

INTER-ACCESSIONAL VARIATION FOR SALT TOLERANCE IN PEA (*PISUM SATIVUM* L.) AT GERMINATION AND SCREENING STAGE

ZAHRA NOREEN^{1*}, MUHAMMAD ASHRAF¹ AND MAHMOOD-UL-HASSAN²

¹*Department of Botany, University of Agriculture, Faisalabad.*

²*Department of Statistics, Islamiya University, Bahawalpur, Pakistan*

**Corresponding author: zohra_noreen@yahoo.com*

Abstract

In order to improve salt tolerance, it is important to explore inter-cultivar genetic variation for salt tolerance, so a greenhouse experiment was conducted to screen 11 local accessions of pea (*Pisum sativum* L.) at the germination and seedling stages. Seeds of 11 local pea accessions were grown at five different levels of salinity (0, 60, 120, 180 and 240 mM NaCl) for two weeks. Both speed of germination and germination percentage of seeds were severely reduced due to increasing intensity of salt stress. Similarly, growth of all pea accessions examined as fresh and dry weights of shoots and roots declined due to salt stress. However, a great magnitude of variation for salt tolerance was observed in the set of pea accessions in terms of all attributes measured. On the basis of salt tolerant indices, the 11 accessions were categorized into three groups i.e., salt sensitive, moderately salt tolerant and salt tolerant. Although, a substantial amount of genetic variation for salt tolerance existed in the available germplasm of cultivars, the germination percentage or speed of germination were not found effective for screening purpose. Overall, cv. Meteor followed by 9200 was found to be salt tolerant which could perform well on saline soils at least at the early growth stages.

Introduction

Exploration of variation for salt tolerance at inter-specific and intra-specific levels is a pre-requisite for improving the trait through a breeding program (Akbar & Yabuno, 1977; Akbar *et al.*, 1986; Azhar & McNeilly, 1988; Al-khatib *et al.*, 1994; Ashraf, 1994; 2004; Takeda *et al.*, 1995; Mano & Takeda, 1997). In view of a number of earlier studies it is evident that a great magnitude of intra-specific variation exists in different crops e.g., wheat (Kingsbury & Epstein, 1984; Ashraf & McNeilly, 1988), cotton (Ashraf & Ahmad, 1999), barley (Belkhodja *et al.*, 1994), lentil (Ashraf & Waheed, 1993). However, variation in different crops has been assessed at a specific growth stage rather than at different growth stages. This causes a problem in assessing the overall degree of salt tolerance of a crop, particularly in that whose degree of salt tolerance varies with the developmental stage. In such crops the plausible way is to assess degree of salt tolerance at each growth stage (Ashraf & Khanum, 1997; Ashraf, 2002).

Pea (*Pisum sativum* L.), is one of predominant export crops in the world trade and it represents about 40% of the total trade in pulses (Oram & Agcaoili, 1994). Although the crop is ranked among the salt sensitive crops like other leguminous crops and produce low yield even at mild salt stress (Francois & Mass, 1994), a detailed information on genetic variability for salt tolerance is still lacking in the literature.

Although our long-term objective is to assess inter-accessions/cultivars variation for salt tolerance in the available accessions/cultivars of pea at different phases of development, in the present study only variation for salt tolerance was assessed at the

germination and seedling stage, because these two initial stages may play a vital role in the ultimate development and survival of a crop under stressful environment. In addition, the accessions were ranked using multivariate analysis of multiple agronomic parameters at the germination and seedling stages.

Materials and Methods

Seeds of nine pea accessions (2001-20, 2001-35, 2001-40, 2001-55, 9800-5, 800-10, 9200, Tere-2 and Climax) were obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad, while those of two cultivars, Indian Azad P-1 and Meteor from the local market. The study was carried out in a growth room of the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The experiment was conducted in a completely randomized design (CRD) in a factorial arrangement with four replications. Four hundred seeds of each pea cultivar/accession were surface sterilized in 5% Sodium hypochlorite solution for 10 min., and then thoroughly rinsed with distilled water. Twenty seeds of each cultivar/accession were allowed to germinate in a Petri plate double lined with filter paper moistened with 10 mL of Hoagland's nutrient solution along with five levels of NaCl (0, 60, 120, 180 and 240 mM). Salt levels were maintained each day by dripping out and applying fresh nutrient solution twice. Germination started after three days of sowing. Germination was recorded daily and a seed was considered germinated when the radicle reached 5 mm in length. The germination results were expressed in terms of a promptness index (PI) following George (1967).

$$PI = nd2(1.00) + nd4(0.75) + nd6(0.50) + nd8(0.25)$$

where nd2, nd4, nd6 and nd8 = number of seeds germinated on the 2nd, 4th, 6th and 8th day, respectively. A germination stress tolerance index (GSTI) was expressed in percentage and calculated as follows:

$$GSTI = \frac{PI \text{ of stressed seeds}}{PI \text{ of control seeds}} \times 100$$

After fifteen days, plant seedlings were removed from the Petri plates and separated into shoots and roots and fresh weights recorded. Then they were oven-dried at 65°C for three days and their dry weights recorded.

