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Screening Ethiopian Lentil (Lens CulinarisM.) for Salt Tolerance at Germination and Early Seedling Stage

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Abstract

To evaluate genetic variation among Ethiopian lentil, laboratory experiment were conducted to screen 12 accessions of lentil (Lens culinaris M.) for salt tolerance. Seeds of 12 Lentil accessions were grown at laboratory (Petri dish) condition with different levels of salinity (0, 2, 4, and 8 dSm^{-1}NaCl) for 4 weeks. The experimental design was completely randomized design (CRD) in factorial combination with three replications. Data analysis was carried out using SAS software. Average germination time, germination percentage, seedling shoot and root traits, seedling shoot and root weight were evaluated. The two way ANOVA for varieties revealed statistically significant variation among lentil accession, NaCl level and their interactions ($p<0.001$) with respect to the entire parameters. It was found that salt stress significantly delays germination rate and decreases germination percentage, shoot and root length, seedling shoot and root weight of lentil accessions. The degree of decrement varied with accessions and salinity levels. Accessions Lent 12, Lent 1 and Lent 2 were better salt tolerant than the other accessions. As the result, it is recommended to be used as a genetic resource for the development of lentil accession and other very salt sensitive crop with improved germination under salt stress condition.

1. Introduction

Salinity is one of the most serious factors that hamper the productivity of agricultural crops, with adverse effects on germination, plant vigor and crop yield [1], particularly in arid and semi-arid regions of the world [2] because of the lack of sufficient amount of rainfall leads to leaching the accumulated salt and also it affects many irrigated areas mainly due to the use of underground water. Salt-affected soils are distributed throughout the world and no continent is free from the problem [3]. In Ethiopia, salt-affected soils are prevalent in the Rift Valley and the lowlands [4]. Salinity stress because a multitude of physiological problems in plant processes [5]. It causes a significant reduction in germination percentage, germination rate, and root length, shoot and root length, root and shoot weight, and dry root and shoot weight, and seed yield which lead to the death of the entire plant [6].

Germination and seedling growth under saline environment are the screening criteria that are widely used to select the salt tolerance genotype [7]. Because of salinity tolerant at this stage was shown to be a heritable trait that enable the crop salt tolerant throughout its growth stage [8], although it is a polygenic character linked to a complex genetic basis [9] and seeds and young seedlings are frequently was affected by much higher salinities than vigorously growing plants because germination usually occurs in surface soils, which accumulate soluble salts because of evaporation and capillary rise of water [10].

Since grain legumes especially lentil are salt sensitive, farmers do not consider growing them in a saline environment, though; there is a considerable difference in salt tolerance among crops/accessions [7]. Screening of available Lentil accessions is important to find a relative salt tolerant accession. Therefore, the general objective of this study was to assess the genetic variability for salinity tolerance among some lentil accessions, specifically to evaluate the effect of salt on germination and early seedling stage of Lentil accessions and to identify salt tolerant Lentil accessions.

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2. Methods and material

2.1 Description of the study area and plant material

The experiment was conducted in the Botanical science laboratory, Department of Biology, Haramaya University, Ethiopia. Seeds of twelve Lentil accessions were obtained from the Ethiopia Institute of Biodiversity (EIB)

2.2 Treatments and Experimental Design

The study was conducted under laboratory condition at room temperature and aimed to assess morphological variation among lentil accessions in terms of seed germination and seedling growth. Four different NaCl solutions with salinity levels of 0 (control), 2, 4, and 8 dS/m [11-12] were prepared by dissolving 1.28, 2.56 and 5.12 gm of NaCl in one liter of water respectively 12 accessions of Lentil. The experiment was laid as a Completely Randomized Design (CRD) in a factorial arrangement and replicated three times. The treatments were assigned randomly to each Petri dish.

