Morpho-Anatomical Changes In Salt Stressed Kallar Grass (Leptochloa fusca L. Kunth)

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ABSTRACT

Growth and morphological responses of Leptochloa fusca grown on sandy soil and irrigated with Hoagland’s nutrient solution containing 0, 100, 200 and 300 mM NaCl were studied in pot experiments. The results showed that no significant differences were found between the control and 100 mM NaCl level in the following characters: lamina and mesophyll thickness, bulliform cell area, percentage of sclerenchyma area, metaxylem and phloem areas in leaf blade; sheath thickness, area of aerenchyma, metaxylem and phloem areas and percentage of sclerenchyma area in leaf sheath and area of stem, percentage of pith cavity area, air spaces area, percentage of aerenchyma area, number and area of stem vascular bundles. Meanwhile, plant height, shoot fresh weight and some anatomical characters were significantly decreased under 100 mM NaCl salinity, i.e., midvein thickness, and air cavity area in leaf blade; sheath thickness, metaxylem and phloem area. Furthermore, salinity treatment over 100 mM significantly induced some anatomical changes such as highly developed bulliform cells intervening the upper epidermal cells and increased sclerification in stem, leaves and stomata number in the lower surface. The number of vascular bundles in stem was increased with increasing the salinity except the 300 mM NaCl. The above mentioned anatomical changes in response to the highest salinity level could be considered as anatomical function to adapt and tolerate kallar grass to salinity.

Key words: Kallar grass, Salt stress, Growth parameters, Anatomical characters.

Introduction

Salinity is an environmental stress that limiting plant growth and productivity around the world. This problem is more severe in arid and semi-arid regions (Munns, 2002). Increasing salinity induces specific changes on cell, tissue and organ levels. These changes are physiological, morphological and anatomical (Shannon, 1997 and Isla et al., 1998). It has been revealed from many studies that high salinity mostly causes anatomical alterations such as reduction of stomata number (Hwang and Chen, 1995 and Çavuşoğlu et al., 2007), leaf thickness (Shennan et al., 1987 and Çavuşoğlu et al., 2008), distance between vascular bundles and epidermis cell number (Çavuşoğlu et al., 2007). However, promoting effects of salt stress on leaf thickness (Hwang and Chen, 1995; Kiliç et al., 2007 and Vijayan et al., 2008), epidermis cell number and stomata number (Kiliç et al., 2007) have also been recorded. Other structural changes occurred in salt stressed plants such as inhibition of differentiation, diameter and number of xylem vessels.

Kallar grass has a C₄ photosynthetic pathway (Zafer and Malik, 1984), is a good animal fodder providing three to four cutting without any N-fertilization. This species has a particular importance, since it acts as a soil fixative species and it can be used as a fodder in dry lands. However, little information is available about the anatomical responses to salt stress in this species. In this study, we examined the effect of different salinity levels on growth and anatomical structures of kallar grass.

Materials And Methods

Pot experiments were carried out in a greenhouse at Department of Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo, Egypt on April 1st 2009 and 2010. Ramets (lateral branches on main tiller) with uniform size were detached from the mother plant and prepared for planting in pots. Stem cuttings 15-20 cm in length contain one or two aerial leaves. The basal nodes of cuttings were soaked in tap water for 4-6 days for rooting. The initial weight of cutting was about 3-4 g. Lastly one rooted cutting was planted per each pot (40 cm in diameter and 33 cm in height, filled with sandy soil), six replicates were planted. Four salinity levels were applied after the establishment of seedlings (one week after planting date). The treatments were control (no salinity added), 100, 200 and 300 mM of NaCl salinity. All treatments were irrigated with Hoagland’s nutrient solution (Arnon and Hoagland, 1940) along the experiment time. The samples were taken 60 days after planting date for examination of morph-anatomical characters. The recorded data include plant

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height and shoot fresh weight. For the anatomical studies, the main tiller of each replicate was selected to study the structure of the third leaf and third internode under the flag leaf. Specimens of leaf, about 5 mm in length, from the mid-portion of the lamina and also from the leaf sheath were taken. The stem samples, on the other hand, were taken from the median parts of the internode of the main tiller. Plant samples were fixed in FAA solution (formaline, acetic acid and 70% ethyl alcohol, 5:5:90/100 ml) for 24 h at room temperature. Free-hand sections were prepared with a razor blade then stained by Tulidine-blue (0.03 % w/v) for leaf blade, sheath and stem (Sakai, 1973). Anatomical examination and measurements were achieved using a Leica light Research Microscope model DM-2500 supplied with a digital camera. The mean area of parenchyma bundle sheath and mesophyll was taken from the cells which surrounding the first largest and a neighbor small bundle on the lamina as following:

\[
\text{parenchymatous bundle sheath area} = \frac{chl_1 + chl_2}{2}
\]
\[
\text{mesophyll area} = \frac{mes_1 + mes_2}{2}
\]

where:

- \(chl_1\) = parenchyma bundle sheath of the first largest bundle in lamina.
- \(chl_2\) = parenchyma bundle sheath of the neighbor small bundle.
- \(mes_1\) = mesophyll area of the first largest vein in lamina.
- \(mes_2\) = mesophyll area of the neighbor small vein.

Mesophyll thickness was measured between the first largest and smallest bundle in the lamina, while the metaxylem, phloem areas and percentage of sclerenchyma were taken in the midvein vascular bundle of leaf blade and sheath. The aerenchyma area of the leaf sheath was taken at whole leaf sheath but the leaf sheath thickness was taken at the midvein of the sheath.

Air spaces, vascular bundles area and the percentage of sclerenchyma were taken at whole stem.

Scanning Electron Microscopy (SEM): fresh materials were fixed in 3% glutaraldehyde for 24h at 4°C (Harley and Ferguson, 1990). The specimens were dehydrated using ascending concentrations of ethanol; critical point dried and finally coated with gold. The morphological examinations were carried out using a Jeol Scanning Electron Microscope (JSE-T330A) equipped with image recording and processing system (Sem Afor).

Statistical analysis: The statistical analysis of the obtained data was done by statistical analysis system (SAS, 1999). Tukey test was used for separation between means.

Results And Discussion

Growth parameters:

The results in Figs. (1 and 2) showed a progressive decrease in plant height and plant fresh weight with increasing salinity level. These results may be attributed to, at high salinity level, growth depression may originate from inhibition of nutrient uptake, transport and utilization in plants (Cramer and Nowak, 1992). Munns (2002) reported that salinity can affect growth via changed water relations, hormonal balance, or carbon supply. According to Flowers et al. (1977); Marschner (1995); Läuchli and Lüttge (2002) and Liu et al. (2006) the reduction in growth and yield under high salinity levels could be due to reduction in photosynthesis, disturbance in mineral uptake, protein synthesis or carbohydrate metabolism. They also, added that in most halophytic species growth decreases gradually with the increase of salt rate in the culture medium a critical threshold specific to each species. Ashour et al. (2004) reported that, the reduction in growth at higher salinity levels is attributed to reduced turgor and high energy cost of massive salt secretion and osmoregulation. Whereas, Tawfik et al. (2006) stated that salinity affect the growth of halophytic plants, this may be attributed to improved shoot osmotic status as a result of increased ion uptake.

Anatomical characteristics:

Examination of the transverse sections of leaves and stems of kallar grass were adversely affected by increasing NaCl salinity level. Leaf blade showed a significant decrease in the midvein thickness, lamina thickness, mesophyll thickness and mesophyll area along the leaf axis with increasing salinity levels comparing with the control treatment. There was insignificant differences in the lamina thickness between the control and 100 mM treatments (189.77 and 170.60 µm, respectively), whereas the other treatments showed a slightly decrease in these characters. The highest level of salinity 300 mM gave considerably thinner lamina (142.17µm) than those recorded in all other treatments (Table. 1 and Fig. 4 a&b). Increasing of salinity levels resulting in a gradual decrease in mesophyll area and a decrease in the parenchyma bundle sheath area. These findings were recorded in all treatments. Leaf sheath thickness gradually decreased with increasing salinity level reaching 401.33 µm under 300 mM NaCl treatment comparing to control (774.0 µm) as shown in Table (2) and Fig. (4 c&d). The observed reduction in mesophyll area (the first largest vein in lamina and the neighbor small vein) may suggest that salinity reduced the capacity for re-translocation of mineral nutrients and assimilates. These
results agree with Hu et al. (2005) who observed reduction in cross-sectional area of wheat leaves in the 120 mM NaCl treatment and explain the reasons for the observed reduction due to a decrease in the size of the midrib and large veins and also in the number of medium and small veins. The different types of vein play different roles in the physiological functions of the leaf, e.g. the large veins are mainly for transporting water and the small veins are mainly for loading and transporting nutrients. Also, Bongi and Loreto (1989) showed that photosynthesis in salt-stressed olive leaves was reduced in part because of the reduced mesophyll conductance caused by leaf thickening.