Ranking of pea cultivars/accessions for salt tolerance: For comparing cultivars/accessions for salt tolerance, all the data were transformed following Zeng *et al.*, (2002) into salt tolerance indices i.e., means of each parameter of salt stressed plants divided by the means of their respective controls (Table 5). Cluster group ranking numbers were assigned to cluster groups based on cluster means, and used to score cultivars using JMP ver. 6, 2005 release software (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). The cluster analysis was based on Ward's minimum variance cluster analysis of the averages of the salt tolerance indices for all parameters. Pea accessions were ranked on the basis of Ward's minimum variance cluster analysis of the averages of the salt tolerance indices of two groups of parameters, one containing cluster group rankings based on indices of germination percentage and promptness index (PI) (Table 10), while the other group based on shoot fresh weight, shoot dry weight, root fresh weight and root dry weight (Table 11). A sum was obtained by adding the number of cluster group

rankings at each salt level in each accession. The accessions were finally ranked on the basis of sums, such that those with the smallest and largest sums were ranked as the tolerant and sensitive cultivars/accessions, respectively in terms of relative salt tolerance.

Statistical analysis of data: The untransformed data for each parameter were subjected to analysis of variance (ANOVA) using the COSTAT v 6.3, statistical software (Cohort Software, Berkeley, California). The mean values were compared with the least significance difference test following Snedecor & Cochran (1980).

Results

A great magnitude of variation was observed in the set of pea lines for relative salt tolerance indices for all measured parameters (Table 9). Analysis of variance of the data for germination percentage of pea cultivars showed that salt stress caused a significant reduction ($p \leq 0.001$) in germination percentage of all cultivars (Table 1 & 3). However, a significant inter-cultivar variation was observed among pea cultivars when exposed to saline conditions. Under mild salt stress (60 mM) Tere-2 had the lowest salt tolerance index (0.85), while Meteor had the maximum salt tolerance index (1.00). In comparison with other cultivars, 2001-35 had the highest salt tolerance index of germination percentage (1.00) at 60 mM NaCl, but it showed the lowest germination percentage (0.28 salt tolerance index) under 240 mM of NaCl.

Promptness index (PI) was significantly ($p \leq 0.001$) reduced in all accessions with increase in salt stress. Maximum PI was found in 2001-20 followed by 9800-5 and 9800-10, while the lowest in accession Tere-2 at 60 mM NaCl (Table 2). However, at the highest salt level (240 mM), accession 9200 ostentatious was the highest while 2001-40 the lowest in PI (Table 9). At the moderate salt level (120 mM), the PI values ranged from 0.45 to 0.69.

The cluster analysis based on germination percentage and PI showed that accessions Meteor and 9800-5 (Fig. 1) were the least affected, while accessions Indian Azad P-1 and 2001-55 the most due to salt stress.

Seedling shoot fresh and dry weights were significantly different among all pea cultivars (Table 4 & 8). Maximum values of salt tolerance indices, worked out using data for shoot fresh and dry weights, were observed in cv. Meteor at the mild salt stress. At 240 NaCl, Tere-2 showed considerable reductions in salt tolerance indices, i.e. 0.29 and 0.37 for shoot fresh and dry weights, respectively, while cv. Meteor exhibited maximum indices, (0.71 and 0.519) at this salt level.

The salt tolerance indices worked out using data for root fresh and dry weights were decreased significantly with increase in external salt regime (Table 6, 7 & 9). For instance, salt tolerance indices for these parameters ranged from 0.68 to 0.97 at low salt level, whereas at the highest salt level (240 mM) the indices for root fresh and dry weights ranged from 0.36 to 0.55 and 0.28 to 0.55, respectively. Based on cluster analysis, the cultivars were divided into five cluster groups at all salt levels and then ranked into three classes, tolerant, moderate and sensitive (Table 10 & 11). Cultivars Meteor, 9200 and 2001-20 were found to be tolerant, whereas 2001-35 and Climax were sensitive (Fig. 2).

Overall cultivar Meteor was tolerant in terms of ranking based on germination percentage and promptness index as well as relative salt tolerance based on seedling biomass, while accession 2001-35 the sensitive among all cultivars/accessions examined. The remaining lines had no consistent pattern in the two different modes of ranking.

