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INTERVARIETAL VARIATION IN SALT TOLERANCE OF LENTIL (*LENS CULINARIS* MEDIK.) IN POT EXPERIMENTS

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Abstract

Seven lentil (*Lens culinaris* Medik.) varieties were grown in pots irrigated with NaCl solution of different concentrations (0, 100, 200 and 300 mM) to assess their salinity tolerance potentials. Data revealed that the highest and the lowest salt tolerance were shown by BARI masur-5 (168.50%) and BARI masur-2 (56.32%), respectively. Cluster analysis based on the salt tolerance indices also showed grouping of the varieties into 4 clusters where BARI masur-5 was found highly salt tolerant, BARI masur-6 was moderately salt tolerant and BARI masur-1 and BARI masur-2 were least tolerant. Although shoot height, fresh weight and water content decreased, root length and root to shoot ratio increased significantly with the increase of salt concentrations in the varieties tested.

Introduction

Soil salinity is one of the main factors that limit crop production in many parts of the world (Shannon 1986). It is estimated that 6% of the world's land surface and 20% of irrigated land area of the world are affected by soil salinity (Chinnusamy *et al*. 2005). It is also reported that the area under salinity is increasing gradually posing threats to agriculture. However, the problem of salinity is more alarming in some countries where food production has to be increased in the limited land area (Ponnamieruma 1984, Yokoi *et al*. 2002). Given the scenarios of increasing trend of soil salinity worldwide, use of salt tolerant crops could be one of the suitable options to cope with the salinity problems because soil reclamation is expensive and not environment friendly (Kökten *et al*. 2010).

Lentil (*Lens culinaris* Medik.) is an important crop in many countries including Bangladesh since it provides protein-rich food for humans and animals and acts as soil quality improver by supplying increased nutrients to soil (Thomson and Siddique 1997, Katerji *et al*. 2001). In Bangladesh, lentil is placed second position among the pulses according to cultivated area and production while first in terms of usage (Afzal *et al.* 1999). However, cultivation of lentil has been challenged in Bangladesh due to increasing trend of soil salinity (Sikder and Elias 1985, Ashraf and Waheed 1990). Although, several disease-resistant varieties of lentil have been developed and released in Bangladesh over the years, information about the salt tolerance of these varieties is not available (Ashraf and Waheed1990, Hossain *et al*. 2016). The main objective of the present study, therefore, was to identify salt tolerant lentil varieties released by the Bangladesh Agricultural Research Institute (BARI).

Materials and Methods

Seeds of seven lentil varieties (BARI masur-1, BARI masur-2, BARI masur-3, BARI masur-4, BARI masur-5, BARI masur-6 and BARI masur-7) were collected from the courtesy of the Pulses Research Centre of BARI,

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Joydevpur, Gazipur. Before germination, seeds were surface-sterilized with 3% sodium hypochlorite and rinsed with distilled water. Then, the seeds were kept for two days for germination in Petri dishes with sterilized distilled water.

Two-day-old germinated seeds were transferred to plastic pots of 500 ml in volume filled with 400 g sterilized sands. Plants were supplied with NaCl solution of four different concentrations (0, 100, 200 and 300 mM). Salt was applied to the plants once a week since the age of seven days and 5 ml sterilized distilled water was applied to each pot once in every three days in order to compensate the amount of water lost by evapotranspiration following the approach as described by Panuccio *et al*. (2014). Each treatment had four replicates. Thus, a total of 112 pots (7 varieties × 4 salt treatments × 4 replicates) were used to grow plants in the growth room at the Department of Botany, University of Dhaka. Twenty plants were allowed to grow in each pot. Plants were grown for 4 weeks at 25ºC under the light intensity of 900 lux measured by Topcon IM-2D, Japan, during day time starting from 6:0 a.m. to 6:0 p.m.

Following harvest, shoots and roots were separated for the determination of height, fresh weight, dry weight, length, and water contents. Dry weight was measured after drying in oven at $80^{o}C$ for 48 hrs until the weight became constant. Fresh leaf of 1.0 g was used for the extraction of proline and optical density was recorded at 520 nm wavelength by using spectrophotometer. Amount of proline was expressed as microgram proline per gram fresh leaf.

