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***Review*:**

Salinity and the growth of non-halophytic grass leaves: the role of mineral nutrient distribution

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Abstract. Salinity is increasingly limiting the production of graminaceous crops constituting the main sources of staple food (rice, wheat, barley, maize and sorghum), primarily through reductions in the expansion and photosynthetic yield of the leaves. In the present review, we summarise current knowledge of the characteristics of the spatial distribution patterns of the mineral elements along the growing grass leaf and of the impact of salinity on these patterns. Although mineral nutrients have a wide range of functions in plant tissues, their functions may differ between growing and non-growing parts of the grass leaf. To identify the physiological processes by which salinity affects leaf elongation in non-halophytic grasses, patterns of mineral nutrient deposition related to developmental and anatomical gradients along the growing grass leaf are discussed. The hypothesis that a causal link exists between ion deficiency and / or toxicity and the inhibition of leaf growth of grasses in a saline environment is tested.

Keywords: grasses, growth zone, leaves, mineral nutrients, net deposition rate, non-halophytes, salinity.

Introduction

Leaf growth of grasses is of central importance to their development. Not only is the expansion of leaves crucial to early seedling establishment by providing a continuous supply of energy and carbon through photosynthesis, it also facilitates development of other organs such as tillers, ears and grains. The key factors that could potentially limit leaf growth are hormones, metabolic energy and building blocks provided through photosynthesis, biophysical properties of the cells, and mineral nutrients. The growing portion of an expanding grass leaf is enclosed in the whorl, which is formed by sheaths of older leaves, and expands predominantly in a longitudinal direction (Dale 1988). Tissue age along the leaf axis is therefore heterogeneous and increases from the basal (youngest tissue) to the distal zones (oldest tissue) of the leaf blade (Davidson and Milthorpe 1966; Kemp 1980). Thus, grass leaves provide a convenient experimental system to study growth processes and nutrient dynamics within growing tissues under stress because growing tissues are located in a well-defined region.

Under saline conditions, soils contain extreme ratios of Na^+ / Ca^{2+}, Na^+ / K^+, Ca^{2+} / Mg^{2+}, and Cl^- / NO_3^- under saline conditions. Therefore, plants growing in saline soils are subjected primarily to four types of stresses acting on biophysical and / or metabolic components of plant growth: osmotic stress, specific ion toxicities (e.g. Na^+ and Cl^-), ionic imbalance, and developmental disturbance (Grattan and Grieve 1999). Despite intense research effort, it remains unclear whether osmotic or ionic effects dominate. Munns (1993) proposed a 'two-phase growth response to salinity' model, in which water deficits inhibit growth shortly after salinisation and then ionic effects occur later. Since then, many studies have been conducted to test the two-phase model. Sumer *et al*. (2004) found that in the first phase of the reduction in maize growth under saline conditions, both osmotic and ion effects were involved. Studies on wheat by Arif and Tomos (1993) or barley by Fricke and Peters (2002) showed no correlation between turgor at the cellular level (as measured with the pressure probe) and cell elongation in the growth zone for a short-term salt stress. In contrast, the growth zone of grass leaves is a strong sink for nutrients, and salinity may affect nutrient availability. The increased levels of carbohydrates in growing and mature leaf regions of grasses exposed to salinity argue against any growth limitations deriving from this variable (Munns *et al*. 1982; Hu *et al*. 2000*b*). Thus, the maintenance of an adequate supply of mineral nutrients to the growth zone should be a key component of the growth response to salinity. Furthermore, in and very near the meristem of growing leaves, cells are not vacuolated (Esau 1977).

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There is also a vascular discontinuity between the stem and the leaf vessels in the cell division zone of the leaf base, requiring that transport through the leaf division zone and into the developing vascular systems of the expanding zone relies either on symplastic transport or transport through the thin cell walls (Lazof and Bernstein 1999). At the base of the growing grass leaves, protoxylem is functional and becomes dysfunctional as the distal end of the elongation zone approaches, while at the same time, metaxylem becomes fully functional only beyond the elongation zone (Fricke and Flowers 1998; Martre *et al*. 2000). This may lead to a hydraulic bottleneck in the leaf base. Salinity reduces size of meta- and protoxylem and the number of small veins that may influence water transport and nutrient retranslocation (Baum *et al*. 2000; Hu *et al*. 2005). As such, the requirement for continuous nutrient supply to maintain the mineral status within rapidly expanding tissues renders the meristematic region highly susceptible to nutrient disturbances. Thus, we hypothesise that the growth of grass leaves under either control or saline conditions should be much more closely associated with nutritional changes within the growth zone than with average changes in the whole plant or changes in non-growing tissues. By relating the spatial distribution of cell elongation along the leaf growth zone to the spatial distribution of essential nutrients in the presence or absence of salinity, it should be possible to identify the physiological processes through which salinity affects leaf elongation in non-halophytic grasses.

The response of expansion in the grass leaf to salinity

The leaf elongation rate of grasses is a function of the length of the elongation zone or the duration of cell elongation and the relative elemental growth rate along the growth zone. The relative elemental growth rate increases from the leaf base and reaches its maximum halfway along the growth zone before decreasing to zero at the distal end of the growth zone (curve a in Fig. 1). The relative elemental growth rate and the length of elongation zone respond in various ways to environmental constraints on the plant. Salinity delays leaf emergence and reduces the leaf size of grasses both longitudinally and laterally (e.g. Rawson *et al*. 1988; Yeo *et al*. 1991; Bernstein *et al*. 1993*a*, *b*; Hu *et al*. 2000*a*). Studies on a range of grass species show that reduction in the leaf elongation rate by salinity is associated with a decrease in relative elemental growth rate along the growth zone (Delane *et al*. 1982; Bernstein *et al*. 1993*a*, *b*; Hu *et al*. 2000*a*; Neves-Piestun and Bernstein 2005). However, reports conflict about the effect of salinity on the length of the elongation zone.

