Screening of local/exotic accessions of lentil *(Lens cufinaris* **Medic.) for salt tolerance at two growth stages**

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Abstract

Screening of available local/exotic germplasm of a crop for salinity tolerance is of considerable value for the economic utilization of salt-affected soils of arid and semi-arid regions.

The response of 133 lentil *(Lens culinaris* Medic.) accessions, to NaC1 at the germination and seedling stage, was examined. A great amount of variation of NaC1 tolerance in lentil was observed at both the growth stages but, in general, there was no consistent relationship between tolerance assessed at germination or at the seedling stage. In the NaCI treatment five accessions, ILL 5845, ILL 6451, ILL 6788, ILL 6793, and ILL 6796 produced significantly greater fresh and dry plant biomass in both absolute and relative terms than the others, but these accessions performed as well as other intermediate or low biomass producing accessions in total germination percentage and rate of germination. In view of the existence of the great amount of variability of tolerance to NaCI among lentil varieties improvement in NaC1 tolerance in this species is possible.

Introduction

Soil salinity is one of the major impediments to agriculture in arid and semi-arid regions of the world (Akbar and Yabuno, 1974; Ashraf *et al.,* 1986; Epstein, 1985; Kingsbury and Epstein, 1984; Shannon, 1985). A biotic approach to overcoming the salinity problem has been considered the most feasible and economic path and it has recently received much attention (Ashraf and McNeilly, 1987; Epstein and Norlyn, 1977).

The different strategies that have been adopted by various plant scientists, in employing a biological approach to overcoming the salinity problem, have been listed (Ashraf and McNeilly, 1988). One of the procedures, which is of prime importance, is the screening of available local/ exotic germplasm of a crop for salt tolerance to identify a tolerant cultivar/line which may, hopefully, sustain a relatively reasonable yield on salt affected soils (Ashraf and McNeilly, 1987; Kingsbury and Epstein, 1984).

Lentil *(Lens culinaris* Medic.) is one of the major pulse crops throughout the world and has considerable importance as food, feed, and forage. The crop is sensitive to salinity, like many other leguminous crops, and may not sustain good yield even if it is grown in soils with low salt concentrations (Ashraf, personal communication). Therefore, the screening of a large number of available germplasm may provide a relatively tolerant cultivar/line.

It is generally accepted that the germination and seedling stages of the plant life cycle are more sensitive than the adult stage (Ashraf *et al.,* 1986). In tomato, Pasternak *et al.* (1979) observed that the seedling stage, of seven cultivars, was more sensitive to salinity stress than their adult stage. Under saline conditions they found a high correlation between early growth stages and yield. Similarly, Greenway (1965) and Blum (1985) considered that the response of seedlings to salinity was highly predictive of the response of the adult plants to salinity. Allen *et al.* (1986), working with alfalfa, showed a high correlation, under saline conditions, between the germination stage and biomass yield.

In contrast, it has been argued, by a number of workers, that in some crops selection for salt tolerance at the early growth stages may not correlate with their tolerance at the later growth stages (Ashraf and McNeilly, 1988; Kingsbury and Epstein, 1984; Shannon, 1978). Nevertheless, seed germination and seedling establishment are the most critical stages in life cycles of plants in a saline environment (Blum, 1985) and are of importance in assessing the overall tolerance of a crop to salinity stress (Akbar and Yabuno, 1974; Ashraf *et al.,* 1986).

Keeping this in mind, the work presented here was carried out to examine the intraspecific variation of salt tolerance in 133 local and exotic accessions/lines of lentil, during their germination and later seedling stage, when exposed to different NaC1 concentrations.

Materials and methods

The 133 accessions used in the study and their identification and sources are given in Table 1. The seed material was mainly obtained through personal contacts. It has been mentioned earlier that the degree of salt tolerance of a plant species varies with the growth changes throughout its life cycle. Therefore, screening of the available germplasm was carried out at the two initial growth stages of germination and seedling.

