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PHENOTYPIC CHARACTERIZATION OF SOME ETHIOPIA LENTIL FOR SALINITY

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ABSTRACT

To evaluate phenotypic variation of lentil for salinity in order to identify salt tolerant accessions at germination and early seedling stage, greenhouse experiment was carried out using twelve lentil accessions and four salinity levels. Seeds of 12 lentil accessions were grown with different levels of salinity (control, 2, 4, and dSm^{-1} NaCl) for one month. The experimental design was Randomized Complete Block Design (RCBD) with three replications. Germination time, germination percentage, salt tolerance index, seedling shoot and root traits were evaluated. The result showed that there were significant variation among and between lentil accessions, NaCl levels and their interaction for the entire traits (p<0.001).and salinity significantly delays and hinders seed germination; formation and the overall growth of seedling shoot and root traits. The degree of decrement was varied with accessions and salinity level. The greatest and the least values of germination percentage, salt tolerance index and length of plumule and radicle were observed at the control and highest salinity level respectively. However, accession Lent 12, Lent 1 and Lent 2 were performed well and gives better result than other accessions even at higher salinity level the entire traits, thus; these accessions are recommended for saline conditions.

Key word: Germination, lentil, Salinity, salt tolerant, Seedling stage

INTRODUCTION

Salinity is the most serious environmental factor that hinders agricultural productivity [12], with adverse effects on germination; plant potency and crop yield which leads to the death of the entire plant [32]. Salinity distributed though out the world and no continents is free from the problem [15] It mainly occur in arid and semi-arid and many irrigated areas regions of the world [3] because of, the lack of adequate amount of rainfall which leads to percolate the accumulated salt, and the use of underground water [11, 12] respectively. In Ethiopia, salt-affected soils are predominantly occurring in the Rift Valley and the lowland regions of the country [11]. Salinity hinders seed germination and yield productivity of the crop; by (i) reducing the osmotic potential, (ii) toxicity of specific ions such as sodium and chlorine and by (iii) reducing essential nutrients such as calcium and potassium [11], [12]. It also,

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affects many physiological, enzymatic and metabolic activities of plant that leads of the alteration of many enzymes to perform their function properly finally leads to reduction seed germination, growth and formation of shoot and root and seed yield production of the plant [27, ,29, 31].

Germination and Seedling growth under saline environment are the ideal screening criteria that are widely used to select the salt tolerance accessions/genotype [9], [11] because of, firstly, salinity tolerant at this stage was shown to be a heritable trait that enable the crop salt tolerant throughout its growth stage although, it links to many polygenetic basis [10] and [28] and Secondly, seeds and young seedlings are frequently 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 [5].

Lentil is good sources of protein, essential nutrient, and the ideal crop to fight malnutrition especially in developing country. Even if lentil is the best, multi-purpose crop they are salt sensitive [9], [11] and [12] hence, farmers do not consider growing them in a saline environment, though; there is a considerable difference in salt tolerance among crops/accessions [9]. Evaluation of lentil accessions to salt stress very important and screening of available lentil accessions is vital to find out salt tolerant accessions. Therefore, the objective of this study was to assess phenotypic variation of lentil accessions for salinity; specifically, to assess the effect of salt on germination and early seedling stage of lentil in order to identify salt tolerant accessions.

MATERIAL AND METHODS

Description of the Study Area and plant materials

The experiment was carried out at greenhouse, Haramaya University, Haramya, Ethiopia, from December to January, 2013. The average temperature and relative humidity of the greenhouse were 25 °C and 60 mg/l respectively and Seeds of twelve Lentil accessions were obtained from the Ethiopian Institute of Biodiversity.