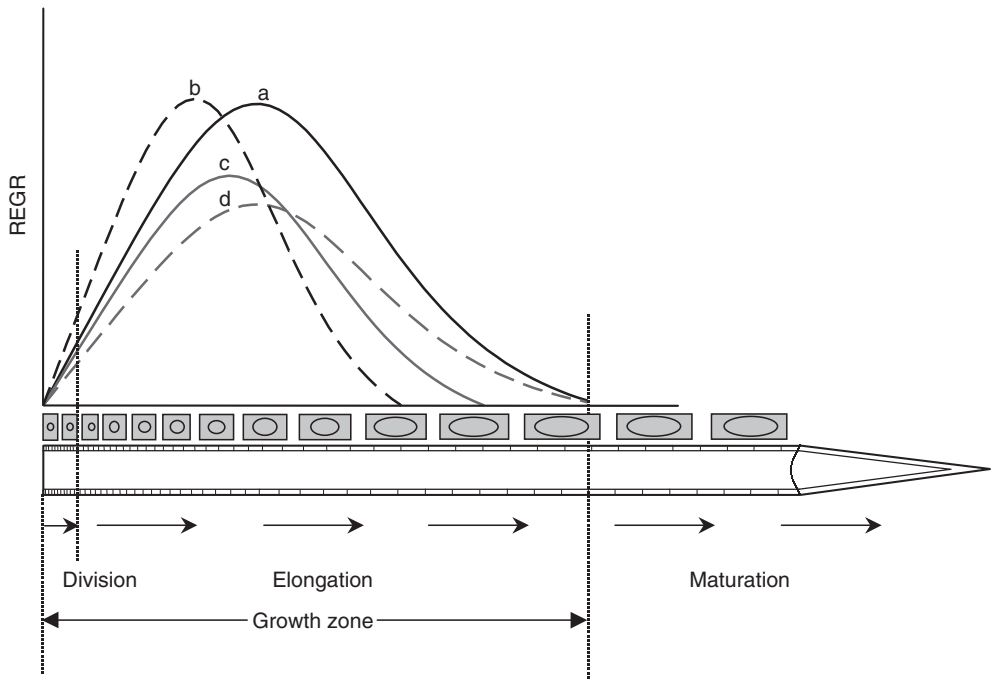

Fig. 1. The spatial distribution of relative elemental growth rates (REGR) (mm mm^{-1} h^{-1}) of grass leaves (*a*) and possible responses (*b*–*d*) of the REGR and the length of growth zone to genetic and environmental constraints. Curve *a*, REGR profile in non-disturbed plants; curve *b*, length of elongation zone is reduced, whereas REGR in some regions is increased; curve *c*, both the length of elongation zone and REGR are reduced; curve *d*, the length of elongation zone does not change with stress conditions, whereas the REGR is reduced. The increase in cell and vacuole size as cells exit the cell division zone and move tip wards through the elongation zone, is indicated.

For example, the studies of Delane *et al*. (1982), Hu *et al*. (2000*a*) and Fricke (2004) demonstrated that there is no effect of salinity on the length of the elongation zone of wheat or barley leaves during the steady phase of growth when leaf elongation is at a (near) maximum. In contrast, Bernstein *et al*. (1993*a*, *b*) and Neves-Piestun and Bernstein (2005) observed that salinity inhibited leaf growth in sorghum and maize by reducing both the relative elemental growth rate and the length of the elongation zone. Cell production and the duration of cell elongation are two processes that influence the relative elemental growth rate and the length of the elongation zone. However, little information exists about the effect of salt stress on cell division or on the duration of elongation. Hu and Schmidhalter (unpubl. data) observed that the shortening in the duration of cell elongation in wheat leaves under saline conditions leads to a reduced leaf elongation rate, which is in agreement with studies for temperature in maize (Ben-Haj-Salah and Tardieu 1995), for soil resistance in wheat (Beemster *et al*. 1996), for nitrogen in tall fescue (Rademacher and Nelson 2001), and for phosphorus in maize (Assuero *et al*. 2004). Hu and Schmidhalter (2001) and Hu *et al*. (2005) showed that salinity reduces the width and cross-sectional area of the growing leaves of wheat at all locations above 5–10 mm from the leaf base during the steady stage of growth.

Therefore, to understand any limitations that salinity imposes on the longitudinal and lateral expansion of grass leaves, all components that are required for the formation of leaf cross-section, cell division or production rate, cell elongation rate and the duration of cell elongation should be considered.

Models for mineral nutrients associated with cell division, cell elongation and maturation in grass leaves

Distinct zones of cell division, cell elongation and maturation along the growing grass leaf are associated with specific distribution patterns of individual mineral nutrients, and a causal link is likely. Four specific patterns of spatial distribution of the mineral nutrients in the growing leaves of grasses can be distinguished (Fig. 2).

(I) Ionic concentrations are highest at the leaf base, reaching a minimum at the end of the elongation zone and remaining nearly unchanged thereafter (e.g. N, P, Mg^{2+}, S and Zn) (Meiri *et al*. 1992; Evéquoz 1993; Gastal and Nelson 1994; Bernstein *et al*. 1995; Hu and Schmidhalter 1998*a*; Hu *et al*. 2000*c*; Neves-Piestun and Bernstein 2005) (curve a in Fig. 2). This pattern most likely reflects that the smaller-sized cells at the leaf base contain a higher proportion of proteins and nucleic acids than the larger-sized cells at more distal locations.

(II) Ionic concentrations are lowest at the leaf base, reaching a maximum at the end of the elongation zone and decreasing with distance thereafter (e.g. K^+ in the growing leaves of wheat, maize and sorghum and NO_3^- in the growing leaves of wheat). This distribution pattern applies to those ions that are preferentially localised in the vacuole and function as osmotica, because vacuoles are small at the leaf base compared to the distal end of the

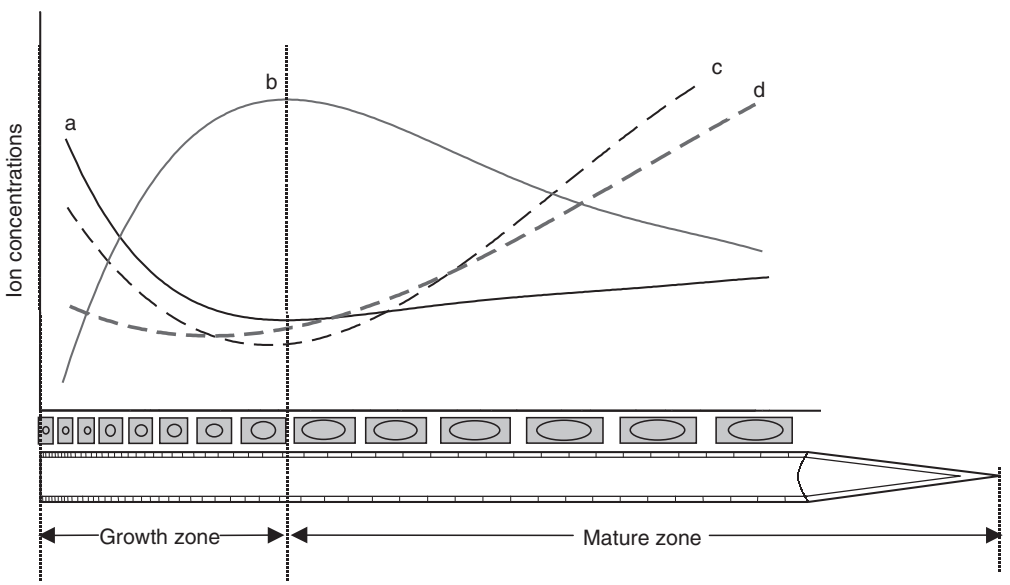

Fig. 2. Four different spatial distribution patterns of mineral nutrients (mmol or mg kg^{-1} fresh weight) in the different zones of grass leaves. Curve *a* represents the distribution of N, P, Mg^{2+} and Zn; curve *b* represents the distribution of K^+ and NO_3^-; curve *c* represents the distribution of Ca^{2+}; curve *d* represents the distribution of Fe and Mn along the leaf axis.

