Some physiological responses of wheat and bean to soil salinity at low matric suctions

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A b s t r a c t. The effect of soil matric suction (2-33 kPa) and salinity (soil solution electrical conductivity 0.7-8 dS m⁻¹ for bean and 2-20 dS m⁻¹ for wheat) on some physiological characteristics of bean and wheat in a clay loam soil under greenhouse condition was investigated. The results showed that the leaf chlorophyll content index and potassium concentration decrease under salinity stress and increase with matric suction from 2 to 33 kPa suction for both plants. The wheat chlorophyll content index declines during the stress spell but bean chlorophyll content index remains nearly constant. The lowest values of the content of soluble sugars and the highest values of leaf proline content are observed at 2 kPa matric suction (highest aeration stress) for bean and wheat. As matric suction increases from 2 to 6 kPa, the soluble sugars increases and proline content decreases significantly and then soluble sugars decreases and proline content increases until 10 kPa suction, and the soluble sugars remains nearly constant at the higher matric suctions for both plants. While the electrical conductivity effect on the soluble sugars is not significant, the values of proline content for both crop increase significantly with electrical conductivity. It was shown that the aeration stress can result in more considerable and rapid physiological responses, in comparison with salinity stress. There is a strong correlation between wheat and bean chlorophyll content index and potassium concentration under salinity and aeration stresses.

K e y w o r d s: aeration, chlorophyll, potassium, proline, salinity, sugars

INTRODUCTION

Plants growing in the natural environment frequently undergo many abiotic stresses. The stresses decrease crop yield through changing plant physiological status and equilibriums (Gaspar et al., 2002). Salinity is an important limiting factor in crop production. During the salinity stress, the chlorophyll content decreases and, subsequently, plant growth declines (Cha-um et al., 2011). Soil salinity raises chlorophyllase activity, which leads to destruction of enzymes, weakness of the protein-pigment-lipid complex, degradation of chlorophyll molecules, inhibition of chlorophyll synthesis, and eventually decline in chlorophyll a and b (Parida et al., 2002). Cha-um et al. (2011) observed that the rice leaf osmotic potential decreases and chlorophyll content degrades under salinity stress. Since the chlorophyll b is more sensitive than chlorophyll a, the chlorophyll a/b ratio increases with soil salinity (Parida et al., 2002). This consequence becomes more considerable when the plant is under salinity stress for a long time, because chlorophyll b is converted to chlorophyll a. Moreover, salinity stress results in a decline in nutrient uptake and imbalance of essential nutrients. For instance potassium concentration (K⁺) and the K/Na ratio decrease with salinity stress (Sholi, 2012).

Salt-affected soils exist in all continents and under almost all climatic conditions. Their distribution, however, is relatively more extensive in regions with a drainage problem or water excess. Therefore, plant growth is often affected by stresses of salinity and aeration deficit as well (Barrett-Lennard, 2003).

Aeration deficit often corresponds to soil matric suction (h) lower than the field capacity coefficient (h<10-30 kPa). Barrett-Lennard (2003) showed that leaf chlorosis (degradation of photosynthetic pigments) and senescence are the main visible responses of plants under stresses of salinity and aeration. Thus, the chlorophyll content, photosynthesis
rate, and root and shoot biomasses decrease significantly (Drew and Sisworo, 1977). The decline in root extension is a consequence of a decrease in the uptake and balance of nutrients. Zeng et al. (2015) showed that, compared to salinity stress, the combined stress of aeration and salinity doubles the Na\(^+\) concentration and decreases K\(^+\)c in barley leaves by 40\%. These effects are influenced by soil redox potential and accumulation of Mn and Fe under waterlogged conditions. Therefore, hypoxia condition is not the main factor determining differential plant growth under stresses, and ion toxicities due to alteration in soil redox potential influence significantly plant physiological and agronomical responses as well.

