

Vascular Architecture Alterations in Expanding Durum Wheat Leaf Under Salinity

Ehsan Bijanzadeh^{1*} and Yahya Emam²

Received: January 30 2016 Accepted: November 19 2016

¹Department of Agroecology, Agriculture College and Natural Resources of Darab, Shiraz University, Iran

²Department of Crop Production and Plant Breeding, College of Agriculture, Shiraz University, Shiraz, Iran

*Corresponding author; Email: bijanzd@shirazu.ac.ir

Abstract

The leaf growth and cross-sectional area of durum wheat during its development may be reduced under salt stress due to vascular architecture alterations of leaves. A hydroponic experiment was conducted to compare growth rate and vascular architecture changes of two durum wheat cultivars including Shabrang and Yavaroos under 0 and 100 mM NaCl. Plants were sampled at the three-leaf stage growth. Results showed that under salt stress at 15 DAG, Shabrang with 0.69 mm/h had a greater elongation rate than Yavaroos (0.27 mm/hr). Likewise, under salt stress at 25 mm above the leaf base, 32 and 37% reductions in cross sectional area were observed in Shabrang and Yavaroos, respectively. In all treatments, maximum leaf width was obtained at the growth zone (25 mm above the leaf base). In both cultivars, the leaf cross-section of the control consisted of one midrib, 5 large veins and 11-21 small veins, while it composed of one midrib, 4 large veins and 3-12 small veins under salt stress. Overall, in both cultivars, comparison of control and salt stress treatments showed that the reduction in protoxylem area at 5 mm was greater than 100 mm above the leaf base. It can be concluded that the reduction in the cross-section of durum wheat is mainly correlated with a decreased number of small veins, and Shabrang cultivar with greater number and area of small veins along the leaf base had higher leaf growth and expansion rate than Yavaroos, when plants exposed to salt stress. This probably can explain why Shabrang cultivar might be more tolerant to salt stress than Yavaroos.

Keywords: Cross section area; Durum wheat; Elongation rate; Metaxylem; Midrib; Protoxylem Salinity

Introduction

Durum wheat (*Triticum durum*) is the only tetraploid species of wheat with commercial importance that is widely cultivated today. Nowadays, there is increasing demand for durum wheat varieties used in bakery purposes, but farmers are unaware about the durum wheat and its importance. Hence the cultivation of durum wheat in Iran is negligible (Alavi Matin *et al.* 2015). In the field, where the salinity rises to 100 mM NaCl (about 10 dS m⁻¹), rice will die before maturity, while wheat will produce a reduced yield. Even barley, the most-tolerant cereal, dies after extended periods at salt concentrations

higher than 250 mM NaCl (Munns *et al.* 2006). Alavi Matin *et al.* (2015) reported that type of cultivar and salinity level had noticeable effect on tolerance of durum wheat to salinity, so that Shabrang durum wheat cultivar was employed as tolerant to salinity and Yavaroos as sensitive.

Salt stress causes a rapid and potentially lasting reduction in the rate of leaf growth. Reduction of leaf elongation rate results from a reduction in the number of elongating cells or a reduction in the rate of cell elongation or from both. From the biophysical point of view, a leaf cell of a NaCl-treated plant can expand at reduced rates because of reduced uptake rates of water,

hardened walls, or lowered turgor (Hu *et al.* 2000b; Fricke and Peters 2002). Leaf growth is often more reduced than root growth under salinity stress, a change which is in common with dry soil conditions (Bijanzadeh and Emam 2015). On the other hand, salinity reduces the ability of plants to uptake water, and this quickly causes reductions in growth rate, along with a suite of metabolic changes identical to those caused by water stress (Hu *et al.* 2000b; Cavusoglu *et al.* 2007 and 2008).

Many researchers reported that high salinity levels reduce the size of the vein segments and number of small veins (Hu *et al.* 2005), stomata number (Hwang and Chen 1995; Cavusoglu *et al.* 2007) and leaf thickness (Cavusoglu *et al.* 2008). Other structural changes such as inhibition of differentiation, diameter and number of xylem vessels also occur in the salt stressed plants (Ola *et al.* 2012; Bijanzadeh and Kazemeini 2014). Under saline conditions, smaller final leaf size is not only due to a shorter leaf length, but also to a narrower leaf (Hu *et al.* 2005). Also, Hu *et al.* (2000a) reported that the length and width of wheat leaves on the main stem reduced approximately by 20–30%, and width reduction mainly occurred at the leaf base under 120 mM NaCl. Hu *et al.* (2005) showed that a total of 34 veins are present in a mature wheat leaf (cv. Thasos) in the region between the ligule and 15 cm above it which 11 of them were large or medium size. Kuo *et al.* (2004) reported that 11 large and medium-size veins in wheat would account for 96% of the total water flow in the cross-section. Trivett and Evert (1998) reported that in barley, the first, large and medium veins

are initiated and differentiate acropetally; small veins are initiated later in development and differentiate basipetally. Most of axial water transport through the elongation zone occurs through protoxylem vessels.

In recent years, considerable attention has been focused on the development of conducting tissue in monocot leaves. However, little information is available about how the veins in durum wheat leave respond to salinity. Studying the relation between the cross-section of durum wheat leaves and the leaf architectural changes under saline conditions will provide an opportunity to obtain new insights into the mechanisms of salt limitation to plant growth because the physiological functions of the leaf are linked to its architectural properties (Hu *et al.* 2000a, 2000b; Munns *et al.* 2006). The main aim of this study was to evaluate the effect of salinity stress on spatial distribution of components in a cross-section of the growing leaf axis including number of veins, the area of midrib, large and small veins and protoxylem and metaxylem vessels of two durum wheat cultivars.