3. Experiment procedure

In order to assess the response of the 12 Lentil accessions under different concentration of NaCl, 12 seeds were first surface sterilized in 5 % sodium hypochlorite solution for 20 minute and washed three times with sterilized distilled water. Prior to experiment 10 cm diameter Petri dishes were thoroughly washed and sterilized in hot air oven at 70 0C for 36 hours and Whatman filter paper for 24 hours at 700°C [4]. After sterilization, Petri dishes were lined with Whatman No 3 filter paper and treated with 10 ml of deionizer water (control), 2, 4, and 8 dS/m of NaCl. Following this, twelve uniform seeds of each Lentil accessions were placed on each Petri dish approximately in uniform distance. The Petri dishes were arranged in a completely randomized design (CRD) in a factorial combination with three replications [13]. Each Petri dish was treated with 10 ml of the respective concentrations of NaCl in every other day. Salt levels were maintained each day by dripping out and applying fresh salt solution. The Petri dishes were put within a glass box to avoid loss of moisture through evaporation. Germination started after two days of sowing and the germination count was continued until the 9th day. Germination was recorded daily and a seed was considered germinated when both plumule and radical had emerged ≥ 0.05 cm [16].

Table1 Descriptions of lentil accessions that were used in the experiment

Accession number	Code	Region/ State/	Zone	Woreda/ District	Latitude	Longitude	Altitude
9235	Lent 1	Oromiya	MisrakHarerge	Meta	09-16-21-N	41-33-45-E	2535
36004	Lent 2	Amara	Semen Shewa	Ankober	09-39-00-N	39-41-00-E	3180
36006	Lent 3	Oromiya	MisrakShewa	Gimbichu	08-57-00-N	39-05-00-E	2370
36019	Lent 4	Oromiya	MirabShewa	AlemGena	08-48-00-N	38-20-00-E	2150
36025	Lent 5	Gumuz	Metekel	Wenbera	Unknown	Unknown	1580
36032	Lent 6	Oromiya	Bale	Ginir	Unknown	Unknown	1520
36064	Lent 7	SNNP	Bench Maji	Dirashe	Unknown	Unknown	Unknown
36093	Lent 8	Oromiya	MirabHarerge	Chiro	09-04-00-N	40-41-00-E	2000
36094	Lent 9	Oromiya	MirabHarerge	Chiro	09-02-00-N	40-44-00-E	1870
36095	Lent 10	Somali	Shinile	Afdem	Unknown	Unknown	1800
36113	Lent 11	Oromiya	MisrakHarerge	Deder	Unknown	Unknown	Unknown
36120	Lent 12	Oromiya	MirabWellega	GawoDale	Unknown	Unknown	1870

3.1 Average germination time: the average number of days needed for plumule or radical emergence calculated following the formula described by [4].

3.2 Germination percentage: Seven days after seeds were put into the Petri dishes, six germinated seeds were counted, and the germination percentage calculated. The germination percentage calculated [14] as:

Germination percentage: $\frac{\text{number of seed germinated}}{\text{number of seed sown}}$*100

3.3 Salt tolerance index was calculated as total plant (shoot + root) dry weight obtained from 6 randomly selected seeds grown on different salt concentrations compared to total plant dry weight obtained on normal concentration. {[STI = (TDW at Sx/TDW at S1) x 100], STI= salt tolerance index, TDW = total dry weight, S1 = control treatment, Sx = x treatment}[14].

3.4 Seedling Shoot Length (cm): Fifteen days after germination, shoot length of 6 randomly picked seedlings from each Petri dish measured in centimeters.

3.5 Seedling Root Length (cm): Fifteen days after germination, root lengths of 6 randomly picked seedlings from each Petri dish were measured in centimeters.

Seedling Shoot-to-Root Ratio: calculated as the ratio of seedling shoot length to seedling root length [15].

3.6 Seedling Fresh Shoot Weight (g) was measured after 30 days of sowing by weighting the mass of shoots of 6 randomly picked seedlings from each Petri dish using sensitive balance.

3.7 Seedling Fresh Root Weight (g): measured by weighting the mass of roots of six randomly picked seedlings after 30 days of sowing from each Petri dish using sensitive balance.

3.8 Seedling Shoot Dry Weight (g): measured by picking six seedlings randomly from each petri dish and oven drying their shoots at 80 0C for 48 hours and weighting them using sensitive balance.

3.9 Seedling Root Dry Weight (g): measured by picking six seedlings randomly from each Petri dish after 30 days of sowing and oven drying their roots at 800°C for 48 hours and weighting them using sensitive balance [17].

3.10 Data Analysis

The data were subjected to analysis of variance using SAS (version 9.1) and the means were separate using the Least Significant Difference (LSD) test at 5% significance level.