To adapt to some abiotic stresses, plants begin to accumulate metabolites with low molecular weights such as carbohydrates, sugars, and proteins (Zhifang et al., 2003). These compounds, maintain cellular turgor pressure, conserve the structure of protein and enzyme complexes, provide biosynthesis of nitrogen compounds, and finally result in plant adaptation to salinity stress (Mansour, 2000). For instance, proline content (Pc) is an essential amino acid for primary metabolisms and increases in plant under environmental stresses such as salinity (Choudhary et al., 2005) and aeration deficit (Vartapetian and Jackson, 1997), especially in sensitive cultivars and long stress spells (Olgun et al., 2008). Under low matric suction or waterlogging, the Pc accumulation in legumes is correlated to the inverse of root porosity and aeration (Striker and Colmer, 2016) and acts as a biomolecule stabilizer, where under salinity stress, the Pc works as an osmolyte (Sami et al., 2016). Watad et al. (1983) showed that proline increases linearly with salinity after a threshold concentration (35 mM NaCl).

Aeration stress can also reduce K\(^+\) uptake by roots and, consequently, decrease shoot K\(^+\)c. Deficit of leaf K\(^+\)c can lead to a several-fold decrease in stomatal conductivity (Shabala and Pottosin, 2014).

The combined effects of aeration or soil low matric suction and salinity on plant physiological characteristics are rarely investigated in wet soil, especially in a close-saturated range to the soil field capacity (FC) and moisture content. The purposes of this study are:
- to evaluate the combined effects of soil aeration and salinity on the chlorophyll content index (CCI), K\(^+\)c, Pc, and soluble sugar (Ss) contents, at FC and higher moisture content and
- to investigate the relationship between the CCI and K\(^+\)c.

The bean and wheat as two main agricultural crops with different stress resistances are selected as test plants.

We hypothesize that:
- the effect of salinity on plant physiological components is less considerable at low matric suction and
- critical aeration porosity traditionally assumed as 0.1 m\(^3\) m\(^{-3}\) cannot be considered as a reliable upper limit of water uptake for all plants.

MATERIALS AND METHODS

The experiments were conducted in greenhouse conditions (20 ± 5°C) with 3 replicates in a factorial arrangement and in the main completely randomized design.

Non-saline agricultural topsoil (0-0.3 m layers) was sampled from the research field of University of Zanjan, north-western Iran. Soil particle size distribution (PSD) was determined using the hydrometer method (Gee and Or, 2002). To measure the soil water characteristic curve (SWCC), repacked small soil cores, 100 cm\(^2\) in size, were prepared in three replicates with considering the initial field bulk density. The soil water content in the lower matric suction range was gravimetrically determined at suction levels equivalent to 0.1, 2, 6, and 10 kPa, respectively. The suction was created by lowering the water level of a sand box apparatus, in the middle of which the core samples were placed. The same samples were thereafter placed in a pressure plate apparatus for measuring soil water content corresponding to the suctions, equivalent to 33 and 100 kPa.

In the next step, disturbed soil samples were placed in a pressure membrane apparatus for determining soil water content at suction equivalent to 200, 300, 500, 1,000, and 1,500 kPa (Dane and Hopmans, 2002). The soil was air-dried and passed through an 8 mm mesh sieve; then it was packed into 120 plastic pots (with 27 cm height and 26 cm diameter) with an initial field bulk density (1.30 Mg m\(^{-3}\)). The wheat (Triticum aestivum cv. Mahdavi) and bean (Phaseolus vulgaris cv. COS16) seeds were soaked in the water for 24 h before sowing into the soil. Ten bean seeds or fifteen wheat seeds were planted in each plastic pot. Once the seeds germinated and emerged, eight wheat and four bean plants were kept, on which the trial was continued.

Soil salinity and suction treatments were begun immediately after third leaf appearance for wheat (L3) and first trifoliate appearance for bean (V1).

The soil moisture treatments were conducted at matric suctions of 2, 6, and 10 kPa with homemade tension tables and at 33 kPa matric suction with standard tensiometers. The homemade tension table consisted of a porous cap and a tube in a bottle of water with a specific base surface. There was a particular height difference between the base surface of water (as the suction regulator) and the middle of the soil surrounding the porous cap, which adjusted the value of matric suction at 2-10 kPa to the pot soils (just as a sand box). The soils rapidly reached equilibrium after each irrigation event at a desired suction head. More details of the homemade tensiometer installation are given by Meskini et al. (2015).

