Doctoral Thesis

Growth response of wheat plant to salinity in hydroponics and soil
II Spatial and temporal distribution of growth and the mineral element and carbohydrate contents in the leaves under saline soil conditions

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Growth Response of Wheat Plant to Salinity in Hydroponics and Soil

I Interactive Effects of Salinity and Macronutrients on the Growth, Yield, and Mineral Element Contents under Hydroponic Conditions

II Spatial and Temporal Distribution of Growth and the Mineral Element and Carbohydrate Contents in the Leaves under Saline Soil Conditions

A dissertation submitted to the
Swiss Federal Institute of Technology Zürich
for the degree of
Doctor of Natural Sciences

presented by
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Acknowledgements
Summary

The tolerance of plants to salinity changes under different soil fertility levels. Most previous investigations have been concerned with only one or two nutrients however, and mechanisms of salt effects on plant growth under different soil conditions are relatively unknown. Therefore, this study aimed to (i) obtain information on the possible role of macronutrient level in salt tolerance of wheat plants grown in a salinized nutrient solution and (ii) identify the mechanisms where salt inhibits the growth of soil-grown wheat plants. To investigate interactive effects of salinity and macronutrients on wheat growth, spring wheat (*Triticum aestivum* L. cv. Lona) was grown in hydroponic culture in growth chambers until grain maturity. Eight salinity levels between 0 and 150 mM NaCl were established and 1, 0.2, and 0.04 strength Hoagland macronutrient solution were taken as the levels of nutrient supply. To better understand mechanisms of salt inhibition on wheat growth, additional experiments were conducted with soil-grown plants in growth chambers. Plants were grown in 1.5-L pots containing an illitic-chloritic silty loam with two or four salt levels ranging from 0 to 120 mM NaCl during the vegetative stage under saline soil conditions. The effects of salinity on the growth of wheat seedlings, leaf water relations, leaf elongation rate, spatial distribution of leaf growth, mineral elements, and carbohydrate components in the elongating and mature zones of leaf 4 of the main stem were measured. The continuity equation was used to calculate the net deposition rate of fresh weight (FW), dry weight (DW), mineral elements, and carbohydrate components in the elongating and mature zones of leaf 4 of the main stem of the wheat plants.

Grain yield was significantly reduced by increasing salinity. Increasing the macronutrient supply significantly increased the plant salt tolerance under low fertility level and under medium macronutrient concentration with high salinity levels. The grain yield of the main spike decreased much less than the investigated vegetative growth parameters. At all investigated macronutrient levels, plant grain yield reductions were closely related to decreased leaf and tiller numbers. This suggests that salinity exerts its main effects during the early growth stages. Na⁺ and Cl⁻ contents in leaves and stems at grain maturity increased significantly, whereas K⁺ and NO₃⁻ decreased significantly with an increase in salinity regardless of the macronutrient level. Extreme Na⁺/Ca²⁺ ratios in plant tissues negatively affected grain yield production at high salinity with medium or
high macronutrient levels and at low macronutrient level together with medium salinity. In contrast to leaves and stems, mineral contents in the grain increased (Na\(^+\), Cl\(^-\)) or decreased (Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\)) only slightly or were not affected (K\(^+\)) by salinity except at high salinity and low macronutrient level. Even though strong and significant correlations between mineral contents at grain maturity in the leaves, stems, grain, and various yield parameters were observed, there is no evidence that toxicity, nutrient imbalance, or nutrient deficiency influenced grain yield to any considerable extent, since salinity mainly affected wheat growth at the early stages of growth.

Leaf production of the main stem with soil-grown wheat was significantly reduced by increasing salinity. Leaf width significantly decreased as well with increasing salinity. Salinity spatially affected cell elongation in the leaf elongation zone. The reduction in leaf elongation rate was due to decreasing segmental elongation rate, but not due to shortening the length of the elongation zone. The effect of salinity on leaf growth became more severe with increasing leaf number. The reduction in cross-sectional area mainly occurred near the ligule of wheat leaves. Under saline conditions, the slower expansion of leaves may be due to the limitation of the local net deposition rate of water. The inhibition of the secondary cell wall production by salinity may be responsible for the greater reduction in dry weight content beyond the elongation zone.

The elongation zone of the wheat leaf is the strongest sink for mineral elements and for carbohydrates. 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 ligule, whereas NO\(_3\) concentration was lower at 120 mM NaCl. Local net deposition rates of Na\(^+\), Cl\(^-\), Ca\(^{2+}\), and Mg\(^{2+}\) (mmol kg\(^{-1}\) FW h\(^{-1}\)) in the most actively elongating region were enhanced by 120 mM NaCl, whereas for nitrate this was depressed. The limitation of NaCl to leaf growth may be due to Cl\(^-\) toxicity in both elongating and mature regions. Low nitrate concentration and net deposition rate that may be caused by a low transport rate beyond about 15 mm from the ligule under saline conditions could also be responsible for the inhibition of leaf growth caused by salinity. Higher tissue Na\(^+\) concentrations may have caused ion imbalance, but did not directly lead to ion toxicity in this study. Glucose and fructose concentrations were low at the base of the elongation zone and greatly increased up to the end of the elongation zone in both treatments at the two harvest times. The increase in hexoses with distance from the ligule may correspond to an increase in the demand for osmotic adjustment and high biosynthesis in the elongation zone and for the synthesis of nonstructural material in the zone located between 30 and 60 mm from the ligule. In contrast, the sucrose concentration in the elongation zone was high at the leaf base and sharply decreased with distance from the ligule to the end of the elongation zone for both treatments, probably due to dilution with water, the rate of hydrolysis into glucose and fructose, and the rate of sucrose transport. Salt limitation to growth may be due to the utilization of carbohydrates in the elongation zone and their supply in the zone of large deposition of secondary cell walls. In this study, there was no evidence to suggest that the reduction of leaf growth under saline conditions is mainly due to a water deficit in leaves.
Zusammenfassung


signifikant erhöht, während K⁺ und NO₃⁻ signifikant reduziert waren mit zunehmender Salinität unabhängig vom Makronährstoffniveau. Extreme Na⁺/Ca²⁺ Verhältnisse in Pflanzengeweben beeinträchtigten die Kornnertragsproduktion negativ bei hoher Salinität bei mittleren oder hohen Makronährstoffniveaus und bei mittlerer Salinität mit niedrigem Makronährstoffniveau. Im Gegensatz zu Blättern und Stengel waren die Mineralstoffgehalte in den Körnern nur wenig erhöht (Na⁺, Cl⁻) oder erniedrigt (Ca²⁺, Mg²⁺, K⁺) oder nicht beeinflusst (K⁺), ausgenommen bei hoher Salinität und niedrigem Makronährstoffniveau. Obschon hochsignifikante und enge Korrelationen zwischen dem Mineralstoffgehalt bei Kornreife in den Blättern, Stengeln, Körnern und verschiedenen Wachstumsparametern beobachtet wurden, gibt es keine Hinweise, dass Toxizität, Nährstoffungleichgewicht oder Nährstoffmangel den Korntrag wesentlich beeinflussten, da Salinität das Wachstum hauptsächlich in einem frühen vegetativen Stadium beeinflusste.


Die Wachstumszone des Weizenblattes stellt den stärksten Sink für Mineralelemente und für Kohlenhydrate dar. Na⁺, K⁺, Cl⁻, Ca²⁺, Mg²⁺, und N-Konzentrationen (mmol kg⁻¹ FW) bei der Ligula entlang der Blattachse waren konsistent höher bei 120 mM NaCl gegenüber 0 mM NaCl, während die NO₃⁻-Konzentrationen bei 120 mM NaCl niedriger waren. Die Nettodepositionsraten von Na⁺, Cl⁻, Ca²⁺ und Mg²⁺ (mmol kg⁻¹ FW h⁻¹) wurden in der Wachstumszone durch 120 mM NaCl erhöht, während diejenigen von NO₃⁻ erniedrigt wurden. Die Hemmung von NaCl im Blattwachstum könnte durch Cl⁻-Toxizität in der Wachstumszone und dem adulten Gewebebereich verursacht sein. Hemmungen des Blattwachstums könnten aber auch durch eine niedrige NO₃⁻-Konzentration und Nettodepositionsrate bedingt sein, verursacht durch eine niedrige Transportrate oberhalb von 15 mm von der Ligula. Hohe Na⁺-Konzentrationen könnten ein Ionenungleichgewicht verursacht haben, resultierten aber nicht in Toxizität. Glukose- und Fruktosekonzentrationen waren niedrig an der Basis der Wachstumszone und nahmen in beiden Verfahren sehr stark bis zum Ende der Wachstumszone zu. Eine Zunahme der Hexosekonzentration mit der Distanz von der Ligula entspricht möglicherweise einem erhöhten Bedarf der osmotischen Anpassung und einer hohen Syntheserate nichtstruktureller Kohlenhydrate in der Wachstumszone sowie einem hohen Bedarf für nichtstrukturelle Verbindungen in der Zone zwischen 30 bis 60 mm von der Ligula. Im Gegensatz dazu war die Saccharosekonzentration in der Wachstumszone in der Blattbasis hoch und nahm in beiden Verfahren stark mit zunehmender Distanz von der Ligula bis zum Ende der Wachstumszone ab. Möglicherweise ist dies auf eine Verdünnung mit Wasser, der Hydrolyserate in Glukose und
Fruktose, und der Saccharosetransportrate zurückzuführen. Wachstumsbeschränkungen durch Salinität könnten auf die limitierte Ausnutzung von Kohlenhydraten in der Wachstumszone und ihre Nachlieferung in die Zone des sekundären Zellwandwachstums zurückzuführen sein. In dieser Arbeit ergeben sich keine Hinweise, dass die Reduktion des Blattwachstums unter Salinität hauptsächlich auf ein Wasserdefizit zurückzuführen ist.
1

General Introduction

1.1 Salinity as Natural Environmental and Man-made Factors

Salinity is one of the major problems in the world. In principle, elevated salinity in soils results mainly from two sources: natural and man-made. Salinity in arid and semi-arid areas is mainly caused by natural causes: low precipitation, high level of evaporation, and existence of saline parent rock (Bresler et al., 1982). However, salinity also results from: mismanaged amelioration systems, irrigation with salinized water, and salt accumulation from high doses of mineral fertilization (Bresler et al., 1982).

1.2 Salinity in Agriculture

Overall estimates of the world's demand for food suggest a growth of 3-4% per annum (Toenniessen, 1984). There are two ways in which such a demand for increased food production might be achieved: an increase in the area under production or an increase in the productivity per unit area. The former depends on the availability of land, the latter on the interaction between yield and resource inputs. Up to the middle of this century, most of the rises in food production resulted from an increase in the area under cultivation. From 1950 to the present, however, the rise has been increasingly due to greater yields per unit area farmed (Brown, 1984).

Recent figures, however, cause concern; the rate of production was lower in the 1970s as compared with the 1960s due to the smaller harvested area (Flowers, 1994) mainly as a result of loss of cultivated land, e.g. land degradation through salinization, erosion, and loss of fertility. Of the world's $1.5 \times 10^9$ ha cultivated land, 23% is considered saline in more than 100 countries (Tanji, 1990; Szabolcs, 1989). The economic importance of salinity is strongly substantiated by the dangerous trend of a 10% per year increase in the saline area throughout the world (Ponnamieruma, 1984).
Soil scientists have devised many reclamation methods and management practices to reduce salt stress (U.S. Salinity Laboratory Staff, 1954). For reclamation of saline soils, leaching of surface salts has been widely recommended because of normal permeability of those soils; the salts are usually leached below the root zone whenever the amount of water infiltrated exceeds that lost by evapotranspiration. In contrast, in arid and semi-arid regions where rainfall is low and irrigation water is saline, it is difficult to achieve adequate leaching (Ashraf, 1994).

Numerous studies have confirmed that fertilization management of saline soils plays a vital role in agricultural economics (Ravikovitch and Yoles, 1971; Bernstein et al., 1974; Feigin, 1985; Kafkafi et al., 1982; Papadopoulos and Rendig, 1983). Addition of nutrients resulted in enhancing, decreasing, or no changes in plant salt tolerance, depending on the level of salt stress. In many cases, salinity-to-fertility relationships may be summarized as follows: (1) under low salt stress, nutrient deficiency limits plant growth to a greater extent than salinity; a positive interaction or increased salt-tolerance results; (2) under moderate salinity, nutrient deficiency and salinity may equally limit plant growth, and no interaction occurs; and (3) under high salinity, salinity limits growth to a greater extent than nutrient deficiency. Therefore, plant performance would always exhibit a negative interaction or decreased salt-tolerance response (Bernstein et al., 1974; Feigin, 1985; Grattan and Grieve, 1992). It is anticipated that experiments will show that, under conditions of different nutrient status (concentration and composition), plant response to salt stress may change.

1.3 Mechanisms of Salt Limitation to Plant Growth

Under saline conditions, soils contain extreme ratios of Na+/Ca2+, Na+/K+, Ca2+/Mg2+, and Cl−/NO3−. The growth inhibition due to salinity may be caused primarily by the osmotic and/or ionic components of salinity, acting on biophysical and/or metabolic components of expansive growth (Thiel et al., 1988).

