Spatial distributions and net deposition rates of mineral elements in the elongating wheat (*Triticum aestivum* L.) leaf under saline soil conditions

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Abstract. Wheat leaf growth is known to be spatially affected by salinity. The altered spatial distribution of leaf growth under saline conditions may be associated with spatial changes in tissue mineral elements. The objective of this study was to evaluate the spatial distributions of mineral elements and their net deposition rates in the elongating and mature zones of leaf 4 of the main stem of spring wheat (*Triticum aestivum* L. cv. Lona) during its linear growth phase under saline soil conditions. Plants were grown in an illitic-chloritic silty loam with 0 and 120 mM NaCl. Three days after emergence of leaf 4, sampling was begun at 3 and 13 h into the 16-h light period. Spatial distributions of fresh weight (FW), dry weight (DW), and Na^+, K^+, Cl^-, NO_3^-, Ca^{2+}, Mg^{2+}, total P, and total N in the elongating and mature tissues were determined on a millimeter scale. The patterns of spatial distribution of Na^+, Cl^-, K^+, NO_3^-, and Ca^{2+} in the growing leaves were affected by salinity, while those of Mg^{2+}, total P, and total N were not. Sodium, K^+, Cl^-, Ca^{2+}, Mg^{2+}, and total N concentrations (mmol · kg^{-1} FW) were consistently higher at 120 mM NaCl than at 0 mM NaCl along the leaf axis from the leaf base, whereas NO_3^- concentration was lower at 120 mM NaCl. Deposition rates of all nutrients were greatest in the elongation zone. The elongation zone was the strongest sink for mineral elements in the leaf tissues. Local net deposition rates of Na^+, Cl^-, Ca^{2+}, and Mg^{2+} (mmol · kg^{-1} FW · h^{-1}) in the most actively elongating zone were enhanced by 120 mM NaCl, whereas for NO_3^- this was depressed. The lower supply of NO_3^- to growing leaves may be responsible for the inhibition of growth under saline conditions. Higher tissue concentrations of Na^+ and Cl^- may cause ion imbalance but probably did not result in ion toxicity in the growing leaves. Potassium, Ca^{2+}, Mg^{2+}, total P, and total N are less plausibly responsible for the reduction in leaf growth in this study. Higher tissue K^+ and Ca^{2+} concentrations at 120 mM NaCl are probably due to the presence of high Ca^{2+} in the soil of this study.

Key words: Leaf elongation – Mineral elements – Net deposition rate (minerals) – Salt stress – Soil salinity – *Triticum* (salt stress)

Introduction

Leaf growth of wheat seedlings is severely inhibited by high concentrations of NaCl (Munns and Termaat 1986; Arif and Tomos 1993). The inhibition of growth under saline soil conditions may be due to water and osmotic stresses, specific ion toxicity, and/or ionic imbalance, acting on biophysical and metabolic processes of growth (Greenway and Munns 1980; Thiel et al. 1988).

Leaf elongation in grasses is restricted to a small region at the base of the blade enclosed by older leaves (Kemp 1980). We have previously observed that wheat leaf growth is spatially affected by salinity. This spatial reduction in leaf growth may be associated with the altered patterns of the spatial distribution of ion uptake and accumulation in the growing leaves. Therefore, an understanding of the physiological processes of leaf growth in saline soils requires knowledge of the spatial distribution of ions and their uptake rates in growing leaves.

Gandar (1980) and Silk (1984) have discussed and demonstrated that the local net deposition rate (D), which may be viewed as a quantitative picture of sink and source relationships, can be calculated according to the continuity equation. Data on the spatial distribution of both growth velocity and concentration of ions are

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Abbreviations: D = local net deposition rate; DV = undisturbed displacement velocity of a segment; total P = total phosphorus; SER = segmental elongation rate

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required for this calculation. Kinetic analyses for ion content have been used for maize leaves (Meiri et al. 1992), sorghum leaves (Bernstein et al. 1995), and cotton roots (Zhong and Läuchli 1994). However, there is no information available on the spatial distributions and net deposition rates of ions in the elongation and mature zones of wheat leaves under saline soil conditions. Spatial distributions of Na^+, K^+, Ca^{2+}, and Mg^{2+} beyond the elongation zone and of Cl^-, NO_3^-, total P, and total N in the whole leaves have not been reported previously for salt-stressed leaves. There is also no information available about the spatial distribution of nutrients in the morning and evening.

The objective of this study was to evaluate quantitatively the spatial distributions of ions, e.g. Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, NO_3^-, total P, etc., and their net deposition rates in the elongating and mature zones of wheat leaf 4 of the main stem during its linear growth phase in soil with 0 and 120 mM NaCl.