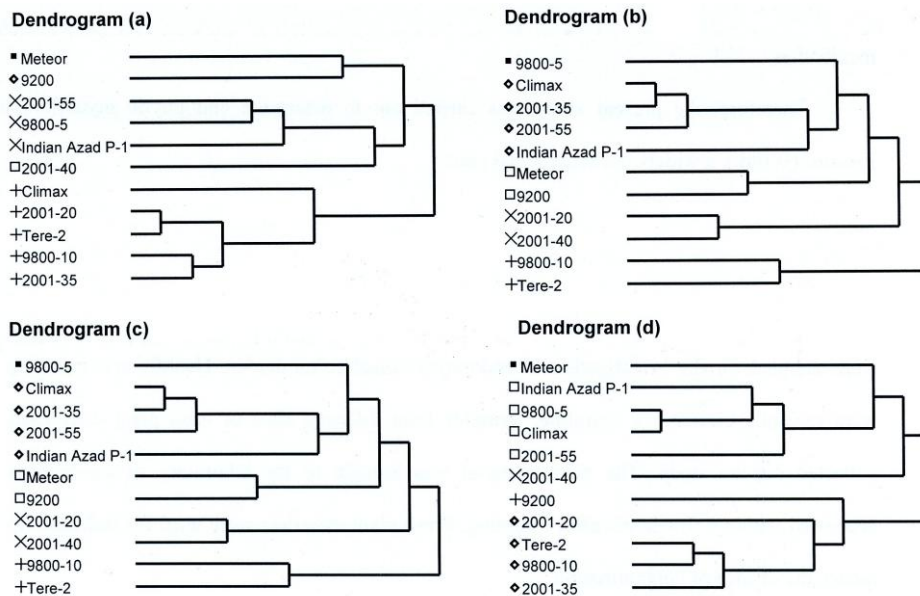


Fig. 1. Dendrograms of pea lines based on Ward's minimum variance cluster analysis of the averages of the salt tolerance indices for two parameters i.e., promptness index (PI) and germination percentage at varying salt levels (60 mM (a), 120 mM (b), 180 mM (c) and 240 mM NaCl (d)). Scores obtained from these dendrograms are used for ranking the lines.

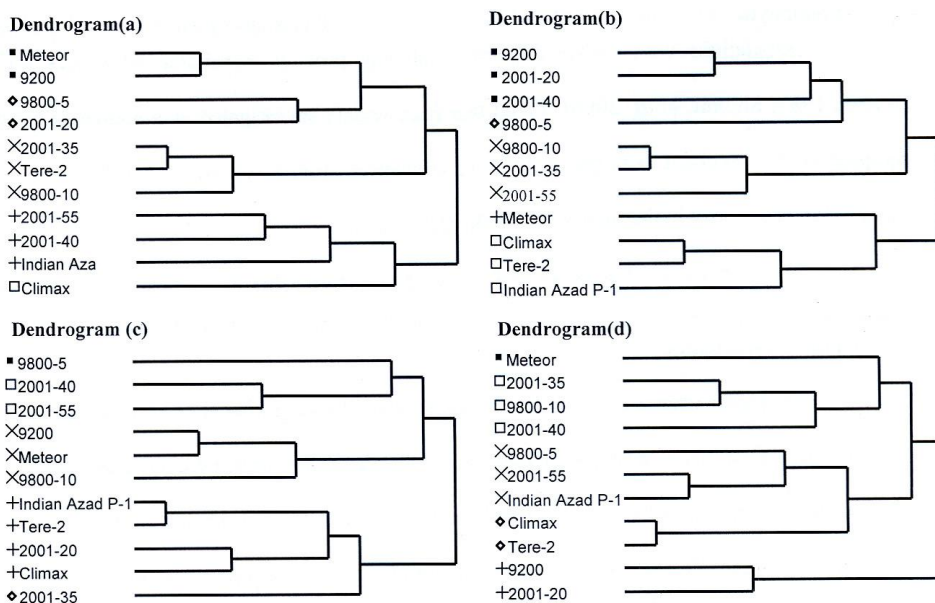


Fig. 2. Dendrograms of pea lines based on Ward's minimum variance cluster analysis of the averages of the salt tolerance indices for four parameters i.e., shoot fresh wt., shoot dry wt., root fresh wt., and root dry wt., per plant at varying salt levels (60 mM (a), 120 mM (b), 180 mM (c) and 240 mM NaCl (d)). Scores obtained from these dendrograms are used for ranking the lines.

Table 1. Germination percentage of different pea lines subjected to varying levels of NaCl at the seedling stage (mean \pm S.E; $n = 4$).

Lines	NaCl level (mM)				
	0	60	120	180	240
2001-20	97.5 \pm 1.44	95.0 \pm 2.04	93.8 \pm 2.39	67.5 \pm 1.44	45.0 \pm 4.56
2001-35	97.5 \pm 1.44	97.5 \pm 1.44	91.3 \pm 1.25	65.0 \pm 2.89	27.5 \pm 3.23
2001-40	96.3 \pm 1.25	92.5 \pm 1.44	90.0 \pm 0.00	75.0 \pm 2.89	43.8 \pm 3.75
2001-55	97.5 \pm 1.44	90.0 \pm 0.00	88.8 \pm 1.25	86.3 \pm 1.25	52.5 \pm 1.44
9800-5	100.0 \pm 0.00	100.0 \pm 0.00	97.5 \pm 1.44	87.5 \pm 3.23	60.0 \pm 2.04
9800-10	92.5 \pm 1.44	90.0 \pm 2.04	75.0 \pm 2.04	62.5 \pm 3.23	32.5 \pm 1.44
9200	95.0 \pm 0.00	93.8 \pm 1.25	91.3 \pm 1.25	83.8 \pm 1.25	52.5 \pm 3.23
Meteor	97.5 \pm 1.44	97.5 \pm 1.44	95.0 \pm 0.00	91.3 \pm 1.25	78.8 \pm 2.39
Indian Azad P-1	85.0 \pm 2.89	78.8 \pm 1.25	75.0 \pm 0.00	70.0 \pm 0.00	57.5 \pm 3.23
Tere-2	87.5 \pm 1.44	75.0 \pm 2.04	70.0 \pm 0.00	60.0 \pm 2.04	35.0 \pm 2.04
Climax	98.8 \pm 1.25	96.3 \pm 1.25	92.5 \pm 1.44	75.0 \pm 0.00	57.5 \pm 3.23

Table 2. Promptness index of different pea lines subjected to varying levels of NaCl at the seedling stage (mean \pm S.E; $n = 4$).

