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Salinity tolerance of lentil genotypes based on stress tolerance indices

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Abstract
In order to identify genotypes for salt tolerance and to investigate the relationship among several salt tolerance/resistance indices a randomized block design with three replications was conducted under normal (0.0 mM NaCl) and saline (40mM NaCl) conditions during seedling stage in petridishes using ten lentil genotypes in laboratory condition where temperature was 24±2 ^{0}C. The experiment was terminated on 8th day and observations on dry weight of seedlings were recorded after drying in hot air oven at 65 ^{0}C for 48 hours. Various stress tolerance indices *viz.,* TOL, SSI, STI, MP, GMP, YI, SSPI and MSTI were calculated to screen the genotypes for salt stress tolerance.The results of pooled ANOVA showed that the genotype (G) environment (E), and genotype × environment (GxE) interaction effects were highly significant for seedling dry weight. The E effect was the most important source of dry weight variation and accounted for 51.65% of total sum of squares (TSS). The mean dry matter yield/dry weight of genotypes decreased under stress condition. On the basis of dry matter yield and stress indices the genotypes SAPNA, RLG-258 and RLG-234 were identified as the most salt tolerant genotypes. The indices STI, MP and GMP exhibited good correlation with dry matter yield under both the conditions therefore selection should be based on these indices in salinity tolerance programmes.

Keywords: Lentil, Salinity, Indices, Seedling, Stress

Introduction
Soil salinity is a condition in which the soluble salt content of the soil reaches a level harmful to crops through the reduced osmotic potential of the soil solution and the toxicity of specific ions. Lentil (*Lens culinaris* M.) is one of the most important grain legume nitrogen fixing crop and it is mainly cultivated in semi-arid regions of the world particularly in the Indian sub-continent and the dry areas of Middle East (Malik) [1]. Salinity is one of the most serious factors that hamper the productivity of agricultural crops (Munns and Tester) [2]. Salinity reduces the ability of plants to take up water, leading to metabolic effect that reduces plant growth. The deleterious consequences of high salt concentrations in the external solution of plant cells are hyper-osmotic shock and ionic imbalance (Begum *et al.*) [3]. Although, salt stress affects all growth stages of a plant but seedling growth stages are known to be more sensitive for most of the plant species. In the absence of an understanding of the special mechanisms of tolerance the quantification of salinity tolerance should be based on the seedling dry weight/dry matter yield in both stress and non-stress conditions that can lead to selection of tolerant genotypes under stress condition (Kokten *et al.*) [4]. The indices are either based on stress resistance or susceptibility of genotypes (Fernandez) [5]. Some researchers believe in selection under non-stress conditions, others in stress condition while others yet have chosen a mid-point and believe in selection under both non-stress and stress conditions (Nouri *et al.*) [6]. Various researchers have used different methods to evaluate genetic differences in salinity tolerance. Rosielle and Hamblin [7] defined stress tolerance (TOL) as the differences in yield between the stress (Y_s) and non-stress (Y_p) environments and mean productivity (MP) as the average yield of genotypes under stress and non-stress conditions. Fischer and Maurer [8] suggested the stress susceptibility index (SSI) for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments. Among the stress tolerance indicators, a larger value of TOL and SSI represent relatively more sensitivity to stress, thus a smaller value of TOL and SSI are favored. Selection based on these two criteria favors genotypes with low yield potential under non-stress conditions and high yield under stress conditions. Guttieri *et al.* [9] using SSI criterion suggested that SSI > 1 indicating above-average susceptibility and SSI < 1 indicated below-average susceptibility to stress. Fernandez [5] defined a new advanced index (STI= stress tolerance index), which can be used to identify genotypes that produce high yield under both stress and non-stress conditions.

