was recorded at 45 and 65 days and earheads, number of spikes and thousand grain weight were recorded at maturity. Na^+/K^+ and proline were measured at heading stage from flag leaf of five randomly selected plants of each RIL and parents. A reduction in tiller number, number of earheads and spike number was observed in all RILs in treatment microplots. An increase in Na^+ concentration and a decrease in K^+ concentration was observed in all RILs. Salt tolerance index (STI) of RILs and parents was calculated for the 14 traits. STI values of the RILs and parents given in supplementary Table 1.

3.1 Correlation between Various Traits

Correlation was interpreted between various parameters for salinity tolerance. Tiller number showed significant positive correlation with number of earheads, plant height, length of earheads, spike number, K^+ concentration and 1000 grain weight. Days to heading and days to anthesis showed negative correlation with most of the traits except with Na^+ concentration showing a positive correlation (Table 1). Chlorophyll content showed positive correlation with plant height, length of earheads and number of earheads. K^+ showed positive correlation with plant height and length of earheads. Proline showed a positive correlation with thousand grain weight.

Table 1. Pearson's correlations between various morphological, physiological and biochemical parameters

	G %	TN 45	TN 65	CC	PH	NE	LE	DTH	DTA	SN	Na^+/g	K^+/g	P	TGW
G%	1													
TN 45	0.227*	1												
TN 65	0.248**	0.818**	1											
CC	-0.060	0.037	0.084	1										
PH	0.089	0.552**	0.676**	0.259**	1									
NE	0.038	0.483**	0.664**	0.219*	0.600**	1								
LE	0.073	0.226*	0.269**	0.263**	0.417**	0.245**	1							
DTH	-0.154	-0.013	0.053	-0.060	0.146	0.001	0.188*	1						
DTA	-0.149	-0.017	0.052	-0.049	0.145	0.007	0.186	0.995**	1					
SN	0.059	0.304**	0.395**	0.117	0.390**	0.331**	0.512**	0.164	0.158	1				
Na^+/g	-0.081	-0.197*	-0.251**	0.026	-0.173	-0.142	-0.085	0.306**	0.305**	-0.124	1			
K^+/g	0.062	0.289**	0.307**	0.109	0.216*	0.344**	0.281**	-0.209*	-0.200*	0.132	-0.150	1		
P	-0.142	-0.001	-0.060	-0.055	0.043	0.059	-0.143	-0.193*	-0.168	0.030	-0.062	0.049	1	
TGW	0.158	0.240*	0.190*	0.171	0.209*	0.142	-0.037	-0.400**	-0.385**	-0.078	-0.310**	0.131	0.284**	1

Note. [1] * and ** indicate the Pearson's correlation coefficient is significant at one and five per cent level of probability (2-tailed).

G%- germination percentage, TN 45- Tiller number at 45 days, TN 65- Tiller number at 65 days, CC- Chlorophyll content, PH- plant height (cm), NE- Number of earheads, LE- Length of earheads (cm), DTH- Days to heading, DTA- days to anthesis, SN- Spike number, Na+/g (mg/gdw), K+/g (mg/gdw), P- Proline (µg/gFW), TGW- Thousand grain weight (gm).

3.2 Principal Component Analysis

All the fourteen parameters were taken for principal component analysis (PCA). PCA analysis reduced the variables from thirteen to four components accounting for 63.35% of the total variations. The first component accounted for 26.82% of the varability. Table 2 shows the eigen values of the first four component and their variability percentage. Table 3 shows the factor loading for the first four components. For the first component the most effective traits were tiller number and plant height. The most effective trait for the second component were days to heading and days to anthesis. For third component chlorophyll content and for fourth component proline were the most effective traits.

Table 2. Eigen values and extraction of variability

Principal component	Eigen value	Variability	Cumulative %
1	3.76	26.82	26.82
2	2.67	19.12	45.94
3	1.26	9.00	54.94
4	1.17	8.40	63.35

Table 3. Factor loading corresponding to four principal components

	F1	F2	F3	F4
G %	0.239	-0.188	-0.588	-0.398
TN 45	**0.783**	-0.031	-0.334	0.153
TN 65	**0.877**	0.053	-0.296	0.125
CC	0.276	-0.009	0.645	-0.284
PH	**0.808**	0.165	0.077	0.159
NE	**0.756**	0.031	0.087	0.179
LE	0.527	0.320	0.327	-0.433
DTH	-0.018	0.933	-0.069	0.194
DTA	-0.015	0.924	-0.057	0.207
SN	0.559	0.293	0.171	-0.122
Na+/g	-0.335	0.428	0.083	0.014
K+/g	0.470	-0.220	0.177	-0.199
P	0.019	-0.326	0.312	0.684
TGW	0.303	-0.600	0.076	0.225

Note. [1] G%- germination percentage, TN 45- Tiller number at 45 days, TN 65- Tiller number at 65 days, CC- Chlorophyll content, PH- plant height (cm), NE- Number of earheads, LE- Length of earheads (cm), DTH- Days to heading, DTA- days to anthesis, SN- Spike number, Na^+/g (mg/gdw), K^+/g (mg/gdw), P- Proline (µg/gFW), TGW- Thousand grain weight (gm).