Accessi	o Code	Region/ Zone	Woreda/	Latitude	Longitude	Altitude
n		State/	District			
9235	Lent 1	Oromiy Misrak	Meta	09-16-21-N	41-33-45-E	2535
36004	Lent 2	Amhara Semen Shew	aAnkober	09-39-00-N	39-41-00-Е	23180
36006	Lent 3	Oromiy Misrak	Gimbichu	08-57-00-N	39-05-00-E	2370
36019	Lent 4	Oromiy Mirab Shewa	a Alem Gena	a 08-48-00-N	38-20-00-Е	2150
36025	Lent 5	Gumuz Metekel	Wenbera	Unknown	Unknown	1580
36032	Lent 6	Oromiy Bale	Ginir	Unknown	Unknown	1520
36064	Lent 7	SNNP Bench Maji	Dirashe	Unknown	Unknown	Unknown
36093	Lent 8	Oromiy Mirab	Chiro	09-04-00-N	40-41-00-Е	E 2000

Table 1 Description of study area that were used in the experiment

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36094	Lent 9 Oromiy Mirab	Chiro	09-02-00-N	40-44-00-E	E 1870
36095	Lent 10 Somali Shinile	Afdem	Unknown	Unknown	1800
36113	Lent 11 Oromiy Misrak	Deder	Unknown	Unknown	Unknown
36120	Lent 12 Oromiy Mirab	Gawo Dal	le Unknown	Unknown	1870

Treatments and Experimental Design

Twelve accessions of Lentil and four NaCl solutions with salinity levels of control (0), 2, 4, and 8 deci Siemen per meter (dSm⁻¹) were prepared by dissolving 1.28, 2.56 and 5.12 gram (gm) of NaCl in one liter of water respectively. No salt was added in the control [7], [30]. The experiment was laid as Randomized Completed Block Design (RCBD) in a factorial arrangement and replicated three times [12]. The treatments were assigned randomly to each pot.

Experiment Procedure: The experiment was carried out using plastic pots with 19 cm diameter at the base and 20 cm at the top and 18 cm height and following the appropriate procedure [3] and [11]. The soil was filled into 144 pots. Then ten surface sterilized uniform seeds of each lentil accessions were sown in the plastic pots at uniform depth and distance. Then, the pot were arranged in RCBD replicated three times, and irrigated with equal 100 ml of NaCl solutions of 2, 4, and 8 dSm⁻¹, and tap water as control [12]. The EC of the tap water was measured by EC meter and it was 1.46 dS/m. Treatment application with the same amount of salt solution continued every one day and germination count started 5th day [12] and continued till 12th day Germination was recorded daily and a seed was considered be to germinated both plumule and radicle had emerged ≥ 0.05 cm [2], [11]. Table 2 soil analysis and its descriptions

Soil type	%	%	%silt	EC	pН
	sand	clay			
Sand clay loam	67.8	29.2	3	0.56	6.8

Data Collected

Germination time: refers to the average number of days needed for plumule and radicle emergence [18].

Germination percentage: fifteen days after sowing, all the germinated seeds were count, and the germination percentage was calculated as:

Germination percentage= $\frac{Number of seed germinated}{Number of seed sown} x 100$ [11]

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 at control after 30 day of sown [25].

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Fifteen days after germination, **shoot length** of 6 randomly selected seedlings from each pot were measured in centimetres and the average recorded as shoot length.

Fifteen days after germination, **root length** of 6 randomly selected seedlings from each pot were measured in centimetres and the average recorded as seedling root length

Seedling shoot to root ratio was calculated as the ratio of seedling shoot length to seedling root length

Seedling fresh shoot and root weight were measured after 30 days of sowing by weighting the mass of shoot and root of 6 randomly picked seedlings from each Pot using sensitive balance respectively.

Seedling dry shoot and root weight were measured after oven drying the seedling fresh shoot and root weight of 6 randomly picked seedlings by 80° C for 48 hours [11] [23].

Data Analysis

The data were analysed using SAS (Version 9.1) software and means were separate using the Least Significant Difference (LSD) test at 5% level of significance [11, 20].

