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ORIGINAL ARTICLE

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Effect of salinity on tissue architecture in expanding wheat leaves

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Abstract Salinity greatly reduces the leaf cross-sectional area of wheat (*Triticum aestivum* L.) during its development, which may lead to variation in the architectural properties of growing leaves that would result in a change in leaf physiological functions. Our objective was to characterize the effect of salinity on the spatial distribution of the cross-sectional area and the anatomy of large and small veins of a growing wheat leaf. Spring wheat was grown in a growth chamber in soils with or without 120 mM NaCl. Leaf 4 in both treatments was harvested 2–3 days after its emergence and then cut into five transverse segments. Examination of the transverse sections revealed that salinity significantly reduced the cross-sectional area, width, and radii of both epidermal and mesophyll cells along the leaf axis. Reduction in the cross-sectional area and width occurred mainly at the leaf base, indicating that these reductions occur during the period of leaf initiation. The reduction in cross-sectional area was attributed to a decrease in the size of the vein segments and a reduced number of medium and small veins. The thickness of the leaf was also reduced under the 120 mM NaCl treatment. A greater intercellular air space in the large vein segments under saline conditions was also found. The approximately 35% reduction observed in the number of veins under saline conditions (mainly in the number of small veins) may suggest that salinity reduces the capacity for re-translocation of mineral nutrients and assimilates. The reduced area of protoxylem and metaxylem in midrib and large vein segments in growing tissues may be responsible for lower water deposition into the growth zone under saline conditions.

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Introduction

Wheat is a major food crop in most of the countries where saline soils exist or may develop (Ashraf and McNeilly 1988) and is reported by Maas and Hoffman (1977) to be moderately tolerant to salinity. However, the mechanisms by which salt affects wheat growth are still poorly understood. In the early stages of wheat development, as in other grasses, leaf growth largely determines the rate of plant growth (Munns and Termaat 1986; Maas and Poss 1989; Arif and Tomos 1993). Under saline conditions, the reduction in final leaf size is due not only to a shorter leaf length, but also to a narrower leaf. Our earlier study (Hu et al. 2000a) showed that the final length and width of wheat leaves on the main stem that were treated with 120 mM NaCl were reduced by about 20–30%, and the reduction in width occurred mainly at the leaf base. Yet, many studies have focussed only on the effect of salinity or other stresses on longitudinal leaf elongation of grasses (e.g. Bernstein et al. 1993; Hu et al. 2000a; Ben-Haj-Salah and Tardieu 1995). Surprisingly few studies have dealt with the effect of salinity on leaf width and anatomy of the leaf cross-section. A study by Hu and Schmidhalter (2001) showed the effect of salinity on leaf width and leaf cellular cross-sectional area estimated by using leaf water content, which could not explain the reasons for the observed changes. Studying the relation between the cross-section of grass 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.

As in other grasses, the growth of wheat leaves is limited to a small region near the leaf base, and the growth zone is enclosed in the older leaf sheath. The

visible leaf of grasses increases in length only because the width remains unchanged once the leaf has emerged from the sheath bundle (Dale 1988). Rademacher and Nelson (2001) demonstrated that the increase in the cross-sectional area along the leaf axis of the growing leaves in tall fescue is most rapid near the base, and is independent of genotype and N treatments. According to Hu and Schmidhalter (2001), the inhibition by salinity of growth in the cross-sectional area or width in a growing wheat leaf occurs mainly between the leaf base and 5 mm above the base.

The vascular systems of a grass leaf consist of a series of roughly parallel longitudinal vein segments that are grouped into dermal, ground and vascular tissues associated with a vein in the cross-section. Each vein segment consists of different types of cells, such as epidermal cells, mesophyll cells, xylem and phloem cells, and intercellular space. Kuo et al. (1974) reported that there are a total of 34 veins in a mature wheat leaf (cv. Heron) present in the region between the ligule and 15 cm above it. Of these 34 longitudinal veins, 11 are of large or medium size, whereas 23 are small. Large and medium veins are initiated first and differentiate acropetally; small veins are initiated later in development and differentiate basipetally (Trivett and Evert 1998). From theoretical considerations, Kuo et al. (1974) concluded that, because they contain the large-diameter vessels, the 11 large and medium-size veins in wheat would account for 96% of the total water flow in the cross-section. Knowledge of the effect of salinity on the number of large or small veins will contribute to a better understanding of the processes of leaf development and characteristics of water transport. However, there is still no information available about how the veins in grass leaves respond to salinity or other stresses.

The leaf growth zone is an active sink for water, assimilates and mineral nutrients, with these elements being spatially distributed along the leaf growth zone of wheat and sorghum and spatially affected by salinity as well (Bernstein et al. 1995; Hu and Schmidhalter 1998; Hu et al. 2000b). Several studies have suggested that most of the axial water transport through the elongation zone occurs through protoxylem vessels (Dannenhoffer and Evert 1994; Fricke and Flowers 1998; Martre et al. 2001; Tang and Boyer 2002). The much larger and faster-conducting metaxylem vessels are found only in mature tissues and beyond the distal end of the elongation zone. The switch from a low-conducting protoxylem path (elongation zone) to a high-conducting metaxylem path (maturation zone) may explain the 10-fold lower axial hydraulic conductance in the elongation zone compared to the mature blade (Martre et al. 2000). Only one study has dealt with the effect of salinity on xylem structure in growing leaves, but this was for sorghum (Baum et al. 2000), which is more tolerant to salt than wheat. Additionally, at saturating irradiance, photosynthesis is generally limited by the rate of CO_2 diffusion into the leaf, where the two most important components controlling this diffusion are stomatal resistance and mesophyll resistance. The studies of Brugnoli and Björkman (1992) and Delfine et al. (1998) have shown that salinity-reduced conductance to CO_2 diffusion caused by stomatal closure accounts for much of the reduction in photosynthesis under saline conditions. Recently, James et al. (2002) showed that salinity also reduced the conductance to CO_2 diffusion in the mature leaves of wheat. Thus, study of the development of mesophyll cells in the growing leaf of grasses may also be important to understand the limitation of photosynthesis under saline conditions.