The salinity treatments were applied at electrical conductivity (EC) values 0.7, 2, 4, 6, and 8 dSm\(^{-1}\) for bean and 2, 4, 8, 16, and 20 dSm\(^{-1}\) for wheat using CaCl\(_2\) and NaCl (3:1) salts as the irrigation water solution. The levels of salinity treatments of bean and wheat were selected according to the range of their resistance to salinity (FAO, 2002).
During the experiment period, the leaf CCI was estimated with a Chlorophyll meter (CCM-200). The CCI was measured for the second matured leaf on days 30, 37, 44, and 50 after application of the treatments. At the end of the growing season, the plant green leaves were picked and used for measurements of plant Pc with the acid-ninhydrin method and a spectrophotometer apparatus (UV/VIS Perkin Elmer-Lambda 25-USA) with a wavelength of 485 nm (Bates et al., 1973). The plant shoots were harvested and washed with distilled water. Afterwards, the samples were dried in an oven at 70°C for 72 h. The soluble sugars (Ss) in the dried samples were measured using the phenol-sulfuric acid method and a spectrophotometer apparatus (UV/VIS Perkin Elmer-Lambda 25-USA) with a wavelength of 520 nm (Kochert, 1978). Also, the dried samples were digested using sulfuric acid-hydrogen peroxide (Cresser and Parsons, 1979). Finally, K⁺ in the extracts were measured with a flame photometer apparatus (Jenway, PFP-7, UK).

All the statistical analyses were conducted using the SAS 9.1.3 statistics software. The effect of the treatments and the interactive effects between them were investigated using Duncan multiple range test. The charts were drawn using Excel 2010 software.

RESULTS AND DISCUSSION

The soil water characteristic curve (SWCC) is depicted in Fig. 1 and some physical and chemical properties of the soil are given in Table 1. The effects of the soil salinity and soil matric suction on the CCI, K⁺, Ss, and Pc for wheat and bean were statistically analysed and summarized in Table 2.

![Moisture characteristic curve of the soil used in the greenhouse experiment.](image)

**Table 1.** Physical and chemical properties of the studied soil (Clay loam)

<table>
<thead>
<tr>
<th>Silt (2×10⁻⁵-5×10⁻⁴ m) (%)</th>
<th>Clay (&lt;2×10⁻⁵ m) (%)</th>
<th>BD (Mg m⁻³)</th>
<th>pH</th>
<th>EC (dS m⁻¹)</th>
<th>N (g kg⁻¹)</th>
<th>P (mg kg⁻¹)</th>
<th>K (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>30</td>
<td>1.30</td>
<td>7.72</td>
<td>1.5</td>
<td>10</td>
<td>14.2</td>
<td>220</td>
</tr>
</tbody>
</table>
Table 2. Variance analysis of the effects of soil salinity EC and matric suction (h) on the CCI, K\(^{+}\)c, Ss and Pc for bean and wheat

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>CCI (wheat)</th>
<th>K(^{+})c (%) (wheat)</th>
<th>Ss (mg g(^{-1}) DW) (wheat)</th>
<th>Pc (mg g(^{-1}) FW) (wheat)</th>
<th>CCI (bean)</th>
<th>K(^{+})c (%) (bean)</th>
<th>Ss (mg g(^{-1}) DW) (bean)</th>
<th>Pc (mg g(^{-1}) FW) (bean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>4</td>
<td>58.17*</td>
<td>443.23**</td>
<td>0.67**</td>
<td>0.64*</td>
<td>0.02*</td>
<td>0.004</td>
<td>0.70**</td>
<td>0.02*</td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>3</td>
<td>227.50**</td>
<td>209.57**</td>
<td>0.34**</td>
<td>1.01**</td>
<td>0.04**</td>
<td>0.06**</td>
<td>0.95**</td>
<td>0.12**</td>
<td></td>
</tr>
<tr>
<td>EC × h</td>
<td>12</td>
<td>3.88</td>
<td>11.86</td>
<td>0.03</td>
<td>0.03</td>
<td>0.002</td>
<td>0.007</td>
<td>0.04</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>16.31</td>
<td>5.90</td>
<td>0.03</td>
<td>0.06</td>
<td>0.007</td>
<td>0.01</td>
<td>0.03</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td></td>
<td>11.55</td>
<td>12.65</td>
<td>10.09</td>
<td>15.35</td>
<td>15.40</td>
<td>19.15</td>
<td>37.37</td>
<td>26.69</td>
<td></td>
</tr>
</tbody>
</table>