Materials and Methods

Plant material

The effect of salt stress (100 mM NaCl) and control (0 mM NaCl) on the vascular tissue architecture of durum wheat leaf was studied in Shabrang (tolerant to salt stress) and Yavaroos (sensitive to salt stress) cultivars, hydroponically. After soaking seeds in distilled water for one day, they were floated in 1.0 mM CaSO₄ solution for better germination during three days (Fricke and Petres 2002).

Hydroponic culture

The germinated seeds were transferred into a hydroponic system, containing a modified half-strength Hoagland nutrient solution [KH_2PO_4 (1.5 mM), KNO_3 (2.0 mM), CaCl_2 (1.0 mM), MgSO_4 (1.0 mM), FeNa_2 (18.0 μM), H_3BO_3 (8.1 μM), MnCl_2 (1.5 μM)] under 0 and 100 mM NaCl (Fricke *et al.* 2014). Four seedlings were grown in 1-liter glass beakers of nutrient solution ventilated by a gas exchange pump at a flow rate of 400 mL min^{-1} in growth chamber at 16/8 hours day/night photoperiod, temperature cycle of 25/15°C and relative humidity of 70%. Plants were sampled from 11 to 18 days after germination (DAG) corresponding to the three leaf stage growth (from ZGS12 to ZGS13; Zadoks *et al.* 1974).

Leaf length determination and elongation rate

Leaf length and elongation rate were measured daily and expressed to the nearest 5 mm from the base of the third leaf to the tip of the leaf according to Bernstein *et al.* (1993).

Sampling from leaf tissue

For each durum wheat cultivar, the third leaves of about 10-12 cm in length were selected for tissue sampling. Leaf elongation is approximately steady during this stage (Hu *et al.* 2000a). The elongating leaf was carefully freed from the surrounding leaf sheaths and then cut from the base with a razor blade. The leaf was sectioned into five 5-mm-long segments above the base: from the leaf base (5 mm above the leaf base), at the middle of the growth zone (25 mm), at the end of the growth zone (50 mm), in the zone of secondary cell wall deposition (75 mm) and photosynthetic tissues

(100 mm); definition of these zones was based on the study by Hu *et al.* (2000b). Samples were taken quickly (within 2-3 min) under low light intensity to prevent disturbances in water status of the tissue. After sampling, segments were immediately transferred to phosphate buffered saline (PBS) supplemented with 3% formaldehyde and incubated overnight. Then samples were washed in PBS and dehydrated in a graded series of ethanol (Hu *et al.* 2005). After embedding in white acrylic resin, semi-thin sections at 5, 25, 50, 75 and 100 mm above the leaf base were cut with a razor blade and stained with toluidine blue for one minute (Brundrett *et al.* 1988; Hachez *et al.* 2006) and leaf components were detected under bright light microscope (Canon 2022, Japan). All measurements were performed in four replicates. The longitudinal veins in durum wheat leaves were classified as large and small veins (Figure 2A). These veins are characterized by differential presence of metaxylems and protoxylems. Large veins have a large metaxylem vessel on either side of the protoxylem while medium veins have smaller metaxylem, and small veins lack protoxylem and the metaxylem vessels. Light micrographs show cross-sections of vascular vessels and xylem vessels (Figures 2 and 3). According to light micrographs, the number and the area of midrib, large and small veins were determined. Also, the areas of vascular bundles were determined for large and small veins and areas of metaxylem and protoxylem in a large vein using Photoshop 15 software.

Statistical analysis

Twelve seedlings of each durum wheat cultivar (Shabrang and Yavaroos) at two concentrations of NaCl (0 and 100 mM) in four replications were compared in a completely randomized design. Collected data were subjected to analysis of variance and the means were compared using least significant (LSD) test ($p \leq 0.05$) by SAS 9.1 software.

Results and Discussion

Leaf length and leaf elongation rate of durum wheat cultivars

Results of non-destructive daily measurements of third leaf length in Shabrang and Yavaroos cultivars under control and salt stress (100 mM NaCl) are presented in Figure 1a. In the control, for both cultivars, the leaf length started to increase sharply from 13 DAG and reached to a maximum value at 16 DAG and remained constant up to 18 DAG. Shabrang had the higher leaf length under salt stress from 12 to 18 DAG. The leaf lengths in Shabrang and Yavaroos were maximized at 16 and 15 DAG, respectively, and remained constant up to 18 DAG. In addition, under salt stress, at 16 DAG the 3rd leaf length decreased 31% in Shabrang and 60% in Yavaroos.

At 14 DAG, the highest leaf elongation rate was obtained in Shabrang (1.25 mm/hr); similarly, under salt stress at 15 DAG, Shabrang with 0.69 mm/hr had the higher elongation rate than Yavaroos (Figure 1b). In Shabrang, conversely to Yavaroos, the development of the third leaf delayed one day under salt stress. The effect of salt stress on durum wheat leaf growth and shoot development was similar to its effect on barley