The objective of this study was to determine the effect of saline conditions on the spatial distribution of components in a cross-section of the growing leaf axis. The components that we examined include the areas or diameters of protoxylem and metaxylem vessels and the cross-sectional area of the epidermal and mesophyll cells.

Materials and methods

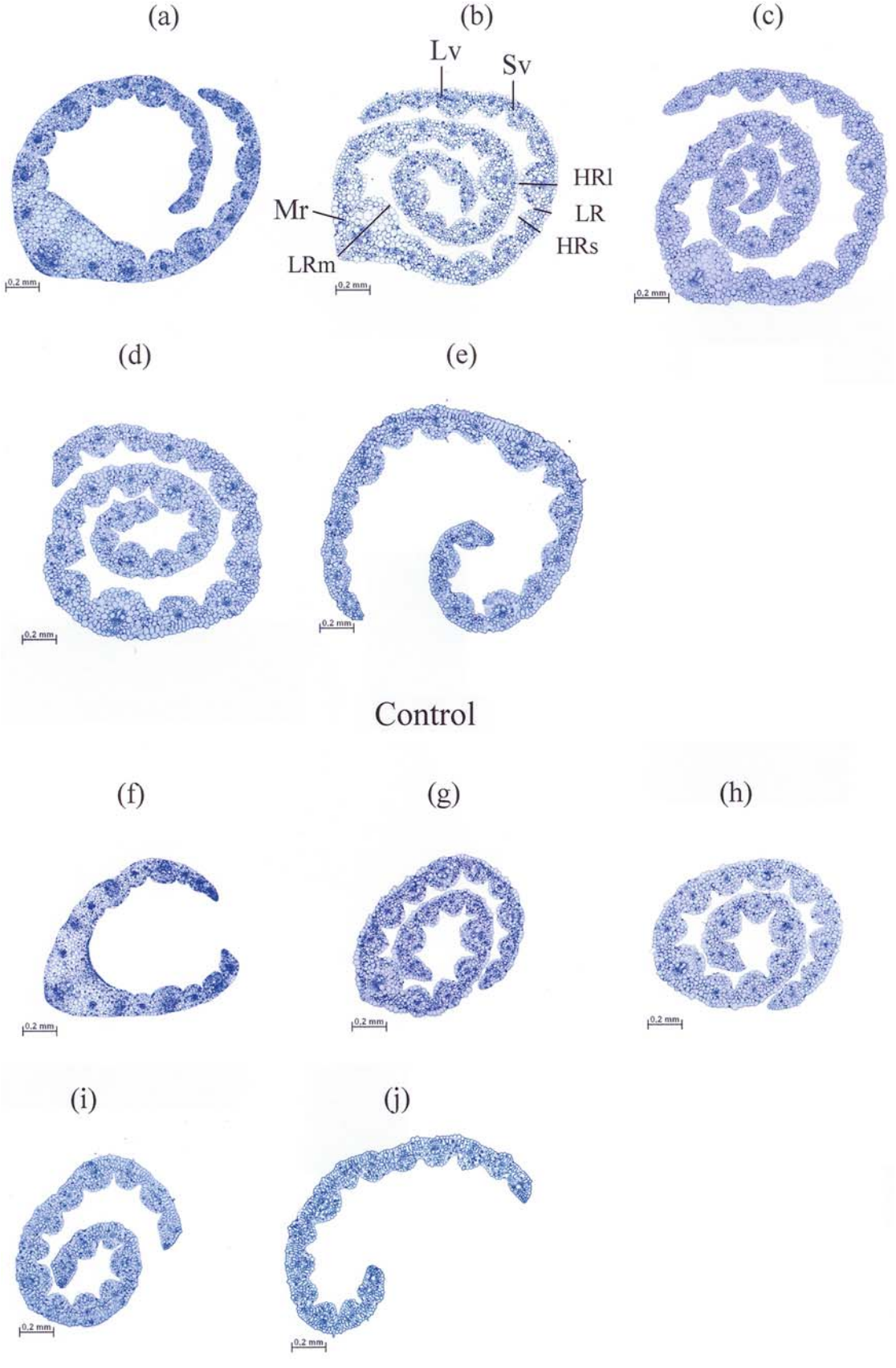
Growth conditions

Six seeds of spring wheat (*Triticum aestivum* L. cv. Thasos), pre-germinated at 20°C for 2 days on filter paper wetted with tap water, were sown in 1.5-l pots (10 cm in diameter and 20 cm high) containing loam soil. The soil was initially watered to 0.25 g H_2O per g of dry soil (which allowed for optimum aeration), with 0.2 g NH_4NO_3 per kg of dry soil. A salt level of 120 mM NaCl was obtained by adding NaCl to the nutrient solution. The soil was thoroughly mixed and kept in tightly closed plastic boxes for 1 week to facilitate equilibration. Thereafter, the soil was sieved and put into pots. Soil moisture content was maintained at the initial content by watering with tap water. To minimize water loss by evaporation, the pots were covered with a perforated plastic film that allowed the plants to grow through the small holes. One week after sowing, the seedlings were thinned to four plants per pot.