Adverse Effects of Salinity on Growth due to Osmotic Effects

Under saline conditions, the low osmotic potentials of soil solutions induce water deficit in plant tissue. As a consequence, cell turgor pressure decreases. Because the growth of cells is correlated with turgor pressure, decreased turgor is the major cause of inhibition of plant growth under saline conditions (Greenway and Munns, 1980). Osmotic adjustment under salt stress may occur due to ion uptake from the soil solution or by internal synthesis of organic solutes. The production of sufficient osmotica is metabolically expensive, potentially limiting the plant by consuming significant quantities of carbon that could otherwise be used for growth (Greenway and Munns, 1980). The alternative to producing organic osmotica is to accumulate a sufficiently high concentration of ions from the external medium. The energetic cost of osmotic adjustment by inorganic ions is much lower than that conferred by organic molecules synthesized in the cell (Wyn Jones, 1981; Yeo, 1983). However, this causes another problem in that such high concentrations of toxic ions may interfere with normal biochemical activities within the cell (Poljakoff-Mayber, 1975).
Ionic Effect of Salinity on Plant Growth

Accumulation of Na\(^+\) and Cl\(^-\) in the leaves, through the transpiration flow, is generally a long-term process occurring in salt-stressed plants (Munns and Termaat, 1986). High internal concentrations of Na\(^+\) and Cl\(^-\) may provide toxic ions in the cellular compartment (Greenway and Munns, 1980). Plant growth is affected by the interactions of Na\(^+\) or Cl\(^-\) and many mineral nutrients, causing imbalances in the nutrient availability, uptake, or distribution within plants and also increasing the plant's requirements for essential elements (Grattan and Grieve, 1992). For example, high concentrations of Na\(^+\) in the external solution caused decreases in K\(^+\) and Ca\(^{2+}\) concentrations in the tissues of many plant species (Greenway and Munns, 1980; Rathert, 1983). The decrease may be due to the antagonism between Na\(^+\) and K\(^+\) or Ca\(^{2+}\) at sites of uptake in roots, to the effect of Na\(^+\) on the K\(^+\) and Ca\(^{2+}\) transport into the xylem (Lynch and Läuchli, 1984; 1985), or to indirect inhibition of the uptake process in other aspects, for example, H\(^+\)-ATPase activity (Gronwald et al., 1990; Suhayda et al., 1990). Because Ca\(^{2+}\) is essential for maintaining selectivity and integrity of cell membranes (Epstein, 1972; Fageria, 1983), the deficiency of Ca\(^{2+}\) could impair both the selectivity and the integrity of the membrane and then accelerate the passive accumulation of Na\(^+\) in plant tissues.

Effect of Salinity on Photosynthesis

At low or moderate soil salinity, decreased growth is primarily associated with a reduction in photosynthetic area rather than a reduction in photosynthesis per unit leaf area (Munns, 1993). At high salinity, however, leaf photosynthesis can be reduced by lowered stomatal conductance as a result of water imbalance (Brugnoli and Lauteri, 1991) or by a change in the ionic relations of the chloroplasts (Long and Baker, 1986). In addition, the transport of photosynthates in the phloem may be inhibited (Iyengar and Reddy, 1994). Thus, the amount of photosynthates reaching the growing region may decrease. The tolerance of photosynthesis systems to salinity is associated with a capacity to control ion distribution into the vacuole, away from cytoplasm and chloroplasts. In salt-sensitive species, this mechanism may break down at high salinity levels when the membrane function is affected (Noble and Rogers, 1994).

Adverse Effect of Salinity on Rheological Properties of the Cell Wall

Growth is cell enlargement, with water absorption and irreversible (plastic) cell wall expansion being critical components of the process. According to the biophysical model of leaf elongation (Lockhart, 1965; Boyer, 1985; Cosgrove, 1986), the rate of leaf elongation (r) is regulated or controlled by alterations in any of several parameters: cell wall extensibility (\(\phi\)), turgor pressure (P), and yield threshold (Y). This relationship of the parameters may be expressed as: \(r = \phi (P - Y)\) (Lockhart, 1965). From this equation, the limitation of growth by salinity is probably due to a decrease in wall extensibility and/or to an increase in yield threshold. The decrease in cell wall extensibility (\(\phi\)) has been reported for maize leaves (Cramer and Bowman, 1992; Neumann, 1993) after long-term salt stress. Several reports suggested that the yield
threshold (Y) of growing leaf tissues may increase in response to osmotic stress or water stress (Hsiao and Jing, 1987; Randall and Sinclair, 1989; Pritchard et al., 1991; Cramer and Bowman, 1992; Neumann et al., 1994), resulting in a decrease in cell elongation.

Enzyme Activity

Most research on the effect of salinity on enzyme activity and protein metabolism has been performed in vitro (Noble and Rogers, 1994). The situation in vivo has been more difficult to determine, and changes in enzyme activity may be attributed to a water stress effect rather than to a toxic ion effect (Winter, 1973). Salinity interferes with protein synthesis and inhibits amino acid incorporation into proteins (Blume, 1988).

Hormone Balance

Discussion of effects of salinity on the role of hormones can be found elsewhere (Jennings, 1976; Munns and Termaat, 1986; Blume, 1988; Pitman et al., 1974). There is little evidence that NaCl directly affects the hormone balance within the plant, and the greatest change in hormone levels caused by saline conditions results from water stress (Jennings, 1976; Blume, 1988).

On the whole, we do not know, however, whether water deficit, specific ion toxicity, nutrient imbalance, cell biophysical properties, or another process is mainly responsible for growth inhibition induced by salt stress and whether the site of action is located at expanded or expanding cells, especially for leaf tissues in wheat plants.

1.4 Response of Wheat to Salinity

Importance of Wheat Production

Plants constitute 93% of the world's diet. Cereals contribute two thirds and wheat is the largest source of cereal nutrition (Hanson et al., 1982). Wheat is important because (i) it is grown on 240 million hectares, an area greater than that of any other crop; (ii) it is the greater source of calories and protein than any other food crop; and (iii) world trade in wheat exceeds trade in all other grains combined (Hanson et al., 1982).

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 to be moderately tolerant to salinity.

Whole-plant Response to Salinity

The effect of salinity on the growth of wheat plants has been reported in a number of studies (Delane et al., 1982; Francois et al., 1986; Maas and Poss, 1989). Salt sensitivity of wheat plants varies with growth stage (Maas and Poss, 1989). The seedling or early vegetative stage
appears to be the most sensitive, with subsequent stages showing increased tolerance (Francois and Maas, 1994). Since the life cycle of wheat is an orderly sequence of development stages, salt stress can have a significant effect on the developmental processes which occur at a particular time. The sequence of events has been separated into three distinct but continuous developmental phases (Francois and Maas, 1994). In the first phase, which encompasses the early vegetative growth stage, leaf and spikelet primordia are initiated, leaf growth occurs, and tiller buds are produced. High soil salinity at this time reduces the number of leaves per culm, number of spikelets per spike, and the number of tillers per plant (Maas and Grieve, 1990). The differentiation of the terminal spikelet signals the completion of this phase. During the second phase, the tillers grow, the main stem and tiller culms elongate, and the final number of florets is set (Kirby, 1988). Salinity stress during this phase may affect tiller survival and reduce the number of functional florets per spikelet. This phase ends with anthesis. Fertilization and grain filling occur during the final phase (Kirby, 1988) when salinity affects seed number and seed size. In general, however, the effect of salinity on spikelet and tiller number established during the first phase has a greater influence on final seed yield than the effects exerted on yield components in the latter two phases (Maas and Poss, 1989).

Salinity significantly alters the pattern of grain distribution along the spike and within the spikelets (Shannon et al., 1994). The spikes on the main stem of salt-stressed plants are shorter, fewer spikelets are produced, and the number of kernels per spike is reduced (Maas and Grieve, 1990). Depending on the level of salinity, the weight of individual kernels may increase. Thus, yield of the main stem may not change or may even increase in response to salinity, since the decrease in kernel number may be completely compensated by an increase in kernel weight (Shannon et al., 1994).

However, no information is available on the interactive effects of salinity and full macronutrients on the morphological parameters (plant height, leaf number, and tiller number), yield components (dry weight of leaves, stems, grains, and above ground plant part), and yield of the main stem (spike length, spikelet number, and kernel number per spikelet) of spring wheat.

1.5 Leaf Growth in Response to Salinity

As mentioned above, the early vegetative stage in wheat plants is more sensitive to salinity than other stages (Arif and Tomos, 1993; Munns and Termaat, 1986; Maas and Poss, 1989). Furthermore, vegetative growth of wheat plants is characterized by the sequential appearance and elongation of leaves on tillers. Therefore, it is necessary to characterize leaf growth and its response to salinity.

The reduction in the final length of the leaves of wheat plants can result from a reduced leaf elongation rate (LER) and/or a shortened elongation period. Under given conditions, leaf elongation rate also varies with leaf age and daily cycles. Different rates of leaf elongation may characterize four distinct stages in the development of grass leaves (Skinner and Nelson, 1995). Initial elongation is characterized by the production of new and uniformly sized cells which continue to divide until the epidermal cell division zone has been established. Once the division zone is established, an increase in the elongation rate is observed, and the leaf reaches its maximum relative LER. Following establishment of the entire elongation zone, the leaf enters a
period of linear growth characterized by high absolute LER. As the supply of new cells for leaf growth stops and older cells reach their final length, the elongation zone shrinks, and the decreasing stage of elongation occurs. Kinetic studies on LER for wheat (Christ, 1978a, b), for tall fescue (Parrish and Wolf, 1983; Schnyder and Nelson, 1988), and for Lolium temulentum (Thomas and Stoddart, 1984) have also shown a distinct diurnal rhythm. Under saline conditions, however, no information is available on the kinetics of leaf growth from leaf emergence to the end of leaf growth and on diurnal growth pattern of leaf elongation.

Leaf elongation in grasses is restricted to a small region at the base of the blade enclosed by older leaves (Kemp, 1980). Although the elongation zone is enclosed, grass leaves present a good opportunity to study leaf growth processes, because the growing zone is so distinct and relatively simply organized (Schnyder and Nelson, 1988). Elongation is largely unidirectional, and a cellular particle is displaced away from the leaf base as a result of production of younger tissue and longitudinal growth. Moreover, elongation is a dynamic process and not uniformly distributed throughout the elongation zone of the leaf, i.e. the leaf elongation rate is spatially distributed along the leaf axis. Previous studies have shown that the spatial distribution of leaf growth is influenced by genotype for tall fescue (Volenc and Nelson, 1981), developmental stage for maize (Meiri et al., 1992), light for tall fescue (Schnyder and Nelson, 1988), and salinity for sorghum (Bernstein et al., 1994). Therefore, it is important to investigate the spatial distribution of leaf growth in wheat plants under saline conditions. Furthermore, the understanding of physiological processes of leaf growth requires knowledge of the spatial distribution of water content, ions, carbohydrates, and their accumulation rate in growing leaves under saline conditions.

1.6 Local Net Deposition Rates in Growing Leaves

The volume of plant cells in growing leaves increases because of water uptake and/or biosynthetic production. To find the rates at which substances are produced and transported it is necessary to characterize the spatial and temporal patterns of both growth and the density of substance of interest (Silk, 1984). A particularly useful formulation from hydrodynamics is the continuity equation. The continuity equation can be expressed for spatial location to determine local biosynthesis or transport rates in the growing leaves (Silk, 1984):

\[
D = (\frac{\partial P}{\partial t}) + DV \cdot (\frac{\partial P}{\partial x}) + (SER \cdot P) \tag{1}
\]

where D is the local net deposition rate (resulting from biosynthesis and/or transport rate). The local net deposition rate (D) may be viewed as quantitative picture of sink and source relationships. P is substance density, t is time, and x is the distance from the ligule of the leaf blade. DV and SER are the displacement velocity of a segment and the segmental elongation rate.

On the right side of the continuity equation [1], the first term (I), \(\frac{\partial P}{\partial t}\), represents the local rate of change (time rate change in substance content at a fixed distance from the ligule).

The second term (II), \(DV \cdot (\frac{\partial P}{\partial x})\), of the Eq. [1] is called "convective rate of change" which represents the change due to movement of cells away from the leaf base and can be considered as the deposition rate needed to maintain any spatial gradient in density (Silk et al.,...
The third term \((\text{III}), (\text{SER} \cdot P)\), on the right 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).

### 1.7 Aims of the Thesis

Objectives of this study were: (1) to obtain fundamental information on improved fertility management under saline conditions as a means of increasing salt tolerance of wheat plants; (2) to identify interactive effects of salinity and macronutrients on the mineral element contents in leaves, stems and grains of spring wheat; (3) to quantitatively evaluate the spatial distribution of growth, ion concentrations, and carbohydrate status and their net deposition rates in the elongating and mature zones of wheat leaf 4 of the main stem during its steady growth under saline soil conditions; and (4) to enhance the understanding of the mechanisms limiting leaf growth of wheat plants under saline conditions.

### 1.8 Outline of the Thesis

The studies are divided into two parts: I. Interactive effects of salinity and macronutrients on growth, yield, and mineral contents of wheat plants under hydroponic conditions and II. Spatial and temporal distributions of growth, mineral elements, and carbohydrate status in wheat leaves under saline soil conditions.

In the first part, the plants were grown in hydroponics with three levels of macronutrients and eight salt levels ranging from 0 to 150 mM NaCl. The parameters of plant growth, yield, parameters of the main spike, and water relations of leaves were determined, and the interactive effects of salinity and macronutrient concentration on the above parameters are discussed (Chapter 2). Chapter 3 presents the interactive effects of salinity and macronutrients on the mineral element contents such as \(\text{Na}^+, \text{Cl}^-, \text{K}^+, \text{Ca}^{2+}, \text{Mg}^{2+}, \text{NO}_3^-,\) total nitrogen, and \(P\) in various tissues of wheat plants.