elongation zone. The decrease in the concentrations of the ions beyond the elongation zone might be due to an increase in their retranslocation (K^+; curve b in Fig. 2) (Bernstein *et al*. 1995; Hu and Schmidhalter 1998*a*; Neves-Piestun and Bernstein 2005).

(III) Ionic concentrations decrease with distance from the leaf base, reaching a minimum at the end of the growth zone before increasing with distance thereafter (curve c in Fig. 2), for example Ca^{2+}, which is predominantly found in the cell walls and associated with the plasma membrane. The continuous increase in Ca^{2+} concentration in maturing leaf tissues may be due to lack of phloem mobility and export (Hu and Schmidhalter 1998*a*).

(IV) Ionic concentrations, compared with the leaf base, decrease slightly or remain unchanged in the elongation zone and increase consistently with distance thereafter (e.g. Fe, Cu, and Mn) (Hu *et al*. 2000*c*; Neves-Piestun and Bernstein 2005) (curve d in Fig. 2). Such a distribution pattern is probably associated with ions with a function during chloroplast development. Although chloroplasts develop progressively along the leaf axis of grasses, chloroplast number increases markedly beyond the end of the growth zone (Nakamura and Hashimoto 1988), coincident with changes in Fe and Mn concentrations.

Studies published to date have shown that salinity stress did not change the distribution patterns of these nutrients (Bernstein *et al*. 1995; Hu and Schmidhalter 1998*a*; Neves-Piestun and Bernstein 2005).

As cells expand, their volume and water content increase continuously such that solutes, including mineral nutrients, have to be accumulated in parallel to maintain adequate concentrations. To determine the rates at which mineral elements are deposited, the continuity equation can be used (Erickson 1976; Silk 1984), where the net deposition rate is viewed as a quantitative picture of sink and source relationships. A positive net deposition rate implies the local net increase for a given substance, whereas a negative net deposition rate implies its local net loss. Along growing grass leaves, the highest net deposition rates of mineral nutrients are found in the growth zone (Fig. 3). The position of the peak in the growth zone varies with individual nutrients. For example, the net deposition rate is highest in the basal half of the leaf growth zone, where cell division is active, for N, P, Mg^{2+}, Ca^{2+} and Zn (mmol kg^{-1} FW h^{-1} or mmol mm^{-1} leaf length h^{-1}) and in the middle of the leaf growth zone for K^+, NO_3^-, Fe and Mn (Gastal and Nelson 1994; Bernstein *et al*. 1995; Hu and Schmidhalter 1998*a*; Hu *et al*. 2000*c*; Neves-Piestun and Bernstein 2005). The net deposition rate for many nutrients can be negative in more mature tissues due to the remobilisation of nutrients through the phloem to younger tissues.

Relationships between ion concentration, net deposition rate and leaf growth under saline conditions

In recent decades, considerable attention has been focused on the hypothesis that Na^+ or Cl^- may be toxic to plants and / or cause ionic imbalance. In support of this hypothesis, positive correlations between salt tolerance and Na^+ exclusion have been reported (e.g. Drew and Läuchli 1987; Schubert and Läuchli 1990; Cramer 1992; Hu and Schmidhalter 1997).

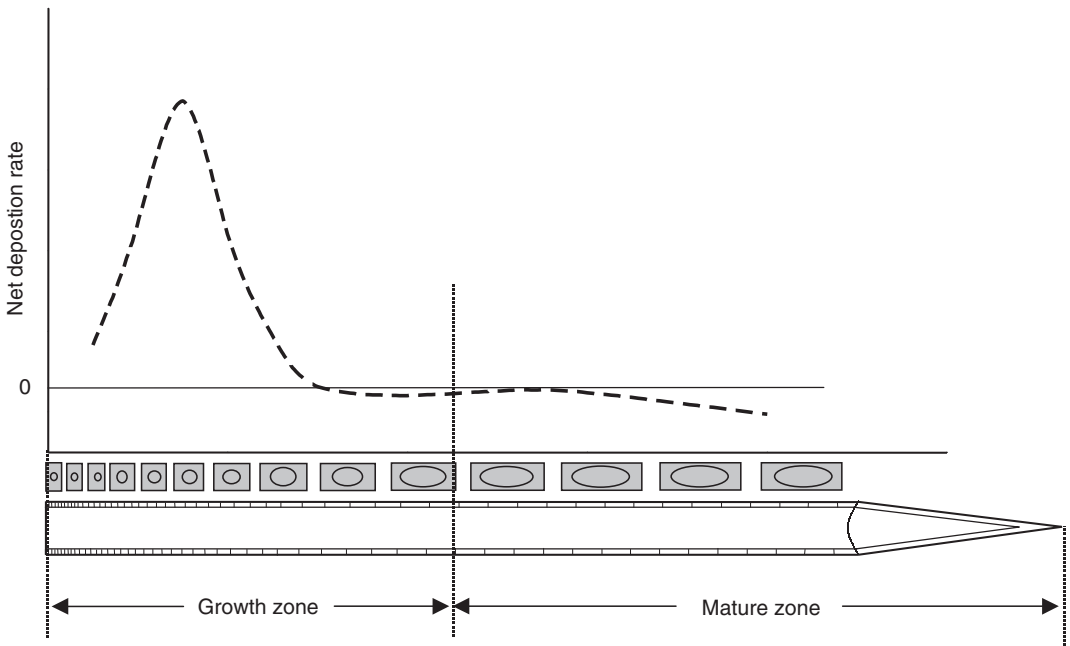

Fig. 3. Spatial distribution of the net deposition rate (NDR, mmol or μg kg^{-1} fresh weight h^{-1} or mmol or μg mm^{-1} leaf length h^{-1}) of inorganic nutrients along the growing leaves of grasses.

However, because the conclusions were derived from the analysis of the whole plant or non-growing tissues, they may not reveal the factors responsible for growth inhibition in growing tissues.