Stems of kallar grass plants were slightly affected with salinity levels. There was insignificant difference between the control and 100 mM treatment in all characters; whereas, there was a significant decrease in these characters between the control and 300 mM treatments. The reduction in stem diameter may be due to reduction in DNA content resulted in reduced cell division and expansion (Wignarajah et al., 1975). The reduction of leaf, sheath thickness and stem area might be attributed to that salinity reduces the ability of plants to take up water and this quickly causes reduction in the growth rate, if excessive amounts of salt enter the plant they will eventually rise to toxic levels in the older transpiring leaves and reduce the photosynthetic capacity of the plant (Munns, 2002).

The bundles in large veins are characterized by the presence of large metaxylem vessels on either side of the protoxylem (Russell and Evert, 1985). The vast bulk of axial water movement occurs through proto- and metaxylem elements in the midrib and large veins. Furthermore, the obtained results showed a significant
decrease in metaxylem and phloem area of main vascular bundle at midvein of leaf blade. The area of metaxylem and phloem recorded 225.47 and 165.73 μm² under 300 mM NaCl comparing with 302.20 and 265.07 μm² under control treatment (Table 1), and a significant decline in xylem and phloem areas of the main vascular bundle of the leaf sheath comparing to the control treatment (Table 2). Also, there was a significant decrease in vascular bundle area of stem between the control and 300 mM treatments (Table 3 and Fig. 4 e&f). Salinity may influence the procambial division during the early stages of growth. The same result was found by Boughalleb et al. (2009). The reduction of xylem vessel diameter under saline conditions was early observed in cotton and tomato plants (Strogonov, 1962) and in wild barley (Huang and Redmann, 1995). According to Hameed et al. (2009) who examined the anatomical adaptation in the leaf of Imperata cylindrica [Faisalabad ecotype] and reported that gradually decrease in vascular bundles area, metaxylem area and phloem area with increasing salinity level in the growth media. The average area of xylem (protoxylem + metaxylem) within a leaf was reduced by 55% under saline conditions in the study of Hu and Schmidhalter (2001). This compares favorably to the reduction in the net deposition rate of water within the growth zone (about 40%) induced by salinity.

The number of vascular bundles were increased with increasing salinity level in the stem, this may compensate the reduction of xylem and phloem areas in the vascular bundles.

Leaf rolling was observed in all treatments whereas, the 300 mM of NaCl treatment showed extensive leaf blade rolling. This feature is affected by the turgidity of the bulliform cells. The bulliform cells area were increased with increasing salinity level (153.67 and 227.70 μm² in control and 300 mM NaCl, respectively) which mean this increase reach 48% over control treatment (Fig. 5 a&b). Bulliform cells play an important role in leaf rolling to avoid water loss during drought stress (Abernethy et al., 1998; Balsamo et al., 2006 and Alvarez et al., 2008). The presence of greatly enlarged bulliform cells in the salt range ecotype is a significant adaptation against water loss under physiological drought conditions due to salt stress. Extensive leaf rolling was observed in the salt range ecotype; therefore, it can safely be referred to as an important adaptive defensive strategy against salt stress (Hameed et al., 2009). According to Gielwanowska et al. (2005) high salinity resulted in well-developed bulliform cells in Deschampsia antarctica plants. In addition, bulliform cells or rolling cells had been botanically described as motor cells responsible for leaf blade rolling under water or heat stresses. Therefore, in some physiological reports leaf rolling was defined as water conservation movement (Srivastava, 2001). The mechanism by which motor cells (bulliform cells) induced leaf roll movement was previously described under plant movement and defines as nastic movement. Nastic movement depends on cell turgor