In the second part, the experiments were conducted in saline soils mixed with 1 strength Hoagland nutrient solution and with two or four salt levels ranging from 0 to 120 mM NaCl during the vegetative stage of wheat plants. The effects of salinity on the growth of wheat seedlings, leaf water relations, and the leaf elongation rate at the whole leaf level were measured by the linear variable differential transformers (LVDT); spatial distributions of leaf growth along the leaf axis were measured by puncturing methods which are described in Chapter 4. Chapters 5 to 7 present spatial and temporal distributions of dry weight and water, mineral elements, and carbohydrate components in wheat leaves. The continuity equation was used to calculate the net deposition rate of substances such as fresh weight, dry weight, mineral elements, and carbohydrate components in the elongating leaves of wheat plants. In Chapter 8, the spatial distribution of osmotica, inorganic and organic solutes, and the inorganic and organic solutes contributing to osmotic adjustment under saline soil conditions are presented and the limiting effect of salinity on growth due to a deficit of water or to other factors is discussed. The general discussion is
presented in the final Chapter 9.

1.9 References


Interactive Effects of Salinity and Macronutrients on Growth, Yield, and Mineral Element Contents of Wheat Plants Grown in Hydroponics
Leer - Vide - Empty
Interactive Effects of Salinity and Macronutrient Level on Growth and Yield of Spring Wheat

Abstract Plant growth response to salinity is known to change under different fertility levels. The objective of this study was to investigate interactive effects of salinity and macronutrient level on growth and yield of spring wheat (Triticum aestivum L. cv. Lona), grown in hydroponic culture in growth chambers until grain maturity. Eight salinity levels, 0 to 150 mM NaCl, were established and 1, 0.2, and 0.04 strength Hoagland macronutrient solution (x HS) were taken as the levels of nutrient supply. Leaf number, tiller number, main stem height, yield components of the main spike, and dry weight of leaves, stems, grains, and above-ground plant parts were determined.

Only small reductions in the grain yield of the main spike were found at 1 and 0.2 x HS with low and medium salinity (0-40 and 40-100 mM NaCl, respectively). Large reductions in the grain yield of the main stem were found at either 0.04 x HS or at high levels of salinity (125-150 mM NaCl), more marked at 0.2 than at 1 x HS. In contrast to the main spike grain yield, which was only slightly affected, increasing salinity strongly decreased plant yield components linearly. The salinity level associated with a 50% grain reduction was about 84, 72, and 31 mM NaCl for 1, 0.2, and 0.04 x HS, respectively. At all investigated macronutrient levels, plant grain yield reductions were mainly and closely related to decreased leaf and tiller numbers. This suggests that salinity exerts its main effects during the early growth stages. The most promising strategies for increasing wheat yields in saline soils will be (i) increasing nutrient supply in nutrient poor soils, (ii) creating favourable conditions in the root zone during germination, seedling stage, and early tillering by salt elimination, and (iii) increasing main stem population density by increasing seed rate. The second and third measures will increase yields to a greater extent at moderate and sufficient nutrient levels than additional supplies of nutrients. In conclusion, salt tolerance of wheat was significantly increased by increasing the macronutrient concentration at low macronutrient level (0.04 x HS) and moderately increased at the intermediate macronutrient level (0.2 x HS) with high salinity (100-150 mM NaCl).
2.1 Introduction

Under saline conditions, characterized by low nutrient activities and high ratios of Na⁺/Ca²⁺, Na⁺/K⁺, Ca²⁺/Mg²⁺, and Cl⁻/NO₃⁻ in the root medium, nutritional disorders and osmotic effects develop and plant growth may be hindered. Nutrient imbalances may result from the effect of nutrient availability, competition or distribution of ions within the plant or may be caused by an increase in the plant's requirement for essential elements (Grattan and Grieve, 1992).

Numerous studies have confirmed that fertilization management of saline soils plays a vital role in agricultural economics (Ravikovitch and Yoles, 1971; Bernstein et al., 1974; Feigin, 1985; Kafkafi et al., 1982; Papadopoulos and Rendig, 1983). Addition of nutrients resulted in either enhancing, decreasing, or in no changes in plant salt tolerance, depending on the level of salt stress. In many cases, salinity-to-fertility relationships can be summarized as follows: 1) under low salt stress, nutrient deficiency limits plant growth more than salinity, and a positive interaction or increased salt-tolerance response occurs, 2) under moderate salinity, nutrient deficiency and salinity may equally limit plant growth, and no interaction occurs, and 3) under high salinity, salinity limits growth to a greater extent than nutrient deficiency. Plant response in relation to the concentration of an essential nutrient in the root medium has often been described, whereas studies on the interactive effects of salinity and nutrients on the ionic relations in plants were concerned only with individual or with two nutrients. Furthermore, plants may not exhibit the same function under saline conditions as under non-saline conditions (Grattan and Grieve, 1992). It is anticipated that, under different nutrient status (concentration and composition), plant response to salt stress may change; experimental evidence is still lacking.

The objective of this study was to obtain information on the interactive effects of salinity and macronutrient level on spring wheat, a nonhalophytic crop. The plants were grown in saline solution culture (0 to 150 mM NaCl) and three levels of all major nutrients whose composition was based on Hoagland's solution (1.0, 0.2, and 0.04 strength Hoagland macronutrients (x HS)). Morphological parameters (plant height, leaf number, and tiller number), yield components (dry weight of leaves, stems, grains, and above-ground plant part) of spring wheat were investigated, and the salt tolerance, as affected by the macronutrient concentration, was identified.

2.2 Materials and Methods

2.2.1 Plant Growth

Seeds of spring wheat (Triticum aestivum L. cv. Lona) were germinated on quartz:soil (2:1) for seven days, and then four seedlings were transplanted to polyethylene containers filled with 30 litres of nutrient solution. The experiment was conducted in growth chambers with a photoperiod of 16 h/day. The light intensity was approximately 450-500 μmol m⁻² s⁻¹ (PPFD) provided by a mixture of 160 watt Sylvania cool white fluorescent and 60 watt Tungsram E27 standard tungsten lamps. The air temperature was 23/13°C (day/night), and the relative humidity was maintained at 55-65%.

Eight salinity levels were established (0, 20, 40, 60, 80, 100, 125, and 150 mM NaCl). The
levels of macronutrients were 1, 0.2, and 0.04 x HS (Table 2.1). Micronutrients were kept at 0.5 strength as recommended by Epstein (1972) for each of the treatment solutions. All treatments were replicated twice. In order to avoid an osmotic shock to plants, salinity was gradually increased in nutrient solutions except at the level of 20 mM NaCl. A final NaCl concentration (150 mM) was reached 17 days after transplanting. Throughout the course of the experiment, the concentration of macroelements was monitored and maintained by adding nutrients or by changing solutions. If necessary, adding nutrients and exchanging solutions were made daily.

Table 2.1 Strength of modified Hoagland macronutrient solution (HS) used to evaluate the effects of macronutrient levels and salinity on growth of spring wheat.

<table>
<thead>
<tr>
<th>Elements</th>
<th>1 x HS</th>
<th>0.2 x HS</th>
<th>0.04 x HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>15.0</td>
<td>3.00</td>
<td>0.60</td>
</tr>
<tr>
<td>P</td>
<td>1.00</td>
<td>0.20</td>
<td>0.04</td>
</tr>
<tr>
<td>K</td>
<td>6.05</td>
<td>1.21</td>
<td>0.24</td>
</tr>
<tr>
<td>Ca</td>
<td>5.00</td>
<td>1.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Mg</td>
<td>2.00</td>
<td>0.40</td>
<td>0.08</td>
</tr>
<tr>
<td>S</td>
<td>2.00</td>
<td>0.50</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The number of leaves and tillers and the height of the main stem were recorded weekly. The leaf water status was determined on days 35 and 70 after transplanting. Water and osmotic potentials from the middle of the second youngest fully developed leaf blades were measured with a pressure bomb (PMS Instrument Co., Model 1002, Corvalis Co., Oregon, USA) (Scholander et al., 1965) and vapour pressure osmometer (Wescor 5100C, Wescor Inc., Logan, USA), respectively. Two plants from each treatment were used for measuring the leaf water status. After measuring the water potential (Ψ), the same leaf was divided into two parts and the osmotic potential (Ψ_o) and relative water content (RWC) were measured. Turgor pressure (P) was calculated according to the equation: P = Ψ - Ψ_o; RWC was calculated from fresh weight (FW) and dry weight (DW) of leaves with the equation: RWC (%) = (FW-DW) x 100 / FW.

Grain maturity was visually estimated according to the complete loss of green colour from the grumes. At grain maturity, plants were harvested and separated into leaves, stems, roots, and ears. Main spikes were separated from the other spikes of plants, dissected, and examined in detail. Each fresh fraction was weighed and then dried at 105°C for one hour and then at 65°C for 48 hours. The dry samples were weighed. Ears were threshed, and the grains were redried at 65°C for 24 hours. The chaff weight and thousand grain weight were determined.

### 2.2.2 Statistics Analysis

Data were analyzed for the correlations between yield parameters of main spike and whole plants and salinity. Linear regression analysis was used to evaluate the salt tolerance of wheat with 1,
0.2, and 0.04 x HS. Data were also analyzed by analysis of variance (ANOVA) to test for the significance of main effects and interactions. Terms were considered significant at P=0.05.

2.3 Results and Discussion

2.3.1 Morphology

Visual observations at early growth stages already revealed signs of injury like chlorosis, necrosis, and burning of the leaf margin, in all salt stressed plants; these injuries were severe at high salt concentrations. The injuries also appeared to be greater in salinized plants provided with low macronutrient concentrations. All plants receiving 100 mM NaCl or higher salt concentrations with 0.04 x HS died within 25 days after transplantation, indicating that an increase in the macronutrient level may increase the tolerance of plants to salinity.

For the early diagnosis of crop salt tolerance easily assessible morphological parameters of plant growth, i.e. leaf number, tiller number, and main stem height of wheat plants, are chosen. The leaf number, tiller number, and height of the main stem of spring wheat were significantly reduced when salt concentration increased (Fig. 2.1). Increasing macronutrient concentration improved the growth of spring wheat. On day 7 after transplantation, leaf number and tiller number of plants provided with 0.04 x HS were affected by salinity; this occurred after only 21 days for plants with 1 and 0.2 x HS (data not shown). With time, numbers of leaves and tillers were more strongly reduced at high salinity levels for plants with 0.2 x HS than with 1 x HS. Results in Figure 2.1 show that the reduction in leaf number was closely related to the reduction in tiller number. Twenty-one days after transplantation, leaf number of plants with 150 mM NaCl was decreased by 61, 48, and 95% for 1, 0.2, and 0.04 x HS, respectively, compared with the non-salinized treatments; tiller number of plants with 150 mM NaCl was reduced by 49, 47, and 83% for 1, 0.2, and 0.04 x HS, respectively (Fig. 2.1). At the high and medium macronutrient levels, the numbers of leaves and tillers differed only slightly among salinity levels before 21 days, since the salt level of 150 mM NaCl was reached only at day 17 after transplantation. These observations suggest that salinity exerts significant effects during the early stages, while enhanced fertilization considerably inhibited the deleterious impact of salinity. Similarly, the height of plants with high salt concentrations and lower macronutrient concentrations decreased most (Fig. 2.1), but the obvious changes in height appeared during the reproductive stages. 150 mM NaCl reduced the height of spring wheat at the final harvest from 79 to 55 cm in plants provided with 1 x HS, while the height of plants receiving 0.2 and 0.04 x HS decreased from 79 to 49 cm and 71 to 1 cm, respectively.

2.3.2 Growth of Main Stem

Growth stages: No effect on the growth stages of plants treated with different concentrations of NaCl and macronutrient concentrations were observed until tillering which occurred on the fifth day after transplantation (Table 2.2). Ear emergence and anthesis stage were observed 43 and
Fig. 2.1 Interactive effects of salinity and macronutrient level on leaf number, tiller number, and height of main stem of wheat plants at various intervals after transplantation. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.
Table 2.2 Interactive effects of salinity and macronutrient levels on the growth stages of spring wheat grown in hydroponics.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth stage</th>
<th>Time of harvesting</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS¹ NaCl</td>
<td></td>
<td>Tillering</td>
</tr>
<tr>
<td>mM</td>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>1.0 0</td>
<td>--------------</td>
<td>5</td>
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<tr>
<td>20</td>
<td>5</td>
<td>42</td>
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<td>40</td>
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<td>60</td>
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<td>80</td>
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<td>39</td>
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<td>100</td>
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<td>125</td>
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<tr>
<td>150</td>
<td>5</td>
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<tr>
<td>0.2 0</td>
<td>5</td>
<td>43</td>
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<tr>
<td>20</td>
<td>5</td>
<td>41</td>
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<td>125</td>
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<tr>
<td>150</td>
<td>5</td>
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</tr>
</tbody>
</table>

¹ Strength of Hoagland macronutrient solution (HS)

49 days after transplantation under non-salinity with 1 x HS, while under 150 mM NaCl, ear emergence and flowering occurred 39 and 43 days after transplantation (Table 2.2). There were similar changes in the growth stages of spring wheat at low and medium salinity levels with 0.2 and 1 x HS, while at 0.04 x HS the growth stages were accelerated with increasing salinity (Table 2.2). A shorter growth period under stress conditions may be one of the reasons for the reduction in the plant yield. Francois et al. (1986) found that, under optimum macronutrient conditions, flowering of wheat occurred approximately 10 days earlier in the high salt treatment than in the control treatment. Cerda and Bingham (1978) also reported that ear emergence and grain maturity were accelerated at high salinity and low P level. The results of the work presented here support the findings that salinity and low macronutrient levels accelerate ear emergence, flowering, and grain maturity.
Leaf water relations: Results in Figure 2.2 show that RWC on days 35 and 70 after transplantation slightly decreased with increasing salt concentration and, to a lesser degree, with time. There was no significant difference in RWC among 1, 0.2, and 0.04 x HS except on day 70 at 0 mM NaCl.

