



Full Length Article

Comparison of Different Tolerance Indices and PCA Biplot Analysis for Assessment of Salinity Tolerance in Lentil (*Lens culinaris*) Genotypes

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Abstract

Lentil is very important legume crop and affected by salinity stress. This study was planned to sort out the lentil germplasm for salinity tolerance. Four different salinity treatments were used (S_{0mM} , S_{50mM} , S_{100mM} , S_{150mM}) in hydroponic culture and root length (RL), shoot length (SL), root weight (RW), shoot weight (SW), total proteins contents (PC), α -amylase (A.A), total soluble sugars (TSS) sodium ions (Na^+), potassium ions (K^+) and sodium to potassium ratio (Na^+/K^+) were used as criteria for selection. Principle component analysis (PCA) based biplot, rank sum (RS) scores, integrated selection index (ISI) and integrated scoring (IS) were used as statistical analysis for sortation of lentil genotypes for salinity tolerance and to compare the results of different indices with each other and with PCA based biplot. Analysis of variance showed that genotypes, salinity treatments and their interactions for all subjected traits were significantly different from each other. PCA based biplot showed that Na^+ , Na^+/K^+ , K^+ and TSS under S_{0mM} , RW, SW, RL, SL, K^+ , Na^+/K^+ and Na^+ under S_{50mM} , SL, SW, RL, RW, Na^+ , K^+ , Na^+/K^+ under S_{100mM} , TSS, SL, RL, RW, Na^+ , K^+ , Na^+/K^+ under S_{150mM} were most discriminating parameters for evaluation of lentil genotypes under respective salinity treatment. PCA biplot showed that Masoor 2002, NL20-3-3, NL9775, NARC11-2 and ILL5888 under S_{0mM} ; Masoor 2002, NL9775, ILL5888 and ILL6024 under S_{50mM} ; Masoor 2002, NL0196, NL20-3-3, NL9775 under S_{100mM} ; Masoor 2002, Masoor 2009, NL9775, NL0196, NL0188 and NL20-3-3 under S_{150mM} were tolerant. Masoor 2002, NL 20-3-3, LN 0188, M93 and NL9775 were declared to be salt tolerant by RS, ISI and IS. Results of RS, ISI, IS and PCA Biplot were comparable and equally valid as these declared unanimously Masoor 2002, NL 20-3-3, LN 0188, M93 and NL9775 as salt tolerant at early growth stages. Genotypes NARC 11-4 and Marka 209 were relatively susceptible against salinity. These tolerant and susceptible lentil genotypes could be used as further evaluation against salinity on physiological and biochemical basis. © 2017 Friends Science Publishers

Keywords: Integrated selection index (ISI); Rank sum (RS) score; Integrated scoring (IS); Principle component analysis

Introduction

Leguminosae is very important family of flowering plants and comprised of four subfamilies named as Caesalpinoideae, Mimosoideae, Papilionoideae and Swartzioideae. Leguminous crops have utmost fame of being inexpensive source of energy, protein, minerals and vitamins. Combination of cereals and legumes make the balanced diet for human. Among legumes, lentil was component part of ancient Egyptian cropping system and as old as emmer and einkorn wheat (Harlan, 1992). Lentil along with other legumes is important food component of the South Asia, Middle East and North Africa.

Area under lentil cultivation is 4.6 m ha with 4.2 m tons production and average yield of 1095 kg ha⁻¹ yield (FAO, 2010). Canada, India, Turkey, Iran, Bangladesh, China, Syria and Nepal are the main lentil producers and it is liked due to

fast cooking ability and nutrient composition being enriched with proteins, fats, iron, cobalt, iodine, lysine and arginine (Bhatty, 1988; Kowieska and Petkov, 2003). Among different leguminous crops, lentil (*Lens culinaris* L.) is also very important crop of Pakistan where, per capita consumption is 15 kg per year. Fixation of atmospheric nitrogen in rhizosphere and improvement of soil fertility are the other important features of lentil (Crook *et al.*, 1999). It is diploid species with estimated haploid genome size of 4063 Mbp (Arumuganathan and Earle, 1991).

Lentil is mainly grown during winter on marginal lands and rainfed areas of Pakistan. Importance of lentil is continuously increasing due to increasing the demand of lentil across the world. In Pakistan, 22,500 ha are under the lentil cultivation with 11,600 tons production during 2012 which was about 12.8% less than the previous year (Government of Pakistan, 2011). Area under lentil is lower

due to its competition with staple cereals and efforts are targeted to enhance the per unit area production. Significant varietal differences were reported in lentil genotypes for number of branches, plant height, number of seeds per pod, number of pods per plant, biological yield and harvest index (Karadavut and Genc, 2010). Differential responses of various lentil varieties for numerous traits showed that existing variability is providing the opportunity of targeted selection.

Different biotic (rust, wilt and blight) and abiotic (drought, low temperature and salinity) stresses are threatening the lentil crop across the globe depending upon the climatic regimes of the concerned regions. Genetic variation among different lentil genotypes was observed for drought, low temperature, salinity, nutrient deficiency and toxicity (Fratini and Pérez De La Vega, 2011). Development of salinity tolerant germplasm is very important to expand the cultivation of lentil in the drier areas with poor soils. Salinity is among the main stresses, which badly affect the seedling growth and development of different crops (Atak *et al.*, 2006; Kaya *et al.*, 2006).