Germination experiment

Seed samples of the 133 accessions of lentil were surface sterilized in 5% sodium hypochlorite solution for five minutes prior to experimentation. Plastic Petri dishes with internal diameter of 6 cm were used. The salt treatments of NaC1 used were 0 (control), 50, and 100 mol m^{-3} dissolved in full strength nutrient solution (attri-

buted to Rorison, in Hewitt, 1966) and given as,
Ca(NO₃), 4H₂O 2 mol m⁻³; K₂HPO₄ $Ca(NO₃)₂$ 4H₂O 2 mol m⁻³; K₂HPO₄ 0.5 mol m⁻³; MgSO₄ 7H₂O, 1 mol m⁻³; KCl, 1 mol m⁻³; Fe-EDTA, Fe $\overline{3}$ ppm; MnSO₄ 4H₂O, Mn 0.5 ppm; H_3BO_3 , B 0.5 ppm; (NH_4) ₆ Mo O_{24} , Mo 0.11 ppm; ZnSO₄ 7H₂O, Zn 0.10 ppm; $CuSO₄$ 5H₂O, Cu 0.10 ppm). Only NaCl was used in this study as it is one of the most common salts occurring in most of the saline areas of Pakistan (Ashraf and Yasmin, unpublished data).

The germination experiment was conducted in a growth room at 26 ± 2 °C, with 12 h day length, at a light intensity of 36 W m^{-2} and relative humidity of 76%. The plastic Petri dishes were arranged in a completely randomized design, with three replicates, three salt treatments and 133 accessions. 17 surface sterilized seeds of each accession were placed on filter paper in each Petri dish. 5 mL of appropriate treatment solution was applied on alternate days to each Petri dish after rinsing out the previous solution.

The number of seeds germinated were counted daily and data were recorded for two weeks. A seed was considered to have germinated when both plumule and radicle had emerged ≥ 0.5 cm. Total germination was expressed as a percentage of that in the control treatment for each accession and then data were arcsine transformed for the statistical analysis. Rate of germination was determined on the basis of days to 50% germination, calculated from the untransformed data.

Seedling experiment

Ordinary river sand was washed thoroughly with tap water, then with distilled water and finally with full strength nutrient solution. Plastic beakers of 500 cm^3 size were filled with the washed dry sand. The experiment was conducted in a greenhouse at 24 ± 3 °C day temperature and 12 ± 1 °C night temperature.

The concentrations of NaCl used were 0 (control), and 50 mol m^{-3} in full strength nutrient solution. The experiment was placed in a completely randomized design, with two replicates, 133 accessions and two salt treatments. Seven two-week-old seedlings of each accession were transplanted, equidistant from each other, into

 $\ddot{\cdot}$ $\frac{1}{2}$ $\overline{\mathbf{r}}$ Ě $\ddot{}$ \ddot{a} each beaker. All the beakers were irrigated for one week with full strength Rorison nutrient solution every alternate day.

NaC1 treatment was begun three weeks after the start of the experiment. The salt concentration was increased in aliquots of 25 mol m^{-3} on alternate days until the appropriate salt treatment was reached. Treatment continued with the addition of 500 mL of the appropriate solution after every 2 days.

The plants were harvested three weeks after the start of salt treatment. Plant roots were removed carefully from the sand. Shoots and roots were washed with distilled water and separated. The fresh weight of all the samples was recorded. Plant material was then dried at 70°C for four days and the dry weight was recorded.