3.3 Grouping of RILs

Based on salt tolerance index of all fourteen traits and first four components of PCA, salt tolerance value were calculated. The salt tolerance value were then used to group RILs, Parents (Kh 65 and HD 2009) and varieties HD 2967 and KRL 1-4 into three groups namely salt tolerant, moderately salt tolerant and salt sensitive. The first group had salt tolerance values greater than or equal to 1.62. For group second the salt tolerance values were between 1.61 to 1.40. The third group had salt tolerance values less than 1.40 (Table 4). In first group RIL 26 and RIL 2 had maximum salt tolerance value 2.19 and 2.10 respectively, so were ranked as most tolerant RILs. The first group also included variety Kh 65 (RIL salt tolerant parent) and 25 RILs. Group second includes maximum number of RILs and variety KRL1-4. Group third included HD 2009 and HD 2967 varieties and 27 RILs.

Biplot based on first two Eigen values was ploted. Biplot analysis showed germination percentage and K^+ concentration were more correlated with each other. Tiller number at 45 and 65 days are correlated with number of earheads, plant height and chlorophyll content (Figure 1). Figure 2 shows biplot based on first two components of PCA showing relation between RILs, Parents and traits for salinity tolerance.

Table 4. Salt tolerance value based grouping (calculated from PCA) of RILs into three groups, tolerant, moderately tolerant and susceptible

Tolerant	Salt tolerance value	Moderately tolerant	Salt tolerance value	Moderately tolerant	Salt tolerance value	Susceptible	Salt tolerance value
RIL-26	2.19	RIL-13	1.61	RIL-4	1.45	RIL-56	1.39
RIL-2	2.10	RIL-25	1.61	RIL-82	1.44	RIL-98	1.39
RIL-75	2.07	RIL-10	1.60	RIL-37	1.43	RIL-88	1.39
RIL-69	2.03	RIL-72	1.60	RIL-105	1.42	RIL-29	1.38
RIL-118	2.02	RIL-15	1.60	RIL-86	1.42	RIL-35	1.37
RIL-32	1.94	RIL-12	1.60	RIL-91	1.42	RIL-94	1.37
RIL-70	1.89	RIL-22	1.59	RIL-51	1.41	RIL-83	1.36
RIL-63	1.86	RIL-73	1.57	RIL-58	1.41	HD-2967	1.35
RIL-117	1.79	RIL-103	1.56	RIL-30	1.41	RIL-96	1.34
KH-65	1.78	RIL-79	1.56	RIL-38	1.40	RIL-33	1.34
RIL-23	1.76	RIL-20	1.56	KRL 1-4	1.40	RIL-47	1.34
RIL-113	1.76	RIL-104	1.55	RIL-112	1.40	RIL-36	1.33
RIL-24	1.75	RIL-71	1.54	RIL-48	1.40	RIL-52	1.33
RIL-9	1.75	RIL-87	1.54	RIL-19	1.40	RIL-14	1.33
RIL-80	1.72	RIL-77	1.53	RIL-8	1.40	RIL-34	1.32
RIL-110	1.71	RIL-119	1.53			RIL-89	1.30
RIL-5	1.71	RIL-64	1.52			RIL-39	1.28
RIL-84	1.69	RIL-90	1.52			RIL-78	1.27
RIL-76	1.67	RIL-85	1.51			RIL-60	1.26
RIL-74	1.67	RIL-17	1.50			RIL-61	1.25
RIL-1	1.66	RIL-16	1.49			RIL-49	1.25
RIL-59	1.64	RIL-115	1.49			RIL-42	1.24
RIL-122	1.63	RIL-109	1.49			RIL-18	1.24
RIL-62	1.63	RIL-65	1.49			RIL-41	1.23
RIL-3	1.63	RIL-67	1.48			HD-2009	1.23
RIL-114	1.62	RIL-100	1.48			RIL-53	1.20
		RIL-66	1.47			RIL-40	1.20
		RIL-93	1.46			RIL-50	1.15
						RIL-57	1.08