How does a high concentration of Na^+ or Cl^- in the growing leaf limit growth?

Many studies have shown that a non-uniform distribution of ions exists within the shoots of plants exposed to salinity (Flowers *et al*. 1977; Greenway and Munns 1980). Comparatively few studies have attempted to identify the mechanisms that underlie the direct effects of toxic ions like Na^+ and Cl^- on cell expansion in the growing leaf tissues of grasses (e.g. Delane *et al*. 1982; Munns *et al*. 1988; Bernstein *et al*. 1995; Hu and Schmidhalter 1998*a*; De Lacerda *et al*. 2003; Neves-Piestun and Bernstein 2005). It is known that whereas Na^+ concentrations are low in young and developing leaves, and high in the mature leaves of both salt-tolerant and -sensitive crop plants, the opposite is true for K^+ (Greenway *et al*. 1965; Delane *et al*. 1982; Yeo and Flowers 1982; Jeschke and Stelter 1983; Jeschke 1984; Jeschke and Wolf 1988; Wolf *et al*. 1991; Salam *et al*. 1999). This observation could be interpreted as meaning that growing tissues are specifically protected from a high accumulation of Na^+ and Cl^-. If this is true, one would expect to find lower Na^+ and Cl^- concentrations in the growth zone than in the mature zones of individual, growing leaves. However, Delane *et al*. (1982) observed that Na^+ and Cl^- concentrations under saline conditions were similar in the growth and mature zones of barley leaves. Moreover, Hu and Schmidhalter (1998*a*) reported that, compared with the mature zone, a higher Na^+ concentration existed in the growth zone of wheat leaves under salinity (Fig. 4). The profiles of Na^+ and / or Cl^- along the growing leaf axis under saline conditions have been studied on a millimetre scale for sorghum (Bernstein *et al*. 1995), wheat (Hu and Schmidhalter 1998*a*) and maize (Neves-Piestun and Bernstein 2005). Both ions have a distribution pattern that is similar to that of K^+ or NO_3^- in the growth zone of grass leaves. In the saline treatments, the Na^+ concentrations in the growing leaves of three species increased rapidly from the leaf base, reached a maximum in the middle of the growth zone, decreased to the end of the growth zone, and then increased in the mature zone with distance; they remained almost unchanged along the growing leaf in non-salinised plants (Fig. 4). Similarly, Cl^- concentrations in the growing leaf of wheat under saline conditions increased rapidly from the leaf base to the end of the leaf growth zone before decreasing slightly with distance in the mature tissues (Fig. 4). In contrast, Cl^- increased slightly in the growth zone and remained almost unchanged in the mature tissue in control treatments.

The high levels of Na^+ or Cl^- in the growth zone may have several causes. One is that the most active growth occurs in such regions under saline conditions, which, in turn, can be explained by the highest net deposition rates of Na^+ and Cl^- occurring in the growth zone compared with zero or negative net deposition rates in mature tissue (Fig. 5). Furthermore, although salinity causes osmotic effects, studies showed no correlation between turgor at the cellular level (as measured with the pressure probe) and cell elongation in the growth

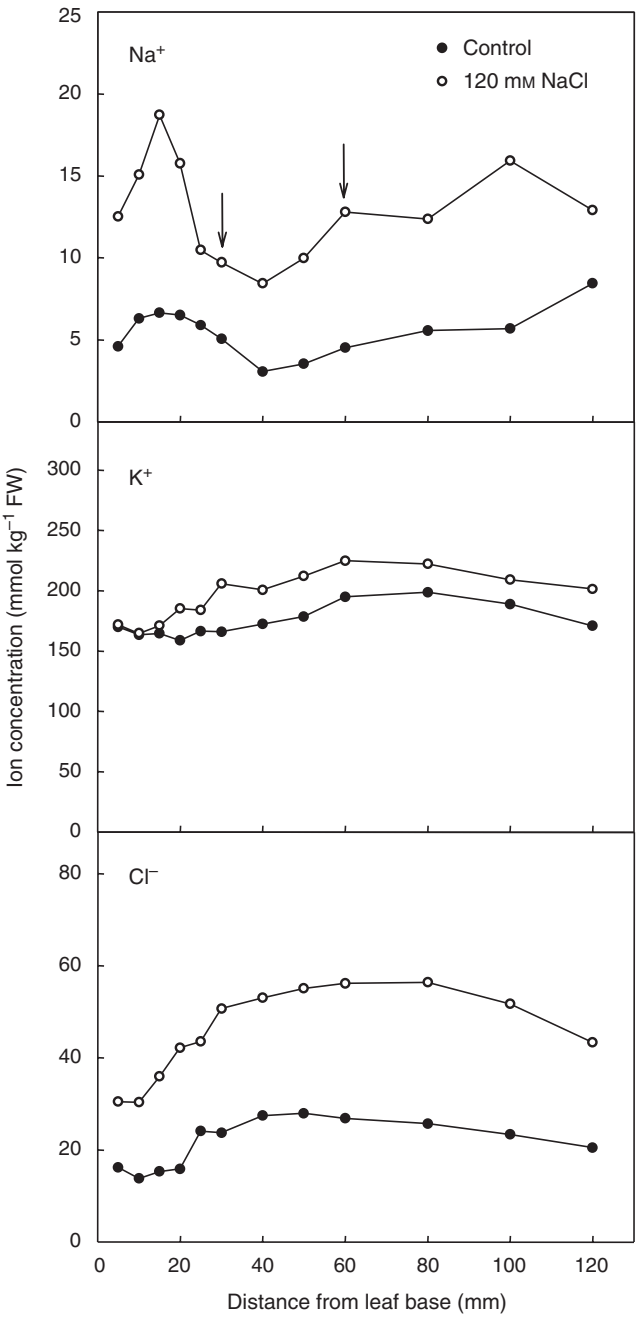

Fig. 4. Spatial distribution of Na^+, K^+ and Cl^- in the growing leaf 4 of the main stem of wheat plants grown in soil with or without 120 mM NaCl (modified from Hu and Schmidhalter 1998*a*). Arrows indicate the positions of the end of the growth zone (left arrow) and of the leaf sheath (right arrow).

