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

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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; Al-khatib et al., 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 & Ageaolo, 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
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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. Dendograms 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 dendograms are used for ranking the lines.

Fig. 2. Dendograms 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 dendograms 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 ± S.E; \( n = 4 \)).

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

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

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

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 ± S.E; \( n = 4 \)).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Germination % age</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main effects</td>
<td></td>
<td>1246.3 ***</td>
<td>16.4 ***</td>
</tr>
<tr>
<td>Lines</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinity</td>
<td>4</td>
<td>15139.3 ***</td>
<td>130.2 ***</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lines x Salinity</td>
<td>40</td>
<td>194.5 ***</td>
<td>2.87 ns</td>
</tr>
<tr>
<td>Error</td>
<td>165</td>
<td>15.2</td>
<td>4.35</td>
</tr>
</tbody>
</table>
Table 4. Shoot fresh weight (mg/seedling) of different pea lines subjected to varying levels of NaCl at the seedling stage (mean ± S.E; n = 4).

<table>
<thead>
<tr>
<th>Lines</th>
<th>NaCl level (mM)</th>
<th>0</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001-20</td>
<td>152.3 ± 19.20</td>
<td>142.0 ± 0.86</td>
<td>137.9 ± 4.96</td>
<td>88.9 ± 2.56</td>
<td>70.7 ± 0.64</td>
<td></td>
</tr>
<tr>
<td>2001-35</td>
<td>109.8 ± 3.09</td>
<td>108.5 ± 0.15</td>
<td>95.2 ± 6.19</td>
<td>52.3 ± 10.01</td>
<td>59.5 ± 1.40</td>
<td></td>
</tr>
<tr>
<td>2001-40</td>
<td>113.8 ± 9.43</td>
<td>104.4 ± 1.19</td>
<td>99.9 ± 4.77</td>
<td>86.9 ± 5.01</td>
<td>51.3 ± 4.63</td>
<td></td>
</tr>
<tr>
<td>2001-55</td>
<td>143.9 ± 3.53</td>
<td>132.4 ± 5.56</td>
<td>124.1 ± 1.53</td>
<td>91.3 ± 3.60</td>
<td>73.3 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>9800-5</td>
<td>151.3 ± 0.17</td>
<td>149.4 ± 2.24</td>
<td>143.6 ± 5.01</td>
<td>125.6 ± 2.89</td>
<td>87.0 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>9800-10</td>
<td>122.7 ± 7.18</td>
<td>116.6 ± 6.76</td>
<td>110.7 ± 0.74</td>
<td>87.0 ± 1.15</td>
<td>54.1 ± 4.07</td>
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<tr>
<td>9200</td>
<td>150.5 ± 1.82</td>
<td>148.4 ± 3.20</td>
<td>147.5 ± 4.79</td>
<td>124.3 ± 0.77</td>
<td>90.6 ± 3.51</td>
<td></td>
</tr>
<tr>
<td>Meteor</td>
<td>165.9 ± 1.24</td>
<td>165.2 ± 0.21</td>
<td>150.2 ± 1.21</td>
<td>135.4 ± 0.34</td>
<td>117.8 ± 0.38</td>
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</tr>
<tr>
<td>Indian Azad P-1</td>
<td>140.0 ± 4.05</td>
<td>121.7 ± 3.18</td>
<td>107.0 ± 0.60</td>
<td>86.5 ± 3.15</td>
<td>63.7 ± 5.87</td>
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</tr>
<tr>
<td>Tere-2</td>
<td>137.0 ± 0.86</td>
<td>134.1 ± 13.01</td>
<td>106.8 ± 6.36</td>
<td>80.5 ± 14.40</td>
<td>51.8 ± 2.79</td>
<td></td>
</tr>
<tr>
<td>Climax</td>
<td>147.4 ± 3.03</td>
<td>121.1 ± 1.55</td>
<td>116.8 ± 2.76</td>
<td>86.0 ± 0.75</td>
<td>59.6 ± 3.96</td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
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<th>Lines</th>
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<th>0</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
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<tbody>
<tr>
<td>2001-20</td>
<td>178.5 ± 4.64</td>
<td>167.1 ± 12.10</td>
<td>131.9 ± 5.28</td>
<td>90.7 ± 3.27</td>
<td>68.3 ± 2.77</td>
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</tr>
<tr>
<td>2001-35</td>
<td>154.9 ± 18.4</td>
<td>149.2 ± 2.37</td>
<td>121.1 ± 3.77</td>
<td>77.0 ± 0.84</td>
<td>72.9 ± 0.48</td>
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</tr>
<tr>
<td>2001-40</td>
<td>140.5 ± 4.38</td>
<td>110.9 ± 1.88</td>
<td>107.9 ± 10.20</td>
<td>98.5 ± 4.60</td>
<td>78.3 ± 1.28</td>
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<tr>
<td>2001-55</td>
<td>166.4 ± 6.43</td>
<td>117.8 ± 5.26</td>
<td>113.7 ± 3.55</td>
<td>106.2 ± 4.85</td>
<td>81.8 ± 3.45</td>
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</tr>
<tr>
<td>9800-5</td>
<td>156.6 ± 20.5</td>
<td>151.5 ± 2.58</td>
<td>138.6 ± 6.72</td>
<td>135.3 ± 5.35</td>
<td>84.1 ± 3.57</td>
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</tr>
<tr>
<td>9800-10</td>
<td>160.4 ± 4.62</td>
<td>151.1 ± 13.30</td>
<td>125.3 ± 0.52</td>
<td>81.4 ± 2.95</td>
<td>69.9 ± 7.47</td>
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</tr>
<tr>
<td>9200</td>
<td>215.5 ± 0.51</td>
<td>172.9 ± 3.01</td>
<td>150.3 ± 6.35</td>
<td>103.4 ± 3.79</td>
<td>79.5 ± 1.42</td>
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</tr>
<tr>
<td>Meteor</td>
<td>248.6 ± 8.97</td>
<td>185.0 ± 4.23</td>
<td>143.7 ± 2.57</td>
<td>135.2 ± 1.69</td>
<td>115.8 ± 4.99</td>
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</tr>
<tr>
<td>Indian Azad P-1</td>
<td>148.3 ± 6.46</td>
<td>135.6 ± 13.30</td>
<td>107.7 ± 12.50</td>
<td>90.6 ± 3.59</td>
<td>70.3 ± 8.45</td>
<td></td>
</tr>
<tr>
<td>Tere-2</td>
<td>120.2 ± 10.40</td>
<td>112.5 ± 16.01</td>
<td>73.1 ± 2.35</td>
<td>69.8 ± 7.74</td>
<td>58.8 ± 4.14</td>
<td></td>
</tr>
<tr>
<td>Climax</td>
<td>155.6 ± 3.79</td>
<td>106.1 ± 4.26</td>
<td>87.5 ± 1.94</td>
<td>72.5 ± 2.41</td>
<td>67.1 ± 7.01</td>
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</tr>
</tbody>
</table>
Table 7. Root dry weight (mg/seedling) of different pea lines subjected to varying levels of NaCl at the seedling stage (mean ± S.E; n = 4).