(Bijan-zadeh and Kazemeini 2014), sorghum (Bernstein *et al.* 1993) and lettuce (Lazof *et al.* 1991). In all these three species salinity stress decreased the rates of leaf elongation, maximal leaf length attained and plastochron duration. Fricke and Petres (2002) declared that the effect of reduced leaf elongation rate was partly compensated by an 0.5- to 2-days longer duration of third leaf when plants exposed to salt stress and final leaf length was 34.8 ± 2.9 cm in control, 33.2 ± 2.7 cm in 75 mM NaCl and 30.5 ± 4.7 cm in 120 mM NaCl plants (means \pm SD of 10–17 plant analyses). Also, they reported that maximal leaf length attained was reduced 20% in the third leaf, and maximal elongation rate of the third leaf was decreased 47% by the salt stress. Between batches of plants, means of maximal elongation rates ranged from 2.57 to 2.98 mm/hr (control), from 2.09 to 2.53 mm/hr (75 mM NaCl) and from 1.79 to 2.12 mm/hr (120 mM NaCl), respectively. Fricke *et al.* (2014) observed that leaf elongation rate decreased by 0.12 mm/hr or 6% less at 11 DAG than control and the elongation rate suddenly recovered to 1.15 mm/hr at 13 DAG, when the wheat (cv. Shiraz) exposed to 100 mM NaCl. Vysotskaya *et al.* (2010) showed that the mean third leaf length declined 20%, compared to the control treatment, in wild barley (*Hordeum spontaneum*) under 75 mM NaCl, and the leaf length started to increase at 12 DAG and maximized at 15 DAG. They concluded that the type of cultivar, salinity level and seedling growth stage had the main role in salt tolerance of wild barley. Opposite to our results, Munns *et al.* (1995) reported no significant difference between

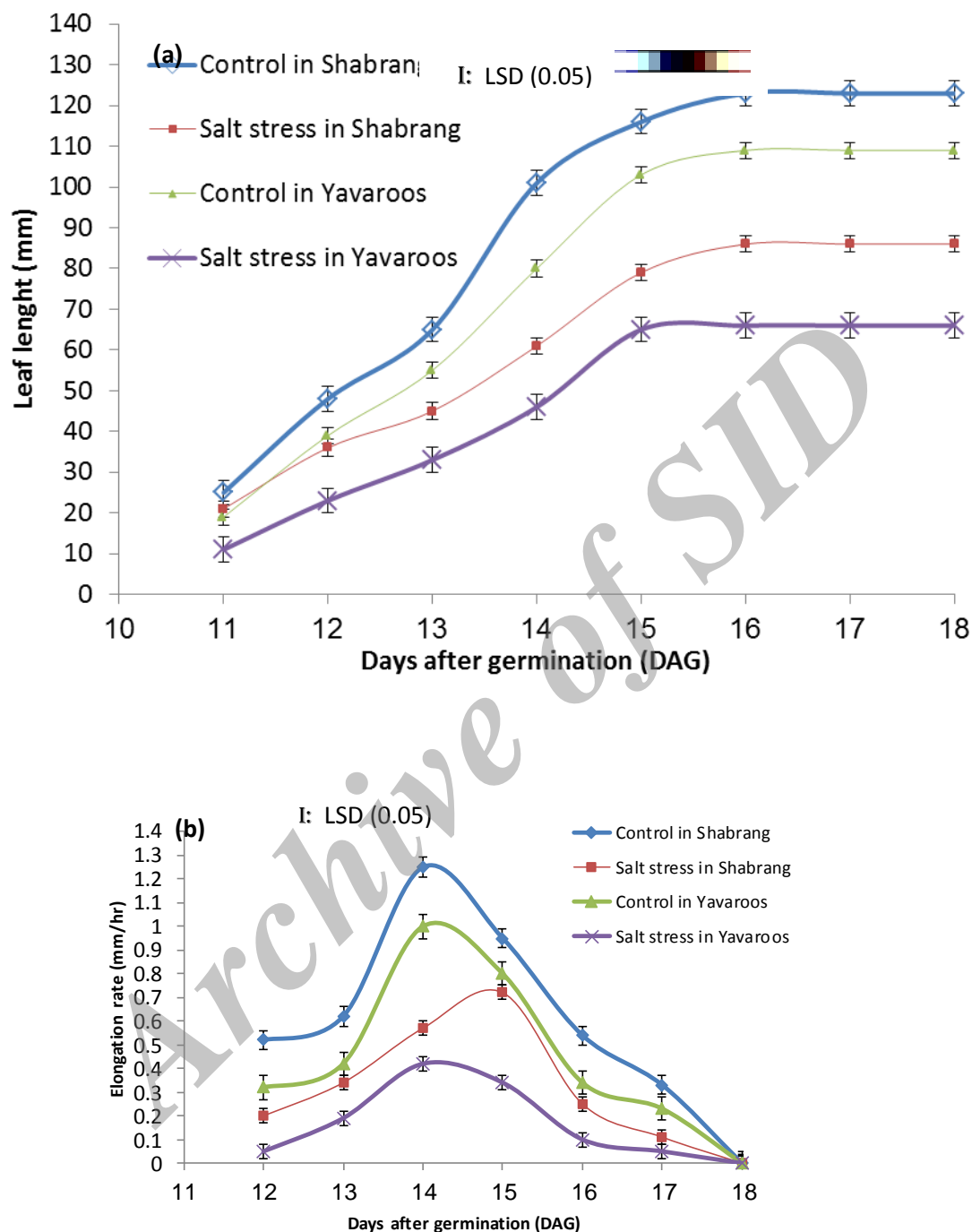


Figure 1. Effect of 100 mM NaCl on leaf length (a) and elongation rate (b) of third leaf durum wheat cultivars. Data represented results of non-destructive daily leaf length measurements. Vertical bars represent standard error and data was compared using LSD test at 5% probability level.

the leaf elongation rates in the first 20 days of applying salinity to durum wheat cultivars.

Third leaf tissue architecture of durum wheat cultivars

The tissue and vascular architecture of durum wheat third leaf are shown using light micrographs of cross-sections at 5, 25, 50, 75 and 100 mm above the leaf base for control and 100 mM NaCl treatments in Figure 2. The following results were obtained from these measurements.