RESULTS AND DISCUSSIONS

Effects of Salinity on Germination of lentil accessions

Germination time is the time need for seed germination and salinity highly significantly influenced germination time of lentil accessions. However, accessions responded differently to different salinity levels. For instance, at 2 dSm⁻¹ salinity level, accession Lent 12 and Lent 2 had the shortest germination time than the other accessions and these accessions were germinated quickly than other accessions while, accession Lent 10 and Lent 9 needed longest germination time and these accessions were germinated slowly. Furthermore, at 4 and 8 dSm⁻¹ salinity levels, accession Lent 1, Lent 12 and Lent 2 had taken the shortest germination time and these accessions were germinated quickly. Whereas, salinity highly delays seed germination of accession Lent 9, Lent 4 and Lent 3 hence, these accessions were germinated slowly (fig 1). The findings revealed that salinity highly delays the seed germination of lentil accessions and increment of salt concentration in the growth media cause a significance delay of seed germination of lentil accessions. The result was in full agreement with the previous studies of [11] [9], [35] and [25] in lentil and [16] in *L. perezii* reported that salinity delay seed germination of these plants.

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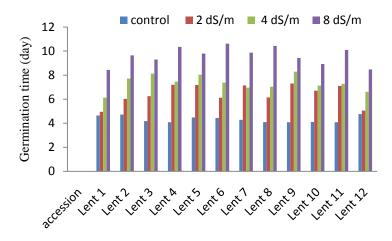


fig 1. Effect of salinity on germination of lentil accessions

Germination percentage analysis of variance showed that salinity highly hinders seed germination of lentil accessions. Among the accessions that were tested, accession Lent 1, Lent 2, Lent 12 achieved the higher germination percentage at 2, 4 and 8 dSm⁻¹ salinity levels while; accession Lent 4 and Lent 9 had the lower germination percentage (fig 2). The result indicates that salinity hinder seed germination of lentil accessions and increasing of salt concentration in the environment cause a significant reduction of germination percentage. The findings of this study were in line with previous research studies reported by [34] in pea, [25] and [11] in lentil and [5] in wheat who reported that a significant decrease in seed germination where observed while increasing salinity level. The reason is assumed to be salinity attributed to osmotic retention of water (water deficiency) and affects many metabolic and physiological activities which lead to seed dormancy of the plant.

Table 3.Analysis of variance for germination and seedling growth of lentil accessions as affected by different salinity levels

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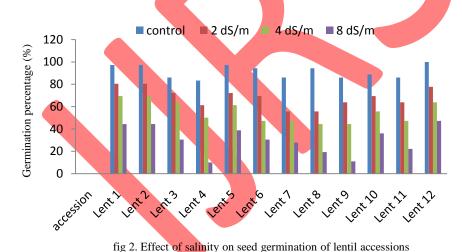
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Mean squares						
	NaCl (N) Accession(A) N x A		Error			
parameter	(df=3)	(df=11)	(df= 33)	(df= 94)	CV (%)	
GT	80.99**	1.08**	0.64**	0.24	9.82	
GP	23482.29**	851.90**	77.52**	26.55	8.52	
STI	66029.1**	265.29**	109.07**	2.13	3.72	
SSL	844.79**	16.19**	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	

*and ** = significantly different at 5% and 0.1% level of probability, respectively; GT= 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 root weight; SDSW=seedling dry shoot weight; SDRW=seedling dry root weight



Salt tolerance index the measure of the ability of the crop to tolerant salinity and analysis of variance (ANOVA) revealed that highly significant difference exist among accessions, salinity levels, and their interaction (Table 3). At 2 dSm⁻¹ salinity levels, the maximum value of salt tolerance index recorded in accession Lent 12, Lent 1 and Lent 2 while, salinity adversely reduced the overall growth of accession Lent 4 and this accession attained the minimum percent of salt tolerance index (fig 3). Furthermore, at 4 dSm⁻¹ salt concentration, accession Lent 1, Lent 12 and Lent 2 performed better than the other accessions and those accessions attained the