![Graph showing relative water content (RWC) of leaves of wheat plants at various intervals after transplantation. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.](image)

Fig. 2.2 Interactive effects of salinity and macronutrient level on the relative water content (RWC) of the leaves of wheat plants at various intervals after transplantation. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

Figure 2.3 shows the results of the interactive effects of salinity and macronutrients on $\Psi$ and $\Psi_s$ 35 and 70 days after transplantation. At all three macronutrient levels, $\Psi$ and $\Psi_s$ were highest in plants grown in lower salt concentrations. Increasing salt concentration decreased both the water potential and the osmotic potential of the leaf. Although $\Psi$ in the root medium was lower with 1 x HS than with 0.2 and 0.04 x HS (data not shown), there were no great differences in $\Psi$ and $\Psi_s$ among 1, 0.2, and 0.04 x HS 35 days after transplantation. On day 35, for instance, $\Psi$ at 40 mM NaCl was -0.36, -0.32, and -0.31 MPa with 1, 0.2, and 0.04 x HS, respectively; $\Psi_s$ was -1.1, -1.0, and -1.1 MPa with 1, 0.2, and 0.04 x HS, respectively. Hoffman and Rawlins (1971) reported that plant age could significantly affect $\Psi$ and $\Psi_s$ in root crops. In this study, $\Psi$ and $\Psi_s$ were measured at the vegetative and reproductive stages. The results show that $\Psi_s$ and $\Psi$ decreased as plants aged (Fig. 2.3).

Figure 2.3 shows that turgor pressure increased slightly with increasing salinity regardless of the macronutrient level. On the whole, turgor pressure increased with time under salt stress, while under non-salinity, turgor pressure remained similar at 1 and 0.2 x HS and decreased at 0.04 x HS. Greenway and Munns (1980) proposed that the reduction in plant growth is due to the decline in turgor pressure with increasing salt stress. In contrast, Munns (1988) reported that there was no relationship between turgor pressure and plant growth under salinity. In this study, the reduction in plant growth under saline conditions is probably not due to the limitation of turgor.
Main spike yield parameters: The main spike of wheat develops through an orderly series of morphogenic events. These events determine, in sequence, the three yield components of the main spike: the number of spikelets, the number of kernels per spikelet, and the dry weight of individual kernels. Spikelet number is determined prior to differentiation of the terminal spikelet. Kernel number is determined during the period of spike emergence to anthesis and maturity (Bingham, 1969). Data in Figure 2.4 show that the effect of salinity on the components of the yield of the main spike was stronger at 0.2 and 0.04 x HS than at 1 x HS. At all three macronutrient levels, spikelet number, kernel number, and total kernel weight per spike responded more strongly to salinity than did spike length and individual kernel weight (Fig. 2.4). Spike length was 10.4 cm at 0 mM NaCl and 9.5 cm at 150 mM NaCl with 1 x HS; and 10 and 8.7 cm with 0.2 x HS. At 150 mM NaCl, the spikelet number of the main spike was reduced by 15.6, 28.3, and 100% for plants provided with 1, 0.2, and 0.04 x HS, respectively. The decrease in kernel number per spike was consistent and significant as salt stress increased from 125 to 150, 100 to 150, and 20 to 60 mM NaCl for plants grown at 1, 0.2, and 0.04 x HS, respectively. Total kernel dry weight of plants grown at 150 mM NaCl with 1, 0.2, and 0.04 x HS was decreased by 35.8, 65.8, and 100% as compared with the control treatment, whereas the thousand kernel weight was reduced by 15.7, 28.4, and 100%, respectively.

Generally, conditions such as high temperature, long days, and water deficit that reduce spikelet number also reduce floret number and grain set per spikelet. Salinity, in common with other stress factors, limits spikelet number (Grieve et al., 1992). However, the difference in spikelet number depends on the level of stress and nutrients. The small reduction in grain yield of the main spike (Fig. 2.4) under high and medium (1 and 0.2 x HS) nutrient supply at low and medium salinity (0-40 and 40-100 mM NaCl) was mainly due to the effect of salinity on spikelet number.
Fig. 2.4 Interactive effects of salinity and macronutrient level on the main spike parameters (spikelet number, kernel number per spikelet, kernel numbers, spike length, thousand grain weight, and grain dry weight) of wheat plants. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.
number. In contrast, stronger reductions in grain yield, found at the low macronutrient level (0.04 x HS) and at high salinity (100-150 mM NaCl), were primarily caused by decreased kernel number per spikelet and decreased thousand grain weight. Therefore, in the latter case, the development of the main spike was also affected from spike emergence to anthesis and maturity.

2.3.3 Plant Yield

The average dry weights of leaves, stems, grains, and above-ground plant parts and spikes per plant are presented in Figure 2.5. Above-ground dry weight is defined as the sum of leaf, stem, chaff, and grain dry weight. The analysis of the correlations between yield parameters and salinity demonstrated a consistent reduction in all yield components with increasing salinity (data not shown). This reduction was partly counteracted in plants provided with high macronutrient levels. Analysis of variance showed that there were no significant differences in the yield components between 1 and 0.2 x HS under low and moderate salinity (Fig. 2.5). At 150 mM NaCl, leaf dry weight decreased by 89, 90, and 92% with 1, 0.2, and 0.04 x HS, respectively; stem dry weight was reduced by 88, 92, and 99%, grain dry weight was reduced by 89, 97, and 100% and above-ground plant dry weight was decreased by 89, 95, and 100%, respectively. The salinity level associated with a decrease of 50% grain yield was about 84, 72, and 31 mM NaCl for 1, 0.2, and 0.04 x HS, respectively. These results suggest that improved fertilization management can alleviate growth inhibition due to salinity (Feigin, 1985). Stronger reductions in dry weight of leaves, stems, and grains as well as in the spike number were observed at high salinity levels (100-150 mM NaCl) at the medium macronutrient level (0.2 x HS) as compared with the high macronutrient level (1 x HS) (Fig. 2.5). These reductions were closely related to those in leaf and tiller number (Fig. 2.1).

Thousand grain weight (TGW) decreased only at the highest salinity level regardless of the macronutrient level (Fig. 2.5). There were no significant differences in TGW of spring wheat under low and medium salinity except in TGW at 0.04 x HS. Because kernel number per spike of plants was not markedly reduced by low and medium levels of salinity (data not shown), this indicates that grain yield was reduced mainly by the spike number, i.e. salt stress during early developmental stages affected spike differentiation under low and moderate saline conditions (Maas and Poss, 1989).

Relative yield (% of control) can be used to assess the sensitivity of plants to salt stress. Relative grain yield and relative straw yield (%) (sum of leaves, stems, and chaffs) decreased linearly with increasing salinity (Fig. 2.6). The slope of the line in Figure 2.6 represents the reduction in relative yield % per unit increase in mM NaCl. There were only small differences in the slope between 1 and 0.2 x HS for grain and straw, while the change in macronutrient concentration from 0.2 to 0.04 x HS exhibited a great effect on the slope for grain and straw (Table 2.3). An increase in each unit mM NaCl reduced the relative yield (%) in plants provided with 1, 0.2, and 0.04 x HS by 0.56, 0.68, and 1.89% (Table 2.3). The increase in each unit of NaCl concentration reduced the relative straw yield (%) in plants with 1, 0.2, and 0.04 x HS by 0.56, 0.67, and 1.86%, respectively. The values for grain were about the same as that of straw at the same macronutrient level, indicating that there is a close relationship between grain yield and straw yield.
Fig. 2.5 Interactive effects of salinity and macronutrient level on plant yield parameters (leaf dry weight, stem dry weight, above ground part dry weight, spikes per plant, thousand grain weight, and grain dry weight). Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.
Fig. 2.6 Interactive effects of salinity and macronutrient level on relative grain yield and relative straw yield in % of the control treatment.

Table 2.3 Equation of linear regressions relating salt stress (in mM NaCl) (x) to plant yield (%) of control (y) where $y = a + bx$.

<table>
<thead>
<tr>
<th>Yield components</th>
<th>Treatment</th>
<th>Parameters of regression</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HS$^1$</td>
<td>NaCl (mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain</td>
<td>1.0</td>
<td>0-150</td>
<td>97.2</td>
<td>-0.56</td>
<td>0.95***</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0-150</td>
<td>98.7</td>
<td>-0.68</td>
<td>0.98***</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0-60</td>
<td>110</td>
<td>-1.89</td>
<td>0.88ns</td>
</tr>
<tr>
<td>Straw</td>
<td>1.0</td>
<td>0-150</td>
<td>91.6</td>
<td>-0.56</td>
<td>0.96***</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0-150</td>
<td>98.8</td>
<td>-0.67</td>
<td>0.97***</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0-60</td>
<td>116</td>
<td>-1.86</td>
<td>0.86ns</td>
</tr>
</tbody>
</table>

$^1$ Strength of Hoagland macronutrient solution.

*** represents significant level at $P < 0.001$, ns not significant at $P > 0.05$.

A number of investigators reported various effects on the salt tolerance of plants grown at different macronutrient levels (Bernstein et al., 1974; Feigin, 1985; Oertli, 1991). Data in Table 2.3 indicate that the salt tolerance of spring wheat was greatly enhanced at low macronutrient levels by increasing the macronutrient concentration (salt tolerance at 0.2 x HS was three times higher than at 0.04 x HS (Table 2.3)), whereas it increased only slightly when the macronutrient level was increased from 0.2 x HS to 1 x HS. Although yield and salt tolerance of spring wheat increased with higher macronutrient concentrations, the degree of increase was different at different macronutrient levels, reflecting that the economic optimum should be considered in the fertility management of saline soils.
2.4 Conclusions

A consistent reduction in growth parameters and yield components was found with increasing salinity. The salt tolerance of wheat was significantly increased by increasing the macronutrient concentration under low fertility level and by using a high macronutrient level as compared with a moderate macronutrient level at high salinity levels (100-150 mM NaCl).

There is a close relationship between vegetative growth parameters, i.e. number of leaves and tillers and dry weight of leaves, stems, and grains, indicating that salinity has significant effects during the early growth stages. At later growth stages higher salt levels in the root zone can be tolerated by wheat plants (Maas and Poss, 1989). Growth stages were accelerated by salinity, especially at the low macronutrient level (0.04 x HS).

The grain yield of the main spike decreased much less than the investigated vegetative growth parameters. Under high nutrient supply and low and medium salinity, the reduction in the final grain yield was mainly due to the effect of salinity on the spike number per plant, while at low macronutrient level or high salinity, the grain yield was reduced by a decrease in the spike number and by the effect on the differentiation of spikelet and the development of the spike from spike emergence to anthesis and maturity, indicating that at the low macronutrient levels salt stress during early developmental stages affected spike differentiation under low and moderate saline stress.

Successful management in saline areas must consider the local conditions and the availability of resources. Optimized strategies would relieve the most severe growth-limiting stress. This study shows that, with low fertility, nutrient supply causes a strong increase in yield. Improved yields due to higher macronutrient supply can also be expected in moderately and highly saline soils with below-optimum nutrient levels. However, relieving salinity stress during the early stages of development will be more successful than increasing nutrient supply. If feasible, salt elimination from the root zone at germination, at the seedling stage, and at early tillering will dramatically increase yields of wheat. If this measure is not applicable, increasing seeding densities, to achieve a higher total plant population per area to replace lost tillers with main stems, seems promising (Francois et al., in press). Reduced energy load per plant can reduce the amount of transpired water. Thus, decreasing the accumulation of toxic ions and further reducing evaporation losses due to a higher leaf area index. At nearly sufficient nutrient levels in soils, increased macronutrient supply to saline soils will not improve yields and is not desirable from an economic and environmental point of view (Grattan and Grieve, 1992).

Salinity decreases yield in wheat plants mainly by decreasing the tiller number independent of the nutrient level. The yield of the main stem is only slightly affected, even at high salinity levels. This indicates that, although symptoms of toxicity are manifest, toxicity is not yield limiting, at least not for the main stem. Tillers which are formed later may be subjected for a longer period of time to increased levels of toxic elements, or non-compatible solutes are preferentially allocated to primary or secondary tillers. The main stem may thus avoid the build-up of harmful concentrations of toxic compounds. In contrast, the chance of an increase in build-up of toxic compounds in non-growing tissues increases the longer transpiration continues in the growing season and could negatively affect, e.g. photosynthesis.

Results of this study suggest that turgor does not limit growth. In fact, we do not expect
to find a direct relation between turgor measured in non-growing tissues and the growth process. The latter is controlled by the effective turgor (turgor minus turgor threshold). Hence, we can not exclude the possibility that growth was limited by the decreased osmotic potential in the nutrient solution. Likewise toxicity, however, low osmotic potentials hardly affected the yield of the main spike.