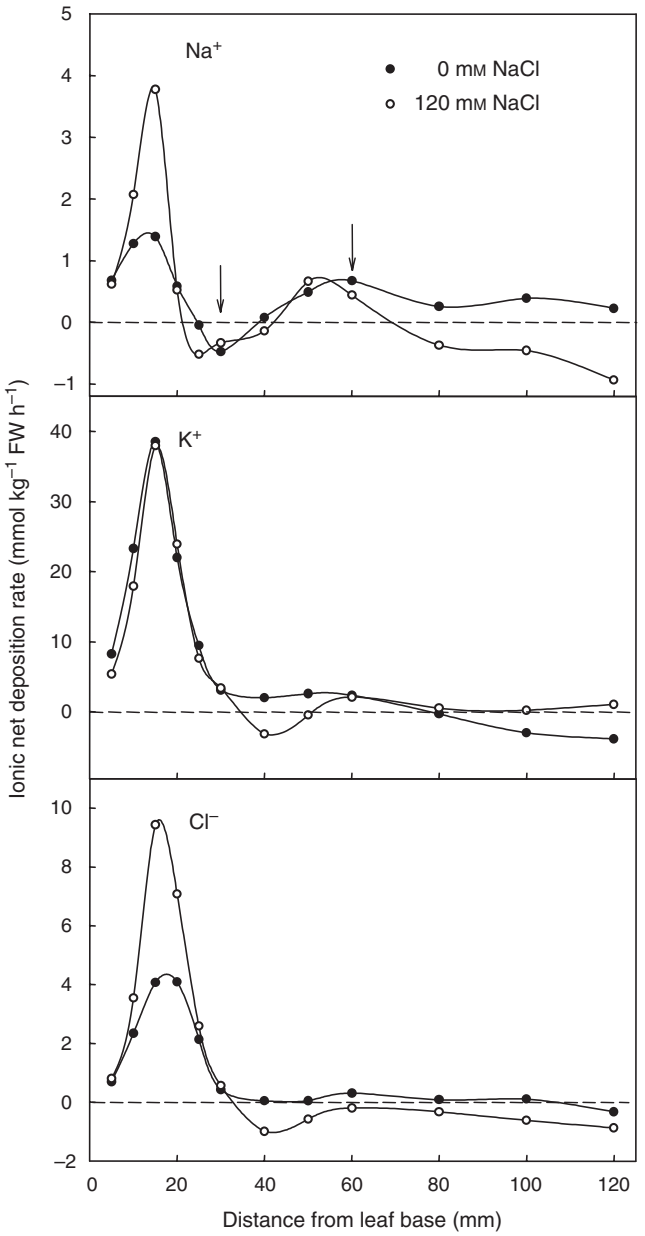

Fig. 5. Spatial distribution of the net deposition rate of Na^+, K^+ and Cl^- in the growing leaf 4 of the main stem of wheat plants grown in soil with or without 120 mM NaCl (modified from Hu and Schmidhalter 1998*a*). Arrows indicate the positions of the end of the growth zone (left arrow) and of the leaf sheath (right arrow).

zone under saline conditions in wheat (Arif and Tomos 1993) or in barley (Fricke and Peters 2002). This observation might be attributable to a sufficient osmotic adjustment being attained by the grasses through the high deposition of both Na^+ and Cl^- into the growing zone of the leaves in addition to the deposition of sugars (Delane *et al*. 1982; Fricke and Flowers 1998; Hu and Schmidhalter 1998*b*). However, salinity imposes very different challenges on the solute relations of the growing grass leaf as concerns osmotic regulation. Although salinity guarantees a sufficient supply of solutes (i.e. Na^+, Cl^-), it also increases the tissue load of Na^+ and Cl^- to potentially toxic levels.

Although peak Na^+ and Cl^- concentrations are found in the middle or at the end of growth zone (Fig. 4), the concentrations of Na^+ and Cl^- in the growing leaves under saline conditions are still much lower than those in the older leaves. Therefore, it has been suggested that the reduction in leaf growth observed under salinity may not be a direct cause of the toxicity of Na^+ and Cl^- in the growing tissues. For example, Bernstein *et al*. (1995) and Hu and Schmidhalter (1998*a*) argued that the spatial distribution pattern of Na^+ and Cl^- in the leaf growth zone of sorghum and wheat under saline conditions was closely related to that of the relative elemental growth rate in the growth zone, which contradicts the behaviour that is expected under the hypothesis of growth inhibition. The final lengths of the epidermal cells also do not necessarily correlate with the leaf elongation rate. Stress can stimulate epidermal cell elongation from the leaf base up to the latter half of the growth zone, even though the final cell length may be comparatively shorter because of a short duration of cell expansion, as has been observed for tall fescue leaves in low N (MacAdam *et al*. 1989) and for maize leaves in P deficiency treatments (Assuero *et al*. 2004). Hu and Schmidhalter (unpubl. data) obtained similar results for wheat leaves under saline conditions (curve b in Fig. 1), further suggesting that high Na^+ or Cl^- levels are not directly linked to the reduction in cell elongation. Furthermore, in a study by Hu and Schmidhalter (1997), Cl^- concentrations in the mature leaves of wheat were ~10 times as high as those in growing leaves under similar conditions, and hardly affected the main stem grain yield. Results from the studies of Delane *et al*. (1982) and Munns *et al*. (1988) also suggested that high Na^+ and Cl^- concentrations in the growth zone of barley under saline conditions were unlikely to have a negative impact on leaf elongation. Therefore, the obvious question remains: how do high Na^+ or Cl^- concentrations in the growing zone of grass leaves limit leaf growth if no direct ion-toxicity of high Na^+ or Cl^- levels on cell elongation exists? At least six answers appear plausible.

(1) Na^+ and Cl^- affect cell division and the duration of cell elongation. The leaf elongation rate of grasses is influenced by the supply of cells (i.e. the number of cells produced per file) and the rate and duration of cell elongation in the epidermal system (MacAdam *et al*. 1989). To date, no information is available on the effect of salinity on cell division and the duration of cell elongation in growing grass leaves. However, shortened cell division zones have been found in maize leaves subjected to low P and drought stresses, indicating a reduction in the number of dividing cells. A shorter cell division zone under saline conditions has been found in the roots of cotton (Kurth *et al.* 1986) and *Arabidopsis* (West *et al*. 2004). Furthermore, a potentially important link between stress and cell division was revealed

by induction of ICK1 in *Arabidopsis* by ABA (Wang *et al*. 1998). ICK1, a cyclin-dependent protein-kinase inhibitor, might hinder cell division by reducing the activities of cyclin-dependent protein kinases that help to drive the cell cycle (Zhu 2001). Salt stress might inhibit cell division by causing the accumulation of ABA, which, in turn, induces ICK1. The increase of ABA concentration in the growth zone of the maize leaves by salt-stress has been observed by Cramer and Quarrie (2002).

