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Assessment of agronomic trait and tolerance indices on yield parameters in eight barley (*Hordeum vulgare* L.) accessions under salt stress

Rajeswari Somasundaram[1]*, A. Somasundaram[2]

[1]Department of Life Sciences, Sri Sathya Sai University for Human Excellence, Kalaburagi, Karnataka, India.
[2]Department of General Sciences, Dubai Campus, BITS Pilani, Dubai, UAE.

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ABSTRACT

Analysing the salt tolerance indexes (mean productivity [MP], geometric MP [GMP], stress tolerance index [STI], stress stability index [SSI], tolerance index [TOL], yield index [YI], and yield stability index [YSI]) on productivity on salt stress are one of the best ways to identify naturally existing variant in the crop accessions. Eight USDA barley accessions (Kindred, Morex, ELS 6302-5, NB 5, MOR 7 / 5, seco, 334 and 1532) were investigated the effect of salt stress at 10 dSm^{-1} on yield parameter compared to non-stressed crops. On grain maturity dry weight, number of leaves, number of tillers, total plant height, number of sterile, and fertile tillers, number of grains in each plant was recorded. In the leaf and leaf sheath, mineral ions such as sodium (Na^+) and potassium (K^+) were estimated using inductively coupled plasma–optical emission spectroscopy. The stem and leaf parts were explored for their calorific value using a bomb calorimeter. Yield under stress showed positive correlations with GMP and STI than TOL, SSI, and YSI based on principal component analysis and cluster analysis. Based on the analysis Kindred, ELS 6302-5, NB 5, and 334 accessions show salt stress tolerance. The salt tolerance accessions are naturally existing high yielding ones. Hence, that can be recommended for cultivation on the farm land affected by salinity.

1. INTRODUCTION

Salinity is abiotic stress that threat the yield in agriculture. Approximately 45 million hectares (20%) of irrigated land, used for agriculture, are affected by salt [1,2]. Worldwide each year 10% of agricultural land is getting affected by salt accumulation and was estimated to be 10 million hectares [3]. Natural environmental factors (mineral leaching, evaporation, etc.) and improper agricultural management practices hasten the rate of salinization. It is predicted that 50% of the world's arable land will be affected by salinity by 2050 [4,24]. In arid and semiarid areas, the problem is more intense due to high temperatures and less rainfall. It is quite challenging to grow crops in these environments. FAO reported that the demand for food, fodder, and industrial crop increases by 0.9% every year [5]. To improve productivity to meet the demand, it is necessary to use the salt-affected marginal land for cultivation. A green way to obtain crop yield in these environments is possible by growing suitable salt-tolerant crop accessions as salt stress affects the agronomy, physiology, biochemistry, and molecular biology of the plant [6].

**Corresponding Author:*
Dr. Rajeswari Somasundaram,
Department of Life Sciences, Sri Sathya Sai University for Human Excellence, Nellakathiran Halli Campus, Chickabellapur, Karnataka, India.
E-mail: rajeswari.s @ sssuhe.ac.in

Barley is the best crop to study salt tolerance due to its genetic simplicity, one among the important cultivated cereal crop (approximately 132 million tons/year) from ages. During the process of domestication, barley has been screened and gradually accumulated desirable agricultural traits [7]. Moreover, barley is a bio-saline agricultural crop as it has vast genetic resources to improve agricultural profit on saline land and/or saline irrigation water [21]. The cops salt tolerance ability makes them survive in marginal agricultural land under stressed conditions without harming much productivity [22]. In this context, it is mandatory to look for suitable crop accessions to obtain sustainable yield.

Agronomic trait serves as useful biological markers to screen desirable accessions when exposed to abiotic stresses [8-10]. Yield stability index, yield index (YI), stress tolerance index (STI), geometric mean productivity (MP), tolerance index, and stress susceptibility index are the commonly used stress tolerance indices to evaluate the accessions' based on their performance [11]. The efficiency of screening procedures to enhance the success in conventional salt-tolerant accession cultivation depends on the correct assessment of agronomic traits and the indexes. The objective of the study is to identify salt-tolerant accession(s) in eight barley accessions based on its agronomic trait at the grain maturation stage under field conditions and to find an effective salt tolerance index based on yield.