Salt tolerance index was defined as the observed value of a target trait under a given salt treatment divided by the mean value for that trait under control and it was calculated for both shoot and root. Two-way ANOVA was performed using JMP statistical software (JMP version 4.0, SAS Institute, NY). Cluster analysis was done by using R-programming. Lentil varieties were classified into various clusters on the basis of their salt tolerance level following the method described by Chunthaburee *et al*. (2016).

Results and Discussion

Two-way ANOVA statistics on the effects of variety, treatments and their interactions are presented in Table 1. Lentil varieties showed significant variation in shoot height ($p < 0.0001$), shoot fresh weight ($p < 0.0001$), shoot dry weight ($p < 0.0001$), shoot water content ($p < 0.0008$), root length ($p < 0.0001$), root fresh weight ($p < 0.0035$), root dry weight ($p < 0.0002$), root water content ($p < 0.05$), proline ($p < 0.0001$) and root **:** shoot ($p < 0.0001$) due to salt treatment. Salt treatment also significantly affected shoot height ($p < 0.0001$), shoot fresh weight ($p < 0.0001$), shoot water content ($p < 0.0001$), root length ($p < 0.0001$), root fresh weight ($p < 0.0001$), root dry weight ($p < 0.0001$), root water content ($p < 0.0001$), proline content ($p < 0.05$) and root : shoot ($p < 0.0001$) except shoot dry weight. Interactions between variety and treatment also showed significant variation on shoot height ($p < 0.0027$), shoot fresh weight ($p < 0.0301$), shoot water content ($p < 0.0072$), root dry weight ($p < 0.0216$), proline content ($p < 0.0001$) and root : shoot ($p < 0.0009$) except shoot dry weight, main root length, root fresh weight and root water content.

Mean values of the effects of salt treatments on the growth parameters of the different varieties of lentil are shown in Table 2. Compared to control, salt treatments of 100, 200 and 300 mM caused a significant reduction in shoot fresh weight, shoot water content, root fresh weight and shoot height and significant increase in main root length in all varieties. Plants treated with salt showed reduced root water content in all varieties except BARI masur-5. Significant decrease in fresh weight under salt treatment might be due to the reduced water uptake by plants. Osmotic stress, occurring in the root medium on exposure to salts, can result in inhibition of water uptake (Munns and Tester 2008). It has been reported that the fresh and dry weight of the shoot and root systems is affected either negatively or positively, due to changes in salinity concentration

(Jimenez *et al*. 2002, Jamil *et al.* 2005, Niaz *et al*. 2005). Fresh and dry weight of both root and leaf and also leaf area of pea plant decreased with salt stress (Hernandez *et al.* 1999). Salt stress significantly reduced the total dry matter of rice cultivars at the seedling stage (Tatar *et al.* 2010). High salinity affects plants negatively in several ways including drought stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization and reduction of cell division and expansion (Hasegawa *et al*. 2000, Munns and Tester 2008, Muscolo *et al*. 2013, Panuccio *et al*. 2014).

Table 1. Two-way ANOVA statistics on the effects of variety, salt treatment and their interactions on the growth parameters of lentil (*Lens culinaris* Medik.).

Response	Source of variation	df	F-ratio	p-value
	Variety	6	9.0273	<.0001
Shoot height (cm)	Treatment	3	38.4328	<.0001
	Variety × treatment	18	2.4914	0.0027
	Variety	6	5.5246	<.0001
Shoot fresh weight (mg/plant)	Treatment	3	41.6168	<.0001
	Variety × treatment	18	1.8665	0.0301
	Variety	6	6.7694	<.0001
Shoot dry weight (mg/plant)	Treatment	3	0.6106	0.610
	Variety × treatment	18	1.2000	0.2801
	Variety	6	4.3039	0.0008
Shoot water content (mg/plant)	Treatment	3	52.6729	<.0001
	Variety × treatment	18	2.2423	0.0072
	Variety	6	58.1985	<.0001
Proline (µg/g leaf)	Treatment	3	2.7424	0.0514
	Variety × treatment	18	4.8268	<.0001
	Variety	6	9.1649	<.0001
Main root length (cm)	Treatment	3	9.8171	<.0001
	Variety × treatment	18	0.5770	0.9067
	Variety	6	3.5479	0.0035
Root fresh weight (mg/ plant)	Treatment	3	15.3478	<.0001
	Variety × treatment	18	1.5349	0.0983
	Variety	6	4.9777	0.0002
Root dry weight (mg/plant)	Treatment	3	10.7837	<.0001
	Variety × treatment	18	1.9549	0.0216
	Variety	6	2.2334	0.0477
Root water content (mg/plant)	Treatment	3	17.413	<.0001
	Variety × treatment	18	1.1884	0.2894
	Variety	6	7.1377	<.0001
Root : Shoot	Treatment	3	18.2778	<.0001
	Variety × treatment	18	2.7595	0.0009