Lines	NaCl level (mM)				
	0	60	120	180	240
2001-20	5.30 \pm 1.85	4.73 \pm 1.51	3.67 \pm 1.73	1.44 \pm 0.73	0.69 \pm 0.53
2001-35	3.88 \pm 1.58	3.22 \pm 1.50	1.73 \pm 1.10	1.06 \pm 0.77	0.47 \pm 0.32
2001-40	1.95 \pm 1.18	1.63 \pm 1.09	1.25 \pm 1.09	0.94 \pm 0.94	0.73 \pm 0.63
2001-55	3.38 \pm 1.15	2.47 \pm 1.13	1.58 \pm 1.05	1.20 \pm 1.04	0.66 \pm 0.66
9800-5	5.95 \pm 1.87	5.09 \pm 1.04	3.08 \pm 1.11	2.38 \pm 1.16	0.88 \pm 0.72
9800-10	4.09 \pm 1.21	3.50 \pm 0.98	1.81 \pm 0.87	1.16 \pm 0.75	0.41 \pm 0.41
9200	8.36 \pm 2.43	5.11 \pm 1.51	2.61 \pm 1.20	1.17 \pm 1.01	0.66 \pm 0.66
Meteor	8.67 \pm 2.58	5.72 \pm 1.10	3.42 \pm 0.84	2.02 \pm 1.03	1.20 \pm 0.93
Indian Azad P-1	4.03 \pm 1.28	2.94 \pm 0.77	2.05 \pm 0.93	1.31 \pm 0.76	0.72 \pm 0.72
Tere-2	4.04 \pm 1.32	2.19 \pm 1.06	1.44 \pm 0.75	1.06 \pm 0.66	0.44 \pm 0.44
Climax	4.61 \pm 1.55	2.91 \pm 1.44	2.09 \pm 1.06	1.13 \pm 0.89	0.72 \pm 0.72

Table 3. Mean squares from analysis of variance (ANOVA) of data for germination percentage and promptness index (PI) of pea seedlings grown under varying levels of NaCl (Mean \pm S.E; $n = 4$).

Source of variation	df	Germination % age	PI
Main effects			
Lines	10	1246.3 ***	16.4 ***
Salinity	4	15139.3 ***	130.2 ***
Interaction			
Lines x Salinity	40	194.5 ***	2.87 ^{ns}
Error	165	15.2	4.35

*** = Significant at 0.001

Table 4. Shoot fresh weight (mg/seedling) of different pea lines subjected to varying levels of NaCl at the seedling stage (mean \pm S.E; $n = 4$).

Lines	NaCl level (mM)				
	0	60	120	180	240
2001-20	152.3 \pm 19.20	142.0 \pm 0.86	137.9 \pm 4.96	88.9 \pm 2.56	70.7 \pm 0.64
2001-35	109.8 \pm 3.09	108.5 \pm 0.15	95.2 \pm 6.19	52.3 \pm 10.01	59.5 \pm 1.40
2001-40	113.8 \pm 9.43	104.4 \pm 1.19	99.9 \pm 4.77	86.9 \pm 5.01	51.3 \pm 4.63
2001-55	143.9 \pm 3.53	132.4 \pm 5.56	124.1 \pm 1.53	91.3 \pm 3.60	73.3 \pm 0.30
9800-5	151.3 \pm 0.17	149.4 \pm 2.24	143.6 \pm 5.01	125.6 \pm 2.89	87.0 \pm 0.50
9800-10	122.7 \pm 7.18	116.6 \pm 6.76	110.7 \pm 0.74	87.0 \pm 1.15	54.1 \pm 4.07
9200	150.5 \pm 1.82	148.4 \pm 3.20	147.5 \pm 4.79	124.3 \pm 0.77	90.6 \pm 3.51
Meteor	165.9 \pm 1.24	165.2 \pm 0.21	150.2 \pm 1.21	135.4 \pm 0.34	117.8 \pm 6.38
Indian Azad P-1	140.0 \pm 4.05	121.7 \pm 3.18	107.0 \pm 6.00	86.5 \pm 3.15	63.7 \pm 5.87
Tere-2	137.0 \pm 0.86	134.1 \pm 13.01	106.8 \pm 6.36	80.5 \pm 14.40	51.8 \pm 2.79
Climax	147.4 \pm 3.03	121.1 \pm 1.55	116.8 \pm 2.76	86.0 \pm 0.75	59.6 \pm 3.96

Table 5. Shoot dry weight (mg/seedling) of different pea lines subjected to varying levels of NaCl at the seedling stage (mean \pm S.E; $n = 4$).