4. Results and Discussions

4.1 Influence of Salinity on Germination of Lentil Accessions

4.1.1 Average germination time Two-way analysis of ANOVA for germination time showed that the NaCl salt levels highly significantly ($p<0.001$) influenced germination rate. Nevertheless, accessions responded differently to different salinity levels. For instance, at 2 dSm^{-1} salinity level, accession Lent 12, Lent 2 and Lent 1 had the shortest germination rate .Thus, those accession had needed shorter time to be germinated than the other accessions while, accession Lent 4, Lent 6 and Lent 7 attained the longest germination rate and needed longer time to be germinated (fig 1). Moreover, at 4 and 8 dSm^{-1} salinity level, accession Lent 12, Lent 1 and Lent 2 had the shortest germination rate and germinated faster than other accessions that were tested as shown in figure 1.Whereas, accession Lent 4, Lent 7 and Lent 8 had longer germination rate and needed more time to be germinated (fig 1). Accessions Lent 12, Lent 1 and Lent 2 germinated faster than the other accession at 2 4 and 8 dSm^{-1} salinity level whereas, salinity delayed seed germination of accession Lent 4, Lent 8, and Lent 5 and those accessions needed longer time to germinated in the entire salt treatments (fig 1). The result revealed that salinity delays the germination rate of lentil accessions and the result was in full agreement with the previous studies of [7, 15] in lentil; Jeannette et al. [18] in Phaseolus species who reported that salinity delay seed germination and decrease germination rate. This might be due to salinity affects germination by facilitating intake of toxic ions and hinder many metabolic, physiological, and enzymatic activities. In addition, salinity may cause osmotic potential which result decreasing absorption of water.

4.2 Germination percentage the two-way analysis of variance (ANOVA) found highly significant variation in germination percentage among accessions, salinity and their interaction ($p < 0.001$). At 2 dSm^{-1} salinity (NaCl) level, accession Lent 1, Lent 2 and Lent 12 performed well and achieved the higher percent of germination whereas, salinity highly affected seed germination of accession Lent 4 (fig 2). Moreover, at 4 and 8 dSm^{-1} salinity level, the highest value of germination percentage was recorded in accession Lent 1, Lent 12 and Lent 2 whereas, the lowest value was recorded in accession Lent 4 and Lent 9 (figure 2). The result indicates that the percent of germination generally decreased with increasing salt concentration The findings of

Table 2 Summary of analysis of variance for germination growth and biomass parameters of lentil accession of NaCl and ** = significantly different at 5% and 0.1% level of probability, respectively; AGT= average germination time; STI=salt tolerance index; SSL=seedling shoot length; SRL=seedling root length; SSR=seedling shoot to root ratio; SFSW=seedling fresh shoot weight; SFRW=seedling fresh

	Mean squares				
parameter	NaCl (N) (df= 3)	Accession (A) (df= 11)	N x A (df= 33)	Error (df= 94)	CV (%)
AGT	80.99**	1.08**	0.64**	0.24	9.82
GP	23482.29**	851.90**	77.52**	26.55	8.52
STI	66029.1**	265.29**	109.07**	2.13	3.72
SSL	844.79**	16.19**	2.30**	0.159	5.90
SRL	179.99**	6.45**	1.06**	0.046	5.40
SRR	2.83**	0.17**	0.08*	0.013	7.16
SFSW	179.99**	6.45**	1.06**	0.046	4.12
SFRW	2.97**	0.05**	0.02**	0.0001	2.53
SDSW	32.90**	0.56**	0.25**	0.0013	5.08
SDRW	0.87**	0.07**	0.05**	0.0008	15.24

root weight; SDSW=seedling dry shoot weight; SDRW=seedling

the study were in line with previous research studies reported by Noreen et al. [19] in P.sativum and Ashraf and Waheed, [7] in lentil who reported that a significant decrease in seed germination were observed while increasing salinity level. The reason is assumed to be due to salinity that attributed to osmotic retention of water. **4.3 Salt tolerance index** Analysis of variance (ANOVA) for salt tolerance index showed that salt tolerance index was highly significant difference among accession, salinity level, and accession*salinity interaction ($p<0.001$). Even though at 2 dSm^{-1} salinity level, salinity reduced the overall