Although fresh weight of both shoot and root of the lentil varieties was negatively affected by the high salt concentrations dry weight, however, did not show the similar pattern of response in the present experiment. Further, data revealed no general pattern of effects of salt application on the dry mass of lentil variety as per degree of salt stress. Dry mass decreased in some varieties such as BARI masur-1 and BARI masur-2 but increased in BARI masur-3, BARI masur-4. BARI masur-5 and BARI masur-6 whereas almost unchanged in BARI masur-7 at salt concentrations between 100 and 300 mM. Such variation in response to salt stress among the lentil varieties might be related with their inherent genotypic variation (Bandeoğlu *et al.* 2004).

Table 2. The effect of salt treatments (0, 100, 200 and 300 mM) on the growth parameters of lentil.

Variety	Treatment	SFW (mg)	SDW (mg)	SWC (mg)	SH (cm)	Proline (µg/g leaf)	RFW (mg)	RDW (mg)	RWC (mg)	MRL (cm)	Root/ shoot	DMC (mg)
BARI masur-1	0	19.58	3.08	16.50	12.20	31.49	7.65	3.49	4.16	10.25	0.84	6.57
	100	9.59	3.30	6.29	8.43	28.34	5.57	1.83	3.74	11.75	1.53	5.13
	200	7.39	4.49	2.90	8.50	24.31	3.54	1.80	1.74	12.00	1.41	6.29
	300	3.48	1.99	1.49	5.50	16.04	3.62	1.71	1.90	11.92	2.49	3.7
BARI masur -2	0	17.93	3.56	14.37	12.53	26.31	5.80	2.01	3.79	6.25	0.49	5.58
	100	9.37	4.07	5.30	7.58	19.72	3.10	1.21	1.89	8.62	1.32	5.28
	200	7.80	3.31	4.48	7.83	19.30	3.65	1.58	2.07	8.85	1.21	4.90
	300	3.35	2.07	1.28	4.05	37.14	3.30	1.72	1.57	10.65	3.03	3.79
BARI masur -3	0	20.75	4.05	16.70	11.85	10.46	10.30	3.94	6.35	7.62	0.64	8.00
	100	11.82	4.55	7.27	8.40	19.34	4.20	1.51	2.68	8.25	1.11	6.07
	200	5.77	3.74	2.03	7.20	15.43	4.18	1.61	2.56	8.50	1.20	5.35
	300	5.77	3.96	1.80	9.75	17.63	5.70	2.85	2.85	8.47	0.87	6.81
BARI masur -4	0	21.73	4.86	16.87	11.68	3.06	7.53	3.40	4.13	8.00	0.68	8.26
	100	16.82	5.54	11.28	9.55	3.32	5.87	2.75	3.11	8.50	0.91	8.29
	200	7.13	5.00	2.13	9.30	4.14	2.11	1.02	1.08	8.82	0.95	6.02
	300	9.70	5.61	4.08	9.08	1.93	4.65	1.86	2.78	10.12	1.11	7.47
BARI masur -5	0	35.25	4.38	30.87	13.15	2.39	7.48	3.24	4.24	7.60	0.58	7.63
	100	29.62	6.03	23.59	11.60	2.03	10.15	5.20	4.95	9.50	0.83	11.23
	200	6.33	4.41	1.91	9.13	5.41	2.70	1.30	1.40	9.85	1.07	5.72
	300	11.80	7.41	4.39	10.80	9.07	10.15	5.43	4.72	10.22	0.94	12.84
BARI masur -6	0	34.86	5.02	29.84	13.18	8.05	8.23	3.32	4.97	7.12	0.54	8.34
	100	14.18	5.05	9.12	11.68	10.23	5.16	1.98	3.17	9.37	0.80	7.04
	200	11.65	6.07	5.57	9.58	12.52	3.40	1.73	1.66	10.47	1.11	7.81
	300	13.25	5.88	7.36	11.70	17.63	7.42	3.62	3.79	12.47	1.08	9.50
BARI masur -7	0	38.06	5.44	32.61	13.55	13.10	7.70	2.97	4.73	5.87	0.42	8.41
	100	8.04	5.66	2.37	8.80	5.12	3.09	1.41	1.68	6.87	0.77	7.07
	200	7.58	4.34	3.23	6.63	14.15	1.73	0.70	1.03	7.35	1.11	5.04
	300	8.97	4.56	4.40	6.80	12.67	4.80	2.40	2.40	7.47	1.18	6.96