Lines	NaCl level (mM)				
	0	60	120	180	240
2001-20	13.40 \pm 0.45	12.85 \pm 0.35	11.23 \pm 0.48	6.45 \pm 0.11	4.64 \pm 0.10
2001-35	9.53 \pm 0.26	9.36 \pm 0.22	8.53 \pm 0.66	4.88 \pm 0.37	4.74 \pm 0.05
2001-40	9.69 \pm 0.23	9.17 \pm 0.16	7.21 \pm 0.74	6.68 \pm 0.44	4.29 \pm 0.41
2001-55	12.51 \pm 0.28	11.41 \pm 0.80	10.94 \pm 0.08	7.64 \pm 0.43	5.65 \pm 0.32
9800-5	13.91 \pm 0.58	13.25 \pm 0.24	12.01 \pm 0.05	6.38 \pm 1.21	4.44 \pm 0.80
9800-10	8.71 \pm 0.01	8.28 \pm 0.19	8.09 \pm 0.24	6.38 \pm 0.20	4.45 \pm 0.35
9200	13.57 \pm 0.11	13.49 \pm 0.00	11.87 \pm 0.12	9.87 \pm 0.18	6.39 \pm 0.08
Meteor	16.03 \pm 0.55	15.79 \pm 0.36	12.27 \pm 0.63	10.94 \pm 0.33	8.32 \pm 0.61
Indian Azad P-1	12.26 \pm 0.12	10.63 \pm 0.05	8.13 \pm 0.68	5.91 \pm 0.47	4.40 \pm 0.48
Tere-2	12.19 \pm 0.09	12.07 \pm 1.49	8.47 \pm 0.61	5.80 \pm 1.02	3.60 \pm 0.38
Climax	12.27 \pm 0.10	9.98 \pm 0.08	9.14 \pm 0.23	6.34 \pm 0.16	4.12 \pm 0.30

Table 6. Root fresh weight (mg/seedling) of different pea lines subjected to varying levels of NaCl at the seedling stage (mean \pm S.E; $n = 4$).

Lines	NaCl level (mM)				
	0	60	120	180	240
2001-20	178.5 \pm 4.64	167.1 \pm 12.10	131.9 \pm 5.28	90.7 \pm 3.27	68.3 \pm 2.77
2001-35	154.9 \pm 18.4	149.2 \pm 2.37	121.1 \pm 3.77	77.0 \pm 0.84	72.9 \pm 0.48
2001-40	140.5 \pm 4.38	110.9 \pm 1.88	107.9 \pm 10.20	98.5 \pm 4.60	78.3 \pm 1.28
2001-55	166.4 \pm 6.43	117.8 \pm 5.26	113.7 \pm 3.55	106.2 \pm 4.85	81.8 \pm 3.45
9800-5	156.6 \pm 20.5	151.5 \pm 2.58	138.6 \pm 6.72	135.3 \pm 5.35	84.1 \pm 3.57
9800-10	160.4 \pm 4.62	151.1 \pm 13.30	125.3 \pm 0.52	81.4 \pm 2.95	69.9 \pm 7.47
9200	215.5 \pm 0.51	172.9 \pm 3.01	150.3 \pm 6.35	103.4 \pm 3.79	79.5 \pm 1.42
Meteor	248.6 \pm 8.97	185.0 \pm 4.23	143.7 \pm 2.57	135.2 \pm 1.69	115.8 \pm 4.99
Indian Azad P-1	148.3 \pm 6.46	135.6 \pm 13.30	107.7 \pm 12.50	90.6 \pm 3.59	70.3 \pm 8.45
Tere-2	120.2 \pm 10.40	112.5 \pm 16.01	73.1 \pm 2.35	69.8 \pm 7.74	58.8 \pm 4.14
Climax	155.6 \pm 3.79	106.1 \pm 4.26	87.5 \pm 1.94	72.5 \pm 2.41	67.1 \pm 7.01

Table 7. Root dry weight (mg/seedling) of different pea lines subjected to varying levels of NaCl at the seedling stage (mean \pm S.E; $n = 4$).

Lines	NaCl level (mM)				
	0	60	120	180	240
2001-20	16.7 \pm 0.68	10.3 \pm 1.34	8.74 \pm 0.34	6.58 \pm 0.09	4.77 \pm 0.18
2001-35	10.7 \pm 1.14	9.62 \pm 0.04	8.14 \pm 0.69	6.30 \pm 0.11	5.94 \pm 0.24
2001-40	12.6 \pm 0.97	7.67 \pm 0.24	7.53 \pm 0.52	6.88 \pm 0.28	6.66 \pm 0.10
2001-55	15.1 \pm 0.80	10.7 \pm 0.42	10.11 \pm 0.20	8.77 \pm 0.40	5.99 \pm 0.31
9800-5	15.6 \pm 1.57	11.3 \pm 0.66	9.19 \pm 0.01	8.85 \pm 0.50	6.80 \pm 0.46
9800-10	11.3 \pm 0.24	10.9 \pm 1.11	8.12 \pm 0.47	5.68 \pm 0.45	5.10 \pm 0.49
9200	18.8 \pm 0.16	15.3 \pm 0.11	10.90 \pm 0.33	8.15 \pm 0.36	5.73 \pm 0.13
Meteor	18.8 \pm 0.40	13.4 \pm 0.01	9.87 \pm 0.32	8.74 \pm 0.22	8.01 \pm 0.09
Indian Azad P-1	11.9 \pm 0.60	8.07 \pm 0.35	7.18 \pm 0.81	5.59 \pm 0.22	4.28 \pm 0.24
Tere-2	8.4 \pm 0.47	8.00 \pm 1.51	4.70 \pm 0.03	4.22 \pm 0.39	3.74 \pm 0.38
Climax	10.6 \pm 0.08	6.83 \pm 0.42	6.46 \pm 0.27	4.98 \pm 0.24	4.59 \pm 0.55

Table 8. Mean squares from analysis of variance (ANOVA) of data for shoot fresh wt., shoot dry wt., root fresh wt. and root dry wt. (mg/seedling) of pea seedlings grown under varying levels of NaCl (Mean \pm S.E; $n = 4$).