The experiment was conducted in a growth chamber with a 16-h photoperiod. The light intensity was approximately 550 μmol photons m^{-2} s^{-1} (PPFD). Air temperature was 20°C (day/night) and the relative humidity was maintained at 55–65%.

Tissue sampling and sample preparation

Because salinity delays the development of grass leaves (Hu et al. 2000a), plants in both treatments were harvested at the same developmental stage: 2–3 days after leaf 4 on the main stem had emerged. Elongating leaves of about 15 cm in total length were selected for sampling. Leaf elongation was 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 of the leaf with a razor blade.


(a)
(b)
(c)
Lv
Sv
HRl
LR
HRs
Mr
LRm
0.2 mm
(d)
(e)
Control
(f)
(g)
(h)
(i)
(j)
120 mM NaCl

◄

Fig. 1a–j Light micrographs showing cross-sections of leaf 4 of wheat (*Triticum aestivum*) at 2 mm (**a,f**), 15 mm (**b,g**), 30 mm (**c,h**), 60 mm (**d,i**), and 90 mm (**e,j**) above the leaf base for control and 120 mM NaCl treatments, respectively. *Mr* Midrib, *Lv* large vein, *Sv* small vein, *LRm* ridge of midrib, *LR* low ridge, *HRm* high ridge of midrib, *HRl* high ridge of large vein segment, *HRs*, high ridge of small vein segment. Bars = 0.2 mm

Beginning at 2 mm above the base, the leaf was then sectioned into five 5-mm-long segments: from the leaf base (2–7 mm above the leaf base), at the middle of the growth zone (15–22 mm), at the end of the growth zone (30–35 mm), in the zone of secondary cell wall deposition (60–65 mm), and photosynthetic tissues (90–95 mm); definition of these zones was based on the study by Hu et al. (2000b). To prevent disturbances in the water status of tissues, each sampling process was finished within 2–3 min and was conducted under low light intensity. After sampling, segments were immediately fixed in a solution of 3% formaldehyde in phosphate-buffered saline (PBS) overnight, and then washed in buffer and dehydrated in a graded series of ethanol. After embedding in LR White acrylic resin, semi-thin sections at 2, 15, 30, 60 and 90 mm above the leaf base were cut with a diamond knife and stained with Toluidine Blue for light microscopy. All measurements were performed with two replicates.

To examine if differential shrinking and swelling of tissues within a gradient of development were a problem during fixation, the spatial distribution of the width of leaf 4 at 2, 15, 30, 60 and 90 mm above the leaf base was measured by using a binocular microscope with scales. Data showed that fixation did not affect the leaf size.