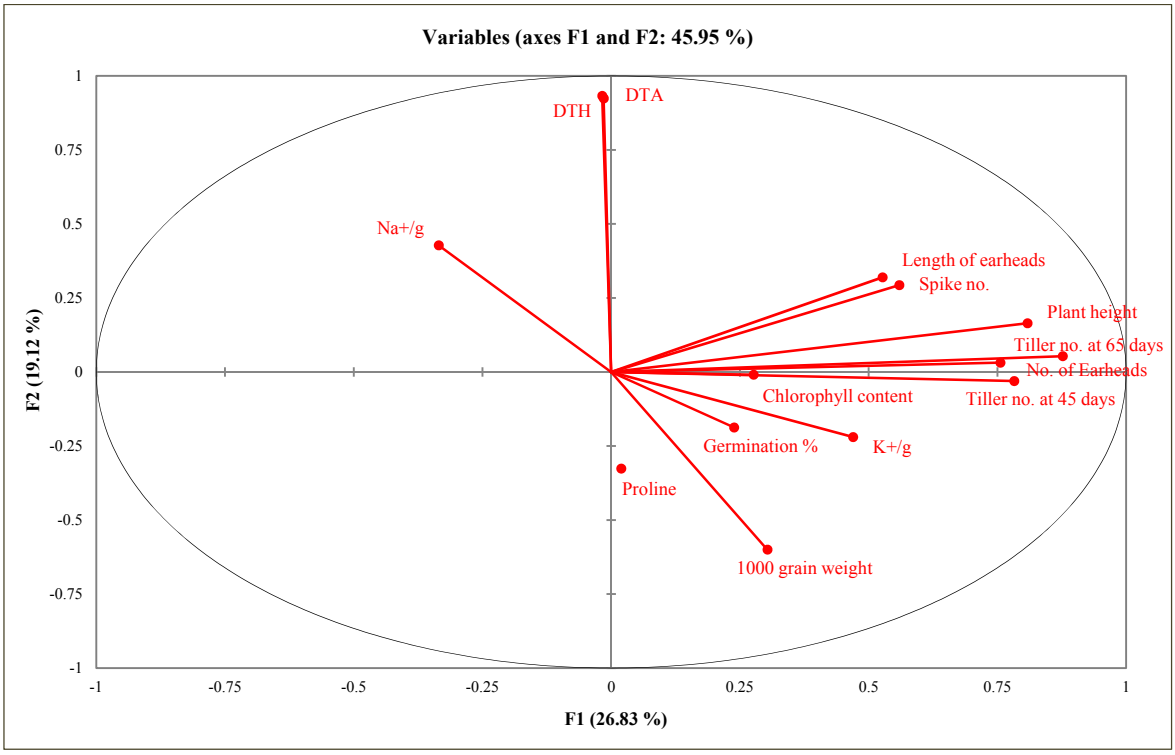

Figure 1. Biplot showing different parameters for salinity

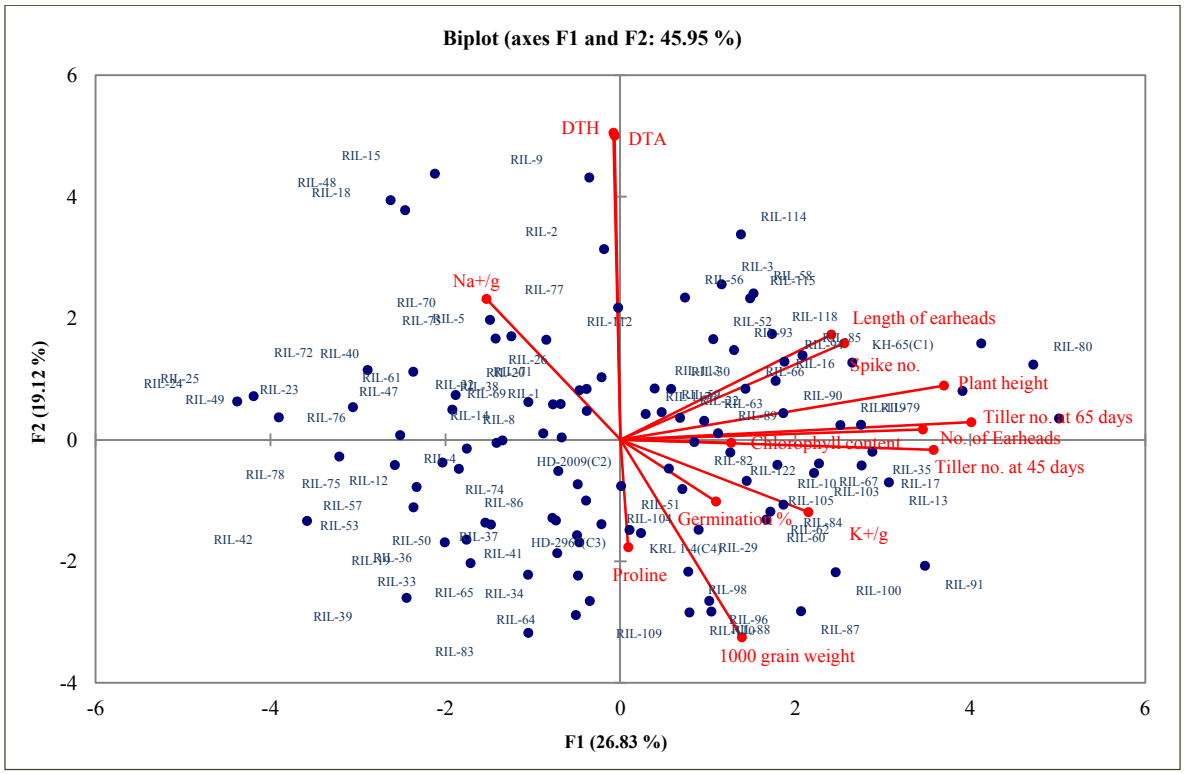

Figure 2. Biplot based on first two components of PCA showing RILs, Parents and traits related to salinity tolerance

4. Discussion

Wheat is considered to be moderately tolerant to salinity among other cereals and wheat cultivars shows considerable variations to salinity stress (Munns et al., 2006). Identification of variability to salinity stress in wheat not only requires study of yield related traits but also various other physiological and biochemical traits like Na^+/K^+ and proline concentration. So the present study evaluated morphological, physiological and biochemical traits related to salinity tolerance. The use of multiple traits for evaluation of salinity tolerance is that it increases the accuracy (Zeng et al., 2002). Zeng et al. (2002) evaluated 12 rice genotypes for salinity tolerance and ranked genotypes for salt tolerance based on means of multiple parameters and found spikelet and tiller number contributing most of the variation to seed yield. El-Hendawy et al. (2005) ranked thirteen wheat genotypes according to cluster analysis with multiple agronomic parameters at all growth stages and found the Egyptian genotypes Sakha 8 and Sakha 93 and the Indian genotype Kharchia as the most tolerant to salinity. Ashraf (2002) also identified Kharchia as highly salt tolerant during his genetic variation evaluation in spring wheat for salinity tolerance.

Correlation of various traits to thousand grain weight and among themselves was studied. Plant height and tiller number were found to be highly correlated with thousand grain weight. Studies conducted by Leon et al. (2011) on ITMA wheat population, also showed that grain yield is positively correlated with tiller number and plant height. Beside these morphological traits some physiological and biochemical parameters like K^+ and proline could be used to identify salt tolerance. Osmolytes like proline and glycine betaine accumulate in response to stress conditions (Ashraf & Foolad, 2005). Various earlier studies have shown a positive role of proline to salinity tolerance (Khan et al., 2009; Ashraf & Foolad, 2005; Munns, 2005). A significant positive correlation of proline with 1000 grain weight in present study coincides with the earlier studies, indicating proline accumulation related with salinity tolerance.