Materials and methods

Growth conditions. Six seeds of spring wheat (*Triticum aestivum* L. cv. Lona; purchased from Volg, Winterthur, Switzerland), pre-germinated for 2 d on filter paper moistened by tap water at 20 °C, were sown in 1.5-l pots (10 cm in diameter and 20 cm high) containing an illitic-chloritic silt loam (fine, mixed, mesic Aquic Ustifluvent; collected locally in Charrat, Switzerland) (Table 1 in Schmidhalter et al. 1994). The calcium concentration, determined in a soil:water saturation extract at 0.37 g · g^{-1} gravimetric water content, was 3 cmol · L^{-1}. The soil was initially watered to 0.25 g H$_2$O · g^{-1} dry soil (soil matric potential: Ψ_m = −0.03 MPa) with Hoagland solution at normal strength for macronutrients, modified by increasing the phosphate concentration tenfold, and 0.5-strength micronutrients as recommended by Epstein (1972). The composition of the modified Hoagland nutrient solution was: 6.05 mM K^+, 15.0 mM NO_3^-, 5.0 mM Ca^{2+}, 2.0 mM Mg^{2+}, 10.0 mM $H_2PO_4^-$, and 2.0 mM SO_4^{2-}. The salt level of 120 mM NaCl was obtained by adding NaCl to the nutrient solution. The soil was mixed thoroughly and kept in tightly closed plastic boxes for one week to facilitate equilibration. Thereafter, the soil was sieved into pots. Soil moisture content was maintained at the initial content by watering with tap water. In order to avoid water loss by evaporation, the pots were covered with a perforated plastic foil, and plants grew through small holes in the foil. 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 photosynthetic photon flux density was approximately 550 μmol · m^{-2} · s^{-1} provided by a mixture of 160-W cool-white fluorescent and 60-W standard tungsten lamps. The air temperature was 20 °C day/night and the relative humidity was maintained at 55–65%.

Tissue sampling. Three days after the emergence of leaf 4, sampling was begun at 3 h (0900 hours) and 13 h (1900 hours) into the 16-h photoperiod. Two replications were harvested successively and the harvest time recorded, sampling being finished within 1 h. Elongating leaves were selected for sampling if the blade was equal or greater than 12 cm long, but less than 14 cm. This ensured that the increase in leaf length was linear. The elongation zone was carefully freed of leaf sheaths, and then cut from the stem at the base of the leaf blade. The blade was cut with a razor blade, beginning at the leaf base, into six segments, 5 mm long, followed by three segments, 10 mm long and three segments, 20 mm long. About 120 leaf segments from the same position were combined into a sample. After fresh weight was determined, the samples were dried

at 65 °C for 48 h and dry weight was determined. Dry plant material was stored for the analysis of ion concentration.

Analysis of ion concentration. Dry samples from different positions in leaf 4 of the main stem were ground by hand with a glass rod into test tubes. The concentrations of ions were measured using the following methods:

Sodium, K^+, Ca^{2+}, Mg^{2+}, and total P: approximately 25-mg plant samples were ashed at 560 °C for 6 h and digested with 1 ml of 20% HCl at 65 °C for 5 min and then diluted to 25 ml. The concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, and total P were determined with an inductively coupled plasma emission spectrometer (ICP model Liberty 200; Varian Australia Pty., Mulgrave, Victoria, Australia).

Chloride, NO_3^- and water-soluble P: 20-mg ground samples were extracted with 2 ml distilled water at 100 °C for 5 min, shaken for about 1 min and then filtered with a Millex-HV$_{13}$ filter unit. Chloride was determined by using a chloride-selective electrode (Chloride analyzer 926; Corning, Halstead, Essex, UK), and NO_3^- and water-soluble P with an HPLC detector (LC 75; Perkin-Elmer Co., Norwalk, Conn. USA)

Total nitrogen: 6-mg plant samples were weighed with a super micro-balance (Sartorius, Goettingen, Germany). Nitrogen was analyzed with a nitrogen analyzer (Carlo ERBA Strumentazione, Nitrogen analyzer 1500; Cable Erbadas, Milan, Italy).

Numerical methods. Local net deposition rates (D, mmol · kg^{-1} FW or DW · h^{-1}) of mineral elements like Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, NO_3^-, total P, etc. were calculated according to the one-dimensional version of the continuity equation as described by Silk (1984):

$$D = (\partial P/\partial t) + DV \cdot (\partial P/\partial x) + (SER \cdot P) \qquad [1]$$

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where P is substance density (e.g. mmol · kg^{-1} FW), t is time (h), and x is distance (mm) from the leaf base of the leaf blade. DV and SER are the undisturbed displacement velocity of a segment (mm · h^{-1}) and the segmental elongation rate (mm · mm^{-1} · h^{-1}), respectively.

On the right side of Eq. 1 the first term (I), $\partial P/\partial t$, represents the local rate of change (temporal rate change in substance content at a fixed distance from the leaf base). It was calculated from data obtained from the tissue sampled at the beginning (t_a) and end (t_b):

$$\partial P/\partial t = (P_b - P_a)/(t_b - t_a)$$

Thus, the local rate of change was assumed to occur at a linear rate between t_a and t_b.