Cross sectional area and leaf width

In all treatments, cross sectional area increased with a distance up to 25 mm above the leaf base and then decreased up to 100 mm above the leaf base (Figures 2 and 3a). Under salt stress at 25 mm above the leaf base, 32 and 37% reduction in cross sectional area were observed in Shabrang and Yavaroos, respectively. In all treatments, the pattern of spatial distribution of width along the leaf axis was similar to that of cross sectional area and maximum leaf width was observed at growth zone (25 mm above the leaf base; Figures 2 and 3b). Under salt stress, leaf width in Yavaroos decreased 38, 50, 53, 64 and 56% in 5, 25, 50, 75 and 100 mm above the leaf base of third leaf compared to the control, respectively. Hu and Schmidhalter (2000) reported that maximum cross sectional area and leaf width along the leaf axis existed at the leaf base of wheat and salinity affects the cross section area of leaf base, possibly by altering the processes of recruiting founder cells from the shoot apical meristem. They concluded that the cellular cross sectional area of

the leaf at 120 mM NaCl was reduced by 52% at 5 mm as compared with about 36% averaged from the region between 5 and 30 mm from the leaf base. Taleisink *et al.* (2009) stated that tissue expansion and cell division in monocotyledons during leaf development tend to become unidirectional, resulting in strap-like organs. Most studies have focused on leaf length expansion under salinity in grasses, although narrower leaves are common under saline conditions and reduction in cross section also occurs at the leaf base (Bijan-zadeh and Kazemeini 2014; Hu *et al.* 2005). Smith and Hake (1992) reported that the orientation of cell division is primarily responsible for the increase in leaf width at early leaf development. In a study on sorghum, Baum *et al.* (2000) reported that salinity stress caused narrower midribs and smaller leaf width and cross sectional area. Under these conditions, only half of the veins were functional in water transport. Our results indicated that the salinity induced the reduction in the cellular cross sectional area occurred mainly at the leaf base where the leaf initiated. This was further supported by reduction in width, which mainly occurred at the leaf base (Figure 3b).

Number and area of midrib and veins

The leaf cross-sectional area is dependent on both the number of veins and the size of vein section (Figure 2). The total number of veins includes midrib and all large, medium and small veins. Figures 4 and 5 showed the trend of spatial distribution of cross-sectional areas of the midrib and large and small vein sections along the leaf