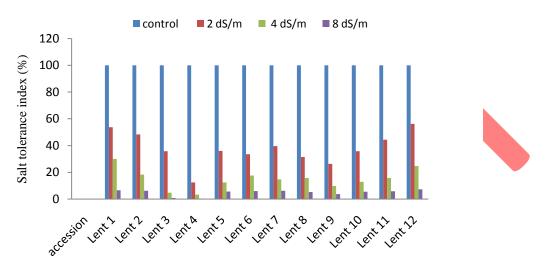
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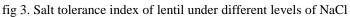
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highest value of salt tolerance index whereas, the minimum value of salt tolerance index was recorded in accession Lent 4 (fig 3). Besides this, at 8 dSm⁻¹ salinity levels, accession Lent 12, followed by accession Lent 2 and Lent 1 achieved the maximum values of salt tolerance index whereas, salinity highly hinders the overall growth of accession Lent 4 as result this accession achieved the lowest value of salt tolerance index (fig 3). The result indicates that salt tolerance index of lentil accessions were significantly reduced as salinity concentration increased (fig 3). The finding was in line with previous studies of [11] [23] who reported the salt tolerance index of lentil decrease with the increment of salinity.





Seedling shoot length (**SSL**) two way analysis of variance (ANOVA) showed that significant variation exists in seedling shoot length among accessions, salinity level and their interaction (Table 3). Accessions responded differently to different salinity levels. For instance, accession Lent 12, Lent 1, and Lent 2 attained the longest shoot length at 2, 4 and 8 dSm⁻¹ salinity levels than other accessions (fig 4). Whereas, the shortest seedling shoot length observed in accession Lent 9, Lent 10 Lent 8 and Lent 4 (fig 4). This result explained that increment of NaCl levels in growth media of cause a significant reduction of shoot formation and growth (fig 4). The findings of this studies show conformity with research result of [25] and [27] who reported that salinity inhibits elongation and growth of shoot of lentil. This reduction is probably because of genetic variation between lentil accessions and excessive accumulation of salts in the cell wall elasticity [4]. 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.

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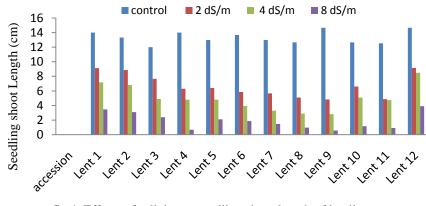


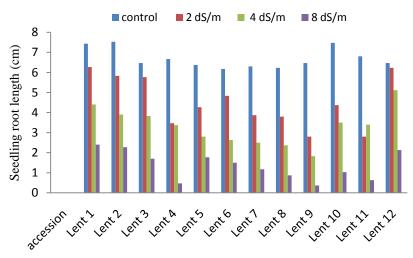
fig 4. Effects of salinity on seedling shoot length of lentil accessions

Seedling root length (**SRL**) The analysis of variance (ANOVA) revealed that there was highly significant variation in seedling root length among lentil accessions, salinity levels and their interaction (Table 3). Salt concentration used in the experiment adversely affect root elongation growth and cause significant reduction of root length but the degree of reduction depend on salt concentrations and accessions. Some accession were performed better that other even at higher salinity levels. For instance accession Lent 1, Lent 12 and Lent 2 attached the longest root length in all salinity levels that other accessions while accession Len 9 achieved the shortest root length in all treatment (fig 5). The result explain that the increment of NaCl concentration in the environment cause a significant reduction of seedling root length of lentil (fig 5). The result is in full agreement with [10], [11] in lentil; [6] in senna plant; [17] in pea who reported that high salinity reduced root length in these plants.