Source of variation	df	Shoot fresh wt.	Shoot dry wt.	Root fresh wt.	Root dry wt.
Main effects					
Lines	10	7185.4 ***	58.1 ***	9575.4 ***	76.9 ***
Salinity	4	35270.9 ***	404.6 ***	56814.2 ***	443.2 ***
Interaction					
Lines x Salinity	40	2260.3 ***	3.79 ***	916.5***	7.10 ***
Error	165	115.4	0.89	205.1	1.26

*** = Significant at 0.001

Discussion

It is now well established that improvement in crop salt tolerance depends upon the existence of genetic variability for salt tolerance at inter-specific and intra-specific level (Ashraf, 1994; Munns, 2007). To explore such type of genetic variability in pea particularly at the intra-specific level, 11 available local pea cultivars were screened at the germination and seedling stages, as salt tolerance throughout these two stages is crucial for the establishment of a crop in a saline environment (Blum, 1985) and is of considerable importance in assessing the overall tolerance of a crop to salinity stress (Akbar & Yabuno, 1974; Ashraf *et al.*, 1986). Extent of salt tolerance of any crop species may be measured as absolute growth at varying levels of salt concentration or in relative terms, i.e., salt tolerance indices at a given salt concentration. Although both modes are equally important to estimate the ultimate tolerance of a cultivar, the relative measure was considered more important (Shannon, 1984; El-Hendawy *et al.*, 2005b; 2007; Ulfat *et al.*, 2007), particularly where growth potential of a cultivar under non-saline conditions is more important.

Table 9. Salt tolerance indices of different parameters of pea cultivars under varying NaCl levels at the seedling stage.

Cultivars	Salt levels (mM)	Promptness index	Germination percentage	Shoot fresh wt.	Shoot dry wt.	Root fresh wt.	Root dry wt.
2001-20	60	0.894	0.974	0.932	0.959	0.936	0.616
	120	0.693	0.962	0.906	0.838	0.739	0.524
	180	0.271	0.692	0.584	0.482	0.508	0.395
	240	0.130	0.462	0.464	0.346	0.382	0.286
2001-35	60	0.831	1.000	0.988	0.982	0.964	0.897
	120	0.448	0.936	0.867	0.896	0.782	0.759
	180	0.274	0.667	0.477	0.512	0.497	0.587
	240	0.121	0.282	0.542	0.497	0.471	0.553
2001-40	60	0.832	0.961	0.917	0.946	0.790	0.607
	120	0.640	0.935	0.878	0.744	0.768	0.596
	180	0.480	0.779	0.763	0.690	0.701	0.545
	240	0.280	0.455	0.451	0.443	0.557	0.527
2001-55	60	0.731	0.923	0.920	0.912	0.708	0.708
	120	0.468	0.910	0.862	0.874	0.683	0.672
	180	0.356	0.885	0.635	0.610	0.638	0.582
	240	0.194	0.538	0.509	0.452	0.492	0.397
9800-5	60	0.856	1.000	0.987	0.953	0.967	0.723
	120	0.517	0.975	0.949	0.863	0.885	0.588
	180	0.399	0.875	0.830	0.459	0.864	0.567
	240	0.147	0.600	0.575	0.319	0.537	0.436
9800-10	60	0.855	0.973	0.950	0.950	0.942	0.961
	120	0.443	0.811	0.902	0.929	0.781	0.722
	180	0.282	0.676	0.709	0.733	0.508	0.505
	240	0.099	0.351	0.440	0.511	0.436	0.453
9200	60	0.611	0.987	0.986	0.995	0.802	0.812
	120	0.312	0.961	0.980	0.875	0.697	0.578
	180	0.140	0.882	0.826	0.727	0.480	0.433
	240	0.079	0.553	0.602	0.471	0.369	0.304
Meteor	60	0.659	1.000	0.996	0.985	0.744	0.713
	120	0.395	0.974	0.905	0.765	0.578	0.524
	180	0.232	0.936	0.816	0.683	0.544	0.464
	240	0.139	0.808	0.710	0.519	0.466	0.425
Indian Azad P-1	60	0.729	0.926	0.869	0.867	0.914	0.680
	120	0.508	0.882	0.764	0.663	0.726	0.606
	180	0.326	0.824	0.618	0.482	0.611	0.472
	240	0.178	0.676	0.455	0.359	0.474	0.361
Tere-2	60	0.542	0.857	0.979	0.990	0.936	0.947
	120	0.356	0.800	0.780	0.695	0.608	0.556
	180	0.263	0.686	0.588	0.476	0.581	0.499
	240	0.108	0.400	0.378	0.295	0.490	0.443
Climax	60	0.631	0.975	0.821	0.813	0.682	0.644
	120	0.454	0.937	0.792	0.745	0.562	0.609
	180	0.244	0.759	0.583	0.516	0.466	0.469
	240	0.156	0.582	0.404	0.335	0.432	0.432

Table 10. Ranking of pea lines for their relative salt tolerance in terms of germination percentage and promptness index (PI) at the seedling stage in a cluster analysis based on Ward's minimum variance analysis.