*, ** – mean significant effects at 0.01 and 0.05 levels of probability, respectively. DW – dry weight, FW – fresh weight.

Fig. 2. Wheat leaf CCI as a function of soil salinity EC under different matric suctions, a – 30, b – 37, c – 44, and d – 55 days after treatments application. Error bars show two standard deviations of uncertainty.
suction increases. Thus, plant activities such as chlorophyll synthesis (and consequently CCI) improve in appropriate soil-air permeability conditions.

Depending on plant species, the anoxia effects on the chlorophyll content begin after a few hours to a few weeks (Smethurst and Shabala, 2003). Figure 2 shows that the wheat CCI decreases with time at all soil salinities and suction treatments. The lowest wheat CCI is observed on the 55th day after the treatments (Fig. 2 d). However, the bean CCI remains nearly constant during the time (Fig. 3). Since bean is sensitive to salinity stresses, its CCI is affected shortly (time course <30 day), while wheat CCI decreases gradually until the 55th day (Figs 2 and 3).

The variations of K\textsuperscript{+}\textsubscript{c} (%) as a function of the soil matric suction and soil salinity are shown in Fig. 4. The lowest wheat K\textsuperscript{+}\textsubscript{c} (1.0%) is observed at ECs 20 dSm\textsuperscript{-1} (Fig. 4a) and, similarly, the lowest bean K\textsuperscript{+}\textsubscript{c} (0.7%) is observed at ECs 8 dSm\textsuperscript{-1} (Fig. 4b) at 2 kPa suction (low aeration). Figure 4 shows that there are almost linear increases of 0.4% in wheat K\textsuperscript{+}\textsubscript{c} and 0.5% in bean K\textsuperscript{+}\textsubscript{c} between suctions 2 and 33 kPa. The decline in K\textsuperscript{+}\textsubscript{c} at low aeration (h=2 kPa) can be attributed to changes in solute movement across cell membranes, cell membrane potentials, and water and nutrient uptake (Chang and Loomis, 1945). Moreover, soil salinity reduces K\textsuperscript{+}\textsubscript{c} at all soil matric suctions. Since the soil available water for plants decreases with salinity, the uptake of nutrients such as K\textsuperscript{+}\textsubscript{c} is influenced (Chaum et al., 2011).

Figure 5 shows the relationships between the K\textsuperscript{+}\textsubscript{c} and the CCI for wheat and bean. The strong correlation coefficients (r=0.79-0.88) between the K\textsuperscript{+}\textsubscript{c} and the CCI with high linear slope values (21.8-19.6) demonstrate a considerable

![Figure 3. Bean leaf CCI as a function of soil salinity EC under different matric suctions, a – 30, b – 37, c – 44, and d – 55 days after treatments application. Error bars show two standard deviations of uncertainty.](image-url)
effect of the K\(^+\) on the CCI. Many investigators have found that the deficiency of K\(^+\) results in chlorophyll breakdown, development of brown spots, and leaf necrosis (Pfluger, and Mengel, 1972).