The geometric mean productivity (GMP) is often used by breeders interested in relative performance since salinity stress can vary in severity in field environment over years (Ramirez and Kelly) [10]. On the other hand, selection based on STI and GMP will be resulted in genotypes with higher stress tolerance and yield potential will be selected (Fernandez) [5]. Gavuzzi *et al.* [11] suggested yield index (YI) in order to evaluate the stability of genotypes in the both stress and non-stress conditions. Moosavi *et al.* [12] introduced stress susceptibility percentage index (SSPI) for screening drought tolerant genotypes in stress and non-stress conditions. To improve the efficiency of STI a modified stress tolerance index (MSTI) was suggested by Farshadfar and Sutka [13] which corrects the STI as a weight. Therefore, the objectives of the present investigation were (i) to identify genotypes for salt tolerance in lentil and (ii) screening dry matter yield based indices of salinity tolerance.

Materials and methods

The laboratory experiment was carried out at Department of Plant Breeding and Genetics, Sri Karan Narendra Agriculture University, Jobner-303329, Rajasthan in December, 2016 at 24±2 ^{0}C. The seeds of ten genotypes of lentil namely, RLG–5, RLG–195, RLG–234, RLG–250, RLG–254, RLG–256, RLG–258, SAPNA, DPL–58 and L–4076 were used for evaluation. Prior to germination, the seeds were surface sterilized with 0.1% mercuric chloride for 1 minute and washed 3 times under running tap water followed by washing with double distilled water. Two salinity levels viz., 0 and 40 mM NaCl were prepared by dissolving 0 and 584.4 mg of NaCl salt in 250 ml of double distilled water, respectively and used in experiment and designated as S_0 and S_1, respectively. The experiment was laid down in randomized block design with three replications. The 15 seeds of each genotype was germinated in sterilized (165 ^{0}C for 4 hours in hot air oven) petridishes of 9 cm diameter layered with autoclaved (15 psi and 121 ^{0}C for 20 minutes) germination papers and then moistened with 3 ml of test solutions daily after removing previous day solution. The set was maintained in dark for first two days. The experiment was terminated on 8th day and dry matter yield per seedling (mg) was determined under both salinity stress (40 mM NaCl) and non-stress (0 mM NaCl) conditions after drying in hot air oven for 48 hours at 65 ^{0}C and denoted as Y_s and Y_p, respectively.

Calculation of tolerance indices

Eight salinity tolerance indices were calculated using the following formulae:

1. Stress tolerance = TOL = $Y_p - Y_s$ (Rosielle and Hamblin) [7]. The genotypes with low values of this index are more stable in two different conditions.
2. Stress susceptibility index = SSI = $[1 - (Y_s / Y_p)] / [1 - (\bar{Y}_s / \bar{Y}_p)]$ (Fischer and Maurer) [8]. The genotypes with SSI< 1 are more resistant to salinity stress.
3. Stress tolerance index = STI = $(Y_p)(Y_s) / (\bar{Y}_p)^2$ (Fernandez) [5]. The genotypes with high STI values will be tolerant to salinity stress.
4. Mean productivity = MP = $(Y_s + Y_p) / 2$ (Rosielle and Hamblin) [7]. The genotypes with high value of this index will be more desirable.
5. Geometric mean productivity = GM = $\sqrt{Y_p \times Y_s}$ (Fernandez) [5]. The genotypes with high value of this index will be more desirable.
6. Yield index = YI = $(Y_s) / (\bar{Y}_s)$ (Gavuzzi *et al.*) [11]. The genotypes with high value of this index will be suitable for stress condition.
7. Stress susceptibility percentage index = SSPI = $[Y_p – Y_s/2 (\bar{Y}_p)] \times 100$ (Moosavi *et al.*) [12]. The genotypes with low values of this index are more stable in two different conditions.
8. Modified stress tolerance index = MSTI = KiSTI, $K_1 = (Y_p)^2 / (\bar{Y}_p)^2$ and $K_2 = (Y_s)^2 / (\bar{Y}_s)^2$ (Farshadfar and Sutka) [13]. The genotypes with high value of this index will be more desirable.