Lines	NaCl levels (mM)				Sum	Ranking of lines	Degree of salt tolerance
	60	120	180	240			
Meteor	1	4	1	1	7	1	Tolerant
9800-5	2	1	3	4	10	2	Tolerant
9200	1	4	5	2	12	3	Moderate
Climax	1	5	2	4	12	3	Moderate
Tere-2	4	2	2	5	13	4	Moderate
2001-35	2	5	3	5	15	6	Sensitive
2001-20	5	3	2	5	15	6	Sensitive
9800-10	5	2	2	5	14	5	Sensitive
2001-40	5	3	4	3	15	6	Sensitive
Indian Azad P-1	3	5	3	4	15	6	Sensitive
2001-55	3	5	3	4	15	6	Sensitive

Table 11. Ranking of pea lines for their relative salt tolerance in terms of shoot fresh wt., shoot dry wt., root fresh wt. and root dry wt. (mg/plant) at the seedling stage in a cluster analysis based on Ward's minimum variance analysis.

Lines	NaCl levels (mM)				Sum	Ranking of lines	Degree of salt tolerance
	60	120	180	240			
Meteor	1	4	1	1	7	1	Tolerant
9200	1	1	3	2	7	1	Tolerant
2001-20	5	1	2	2	10	2	Tolerant
2001-55	2	3	4	3	12	3	Moderate
2001-40	2	1	4	4	11	3	Moderate
Indian Azad P-1	2	4	2	3	11	3	Moderate
9800-10	3	3	3	4	13	4	Moderate
9800-5	5	5	1	3	14	5	Sensitive
Tere-2	3	4	2	5	14	5	Sensitive
2001-35	3	3	5	4	15	6	Sensitive
Climax	4	4	2	5	15	6	Sensitive

In the present study, 11 available local pea cultivars were assessed for their ability to germinate and sustain growth at varying levels of NaCl in relative terms following modified methods described in some available reports in the literature (Shannon, 1984; El-Hendawy *et al.*, 2005b; 2007; Ulfat *et al.*, 2007). From the results of present study, it is evident that germination, speed of germination and seedling growth of all pea cultivars were significantly reduced with increasing salinity stress. However, a great magnitude of inter-cultivar variation for germination at varying levels of salt stress was observed even within a small number of available pea accessions. Variation in pea cultivars in response to varying salinity levels was also found at the seedling stage. However, no consistent relationship was found between tolerance assessed at the germination and seedling stages. For instance, of 11 pea cultivars examined in the present study, particularly, cvs. 2001-40, 2001-55 and Indian Azad were highly salt sensitive accessions. Tere-2, 2001-35 and Climax were ranked as moderately salt tolerant, while one cultivar Meteor followed by cv. 9800-5 exhibited high salt tolerance at germination. In contrast, cultivar ranking for salt tolerance in terms of seedling growth was altogether different, except cv. Meteor that showed a consistent degree of salt tolerance at both growth stages. Thus, most of the pea

cultivars having high germination salt tolerance index exhibited poor performance at the seedling stage. Similarly, parallels cannot be drawn between total germination percentage and rate of germination (Table 10). From these findings of the present study, it is clear that tolerance to salt stress cannot be predicted from germination tolerance index. However, this is in contrast to the findings of Riga & Vertanian (1999) who found a positive association between tolerance at germination and at a later stage in tobacco and wheat and concluded that germination ability under salt stress could be useful in screening for stress tolerance.

For ranking of cultivars for salt tolerance, scientists usually use a single agronomic or physiological parameter. A few years back, while working with wheat El-Hendawy *et al.*, (2005b) proposed that the screening for salt tolerance should be based on multiple parameters. Similarly, while identifying physiological selection criteria for salt tolerance in 34 canola cultivars, Ulfat *et al.*, (2007) suggested that ranking for salt tolerance based on multiple parameters is very useful. Likewise, all parameters examined in the present investigation appeared to be equally useful for screening pea cultivars for salt tolerance. However, in the present study, ranking of pea cultivars using all parameters did not correspond to the degree of salt tolerance of cultivars with reference to their growth potential under normal growth conditions.

In conclusion, a considerable amount of genetic variation for salt tolerance existed in the available germplasm of pea. However, germination percentage or speed of germination was not found effective for screening purpose. In addition, screening for salt tolerance based on multiple parameters was also not applicable. Screening based on seedling growth showed that cv. Meteor followed by 9200 was salt tolerant and could perform well on saline soils, at least at early growth stages.

Acknowledgement

The results presented in this paper are a part of PhD studies of Miss Zahra Noreen. The corresponding author gratefully acknowledges the funding from the Higher Education Commission (HEC) for Miss Zahra Noreen under Indigenous PhD 5000 Fellowship programme Phase I. Mr. Mahmood-ul-Hassan, Statistics Department, Islamiya University, Bahawalpur, Pakistan helped a lot for carrying out cluster analysis, thus he was included as one of the co-authors of the present work.