2. MATERIALS AND METHODS

2.1. Experimental Arrangement and Germplasm

Barley accessions, namely, Kindred, Morex, ELS 6302-5, NB 5, MOR 7 / 5, seco, 334, and 1532 [Table 1], were obtained from the gene pool of GRIN (USDA). All the selected eight accessions were spring barley, awned, annual life form, and six-rowed with the seeds covered with the hull.

The field testing was laid out at the field at BITS Pilani, Dubai Campus, Dubai, during the winter months (November–January) 2014–15. The field trial was performed in a complete randomized design (CRD) for two sets with triplicate. Sandy loam commercial sweet soil mixed with potting soil in the ratio of 100:1 was used for planting. The initial electrical conductivity (ECe) of the saturated soil paste water extract was 0.0708 dSm^{-1} and irrigating water was 0.0671 dSm^{-1}. The experiment was executed with two treatments, namely, control (non-stressed; irrigation water with 0.03 dSm^{-1}) and saline condition (10 dSm^{-1}). To make the irrigation water with 10 dSm^{-1} salinities, the required quantity of sodium chloride was used. All the seedlings were pre-propagated with normal irrigation water. The non-stressed plants were propagated with the same condition until grain maturation. Irrigation with 10 dSm^{-1} water was started at the three-leaf stage for saline condition and the treatment was continued until grain maturation.

Throughout the growth period, the plants were monitored for their visual agronomic traits such as chlorosis, necrosis, and tip drying on the leaf. The height of the plant was measured on the main culm from the base to the tip of the latest blade. On grain maturity dry weight, number of leaves, number of tillers, total plant height, number of sterile, and fertile tillers, number of grains in each plant was recorded. In the leaf and leaf sheath, mineral ions such as Sodium (Na^+) and potassium (K^+) were estimated using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES). Chloride (Cl^-) ion concentration was determined by Mohr's method using an aqueous extract of 1 gm of dried plant tissue. The stem and leaf parts were studied for their calorific value using a bomb calorimeter.

Salt tolerance indices [11] such as Yield stability index, YI, STI, geometric MP (GMP), tolerance index, and stress susceptibility index for yield were calculated using the following formulas:

Yield stability inder (YSI) = Ys/Yp [12];

YI = Ys/Y^p [13];

STI = (Yp X Ys)/Y^p^2 [9];

Tolerance index (TOL) = Yp – Ys [15];

GMP = √(Yp * Y^s) [9];

Stress susceptibility index (SSI) = (1- (Ys/Yp))/SI; SI=1-(Y^s/Y^p) [14]

Where Ys and Yp are the yields of accessions evaluated under salinity and control. Y^s is the mean yields of all accessions under salinity and Y^p is the mean yields of all accessions under control.

2.2. Statistical Analysis

Mean, standard error, and simple correlation of grain yield under salt stress and control conditions were imperilled to statistical analysis [26]. Software program IBM SPSS statistics v 22.0 (SPSS Inc., 2007, Chicago, IL, USA) for windows was applied to evaluate the accessions using cluster analysis, principal component analysis (PCA), and a Scree plot [16].

3. RESULTS AND DISCUSSION

A field experiment was performed to evaluate eight barley accession for salt tolerance in an arid environment based on salt tolerance indexes. The leaf width was drastically decreased, and salt stress-induced damages such as necrosis and chlorosis were also noted in all the accessions. The total number of leaves was reduced in five accessions and Morex was affected more (43%). MOR 7 / 5 and seco were unaffected by salt stress and produced the same number of leaves. Kindred produced 20% more leaves on salt treatment. Plant height showed a mixed response to salt stress. The decrease in the plant height was noted in Kindred, Morex, and seco (7–20%), whereas other accessions showed an increase (15–59%) on salt treatment [Table 2]. The number of tillers in NB 5 was 21 on salt treatment which was a 110% increase compared to non-stressed conditions.

All eight accessions produced tillers under stress and non-stressed conditions. Kindred, ELS 6302-5, NB 5, and MOR 7 / 5 produced a greater number of tillers (50%, 50%, 122%, and 33%, respectively) on salinity treatment. Tillers include both fertile (bearing spikes) and non-fertile. The fertile tillers were further divided into those which produced grain and did not produce grain. The accessions seco and 334 produced the same number of tillers on both treatments. The accessions Morex and 1532 were affected by salt treatment and produced 61% and 89% a smaller number of tillers [Table 2]. Due to genetic predisposition Morex had a greater number of grains/spike due to lengthier spike.