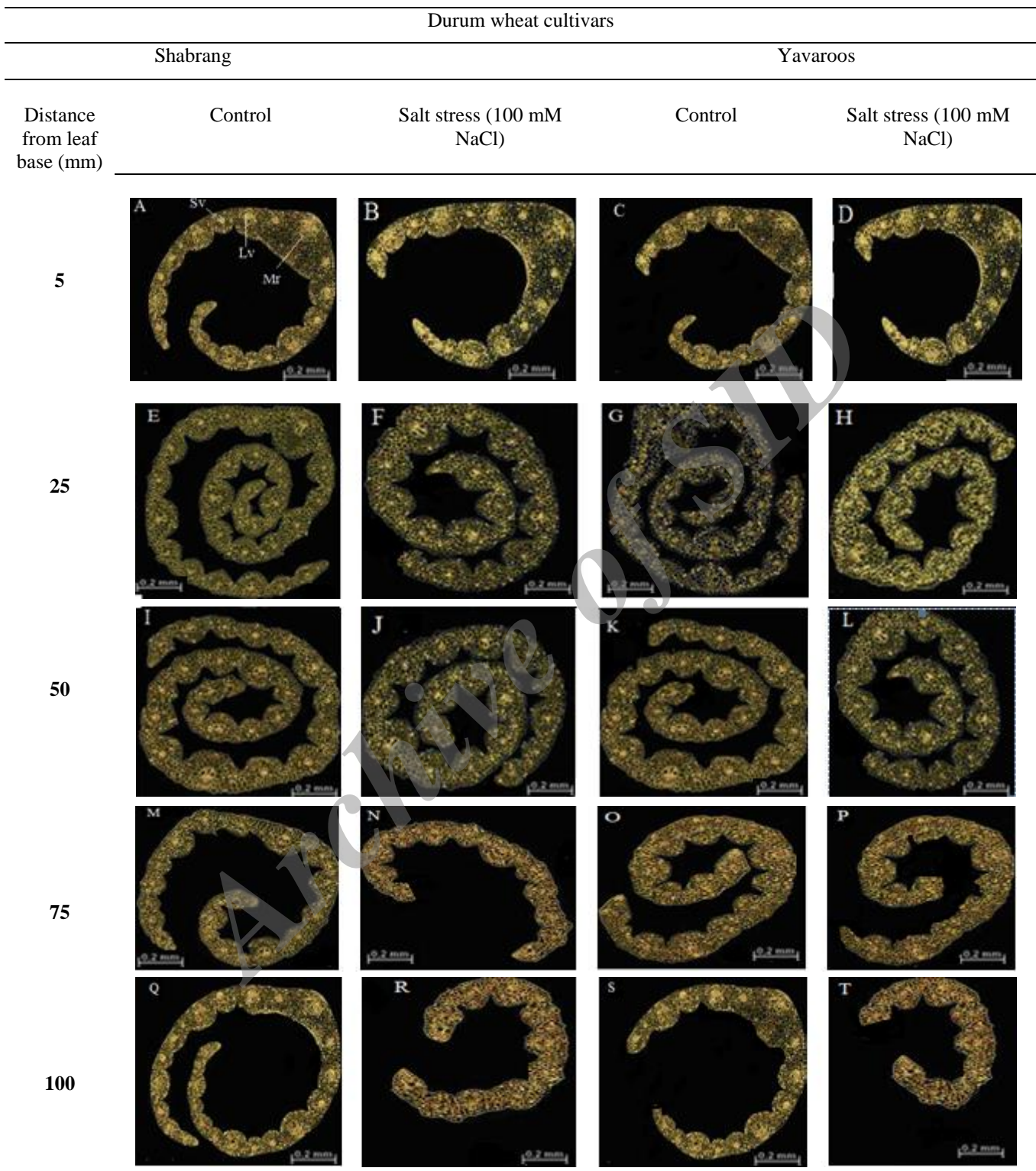


Figure 2. Cross-sections micrographs of third leaf of durum wheat cultivars at 5 mm (start of elongation zone; A, B, C, D), 25 mm (middle of the growth zone; E, F, G, H), 50 mm (end of the growth zone; I, J, K, L), 75 mm (zone of secondary cell wall deposition; M, N, O, P) and 100 mm (photosynthetic tissues; Q, R, S, T) above the leaf base for 0 and 100 mM NaCl. The structures of midrib, large veins and small veins were indicated with arrows in the cross section (A). Mr: midrib, Lv: large vein, Sv: small vein. Scale bar is 0.2 mm.

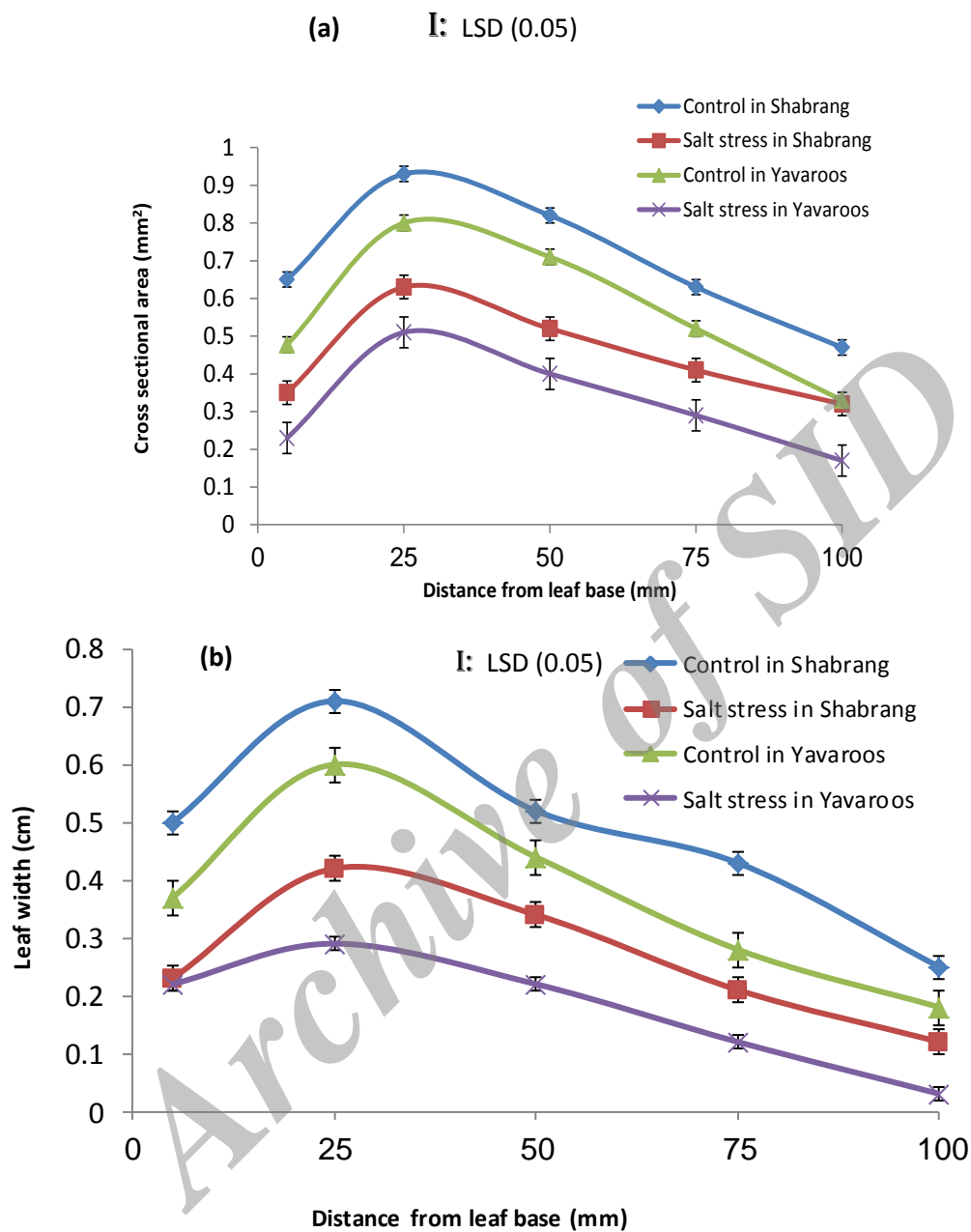


Figure 3. The cross-sectional area (a) and width (b) of third leaf of durum wheat cultivars grown under 0 and 100 mM NaCl treatments. The measurements were based on light micrographs. Vertical bars represent standard error and data was compared using LSD at 5% probability level.