The second term (II), $DV \cdot (\partial P/\partial x)$, in Eq. 1 is called the "convective rate of change" which represents the change due to a movement of cells away from the leaf base and can be considered as the deposition rate needed to maintain a spatial gradient in density (Silk et al. 1984). It was calculated according to the following equation (Schnyder and Nelson 1987):

$$\partial P_i/\partial x_i = 0.5[(P_i - P_{i-1})(x_i - x_{i-1})^{-1} + (P_{i+1} - P_i)(x_{i+1} - x_i)^{-1}]$$

where P_i is the substance content in the segment i and x_i is the distance (mm) from the leaf base of the leaf. For the first or last segment, $\partial P_i/\partial x_i$ was calculated according to

$$(P_{i+1} - P_i)(x_{i+1} - x_i)^{-1} \text{ or } (P_i - P_{i-1})(x_i - x_{i-1})^{-1}$$

The third term (III · SER · P), on the right-hand side of the equation is the "stretch rate" or the "growth dilution term" which represents the deposition rate needed to maintain a constant local density to avoid dilution due to tissue expansion (Silk et al. 1986). The values of DV and SER were taken from a previous study of ours on growth analysis (data not shown). The position of the growing zone was determined by measuring displacement rates along the leaf axis by the puncture method (Kemp 1980). The effect of the puncture on leaf elongation was evaluated by using a ruler to record the change in leaf length compared with other non-punctured leaves over a 10-h period immediately following

puncturing. Since the leaf elongation rate (LER) was reduced by puncturing, a correction factor was applied to the values for DV and SER used in Eq. 1. The correction factor was equal to the ratio of the LER of punctured leaves to the LER of non-punctured leaves. The latter value was obtained using a linear variable displacement transducer, and both rates were measured during the linear phase of growth in the daytime.

Statistical analysis. A randomized complete block design was used. Effects of salinity (0 and 120 mmol NaCl), harvest time (0900 hours and 1900 hours) and the interaction were evaluated by analysis of variance for each location along the leaf axis. Due to the high number of plants and homogeneous growing conditions the block effect was never significant. Hence, we assumed the different batches of plants harvested within the different treatments not to differ except for treatment differences and included the distance from the leaf base and the interaction with salinity and time of

harvest as subhierarchical effects in the model of analysis of variance.

Results

Spatial distribution of the concentration of mineral elements. Data in Figs. 1 and 2 show that the patterns of spatial distribution of Na^+, Cl^-, K^+, NO_3^-, and Ca^{2+} along the leaf axis altered with salinity, while those of Mg^{2+}, total P, and total N changed only slightly or not at all.

The sodium concentration (mmol · kg^{-1} FW) at 0 mM NaCl was fairly constant along the leaf axis being slightly lower between 30 and 50 mm from the leaf

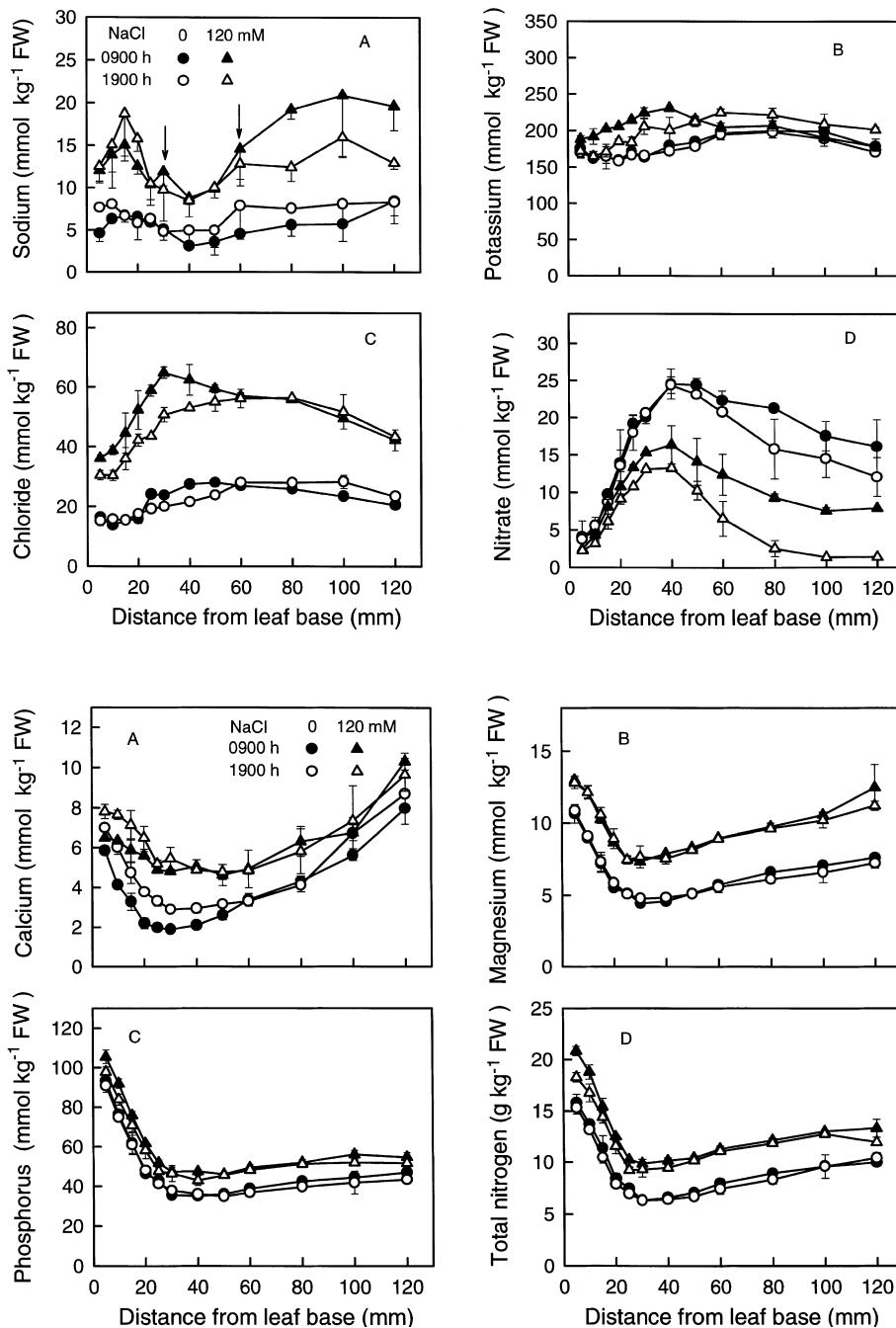**Fig. 1A–D.** Spatial distributions of sodium (**A**), potassium (**B**), chloride (**C**), and nitrate (**D**) concentrations (mmol · kg^{-1} FW) in the growing leaf 4 of the main stem of wheat plants grown in soil with 0 and 120 mM NaCl at two harvest times (at 0900 and 1900 hours). *Error bars* represent standard deviations. Error bars ($n = 2$; each replicate consisted of 120 subsamples from different plants) fit within the plot symbol if not otherwise shown. *Arrows* indicate the length of the elongation zone and the position of the end of the leaf sheath