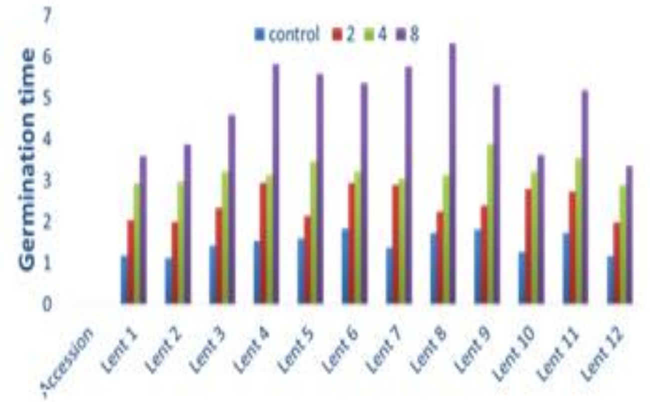

Fig1: Effect of Salinity on germination time of lentil accessions

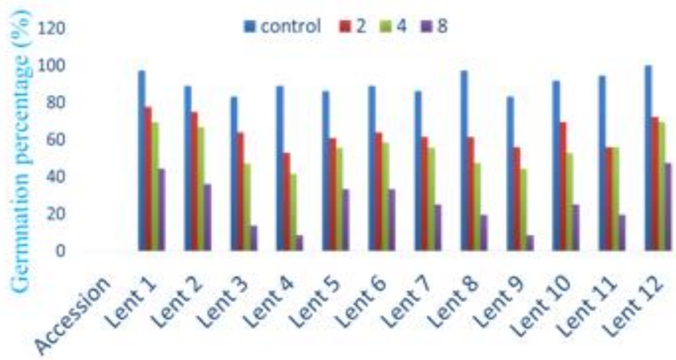

Fig. 2: Effect of salinity on seed germination of lentil accessions

growth of accession Lent 1, Lent 2 and Lent 12, the degree of reduction was lesser on those accession, hence, the maximum salt tolerance index were observed at those accession while, salinity highly reduced the overall growth of accession Lent 4. Thus, accession Lent 4 exhibited the minimum percent of salt tolerance index than the rest of the accessions (figure 3). Moreover, at 4 and 8 dSm^{-1} salinity level, salinity easily hampered the overall growth of accession Lent 1, Lent 12 and Lent 2 than the other accession while, the overall growth of accession Lent 4 were highly hindered by salinity and as result; this accession attained the minimum value of salt tolerance index (figure 3). The result indicates that salt tolerance index of lentil accession were significantly reduced as salinity concentration increased (fig 3). The result was in line with previous studies of Kaganet al. [17] reported the salt tolerance index of lentil decrease when salinity levels become increased.

4.4 Influence of Salinity on Seedling Shoot and Root traits of Lentil Accessions

4.4.1 Seedling Shoot Length (SSL) (cm)

The analysis of variance (ANOVA) for seedling shoot length data showed**4.4 Influence of Salinity on Seedling Shoot and Root traits of Lentil Accessions**