The number of leaves in Kindred, ELS 6302-5, and MOR 7 / 5 was increased on salt treatment [Table 2] and Kindred and MOR 7 / 5 did not produce a high grain yield, so it can be used as a fodder crop in the salt-affected area. A higher K^+/Na^+ ratio is regarded as one of the essential parameters for salt stress tolerance due to the biocompatibility nature of K + . As barley is the most salt-tolerant cereal crop, it maintains higher K^+ content in the leaf. ELS 6302- had a higher K^+/Na^+ ratio. Hence, K^+ minimizes the Na^+ induced damages in the cell [17].

Table 1: The detailed information about the accessions used for the study.

Name	USDA Accession ID	Collection site	Accession status	Lemma colour	Year of collection
Kindred	CIHO 6969	North Dakota, US	Cultivar	Tan	1941
Morex	CIHO 15773	Minnesota, US	Cultivar	White/Amber	1978
ELS 6302-5	PI 295373	Shewa, Ethiopia	Landrace	Brown	1964
NB 5	PI 295960	Israel	Cultivar	White/Amber	1964
MOR 7/5	PI 356696	Morocco	Landrace	Tan	1970
Seco	PI 508552	Arizona, US	Cultivar	Tan	1987
334	PI 531877	Egypt	Undefined	Tan	1988
1532	PI 531907	Kafr Al Sheikh, Egypt	Landrace	Tan	1988

The data showed the mean grain yield (grain weight) in the different barley accessions distinct under stress conditions from 0.50 to 9.38 gm/plant, though the mean yield of accessions under control conditions ranged from 0.97 to 12.07 gm/plant. The accessions ELS 6302-5, NB 5, seco, and 334, gave better yield under salinity conditions, but Morex performed better under control. The mean yield of seco, Kindred, NB 5, and 1532 on salt stress was increased by 20% more compared to the non-stressed condition [Table 3].

Table 4 represents the salt tolerance indices of all eight accessions. The highest TOL was obtained for accessions showed reduced grain yield under salinity stress. It indicates that these accessions are sensitive to salt stress in grain yield. Based on the agronomic traits, Morex was considered a salt-sensitive accession. The lowest TOL value was found in Kindred and NB 5. It indicates that these accessions had the lowest yield reduction under stress. Seco and 1532 had the lowest SSI and were considered as highly susceptible to stress and they were poor performers even in the control treatment. Based on GMP and STI indices, ELS 6302-5, seco, and 334 had the highest values, whereas accessions 1532, Kindred, and Morex had the lowest values.

Ward linkage – proximity matrix using squared Euclidean distance and correlation matrix for the eight barley accessions are presented in Tables 5 and 6. The accession Morex yielded the maximum under control but did not give credible yields under salt stress. Identical outcomes were stated in wheat genotypes on drought stress tolerance earlier [11]. TOL negatively correlated with Ys. Yp correlated positively with all indices except for SI [Table 5]. These outcomes shoes that low

Table 2: Agronomic trait and K^+/Na^+ratio in barley accession on salt-stressed and non-stressed condition.

Accession	Control			Salinity treatment					
	Plant height (cm)	Average No. of leaves/tiller	No. of tiller	Concentration of Na^+ (mg/g)	Concentration of K^+ (mg/g)	K^+/Na^{+r}atio	Plant height (cm)	Average No. of leaves/tiller	No. of tiller
Kindred	49.33	4	9	3.370	22.90	6.790	41.69	5	12
Morex	67.33	7	18	1.511	4.391	2.910	53.00	4	8
ELS6302-5	54.67	6	8	1.294	28.71	22.19	65.00	4	13
NB 5	45.33	4	10	4.390	55.11	12.55	54.50	3	21
MOR 7/5	34.00	5	12	2.603	29.47	11.32	54.67	5	16
Seco	47.00	5	5	7.205	50.89	7.060	42.67	5	5
334	38.67	6	11	1.455	23.79	16.35	44.33	5	11
1532	30.00	5	12	3.810	10.81	2.840	38.00	4	3

Table 3: Yield in barley accession on control and salinity treatment.