Data for mean plant dry weight was expressed as relative dry weight as follows:

Relative plant dry weight

 $=\frac{\text{Plant dry weight at salt concentration}}{\text{Plant dry weight at control treatment}} \times 100$

Results

Germination experiment

Analyses of variance of the data for germination percentage (Arcsine transformed data) and rate of germination of the 133 accessions of lentil show that salt treatment had a significantly adverse effect on total germination percentage and rate of germination (total germination percentage $p \le 0.05$; rate of germination $p \le 0.001$). Accessions differed significantly in both characteristics (both $p \le 0.001$). Increasing NaCl concentration markedly reduced total germination percentage and also decreased the speed of germination of all accessions. However, the interaction terms, cultivars \times NaCl concentrations were also significant for the two characteristics $(p \le 0.001)$, showing that different accessions responded differently to increasing NaCI concentration in the rooting medium.

All the accessions tested in this study were classified into six groups depending on their ability to germinate in saline medium (Table 2).

Most of the accessions had total germination percentages greater than 90% in saline medium. But there were two accessions, which showed very poor germination (40 to 50%) in NaC1 solution. However, classification of the accessions showed that there was tremendous variability of germination in the presence of NaC1 salinity.

In order to simplify data presentation and interpretation, a subsample of the data of 11 accessions out of the 133 assessed has been extracted for more detailed consideration, on the basis of their performance in biomass production at the seedling stage. The 11 include the 5 most tolerant $(1, 2, 3, 4, 5)$, 3 intermediate $(6, 7, 8)$ and 3 least tolerant (9, 10, 11). The accessions, ILL 6451, ILL 6788, ILL 6431 and ILL 6439, each had a total germination percentage greater than 100. This may have been due to either the regulatory effect of salt on germination, or variability in the seed sample reducing control values.

Data for total germination percentage and rate of germination, presented in Table 3, shows that ILL 6210 (No. 9) had the lowest germination percentage in both salt treatments, but had a higher rate of germination as compared to the other 10 accessions. The other 10 accessions did not differ significantly in germination percentage and rate of germination.

Seedling experiment

Analyses of variance of the data for shoot and root water content, and shoot/root ratio for the 133 accessions show that NaCI treatment had a significantly adverse effect on all characteristics measured (shoot and root water content $p \leq$ 0.05; shoot/root ratio $p \le 0.01$). Cultivars also differed significantly (all $p \le 0.001$). The interaction terms, accessions \times NaCl, are also significant for all characteristics (all $p \le 0.001$) which again showed that different accessions responded differently to NaCI treatment.

All the accessions examined in this study were classified into four groups on the basis of their performance in relative dry shoot biomass production (Table 4). There were only seven accessions, ILL 6196, ILL 6205, ILL 6430, ILL 6434, ILL 6765, ILL 6793, and ILL 6796 that produced

172 *Ashraf and Waheed*

Code No.	Accession No.	Total germination percentage		Rate of germination			
		NaCl concentrations mol m^{-3}					
		50	100	Cont.	50	100	
	ILL 5845	100	100	2.8	3.5	4.3	
	ILL 6451	116.7	108.9	3.9	4.2	5.8	
3.	ILL 6788	105.0	98.0	3.2	3.2	4.6	
4.	ILL 6793	100	100	3.6	3.7	4.3	
5.	ILL 6796	100	100	3.9	4.5	5.0	
6.	ILL 6431	107.7	129.2	4.2	4.2	5.5	
7.	ILL 6770	100	100	3.9	4.5	4.5	
8.	ILL 6784	100	86.66	3.6	3.7	5.2	
9.	ILL 6210	62.2	85.6	3.2	2.8	3.5	
10.	ILL 6439	105.0	98.0	2.4	3.3	5.2	
11.	ILL 6778	100	100	3.7	4.2	4.5	
	LSD $5%$	30.6			1.21		

Table 3. Mean germination percentage (percent of control) and rate of germination (days to 50% germination) of 11 accessions of lentil at different NaC1 concentrations in full strength nutrient solution

greater than 120 percent dry shoot biomass. However, in the second group (80-120%), there were 20 accessions that had relative shoot dry matter in the range of 80-120%. But in the third and fourth groups, there were 63 and 43 accessions that produced shoot dry matter in the ranges of 40-80 and 0-40% respectively.