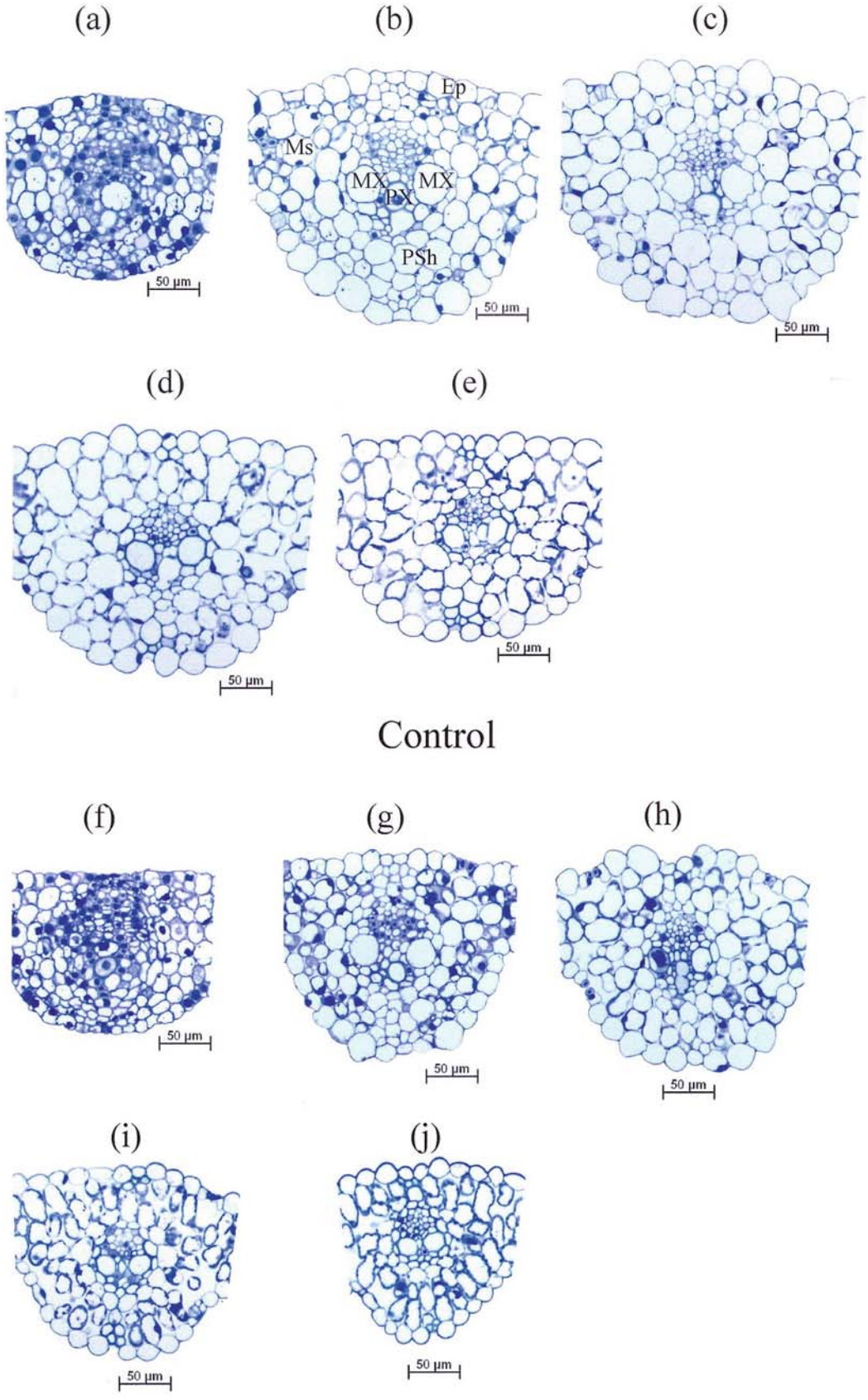
Quantitative microscopy

All anatomical parameters for the transverse sections were determined using a light microscope (Zeiss Axioscope) connected to a PC-based image-processing system. Images were captured using a Zeiss Axio Vision system mounted on top of the microscope and analyzed using SigmaScan Pro 5 (SYSTAT Software, Point Richmond, CA, USA). Besides the midrib, the longitudinal veins in a wheat leaf can be classified by size as large, intermediate and small. According to their appearance and functions and for the purpose of this study, however, the large bundles are referred to as "large vein segments", the intermediate bundles as "medium vein segments" and small bundles as "small vein segments". Large, medium and small veins are characterized by the differential presence of a large metaxylem and protoxylem. Large veins have a large metaxylem vessel on each side of the protoxylem or protoxylem lacuna, medium veins have metaxylem vessels that are much smaller than those in the large veins, and small veins lack protoxylem and the large metaxylem vessels. From the images of transverse sections, cross-sectional area and the width at a given position of the leaf were determined. The mean radii of epidermal and mesophyll cells were determined by measuring 30 individual cells each. The number of epidermal cells was counted separately at both adaxial and abaxial sites. The numbers of mesophyll cells in the midrib, and in large, medium and small veins were counted. The area of mesophyll cells at different veins was calculated according to the number and the mean radii of mesophyll cells. The combined area of mesophyll cells plus intercellular air space was measured, and the intercellular air space was estimated by subtracting the area of mesophyll cells within a vein segment from the combined area of the cells and intercellular air space. The areas of vascular bundles for large and small veins (parenchymatous bundle sheath was included), and areas of metaxylem and protoxylem in a large vein were also determined. Thickness of the high ridges of the midrib, of large and small vein segments and also of the low ridges was determined as well.

Results

Figures 1 and 2 present light micrographs of cross-sections of leaf 4 of wheat at 2, 15, 30, 60, and 90 mm above the leaf base for control and 120 mM NaCl treatments. The following results were derived from the measurements based on these light micrographs. Cross-sectional area increased with distance up to 30 mm above the leaf base and then decreased for both treatments (Fig. 3). However, the decrease in cross-sectional area in the control was sharper than for the 120 mM NaCl treatment. The pattern of spatial distribution of width along the leaf axis was similar to that for cross-sectional area. Salinity significantly reduced the cross-sectional area and leaf width at the leaf base by around 45%, at 20–30 mm above the base by around 38%, and beyond 60 mm above the base by approximately 32%, showing a higher reduction in the growth zone and less reduction in the leaf mature zone for the 120 mM NaCl treatment.

The cross-sectional area of a leaf is dependent on both the number of veins and the size of each vein segment. The total number of veins includes midrib and all large, medium and small veins. In this study, only 1–3 medium veins out of a total 21–31 along the leaf axis were found in the control treatment. Figure 4 shows that besides medium veins, the leaf cross-section of the control consisted of 1 midrib, 4 large and 14–23 small veins, whereas it contained 1 midrib, 4 large and 11–15 small veins (total 16–20 veins) for the 120 mM NaCl treatment. The number of large veins did not change with distance for either treatment and was not affected by salinity (Fig. 4). The number of small veins increased or remained unchanged from 2–15 mm above the leaf base and then decreased with distance for both treatments. Figure 4 demonstrates that the significant reduction observed in total vein number was due to a reduction in the number of medium and, especially, small veins.


(a)
(b)
(c)
Ep
Ms
MX
MX
PX
PSh
50 µm
(d)
(e)
Control
(f)
(g)
(h)
(i)
(j)
120 mM NaCl

◀

Fig. 2a–j Light micrographs showing cross-sections of vascular vessels, xylem vessels, and epidermal and mesophyll cells for leaf 4 of wheat at 2 mm (**a,f**), 15 mm (**b,g**), 30 mm (**c,h**), 60 mm (**d,i**), and 90 mm (**e,j**) above the leaf base for control and 120 mM NaCl treatments, respectively. *Ep* Epidermal cell, *Ms* mesophyll cell, *PSh* parenchymatous bundle sheath, *MX* metaxylem, *PX* protoxylem. Bars = 50 μm

Because leaf cross-sections of the 120 mM NaCl treatments did not contain medium veins and the size of the medium vein segment was similar to that of the small vein segments (data not shown), Fig. 5 presents only the patterns of spatial distribution of cross-sectional areas of the midrib, and large and small vein segments along the leaf axis. The size of large vein segments increased with distance up to the end of the growth zone and then slightly decreased, whereas the size of small vein segments increased until 90 mm above the leaf base for the salt treatment and increased up to 60 mm for the control. In contrast to the large and small vein segments, the size of the midrib decreased up to 90 mm above the leaf base. Salinity significantly reduced the area of the midrib and a large vein segment along the leaf axis, whereas there was no difference for small vein segments between the treatments.