PCA is multivariate data analysis technique used to know the relationship, similarities and dissimilarities among various parameters for salinity tolerance. It develops a set of principal components (orthogonals) that explains the maximum variation contributed by the traits.

Gholizadeh et al. (2014) evaluated 41 genotypes of bread wheat. They observed five components accounting 75.5% variability. Mohamed (2013) did PCA using 10 wheat genotypes. Choukan (2010) used PCA for checking the variability in maize. However, the components derived from PCA shows a lesser variation among RILs in the present study as compared to evaluation of components in different genotypes, as RILs is a fixed population derived from cross between salt tolerant, low yielding and salt susceptible, high yielding genotypes. Factor loading based on the four components clearly indicate tiller number and plant height as major contributors to 1000 grain weight.

Grouping of RILs based on salt tolerance value derived from STI values and PCA increases the accuracy and helped easy classification of RILs into three groups. Ayyoob et al. (2013) used PCA based grouping to study inter-specific disparity in agriculture development of Kerala. Yasir et al. (2013) also used PCA based grouping to group 46 Chinese bread wheat genotypes into three groups. First group drought susceptible, second drought tolerant and third group included genotypes with low yields under drought and normal conditions.

5. Conclusion

The present study concluded that tiller number is a good parameter for evaluating salinity tolerance at early growth stages in plants and is highly correlated with yield. Proline estimation in flag leaf is an important biochemical parameter to identify salt tolerant lines, as its plays an important role in salinity tolerance. Cluster based ranking along with Principal component analysis are effective statistical tools for identifying tolerant genotypes. The identified tolerant genotypes could further be used in breeding programmes. Further molecular studies could be conducted to know the molecular mechanism for salinity tolerance in tolerant genotypes.

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