Fig. 2A–D. Spatial distributions of calcium (**A**), magnesium (**B**), total phosphorus (**C**), and total nitrogen (**D**) and (mmol · kg^{-1} FW) in the growing leaf 4 of the main stem of wheat plants grown in soil with 0 and 120 mM NaCl at both harvest times (at 0900 and 1900 hours). *Error bars* represent standard deviations ($n = 2$). Error bars fit within the plot symbol if not otherwise shown

base, while the Cl^- concentration was slightly lower near the leaf base. At 120 mM NaCl, however, the Na^+ concentration increased dramatically with distance from the leaf base, reaching a maximum in the middle of the elongation zone at both harvests (Fig. 1A). There was a rapid decrease in Na^+ concentration from 15 mm above the leaf base, with a minimum at about 40 mm, and then a continuous increase with distance again. A sharp increase in Cl^- concentration was found in the elongation zone and a slight decrease with distance in the mature part of the leaf (Fig. 1C). Sodium and Cl^- concentrations were statistically significantly increased by 120 mM NaCl.

The potassium concentration at 0 mM NaCl was higher in the mature zone than in the elongation zone (Fig. 1B), while at 120 mM NaCl it increased with distance in the elongation zone and decreased in the mature zone, especially at 0900 hours. The increase in K^+ concentration at 120 mM NaCl was found in the region approximately 10–50 mm from the leaf base.

The nitrate concentration sharply increased to a maximum at about 40 mm from the leaf base in both treatments (Fig. 1D), and then decreased with distance. Salinity only slightly affected the NO_3^- concentration up to 20 mm from the leaf base. However, there was a highly significant decrease in NO_3^- concentration beyond this region at 120 mM NaCl. The mean NO_3^- concentration from 30 to 120 mm from the leaf base was about two to three times higher for plants with 0 mM NaCl than for those with 120 mM NaCl.

Concentrations of water-soluble P were only slightly increased by about 10–15 mmol · kg^{-1} FW towards the leaf base in the growing zone as compared with the mature zone where average concentrations of 40 mmol · kg^{-1} FW were found. The salinized treatment showed slightly higher concentrations than the non-salinized treatment.

The calcium concentration decreased with distance from the leaf base to reach a minimum in the region between 25 to 50 mm and then increased in both treatments; the increase and/or decrease in Ca^{2+} concentration was sharper at 0 mM NaCl than at 120 mM NaCl (Fig. 2A). The calcium concentration was significantly enhanced by salinity at all locations along the zone of elongation.

Magnesium, total-P, and total-N concentrations decreased with distance from the leaf base to reach a minimum at the end of the elongation zone and then slightly increased or remained constant in both treat-
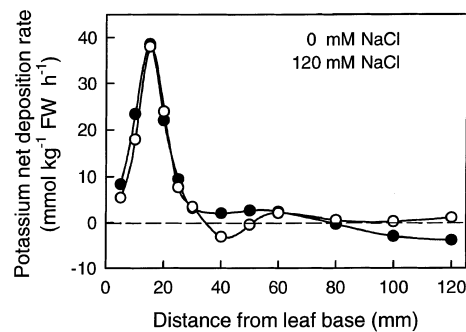

Fig. 3. Spatial distribution of net deposition rates of potassium (mmol · kg^{-1} FW · h^{-1}) in the growing leaf 4 of wheat plants grown in the soil with 0 and 120 mM NaCl

ments. Magnesium, total-P, and total-N contents were consistently higher at 120 mM NaCl than at 0 mM NaCl.