4.4.1 Seedling Shoot Length (SSL) (cm) The analysis of variance (ANOVA) for seedling shoot length data showed

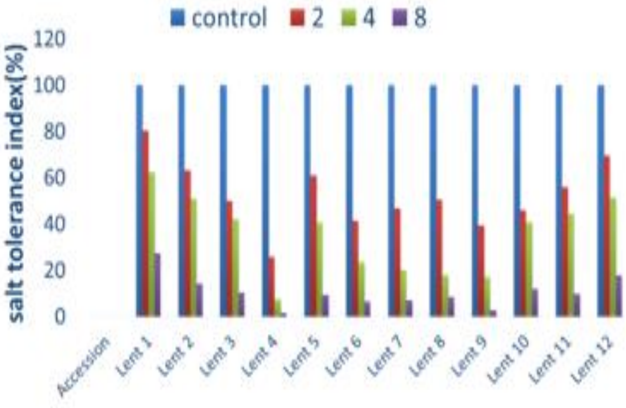

Fig 3: Sensitivity and tolerance of lentil for salinity

that highly significant variation in seedling shoot length ($p < 0.001$) among accessions, salinity level and their interaction. Seedling shoot length of lentil accessions varied among accessions and salinity level (figure 4). At 2 dSm^{-1} salinity level, accession Lent 12 followed by accession Lent 1 and Lent 2 attained the longest shoot length while, accession Lent 9 had the shortest shoot length (fig 4). Moreover, accession Lent 12, Lent 1 and Lent 2 performed well and attained the longest shoot length at 4 and 8 dSm^{-1} salinity levels (figure 4) whereas, the shortest shoot growth was observed in accession Lent 4, Lent 9 and Lent 11 (fig 4). This result showed that increment of NaCl treatments resulted in a significant reduction in shoot growth (fig 4). The findings of this studies show conformity with research result of Kaganet al. [17] and Mane et al. [19] who reported that salinity inhibits elongation of shoot in lentil and increasing NaCl treatment results significant reduction of shoot growth. The reduction in shoot length probably because genetic variation among lentil accession and excessive accumulation of salts in the cell wall elasticity, thus, secondary cell appears sooner and cell wall becomes rigid as a consequence the turgid pressure efficiency in cell enlargement decreases that result in short shoot growth.

4.5 Seedling Root Length (SRL) The analysis of variance (ANOVA) for seedling root length indicates that there was highly significant variation in seedling root length among lentil accessions, salinity levels and their interaction ($p < 0.001$). At 2 dSm^{-1} salt concentration, accession Lent 12, Lent 1 and Lent 2 attained the longest root length than other accession while, salinity adversely reduced root growth in accession Lent 9 and this accession had shortest root length (figure 5). Furthermore, at 4 and 8 dSm^{-1} salt concentration, the longest root length was recorded in accession Lent 12, Lent 1 and Lent 2 than the rest of the accessions whereas; the shortest root length were observed in accession Lent 9, Lent 11, Lent 8, and Lent 4 (figure 5). The result elucidate that the increment of NaCl concentration was cause the reduction of seedling root length of lentil accession (figure 5) The result is in full agreement with Ashraf et al. [7] in lentil; Arshi [20] in senna plant; Duzdemiret al. [21] in pea who reported that high salinity reduced root length in lentil.