Plant seedlings are more severely affected by salinity stress because seed germination and seedling roots are present in the upper layer of soil (Almansouri *et al.*, 2001). Sodium chloride causes the severely harmful effects on the morphological, physiological and biochemical characteristics of crop plants (Arshi *et al.*, 2002; Sairam and Tyagi, 2004; Parida and Das, 2005). With the extensive use of irrigation system, salt affected area is increasing day by day. Evaluation of seedlings for salinity tolerance is effective due to following critical reasons; seedling parameters have higher heritability relative to other stages (Ashraf, 1994), salt accumulations mostly occurred in the upper soil layer due to capillary rise of water and evaporation. Mechanism of salinity tolerance is variable even with in species or between genotypes thus, there is dire need to evaluate the lentil germplasm for salinity tolerance preferably at seedling stage (Lutts *et al.*, 1995; Almansouri *et al.*, 2001). Different biometrical tools have been used for the evaluation of lentil genotypes for salinity tolerance. Use of more than one biometrical tool provides precise, validated and reliable results. Therefore, present study, biplot analysis, ranking scores, integrated selection index (ISI) and integrated scoring (IS) were used for evaluation of lentil genotypes for salinity tolerance.

Materials and Methods

Plant Growth Conditions and Salinity Treatments

Present research experiment was conducted in the research area of Plant Breeding and Genetics Department, University of Agriculture Faisalabad, Pakistan during 2014. Greenhouse experiment was conducted using triplicated completely randomized design. Total fifteen lentil genotypes were used in current study including; NL 96476, Masoor 2002, NL 20-

3-3, NL 96700, M 93, Masoor 2009, NL 0196, ILL 5888, NL 0188, NARC 11-2, Marka 209, NARC 11-4, NL 96505, ILL 6024 and NL 9775. Seeds of each cultivar were grown in sand filled trays and later on transferred to the hydroponic growth culture after seedling establishment for application of salinity treatments. Hydroponic growth culture was supplemented with full strength Hoagland's growth solution (Hoagland and Arnon, 1950) and plants were stabilized for 2 days in growth media. Hoagland's solution was renewed after every fifteen days. Four different salinity treatments used are as following:

S_{0mM} = 0 mM NaCl Solution,
 S_{50mM} = 50 mM NaCl solution,
 S_{100mM} = 100 mM NaCl solution,
 S_{150mM} = 150 mM NaCl solution.

Salinity treatments were applied two days after transplanting in hydroponic culture with an incremental dose of 25 mM on daily basis until or unless desired level is attained in separate treatments. Experiment was continued for 40 days after transplantation in hydroponic growth media and then crop was harvested. After harvesting, data were recorded for following morphological and biochemical traits; root length (RL; cm), shoot length (SL; cm), root weight (RW; g), shoot weight (SW; g), α -amylase (A.A; $mg\ g^{-1}$; Rick and Stegbauer, 1974), total soluble protein contents (PC; $mg\ g^{-1}$; Bradford, 1976), total soluble sugars (TSS; $mg\ g^{-1}$; Dubois *et al.*, 1956), K ions (K^+ ; ppm; Hald, 1947), Na ions (Na^+ ; ppm; Hald, 1947) and Na/K ratio (Na/K) were recorded.

Statistical Analysis

Lentil genotypes and different salinity treatments were two different factors therefore; two factor factorial treatment structure under completely randomized design (CRD) was used for analysis of variance (Steel and Torrie, 1997) for estimation of treatments, genotypes and their interaction effects. Principle Component Analysis (PCA) based Biplots (Gabriel, 1971) were made for each salinity treatment separately. PCA transformed the raw data into unit-less variables and also distribute variability into different factors or principle components. Among different factors, only those factors were considered for further studies which have eigenvector value greater than one. Biplot was drawn by using principle factors, which have most of variability. Biplot was two dimensional scatter diagram which depicted the scattering pattern of genotypes and traits.

Salinity Tolerance Indices

Three different tolerance indices i.e. Ranked scoring (RS; Farshadfar, 2012), Integrated Scoring Index (ISI; Farshadfar *et al.*, 2012a; Farshadfar, 2012; Khalili *et al.*, 2012) and Integrated Scoring (IS; Ahmed *et al.*, 2013) were used in current study for evaluation of relative performance of lentil genotypes under four different salinity treatments.

Ranked Scoring (RS)

Ranked scoring was estimated for each genotype on the basis of all studied traits under all treatments separately. Genotypes having the highest mean values were given highest rank scores whereas, genotypes having lowest means as lowest rank scores. Mean rank scores were estimated from the rank scores of four salinity treatments. Standard deviation of ranks (SDR; S_i^2) were estimated with following formula:

$$S_i^2 = \frac{\sum_{j=1}^m (R_{ij} - \bar{R}_i)^2}{l-1}$$

Where, R_{ij} is the rank of salinity tolerance indicator, \bar{R}_i is the mean rank across all salinity tolerance indicators for the each genotype, l is number of variables.

Rank sum for genotypes were estimated by using rank mean and standard deviation of rank with the help of following formula:

Rank Sum (RS) = Rank mean (\bar{R}) + Standard deviation of rank (SDR).

Integrated Selection Index (ISI)

This index is based on the factor analysis whose factor values are used for estimation of integrated selection index (Farshadfar *et al.*, 2012a; Farshadfar, 2012; Khalili *et al.*, 2012; Khalili *et al.*, 2013).