SFW - Shoot fresh weight, SDW - Shoot dry weight, SWC - Shoot water content, SH - Shoot height, RFW- Root fresh weight, RDW - Root dry weight, RWC - Root water content, MRL - Main root length, DMC - Dry matter content.

Lentil varieties under the present investigation showed increased value of root to shoot length ratio with the increase of salt concentrations. Results thus indicated that roots of lentil plants were more affected by salt stress than shoots. These results corroborated with the findings of other studies (Keiffer and Unger 1997, Kaya *et al.* 2008). At 200 mM salt content, more growth retardation was observed in leaf tissues when compared to root tissues in lentil (Bandeoğlu *et al.*

2004). Increased root to shoot length ratio in tomato was noted with the increased salinity (Shannon *et al.* 1987). Data also suggest that plants invest more resources to root growth than to shoot growth during salt stress condition (Papadopolous and Rending 1983, Snapp *et al.* 1991, Hossain *et al.* 2016).

Table 3 shows a range of variation in salt tolerance efficiency among the lentil varieties at the three concentrations of NaCl (100, 200 and 300 mM). The highest salt tolerance efficiency was shown by BARI masur-5 (168.50%) followed by BARI masur-6 (113.91%) and the lowest was found in BARI masur-1 (56.32%) at 300 mM NaCl. Such variation in salt tolerance efficiency among the varieties might be related with the genotypic variation among them (Ali *et al.* 2007). Except BARI masur-5 and BARI masur-6, all other varieties showed a gradual decrease in salt tolerance efficiency with the increase of NaCl concentrations. Other study also reported that salt tolerance level decreased as the NaCl concentration increased in rice (Amirjani 2010).

Table 3. Salt tolerance indices of seven lentil (*Lens culinaris* Medik.) varieties.

Variety	Treatment	SFW (mg)	SDW (mg)	SWC (mg)	SH (cm)	Proline (µg/g leaf)	RFW (mg)	RDW (mg)	RWC (mg)	MRL (cm)	Root/ Shoot	DMC	STE%
BARI masur-1	100	0.49	1.07	0.38	0.69	0.90	0.73	0.53	0.90	1.15	1.81	0.78	78.06
	200	0.38	1.46	0.18	0.70	0.77	0.46	0.52	0.42	1.17	1.67	0.96	95.79
	300	0.18	0.65	0.09	0.45	0.51	0.47	0.49	0.46	1.16	2.96	0.56	56.32
BARI masur-2	100	0.52	1.14	0.37	0.60	0.75	0.54	0.60	0.50	1.38	2.68	0.95	94.72
	200	0.43	0.93	0.31	0.62	0.73	0.63	0.79	0.55	1.42	2.46	0.88	87.87
	300	0.19	0.58	0.09	0.32	1.41	0.57	0.86	0.41	1.70	6.18	0.68	68.00
BARI masur-3	100	0.57	1.12	0.44	0.71	1.85	0.41	0.38	0.42	1.08	1.72	0.76	75.87
	200	0.28	0.92	0.12	0.61	1.47	0.41	0.41	0.40	1.11	1.86	0.67	66.96
	300	0.28	0.98	0.11	0.82	1.69	0.55	0.72	0.45	1.11	1.36	0.85	85.00
BARI masur-4	100	0.77	1.14	0.67	0.82	1.09	0.78	0.81	0.75	1.06	1.33	1.00	100.40
	200	0.33	1.03	0.13	0.80	1.36	0.28	0.30	0.26	1.10	1.38	0.73	72.91
	300	0.42	0.90	0.19	0.97	1.25	0.36	0.37	0.35	1.04	1.04	0.90	90.00
BARI masur-5	100	0.84	1.38	0.76	0.88	0.85	1.36	1.60	1.17	1.25	1.42	1.47	147.22
	200	0.18	1.01	0.06	0.69	2.26	0.36	0.40	0.33	1.30	1.84	0.75	74.97
	300	0.33	1.69	0.14	0.82	3.79	1.36	1.68	1.11	1.34	1.62	1.68	168.00
BARI masur-6	100	0.41	1.01	0.31	0.89	1.27	0.63	0.60	0.65	1.32	1.49	0.84	84.36
	200	0.33	1.21	0.19	0.73	1.56	0.41	0.52	0.34	1.47	2.06	0.94	93.58
	300	0.38	1.17	0.25	0.89	2.19	0.90	1.09	0.76	1.75	2.00	1.14	114.00
BARI masur-7	100	0.21	1.04	0.07	0.65	0.39	0.40	0.47	0.36	1.17	1.85	0.84	84.07
	200	0.20	0.80	0.10	0.49	1.08	0.23	0.24	0.22	1.25	2.66	0.60	59.99
	300	0.24	0.84	0.13	0.50	0.97	0.62	0.81	0.51	1.27	2.81	0.83	83.00