On a DW basis, the patterns of spatial distribution for all elements were generally similar to those on a FW basis in both treatments at both harvest times (data not shown). However, the K^+ concentration on a DW basis was higher at 0 mM NaCl than at 120 mM NaCl as compared with a FW basis. Enhancement of calcium content by 120 mM NaCl was found only up to about 50 mm from the leaf base. Magnesium, total-P, and total-N concentrations (mmol · kg^{-1} DW) were not affected by salinity.

Effect of time on the spatial distributions of mineral element contents. The Na^+ concentration at 120 mM NaCl was higher at 0900 hours than at 1900 hours beyond 50 mm (Fig. 1A), whereas at 0 mM NaCl this was reversed, although differences were in general not significant. The effect of time on K^+ concentration at 120 mM NaCl varied with the location in the leaf (Fig. 1B), e.g. the K^+ concentration in the region between 10 and 50 mm from the leaf base was slightly higher at 0900 hours than at 1900 hours and slightly lower at 1900 hours in the region between 80 and 120 mm. Time affected Ca^{2+} and Cl^- concentrations in the elongation zone only slightly (Figs. 2A, 1C). In the morning, the NO_3^- concentration in the mature zone was significantly higher, especially at 120 mM NaCl (Fig. 1D), and in the elongation zone at 120 mM NaCl (Fig. 1D). No effect of time on Mg^{2+}, total-P, and total-N contents was observed along the leaf axis at the two NaCl concentrations (Figs. 2B–D).
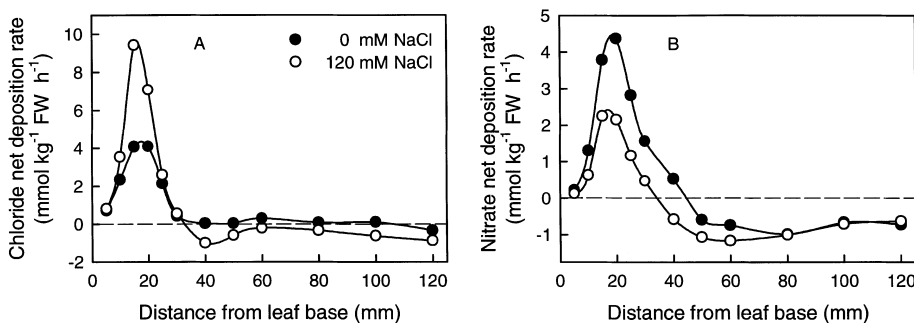

Fig. 4A,B. Spatial distributions of net deposition rates of chloride (**A**) and nitrate (**B**) (mmol · kg^{-1} FW · h^{-1}) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl

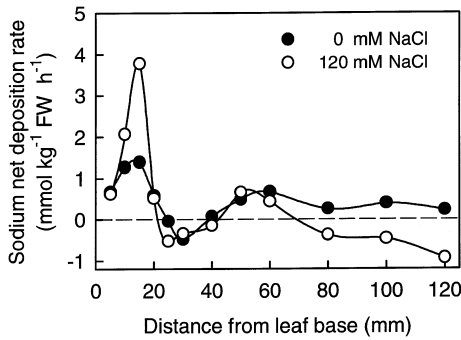**Fig. 5.** Spatial distribution of net deposition rates of sodium (mmol · kg^{-1} FW · h^{-1}) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl

Net deposition rates of mineral elements. The net deposition rate profiles can be described as the quantitative result of sink and source relationships. A positive net deposition rate for a substance can be viewed as indicating the presence of a sink for that substance, while a negative net deposition rate indicates the source of the substance (Silk et al. 1986). Net deposition rates of mineral elements, as shown in Figs. 3–7, were obtained from the average of the two harvest times. The spatial distributions of net deposition rates for all elements in leaf 4 of the main stem in both treatments had a similar pattern in the elongation zone which demonstrated the strongest sink for the mineral elements, while the distribution patterns varied with different elements in the more mature region of the leaf (Figs. 3–7).

The maximum of net deposition rates of K$^+$, Cl$^-$, and NO$_3^-$ (mmol · kg^{-1} FW · h^{-1}) occurred at about 15–20 mm above the leaf base (Figs. 3; 4A,B). Net deposition rates of K$^+$ and Cl$^-$ in the mature zone remained constant (near zero or slightly negative). A negative NO$_3^-$ deposition rate was found in the mature zone in both treatments.

The greatest effect of salinity on net deposition rates of Cl$^-$ and NO$_3^-$ occurred in the region between 10–25 mm and 15–50 mm, respectively (Figs. 4A,B). The mean value of Cl$^-$ net deposition rate in the elongation zone was about 40% higher at 120 mM NaCl than at 0 mM NaCl, while that of NO$_3^-$ in the same zone was 52% greater at 0 mM NaCl than at 120 mM NaCl. In contrast, the net deposition rate of K$^+$ was not affected by 120 mM NaCl (Fig. 3).