Formula(1): $S_{ij} = (X_{ij} - \mu_j) / \sigma_j$

Formula.....(2): $MP_{ij} = (S_{ijd} + S_{ijw}) / 2$

Formula.....(3): $ISI_i = b_1 MP_{i1} + b_2 MP_{i2} + \dots + b_j MP_{ij}$

Formula-1: standardized the values of different traits to the unit value,

Formula-2: estimate the appearance of genotype for each parameter,

Formula-3: integrates the performance of genotypes for all traits.

Where, S_{ij} is the standardized value of trait j ($j=1$ to 10) in cultivar i under normal and salinity stress, X_{ij} = measured value of cultivar i for trait j , μ_j = mean value of trait j for all genotypes, σ_j = the standard deviation of parameter j , MP_{ij} = the mean productivity of parameter j for genotype i ,

b_j = weight value of parameter j , b_j = was measured from the average contribution to factor 1, ISI = integrated selection index.

Integrated Scoring (IS)

Integrated scoring was reported by Ahmed *et al.* (2013) and used 0.125 as factor for standardization or normalization of data because they studied 8 parameters. We modified integrated scoring formula regarding our parameters, as we have 10 traits thus, we used 0.10 as factor for standardization. However, for weighting different traits

differently, normalization factor can further be modified accordingly. Modified formula for integrated scoring is as following:

Integrated score = absolute values of [(Shoot length \times 0.10) + (Root length \times 0.10) + (Shoot weight \times 0.10) + (Root weight \times 0.10) + (α -amylase contents \times 0.10) + (Total protein contents \times 0.10) + (Total soluble sugars \times 0.10) + (K ions \times 0.10) + (Na ions \times 0.10) + (Na/K \times 0.10)].

Results

Analysis of Variance

Genotypes and salinity treatments were two distinct factors to access the significant differences among genotypes (G) and treatments (T). Thus, two factor factorial analysis of variance revealed significant differences among genotypes for shoot length (SL), root length (RL), shoot weight (SW), root weight (RW), α -amylase (A.A), total protein contents (PC), total soluble sugars (TSS), potassium ions (K^+), sodium ions (Na^+) and sodium to potassium ratio (Na^+/K^+). Salinity treatments (S_{0mM} : 0.00 mM, S_{50mM} : 50 mM, S_{100mM} : 100 mM, S_{150mM} : 150 mM) were also significantly different in their effects on lentil genotypes and interaction (G \times T) were also significantly different from each other for subjected traits (Table 1).

Principle Component Analysis (PCA)

Principle component analysis (PCA) was used for data reduction and transforming the raw data into principle components/principal factors. Principal component analysis transformed the raw variable data into distinct principal factors representing the different proportions of the data variability. These factors (F_s) are equal to the number of studied variables thus, in current study PCA transformed the raw data into 10 factors (F_s) with the pattern that first factor (F_1) contributed the most variability and last factor (F_n) contributed the least variability. Factor 1, 2 and 3 have effectively transformed the raw data, extracted significant variability and considerable for further data analysis due to having more than 1 eigenvector value. Factor 4 to 10 had eigenvector value less than 1 for most of factors and therefore, not effective for further consideration in order to interpret the results. Whole variability of the data was partitioned into different factors which could be visualized by cumulative variability. Cumulative variability of three factors (F_1 , 2 and 3) was 74.56%, 75.50%, 80.25% and 72.03% under S_{0mM} , S_{50mM} , S_{100mM} and S_{150mM} , respectively (Table 2).

Different traits have different pattern of contribution for principal factors. For F_1 , all traits were negatively contributing except PC, K^+ , Na^+ , Na^+/K^+ , whereas for F_2 only A.A and K^+ were negatively contributing, whereas for F_3 RL, A.A, TSS and Na^+/K^+ were positively contributing under S_{0mM} (Table 3). PC, TSS, Na^+ and Na^+/K^+ were

Table 1: Mean squares for morphological and biochemical parameters of lentil based on two factor factorial analysis of variance

SOV	DF	SL (cm)	RL (cm)	SW (g)	RW (g)	A.A (mg g ⁻¹)	PC (mg g ⁻¹)	TSS (mg g ⁻¹)	K ⁺ (ppm)	Na ⁺ (ppm)	Na ⁺ /K ⁺
Replication (R)	2	3.41**	15.35**	0.006**	0.025**	0.0027**	0.014**	0.013**	3811.5	1054.4	0.00042
Genotypes (G)	14	44.57**	47.56**	0.0142**	0.035**	0.0141**	0.267**	0.239**	11610**	2795**	0.8532**
Treatments (T)	3	56.64**	145.62**	0.0629**	0.552**	0.0521**	0.202**	0.699**	59653**	48929**	9.6401**
G×T	42	15.25**	18.50**	0.0149**	0.013**	0.0143**	0.338**	0.279**	233.5**	184**	0.1183**
Error	118	3.17**	8.71**	0.0070**	0.009**	0.050**	0.07**	0.021**	15.2	7.1	0.00112

SL: shoot length, RL: root length, RW: root weight, SW: shoot weight, A.A: α -amylase, PC: total soluble protein contents, TSS: total soluble sugars. *: significant at 5% level of significance, **: significant at 1% level of significance

Table 2: Principle factors of principle component analysis and their eigenvalues, variability and cumulative variability for four different salinity treatments