Because both large and small vein segments consist of epidermal cells, mesophyll cells, intercellular air space and vascular bundle, the contribution of each of these components to the area of a vein segment was expressed as percentage (Fig. 6). In large vein segments, the relative contributions of mesophyll cells and intercellular air space to the size of a vein segment increased slightly with distance, whereas those of epidermal cells and vascular bundle decreased. The proportion of the areas of mesophyll and epidermal cells accounted for about 40–50% and 20–25%, respectively, of the cross-section regardless of location and treatments (Fig. 6). The patterns of the relative contributions of vascular bundle and intercellular air space along the leaf axis in small vein segments were similar to those in large ones (Fig. 6). In contrast to the observations in large vein segments, however, the relative contribution in small vein segments of mesophyll cells along the leaf axis decreased up to 20 mm above the leaf base and then remained almost unchanged, whereas that of epidermal cells increased slightly from the leaf base to 30 mm above the leaf base and then decreased with distance (Fig. 6). Salinity reduced the contribution of epidermal cells to the area of large vein segments and increased the relative contribution of the intercellular air space. However, these differences were not significant. In contrast to the results for large vein segments, no difference was observed for any of the parameters for small segments (Fig. 6).

Figure 7 shows that the radii of epidermal cells increased with distance up to 40 mm above the leaf base and then decreased regardless of treatment, whereas the radii of mesophyll cells increased up to 90 mm. Salinity reduced the radii of both epidermal and mesophyll cells along the leaf axis.

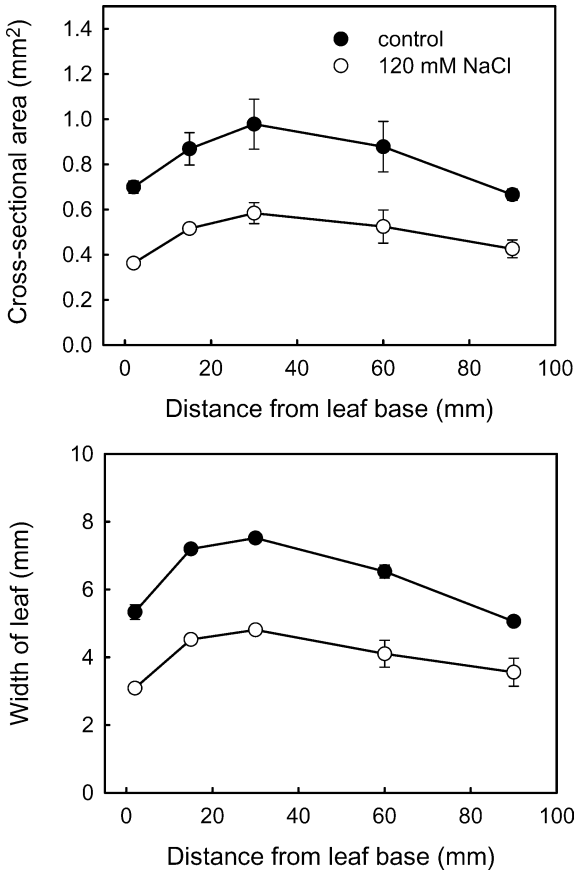


Fig. 3 Spatial distribution of the cross-sectional area and width of growing leaf 4 of wheat grown in soil for control and 120 mM NaCl treatments. The measurements were based on light micrographs. Error bars represent standard errors and fit within the plot symbol if not shown

The area of metaxylem in large veins increased with distance initially and decreased thereafter for both treatments (Fig. 8). However, the increase in the size of metaxylem was up to 15 mm along the leaf axis for the control treatment, but up to 30 mm for the salt treatment. Thereafter, a sharp decrease for the control and only a slight decrease for the salt treatment were observed (Fig. 8). The area of metaxylem was greatly reduced by the 120 mM NaCl treatment along the leaf axis (Fig. 8). Protoxylem area in large veins decreased with distance for both treatments (Fig. 8), and was greatly reduced by salinity except for at 90 mm above the leaf base. The reduction in protoxylem area was greater at the leaf base than at 90 mm, whereas the opposite was true for the metaxylem. The average value of the reduction within the growing leaf for the 120 mM NaCl treatment was approximately 50% and 60% for the metaxylem and protoxylem, respectively.

Thickness of the high ridges of midrib, large and small vein segments and of low ridges is presented in Fig. 9. Low ridge thickness increased slightly with distance for both treatments. The thickness of the high ridge of the midrib decreased along the leaf axis in both

treatments. By contrast, the thickness of the high ridge of the large and small veins increased from the leaf base up to 30 mm and then decreased slightly regardless of treatments. Salinity did not affect the thickness of either the low or high ridges of the small veins, but reduced the thickness of the high ridges of the large veins only at 2 mm above the leaf base and of the midrib at all locations.

Discussion

To understand how salinity affects the architecture of growing leaves and how the changes in leaf architecture are related to physiological functions, the effect of salinity on the architecture of the meristem, expanding and mature tissues in a growing leaf was studied. A growing wheat leaf consists of functionally distinct zones (i.e., cell division, expanding and mature zones), which provides a good opportunity for studying the development of its architecture along its axis. According to Hu et al. (2000a), the growing leaf 4 of wheat during the period of steady growth presents three functionally distinct zones: the cell division and elongation zone (0–30 mm above the leaf base), the secondary cell wall deposition zone (30–60 mm), and the exposed photosynthetically active zone (>60 mm; Hu et al. 2000a). The zones of elongation and secondary cell wall deposition are the regions of the highest biosynthetic activity, and represent strong sinks for carbon photosynthate and nutrients (Allard and Nelson 1991; Hu and Schmidhalter 1998).