References

- Akbar, M. and T. Yabuno. 1974. Breeding for saline resistant varieties of rice. I-Comparative performance of some rice varieties to salinity during early development stages. *Japan. J. Breed.*, 24: 176-181.
- Akbar, M. and T. Yabuno. 1977. Breeding saline-resistant varieties of rice. IV. Inheritance of delayed type panicle sterility induced by salinity. *Japan. J. Breed.*, 27(3): 237-240.
- Akbar, M., G.S. Khush and D. Hillerislamders. 1986. Genetics of salt tolerance in rice. In: *Rice Genetics; Proceedings of International Rice Genetics Symposium*, IRRI, May 1985, pp. 399-409.
- Al-Khatib, M., T. McNeilly and J.C. Collins. 1994. The genetic basis of salt tolerance in Lucerne (*Medicago sativa* L.). *J. Genet. Breed.*, 48: 169-174.
- Ashraf, M. 1994. Breeding for salinity tolerance in plants. *Crit. Rev. Plant Sci.*, 13: 17-42.
- Ashraf, M. 2002. Salt tolerance of cotton, some new advances. *Crit. Rev. Plant Sci.*, 21: 1-30.
- Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora*, 199: 361-376.

- Ashraf, M. and A. Khanum. 1997. Relationship between ion accumulation and growth in two-spring wheat lines differing in salt tolerance at different growth stages. *J. Agron. Crop Sci.*, 178: 39-51.
- Ashraf, M. and A. Waheed. 1993. Screening of local/exotic accessions of lentil (*Lens culinaris* Medic.) for salt tolerance at two growth stages. *Plant Soil*, 128: 167-176.
- Ashraf, M. and S. Ahmad. 1999. Exploitation of intra-specific genetic variation for improvement of salt (NaCl) tolerance in upland cotton (*Gossypium hirsutum* L.). *Hereditas*, 131: 253-256.
- Ashraf, M. and T. McNeilly. 1988. Variability in salt tolerance of nine spring wheat cultivars. *J. Agron. Crop Sci.*, 160: 14-21.
- Ashraf, M., T. McNeilly and A.D. Bradshaw. 1986. The response of selected salt-tolerant and normal lines of four grass species to NaCl in sand culture. *New Phytol.*, 104: 453-461.
- Azhar, F.M. and T. McNeilly. 1988. The genetic basis of variation for salt tolerance in *Sorghum bicolor* (L.) Moench seedlings. *Plant Breed.*, 101: 114-121.
- Belkhodja, R., F. Morales, A. Abadia, J. Gomez-Aparisi and J. Abadia. 1994. Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). *Plant Physiol.*, 104: 667-673.
- Blum, A. 1985. Breeding for crop varieties for stress environments. *Crit. Rev. Plant Sci.*, 2: 199-238.
- El-Hendawy, S.E., Y. Hu and U. Schmidhalter. 2007. Assessing the suitability of various physiological traits to screen wheat genotypes for salt tolerance. *J. Integ. Plant Biol.*, 49(9): 1352-1360.
- El-Hendawy, S.E., Y. Hu, G. M. Yakout, A. M. Awad, S.E. Hafiz and U. Schmidhalter. 2005. Evaluating salt tolerance of wheat genotypes using multiple parameters. *Eur. J. Agron.*, 22: 243-253.
- Francois, L.E., and E.V. Maas. 1994. Crop response to management on salt-affected soils. In: *Handbook of plant and crop stress*. Marcel Dekker, (Ed.): M. Pessarakli. Inc., New York, pp. 149-181.
- George, D.W. 1967. High temperature seed dormancy in wheat (*Triticum aestivum* L.). *Crop Sci.*, 7: 249-253.
- Kingsbury, R.W. and E. Epstein. 1984. Selection for salt resistance spring wheat. *Crop Sci.*, 4: 310-315.
- Mano, Y. and K. Takeda. 1997. Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). *Euphytica*, 94: 263-272.
- Munns, R. 2007. Utilizing genetic resources to enhance productivity of salt-prone land. *CAB Rev.: Perspectives in Agric. Veterinary Sci. Nutr. Nat. Res.*, 2. No. 009.
- Oram, P.A. and M. Agcaoili. 1988. Current status and future trends in supply and demand of cool season food legumes. In: *World Crops: Cool Season Food Legumes*. (Ed.): R.J. Summerfield, Kluwer Academic Publishers, Dordrecht, pp. 3-49.
- Riga, P. and N. Vertanian. 1999. Sequential expression of adaptive mechanism is responsible for drought resistance in tobacco. *Aust. J. Plant Physiol.*, 26: 211-220.
- Shannon, M.C. 1984. Breeding, selection, and the genetics of salt tolerance. In: *Salinity tolerance in plants*. John Wiley & Sons, (Ed.): R.C. Staples and G.H. Toeniessen. New York, pp. 231-254.
- Snedecor, G.W. and W.G. Cochran. 1980. *Statistical methods*. 7th Edition Iowa State University Press, AMES, Iowa.
- Takeda, K., I. Zhao, J. Zhong, U. Liu and Y. Mano. 1995. Selection test of barley and wheat varieties in the Heilonggang region of China. *Proceedings of China-Japan joint Symposium. Impact of salinization and acidification on terrestrial ecosystems and their rehabilitation in East Asia*. Beijing, China, November 3-5, pp. 87-90.
- Ulfat, M., H.R. Athar, M. Ashraf, N.A. Akram and A. Jamil. 2007. Appraisal of physiological and biochemical selection criteria for evaluation of salt tolerance in canola (*Brassica napus* L.). *Pak. J. Bot.*, in press.
- Zeng, L., M.C. Shannon and C.M. Grieve. 2002. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. *Euphytica*, 127: 235-245.