Accession	Control					Salinity treated				
	Fertile spike	Non-fertile spike	No of grains/spike	Total grain weight (g)	Calory (MJ/Kg)	Fertile spike	Non-fertile spike	No of grains/spike	Total grain weight (g)	Calory (MJ/Kg)
Kindred	2	6	19	1.78	15.71	5	7	24	5.61	16.79
Morex	11	5	27	12.07	16.88	3	2	36	4.39	16.97
ELS 6302-5	8	0	11	6.88	15.23	10	2	12	9.38	16.41
NB 5	8	1	11	3.55	14.79	13	6	15	7.86	15.00
MOR 7/5	11	1	13	6.27	16.77	10	6	12	5.26	17.01
Seco	4	0	32	6.60	16.21	4	1	36	7.42	18.19
334	10	1	19	8.05	17.41	6	4	31	7.88	18.00
1532	5	6	5	0.97	14.32	1	1	13	0.50	14.50

Table 4: The salt tolerance indices based on the mean grain yield (gram/plant) of eight barley accessions under salt stress and control treatment.

Accession	Yp	Ys	YSI	YI	TOL	GMP	SSI	STI	SI
Kindred	1.78	5.61	3.16	3	0.32	7.47	1.77	28.42	-1.47
Morex	12.07	4.39	0.36	2	2.75	15.25	0.71	5.82	-0.4
ELS 6302-5	6.88	9.38	1.36	3	0.73	24.58	1.31	12.27	-0.93
NB 5	3.55	7.86	2.22	3	0.45	14.81	0.85	55.4	-2.28
MOR 7/5	6.27	5.26	0.84	2	1.19	13.18	0.87	7.55	-0.78
Seco	6.6	7.42	1.13	1	0.89	19.07	0.25	18	-3.89
334	8.05	7.88	0.98	1	1.02	22.36	1.41	8.81	-0.61
1532	0.97	0.5	0.52	1	1.92	0.5	0.33	4.68	1.54
Std. Error	1.28	0.98	0.33	0.31	0.29	2.78	0.19	6.06	0.55

Yp: Grain yield under non-stressed conditions, Ys: Grain yield under salt-stress, YSI: Yield stability index, YI: Yield index, TOL: Tolerance index, GMP: Geometric mean productivity, SSI: Stress susceptibility index, and STI: Stress tolerance index.

Table 5: Ward's linkage – Proximity matrix using squared Euclidean distance for the eight barley accessions.

	YP	Ys	YSI	YI	TOL	GMP	SSI	STI
Yp	1.000							
Ys	0.322	1.000						
YSI	−0.536	0.356	1.000					
YI	0.051	0.623	0.590	1.000				
TOL	0.503	−0.626	−0.801	−0.519	1.000			
GMP	0.664	0.887	−0.091	0.417	−0.235	1.000		
SSI	−0.057	0.432	0.627	0.879	−0.501	0.236	1.000	
STI	−0.393	0.389	0.739	0.442	−0.631	0.000	0.165	1.000

Table 6: Correlation matrix for the indices.

Case	Accession	Squared Euclidean Distance							
		Kindred	Morex	ELS6302-5	NB 5	MOR 7/5	Seco	334	1532
1	Kindred	0.000							
2	Morex	121.249	0.000						
3	ELS6302-5	43.590	56.936	0.000					
4	NB 5	9.135	93.446	14.159	0.000				
5	MOR 7/5	26.477	37.071	17.756	16.620	0.000			
6	Seco	31.009	43.217	3.969	10.875	4.943	0.000		
7	334	49.793	31.733	3.845	22.128	10.068	2.360	0.000	
8	1532	36.237	139.115	115.705	65.851	51.409	80.990	105.603	0.000

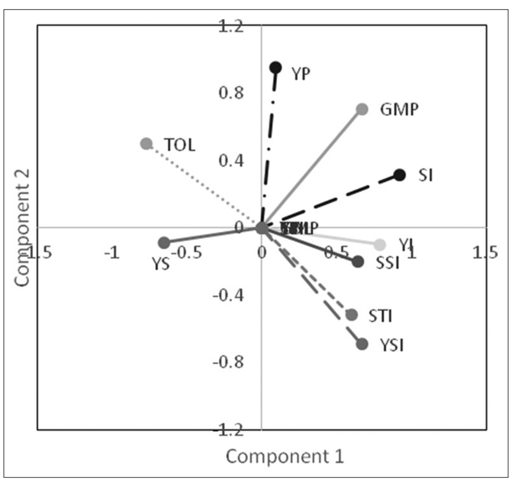

Figure 1: Principal component analysis for barley accessions according to Rotated Component Matrix – Varimax with Kaiser Normalization on the salt tolerance indices.