Where, Y_s and Y_p represent dry matter yield for each genotype in stress and non-stress conditions, respectively and $\bar{Y}_s$ and $\bar{Y}_p$ are mean dry matter yield in stress and non-stress conditions respectively for all genotypes.

Statistical analysis

The mean data of seedling dry weigh were subjected to analysis of variance following Panse and Sukhatme [14]. Besides, the correlation coefficient between Y_p, Y_s and other quantitative indices of salinity tolerance were estimated using online statistical software (OPSTAT). Ranks were assigned to each genotype for each index. Based on indices formula, the genotype with the highest value for Y_s, Y_p, MP, GMP, STI, MSTIk1, MSTIk2 and YI and the lowest value for SSI, TOL and SSPI received a rank 1.

Results and discussion

Analysis of variance

The Pooled ANOVA for dry matter yield over two environments is given in Table 1. The main effects due to the genotype (G), environment (E), and GxE interaction were found to be significant. The E effect was the most important source of dry matter yield variation; accounted for 51.65% of total sum of squares (TSS) followed by G and GxE interaction effects which accounted for 22.07% and 8.94% of TSS, respectively. This indicated differential response of genotypes to salinity for dry matter yield.

Table 1: The Pooled ANOVA for dry mater yield of lentil genotypes tested across non-stress (S_0) and stress (S_1) conditions

Source of variation	df	SS	MSS	% TSS
Genotype (G)	9	24.55	2.728**	22.07
Environment (E)	1	57.46	57.46**	51.65
Replication in environment	4	1.53	0.383	1.38
G X E	9	9.95	1.106*	8.94
Error	36	17.76	0.493	15.96

* and ** represent significant at 5% and 1% level of significance, respectively

Dry matter yield and stress indices

Mean dry matter yield in non-stress condition (Y_p) was 9.05 mg and ranged from 7.82 mg (RLG-254) to 10.93 mg (DPL-58).While, mean dry matter yield in stress condition (Y_s) was 7.09 mg and ranged from 6.31 mg (RLG-250) to 8.22 mg (SAPNA). Thus the data indicated that mean dry matter yield decreased under stress. The genotypes DPL-58, RLG-234 and SAPNA showed higher dry matter yield and genotypes RLG-254, L-4076 and RLG - 250 showed lower dry matter yield in S_0. Whereas, genotypes SAPNA, RLG-258 and RLG-234 obtained higher dry matter yield and genotypes RLG-250, RLG-5 and RLG-254 showed lower dry matter yield in S_1 (Table 2). To evaluate salinity tolerant genotypes using TOL index, higher value of TOL demonstrates more changes of genotype yield in stress and non-stress conditions and shows the susceptibility to non-stress condition. Fernandez [5] and Rosielli and Hamblin [7] stated that selection based on TOL index leads to selection of genotypes which their yields in non-stress condition are low and have lower MP. The results of this experiment showed that genotypes L-4076, RLG-254 and RLG-258 were the most tolerant and DPL-58, RLG-5 and RLG-234 were the most sensitive genotypes based on TOL index to the salinity. For SSI, the higher value refer to more susceptible to salinity, therefore, the genotypes DPL-58, RLG-5 and RLG-250 were the least relative tolerant genotypes and RLG-258, L-4076 and RLG-254 were more tolerant genotypes. The SSPI resulted the same genotype ranking as TOL. Mean productivity (MP), geometric mean productivity (GMP) and stress tolerance index (STI) showed similar ranking of genotypes relative to salt tolerance (Table 2). Based on STI, the greater the difference between the dry matter yields found in normal and stress conditions, the smaller the amount of stress tolerance index and vice versa. Thus, genotypes SAPNA, RLG-234 and DPL-58 were found salt tolerant with high STI and dry matter yield under non-stress and stress conditions, while genotypes RLG-254, RLG-250 and RLG-5 exhibited the lowest amount of STI and dry matter yield under non-stress and stress conditions. The GMP resulted the same genotype ranking as STI. For MP, the higher value refers to more tolerant to salinity; therefore, the genotypes SAPNA, DPL-58 and RLG-234 were more tolerant whereas, the genotypes RLG-254, RLG-250 and RLG-5 were least tolerant to salinity. YI can be used as a selection criterion, although it only ranks cultivars on the basis of Y_s. Based on YI, genotypes SAPNA, RLG-258 and RLG-234 had the highest YI and Ys, hence more tolerant whereas, RLG-250, RLG-5 and RLG-254 had the lower YI and Y_s. According to K_1STI, the genotypes DPL-58, RLG-234 and SAPNA and according to K_2STI, the genotypes SAPNA, RLG-258 and RLG-234 were the most relative tolerant. It was concluded that MP, GMP and STI values are convenient parameters to select high yielding genotypes in both stress and non-stress conditions whereas relative decrease in yield, TOL, SSI and SSPI values are better indices to determine tolerance levels.