SFW - Shoot fresh weight, SDW - Shoot dry weight, SWC - Shoot water content, SH - Shoot height, RFW - Root fresh weight, RDW - Root dry weight, RWC - Root water content, MRL - Main root length, DMC - Dry matter content.

Except BARI masur-1, a general tendency of increase in proline content with the increase of salt treatment was observed in all varieties. Proline content was maximum in BARI masur-1 and minimum in BARI masur-4 and BARI masur-5. Previous studies also reported remarkable increase of proline content in the tissues of leguminous plants under salt stress condition (Tramontano and Jouve 1997, Bandeoğlu *et al.* 2004). Since proline is regarded as an important osmoprotectant in plants, salt tolerance has often been attributed to the accumulation of osmoprotectants (Santa-Cruz *et al.* 1998). However, in the present study, most of the varieties showed higher levels of proline and lower level of dry and fresh biomass under salt stress condition (200 mM and 300 mM). These findings are consistent with Kanawapee *et al.* (2013) who reported that under salt stress condition the highly susceptible cultivars accumulated the highest level of proline than the tolerant cultivars. This might indicate that over accumulation of proline was related to a symptom of salt injury rather than an indicator of salt resistance (Lutts *et al.* 1999).

Cluster analysis done using salt tolerance indices measured at 300 mM salt treatment (Table 3) revealed that all seven lentil varieties were grouped into four main clusters (Fig. 1). Cluster I represented the salt-tolerant group and BARI masur-5 was the only lentil variety in this cluster. Cluster II was considered as highly salt-sensitive group which included BARI masur-1 and BARI masur-2. BARI masur-6 was included in Cluster III and was denoted as moderately tolerant. Cluster lV represented the salt-sensitive group including BARI masur-3, BARI masur-4 and BARI masur-7.

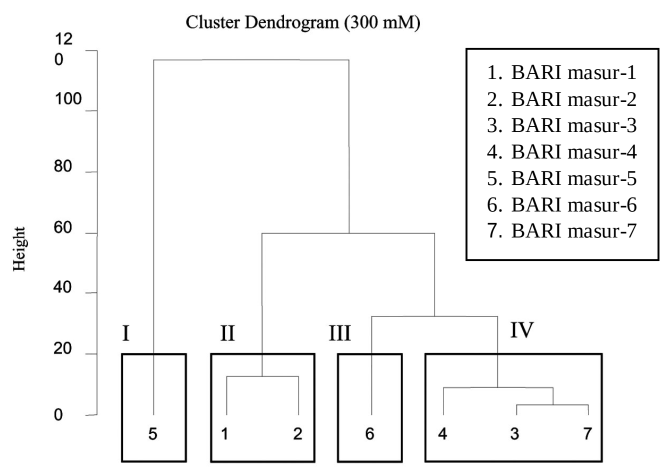

Fig.1. Dendrogram resulting from cluster analysis based on growth parameters of seven lentil varieties at salt concentration (300 mM) (Factor analysis and data mining with statistical software package R).

Grouping of lentil varieties on the basis of growth responses to salt treatments indicate the variation in salt tolerance among the lentil varieties and also the potentials of screening tolerant varieties to be used during cultivation in the salt affected soils. Variability in salt tolerance among the lentil varieties has also been observed by Sorkheh *et al.* (2012). However, although the seven varieties showed difference in salt tolerance at the age of four weeks, further study is needed to assess whether salt tolerance level among them is maintained till their mature phenological stages of flowering and fruiting.

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