axis. In both cultivars, the leaf cross-section of the control consisted of 1 midrib, 5 large veins and 11-21 small veins, while it included 1 midrib, 4 large veins and 3-12 small veins under salt stress (Figures 2 and 4). Interestingly, under control and salinity treatments, no significant difference was observed between the number of large veins of Shabrang and Yavaroos (Figure 4b). Overall, under salt stress a significant reduction was observed in total vein number due to a reduction in the number of small veins (Figures 4a and 4c). Similar to total number of veins, in the two durum wheat cultivars, the number of small veins increased up to 25 mm above the leaf base and then decreased with distance from the leaf base (Figure 4c). Hu *et al.* (2005) reported that the leaf cross-section of control in wheat consisted of one midrib, 4 large and 14–23 small veins in addition to medium veins whereas it contained one midrib, 5 large and 11–15 small veins (totaling 16–20 veins) for 120 mM NaCl treatment. This indicated a 30% reduction in the number of small veins under salt stress. In our study, in both cultivars, between 36 to 53% reduction was observed in small veins under salt stress (Figure 4c). Trivett and Evert (1998) reported that, large veins are initiated and differentiated acropetally in monocotyledons, after which medium and small veins are initiated basipetally. Thus the greater reduction in the number of small veins suggests that salinity affects the development of cross section well after the formation of large veins. In a similar study, Kuo *et al.* (2004) reported that the total number of veins in the transverse leaf section was about 32 in the control plants and remained constant up to 25 mm above the leaf base.

In all treatments, the area of the midrib decreased sharply from 5 to 100 mm above the leaf base in spite of large and small veins (Figure 5a). Also, in both cultivars, salt stress reduced the area of the midrib between 30 to 55% along the leaf axis. In both cultivars, the maximum area of large veins was observed in 25 mm above the leaf base and decreased sharply up to the end of the growth zone (Figure 5b). Also, in Shabrang in spite of Yavaroos, the area of large veins was not affected by salinity from the 25 to 100 mm above the leaf base. In the two cultivars, the size of small veins increased up to 50 and 75 mm for the control and salt stress treatments, respectively. Generally, salt stress affected the area of small veins more than large veins especially in Yavaroos (Figures 5b, 5c). In a similar study, Hu *et al.* (2005) attributed the reduction in cross-sectional area to a decrease in the area of the vein segments and a reduced number of medium and small veins of wheat leaf. They approximately observed 35% reduction in the number of veins under saline conditions (mainly in the number of small veins) which likely suggest the reduction in the capacity of re-translocation of mineral nutrients and assimilates due to salinity.

Area of metaxylem and protoxylem of large vein

The areas of metaxylem and protoxylem from a large vascular bundle in the third leaf of durum wheat cultivars grown under control and 100 mM NaCl treatments are shown in Figure 6. In the control, the area of metaxylem in large veins maximized at 25 mm above the leaf base of Shabrang which was significantly higher than