Table 1: Effect of NaCl salinity levels on some anatomical measurements in leaf blade of kallar grass (Leptochloa fusca (L.).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>100 mM</th>
<th>200 mM</th>
<th>300 mM</th>
<th>M.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midvein thickness (μm)</td>
<td>755.44</td>
<td>566.58</td>
<td>460.08</td>
<td>418.90</td>
<td>90.605</td>
</tr>
<tr>
<td>Lamina thickness (μm)</td>
<td>189.77</td>
<td>170.60</td>
<td>153.54</td>
<td>142.17</td>
<td>21.35</td>
</tr>
<tr>
<td>Mesophyll thickness (μm)</td>
<td>76.77</td>
<td>62.55</td>
<td>59.53</td>
<td>39.81</td>
<td>14.403</td>
</tr>
<tr>
<td>Parenchyma bundle sheath area (μm²)</td>
<td>650.96</td>
<td>539.60</td>
<td>472.55</td>
<td>428.60</td>
<td>18.05</td>
</tr>
<tr>
<td>Mesophyll area (μm²)</td>
<td>790.15</td>
<td>709.90</td>
<td>595.35</td>
<td>533.60</td>
<td>15.92</td>
</tr>
<tr>
<td>Bulliform area (μm³)</td>
<td>153.67</td>
<td>170.83</td>
<td>188.33</td>
<td>227.70</td>
<td>52.195</td>
</tr>
<tr>
<td>Metaxylem area (μm²)</td>
<td>302.20</td>
<td>288.50</td>
<td>269.43</td>
<td>225.47</td>
<td>46.29</td>
</tr>
<tr>
<td>Phloem area (μm²)</td>
<td>265.07</td>
<td>237.87</td>
<td>202.51</td>
<td>165.73</td>
<td>76.789</td>
</tr>
<tr>
<td>Aircavity area (μm²)</td>
<td>1213.3</td>
<td>1263.4</td>
<td>1563.7</td>
<td>2416.6</td>
<td>104.04</td>
</tr>
<tr>
<td>% Sclerenchyma area</td>
<td>26.70</td>
<td>12.67</td>
<td>17.64</td>
<td>24.50</td>
<td>4.69</td>
</tr>
</tbody>
</table>

Table 2: Effect of NaCl salinity levels on some anatomical measurements of different tissues in leaf sheath of kallar grass (Leptochloa fusca (L.) at 60 days after planting date.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sheath thickness (μm)</th>
<th>Aerenchyma area (μm²)</th>
<th>Metaxylem area (μm²)</th>
<th>Phloem area (μm²)</th>
<th>% Sclerenchyma area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>774.00</td>
<td>31153</td>
<td>358.77</td>
<td>262.00</td>
<td>13.83</td>
</tr>
<tr>
<td>100 mM</td>
<td>630.67</td>
<td>24783.40</td>
<td>255.53</td>
<td>162.23</td>
<td>14.71</td>
</tr>
<tr>
<td>200 mM</td>
<td>521.73</td>
<td>16511.67</td>
<td>218.77</td>
<td>123.97</td>
<td>15.81</td>
</tr>
<tr>
<td>300 mM</td>
<td>401.33</td>
<td>5911.13</td>
<td>136.83</td>
<td>112.53</td>
<td>19.60</td>
</tr>
<tr>
<td>M.S.D: 0.05</td>
<td>63.601</td>
<td>8889.9</td>
<td>81.04</td>
<td>42.115</td>
<td>4.78</td>
</tr>
</tbody>
</table>
Table 3: Effect of NaCl salinity levels on some anatomical measurements in stem of kallar grass (Leptochloa fusca (L.)).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Stem area (mm²)</th>
<th>% Pith cavity</th>
<th>Air spaces area (µm²)</th>
<th>% Sclerenchyma</th>
<th>Number of vascular bundles</th>
<th>vascular bundles area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.38</td>
<td>32.21</td>
<td>17446.53</td>
<td>4.29</td>
<td>49.32</td>
<td>0.029</td>
</tr>
<tr>
<td>100 mM</td>
<td>10.74</td>
<td>30.17</td>
<td>16997.07</td>
<td>4.54</td>
<td>54.66</td>
<td>0.024</td>
</tr>
<tr>
<td>200 mM</td>
<td>7.09</td>
<td>28.01</td>
<td>9878.27</td>
<td>5.69</td>
<td>66.00</td>
<td>0.023</td>
</tr>
<tr>
<td>300 mM</td>
<td>4.53</td>
<td>16.08</td>
<td>4648.67</td>
<td>6.80</td>
<td>60.66</td>
<td>0.017</td>
</tr>
<tr>
<td>M.S.D: ≤ 0.05</td>
<td>2.899</td>
<td>14.61</td>
<td>8062.8</td>
<td>1.99</td>
<td>5.84</td>
<td>0.0097</td>
</tr>
</tbody>
</table>