Growth and yield in salinized wheat plants were probably not limited at 0.2 and 1 x HS by nutrients. Several studies also showed only a slight improvement in yield in saline soils as a result of increasing the nutrient level (Feigin, 1985; Grattan and Grieve, 1992). This, however, does not necessarily exclude inadequate nutrition. Even with an increased nutrient level, antagonistic action of certain ions might lead to nutrient imbalances or nutrient deficiencies. Preliminary evidence from studies of wheat leaves indicates that chloride ions inhibit net deposition of nitrate in the growth zone and thus might limit leaf growth (Chapter 6). Tillering is closely related to leaf expansion during early growth and is the main determinant of the number of ears, the component most closely correlated with yield (Roy and Gallagher, 1985). Tillers compete with each other. Generally, tiller roots begin to grow after the tiller has at least two leaves (Rickmann et al., 1985). Before this stage, nutrient elements are provided by older tillers. Therefore, even if nutrients are found in sufficient concentrations in older tillers, this does not necessarily imply that enough resources are allocated to younger tillers. Increased solute accumulation may result from inhibited growth (Munns, 1988) and can easily be misinterpreted. Growth is better described by a kinematic approach than by nutrient concentrations which do not adequately reflect the dynamic behaviour of the growth process (Schmidhalter, 1995). Growth could be limited by inorganic as well as by organic nutrients.

Surprisingly little or no efforts have been made to understand the mechanisms controlling the effect of salinity on the tiller number, a main determinant of yield. Detailed studies of the effects of salinity on leaf emergence and tiller primordia initiation and development will improve substantially our understanding of these processes. Toxicity, osmotic effects, and nutrient imbalances will have to be investigated with respect to their effects on these parameters. Without a doubt, studies will have to concentrate on the early developmental stages of wheat plants. If a wheat plant can manage to develop a few leaves on new tillers, this will probably ensure an increase in yield.

2.5 References


Interactive Effects of Salinity and Macronutrient Level on Ion Contents in Leaves, Stems, and Grains of Spring Wheat

Abstract Results of several studies show interactive effects of salinity and macronutrients on the growth of wheat plants. These effects may be associated with the nutrient status in plant tissues. The objective of this study was to investigate interactive effects of salinity and macronutrients on mineral element contents in leaves, stems, and grains of spring wheat (Triticum aestivum L. cv. Lona), grown in hydroponics, and the relation of these effects to yield components. Eight salinity levels were established from 0 to 150 mM NaCl, and 1, 0.2, and 0.04 strength Hoagland macronutrient solution (x HS) were used as the macronutrient levels. Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, and P in leaves, stems, and grains, NO₃⁻ in leaves and stems, and total nitrogen in grams were determined.

Supplemental Ca²⁺, Mg²⁺, K⁺, and NO₃⁻ added to 0.2 x HS increased mineral contents in leaves and stems but did not improve growth or yield in salinized wheat plants except moderately at 100-150 mM. In contrast, growth or yield was improved significantly when the concentration of macronutrients was increased from 0.04 to 0.2 x HS. In contrast to leaves and stems, mineral contents in grains increased (Na⁺, Cl⁻) or decreased (Ca²⁺, Mg²⁺, K⁺) only slightly or were not affected (K⁺) by salinity except at high salinity and low macronutrient level. Nitrogen and P contents in grains were not affected by salinity. Na⁺ and Cl⁻ contents in leaves and stems increased significantly, whereas K⁺ and NO₃⁻ decreased significantly, with an increase in salinity regardless of the macronutrient level. The latter was also observed for Ca²⁺ and Mg²⁺ in leaves. Extreme Na⁺/Ca²⁺ ratios in plant tissues negatively affected grain yield production at high salinity with medium or high macronutrient levels and at low macronutrient level together with medium salinity. In all other cases, even though strong and significant correlations between mineral content at grain maturity in leaves, stems, and grains and various yield parameters were observed, there is no evidence that toxicity, nutrient imbalance, or nutrient deficiency influenced grain yield to a considerable extent. However, it is possible that tiller number, the main yield determinant in this experiment, was influenced by one of these factors during early growth stages.
3.1 Introduction

Activity and concentration of nutrients in the soil solution are affected by high concentrations of salt ions, usually sodium and chloride, resulting in a nutritional disorder in plants (Grattan and Grieve, 1992). Addition of nutrients can result in an enhancement, a decrease, or no change at all in plant salt tolerance, depending on whether salt or nutrient level is the more limiting factor. When nutrient deficiency limits plant growth more than salinity, for example, an increase in the fertility level enhances the plant’s tolerance. The results reported in Chapter 2 showed that the increase in the macronutrient level from 0.2 to 1 strength Hoagland solution (x HS) did not markedly change the tolerance of wheat plants, whereas there was a significant increase in salt tolerance as macronutrients were increased from 0.04 to 0.2 x HS. Interactive effects of salinity and nutrients on growth may be associated with changes in the nutrient status in plant tissues. There is only little information available on the interactive effects of salinity and nutrient level on the mineral element content in plants. Therefore, this study aimed to identify limiting factors in salt stressed wheat plants, subjected to different macronutrient levels, by analysing mineral contents in various plant tissues and their association with yield parameters.

Accumulation of Na⁺ and Cl⁻ in leaves, through the transpiration flow, is a general, long-term process occurring in salt-stressed plants (Munns and Termaat, 1986). High internal concentrations of Na⁺ and Cl⁻ may provide a means of low energy osmotic adjustment for salt-tolerant plants, which at the same time must be capable of cellular compartmentation of toxic ions (Greenway and Munns, 1980). Plant growth is likely to be affected by the interactions of Na⁺ or Cl⁻ and by many mineral nutrients, causing imbalance in nutrient availability, uptake, or distribution within plants, and also increasing the plant requirements for essential elements (Grattan and Grieve, 1992). For example, high concentrations of Na⁺ in the external solution caused decreases in K⁺ and Ca²⁺ concentrations in the tissue of many plant species (Greenway and Munns, 1980; Rathert, 1983). The decrease could be due to the antagonism of Na⁺ and K⁺ or Ca²⁺ at sites of uptake in roots, to the effect of Na⁺ on the K⁺ and Ca²⁺ transport into xylem (Lynch and Läuchli, 1984) or to the indirect inhibition of uptake processes in other aspects, for example, H⁺-ATPase activity (Gronwald et al., 1990, Suhaya et al., 1990). Because Ca²⁺ is essential for maintaining the selectivity and integrity of the cell membrane (Epstein, 1972; Fageria, 1983), Ca²⁺ deficiency could impair both the selectivity and the integrity of the membrane and accelerate the passive accumulation of Na⁺ in plant tissues. Supplemental Ca²⁺ in the growth medium increased the relative growth rate of barley under saline conditions (Cramer et al., 1990). Similarly, shoot and root growth of rice changed with the alteration of Na⁺/Ca²⁺ and Ca²⁺/K⁺ ratios in the external solution (Muhammed et al., 1987). Previous studies also demonstrated that increased NO₃⁻ levels in the growth medium decreased Cl⁻ uptake and accumulation (Bernstein et al., 1974; Kafkači et al., 1982; Feigin et al., 1987; Martinez and Cerda, 1989). However, most of the studies on the interactive effects of salinity and nutrients on the ionic relations in plants were concerned only with single or with two nutrients.

The objective of this study was to investigate the interactive effect of salinity and macronutrients on the mineral element contents in leaves, stems, and grains of spring wheat, a nonhalophytic crop. The plants were grown in saline solution cultures (0 to 150 mM NaCl) and
with three concentrations of all major nutrients (1, 0.2, and 0.04 strength Hoagland macronutrient solution (x HS)).

3.2 Materials and Methods

3.2.1 Plant Growth

Seeds of spring wheat (Triticum aestivum L. cv. Lona) were germinated on quartz:soil (2:1) for seven days. Four seedlings were transplanted to polyethylene containers filled with 30 litres nutrient solution. The experiment was conducted in growth chambers with a photoperiod of 16 h/day. The light intensity was approximately 450-500 µmol m⁻² s⁻¹ provided by a mixture of 160 watt Sylvania cool white fluorescent and 60 watt Tungsram E27 standard tungsten lamps. The air temperature was 23/13°C (day/night), and the relative humidity was maintained at 55-65%.

Eight salinity levels were established at 0, 20, 40, 60, 80, 100, 125, and 150 mM. 1, 0.2, and 0.04 x HS were used as the macronutrient levels (Table 2.1). Micronutrients were kept at 0.5 strengths as recommended by Epstein (1972) for each treatment solution. All treatments were replicated twice. In order to avoid an osmotic shock to plants, salt concentrations were increased gradually in nutrient solutions except at 20 mM NaCl. A final NaCl concentration (150 mM) was obtained 17 days after transplanting. Throughout the course of the experiment, the concentration of macroelements was monitored periodically and maintained by means of adding nutrients or changing nutrient solutions. If necessary, nutrients were added or solutions were changed daily.

Grain maturity was estimated visually as the complete loss of green colour from the grumes. At grain maturity, plants were harvested and separated into leaves, stems, roots, and ears. Each fresh fraction was weighed. Samples were dried at 105°C for one hour and at 65°C for 48 hours. Dried ears were threshed and then the grains redried at 65°C for 24 hours. Dried samples were weighed and stored for ion analysis.

3.2.2 Analysis of Ion Concentration

Dried flag and second leaves from the top of plant, stems, and grains of each plant were ground with a centrifugal mill (Cyclotec, Sampling mill 1093, Instrumenten-Gesellschaft AG, Zürich, Switzerland) to pass a 0.5-mm diameter sieve. The concentrations of mineral elements in leaves, stems, and grains of spring wheat were measured as follows:

- Na⁺, K⁺, Ca²⁺, Mg²⁺, and P: plant samples (250 mg) were ashed at 560°C for 6 h and were digested with 2 ml of 20% HCl at 60°C for five minutes. The concentrations of Na⁺, K⁺, Ca²⁺, Mg²⁺, and P were determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty. Ltd., Mulgrave Victoria, Australia).

- Cl⁻ and NO₃⁻: 50 mg ground plant samples were extracted with 10 ml distilled water for 15 minutes at 20°C. Within 15 minutes they were shaken twice with a Vortex (Vortex-Genie, K-550-GE, Bender & Hobein AG, Zürich, Switzerland) and then filtered. Chloride was determined using a chloride-selective electrode (Chloride Analyser 926, Corning Ltd., Halstead, Essex, England). NO₃⁻ was analysed with a HPLC detector (LC 75, Perkin-Elmer Co., Norwalk,
Total nitrogen: 6 mg plant samples were weighed with a supermicro-balance (Sartorius, GMBH, Goettingen, Germany). Nitrogen was analysed with a nitrogen analyser (Carlo ERBA Strumentazione, Nitrogen analyser 1500, Cable Erbadas, Milan, Italy).

3.2.3 Statistics Analysis

Data were analysed for the correlations between the mineral element contents in various tissues and the yield parameters of the main spike and whole plants. Data were also analysed by using analysis of variance (ANOVA) to test for significance of main effects and interactions. Terms were considered significant at $P = 0.05$.

3.3 Results

**Sodium:** Salinity increased the content of sodium in leaves, stems, and grains (Fig. 3.1). Na$^+$ content in leaves increased about 50-fold at 150 mM NaCl for plants with 1 and 0.2 x HS and about 90-fold at 60 mM NaCl for the plants with 0.04 x HS as compared with the control treatments. The increase in Na$^+$ content in stems was smaller than in leaves and much lower in grains than in leaves and stems, irrespective of the macronutrient level. Na$^+$ content in grains did not differ significantly ($P<0.05$) up to 125 mM NaCl at 1 and 0.2 x HS and up to 40 mM NaCl at 0.04 x HS. For any given salinity higher than 20 mM NaCl, decreasing the macronutrient concentration in the root medium from 0.2 to 0.04 x HS significantly increased Na$^+$ accumulation in leaves, stems, and grains, while increasing the macronutrient level from 0.2 to 1 x HS did not or only slightly affect Na$^+$ content in leaves, stems, and grains.

**Potassium:** In contrast to Na$^+$, K$^+$ content decreased in leaves and very markedly in stems with increasing salinity (Fig. 3.2). Raising the macronutrient level from 0.04 to 0.2 x HS significantly increased K$^+$ content in leaves and stems under saline conditions. Potassium

![Fig. 3.1 Interactive effects of salinity and macronutrients on the sodium content in leaves, stems, and grains of wheat plants. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.](image-url)
contents in leaves and stems were slightly increased at 1 x HS as compared with 0.2 x HS. No effect of salinity and macronutrient level on K⁺ content in grains was observed (Fig. 3.2). Na⁺/K⁺ ratios increased between 0 and 150 mM NaCl in plants provided with 1 and 0.2 x HS from 0.05 to 3-5, 0.02 to 3-6, and 0.02 to 0.5 in leaves, stems, and grains, respectively. This increase was even more pronounced at 0.04 x HS between 0 and 40 mM NaCl.