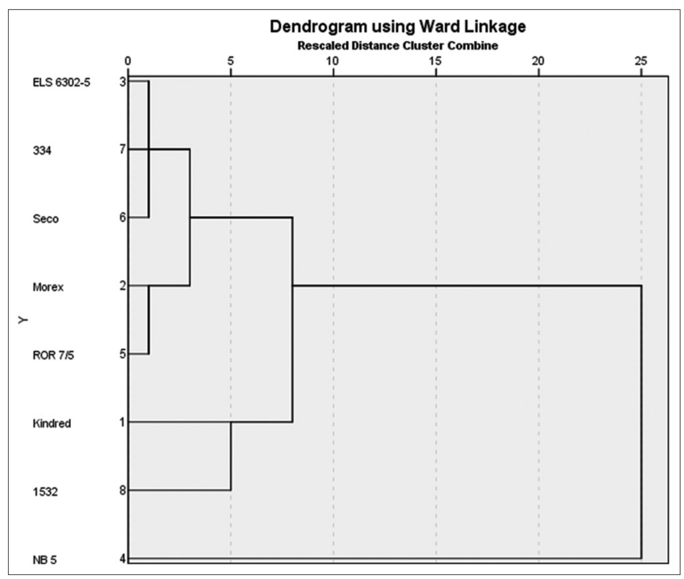

Figure 2: Dendrogram using ward linkage for barley accessions based on tolerance indexes.

TOL bring about better outcome under stress condition [8]. GMP and STI help to recognize and detect better barley accessions based on yield under control and salt stress conditions [9,10]. YI is useful to discriminate salt-tolerant, salt-sensitive, and yield stable accessions and had numerical values such as 3, 2, and 1, respectively. STI can be used as a constant parameter to identify salt-tolerant accession [18].

Based on the results, seco gave the smallest SSI. Hence, it has minimal susceptibility to salt stress which means tolerated the salt stress and produced more grain irrespective of its condition, whereas 1532 has high salt susceptibility and a low yielding accession in both circumstances. Hence, for the identification of salt-tolerant accessions, the SSI result must be carefully interpreted otherwise may mislead the researcher.

PCA on salt tolerance indices was built to detect its relation to salt-stressed and non-stressed conditions [25]. It clustered the salt stress indices for eight barley accessions into two principal components. About 4.09% and 3.62% variation in salt tolerance indices described by PC1 and PC2, respectively. The details of PC1 And PC2 are depicted in Figure 1. PC1 is negatively correlated to TOL and GMP, while PC2 is negatively correlated to YSI, TOL, STI, and GMP. GMP may have less weightage, whereas STI, YI, and SSI can be considered for the selection of tolerant accessions, but YSI has a greater impact [1].

Cluster analysis sequestrates the accessions based on homogeneity [20] (Ward's method), classified the eight accessions into two groups [Figure 2] with seven and one, respectively, based on grain yield. Proximity Matrix using Squared Euclidean Distance for the eight barley accessions are represented in Table 5. The correlation matrix for the indices is presented in Table 6. Identification of salt-tolerant high yielding accessions from the germplasm is important for the improvement and establishment of genetic diversity in crops [23]. It is also crucial for the reliable and sustainable production of crops. It is essential to quantitate the genetic diversity of accessions for successful assessment [19]. An important strategy to analyze the genetic relationship is multivariate statistical algorithms [10].

4. CONCLUSION

Based on the research outcomes, salt stress drastically lowered the yield in a few barley accessions. Although the salt-tolerant accessions indicate that they possess adequate genetic variability to give more yield. STI and GMP are the best indices to evaluate accessions based on yield under stress and control based on PCA. It can be concluded that these indices had positive and important associations with yield. ELS6302-5, 334 showed better performance on yield under salt stress. Based on the agronomic trait, analysis and K^+/Na^+ ratio ELS 6302-5 and 334 could be salt-tolerant accessions.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

Both the authors (S. Rajeswari and A. Somasundaram) have no conflicts of interest.

9. ETHICAL APPROVALS

The research was approved by ethical committee of BITS Pilani.

10. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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