The duration of cell elongation in relation to the length of the growth zone is also important because a shortened duration of individual cell expansion might reduce the leaf elongation rate without any changes in cell division or cell elongation rate (MacAdam *et al*. 1989). The duration of cell elongation may be determined by the chemical composition and properties of the cell wall, with the deposition of the secondary cell wall following cell elongation, which may thus prevent further cell expansion (MacAdam *et al*. 1989). Neumann (1993) reported that salinity induced changes in cell wall extensibility of maize growing leaves. The factors that may also contribute to the cessation of cell elongation have also been investigated. Extensibility of the cell wall is thought to be reduced by the formation of covalent bonds between phenolic residues of pectins, hemicelluose, and structural proteins of cell wall (Fry 1986). Peroxidase promotes this bonding by catalysing the formation of free radicals of the residues (De Souza and MacAdam 2001). Recent reports have also highlighted the biochemical regulation of cell wall extensibility, for example through expansion, as a key process in controlling growth in plants and have led to the identification of several proteins that are potentially involved in this process (Cosgrove 1999). The XET-related gene, *Fp*XET1, is a potential marker for leaf elongation in the growth zone of grasses (Reidy *et al*. 2001). The activity of proton pumps is of primary importance for growth, which may lower cell wall pH and contribute to wall loosening (Van Volkenburgh 1999). Several environmental conditions that affect growth were shown to alter apoplast acidification. For example, growth inhibition by water stress is accompanied by an increase in apoplastic pH and a decrease in acidification rate (Van Volkenburgh and Boyer 1985; Hartung *et al*. 1988). However, Neves-Piestun and Bernstein (2001) reported that salinity-induced inhibition of leaf elongation in maize is not mediated by changes in cell wall acidification capacity in growing tissues.

(2) The reduction in cross-sectional area mediated by Na^+ and Cl^- in older leaves. The reduced width of grass leaves contributes to the 50% reduction in leaf area observed under salinity (Hu and Schmidhalter 2001). Furthermore, the reduction in leaf width or cross-sectional area is associated with a decrease in the area and number of vascular vessels, which may, in turn, inhibit the translocation of water and nutrients into other sink tissues (Hu *et al*. 2005). Because the width of the grass leaves remains unchanged once they have emerged from the sheath (Dale 1988), higher Na^+ or Cl^- levels in the growing leaves cannot cause the major reduction in the width or cross-sectional area of the growth zones. Therefore, we hypothesise that higher Na^+ or Cl^- concentrations may limit the cross-sectional area in the expanding leaves through effects on the Na^+ and / or Cl^- levels in the older leaves. In grasses, expanded and expanding leaves are closely coordinated to each other. Generally, the expanded leaves of grasses are the source for carbohydrates and nutrients and expanding leaves are the sink. Bregard and Allard (1999) reported that the expanding blade of tall fescue imports photosynthate from mature leaves until it reaches 80% of its final length, and then exportation may begin and importation may cease. Although expanding grass leaves have relatively low levels of Na^+ or Cl^-, a rapid increase in Na^+ and Cl^- concentrations occurs after the leaf emerges (Munns *et al*. 1988). As such, Na^+ or Cl^- may first become toxic in the older leaves, resulting in a nutrient deficiency and a reduction in photosynthesis in the older leaves (Munns and Termaat 1986; Munns 1993). Thus, a greater reduction occurs in the younger leaves than in the old leaves. This is supported by the findings that the leaf area of grasses under saline conditions was increasingly reduced with an increase in leaf number from the bottom of plants of both barley (Rawson *et al*. 1988) and wheat (Hu and Schmidhalter 1997; Hu *et al*. 2000*a*).

(3) Fewer and smaller vascular vessels leading to lower water and nutrient transport. At the base of the growing grass leaves, protoxylem is functional and becomes dysfunctional as the distal end of the elongation zone approaches, while at the same time, metaxylem becomes fully functional only beyond the elongation zone (Fricke and Flowers 1998; Martre *et al.* 2000). This may lead to a hydraulic bottleneck in the leaf base. Baum *et al*. (2000) and Hu *et al*. (2005) observed that the reduction in leaf cross-sectional area during leaf initiation was associated with a reduction in the number of vascular vessels. The latter study, in particular, analysed in detail the effects of salinity on tissue architecture in expanding wheat leaves. The authors suggested that salinity reduces the capacity for retranslocation of mineral nutrients and assimilates in grass leaves because of the ~35% reduction observed in the number of veins (and mainly in the number of small veins). The reduced area of the protoxylem and metaxylem in midrib and large vein segments in growing tissues may therefore be responsible for a lower water deposition into the growth zone under saline conditions.

(4) Na^+ or Cl^- cause toxicity in specific types of cells. Leaf cells accumulate Na^+ or Cl^- differently depending on their cell type. Data show that Cl^- is maintained at lower levels in mesophyll compared with epidermal tissue, whereas Na^+ is distributed more evenly between the two leaf tissues (Boursier and Läuchli 1989; Huang and Van Steveninck

1989; Fricke *et al*. 1996; Dietz *et al*. 1992; Cuin *et al*. 2003). Similarly, mesophyll and epidermis, epidermal cell types, and vacuolar and cytosolic cell compartments differ in their ion relations and in their response to salinity. The division zone of mesophyll cells is much longer than that of epidermal cells and can reach up to one third of the length of the elongation zone (MacAdam *et al*. 1989; Skinner and Nelson 1995), which may result in a further increase in Cl^- levels with distance within the length of elongation zone, compared with that for Na^+ (Fig. 4).

(5) Toxicity of Na^+ or Cl^- in the cytoplasm. Within the plant cell various compartments exist, to which ions may be confined by different means, and the discontinuous distributions between compartments may explain differences in plant responses to salinity at the same average tissue concentration of salt. Because rapidly expanding tissues of grass leaves are also rapidly undergoing vacuolisation along the leaf axis, it may be necessary to consider compartmentalisation of nutrients within the growing tissues before any meaningful interpretation can be made about nutrient disturbance, or modifications from the control levels in the most relevant metabolic cell phases in the cytoplasm in expanding cells. At the cellular level, mechanisms of salt tolerance involve excluding salt from the cytoplasm and sequestering it in vacuoles (Zhang and Blumwald 2001). Molecules that function as Na^+ and proton exchangers (or antiporters) contribute towards regulating cytoplasmic concentration by controlling the efflux of Na^+ from the cytoplasm into the vacuole or across the plasma membrane out of the cell (Schachtman and Liu 1999). However, along the grass leaf axis, there is a gradient in vacuolisation and vacuoles occupy a greater cell volume as cells elongate. Because Na^+ and Cl^- concentrations increase with increasing cell size (Bernstein *et al*. 1995; Hu and Schmidhalter 1998*a*), it is unlikely that the increase in the degree of tissue vacuolisation dilutes Na^+ concentrations in the vacuoles. Unicellular vacuolar sap concentrations of Na^+ and Cl^- under saline conditions (Lazof and Bernstein 1999) showed no difference in Na^+ and Cl^- vacuolar concentrations between the middle and end of the leaf growth zone of sorghum. This may suggest a high accumulation of Na^+ and Cl^- in the cytoplasm, and especially for Cl^- because it had a higher concentration at the end of the growth zone than in the middle (Hu and Schmidhalter 1998*a*).