**Calcium and Magnesium:** Ca²⁺ content in leaves decreased with increasing salinity and decreasing macronutrient level (Fig. 3.3). Ca²⁺ content in stems was enhanced or unaffected by salinity at 1 x HS and decreased with 0.2 and 0.04 x HS. Ca²⁺ content was higher in leaves than in stems. The decrease in Ca²⁺ content at 0.2 and 0.04 x HS was less pronounced in stems than in leaves. Ca²⁺ contents in grains with 1 and 0.2 x HS were hardly affected by salinity up to 100 mM NaCl. Strong increases in the Na⁺/Ca²⁺ ratio in leaves, stems, and grains were observed only

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**Fig. 3.2** Interactive effects of salinity and macronutrients on the potassium content in leaves, stems, and grains of wheat plants. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

**Fig. 3.3** Interactive effects of salinity and macronutrients on the Calcium content in leaves, stems, and grains of wheat plants. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.
at salt levels higher than 100 mM NaCl at 0.2 x HS and with 20 mM NaCl at 0.04 x HS (data not shown).

Mg$^{2+}$ content in leaves decreased with increasing salinity and decreasing macronutrient level (Fig. 3.4). The behaviour of Mg$^{2+}$ was very similar to that of Ca$^{2+}$ in leaves, stems, and grains.

**Chloride:** Salinity increased Cl$^{-}$ contents in leaves, stems, and grains with increasing salinity (Fig. 3.5). Chloride accumulation was higher in leaves than in stems and grains. Unlike Na$^{+}$, Cl$^{-}$ content was not significantly enhanced at 0.04 x HS at a given salt level as compared with higher macronutrient levels.

**Nitrate:** Inversely, nitrate contents in leaves and stems of plants provided with 1 and 0.2 x HS declined significantly with an increase in salinity between 0 and 40 mM NaCl (Fig. 3.6).

![Fig. 3.4 Interactive effects of salinity and macronutrients on the magnesium content in leaves, stems, and grains of wheat plants. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.](image1)

![Fig. 3.5 Interactive effects of salinity and macronutrients on the chloride content in leaves, stems, and grains of wheat plants. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.](image2)
After that, nitrate content decreased slightly or remained constant with increasing salinity at 1 and 0.2 x HS. At 0.04 x HS, however, nitrate content in leaves and stems was unaffected by salinity except at 60 mM NaCl. Raising the macronutrient level from 0.2 to 1 x HS increased nitrate contents in leaves and stems in the range from 0 to 80 mM NaCl and from 0 to 150 mM NaCl, respectively.

**Phosphorus and Total Nitrogen:** Phosphorus contents in leaves were higher at 0.2 x HS than at 1 x HS regardless of the salinity level and were also higher in stems at salt levels higher than 80 mM NaCl (Fig. 3.7). At these two macronutrient levels, P contents decreased between 0 and 80 mM NaCl in leaves and stems. Beyond 80 mM NaCl, however, P contents either increased at 0.2 x HS or remained steady at 1 x HS. P contents in grains did not differ, irrespective of the salinity and the macronutrient levels.

Total nitrogen content in grains like P was not influenced by the salinity or macronutrient level (Fig. 3.8).

### 3.4 Discussion

Increasing salinity linearly reduced yield components of spring wheat such as dry weight of leaves, stems, and grains, irrespective of the macronutrient level (Chapter 2). Dry weight of leaves, stems, straw, and grains and spike number were highly significant and positively correlated with each other \( r > 0.97^{***} \) at all investigated macronutrient levels.

#### 3.4.1 Relationship between Mineral Element Contents and Yield Parameters

The correlation analysis between yield parameters and mineral contents in different organs showed that yield parameters (main spike and whole plant) were more closely correlated to the mineral element contents in leaves and stems than in grains (data not shown). Yield parameters (leaf, stem, grain, straw, above-ground dry weight, and spike number per plant) were significantly correlated with Na\(^+\) \( r < -0.82 \), Cl\(^-\) \( r < -0.79 \), and K\(^+\) \( r > 0.62 \) in leaves and stems regardless of the macronutrient level. These parameters were significantly correlated with NO\(_3\)^- \( r > 0.73 \) contents.
in leaves and stems at 0.2 and 1 x HS, and not significantly correlated with Ca\(^{2+}\) and Mg\(^{2+}\) contents in stems and grains at 1 x HS. However, Ca\(^{2+}\) (r>0.76) and Mg\(^{2+}\) (r>0.75) contents of leaves at 1, 0.2, and 0.04 x HS and of stems at 0.2 and 0.04 x HS were found to be highly significantly correlated to yield parameters. No significant correlations were found between yield parameters and P contents in leaves at 0.2 and 0.04 x HS, P contents in grains at 1 and 0.04 x HS and K\(^+\) content in grains, regardless of the macronutrient level. Correlations between yield parameters of the main spike and mineral contents in different organs of plants were similar to those of yield parameters in the whole plant.

In contrast to leaves and stems, mineral contents in grains were increased (Na\(^+\), Cl\(^-\)) or decreased (Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\)) only slightly or did not change (K\(^+\)) as a result of increasing salinity. Nitrogen and P contents in grains were not affected by salinity. Therefore, mineral contents in grains, although sometimes strongly and significantly correlated with yield parameters, do not reflect a serious growth limitation except at very high salinity and low macronutrient level. However, Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Cl\(^-\), and NO\(_3^-\) contents in leaves and/or stems may be associated or even related to yield reductions.

3.4.2 Is Grain Yield Reduced by Ionic Toxicity, Nutrient Imbalance, and/or Nutrient Deficiency?

Did salinity reduce grain yield by toxicity, nutrient imbalance, nutrient deficiency, or in another way? Because grain yield of the main spike was only slightly affected by salinity,
except at a high level (>120 mM NaCl) with 1 and 0.2 x HS (Chapter 2), this suggests that, at least with regard to the grain yield of the main spike none of the above factors limited yield to a great extent. Why then did yield decrease so drastically with increasing salinity? Chapter 2 showed that salinity reduced yield mainly by reducing the tiller number. Did salinity increase competition among tillers by decreasing the availability of a growth (tiller) limiting nutrient? We found that chloride decreased the availability of nitrate in the growth zone of the main stem leaf blades (Chapter 6). This may affect leaf and tiller formation and development. If salinity occurs after tiller formation, yield in wheat plants is hardly reduced (Maas and Poss, 1989). Therefore, it is not very likely that tillers which develop later will react more sensitively to salinity than do the main or primary tillers. However, tillers which develop later are at greater risk because salts continue to accumulate in the already developed organs and these tillers depend initially on their subtending tillers. This may be caused by toxic compounds which are derived from source tillers, by nutrient antagonism due to, for example, non-optimal Na⁺/K⁺, Na⁺/Ca²⁺, K⁺/Ca²⁺ supply, or by a deficiency caused by insufficient Ca²⁺ or NO₃⁻ supply. Another possibility may be disturbances in the hormonal balance. To our knowledge, none of these factors has been adequately investigated in relation to formation and development of tillers.

Yield reductions in spring wheat caused by salinity cannot be explained based on mineral contents measured at grain maturity in leaves, stems or grains. However, it could be argued that the final mineral contents not only accidentally correlate with yield. And nutrient imbalances, deficiencies or toxicities observed at grain maturity might also indicate the likelihood of their occurrence at earlier growing stages.

**Sodium and Potassium:** It is generally accepted that Na⁺ disturbs the nutrient balance and causes specific toxicity. In this study, salinity significantly increased sodium contents in leaves and stems (Fig. 3.1). This increase was accompanied by a decline in the K⁺ content, especially in the stem (Fig. 3.2), indicating an apparent antagonism between K⁺ and Na⁺. This antagonism may be due to the direct competition between K⁺ and Na⁺ at a site of ion uptake in the plasmalemma (Epstein, 1966). Sodium may also enhance the efflux of K⁺ into the growth medium, probably resulting from disturbed membrane integrity (Cramer et al., 1985).

Na⁺ content in leaves was of similar magnitude to that in stems (Fig. 3.1), whereas the K⁺ content at low salinity was much lower in leaves than in stems, and similar K⁺ contents were found at high salinity (Fig. 3.2). K⁺ retranslocation from leaves to other organs may be more active at low and moderate salinity than at high salinity. Kalaji and Piekiewicz (1993) showed that retranslocation of Na⁺ and Cl⁻ is limited, and only minor concentrations are found in the phloem sap.

At any given salinity level, the Na⁺ content in leaves and stems did not differ at 1 and 0.2 x HS, whereas the K⁺ content was slightly lower with 0.2 x HS at 0-90 mM NaCl (Figs. 3.1, 3.2). Na⁺/K⁺ ratios in plant tissues increased with salinity, markedly only at 0.04 x HS. Difference in Na⁺/K⁺ ratios in plant tissues between 1 and 0.2 x HS was not associated with the change in external Na⁺/K⁺ ratios. The main spike grain yield in these two treatments was only slightly affected by increasing external Na⁺/K⁺ ratios. Therefore, we conclude that grain production in the whole plant was probably not affected. The small differences in plant grain yield which were observed at >100 mM NaCl between 0.2 and 1 x HS could not be accounted for by the Na⁺/K⁺
ratio in plant tissues.

**Calcium and Magnesium:** There was no relationship between Ca\(^{2+}\) content in stems and final grain yield at 1 x HS (data not shown). Ca\(^{2+}\) content in leaves declined with increasing salinity (Fig. 3.3). High Na\(^{+}\) levels in the external medium may have greatly reduced the activity of Ca\(^{2+}\) in the solution and may have resulted in a decrease in the amount of Ca\(^{2+}\) available for uptake by the plants (Alam, 1994; Grattan and Grieve, 1992). Root growth and function may be inhibited by a high Na\(^{+}\)/Ca\(^{2+}\) ratio (Kent and Läuchli, 1985), and processes whereby Ca\(^{2+}\) is transported from the root to the shoot may be impaired. Cereals are particularly prone to Ca\(^{2+}\) deficiency at high external Na\(^{+}\)/Ca\(^{2+}\) ratios (Ehret et al., 1990; Maas and Grieve, 1987). The Ca\(^{2+}\) disorder was eliminated when external Na\(^{+}\)/Ca\(^{2+}\) was reduced to 18 (Grieve and Fujiyama, 1987). Na\(^{+}\) displaces membrane-associated Ca\(^{2+}\). Ca\(^{2+}\) has been found to reduce the permeability of Na\(^{+}\) through the plasma membrane and to prevent the loss of the K\(^{+}\)/Na\(^{+}\) selectivity (Cramer et al., 1985; 1987). Numerous studies have shown that the K\(^{+}\) content in plant tissues is reduced as the Na\(^{+}\) or Na\(^{+}\)/Ca\(^{2+}\) ratio in the root medium increases (e.g. Okusanya and Ungar, 1984, Cramer et al., 1985; Janzen and Chang, 1987; Subbarao et al., 1990). In this study, however, multiplying the Ca\(^{2+}\) and K\(^{+}\) concentrations in the nutrient solution by five did not influence Na\(^{+}\) contents in leaves and stems at 1 x HS as compared with 0.2 x HS. Although Na\(^{+}\)/Ca\(^{2+}\) ratios in leaves and stems steadily increased with increasing salinity in all treatments, they hardly influenced main spike grain yield, and probably, most likely also not plant grain yield except at very high salinity (>120 mM NaCl) and in the treatment with 0.04 x HS.

Salinity affected Mg\(^{2+}\) accumulation in leaves and stems similar to Ca\(^{2+}\) (Figs. 3.3, 3.4). The decrease in the Mg\(^{2+}\) content seems to be due mainly to ion competition between Na\(^{+}\) and Mg\(^{2+}\). Calcium is strongly competitive with Mg\(^{2+}\). The binding sites on the root plasma membranes appear to have less affinity for the highly hydrated Mg\(^{2+}\) than for Ca\(^{2+}\) (Marschner, 1995). Competition between Ca\(^{2+}\) and Mg\(^{2+}\) may have occurred in this study too. However, Ca\(^{2+}\)/Mg\(^{2+}\) ratios in leaves showed significant changes only at 40-60 mM NaCl in 0.04 x HS and at 125 and 150 mM NaCl in plants with 0.2 x HS, while the ratio did not change in the treatment with 1 x HS.

**Chloride and Nitrate:** Chloride is a more sensitive indicator of salt damage than Na\(^{+}\), since, generally, more Cl\(^{-}\) than Na\(^{+}\) is stored in plants (Alam, 1994). In general, higher Cl\(^{-}\) than Na\(^{+}\) contents were found in leaves but not in stems. Macronutrient levels of 0.2 and 1 x HS did not affect Cl\(^{-}\) accumulation differently in leaves and stems. Chloride contents in leaves and stems increased with higher salinity (Fig. 3.5). This increase may result from the reduction in the availability of Ca\(^{2+}\) causing an increase in root permeability (Grattan and Grieve, 1992). Accumulated Cl\(^{-}\) may cause leaf injury, thereby decreasing photosynthesis and productivity (Greenway and Munns, 1980). Although chloride concentrations in leaves and stems strongly increased with increasing salinity main spike yield was hardly affected. Therefore, it is rather unlikely that a relation existed between plant grain yield and chloride concentrations in leaves and stems, even though highly significant strong correlations were observed.