Ranking method

The estimated values of salt tolerance indices (Table 2) indicated that the identification of salinity tolerant genotypes based on a single criterion was contradictory. Different indices introduced different genotypes as salinity tolerant. To determine the most desirable salt tolerant genotype according to the all indices, rank total of all salt tolerance indices were calculated and based on this criterion the most desirable salt tolerant genotype were identified. In consideration to all indices, genotypes SAPNA, RLG-258, RLG-234, RLG-195 and RLG-256 were identified as the most salt tolerant genotypes, while genotypes RLG-250, RLG-5, RLG-254, L-4076 and DPL-58 as the most sensitive. Such strategies of using different tolerance indices and ranking pattern for screening of tolerant genotypes were used by several other workers such as Farshadfar *et al.* [15], Farshadfar *et al.* [16] and Mohammed and Kadhem [17] in wheat.

Correlation coefficient

To determine the most desirable salt tolerance index, the correlation coefficient between Y_p, Y_s and other indices of salt tolerance were calculated (Table 3). The best indices are those which have high correlation with dry matter yield in both non-stress and stress conditions and would be able to identify potential upper yielding and salt tolerant genotypes (Talebi *et al.*) [18]. Dry matter yield under stress condition (Y_s) had a weak positive association (r = 0.46) with dry matter yield under non-stress condition (Y_p), indicating that high potential yield under optimal conditions does not necessarily result in improved yield in a salinity stress environment (and the opposite is true) because the genes controlling yield and stress tolerance are different (Rosielle and Hamblin) [7]. Similar findings were also reported by Fernandez [5], Mohammadi *et al.* [19], Farshadfar *et al.* [20] and Sahar *et al.* [21]. The dry matter yield under non-stress condition (Y_p) had significant positive association with YI (1.000), K_2STI (0.999), GMP (0.838), STI (0.834) and MP (0.787), whereas non-significant and positive association with K_1STI (0.431). The dry matter yield under stress condition (Y_s) had significant positive association with K_1STI (0.998), MP (0.910), STI (0.873), GMP (0.869), TOL (0.761) and SSPI (0.761), while YI (0.466) and K_2STI (0.449) exhibited non-significant and positive association. In addition, TOL (-0.227), SSPI (-0.227) and SSI (-0.408) had a negative association with dry matter yield under non-stress condition (Y_p). The indices STI, MP and GMP exhibited good correlation with dry matter yield under both the conditions therefore selection based on MP, GMP and STI will help in the selection of genotypes with higher salinity tolerance and yield potential while TOL, SSPI, and K_1STI exhibited good correlation with dry matter yield under salinity stress condition. Similar findings were also reported by Siahsar *et al.* [22] in lentil, Zare [23] and Saeidi *et al.* [24] in barley, Singh *et al.* [25] and Mohammed and Kadhem [17] in wheat for seed yield. Thus, these indices may be used as selection criteria for salinity stress tolerance breeding programmes.