(6) Na^+ and Cl^- in the apoplastic space. An explanation of the salt effect in compartmentalisation proposed by Oertli (1968) could be a build-up of high salt concentrations in the apoplastic space of the leaves. If the transport of salts into the leaf exceeds its uptake by the cells, apoplastic salt concentrations will rise. In turn, increased apoplastic salt concentrations may damage the plasma membrane surface or lower the apoplastic water potential, which can cause symplastic dehydration. Strong evidence that ion accumulation in cell walls is an important aspect of plant response to salt is the observation of leaf rolling in rice, a typical response to drought (O'Toole *et al.* 1979). Flowers *et al*. (1991) also cited the salt damage caused in rice through dehydration due to the extracellular accumulation of salts as evidence supporting Oertli's hypothesis. Additional experimental support was given by Munns and Passioura (1984), who analysed the osmotic pressure of xylem sap from the tip of the primary leaf of an intact transpiring barley plant under salt stress. They observed that the osmotic potential of the xylem sap decreased dramatically about one week after the highest external salt concentration was reached, after which the leaf died. In contrast, recent studies by Lohaus *et al*. (2000) and Mühling and Läuchli (2001) showed that the apoplastic Na^+ concentrations in maize leaves did not exceed ~20 mM at concentrations of 100 or 150 mM NaCl in the growth medium. These Na^+ concentrations in the leaf apoplast are not high enough to be responsible for the inhibition of leaf growth. However, the thickness of the cell wall in the meristem is much thinner than in mature cells (Lazof and Läuchli 1991), causing us to speculate that there might be much higher concentrations of Na^+ and / or Cl^- in the walls of younger cells than in those of older ones.

Availability of K^+, Ca^{2+}, Mg^{2+}, P, and N to growing grass leaves under salinity

Plant growth is affected by the interaction of Na^+ and / or Cl^- with other mineral nutrients, causing imbalances in the availability of the nutrients (Grattan and Grieve 1999). For example, high Na^+ concentrations in the external solution cause decreases in K^+ and Ca^{2+} concentrations in leaf tissues (Greenway and Munns 1980; Cramer 2002). This decrease may be due to any of (1) the antagonism between Na^+ and K^+ or Ca^{2+} at the sites of uptake in the roots, (2) the effect of Na^+ on the transport of K^+ and Ca^{2+} into the xylem (Lynch and Läuchli 1984, 1985), or (3) the indirect inhibition of the uptake process in other aspects such as H^+-ATPase activity tuned by auxins (Aducci *et al*. 1986; Gronwald *et al*. 1990; Suhayda *et al*. 1990) and long-distance K^+ transport and integration K^+ fluxes at the whole-plant level mainly regulated by ABA and cytokinins (Van Steveninck 1972). Direct elemental analyses of K^+, Ca^{2+} and Mg^{2+} in the growing leaves of wheat (e.g. Figs 4, 5; Hu and Schmidhalter 1998*a*) and sorghum (Bernstein *et al*. 1995) showed that salinity did not affect the distribution patterns of K^+, Ca^{2+} and Mg^{2+}. By contrast, Neves-Piestun and Bernstein (2005) observed that reduced level of K^+ and Ca^{2+} in growing leaves caused leaf growth inhibition. Furthermore, Hu and Schmidhalter (1998*a*) reported that K^+, Ca^{2+} and Mg^{2+} concentrations were increased by salinity along the entire growing leaf, which was in contrast to the findings in the growing leaves of sorghum (Bernstein *et al*. 1995), barley (Delane *et al*. 1982; Wolf and Jeschke 1987)

and maize (Maas and Grieve 1987). The different findings suggest that the effect of salinity on K^+, Ca^{2+} and Mg^{2+} in young tissue varies according to the plant species and growth media. Although nutrients such as K^+ and Ca^{2+} were lower in the growing leaves for saline treatments than in those for the controls, it is difficult to find a correlation between the reductions in the relative elemental growth rate and their net deposition rates throughout the growth zone (Bernstein *et al*. 1995). Data obtained by Bernstein *et al*. (1995) showed that the reduction in the relative elemental growth rate in the sorghum leaf decreased with distance from the leaf base, whereas the reduction in the net deposition rate of Ca^{2+} was constant throughout the growth zone of the young leaf, and the reduction in the net deposition rate of K^+ increased with distance. Thus, the lowered accumulations of K^+ and Ca^{2+} under salinity may arise from the low supply of these nutrients and / or the special architecture of growing tissues, which is indicated by the greater reduction in the net deposition rates of K^+ and Ca^{2+} with distance above the leaf base than in the relative elemental growth rate of sorghum under salinity.

Rapid plant growth and development require large K^+ fluxes to provide this ion to the growing tissues (Fig. 5). There are several routes of nutrient supply to the growing leaves where transport might be disrupted by salinity, including root uptake, xylem loading, long-distance transport, partitioning within the plant, and retranslocation. However, in the very early stages of growth, the leaf depends on the export of nutrients from older leaves via the phloem for its supply such as K^+ and carbohydrates. Data from Fricke (2004) showed an increase in K^+ in the growing leaf during the first 20 h following addition of 100 mM NaCl, suggesting increased K^+ supply via phloem from older source leaves. There is also evidence to show that phloem transport systems have a higher selectivity of K^+ over Na^+ in maize leaves (Lohaus *et al*. 2000). Lacombe *et al*. (2000) and Philippar *et al*. (2004) suggested that K^+ supply via the phloem might be controlled by sugar loading. The understanding of plant K^+ transport has increased in the past decade through the application of molecular biological techniques. The molecular studies suggested that the major type of K^+ channels involved in K^+ loading into the phloem sap of vascular tissues in leaves are AKT2 and AKT3 (Marten *et al*. 1999; Pilot *et al*. 2001; Cherel *et al*. 2002). Plants grown in saline conditions involve significant modification of K^+-channel gene expression, especially in leaves (Golldack *et al*. 2003; Pilot *et al*. 2003) and AKT2 mRNA accumulation is decreased. This is especially pronounced in the leaf epidermis (Dennison *et al*. 2001) and was specific to Na^+ toxicity.