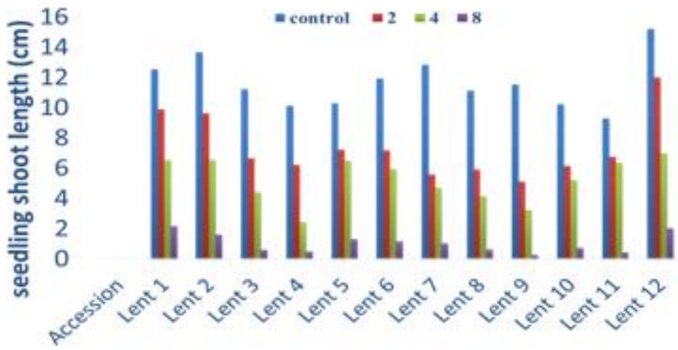

Fig 4: Effect of salinity on seedling shoots length of lentil

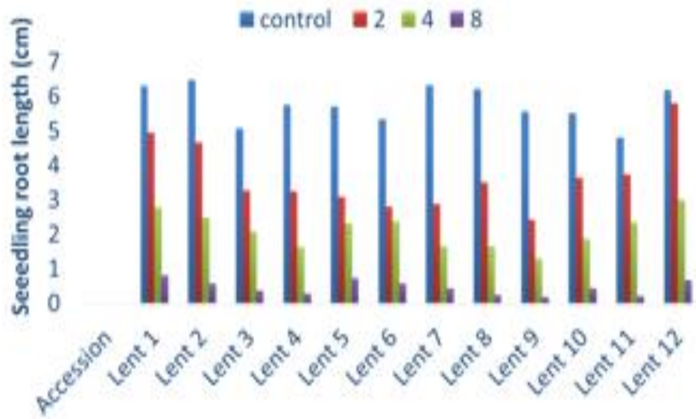

Fig. 5: Effect of salinity on seedling root length of lentil

4.6 Shoot to Root length Ratio (SRR) The two-way analysis of variance (ANOVA) for seedling shoot-to-root length ratio (SRR) showed significant variations for accessions, salinity level and accession salinity interaction ($p < 0.001$). At 2 dSm^{-1}salinity level, accession Lent 8 and Lent 10 had the lower value of shoot to root ratio than the other accessions (figure 6). Moreover, at 4 dSm^{-1}NaCl level, accession Lent 7 followed by Lent 5 and Lent 10 showed the higher seedling shoot to root ratio. However, accession Lent 4 attained significantly lower values seedling shoot to root ratio than the other accession (figure 6). At 8 dSm^{-1}NaCl level, accession Lent 12, Lent 2 and Lent 1 attained significantly higher mean seedling shoot to root ratio in contrast to this, accession Lent 4 showed the lowest value (figure 6). The result indicates that some accessions show significantly higher reduction in SRR as increment of NaCl concentration in the growth media. In contrast to this decrement of SRR in some accession were observed as the salinity level increased. The findings of this study showed conformity with the previous studies on other legumes [4] in haricot bean, Abdelhamid et al., [22] in faba bean who reported that SRR was highly reduced at higher salinity levels for some accessions and the accessions showed significant variation in their response to salinity.

4.7 Seedling fresh Shoot weight (SFSW) (g) Two way of analysis of ANOVA seedling shoot fresh weight confirmed that there were highly significant differences among all accessions, NaCl treatments and their interaction ($p < 0.001$). The NaCl treatments used in caused significant reduced in seedling fresh shoot weight. Some accession attained the maximum value of seedling fresh shoot weight while other attained the minimum value of seedling fresh shoot weight.

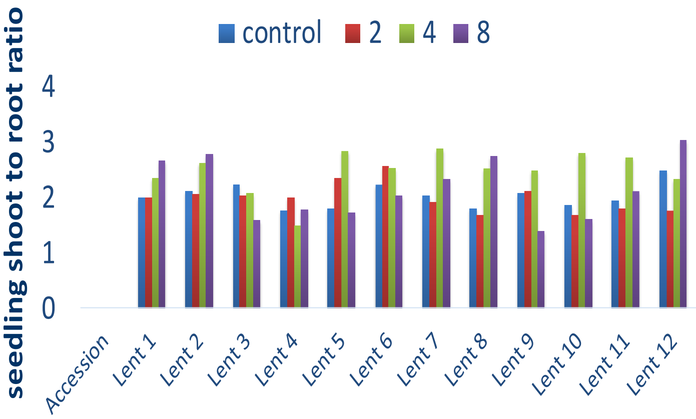

Fig. 6: Effect of salinity on seedling shoots to root ratio of lentil

For instance, accession Lent 1, Lent 12 and Lent 2 attained the longest shoot length and produced the maximum value of seedling fresh shoot weight at 2, 4 and 8 dSm^{-1} salinity level (figure 7) while, minimum value of seedling fresh shoot weight was recorded in accession Lent 4, Lent 9, and Lent 8 (figure7). Salinity reduced shoot growth of all accession as compared to the control but the degree of reduction was varied between accessions and salt concentration. For instance, accession Lent 12, Lent 1 and Lent 2 attained relatively the maximum value of seedling fresh shoot weight even at higher salinity level than the other accessions and considered as salt tolerant while, salinity adversely reduced shoot growth of accession Lent 4, Lent 9, and Lent 8. As result, those accessions exhibited the minimum value of seedling fresh shoot weight even at lower salt concentration (figure7). The result showed that, seedling fresh shoot weights of lentil accessions significantly reduced with increment salinity level (figure 7). The result was in line with previous research findings of Stoeva and Kaymakanova [23] who reported as that there was a rapid decrease in seedling fresh shoot weight of leguminous plants under saline environment. This reduction may be due to limited supply of metabolites to young growing tissues, because metabolic production takes place within in the leaves and is significantly perturbed at high salt stress, either due to the low water uptake or toxic effect of NaCl concentration [24-25].