Variable		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	S _{0mM}	4.571	1.586	1.298	1.067	0.650	0.394	0.252	0.123	0.050	0.009
	S _{50mM}	4.251	2.054	1.245	0.833	0.639	0.621	0.253	0.079	0.022	0.003
	S _{100mM}	3.543	3.101	1.382	0.861	0.477	0.309	0.244	0.070	0.009	0.004
	S _{150mM}	3.816	2.022	1.365	1.001	0.771	0.432	0.385	0.191	0.015	0.001
Variability (%)	S _{0mM}	45.71	15.87	12.98	10.67	6.496	3.944	2.519	1.229	0.496	0.091
	S _{50mM}	42.51	20.54	12.45	8.333	6.394	6.206	2.527	0.793	0.222	0.026
	S _{100mM}	35.43	31.02	13.82	8.606	4.773	3.088	2.440	0.701	0.093	0.045
	S _{150mM}	38.16	20.22	13.65	10.01	7.710	4.322	3.850	1.911	0.153	0.014
Cumulative variability %	S _{0mM}	45.71	61.57	74.56	85.23	91.72	95.66	98.18	99.41	99.91	100.0
	S _{50mM}	42.51	63.05	75.50	83.83	90.22	96.43	98.96	99.75	99.97	100.0
	S _{100mM}	35.43	66.44	80.25	88.86	93.63	96.72	99.16	99.86	99.96	100.0
	S _{150mM}	38.17	58.39	72.03	82.04	89.75	94.07	97.92	99.83	99.99	100.0

S_{0mM}: normal conditions, S_{50mM}: 50mM NaCl solution, S_{100mM}: 100mM NaCl solution, S_{150mM}: 150mM NaCl solution, F1: Principle factor1, F2: Principle factor2, F3: Principle factor3, F4: Principle factor4, F5: Principle factor5, F6: Principle factor6, F7: Principle factor7, F8: Principle factor 8, F9: Principle factor 9, F10: Principle factor10

Table 3: Contribution of morphological and biochemical traits in the principle factors under different salinity treatments (Factor Loading values)

Character	S _{0mM}			S _{50mM}			S _{100mM}			S _{150mM}		
	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
SL	-0.813	0.267	-0.114	-0.746	-0.068	-0.028	-0.282	0.797	-0.305	0.556	0.266	0.616
RL	-0.347	0.504	0.443	-0.152	0.829	0.256	-0.164	0.742	0.573	-0.459	0.622	-0.150
SW	-0.802	0.169	-0.211	-0.673	0.454	0.287	-0.455	0.748	0.052	0.328	0.389	0.735
RW	-0.778	0.359	-0.162	-0.498	0.779	0.119	-0.410	0.641	0.430	-0.039	0.735	-0.386
A.A	-0.258	-0.139	0.722	-0.365	-0.284	0.733	-0.333	-0.744	0.242	-0.602	0.083	0.368
PC	0.125	0.275	-0.667	0.504	-0.235	0.638	0.486	0.378	-0.480	-0.360	-0.470	-0.076
TSS	-0.241	0.798	0.187	0.321	0.489	-0.304	-0.184	-0.411	0.580	0.104	0.791	-0.216
K ⁺	-0.861	-0.427	-0.051	-0.932	-0.154	-0.151	-0.937	-0.218	-0.232	-0.942	-0.027	0.121
Na ⁺	0.922	0.304	-0.062	0.862	0.315	0.049	0.949	0.143	0.194	0.963	-0.112	-0.121
Na ⁺ /K ⁺	0.900	0.312	0.104	0.932	0.227	0.140	0.949	0.107	0.239	0.934	-0.087	-0.236

SL: shoot length, RL: root length, RW: root weight, SW: shoot weight, A.A: α -amylase, PC: total soluble protein contents, TSS: total soluble sugars, S_{0mM}: normal conditions, S_{50mM}: 50mM NaCl solution, S_{100mM}: 100mM NaCl solution, S_{150mM}: 150mM NaCl solution, F1: Principle factor1, F2: Principle factor2, F3: Principle factor3

positively contributing for F1; RL, SW, RW, TSS, Na⁺ and Na⁺/K⁺ were positively contributing for F2; SL, TSS and K⁺ were having negative contribution for F3 while all other traits were positively contributing under S_{50mM} (Table 3). All studied traits were negatively contributing for F1 except PC, Na⁺ and Na⁺/K⁺; only A.A, TSS and K⁺ were negatively contributing for F2; SL, PC and K⁺ were negatively contributing for F3 under S_{100mM} (Table 3). Similarly positive and negative contribution of studied traits for three main factors F1, F2 and F3 under S_{150mM} was also given in found (Table 3).

Biplot 2-D Graphical Analysis

Biplot analysis was accomplished with the help of two main principle factors (F1 and F2) for each salinity treatment (S_{0mM}, S_{50mM}, S_{100mM} and S_{150mM}). Genotypes and variables were merged in single biplot graph to further facilitate the visualization. PCA biplot for S_{0mM} explained the 61.57% of total variability and depicted that Na⁺, Na⁺/K⁺, K⁺ and TSS were most discriminating traits (Fig. 1-S_{0mM}). PCA biplot for S_{50mM} explained the 63.05% of total variability, showing that RW, SW, RL, SL, K⁺, Na⁺/K⁺ and Na⁺ were most discriminating parameters (Fig. 1- S_{50mM}). PCA biplot for