The spatial distribution of cross-sectional area along the leaf axis under control and saline conditions is similar to earlier findings estimated using the tissue cellular water contents of the growing leaves of wheat (Hu and Schmidhalter 2001). The observed differences in cross-sectional area or width along the leaf axis between the two treatments occurred mainly at the leaf base, indicating that salinity greatly affects the initiation of the leaf cross-section, possibly by altering the process of recruiting founder cells from the shoot apical meristem. This confirms our earlier conclusions (Hu and Schmidhalter 2001). However, our earlier analyses of the effect of salinity on the cross-sectional area of leaves based on data from the whole-tissue level could not explain the reasons for the observed reductions. According to Figs. 5 and 6, the reduction in cross-sectional area in the 120 mM NaCl treatment is 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.

The development of the leaf cross-section can be divided into two major stages: initiation and further expansion after the initiation. The first stage involves the formation of a primordium that encircles the shoot apical stem before undergoing elongation growth (Sharman 1942; Sylvester et al. 1990; Timmermans et al. 1998). Cell division is initially distributed uniformly throughout the primordium (Sharman 1942; Sylvester et al. 1990). Early in stage one, cross-sectional development and the orientation of these cell divisions contribute primarily to the increase in leaf width (Sylvester et al. 1996), which also plays a formative role in producing the appropriate spatial arrangement of the internal leaf tissues and their component cells (Dengler et al. 1985). The veins are formed at this stage. Therefore, the effect of salinity on the number of veins at the leaf base may explain why the reduction in leaf cross-sectional area occurs mainly at the initiation stage. During the second stage, cell divisions in the distal

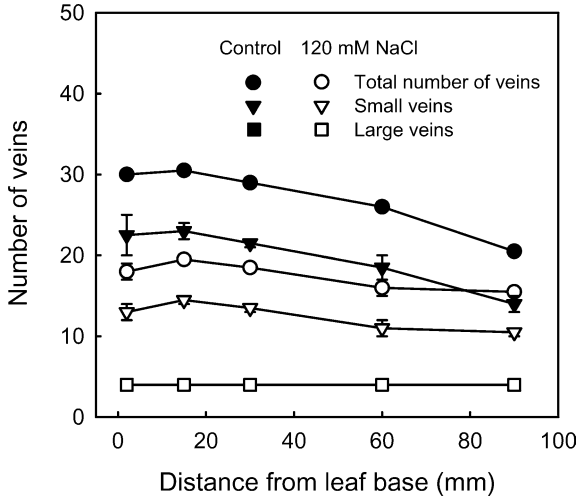


Fig. 4 Spatial distribution of the number of veins in growing leaf 4 of wheat grown in soil for control and 120 mM NaCl treatments. The measurements were based on the light micrographs. Error bars represent standard errors and fit within the plot symbol if not shown

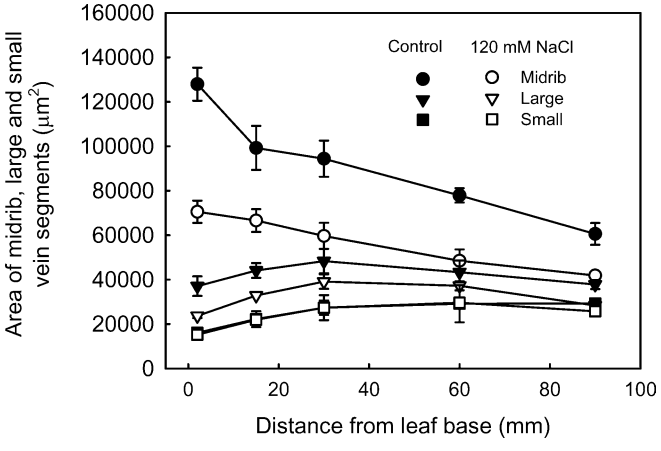


Fig. 5 Spatial distribution of the area of a midrib, a large vein segment and a small vein segment in growing leaf 4 of wheat grown for control and 120 mM NaCl treatments. The measurements were based on light micrographs. Error bars represent standard errors and fit within the plot symbol if not shown