Table 2: Dry matter yield and various indices and their ranks for each genotype under non-stress (S_0) and stress (S_1) conditions

Genotypes	Dry matter yield per seedling				Stress Indices																		Rank Total	Overall Rank
	Y_p	Rank	Y_s	Rank	TOL	Rank	SSI	Rank	STI	Rank	MP	Rank	GMP	Rank	YI	Rank	SSPI	Rank	MSTI (K_1)	Rank	MSTI (K_2)	Rank		
RLG - 195	9.22	4	7.13	4	2.09	7	1.05	7	0.80	5	8.18	5	8.11	5	1.01	4	11.55	7	1.04	4	1.01	4	56	4
RLG - 254	7.82	10	6.57	8	1.25	2	0.74	3	0.63	10	7.20	10	7.17	10	0.93	8	6.91	2	0.75	10	0.86	8	81	8
RLG - 5	8.73	6	6.38	9	2.35	9	1.24	9	0.68	8	7.56	8	7.46	8	0.90	9	12.99	9	0.93	6	0.81	9	90	9
SAPNA	9.79	3	8.22	1	1.57	5	0.74	4	0.98	1	9.01	1	8.97	1	1.16	1	8.68	5	1.17	3	1.34	1	26	1
RLG - 256	8.47	7	7.01	5	1.46	4	0.80	5	0.73	6	7.74	6	7.71	6	0.99	5	8.07	4	0.88	7	0.98	5	60	5
RLG - 250	8.35	8	6.31	10	2.04	6	1.13	8	0.64	9	7.33	9	7.26	9	0.89	10	11.28	6	0.85	8	0.79	10	93	10
DPL - 58	10.93	1	6.85	7	4.08	10	1.73	10	0.92	3	8.89	2	8.65	3	0.97	7	22.55	10	1.46	1	0.93	7	61	6
RLG - 258	9.06	5	7.77	2	1.29	3	0.66	1	0.86	4	8.42	4	8.39	4	1.10	2	7.13	3	1.00	5	1.20	2	35	2
L - 4076	8.20	9	6.97	6	1.23	1	0.69	2	0.70	7	7.59	7	7.56	7	0.98	6	6.80	1	0.82	9	0.97	6	61	6
RLG - 234	9.88	2	7.67	3	2.21	8	1.03	6	0.93	2	8.78	3	8.71	2	1.08	3	12.22	8	1.19	2	1.17	3	42	3

Table 3: Correlation coefficients between dry matter yield and various salt tolerance indices

Tolerance indices	Ys	Yp	TOL	SSI	STI	MP	GMP	YI	SSPI	K_1STI	K_2STI
Ys	1										
Yp	0.46	1									
TOL	0.761*	-0.227	1								
SSI	0.619	-0.408	0.977**	1							
STI	0.873**	0.834**	0.347	0.159	1						
MP	0.910**	0.787**	0.423	0.24	0.996**	1					
GMP	0.869**	0.838**	0.34	0.154	0.999**	0.996**	1				
YI	0.466	1.000**	-0.22	-0.402	0.837**	0.791**	0.842**	1			
SSPI	0.761*	-0.227	1.000**	0.977**	0.347	0.423	0.34	-0.22	1		
K_1STI	0.998**	0.431	0.780**	0.638*	0.856**	0.895**	0.852**	0.437	0.780**	1	
K_2STI	0.449	0.999**	-0.239	-0.417	0.827**	0.779**	0.831**	0.999**	-0.239	0.419	1

* and ** represent significant at 5% and 1% level of significance, respectively

Conclusion

When a breeder is looking for the genotype adapted for a wide range of environments, it is concluded that selection should be based on salt tolerance indices calculated from the dry matter yield under both stress and non-stress conditions during seedling stage. On the basis of rank total of dry matter yield under both conditions and all the stress indices the genotypes SAPNA, RLG-258 and RLG-234 were identified as the most salt tolerant genotypes hence direct or indirect exploitation of these genotypes is essential for breeding of genotypes for salt tolerance. The indices STI, MP and GMP exhibited good correlation with dry matter yield under both the conditions therefore selection based on MP, GMP and STI will help in the development of genotypes with higher salinity tolerance and yield potential.

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