Nitrate concentrations at 1 and 0.2 x HS were comparatively reduced by decreasing the macronutrient level and by increasing salinity up to 90 mM NaCl. Nitrate concentrations in leaves
and stems strongly decreased at 0 mM NaCl with decreasing macronutrient level. Results from the companion paper showed that increasing the external NO$_3^-$ concentration fivefold in 0.2 x HS did not influence plant grain yield at low and medium salinity (0-90 mM NaCl) (Chapter 1). Only a few studies showed an increase in crop yield under saline conditions where N was applied above a level considered optimal under non-saline conditions (Ravikovitch and Yoles, 1971). Reduction in nitrate content may be associated with a rapid increase in Cl$^-$ content. Chloride had an antagonistic effect on nitrate uptake resulting in a suppression of nitrate with increasing salinity (Greenway and Munns, 1980; Torres and Bingham, 1973). The reduction in the nitrate content in leaves may also be attributable to the accelerated reduction of nitrate under salt stress. This conclusion is supported by Munns and Termaat (1986) and Oertli (1991). In contrary, Abdul-Kadir and Paulsen (1982) attributed the decrease in nitrate reductase activity in salt stressed wheat plants to inhibition of NO$_3^-$ uptake by Cl$^-$, which is in agreement with the reports of Imsande and Touraine (1994) and Wilkinson and Crawford (1993).

### 3.5 Conclusions

Together with the results in Chapter 2, there is no evidence that toxicity, nutrient imbalance, and/or nutrient deficiency had a strong influence on grain yield after tillers had been formed in the treatments with 1 and 0.2 x HS at low to moderate salinity levels (0 to 100 mM NaCl). However, either one of these factors may have influenced the tiller number, the main determinant of yield in spring wheat in this experiment. In contrast, mineral contents measured at grain maturity at salinity levels higher than 120 mM NaCl with 1 and 0.2 x HS as well as with 0.04 x HS and >20 mM NaCl most likely reflect more than mere correlations to grain yield. In the latter case, the effect of salinity is not due to lowering osmotic potential. Increased Na$^+$ contents and decreased Ca$^{2+}$ contents in the grains suggest that grain yield might be limited by Na$^+$/Ca$^{2+}$ disturbance. Further work should focus on the relationship between plant mineral content in early vegetative stages like the tiller formation. We expect that these investigations will elucidate whether toxicity, nutrient imbalance, and/or nutrient deficiency mainly limits grain yields in salinized wheat plants.

### 3.6 References


II

Spatial and Temporal Distribution of Growth and the Mineral Element and Carbohydrate Contents in Wheat Leaves under Saline Soil Conditions
Leer - Vide - Empty
Spatial Distribution and Kinetics of Leaf Elongation of Wheat Plants under Saline Soil Conditions

Abstract The leaf growth of wheat plants is one of the most sensitive processes to salinity. Knowledge of the spatial distribution and kinetics of leaf growth is essential for a better understanding of salinity effects on the leaf growth. The objective of this study was to investigate the spatial distribution and kinetics of leaf elongation of spring wheat (Triticum aestivum L. cv. Lona) during linear growth phase under saline soil conditions. The experiment was conducted in growth chambers. Plants were grown in 1.5-L pots containing an illitic-chloritic silty loam with four salinity levels of 0, 40, 80, and 120 mM NaCl. The fresh weight (FW), dry weight (DW), and the tiller number were measured when the plants were harvested on days 12 and 18 after sowing. The length of the elongation zone and the spatial distribution of elongation of leaves 3, 4, and 5 of the main stem were determined by measuring displacement rates along the leaf axis with a puncturing method. Instantaneous measurements of leaf elongation rate (LER) were made by using linear variable differential transformers when leaves 3, 4, and 5 of the main stem were 1-2 cm long. FW and DW were significantly affected even at low salinity levels and decreased linearly with increasing salt concentration from 0 to 120 mM NaCl. At 120 mM NaCl, FW was reduced by 66 and 75% at 12 and 18 days after sowing respectively, and DW was reduced by 61 and 63% respectively. Salinity delayed the appearance of leaves and tillers. Mean leaf elongation rate and final leaf length were reduced by 14, 22, and 31% in leaves 3, 4, and 5, respectively, at 120 mM NaCl. The width of leaves also decreased with an increase in salinity. Leaves 3, 4, and 5 exhibited a distinct diurnal variation during leaf elongation. The reduction in leaf elongation rate was more pronounced at daytime than at night during the linear growth phase. The length of the leaf elongation zone (LEZ) was unaffected by salinity. The reduction in leaf elongation rate caused by salinity is due to decreasing segmental elongation rate.
4.1 Introduction

The inhibition of growth of plants in saline soil conditions may be due to either water and osmotic stresses, specific ion toxicity, or ionic imbalance, acting on biophysical and metabolic processes of growth (Greenway and Munns, 1980; Thiel et al., 1988). Although salinity affects all growth stages of plants, the vegetative stage in wheat plants is most sensitive to salinity (Arif and Tomos, 1993; Munns and Termaat, 1986; Maas and Poss, 1989). Furthermore, vegetative growth of wheat plants is characterized by sequential appearance and elongation of leaves on tillers. Therefore, it is necessary to characterize leaf growth and its response to salinity.

Leaf elongation in grasses is restricted to a small region at the base of the blade enclosed by older leaves (Kemp, 1980). Although the elongation zone is enclosed, grass leaves present a good opportunity to study leaf growth processes because the growing zone is so distinct and relatively simply organized (Schnyder and Nelson, 1988). Elongation is largely unidirectional, and a cellular particle is displaced away from the leaf base as a result of production of younger tissue and longitudinal growth. Moreover, elongation is a dynamic process and not uniformly distributed throughout the elongation zone of the leaf, i.e. the leaf elongation rate is spatially distributed along the leaf axis. Previous studies have shown that the spatial distribution of leaf growth is influenced by genotype (Volene and Nelson, 1981), developmental stage (Schnyder et al., 1990; Meiri et al., 1992), light (Schnyder and Nelson, 1989), and salinity (Bernstein et al., 1994).

The reduction in final leaf length of wheat plants can result from a reduced leaf elongation rate and/or a shortened elongation period. Under given conditions, the elongation rate of a leaf also varies with leaf age and diurnal course. Different leaf elongation rates may characterize four distinct stages in grass leaf development (Skinner and Nelson, 1995). Initial elongation is characterized by production of new and uniformly sized cells which continue to divide until the epidermal cell division zone has been established. Once the division zone has been established, an increase in elongation rate is observed and the leaf reaches its maximum relative LER. Following establishment of the entire elongation zone, the leaf enters a period of linear growth characterized by high absolute LER. As the supply of new cells for leaf growth stops and older cells reach their final length, the elongation zone shrinks and the stage of decreasing elongation is reached. Kinetic studies on LER for wheat (Christ, 1978a, b), for tall fescue (Parrish and Wolf, 1983), and for Lolium temulentum (Thomas, 1984) have also shown a distinct diurnal rhythm. Under saline conditions, however, no information is available on the kinetics of leaf growth from leaf emergence to the end of leaf growth, and on the diurnal growth pattern of leaf elongation.

The objective of this study was to investigate the spatial distribution of leaf elongation during the linear growth phase, and the kinetics of leaf elongation measured by linear variable differential transformers (LVDT) for leaves 3, 4, and 5 of the main stem of wheat plants grown in soil with 0, 40, 80, and 120 mM NaCl.
4.2 Materials and Methods

4.2.1 Growth Conditions

Two days after seeds of spring wheat (*Triticum aestivum* L. cv. Lona) were pregerminated on filter paper wetted by tap water at 20°C, six seeds were sown in 1.5-L pots (10 cm in diameter and 20 cm high) containing an illitic-chloritic silty loam (fine mesic Aquic Ustifluvent) of which characteristics are shown in Table 4.1 (Schmidhalter et al., 1994). The soil was initially watered to 0.25 g H$_2$O g$^{-1}$ dry soil (soil matric potential: $\Psi_m = -0.03$ MPa) with 1 strength Hoagland solution for macronutrients, modified by increasing the phosphate concentration by tenfold, and 0.5 strength micronutrients as recommended by Epstein (1972). The composition of 1 strength modified Hoagland nutrient solution was (in mol m$^{-3}$): 6.05 K$^+$, 15.0 NO$_3^-$, 5.0 Ca$^{2+}$, 2.0 Mg$^{2+}$, 10.0 H$_2$PO$_4^-$, and 2.0 SO$_4^{2-}$. The salt levels of 40, 80, and 120 mM NaCl were obtained by adding NaCl to the nutrient solution. The soil was thoroughly mixed and kept in tightly closed plastic boxes for one week to facilitate equilibration. Thereafter, the soil was sieved and placed into pots. Soil water levels were 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 film, where plants could grow through 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 photon m$^{-2}$ s$^{-1}$ (PPFD) provided by a mixture of 160 watt Sylvania cool white fluorescent and 60 watt Tungsram E27 standard tungsten lamps. The air temperature was 20°C day/night and the relative humidity was maintained at 55-65%.

Table 4.1 Physical and chemical properties of the soil.

<table>
<thead>
<tr>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
<th>Organic matter</th>
<th>pH(H$_2$O)</th>
<th>Cation-exchange capacity</th>
<th>Ca$_{0.37}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>g kg$^{-1}$</td>
<td>g kg$^{-1}$</td>
<td>cmolc kg$^{-1}$</td>
<td>cmolc L$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.09</td>
<td>0.60</td>
<td>0.31</td>
<td>8.5</td>
<td>8.2</td>
<td>4.8</td>
<td>3</td>
</tr>
</tbody>
</table>

$^1$Ca concentration determined in a soil saturation extract at 0.37 g g$^{-1}$ gravimetric water content.

Experiment I

4.2.2 Determination of Growth, Leaf Water Relations, and Leaf Transpiration Rate

Four replicate plants were harvested on days 12 and 18 after sowing. Tiller number per plant was recorded. Leaf length, leaf width, and leaf area were determined with a portable leaf area meter.
Fresh weight (FW) of the whole plant and of each leaf of the main stem was determined. After drying at 105°C for one hour followed by 24 hours at 65°C, dry weight (DW) was determined. Relative water content (RWC) was calculated from the FW and DW of the leaf by using the following equation: RWC % = (FW - DW) x 100 / FW.

At midday on days 12 and 18 after sowing, leaf water potential (Ψ) and osmotic potential (Ψs) were determined in the middle of leaf 4 of the main stem, with four replications. Ψ and Ψs were measured with a pressure bomb (PMS Instrument CO., Model 1002, Corvalis Co., Oregon, USA) (Scholander et al., 1965) and a vapour pressure osmometer (Wescor 5500, Wescor INC., Logan, USA), respectively. Turgor pressure (P) was calculated from Ψ and Ψs according to the equation: P = Ψ - Ψs.

At day 17 after sowing, the diurnal course of leaf transpiration was measured by weighing the pots. The measurements were made at (i) 1 h before the photoperiod commenced, (ii) at 1, 2, 3, 4, 6, 8, 10, 12, 14, 15, and 16 h after the photoperiod commenced, and (iii) at 1 h after the end of the photoperiod. Leaf transpiration rate (LTR) was calculated from the change in weight (ΔW) of the pots between two successive measurements, the time interval (Δt) and the total leaf area (A) per pot which was measured at day 18 after sowing according to the equation:

\[
LTR = \frac{ΔW}{Δt \times A} \quad (mg \ H_2O \ cm^{-2} \ h^{-1})
\]

After each harvest, the soil was carefully removed from the pots and cut horizontally into three 5 cm sections. Gravimetric soil water content (θg) of each section was determined by weighing before and after drying at 105°C for 36 h. The electrical conductivity of the soil solution in a 0.25:1 water-soil extract (EC0.25:1) was measured with a conductometer (Conductometer, E 518, Metrohm, Herisau, Switzerland) on days 0, 12, and 18 after sowing (Table 4.2). There was no significant change in the salt concentration in all treatments. Only a slight decrease in salinity was observed in the top section of the pot at day 18 after sowing.

**Experiment II**

### 4.2.3 Instantaneous Measurements of Leaf Length and Leaf Elongation Rate

Instantaneous measurements of leaf growth were made by using the LVDT when leaves 3, 4, and 5 of the main stem were 1-2 cm long (about one day after leaf emergence) in all treatments. The tip of the leaf was connected with the LVDT by a fishing line (0.22 mm diameter), which was attached to the leaf tip using a small clamp cushioned with mounting rubber to avoid damaging the leaf. The force on the fishing line was 10 g to eliminate oscillations in the LVDT output resulting from slippage and friction in the measurement system. This force did not affect leaf elongation rates during measurement. A reading was taken from each transducer at 30 min time interval. Over this period of 30 min, six values were averaged and this single value was stored by a logger (Delta-T Device, Cambridge, UK). The measurements of leaf elongation were made until
Table 4.2 Gravimetric soil water content ($\theta_g$) and EC$_{0.25:1}$ in 0-5 cm, 5-10 cm, and 10-15 cm sections beginning at the top of the soil column at days 12 and 18 after sowing in treatments with 0, 40, 80, and 120 mM NaCl.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>$\theta_g$ (g g$^{-1}$)</th>
<th>EC$_{0.25:1}$ (dS m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl mM</td>
<td>Days</td>
<td>0-5 cm</td>
<td>5-10 cm</td>
</tr>
<tr>
<td>0 0</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>40 0</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>80 0</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>120 0</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>0 12</td>
<td></td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>40 12</td>
<td></td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>80 12</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>120 12</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>0 18</td>
<td></td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>40 18</td>
<td></td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>80 18</td>
<td></td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>120 18</td>
<td></td>
<td>0.24</td>
<td>0.24</td>
</tr>
</tbody>
</table>

The elongation rate (mm h$^{-1}$) was approximately zero. Leaf elongation rate was calculated by dividing the increase in length by the time interval. All measurements of the leaf elongation rate (LER) were performed with 8 replications.