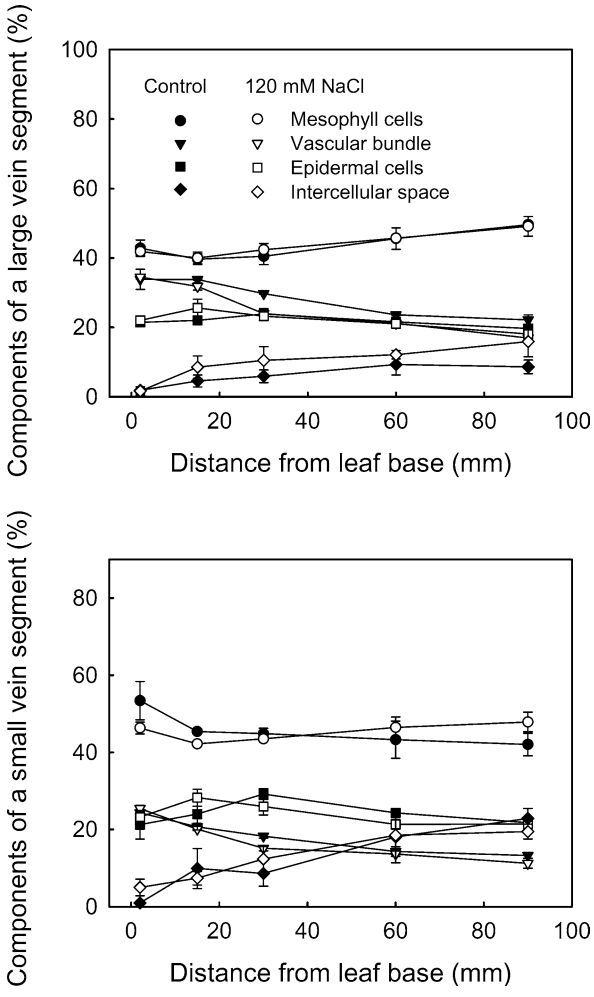


Fig. 6 Areas of mesophyll cells, vascular bundles, air space and epidermal cells as a percentage of the total area of a large vein segment and a small vein segment along the growing leaf 4 of wheat grown in soil for control and 120 mM NaCl treatments. The measurements were based on light micrographs. Error bars represent standard errors and fit within the plot symbol if not shown

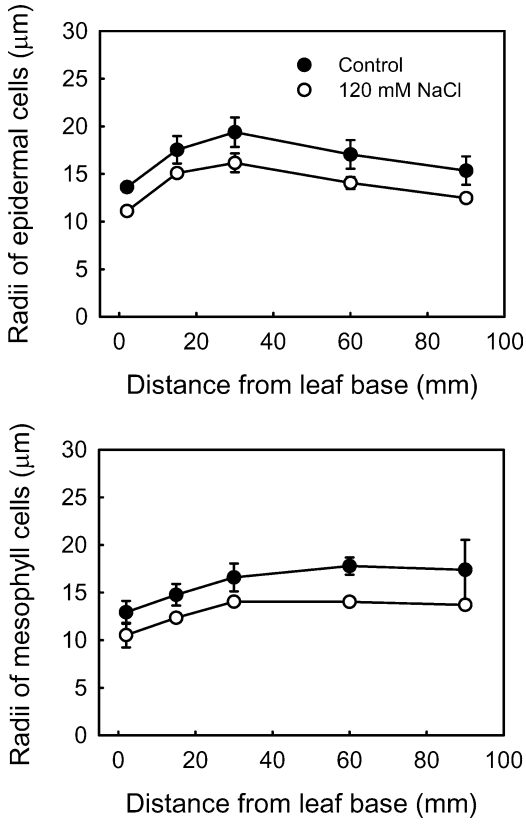


Fig. 7 Spatial distribution of the radii of epidermal cells and mesophyll cells in growing leaf 4 of wheat grown in soil for control and 120 mM NaCl treatments. The measurements were based on light micrographs. Error bars represent standard errors and fit within the plot symbol if not shown

portion of the leaf are predominantly horizontal in orientation and serve to perpetuate cell files originating in the zone of formative divisions at the leaf base. Cell differentiation occurs basipetally along these cell files. Thus, grass leaves display a longitudinal gradient of development. Although leaf expansion of grasses is largely unidirectional at this stage, the leaf expands slightly in the lateral and vertical dimensions as well (Fig. 3), which may be attributed to a further expansion of the mesophyll cells and intercellular air space.

The total number of veins in the transverse leaf section was about 32 in the control plants and remained almost constant up to 15 mm above the leaf base (Fig. 4). A similar number of vascular bundles was reported in mature wheat (cv. Heron) leaves by Kuo et al. (1974). Interestingly, salinity only reduced the cross-sectional area of the midrib and large veins. The average area of cross-section per vein within the leaf was reduced by 10%, whereas the total number of veins was reduced by 35%, which accounted for the major reduction in leaf cross-sectional area. For a better understanding of the effect of salinity on the cross-section, therefore, it is important to know what controls the formation and development of veins, especially the small ones. In recent years, considerable attention has been focussed on this area (e.g. Nelson and Dengler 1997; Sylvester et al. 1996; Ye 2002; Trivett and Evert 1998). Auxin and cytokinin have been considered essential for vascular tissue differentiation and this supposition is supported by recent molecular and genetic analyses (Ye 2002). Several genes that reduce the number of veins—for example *pin1* (Berleth et al. 2000), *hve* and *ixa* (Candela et al. 1999), and *ifl1* (Zhong and Ye 1999)—have been identified. Additionally, *knos* (*knotted 1*-like homeobox) genes (Smith et al. 1992; Kerstetter et al. 1994) belong to the genes controlling the appearance of founder cells at leaf initiation.

According to Trivett and Evert (1998), the regular parallel veins of the leaves of most monocots arise in a hierarchical sequence. First, large veins are initiated and differentiate acropetally, and then medium and small veins are initiated later in development and differentiate basipetally. Thus, the larger reduction observed in the

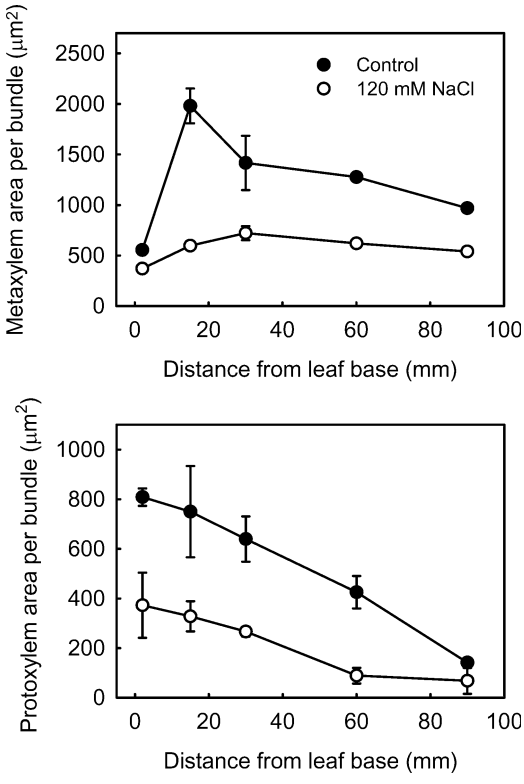


Fig. 8 Spatial distribution of the areas of the metaxylem and protoxylem from a large vascular bundle in growing leaf 4 of wheat grown in soil for control and 120 mM NaCl treatments. The measurements were based on light micrographs. Error bars represent standard errors and fit within the plot symbol if not shown