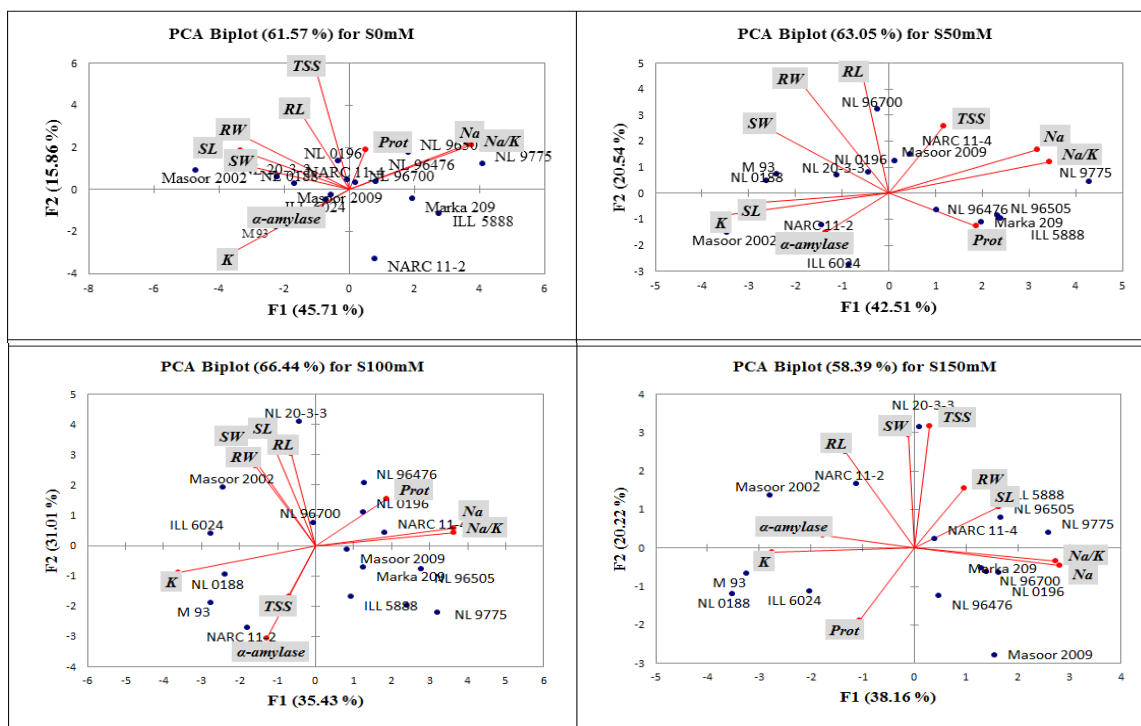


Fig. 1: Principal component 2-D biplot graphs for various traits of lentil genotypes under S_{0mM} , S_{50mM} , S_{100mM} and S_{150mM} . SL: shoot length, RL: root length, RW: root weight, SW: shoot weight, α -amylase: alpha amylase, Prot: total soluble protein contents, TSS: total soluble sugars, K: potassium ions, Na: Sodium ions, Na/K: sodium to potassium ratio

S_{100mM} showed the 66.44% of total variability, reflecting that SL, SW, RL, RW, Na^+ , K^+ , Na^+/K^+ were most discriminating variables among all studied traits (Fig. 1- S_{100mM}). PCA biplot for S_{150mM} explained 58.39% of total variability and represented that TSS, SL, RL, RW, Na^+ , K^+ , Na^+/K^+ were most discriminating traits (Fig. 1- S_{150mM}).

Genotypes NL 20-3-3, Masoor 2002, NL 9775, NARC11-2 ILL5888 were present farthest away from the biplot origin showing better performance with reference to other genotypes under S_{0mM} . NL96700, Masoor 2009 and NARC11-4 were present closer to biplot origin and reflecting that these genotypes have least variability for studied traits under S_{0mM} (Fig. 1- S_{0mM}). Masoor 2002, NL 9775 and ILL5888, ILL6024 were present farthest away from the biplot origin, showing better performance relative to other genotypes under S_{50mM} (Fig. 1- S_{50mM}).

Genotypes Masoor 2002, NL 0196, NL 20-3-3 and NL9775 were most distinct or farthest away from the biplot origin reflecting much better performance compared to the rest of genotypes. NL96700 was located in the biplot origin and reflecting the least variability under S_{100mM} (Fig. 1- S_{100mM}). Genotypes NL9775, Masoor 2002, Masoor 2009, NL 0196, NL 0188 and NL 20-3-3 were located farthest away from biplot origin showing most variability and performed much better compared to other genotypes under S_{150mM} . Genotypes NARC 11-4 were irresponsive genotype against studied variables under S_{150mM} due to presence at the origin of biplot graph (Fig. 1- S_{150mM}).

Salinity Tolerance Indices

Genotypes were ranked on the basis of single trait for each salinity treatment separately. Ranking scores of all traits for particular salinity treatment was subjected to average ranking score showing the mean performance of genotypes (Table 4 and 5). This showed that no genotype was consistent for ranking scores across all studied morphological and biochemical traits. For example; Masoor 2002 had highest score for SL under S_{0mM} , S_{50mM} and S_{100mM} but not under S_{150mM} . In case of RL, this genotype had highest score only under S_{0mM} but lowered under S_{50mM} , S_{100mM} and S_{150mM} . Masoor 2002 ranged from 11 to 14 scores for different salinity treatments, in case of SW. Scores for Masoor 2002 were high for RW (3 to 14), A.A (5 to 14), PC (1 to 13), TSS (1 to 14), K^+ (11 to 14), Na^+ (1 to 5) and Na^+/K^+ (1 to 5) under subjected four different salinity treatments (Table 4). Similarly, the results of other genotypes for studied traits on the basis of four salinity treatments were not consistent. Thus, evaluation of genotypes on the basis of individual traits was not feasible (Table 5).