Figure 6 shows the effect of the soil matric suction on the soluble sugar content Ss under different soil salinities. The minimum values of wheat and bean Ss are about 0.4 mg g\(^{-1}\) DW at 2 kPa suction for all soil salinities. At low matric suction of soil (2 kPa), gas diffusion and transfer is insufficient for aerobic respiration; thus, plants supply their energy demand through fermentation of sugars (Saglio, 1985) and, subsequently, the Ss decrease in both wheat and bean (Fig. 6). The Ss decrease can also be attributed to a decrease in metabolic activity and transfer of carbohydrates from roots (Saglio, 1985). Moreover, under anoxia conditions, the photosynthetic rate in plants and, subsequently, the content of sugars decrease significantly (Peterson and Bazzaz, 1984). As the soil suction increases from 2 to 6 kPa, aeration improves, and anaerobic respiration and fermentation of sugars becomes insignificant and the average values of Ss reach a peak at 0.57 mg g\(^{-1}\) DW for wheat and at 0.64 mg g\(^{-1}\) DW for bean at 6 kPa matric suction. This matric suction corresponds with aeration porosity of 0.16 m\(^{3}\) m\(^{-3}\) (Fig. 1), where there is a balance between the amount of water and air, resulting in reasonable respiration and water uptake as well.

Figure 6 shows that the mean wheat and bean Ss decrease, respectively, to 0.48 and 0.55 mg g\(^{-1}\) DW at matric suction of 10 kPa and then remain nearly constant at higher suctions (h>10 kPa) for wheat. However, there is
a slight decrease of about 0.04 mg g\(^{-1}\) DW in Ss between 10 and 33 kPa matric suction for bean (Fig. 6b). The EC effect on the Ss is not consistent for both wheat and bean (Fig. 6).

Figure 7 shows the variation of the proline content as a function of soil matric suction and salinity. The highest values of the Pc are observed at the lowest matric suction (2 kPa) and the highest soil salinity (EC = 20 dS m\(^{-1}\) for wheat and EC = 8 dS m\(^{-1}\) for bean). Proline is a regulatory molecule accumulated in plants growing under stress conditions (Kaya et al., 2006). We can conclude that the aeration stress occurring at 2 kPa suction results in a partial rise in the proline content and the salinity stress, which is considerable at higher ECs, amplifies the effect of aeration stress and leads to greater proline accumulation in bean and wheat. Figure 8 demonstrates the salinity effect on Pc at different soil suction heads and confirms the synergic effect of aeration and salinity stresses. The lowest values of Pc (0.1 mg g\(^{-1}\) FW for wheat and 0.05 mg g\(^{-1}\) FW for bean) are observed at the lowest salinity level at 6 kPa matric suction. The decrease in Pc with aeration improvement has been observed by many researchers (Carter et al., 2006). The Pc increases with matric suctions between 6 and 10 kPa and then remains nearly constant, for both crops. Overall, the variation of Pc with matric suction is conversely comparable with the variation of Ss with the matric suction.
Wheat and bean Pc increase almost linearly with the soil EC (Fig. 8). The Pc-EC relationships in bean remain approximately consistent for all matric suctions; however, the wheat Pc-EC curves for different matric suctions diverge with EC (Fig. 8).

**CONCLUSIONS**

1. In the wet saline soil, the wheat and bean physiological characteristics are influenced by both the aeration porosity and soil salinity. However, the relative importance of these stress factors depends on the stress level; anoxia conditions may result in more rapid and considerable responses.
2. At low aeration porosity, potassium concentration, chlorophyll content index, and soluble sugar decrease and proline content increases severely and salinity amplifies the effect of the aeration stress.
3. The highest values of soluble sugar and the lowest values of proline content are observed at aeration porosity of 0.16 m³ m⁻³.
4. The critical aeration porosity traditionally assumed as 0.1 m³ m⁻³ cannot be considered as a reliable upper limit of water uptake for all plants.
5. There are high correlations between the salinity stress and the proline content, potassium concentration and chlorophyll content index.
6. There are high correlations between the chlorophyll content index and the potassium concentration where the slope of the best lines are dependent on the type of plant.
7. Comparison of the two plants shows that, despite the sensitivity variations, their physiological responses to soil salinity and matric suction are consistent.

**Conflict of interest:** The Authors do not declare conflict of interest.

**REFERENCES**


PHYSIOLOGICAL RESPONSES OF WHEAT AND BEAN TO SOIL SALINITY