4.8 Seedling root fresh weight (SRFW) (g) Two way analysis of ANOVA for seedling fresh root weight confirmed that there were highly significant differences among lentil accessions NaCl treatments and accessions * treatment interaction ($p < 0.001$). At 2 dSm^{-1} salinity level, accession Lent 1 followed by Lent 12 and Lent 2 achieved the maximum value of seedling fresh root weight whereas, the minimum value of seedling fresh root weight was recorded in accession Lent 4 (figure 8). Moreover, at 4 and 8 dSm^{-1}salinity level, some accession performed well and attained the maximum value of seedling fresh root length while, other accession had the minimum value of seedling fresh root weight. For instance, accession Lent 12, Lent 1 and Lent 2 achieved the maximum value of seedling fresh root weight than the other accession whereas; accession Lent 4 attained the minimum value of seedling fresh root weight (figure 8). Salinity reduced growth of root in accession Lent 12, Lent 2 and Lent 1 but; the degree of reduction of seedling fresh root weight in those accessions was less than the other accessions. Consequently, these accessions had the maximum value of seedling fresh root weight even at higher salinity level than the other accessions. On the other hand, salinity highly inhibited the root elongation of accession Lent 4 and this accession had minimum value of the seedling fresh root weight. The result justified that salinity reduced seedling fresh root weight of lentil accessions (figure 8). The findings of this result in line with the result of findings of kaganet al. [17-18] and Jeannette et al. reported salinity increment significantly reduced fresh root weight in Lentil and phaseolus species, respectively.