Genotypes NL 9775, Masoor 2002, NL0188 and M 93 had highest values for ranking scores showing that these genotypes were relatively salt tolerant. Highest ranking scores of these genotypes shows higher mean values for the studied traits under four salinity (S_{0mM} , S_{50mM} , S_{100mM} and S_{150mM}) treatments. NL 96476, IL6024 and NARC 11-4 had lowest ranking scores which showed that these genotypes

Table 4: Ranking score of 15 lentil genotypes for morphological and biochemical traits under four different salinity treatments

Genotype	SL (cm)				RL (cm)				SW (g)				RW (g)				A.A (mg g ⁻¹)			
	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀
NL 96476	6	10	11	10	12	7	10	13	1	4	13	7	7	3	9	13	5	2	2	10
Masoor 2002	15	15	14	3	15	9	12	14	14	14	12	11	14	8	13	3	14	12	5	13
NL 20-3-3	14	14	15	9	9	10	13	15	13	12	15	14	12	9	14	9	1	13	1	5
NL 96700	3	3	4	12	14	15	14	4	6	13	9	4	9	15	15	10	6	10	14	6
M 93	10	13	12	5	8	12	3	11	12	6	5	10	10	13	6	4	12	4	12	7
Masoor 2009	12	9	10	4	10	11	9	2	10	10	6	3	6	12	8	1	15	8	6	1
NL 0196	11	12	13	15	5	8	5	3	15	11	11	6	13	14	10	6	2	9	7	4
ILL 5888	9	5	3	14	4	1	4	8	3	1	10	9	2	1	1	15	3	3	13	3
NL 0188	13	11	7	1	2	6	6	9	11	15	8	2	11	11	12	2	11	7	11	14
NARC 11-2	2	8	2	11	1	5	1	10	4	9	7	12	1	6	3	14	10	11	15	8
Marka 209	4	6	9	8	3	2	7	5	7	8	4	1	3	2	7	11	7	14	10	11
NARC 11-4	5	7	6	2	7	14	11	7	9	7	3	15	8	10	5	7	9	5	3	12
NL 96505	7	4	8	6	13	3	2	12	5	5	2	8	5	5	2	8	8	1	4	9
ILL 6024	8	2	5	7	11	4	15	6	8	2	14	5	15	7	11	12	4	15	8	15
NL 9775	1	1	1	13	6	13	8	1	2	3	1	13	4	4	4	5	13	6	9	2
Genotype	PC (mg g ⁻¹)				TSS (mg g ⁻¹)				K ⁺ (ppm)				Na ⁺ (ppm)				Na ⁺ /K ⁺ (ppm)			
	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀	S ₀	S ₅₀	S ₁₀₀	S ₁₅₀
NL 96476	10	6	15	15	9	6	5	2	7	7	5	6	9	7	7	7	8	7	7	10
Masoor 2002	2	13	4	1	14	1	6	9	14	13	12	11	1	1	5	2	1	2	5	4
NL 20-3-3	7	4	14	9	7	14	3	15	9	10	10	10	3	6	6	6	5	6	6	6
NL 96700	11	7	2	5	5	12	7	4	8	6	9	7	7	9	10	10	10	8	11	7
M 93	6	8	5	14	4	11	8	6	15	15	15	15	2	2	1	1	2	1	1	1
Masoor 2009	1	10	10	12	8	5	13	3	10	9	6	3	8	14	8	13	9	10	8	13
NL 0196	12	1	7	8	10	3	1	8	5	5	7	8	11	10	13	11	11	11	12	9
ILL 5888	13	3	9	6	1	15	9	12	3	3	8	9	13	11	9	8	14	12	9	8
NL 0188	15	2	12	13	15	8	12	10	13	14	14	14	4	3	2	3	3	3	2	2
NARC 11-2	8	9	3	4	2	7	10	11	12	12	13	13	5	4	3	4	4	4	3	3
Marka 209	5	14	6	3	6	10	11	7	4	2	4	4	12	12	11	12	13	13	13	11
NARC 11-4	9	5	11	11	12	9	2	5	6	8	3	5	10	8	12	9	7	9	10	12
NL 96505	14	11	13	2	11	4	4	14	2	4	2	2	14	13	15	14	12	14	14	14
ILL 6024	4	12	1	10	3	2	14	1	11	11	11	12	6	5	4	5	6	5	4	5
NL 9775	3	15	8	7	13	13	15	13	1	1	1	1	15	15	14	14	15	15	15	15

SL: shoot length, RL: root length, RW: root weight, SW: shoot weight, A.A: α-amylase, PC: total soluble protein contents, TS: total soluble sugars, S_{0mM}: normal conditions, S_{50mM}: 50mM NaCl solution, S_{100mM}: 100mM NaCl solution, S_{150mM}: 150mM NaCl solution

Table 5: Ranking scores, Integrated Selection Index and Integrated Scores for lentil genotypes