The patterns of the net deposition rates of Na$^+$, Ca^{2+}, Mg^{2+}, total P, and total N were slightly different from those of K$^+$, Cl$^-$, and NO$_3^-$ in the elongation zone (Figs. 5; 6A,B; 7A,B). The maximum net deposition rate occurred at the middle of the elongation zone (at about 15 mm), followed by a sharp decrease to zero before the elongation zone ended (at about 20 to 25 mm). Net deposition rates of Na$^+$, Ca^{2+}, Mg^{2+}, and total N in the elongation zone were significantly increased by 120 mM NaCl (Figs. 5; 6A,B; 7B). Mean values of Na$^+$, Ca^{2+}, Mg^{2+}, and total-N net deposition rates in the elongation zone were about 40%, 31%, 27%, and 12% higher, respectively, at 120 mM NaCl than at 0 mM NaCl (Figs. 5; 6A,B; 7B). Negative rates of Na$^+$ deposition at 120 mM NaCl occurred in two regions, between 25 and 40 mm and between 80 and 120 mm from the leaf base. The net deposition rate of Ca^{2+} at 120 mM NaCl was reduced by about 53% in the mature zone. In contrast, the net deposition rate of total P remained constant in the mature zone and was not affected by salinity, regardless of the location in the leaf (Fig. 7A).
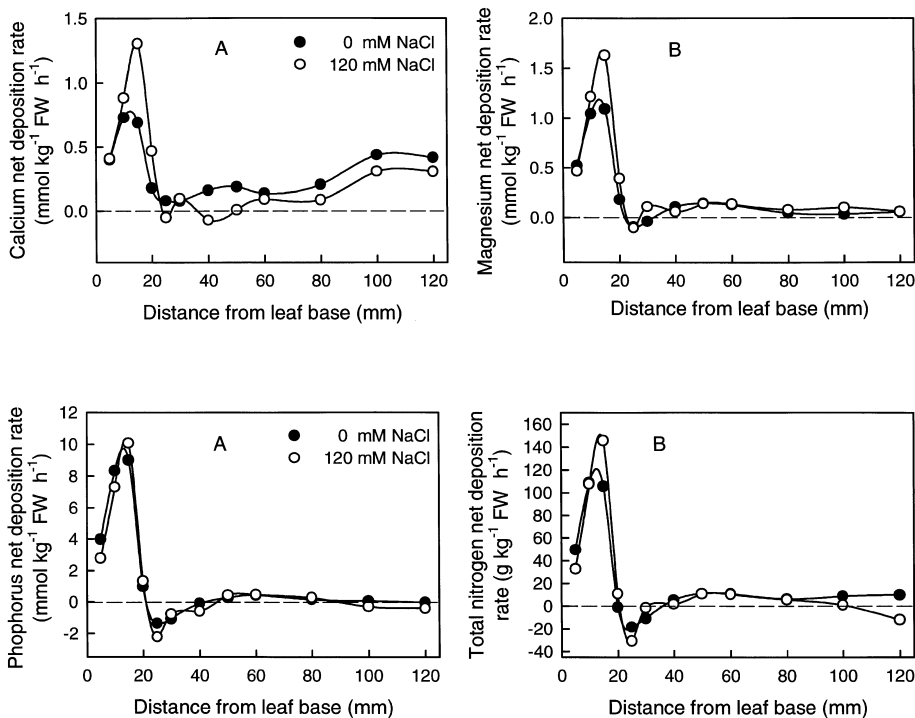

Fig. 6A,B. Spatial distributions of net deposition rates of calcium (**A**) and magnesium (**B**) (mmol · kg^{-1} FW · h^{-1}) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl

Fig. 7A,B. Spatial distributions of net deposition rates of total phosphorus (**A**) and total nitrogen (**B**) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl

Discussion

The results from the spatial distributions of ions and their net deposition rates in the growing leaf of the main stem of wheat show that effects of increased Na^+ and Cl^- levels varied with distance along the leaf axis (Figs. 1–7). This indicates that the location at which specific ion toxicity, ion deficiency, and/or ionic imbalance may occur along the leaf axis, should be considered.