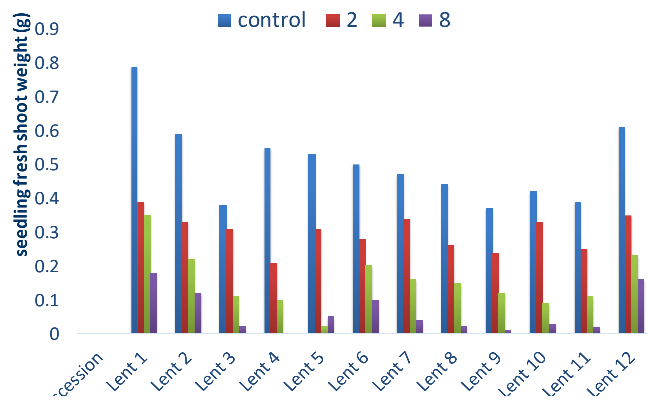

Fig. 7: Effect of salinity on seedling fresh shoot weight of lentil

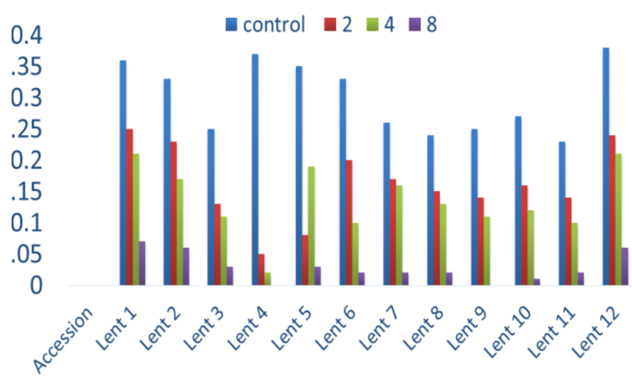

Fig. 8 Effect of salinity on seedling fresh root weight of lentil

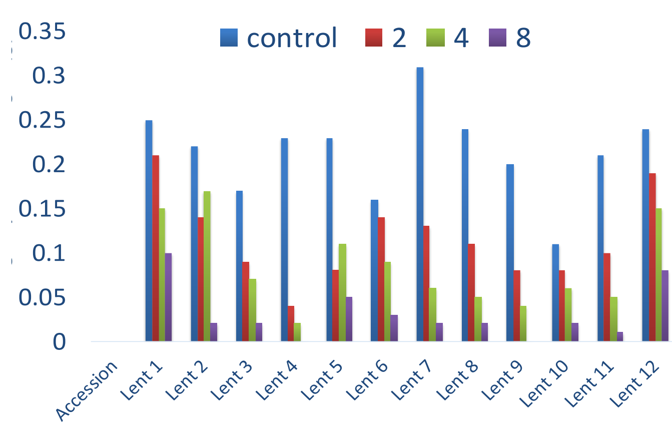

Fig 9.: Effect of salinity on seedling dry shoot weight

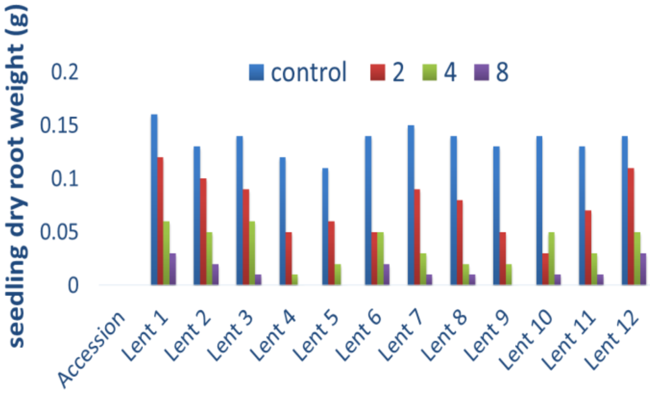

Fig. 10: Effect of salinity on seedling dry root weight of lentil

4.9 Seedling Dry Shoot Weight (SDSW) (g) Statistical analysis for seedling shoot dry weight revealed that there were highly significant differences among all accessions, NaCl treatments and accessions treatment interaction ($p < 0.001$). At 2 dSm^{-1} salinity level, accession Lent 1, Lent 12 and Lent 2 achieved maximum value of seedling dry shoot weight than the other accession (fig 9) whereas, the minimum value of seedling dry shoot weight was scored in accession Lent 4 (fig 9). Moreover, at 4 and 8 dSm^{-1} salt concentration, accession Lent 1 and Lent 12 achieved the maximum value of seedling dry shoot weight than the rest of the accessions that were tested while, accession Lent 4 and Lent 11 attained the minimum value of seedling dry shoot weigh (figure 9). This result revealed that salinity antagonistically reduced in seedling dry shoot weight (figure 9&10). The result show full agreement with previous research result reported of Turanet al. [26] and Islam [27] on lentil; Shereenet al. [28] rice, Bayuelo et al. [29] in phaseolus species who reported that increasing the concentration of salinity cause significant reduction in shoot growth consequently reduced seedling dry shoot weight.

4.10 Seedling Root Dry Weight (SRDW (g) Analysis of variance for seedling dry root weight exhibited that highly significant variations among NaCl treatments, accessions and their interaction ($p<0.001$). accession Lent 1, Lent 12 and Lent 2 had the maximum weight of seedling dry root weight than the other accession that were tested at 2 ,4 and 8 dSm^{-1} salinity level, (figure10), in contrast to this, accession Lent 10, Lent 4 and Lent 5 attained the minimum value of seedling dry root weight (figure 10). The result showed that significantly reduction in mean root dry weight when the salinity concentration increase (figure10). This result showed analogous to earlier studies [30] on Lentil Akhtar and Azhar [31-32] on Gossypiumhirsutum, who reported that salt stress caused a significant decrease dry weight of root tissues.

5. Conclusions

Salinity is a continuing problem in the arid and semi-arid tracts of the world. It could be alleviate during irrigation management and/or crop management. However, the former approach is outdated and very expensive. Nevertheless, the latter is economical as well as efficient and it enables to produce salt tolerant crop lines. However, prior to that there is a need to confirm the presence of genetically based variation for salt tolerance among different species or varieties of a particular crop at different growth stages. The presence of genetic variation offers a basic tool for evaluating effect of salinity on lentil accessions and to overcome the presence of large number of variation for relatively salt tolerant lentil accession and it will appreciated to find accession with gene tolerant to salinity. Screening of salinity tolerance under field condition involves many environmental factors that affect genetic and phenotypic expression of accessions. Hence, controlled environment, Laboratory and greenhouse screening method indicate to be an ideal method to screen large amount of accessions with less efforts and accurately. Thus, the correct and clear expression of Lentil accessions for salt tolerant can be evaluated by this method using different NaCl level. The findings of this work confirmed that response of lentil accession to salinity show significant variation as their expose to different salinity level. The result explain that most out that all of the morpho-physiological and yield and yield related traits considered were significantly decreased with higher levels of salinity. Out of twelve lentil accession, accession Lent 12, Lent 2 and Lent 1 performed well under salt stress conditions in most of the parameter for both laboratory and greenhouse experiment as result those accession were recommended to be sown in saline condition.

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