As stated earlier a subsample of 11 accessions was extracted, for detailed consideration, from the 133 listed in Table 1. The data for relative shoot and root dry weight, of the subsamples selected, are presented in Table 5. Although the growth of the 11 accessions was markedly reduced at 50 mol m^{-3} NaCl, ILL 5845, ILL 6451, ILL 6788, ILL 6793, and ILL 6796 produced significantly greater relative shoot and root dry biomass than the other six intermediate and sensitive accessions. The two sensitive accessions, ILL 6210 and ILL 6439, died at 50 mol m^{-3} NaCl, whereas the other 4 intermediate and sensitive accessions (No. 6, 7, 8, and 11), had very low relative shoot and root dry biomass at that salt treatment.

There was not any significant effect of NaC1 treatment on the shoot and root water content of the 5 tolerant accessions (Table 6). However the shoot and root water content, of the intermediate and sensitive accessions, decreased at 50 mol $m⁻³$ as compared to that of the control treatment.

Although there was considerable variation among the 133 accessions for shoot/root ratio (Table 6), there was no consistent pattern of increase or decrease in shoot/root ratio in the subsample of 11 accessions. Only the intermediate accession, ILL 6784 had a significantly ($p \le 0.05$) greater shoot/root ratio than the other accessions at 50 mol $m⁻³$ NaCl.

Discussion

It is now evident that the existence of the genetic variation in salt tolerance is a pre-requisite for the development of salt tolerant cultivars through selection and/or breeding. To explore such variation in lentil, 133 accessions were screened at the two early growth stages of germination and seedling, as salt tolerance throughout these two stages are crucial for establishment of a crop in a saline environment.

Salt tolerance may be measured in terms of absolute growth at a given salt concentration, or in relative terms depending upon the growth potential of a particular accession under nonsaline conditions. However, both the methods are equally important to know the ultimate tolerance of a cultivar. The relative measure was considered especially important in a study in which diverse plant material had been used

Code No.	Accession No.	Relative shoot dry weight	Relative root dry weight	
	ILL 5845	110.3	81.05	
2.	ILL 6451	82.7	41.6	
3.	ILL 6788	72.7	62.3	
4.	ILL 6793	141.8	135.5	
5.	ILL 6796	145.0	170.4	
6.	ILL 6431	36.1	88.0	
7.	ILL 6770	35.0	22.0	
8.	ILL 6784	59.4	33.5	
-9.	ILL 6210	0.0	0.0	
10.	ILL 6439	0.0	0.0	
11.	ILL 6778	23.5	23.0	

Table 5. Relative dry weights (%) of shoots and roots of 11 accessions of lentil after 21 days growth in sand culture salinized with 50 mol $m⁻³$ NaCl in nutrient solution

(Shannon, 1984), whereas in contrast, Dewey (1960) considered absolute growth important under salinity stress regardless of growth in controlled conditions while screening 25 strains of *Agropyron* species at germination. In our study 133 accessions have been assessed for their ability to sustain growth in NaCl in relative terms as in Shannon (1984).

Data for different growth parameters, at the seedling stage, presented in Tables 5 and 6, clearly show that only five accessions, ILL 5845 (110.3%), ILL 6451 (82.8%) ILL 6788 (72.7%), ILL 6793 (141.5%), and ILL 6796 (145%) produced significantly greater biomass in relative terms and had a higher percent plant water content than all the other accessions. Therefore, the five accessions were categorized as the most NaC1 tolerant among the 133 accessions appraised in this study.

In the germination experiment, no consistent correlation was found between data for total germination percentage and rate of germination. For instance, accession ILL 6431, an intermediate in seedling biomass production in NaC1, had the highest total germination percentage (Table 3), but was slower in germination as compared to the other accessions. Similar results were also determined in some cultivars of wheat (Ashraf and McNeilly, 1988), mash (Ashraf *et al.,* 1988), maize (Ashraf and McNeilly, 1989).