Genotype	Rank Scoring (RS)						Integrated Selection Index (ISI)					Integrated Scoring (IS)				
	S _{0mM}	S _{50mM}	S _{100mM}	S _{150mM}	\bar{R}	SDR	RS	S _{50mM}	S _{100mM}	S _{150mM}	\bar{ISI}	S _{0mM}	S _{50mM}	S _{100mM}	S _{150mM}	\bar{IS}
NL 96476	7.40	5.90	8.40	9.30	7.75	0.189	7.94	8.76	7.77	9.99	8.84	27.03	25.83	24.89	25.43	25.79
Masoor 2002	10.40	8.80	8.80	7.10	8.78	3.629	12.38	16.6	15.8	16.33	16.24	31.73	28.20	27.52	26.74	28.55
NL 20-3-3	8.00	9.80	9.70	9.80	9.33	0.004	9.33	14	15	15	14.67	27.78	26.53	25.28	25.53	26.28
NL 96700	7.90	9.80	9.50	6.90	8.53	0.042	8.57	11	11	12	11.33	27.27	26.24	26.11	25.61	26.31
M 93	8.10	8.50	6.80	7.40	7.70	2.920	10.62	16	14.54	14.33	14.96	30.87	29.69	27.29	29.43	29.32
Masoor 2009	8.90	9.80	8.40	5.50	8.15	0.106	8.26	11	10	11.4	10.80	28.22	27.60	25.03	24.90	26.44
NL 0196	9.50	8.40	8.60	7.80	8.58	0.096	8.67	13	12	13	12.67	26.45	25.37	25.81	25.81	25.86
ILL 5888	6.50	5.50	7.50	9.20	7.18	1.932	9.11	11.3	12.7	11.7	11.90	25.33	23.43	24.77	25.96	24.87
NL 0188	9.80	8.00	8.60	7.00	8.35	3.858	12.21	12	13.4	13.2	12.87	30.53	29.41	27.16	28.89	29.00
NARC 11-2	4.90	7.50	6.00	9.00	6.85	0.274	7.12	11.2	10.5	12.3	11.33	28.86	28.23	25.46	28.29	27.71
Marka 209	6.40	8.30	8.20	7.30	7.55	1.875	9.42	9.87	9.8	8.88	9.52	25.04	23.40	25.47	25.40	24.83
NARC 11-4	8.20	8.20	6.60	8.50	7.88	0.073	7.95	8.66	7.65	7.89	8.07	26.78	26.28	25.47	24.77	25.83
NL 96505	9.10	6.40	6.60	8.90	7.75	0.616	8.37	7.5	8.4	7.9	7.93	25.79	24.35	24.55	25.30	24.99
ILL 6024	7.60	6.50	8.70	7.80	7.65	0.636	8.29	8.7	8.9	9.6	9.07	29.33	26.93	25.85	27.00	27.28
NL 9775	7.30	8.60	7.60	8.40	7.98	5.236	12.98	15.2	15.7	14.8	15.23	29.32	27.86	28.73	27.67	28.29

\bar{R} : Mean rank of four treatment ranks, SDR: standard deviation of ranks, RS: rank sum, \bar{ISI} : mean integrated selection index for three salinity treatments, \bar{IS} : mean integrated scoring for four treatments

were relatively susceptible to the salinity stress. These genotypes had lowest mean values for subjected traits under four salinity treatments and got lowest ranking scores showing susceptibility of these to salinity stress (Table 5).

Integrated selection index (ISI) was used for sortation of genotypes as tolerant and susceptible against salinity stress. Masoor 2002, M93, NL9775, NL 20-3-3 and NL 0188 were found to be tolerant due to highest values for ISI scores.

NL 96476, NARC 11-4, NL 96505 and ILL6024 were susceptible to salt stress due to least ISI scores (Table 5). Integrated scoring (IS) was also used for categorization of lentil genotypes as tolerant or susceptible. M 93, NL 9775, NL 0188 and Masoor 2002 scored the highest values for IS compared to other genotypes and declared as tolerant to wide range of salinity levels. ILL 5888, Marka 209 and NL 96505 scored the lowest IS and classified as susceptible to salinity stress among all studied lentil genotypes (Table 5).

Discussion

With the extensive increase in global population demand for cost effective protein sources has dramatically increased. Lentil being the pulse crop is rich source of proteins of plant origin. As with the extensive use of irrigation water in agriculture and global climatic changes, salt affected area is increasing with the passage of time. The evaluation of lentil germplasm against salinity stress was very effective tool to cope the salinity stress and to equilibrate the demand supply curve of protein from lentil crop by exploiting the saline area. Plant growth and development in terms of morphological and biochemical parameters is adversely affected in response to stressful environments at different stages of growth and development (Anjum *et al.*, 2011; Aslam *et al.*, 2013, 2014; Naveed *et al.*, 2014). Thus, choice of morphological and biochemical traits in present study can be effective and efficient for evaluation of lentil genotypes. Diverse salinity treatments (S_{0mM} , S_{50mM} , S_{100mM} and S_{150mM}) were used for evaluation of lentil genotypes for selection of tolerant genotypes for broader range of saline conditions. Significant differences were found for morphological and biochemical traits among genotypes, salinity treatments and their interactions of present study have been also reported by El-Hendawy *et al.* (2005) and Kausar *et al.* (2012).

Multivariate analysis has numerous advantages such as accuracy for genotypic ranking is increased as multiple traits are subjected to analysis simultaneously. Ranking of genotypes is also made simultaneously under several salinity treatments for assessment of salinity tolerance which gives generalized tolerance over broader salinity levels (Zeng *et al.*, 2002). In current study, use of more than one statistical tools also validated the results for salinity tolerance of lentil genotypes.