<table>
<thead>
<tr>
<th>Lines</th>
<th>NaCl level (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2001-20</td>
<td>16.7 ± 0.68</td>
</tr>
<tr>
<td>2001-35</td>
<td>10.7 ± 1.14</td>
</tr>
<tr>
<td>2001-40</td>
<td>12.6 ± 0.97</td>
</tr>
<tr>
<td>2001-55</td>
<td>15.1 ± 0.80</td>
</tr>
<tr>
<td>9800-5</td>
<td>15.6 ± 1.57</td>
</tr>
<tr>
<td>9800-10</td>
<td>11.3 ± 0.24</td>
</tr>
<tr>
<td>9200</td>
<td>18.8 ± 0.40</td>
</tr>
<tr>
<td>Meteor</td>
<td>18.8 ± 0.40</td>
</tr>
<tr>
<td>Indian Azad P-1</td>
<td>11.9 ± 0.60</td>
</tr>
<tr>
<td>Tere-2</td>
<td>8.4 ± 0.47</td>
</tr>
<tr>
<td>Climax</td>
<td>10.6 ± 0.08</td>
</tr>
</tbody>
</table>

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 ± S.E; n = 4).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines</td>
<td>10</td>
<td>7185.4 ***</td>
<td>58.1 ***</td>
<td>9575.4 ***</td>
<td>76.9 ***</td>
</tr>
<tr>
<td>Salinity</td>
<td>4</td>
<td>35270.9 ***</td>
<td>404.6 ***</td>
<td>56814.2 ***</td>
<td>443.2 ***</td>
</tr>
<tr>
<td>Interaction Lines x Salinity</td>
<td>40</td>
<td>2260.3 ***</td>
<td>3.79 ***</td>
<td>916.5***</td>
<td>7.10 ***</td>
</tr>
<tr>
<td>Error</td>
<td>165</td>
<td>115.4</td>
<td>0.89</td>
<td>205.1</td>
<td>1.26</td>
</tr>
</tbody>
</table>