If parallels are drawn between data for two initial growth stages, the tolerance observed in 5 accessions, ILL 5845, ILL 6451, ILL 6788, ILL 6793, and ILL 6796, at the seedling stage, was

Code No.	Accession No.		Shoot moisture content		Root moisture content	Shoot/root ratio		
		NaCl concentrations mol m^{-3}						
		Cont.	50	Cont.	50	Cont.	50	
ı.	ILL 5845	86.32	87.39	92.55	94.5	1.22	1.87	
2.	ILL 6451	84.5	81.38	90.05	90.56	1.32	2.77	
3.	ILL 6788	85.43	87.44	88.73	90.40	1.27	1.48	
4.	ILL 6793	83.62	84.66	89.94	90.64	1.86	2.005	
5.	ILL 6796	85.53	84.29	92.89	89.00	1.96	1.76	
6.	ILL 6431	84.89	72.56	89.39	60.55	1.13	0.51	
7.	ILL 6770	85.94	80.71	91.46	90.16	1.12	1.87	
8.	ILL 6784	84.88	79.7	91.74	83.33	2.26	5.16	
9.	ILL 6210	83.33	$\bf{0}$	91.25	$\bf{0}$	1.75	$\bf{0}$	
10.	ILL 6439	85.71	$\bf{0}$	93.21	0	1.53	$\bf{0}$	
11.	ILL 6778	84.47	72.41	89.9	87.68	1.14	1.16	
	LSD $5%$		8.2		9.1	0.86		

Table 6. Mean percent plant water content, and shoot/root ratio of 11 accessions of lentil after 21 days growth at 0, and 50 mol m $^{-3}$ NaCl in full strength nutrient solution

not conferred at the germination stage, because the 5 accessions, which were highly tolerant at the seedling stage, did not differ from the intermediate and sensitive accessions in total germination percentage and rate of germination. These results are in close conformity with the early findings for rice (Akbar and Yabuno, 1974) and for wheat (Kingsbury and Epstein, 1984; Ashraf and McNeilly, 1988) which also reported that salt tolerance varies with the different growth stages of the crops.

It has been argued that selection for salinity tolerance at the seedling stage may not produce tolerant adult plants (Kingsbury and Epstein, 1984; Shannon, 1979). In contrast, the performance of seedlings under saline conditions has been considered highly predictive of the response of adult plants to salinity (Azhar and McNeilly, 1987; Blum, 1985; Greenway, 1965). Norlyn (1980), Kingsbury and Epstein (1984), and Ashraf *et al.* (1986) working with barley, wheat, and seven grass species, screened seedlings of these species and derived plants that showed considerable tolerance to salinity at the adult stage.

The results presented in this study deal with the salt tolerance of the accessions at the two initial growth stages. The tolerance observed in the five accessions, ILL 5845, ILL 6451, ILL 6788, ILL 6793, and ILL 6796 may or may not be conferred at the adult stage. Nevertheless tolerance observed at the two first stages is of great importance because it has been emphasized by many workers that the assessment of salt tolerance at every growth stage of a plant species is of considerable value in determining the ultimate tolerance of the species (Akbar and Yabuno, 1974; Ashraf and McNeilly, 1987). In consideration of the severe effect of salt on early growth stages, the growth of a crop cultivar in Pakistan is facilitated by leaching the salt and also by other management practices. Therefore, knowing the tolerance which was observed at the germination and seedling stage of some accessions would be of considerable economic value for crop establishment on salt-affected soils.

The highly salt tolerant accessions found in the diverse germplasm of lentil, examined in this study, could be of considerable economic value in increasing yield on saline areas with moderate salinity, provided the lines are still tolerant when adult and also have adaptability to other factors encountered in salt-affected soils. Information about the responses of the 11 accessions at the adult stage is of considerable interest and will be reported in a subsequent paper.

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176 *Salt tolerance of lentil*

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