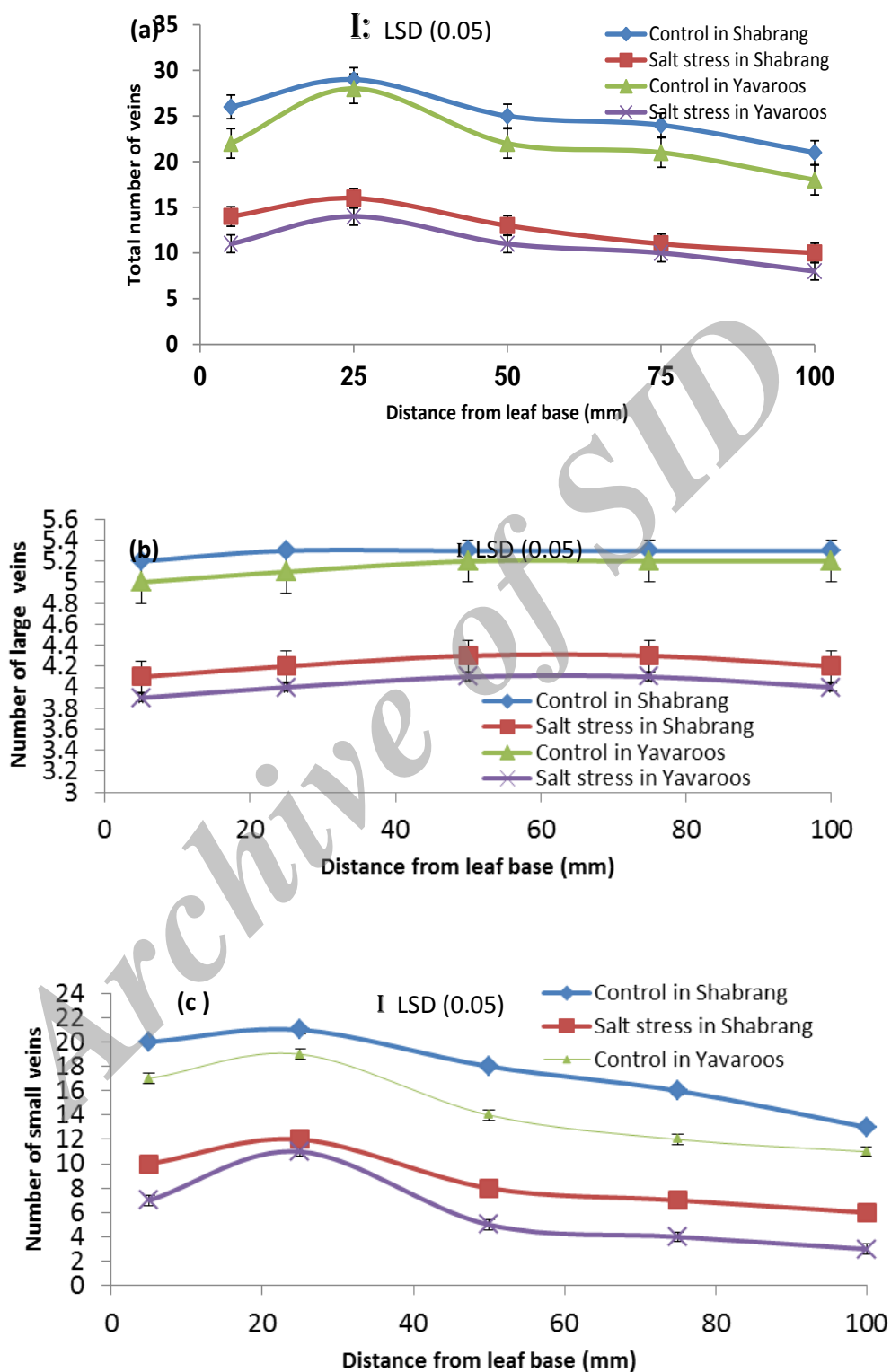


Figure 4. The numbers of veins in third leaf of durum wheat cultivars grown under 0 and 100 mM NaCl. The measurements were based on the light micrographs. Vertical bars represent standard error and data was compared using LSD at 5% probability level.

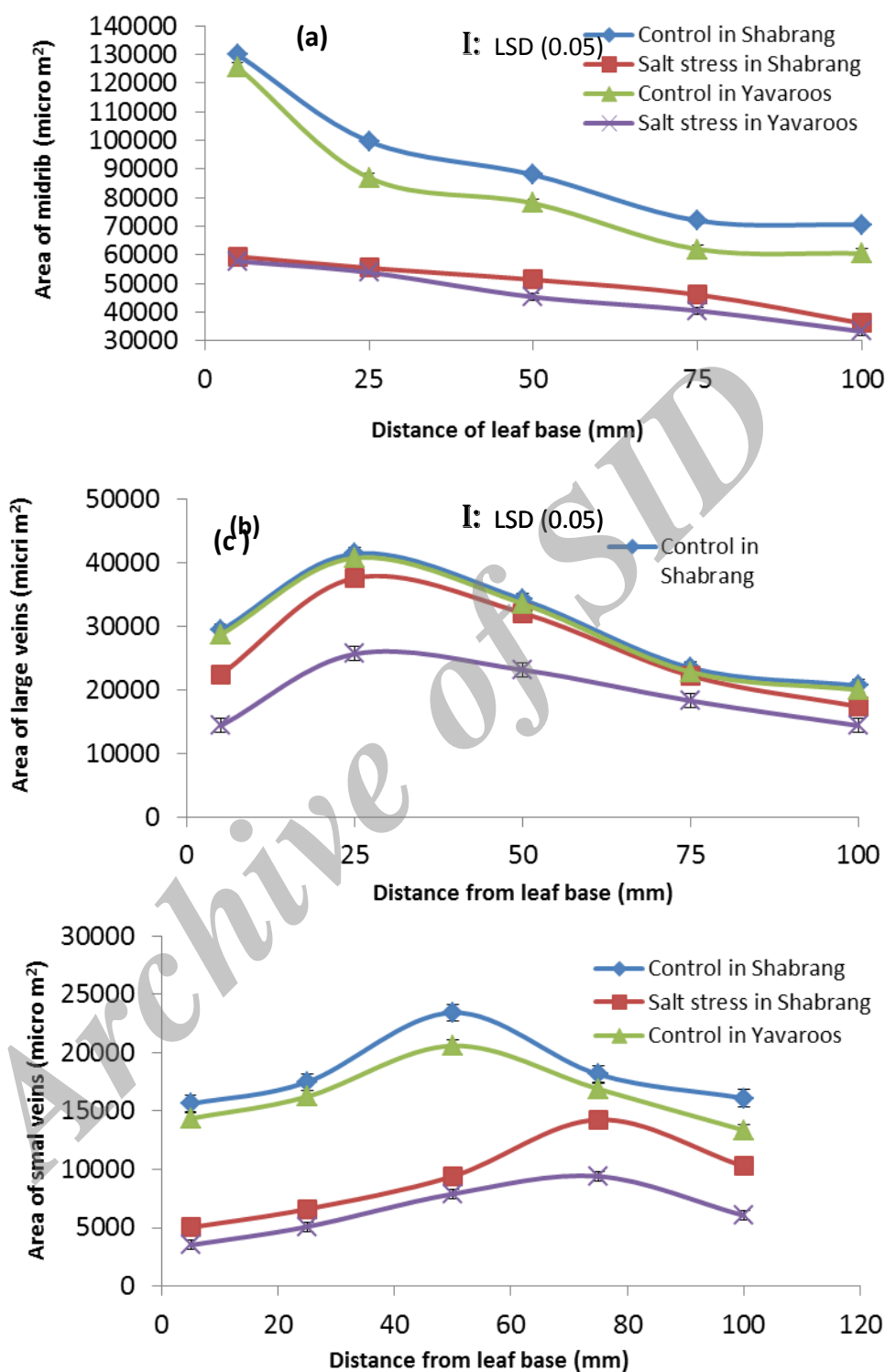


Figure 5. The area of a midrib, large vein and small vein segment in the third leaf of durum wheat cultivars grown under 0 and 100 mM NaCl. The measurements were based on light micrographs. Vertical bars represent standard error and data was compared using LSD at 5% probability level.

Yavaroos (Figure 6a). Under salt stress conditions, in both cultivars, metaxylem area per bundle reduced up to 73% compared to the control. Overall, in both cultivars, comparison of the control and salt stress treatments showed that the reduction in protoxylem area at 5 mm was greater than at 100 mm above the leaf base (Figure 6b). Also, protoxylem area per bundle in Yavaroos decreased more than Shabrang along the leaf base. Baum *et al.* (2000) reported that within the growth zone, salt-affected leaves of sorghum had narrower protoxylem and metaxylem cells than the control and in both salt-treated and control plants; the protoxylem was mature at 13 mm from the base of the leaf while the metaxylem was immature. Hu *et al.* (2005) reported that the reduced area of protoxylem and metaxylem in midrib and large vein segments in growing tissues may be responsible for lower shoot growth under saline conditions. Our results were in disagreement with those reported by Ola *et al.* (2012) in Kallar grass (*Leptochloa fusca* L. Kunth) who reported that metaxylem area and number of veins were not affected by salinity up to 100 mM NaCl. Generally, some researchers suggested that tissue architecture changes depended on salinity level and type of crop and cultivar (Hu and Schmidhalter 2000; Martre *et al.* 2000; Munns 2005).