The special characters of architecture in the growing tissues may also have an effect on the import of nutrients. The primary walls within the apical 50 μm of a dicot shoot apical meristem are very thin and of similar dimension close to the monocot leaf base (Lazof and Läuchli 1991; Lazof and Bernstein 1999). This implies that apoplastic flow must be more restricted in these tissues than in much of the shoot (Esau 1977; Bernstein *et al*. 1995). In and very near the meristem, cells are not vacuolated. There is also a vascular discontinuity between the stem and the leaf vessels in the cell division zone of the leaf base, requiring that transport through the leaf division zone and into the developing vascular systems of the expanding zone relies either on symplastic transport or transport through the thin cell walls (Lazof and Bernstein 1999). As such, the requirement for continuous nutrient supply to maintain the mineral status within rapidly expanding tissues renders the meristematic region highly susceptible to nutrient disturbances. This necessitates that the levels being maintained are entirely cytoplasmic and that they exist without any local mineral reserves. Supply, then, must closely match the needs of cell division and the mineral composition of each daughter cell. Because of this, meristematic nutrient transport is challenging even under optimal conditions, and it can be speculated that salinity could significantly alter those structures that may inhibit the nutrient transport. For instance, all of the small vascular bundles lack protoxylem and large metaxylem elements, and in most of these bundles, the phloem consists entirely of metaphloem (Russell and Evert 1985). The role of these small veins is mainly for loading and transporting nutrients (both organic and inorganic). Under salinity, however, the overall reduction in the cross-sectional area of the leaf is mainly due to a reduction in the number of small veins (Hu *et al*. 2005), which may, in turn, cause a greater inhibition of the translocation of mineral nutrients into the growing leaf than does the reduction in the relative elemental growth rate.

Interestingly, salinity did not alter the distribution patterns of P and total N in the growing leaf of wheat (Hu and Schmidhalter 1998*a*) and even increased the accumulation of these elements at any given location. The higher K^+, Ca^{2+}, Mg^{2+}, P and N levels in wheat can be explained simply by the ratios of the net deposition rate of these nutrients to that of water. Compared to the control plants, higher ratios of the net deposition rate of the ions K^+, Ca^{2+}, Mg^{2+}, P and total N to that of water were found under saline conditions (Hu and Schmidhalter 1998*a*), implying that the reduction in cell (water volume) expansion was greater than the net import of these nutrients. This result also suggests that a higher accumulation of a given nutrient does not indicate a greater supply of it.

The overall influence of mineral nutrient supply on leaf growth is complicated by the fact that many mineral elements are redistributed between different parts of the growing plant. Although a young growing leaf is a net importer of mineral nutrients, it may export certain nutrients via the phloem to the growing tissues of other leaves early in its ontogeny. Several studies (e.g. Graham and Ulrich 1972) have shown that K^+ may be readily exported and ~50% of the K^+ taken

up by rapidly developing young leaves may be derived from elsewhere within the plant (particularly older leaves) rather than from external sources (Greenway *et al*. 1965). Thus, the retranslocation of nutrients from the older leaves may cause the major reduction in cross-sectional area formation observed under salinity.

Barnal *et al*. (1974) proposed that the increased uptake of Cl^- in salt-stressed plants may also be responsible for the reduction in growth by depressing the uptake of other anions such as NO_3^-. Although the effect of salinity on NO_3^- levels in the whole plant or mature tissues is well described, much less is known about the effect of salinity on NO_3^- concentrations in growing tissues. In fact, to our knowledge, there is only one study available on the effect of salinity on the NO_3^- content in the growth zone of the leaf (in wheat; Hu and Schmidhalter 1998*a*), which showed that salinity decreased NO_3^- accumulation beyond the growth zone. Furthermore, the difference between the control and saline treatments was greater in mature tissues, and the net deposition rate of NO_3^- was negative in these same tissues (Hu and Schmidhalter 1998*a*). Because NO_3^- is not translocated in the phloem (Imsande and Touraine 1994), the negative net deposition rate of NO_3^- in the mature zone of the leaves can only derive from the rate of NO_3^- reduction, not from the rate of its export. Trewavas (1985) proposed that NO_3^- might be a plant growth regulator, which affects metabolism and development. He argued that this system could operate via an effect on the Ca^{2+} concentration of the cytoplasm; energy is directed towards NO_3^- reduction when NO_3^- enters a cell, so that a change in NO_3^- uptake would change the energy available for Ca^{2+} expulsion.

Very few data exist on the effect of salinity on micronutrients in the growing leaves of grasses. Hu *et al*. (2000*c*) reported that salinity affected the distribution pattern of Fe concentration on a FW basis, whereas it did not affect those of either Zn or Mn. Therefore, they concluded that the decrease observed in leaf growth was probably not due to either toxicity or deficiency of these micronutrients in the growing leaves of wheat (Hu *et al*. 2000*c*). However, a recent study by Neves-Piestun and Bernstein (2005) demonstrated that although Zn and Mn might not affect leaf growth of maize in the salt stress treatments, toxic level of Fe may be responsible for the reduction in leaf elongation.

Conclusions

Growing grass leaves present a suitable experimental system to study leaf growth processes because they contain distinct zones of cell division, elongation and mature cells. Mineral nutrients, with their special metabolic functions in the different zones, display specific distribution patterns. This review indicates that under saline conditions, elemental analyses of the toxic ions Na^+ and Cl^- and of the nutrients performed on the same scale as the growth analysis for the growing grass leaves rule out direct effects of Na^+ and Cl^- toxicity and of nutrient deficiency or ionic imbalance on both cell expansion and formation of the leaf cross-sectional area. However, it is still unclear whether or not Na^+ or Cl^- at least partially inhibit cell division and the duration of cell elongation. Cell enlargement and maturation is accompanied by synthesis of proteins, nucleic acids, and other cytoplasmic constituents, as well as by the synthesis of components of the cell wall. Yet the changes in such components, in the processes of biological synthesis and ion compartmentation, and in the properties of the cell wall under salinity, all of which might be significant for the functioning of growing tissues, remain largely unknown and have to be investigated. The significant reduction in the number of veins (and mainly in the number of small veins) under saline conditions may be responsible for the reduced retranslocation capacity of mineral nutrients and assimilates in grass leaves. Similarly, the reduced area of protoxylem and metaxylem in the large vein segments in growing tissues may be responsible for lower water deposition into the growth zone under saline conditions. A vascular discontinuity between the stem and the leaf vessels in the cell division zone of the leaf base requires that transport through the leaf division zone and into the developing vascular systems of the expanding zone relies either on symplastic transport or transport through the thin cell walls. Thus, the requirement for continuous nutrient supply to maintain the mineral status within rapidly expanding tissues renders the meristematic region highly susceptible to nutrient disturbances.

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