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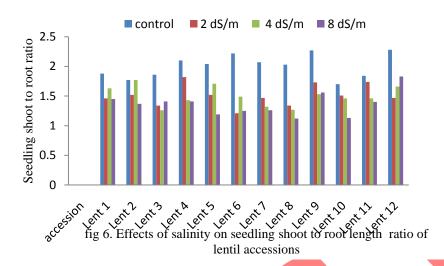
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fig 5. Effects of salinity on seedling root length of lentil accessions

Shoot to root length ratio (SRR) the two-way analysis of variance (ANOVA) showed that significant variations exist among accessions, salinity levels and their interaction (p < 0.001) (Table 3). Accession Lent 4, Lent 10, Lent 2, Lent 12, and Lent 9 exhibited relative higher value of seedling shoot length as result the significantly higher mean seedling shoot to root ratio were observed in these accessions in the 2, 4, and 8 dSm⁻¹NaCl levels (fig 6) whereas, the lower values of seedling shoot to root length ratio were observed in accession Lent 6 and Lent 8. 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 [19 & 26] in haricot bean, [1] in faba bean reported that SRR was highly reduced at higher salinity levels for some accessions and the accessions showed significant variation in their response to salinity.

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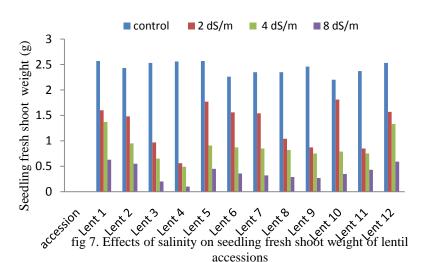
Seedling fresh shoot weight (SFSW) Two way of analysis of ANOVA confirmed that there were highly significant differences among accessions. NaCl treatments and their interaction (p < p0.001) (Table 3). Salinity cause a significant reduction of seedling fresh shoot weight of lentil accessions. At 2 dSm⁻¹ salinity level, accession Lent 1 followed by accession Lent 12 and Lent 2 attained the maximum value of seedling fresh shoot weight than the rest of the accessions (fig 7) while, accession Lent 11 and Lent 4 had the minimum value of seedling fresh shoot weight (fig 7). Moreover, at 4 and 8 dSm⁻¹ salinity level, accession Lent 1, Lent 12 and Lent 2 performed well and achieved the maximum value of seedling fresh shoot weight than the other accessions whereas, salinity inhibited shoot growth of accession Lent 4 (fig 7). Salinity reduced shoot growth of all accessions 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 the maximum value of seedling fresh shoot weight even at higher salinity level than the other accessions (fig 7). The result showed that the seedling fresh shoot weights of lentil accessions significantly reduced with increment salinity level (fig 7). The result was in line with previous research findings of [34] who reported as that salinity adversely reduced seedling fresh shoot weight of leguminous plants under saline environment. This reduction maybe 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 [21, 38].

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Seedling root fresh weight (SRFW) Two way analysis of ANOVA for seedling fresh root weight revealed that there were highly significant difference was occurred among lentil

accessions, NaCl treatments and their interaction (p <0.001) (Table 2). Salinity cause a significant reduction of seedling fresh root weight of lentil accessions but the reduction was depend on salinity levels and genetic makeup of the accessions. Accession .Lent 12, Lent 1 and Lent 2 performed well under salinity conditions and attained the highest value of seedling fresh root weight in all salinity levels (fig 8) whereas, salinity hinders the root growth of accession Lent 4 and thus, this accession attained the lowest value of seedling fresh root weight (fig 8). This result explained that salinity reduced seedling fresh root weight of lentil accessions (fig 8). The finding of this result is in line with the result of findings of [11, 25] and [26] who reported salinity increment significantly reduced fresh root weight in Lentil and phaseolus species, respectively.