Conclusion

It can be concluded that the reduction in the cross-sectional area of durum wheat is mainly correlated to a decreased number of small veins and Shabrang cultivar with greater number and area of small veins along the leaf base had higher leaf growth and expansion rate compared to Yavaroos, when plants were exposed to salt stress. On the other hand, the reduced area of protoxylem and metaxylem in midrib and veins especially at 0-25 mm above the leaf base may be responsible for lower leaf growth rate under salinity conditions. Since the different architectural properties of the leaf vein system are related to physiological leaf functions, further works is needed to determine the water flow of nutrients in relation to leaf anatomical structure in the growing leaves using different salinity levels and durum wheat cultivars. Overall, the leaf vascular architecture studies can be used as a useful tool for selecting and breeding of salt tolerated durum wheat cultivars in the near future.

Acknowledgments

We would like to thank the Research Council of Shiraz University in Iran for financial support of this research project.

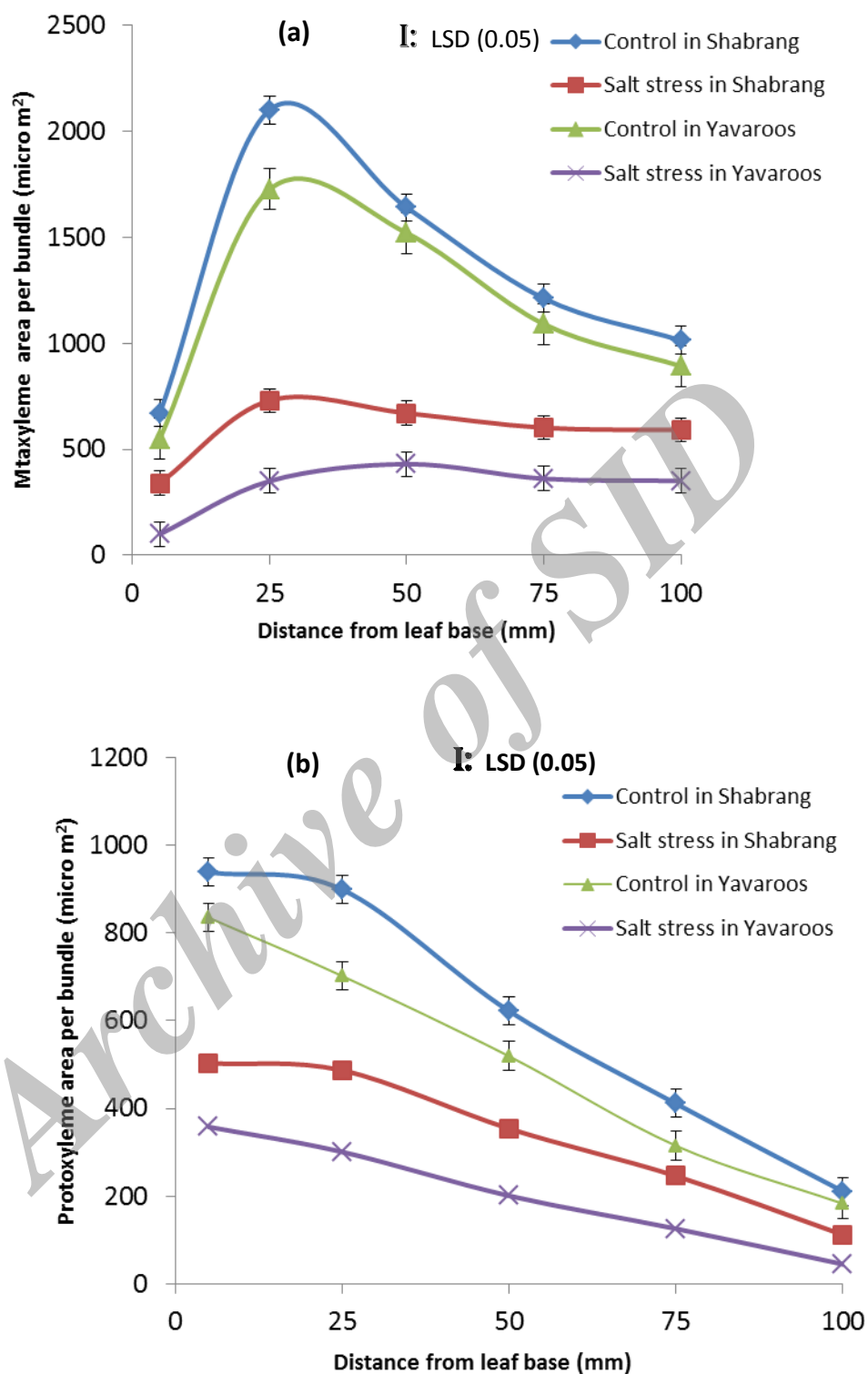


Figure 6. The areas of the metaxylem (a) and protoxylem (b) from a large vascular bundle in third leaf of durum wheat cultivars grown under 0 and 100 mM NaCl. The measurements were based on light micrographs. Vertical bars represent standard error and data was compared using LSD at 5% probability level.

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