*** = 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.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Salt levels (mM)</th>
<th>Promptness index</th>
<th>Germination percentage</th>
<th>Shoot fresh wt.</th>
<th>Shoot dry wt.</th>
<th>Root fresh wt.</th>
<th>Root dry wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001-20</td>
<td>60</td>
<td>0.894</td>
<td>0.974</td>
<td>0.932</td>
<td>0.959</td>
<td>0.936</td>
<td>0.616</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.693</td>
<td>0.962</td>
<td>0.906</td>
<td>0.838</td>
<td>0.739</td>
<td>0.524</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.271</td>
<td>0.692</td>
<td>0.584</td>
<td>0.482</td>
<td>0.508</td>
<td>0.395</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>0.130</td>
<td>0.462</td>
<td>0.464</td>
<td>0.346</td>
<td>0.382</td>
<td>0.286</td>
</tr>
<tr>
<td>2001-35</td>
<td>60</td>
<td>0.831</td>
<td>1.000</td>
<td>0.988</td>
<td>0.982</td>
<td>0.964</td>
<td>0.897</td>
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<td>0.896</td>
<td>0.782</td>
<td>0.759</td>
</tr>
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<td>0.667</td>
<td>0.477</td>
<td>0.512</td>
<td>0.497</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>0.121</td>
<td>0.282</td>
<td>0.542</td>
<td>0.497</td>
<td>0.471</td>
<td>0.553</td>
</tr>
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<td>2001-40</td>
<td>60</td>
<td>0.832</td>
<td>0.961</td>
<td>0.917</td>
<td>0.946</td>
<td>0.790</td>
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<tr>
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<td>0.935</td>
<td>0.878</td>
<td>0.744</td>
<td>0.768</td>
<td>0.596</td>
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<tr>
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<td>0.779</td>
<td>0.763</td>
<td>0.690</td>
<td>0.701</td>
<td>0.545</td>
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<td></td>
<td>240</td>
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<td>0.451</td>
<td>0.443</td>
<td>0.557</td>
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<tr>
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<tr>
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<td>0.610</td>
<td>0.638</td>
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<td>0.802</td>
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<td>0.980</td>
<td>0.875</td>
<td>0.697</td>
<td>0.578</td>
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<tr>
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<td>0.140</td>
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<td>0.480</td>
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<td>0.553</td>
<td>0.602</td>
<td>0.471</td>
<td>0.369</td>
<td>0.304</td>
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<td>0.996</td>
<td>0.985</td>
<td>0.744</td>
<td>0.713</td>
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<td>0.578</td>
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<td>0.466</td>
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<td>Indian Azad P-1</td>
<td>60</td>
<td>0.729</td>
<td>0.926</td>
<td>0.869</td>
<td>0.867</td>
<td>0.914</td>
<td>0.680</td>
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<td>0.508</td>
<td>0.882</td>
<td>0.764</td>
<td>0.663</td>
<td>0.726</td>
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<td>0.455</td>
<td>0.359</td>
<td>0.474</td>
<td>0.361</td>
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<tr>
<td>Tere-2</td>
<td>60</td>
<td>0.542</td>
<td>0.857</td>
<td>0.979</td>
<td>0.990</td>
<td>0.936</td>
<td>0.947</td>
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<td>120</td>
<td>0.356</td>
<td>0.800</td>
<td>0.780</td>
<td>0.695</td>
<td>0.608</td>
<td>0.556</td>
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<td>0.499</td>
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<td></td>
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<td>0.400</td>
<td>0.378</td>
<td>0.295</td>
<td>0.490</td>
<td>0.443</td>
</tr>
<tr>
<td>Climax</td>
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<td>0.631</td>
<td>0.975</td>
<td>0.821</td>
<td>0.813</td>
<td>0.682</td>
<td>0.644</td>
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<td>0.937</td>
<td>0.792</td>
<td>0.745</td>
<td>0.562</td>
<td>0.609</td>
</tr>
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<td>0.583</td>
<td>0.516</td>
<td>0.466</td>
<td>0.469</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>0.156</td>
<td>0.582</td>
<td>0.404</td>
<td>0.335</td>
<td>0.432</td>
<td>0.432</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Lines</th>
<th>NaCl levels (mM)</th>
<th>Sum</th>
<th>Ranking of lines</th>
<th>Degree of salt tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>120</td>
<td>180</td>
<td>240</td>
</tr>
<tr>
<td>Meteor</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9800-5</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9200</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Climax</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Tere-2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>2001-35</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>2001-20</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
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<td>9800-10</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>2001-40</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
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<td>Indian Azad P-1</td>
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<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2001-55</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

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.

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