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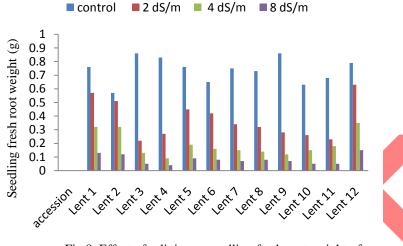


Fig 8. Effect of salinity on seedling fresh root weight of lentil accessions

Seedling dry shoot weight (SDSW) two way analysis of variance (ANOVA) for seedling shoot dry weight revealed that there were highly significant differences among accessions, NaCl treatments and accessions * treatment interaction (p < 0.001) (Table 2). Variation was occurred for the formation and growth of seedling shoot among accessions and salt concentration. For instance, accession Lent 1 Lent 12 and Lent 2 performed well and achieved the maximum value of seedling dry shoot weight than the other accessions (fig 9) whereas, accession Lent 4 showed the minimum value of seedling dry shoot weight in all salt concentrations (fig 9). This result revealed that salinity antagonistically reduced seedling dry shoot weight of lentil (fig 9). The findings of the current study were full agreement with previous research result of [39] and [23] on lentil, [14] in *phaseolus* species, reported that increasing the concentration of salinity cause significant reduction in shoot growth consequently reduced seedling dry shoot weight.

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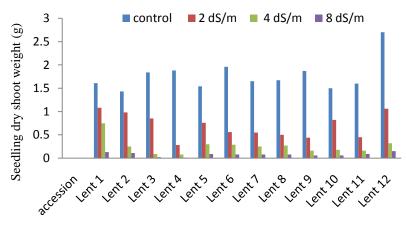


fig 9. Effects of salinity on Seedling dry shoot weight of lentil accessions

Seedling root dry weight (SRDW) two way analysis of variance for seedling dry root weight showed that highly significant difference were occurred among accessions, salinity levels and their interactions (p<0.001) (Table 3). Some accession achieved the highest value of seedling dry root weight in contrast to this; some accessions were performed poorly and achieved the lowest value of seedling dry root weight. For instance accession Lent 12, Lent 1 and Lent 2 achieved significantly higher mean seedling dry root weight than the other accession in all salt concentrations (fig 10). In contrast to this, the minimum value of seedling dry root length was recorded in accession Lent 9 and Lent 4 (fig 10). The result showed that salinity causes significantly reduction in mean root dry weight of lentil (fig 10). This result was in line with the previous studies by [13] on Lentil and [4-8] on different crops, who reported that salt stress caused a significant decrease dry weight of root tissues.

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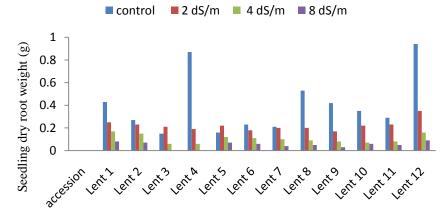


fig 10. Effects of salinity on seedling dry root weight of lentil accessions

CONCLUSION

Salinity is the current most serious problem across the globe that hinders agricultural productivity. It could be alleviate during irrigation and/or crop management. However, the former approach is outdate and very expensive. Nevertheless, the latter is cost-effective as well as efficient and it enables to produce salt tolerant crop. Though, confirming the presence of genetically based variation for salt tolerance among different varieties /accessions of a particular crop at each specific stage is crucial for breeding, and conservation program. The existence of genetic variation offers a basic tool for evaluating the effect of salinity on lentil accessions and to overcome the presence of large number of variation for relatively salt tolerant lentil accessions in order to find out accessions with genetic potential for salt tolerance. Screening of salinity tolerance under uncontrolled condition involves many environmental factors that affect genetic and phenotypic expression of accessions. Hence, greenhouse experiment screening method indicates 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 levels. The findings of the present work elucidate that response of lentil accession to salinity show significant variation as their expose to different salinity level. The result explains that most out that all of the morpho-physiological traits considered were significantly decreased with higher levels of salinity. Out of twelve lentil accessions, accession Lent 12, Lent 2 and Lent 1 performed well under salt stress conditions in most of the parameter; as result these accession were recommended to be sown in saline conditions.

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