Effect of salinity on nutrient allocation and translocation in the growing leaves. In general, there were two patterns of spatial distributions of mineral element contents in the elongation zone, regardless of the treatment: (i) contents of ions were lower at the leaf base and then increased with distance (Cl^-, NO_3^-, salinized treatments for Na^+ and K^+; Fig. 1) and (ii) contents of ions were higher at the leaf base and decreased with distance (Ca^{2+}, Mg^{2+}, total P, and total N; Fig. 2). These findings are consistent with the reports on those of Cl^-, K^+, Ca^{2+}, Mg^{2+}, and total P in maize leaves (Meiri et al. 1992; Evéquoz 1993), K^+ and Ca^{2+} in maize roots (Sharp et al. 1990; Zhong and Läuchli 1994), Na^+, K^+, Ca^{2+}, and Mg^{2+} in sorghum leaves (Bernstein et al. 1995), and NO_3^- and total N in tall-fescue leaves (Gastal and Nelson 1994). The first pattern of spatial distribution may be a consequence of cell vacuole size which is relatively small in the cells at the leaf base and increases with distance from the base, indicating that Cl^-, NO_3^-, K^+, and Na^+ accumulate mainly in vacuoles. Since, in contrast to more distal cells, the small cells at the base contain a large proportion of the protein, RNA, and nucleic acids, the high Mg^{2+}, total-P, and total-N contents near the leaf base indicate that high rates of protein and RNA synthesis may exist in this region. The higher Ca^{2+} content in meristematic tissue (Fig. 2A) could be readily explained if it is true that younger cells contain more pectins in their walls, since the polyuronides of pectins are the major Ca^{2+}-binding sites of the cell wall (Zhong and Läuchli 1994). Throughout the elongation zone, Ca^{2+}, Mg^{2+}, total-P, and total-N concentrations rapidly declined with distance in the two treatments (Fig. 2). This may be explained in part by extensive dilution and vacuolation in the elongation zone, indicating that Ca^{2+}, Mg^{2+}, total P, and total N accumulate less rapidly than water in the growing tissue.

The results (Figs. 1, 2) show that the patterns of spatial distribution of Na^+, Cl^-, K^+, NO_3^-, and Ca^{2+} in the growing leaves were affected by salinity, while those of Mg^{2+}, total P, and total N were not. The effects of salinity on the patterns of spatial distribution of Na^+ and Mg^{2+} in the elongation zone were consistent with a previous report on the leaves of sorghum plants grown hydroponically (Bernstein et al. 1995), while those on the spatial distributions of K^+ and Ca^{2+} were different. Spatial distributions of Na^+, K^+, Ca^{2+}, and Mg^{2+} beyond the elongation zone and of Cl^-, NO_3^-, total P, and total N in the whole leaves have not been reported previously for salt-stressed leaves.

The results in this study show that the toxic effects of Na^+ and Cl^- accumulation under saline conditions are hardly plausible. The pattern of spatial distribution of Na^+ at 120 mM NaCl was closely related to that of the SER (Fig. 1A; Hu 1996), which contradicts the behaviour expected from growth inhibition. This supports suggestions (Munns et al. 1988; Bernstein et al. 1995) that the growth of leaves is not directly controlled by the local concentration of Na^+ in growing tissues or by specific localization and/or compartmentation of Na^+. Whereas the maximum Na^+ content in the elongation zone of sorghum leaves was about 65 mmol · kg^{-1} FW (Bernstein et al. 1995), this value was lower than 20 mmol · kg^{-1} FW in our study with maize leaves. The maximum Na^+ content at the site of the most active elongation is probably needed to meet the demand for osmotic adjustment under saline conditions (Fig. 1A). Although Cl^- was about four times higher than Na^+ in the growing zone under saline conditions (Figs. 1A,C), it is also unlikely to be toxic. We advance two arguments. First, the maximum Cl^- content at the end of the elongating zone reached only 50–60 mmol · kg^{-1} FW, and this concentration did not inhibit in-vitro protein synthesis in a wheatgerm system (Wyn Jones et al. 1979). Second, Cl^- accumulation decreased in the elongation zone after a 10-h light period and remained nearly constant in the mature zone. In our previous study, the Cl^- concentration in the leaves at the final harvest time was about 10 times as high as that in growing leaves under similar conditions, which affected main stem grain yield only a little (Hu and Schmidhalter 1997). These arguments indicate that the growing leaves may be able to regulate the Cl^- concentration to avoid an excessive accumulation of ions. However, there is a probability that in the non-fully vacuolated small cells of the growing zone the cytosolic enzymes may be exposed to higher concentrations of Na^+ and Cl^-. Chloride is very mobile, and plants tolerate it at high concentration (Fixen 1993). It preferentially accumulates in the old leaves and in the leaf sheath (Greenway and Munns 1980; Boursier et al. 1987). A consistent negative net deposition rate of Cl^- in the mature zone in the NaCl treatment reflects the retranslocation of chloride in the phloem (Fig. 4A).

Beyond the elongation zone, the Na^+ concentration increased with distance from the leaf base (Fig. 1A). Munns et al. (1986) reported that the efflux of Na^+ in the phloem from the leaves played only a small role in controlling the Na^+ content of barley leaves. Thus, an increase in Na^+ accumulation, especially in the exposed region, may have resulted from the high proportion of Na^+ transport in the xylem. Negative net deposition rates of Na^+ occurred in two regions, i.e. 20–40 mm and 70–120 mm above the leaf base (Fig. 5), indicating that a limited export of Na^+ transport in the phloem to the most active growing zone occurs. Unlike Na^+, the Cl^- concentration decreased slightly with increasing distance from the elongation zone (Fig. 1C), reflecting the possibility that Cl^- may have undergone considerable retranslocation in the phloem.