The percentage of sclerenchyma area generally increased with increasing salinity levels in leaf blade, leaf sheath and stem (Fig. 5 c&d) and Tables (1, 2 and 3). The increase of sclerenchyma cells in the leaf appeared to be useful for diminution of water loss in Festuca nove – zelandiae (Aberenthy et al., 1998). Also, increased sclerification in the leaves (blade and sheath) as well as in the stems under the highest salinity level may be an importance as it would provide rigidity to these organs. These results are in accordance with some reports of salt induced sclerification in other plants species, e.g., Spartina alterniflora (Walsh, 1990), Kandelia candel (Hwang and Chen, 1995), cotton (Reinhardt and Rost, 1995), Puccinellia tenuiflora (Yujing et al., 2000), and Prosopis strombulifera (Reinoso et al., 2004). Grigore and Toma (2007) reported that the lignin might confer resistance to the cell walls. This resistance is also involved in supporting and counteracting the high osmotic pressure which the halophytes have to face at the level of the rhizosphere.

Air cavity area was decreased consistently in all treatments comparing with the control in leaf blade (Table). There was a significant difference in leaf sheath between control and 300 mM NaCl in aerenchyma area (17446.53 and 4648.67µm², respectively). On the other hand, there was no significant difference between control and 100 mM NaCl treatment (17446.53 and 16997.07 µm², respectively). The same result was observed in stem. Stem airspaces is a characteristic feature of water logged plants. For example, Clomer and Flowers (2008) summarized reports of aerenchyma in halophytes under water logged conditions. Aerenchyma has also been previously reported in Leptochloa fusca (Metcalf, 1960). The treated plants showed a considerable decrease in airspaces formation with increasing salinity level 100 mM (17446.53 µm²). At salinity level of 200 mM airspaces were occupied by parenchyma (9878.27 µm²), and at the highest salinity level this parenchyma was quite tightly packed. This may increase the area of storage tissue with increased vacuolar volume for storing toxic ions and hence represent an important strategy to cope with high salinities (Akhtar et al., 1998).

Fig. (5 e) illustrated that high density of storage starch granules were observed in the parenchyma tissues of the stem. High densities of granules in stem might be related to the fact of that accumulation carbohydrates in response to salinity stress is thought to have important role in the osmotic adjustment in the salt-tolerant plants (Ashraf and Tufail, 1995 and Murakeozy et al., 2003).

Salinity level of 100 mM slightly increased stomata number, but this increment did not show a significant effect, on the lower surface (abaxial side) comparing with the control (Table 1). The obtained results concluded that low salinity increases stomata number (Curtis and Lauchli, 1987). On the other hand, at the same salinity level the stomata area decreased to achieve 47.45% comparing to the control treatment. Kallar grass plants provide adaptation to saline 100 mM by decreasing stomata area and so decrease transpiration and water loss. This is agree with our results that 100 mM NaCl salinity level showed a slightly decrease in plant height and plant fresh weight It is known for a long time that high salinity caused a decreasing effect on stomata number (Flowers et al., 1986). On the other hand, data obtained revealed a substantial increase in stomata number occurred with increasing salinity levels (200 or 300 mM NaCl) as shown in Fig. 6 (a&b). David and Nobel (1979) reported that salinity can affect photosynthesis at stomata and / or mesophyll levels depending on type of salinity, duration of treatment, species and plant age. The results showed that high levels of salinity 200 or 300 mM increased the stomata number, decreasing in stomata area and mesophyll area. All these characters were responsible for the reduction in photosynthesis in kallar grass plants and were reflect as a progressive reduction in plant height, plant fresh weight as shown in Figs. 1&2.
Fig. 4: Transverse sections of leaf blade (a&amp;b), leaf sheath (c&amp;d) and stem (e&amp;f) of kallar grass plant showing the effect of salinity on anatomical features under salinity stress. (a, c & e) 0 mM of NaCl and (b, d & f) 300 mM of NaCl. Bar= 200µm.

Fig. 5: Anatomical modifications in kallar grass plant to salinity stress: (a&amp;b) leaf blade note the well development of bulliform cells in (b) 300 mM NaCl compairing to the control (a) 0 mM NaCl. Bar = (100 µm). (c&amp;d) stem note the increase in sclerification in (d) 300 mM NaCl comparing to the control (c) 0 mM NaCl. Bar= (100 µm). (e) storage starch granules in stem at 300 mM NaCl.
Fig. 6: Scanning electron micrographs showing the abaxial side of kallar grass leaf and reveal the effect of NaCl salinity on the number of stomata (a) the control plant, (b) the 300 mM NaCl level.

References