Principle component analysis has been extensively used in research to partition the observed variability of data into principle factors by data transformation. This analysis is very effective for selection of genotypes under salinity stress. Biplots and genotypic selections were made separately for four different salinity treatments. Yan and Tinker (2005; 2006) and (Maqbool *et al.*, 2015a, b; 2016) also evaluated the manipulation of biplots for evaluation of crop plants across diverse environmental conditions. Most of the variability was demonstrated by three factors (F1, F2 and F3). Among these three factors, only F1 and F2 depicted the highest variability thus, biplots were made by using F1 and

F2 factors. It was also previously reported by (Maqbool *et al.*, 2015a, b; 2016) that first and second principal factors were representing the most of data variability. PCA biplots for S_{0mM} , S_{50mM} , S_{100mM} and S_{150mM} unanimously declared that NL20-3-3, Masoor2002 and NL9775 were tolerant against wide range of salinity stress. Genotype Masoor 2009 showed susceptibility under lower stress levels whereas; it was tolerant against extreme salt level. It showed that Masoor 2009 modulated the tolerance mechanism against extreme salt stress rather than lower level of stress.

Use of integrated selection index (ISI), integrated scoring (IS) and ranked sum (RS) scoring were proved effective to evaluate the lentil genotypes under four different salinity treatments due to inconsistent and differential responses of genotypic traits. Thus, it is validated that morphological and biochemical parameters are effective criteria for evaluation of genotypes at early growth stages with exploitation of integrated indices (Khalili *et al.*, 2013). In the integrated indices for salinity tolerance, lentil genotypes were subjected to comparative evaluation under normal and stressful conditions, which is very effective tool for evaluation. Whereas, some researchers advocated that selection of stress tolerant genotypes should be made under normal environment only (Rajaram and Van Ginkle, 2001; Betran *et al.*, 2003) or under stressful environment only (Rathjen, 1994; Ceccarelli and Grando, 2000). Evaluation of genotypes under normal and stress condition then subjecting the data for evaluation of comparative performance is essence of this experiment, which made the selection effective.

Rank sum (RS) scores were based on the average rank of the genotypes for all traits and treatments. Every trait is given importance for evaluation of genotypes and this is effective than using just crop yield which ignored all other traits. NL9775, Masoor 2002, NL0188 and M93 were tolerant to salt stress due to highest mean ranks on basis of all studied trait and all of four salinity treatments. NL 96505, Marka 209 and IL5888 were found to be susceptible to salt stress as these genotypes have lowest mean ranks. These genotypes might have higher mean value for particular trait under specific salinity treatment but these scored lowest ranks on the basis of all traits and treatments. Ranking scores were also manipulated by several researchers for evaluation of different crops (Khalili *et al.*, 2012; Farshadfar and Elyasi, 2012; Farshadfar *et al.*, 2012a).

Masoor 2002, M93, NL9775, NL 20-3-3 and NL0188 were found to be salt tolerant as these genotypes had highest ISI value whereas, NARC 11-4, ILL5888 and Marka 209 were susceptible with lowest ISI values. ISI comprised of multiple formulae which subjected the data to standardization, performance of genotypes for each trait and followed by integration of performance. ISI is effectively used by numerous researchers for assessment of tolerance against different stresses (Farshadfar *et al.*, 2012a; Farshadfar, 2012; Khalil *et al.*, 2013).

Integrating scoring (IS) was used by Ahmed *et al.*

(2013) for evaluation of wild and cultivated barley genotypes against combined effects of drought and salinity tolerance. IS gives the equal importance to every trait and different factors are used for multiplication depending upon the number of traits. M93, NL9775, NL0188 and Masoor 2002 were relatively salt tolerant lentil genotypes as these scored the highest IS values whereas, N96505, IL 5888 and Marka 209 were relatively susceptible against salinity stress due to lower IS scores.

Different abiotic stress tolerance indices like, superiority index (Pi), stress tolerance index (STI), geometric mean productivity (GMP), stress susceptibility index (SSI), mean productivity (MP), stress tolerance (TOL) and harmonic mean (HM) were manipulated and compared by different researchers for different crop plants (Akçura *et al.*, 2011; Farshadfar *et al.*, 2012b; Esmailpour *et al.*, 2015; Maqbool *et al.*, 2015a; 2016). However, comparison of PCA biplot, rank sum (RS) score, integrated scoring index (ISI) and integrating scoring (IS) for evaluation of salinity tolerance was distinct and novel attempt in present study. Results of these statistical tools were also comparable for wide range of salinity stress in lentil genotypes. PCA biplot and studied indices unanimously and comparably selected the Masoor 2002, NL 20-3-3, LN 0188, M93 and NL9775 as salt tolerant genotypes.

Conclusion

Morphological (RL, SL, RW, SW), biochemical (A.A, PC, TS) and mineral traits (Na^+ , K^+ and Na^+/K^+) of lentil genotypes were significantly affected by salinity stress. Effects of four salinity treatments ($S_{0\text{mM}}$, $S_{50\text{mM}}$, $S_{100\text{mM}}$, $S_{150\text{mM}}$ NaCl solution) were also significantly different on the performance of lentil genotypes. Change of genotypic ranking for different traits showed that use of univariate statistical tools was ineffective for evaluation of lentil performance across different salinity treatments. Use of several tolerance indices and multivariate analysis for evaluation of lentil genotypes were proved effective for concise selection of salt tolerant genotypes. Masoor 2002, NL 20-3-3, LN 0188, M93 and NL9775 were unanimously declared salt tolerant by PCA based biplot, ISI, IS and RS. Salt tolerance of these lentil genotypes at seedling stage will facilitate the establishment of plants at early growth stage and can tolerate the uppermost salt effected soil layers. Genotypes NARC 11-4 and Marka 209 were relatively susceptible against salinity. These tolerant and susceptible lentil genotypes could be used as contrasting parent in hybridization breeding program for genetic improvement of lentil against salinity tolerance.

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(Received 06 April 2016; Accepted 17 January 2017)