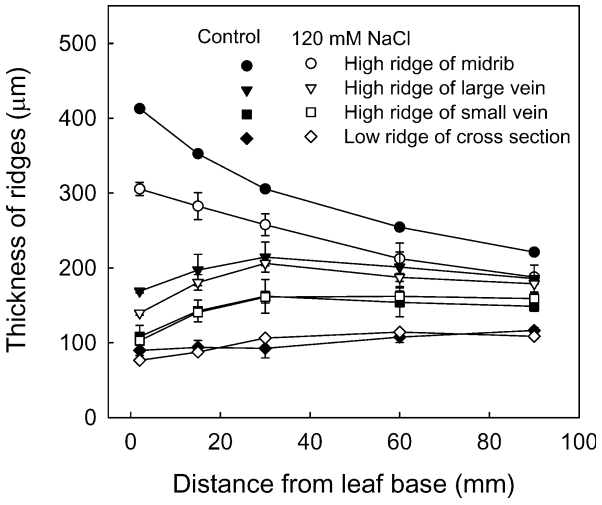


Fig. 9 Spatial distribution of the thickness of the high ridges of midrib, large and small vein segments, and low ridge in growing leaf 4 of wheat grown in soil for control and 120 mM NaCl treatments. The measurements were based on light micrographs. Error bars represent standard errors and fit within the plot symbol if not shown

number of medium and small veins suggests that the effect of salinity on the development of the cross-section occurs mainly after the large veins are formed. All the small bundles lack protoxylem and large metaxylem elements, and, in most, the phloem consists entirely of metaphloem (Russell and Evert 1985). Given that the role of small veins is mainly for loading and transporting nutrients, the reduction in their number under saline conditions may inhibit the re-translocation of assimilates and mineral nutrients. The resulting inhibition of phloem transport capacity in the leaves would explain the observation that there is usually a high accumulation of carbohydrates in plants under saline conditions (Hu et al. 2000b). The similar accumulation of other assimilates under saline conditions is presumably due to the same phenomenon.

According to Sharman (1942), the large veins develop acropetally within the blade. 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 through the elongation zone occurs through proto- and metaxylem elements in the midrib and large veins. In this study, the observed changes in the size of proto- and metaxylem along the growing wheat leaf agree with the detailed study of xylem maturation with respect to water transport along the axis of growing leaves of tall fescue (Martre et al. 2000) and barley (Fricke and Flowers 1998). Starting at the base, protoxylem vessels and narrow and large metaxylem vessels contributed sequentially. However, studies suggest that most of the axial water transport through the elongation zone occurs through protoxylem vessels (Dannenhoffer and Evert 1994; Fricke and Flowers 1998; Martre et al. 2001; Fricke 2002; Tang and Boyer 2002). The average area of xylem (protoxylem + metaxylem) within a leaf was reduced by 55% under saline conditions in this study. This compares favourably to the reduction in the net deposition rate of water within the growth zone (about 40%) induced by salinity (Hu and Schmidhalter 2001). Baum et al. (2000) reported that, in contrast to the lack of correlation between transpiration rate and xylem anatomy, water deposition rate in the growth zone of the sorghum leaves showed a better correlation with the diameter of the protoxylem under saline conditions, which agrees well with our current observations.

Both intercellular air space and the density of mesophyll, as well as the size, shape and distribution of mesophyll cells, are important features for leaf gas exchange (Wilson and Cooper 1970; Sasahara 1982). There is also some evidence that the formation of intercellular air space is spatially and temporally co-ordinated with maturation and initiation of the function of stomatal complexes in the epidermis (Xu et al. 1996; Rademacher and Nelson 2001). 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. The studies by Brugnoli and Björkman (1992) and Delfine et al. (1998) have shown that the salinity-reduced conductance to CO_2 diffusion caused by stomatal closure accounts for much of the

reduction in photosynthesis under saline conditions. Recently, James et al. (2002) showed that conductance to CO_2 diffusion in mature wheat leaves was also reduced by salinity. In this study, the relative proportion of intercellular air space in the large veins was increased, whereas the proportion of mesophyll cells remained almost unchanged for all vein segments and the thickness of leaf was reduced along the leaf axis (Figs. 6, 9). Together, these changes indicate that there might not be a reduction in photosynthesis in wheat under increasing salinity. However, contradictory reports in the literature show that the correlation between mesophyll cell density and leaf photosynthesis was negative among genotypes of perennial ryegrass (Wilson and Cooper 1970), but positive among ploidy levels in tall fescue (Byrne et al. 1981).

Our results provide strong evidence that the observed reduction in the cross-section of wheat is due mainly to a decreased number of medium and small veins. The resulting reduction in the area of metaxylem and protoxylem may result in a reduction in net deposition of water into the growth zone of the growing wheat leaf. Because the architectural properties of the leaf vein system are related to physiological leaf functions (Rot-Nebelstik et al. 2001), further work is needed to determine the transport and redistribution of nutrients in relation to leaf anatomical structure in the growing tissues, which remains an unexplored area in plant physiology.

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