Our results describe solute contents for bulk tissue samples. Leaf solutes are compartmentalized not only at the subcellular level (i.e. between vacuole and cytoplasm)

but also at the intercellular level (i.e. between the epidermis and the remaining leaf, symplast; Fricke et al. 1994). These authors demonstrated for mature cells that epidermal concentrations of Cl^- always exceeded those of the bulk extract, while Na^+ concentrations were similar. Differences in Cl^- distributions may be related to water loss at terminal sites of transpiration. This effect will be negligible in the growth zone where leaves are enclosed within older sheaths. We do not have information about different distributions of inorganic elements within the growth zone. However, it seems that eventual differences might not dramatically affect our previous conclusions about a non-toxic effect of Na^+ and Cl^- because the bulk tissue concentrations were in general fairly low.

Our previous study showed that about 90% of the reduced width, which accounted for about 40% of the reduction in leaf area of wheat, occurred near the leaf base (Hu 1996). This may indicate that, in order for plants to adapt to the stress condition, a signal or feedback due to the higher accumulation of Cl^- and Na^+ in the growing leaves and in other organs of plants (e.g. roots) may control the initial size of the leaf under saline conditions.

Barnal et al. (1974) proposed that the relatively greater uptake of Cl^- than Na^+ in salt-stressed plants may also be responsible for the growth reduction by depressing the uptake of other anions such as nitrate. The lower supply of NO_3^- to growing leaves may be responsible for the inhibition of growth under saline conditions. Although there was no significant difference in NO_3^- accumulation between the two treatments in the region between 0 and 15 mm above the leaf base, the NO_3^- concentration at 120 mM NaCl fell to about zero in the more mature zone at 1900 hours (Fig. 1D). Since 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 leaves can only be considered as the rate of NO_3^- reduction, but not as the export rate. The decreased net deposition rate of NO_3^-, by 120 mM NaCl, is due to the low influx rate of NO_3^- into the elongation zone (Fig. 4B), which may be caused by the low uptake rate of NO_3^- by roots (Rush and Epstein 1976). The decrease in NO_3^- concentration with distance beyond the elongation zone may be due to the greater reduction of NO_3^- in the exposed part of the leaf, since lights stimulate the NO_3^- reduction, and/or due to the decreased NO_3^- uptake under saline conditions. There is an interesting negative correlation between the peak deposition rates of NO_3^- and Cl^- in the control and 120 mM NaCl treatments. Cram (1973) showed that net NO_3^- influx was reduced by a high Cl^- concentration in root tissue, such that, if the abundance of the latter increased, the concentration of the former decreased.

The K^+ and Ca^{2+} concentrations were increased by salinity, especially in the elongation zone (Figs. 1B; 2A); this was in contrast to the study of sorghum leaves under saline conditions (Bernstein et al. 1995) and on maize roots (Zhong and Läuchli 1994). This was most likely due to the high Ca^{2+} content in this soil. Calcium enhanced the uptake of K^+ in pigeon pea (*Cajanus cajan* L. Huth) resulting in a higher concentration of K^+ in plants grown under saline conditions (Subbarao et al. 1990). Cramer et al. (1990) reported higher concentrations of K^+ in the root tips of two corn cultivars in the presence of calcium. Similarly, higher external Ca^{2+} may cause a higher Ca^{2+} concentration under saline conditions. A comparison of concentrations of Mg^{2+}, total P, and total N on the basis of DW and FW (Fig. 2B–D) indicates that the higher concentration of Mg^{2+}, total P, and total N at 120 mM NaCl on the basis of FW as compared to 0 mM NaCl resulted from the lower water content of the plants at 120 mM NaCl. Nevertheless, K^+, Ca^{2+}, Mg^{2+}, total-P, and total-N contents in the leaf tissue probably do not limit leaf growth under saline conditions.

The effect of time on nutrient allocation. Faster growth during the light period enhances the sink strength in the elongation zone and/or in other new leaves. Therefore, the greater sink strength may cause the higher proportion of Na^+ retranslocation in the phloem after 10 h of light (Fig. 1A), resulting in a decrease in Na^+ accumulation in the mature zone. The decrease in NO_3^- content in the mature zone after 10 h of light may be due to a light-stimulated reduction of NO_3^- in leaf tissue in both treatments (Fig. 1D). However, the decrease in the NO_3^- content at 120 mM NaCl (Fig. 1D) was greater, indicating that reductase activity of NO_3^- in the mature part of leaves may be higher and/or the supply of NO_3^- may be lower under saline conditions.

In conclusion, the patterns of spatial distribution of Na^+, Cl^-, K^+, NO_3^-, and Ca^{2+} in the growing leaves were affected by salinity, while those of Mg^{2+}, total P, and total N were not. This study suggests that, under saline conditions, the lower supply of NO_3^- to growing leaves may be responsible for the inhibition of growth. Higher tissue Na^+ and Cl^- may cause ion imbalance but did not result in ion toxicity in the growing leaves. Potassium, Ca^{2+}, Mg^{2+}, total P, and total N are less plausibly responsible for the reduction in leaf growth in this study. Higher tissue K^+ and Ca^{2+} concentrations at 120 mM NaCl are probably due to the presence of high Ca^{2+} in the soil of this study.

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