Leaf length can be viewed as the integral of LER. Once the measurement for each leaf was finished, the final leaf length was also recorded by using a ruler in order to compare the results from the LVDT method.

Experiment III

4.2.4 Elongation Zone Determination

The position of the growing zone for the 3rd, 4th, and 5th leaves of the main stem was determined by measuring displacement rates along the leaf axis with the puncture method (Kemp, 1980). Each treatment had 16 replications. The first puncture was made from the base of the plant. In total, 15 punctures were made at 3 mm intervals by inserting a small needle ($\phi$ 0.2 mm) through the enclosing leaf sheaths, 3 h after the photoperiod was initiated. Ten hours after puncturing, the leaf was removed from the plant and displacement of punctures was measured with a binocular. All measurements were performed at approximately 2 days after leaf emergence, i.e. measurements of the leaf elongation zone were taken during the linear phase of leaf growth. The effect of the puncture on leaf elongation was evaluated by using a ruler to record the change in leaf length from other plants over a 10 h period immediately following puncturing.
Segmental elongation rate (SER) is the relative elongation rate of a leaf segment in one dimension, i.e. the change that occurs in a length of a leaf segment per unit length per unit time. SER was calculated from the following equation:

\[ \text{SER}_i = (D_{i2} - D_{i1}) \cdot D_{i1}^{-1} \cdot (t_{i2} - t_{i1})^{-1} \]

where \( D_{i1} \) represents the initial distance in mm between neighbouring holes defining segment \( i \) (\( i = 1, 2, \ldots, 15 \)) at the time \( t_{i1} \) of making punctures, and \( D_{i2} \) represents the distance between these same punctures after a period \( (t_{i2} - t_{i1}) \) of growth.

Displacement velocity (DV) (mm h\(^{-1}\)), the displacement rate of a particle from the ligule, was calculated from the equation:

\[ \text{DV}_i = (\text{SER}_1 \cdot D_{i1} + \text{SER}_2 \cdot D_{i2} + \ldots + \text{SER}_i \cdot D_{ii}) \]

The length of the elongation zone (LEZ) was defined as the distance from the leaf ligule to the middle of the last segment when SER was less than 0.005 h\(^{-1}\).

The time course of displacement of a given cellular particle through the elongation zone is given by the growth trajectory. The growth trajectory of a cellular particle was estimated by numerical integration of the fitted displacement velocity equations (Gandar, 1980; Morris and Silk, 1992).

4.3 Results

4.3.1 Shoot Fresh Weight and Dry Weight

Mean values of shoot fresh weight (FW) and dry weight (DW) of wheat plants decreased linearly with an increase in external NaCl concentration (Fig. 4.1). Differences in FW and DW between control and saline treatments increased with time. At 120 mM NaCl, FW was reduced by 66 and 70.5% at 12 and 18 days after sowing respectively, and DW was reduced by 61 and 63 respectively, as compared with the control. Furthermore, 120 mM NaCl had a significant effect on tiller number per plant (data not shown). Mean values of tiller number per plant varied from 2 to 1 for the plants grown in soil with 0 and 120 mM NaCl at day 12 and from 5 to 3.1 at day 18 after sowing.

4.3.2 Leaf Length and Leaf Elongation Rate

Final leaf length decreased as the external NaCl concentration increased (Fig. 4.2). With an increase in leaf number, the reduction in final leaf length for plants grown in soil with 120 mM NaCl became more pronounced. For example, final leaf length for plants at 120 mM NaCl was reduced by 14, 22, and 31% in leaves 3, 4, and 5, respectively.

There was a similar pattern of leaf elongation rate (LER) with time for leaves 3, 4, and 5 in all treatments (Fig. 4.3). LER remained steady for a few days before decreasing in all
Fig. 4.1 Effect of salinity on fresh weight (FW) and dry weight (DW) production of wheat plants grown in soil with 0, 40, 80, and 120 mM NaCl at days 12 and 18 after sowing. Error bars represent standard deviations. Error bars fit within the plot symbol if not otherwise shown.

Fig. 4.2 Leaf length of leaves 3, 4, and 5 of the main stem of wheat plants grown in soil with 0, 40, 80, and 120 mM NaCl.

Fig. 4.3 Leaf elongation rates of leaves 3, 4, and 5 of the main stem of wheat plants grown in soil with 0, 40, 80, and 120 mM NaCl.
treatments. With an increase in leaf number, the difference in LER between the plants with and without NaCl was greater. At 120 mM NaCl, the average LER during the entire period of leaf growth was reduced by approximately 14, 22, and 31% for leaves 3, 4, and 5, respectively. For any given leaf, LER decreased as the external NaCl concentration increased. Under saline conditions, the reduction in leaf growth was greater during the linear phase than during the later stages for leaves 3 and 4, but not for leaf 5. For example, about 60% of the reduction in length of leaves 3 and 4 at 120 mM NaCl occurred during the 3-d linear phase of growth, whereas this was only 45% in leaf 5 during the 3-d linear growth phase. The duration of the linear phase of leaf growth for leaf 5 was significantly affected by 120 mM NaCl, whereas that for leaves 3 and 4 was not. No significant differences in the entire duration of leaf growth for leaves 3 and 4 were observed under salt treatments, whereas for leaf 5 the duration of leaf elongation was about one day less for plants with 120 mM NaCl than without NaCl.

Leaves 3, 4, and 5 exhibited a distinct diurnal variation during the period of leaf elongation. LER data of leaf 4 are presented in Figure 4.4. In a diurnal cycle, LER changed between the light and dark period, and when lights were turned off and on. Generally, LER was higher during the daytime than during the night (Table 4.3) and the ratios of LER at night to those at daytime decreased with time. There was a greater effect of NaCl on elongation rate during the light period than during the dark period. For instance, the mean value of elongation rate of leaf 4 within the first 3 day measurements was decreased by 22 and 15% during daytime and darkness, respectively.

LER was not fully constant throughout the period of light and darkness (Fig. 4.4 inset). During the linear phase of growth, LER was slightly lower in the second part of the light period. Each day when the light period commenced, LER immediately decreased until it reached a
Table 4.3 Average elongation rates of leaf 4 with 0 mM and 120 mM NaCl.

<table>
<thead>
<tr>
<th>Days</th>
<th>0 mM NaCl</th>
<th></th>
<th>(%)</th>
<th>120 mM NaCl</th>
<th></th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
<td>Dark</td>
<td>D/L</td>
<td>Light</td>
<td>Dark</td>
<td>D/L</td>
</tr>
<tr>
<td></td>
<td>mm h⁻¹</td>
<td>mm h⁻¹</td>
<td></td>
<td>mm h⁻¹</td>
<td>mm h⁻¹</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.79</td>
<td>2.33</td>
<td>83.5</td>
<td>2.10</td>
<td>1.97</td>
<td>93.8</td>
</tr>
<tr>
<td>3</td>
<td>3.24</td>
<td>2.51</td>
<td>77.5</td>
<td>2.59</td>
<td>2.13</td>
<td>82.2</td>
</tr>
<tr>
<td>4</td>
<td>3.45</td>
<td>2.27</td>
<td>65.8</td>
<td>2.78</td>
<td>1.96</td>
<td>70.5</td>
</tr>
<tr>
<td>5</td>
<td>2.95</td>
<td>1.76</td>
<td>60.0</td>
<td>2.31</td>
<td>1.70</td>
<td>73.4</td>
</tr>
<tr>
<td>6</td>
<td>2.18</td>
<td>1.12</td>
<td>51.4</td>
<td>1.90</td>
<td>1.25</td>
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<tr>
<td>7</td>
<td>1.03</td>
<td>0.42</td>
<td>40.8</td>
<td>1.06</td>
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</tr>
<tr>
<td>8</td>
<td>0.14</td>
<td>0.05</td>
<td>35.7</td>
<td>0.16</td>
<td>0.05</td>
<td>31.3</td>
</tr>
</tbody>
</table>

1Night value of leaf elongation rate as % of the preceding day value.

minimum value. About thirty minutes later, LER increased again to reach the average value. As soon as lights were switched off, LER increased rapidly during the first half hour, followed by a sharp decline within the next half hour.

### 4.3.3 Spatial Distribution of Leaf Growth along the Elongation Zone

The spatial distribution of leaf elongation was characterized by segmental elongation rate (SER) and displacement velocity (DV). At any given location of the elongation zone, cells elongated faster in control treatments than in saline treatments for leaves 3, 4, and 5 (Fig. 4.5). Maximum values of SER in the growing zone decreased with an increase in external NaCl concentration. At 120 mM, the maximum value of SER located in the middle of the leaf growing zone was reduced by 33.7, 15.2, and 27.2% for leaves 3, 4, and 5, respectively, as compared with the control. The maximum value of SER decreased with increasing leaf number, e.g. at 0 mM NaCl the maximum SER from leaves 3 to 5 varied from 0.13 to 0.071 h⁻¹. Although length of the leaf elongation zone (LEZ) increased with leaf number, LEZ was unaffected by salt stress (Fig. 4.5). LEZ was approximately 2.7, 3.3, and 3.6 cm for leaves 3, 4, and 5, respectively.

Displacement velocity (DV), the displacement rate of a particle from the leaf base, increased with distance from the ligule to the end of the elongation zone, where DV became constant (Fig. 4.6). DV beyond the elongation zone in Figure 4.6 is equal to LER.

The time course of the displacement of a given cellular particle through the elongation zone is given by the growth trajectory. Estimates based on the undisturbed DV (Chapter 5) during the linear phase of growth and light period showed that cellular particles under saline conditions needed more time to move from 2.5 mm above the ligule to the end of the elongation zone as compared with non-saline conditions. For instance, displacement in leaf 4 from 2.5 mm location above the ligule through the elongation zone took about 1.5 and 2 d for the plants with 0 and 120 mM NaCl, respectively (Fig. 4.7).
4.3.4 Diurnal Course of Transpiration Rate

Turning on lights in the growth chamber resulted in a sharp increase in leaf transpiration rate (LTR) (mg cm$^{-2}$ h$^{-1}$) within a few minutes and continued to increase slowly to reach its maximum (Fig. 4.8). After the maximum LTR was maintained for a few hours, LTR slightly decreased until the light period ended. When lights were switched off, a rapid decrease in LTR to near zero occurred. Leaf transpiration rate decreased with an increase in external NaCl concentrations. At 120 mM NaCl, the mean value of transpiration rate was decreased by about 15% during the light period.
4.3.5 Water Relations

Water and osmotic potentials decreased linearly with an increase in external NaCl concentrations (Fig. 4.9). Water potential was -0.78 to -1.22 MPa and osmotic potential was -1.15 to -1.55 MPa for the plants grown in soils with 0 and 120 mM NaCl, respectively. Turgor pressures in this study were not affected by NaCl (Fig. 4.9). Turgor pressure of leaf 4 at day 18 after sowing ranged from 0.33 to 0.37 MPa in all treatments.

Relative water content of leaf 4 at day 18 after sowing varied from 84.1 to 80.3% for plants grown at 0 and 120 mM NaCl, respectively.
4.4 Discussion

4.4.1 Salinity Effect on Plant Growth

The effect of salinity on the growth of wheat plants has been reported in a number of studies (Delane, et al., 1982; Francois et al., 1986; Maas and Poss, 1989). Our results show that FW and DW of shoots decreased linearly with increasing salt concentration from 0 to 120 mM NaCl (Fig. 4.1). FW and DW were significantly affected even at low salinity levels (Fig. 4.1). This suggests that this variety reacts sensitively to salinity.

The tiller number per plant, leaf number per tiller and the size of individual leaves are the constituents of total FW or DW per plant. Results in our study show that the reduction in FW and DW of shoots is mainly due to a decrease in leaf number per tiller, tiller number per plant, and leaf size at the two harvest times (data not shown). Salinity significantly delays leaf appearance in wheat (Maas and Grieve, 1990; Maas et al., 1994; Maas and Poss, 1989). In this study, leaves 3, 4, and 5 emerged about 3 to 4 days later at 120 mM NaCl than at 0 mM NaCl. Previous studies have shown that tiller appearance, tiller abortion, or both are affected by water stress (Schonfeld et al., 1989) and salt stress (Maas and Grieve, 1990; Nicolas et al., 1993). For the measurements of FW or DW plants in all treatments were harvested at the same time after sowing. This can possibly result from the lower tiller number per plant, leaf number per tiller, and/or much smaller leaf size of the same leaf as compared with non-salinized plants in this study.

4.4.2 Spatial Distribution of Leaf Elongation

The final length of leaves 3, 4, and 5 of the main stem was reduced at 120 mM NaCl by about 14, 22, and 31%, respectively, as compared with the control treatment (Fig. 4.2). Since the width of leaves in wheat plants also decreased with an increase in salinity (about 30% reduction by 120 mM NaCl) (data not shown), the reduction in leaf growth in two dimensions confirms that leaf growth is one of the most sensitive processes to salinity.

The reduction in final leaf length of wheat can result from a reduced leaf elongation rate. The elongation rate is a function of the length of the elongation zone and the segmental elongation rate (SER) in the elongation zone (Fig. 4.5). These parameters, in turn, are the result of cell
division, cell extension, and duration of elongation of individual cells. There was no effect of salinity on the length of the leaf elongation zone during the linear phase of leaf growth for a given leaf. Therefore, the reduction in leaf elongation caused by salinity is due to decreasing segmental elongation rate. This finding is consistent with other reports of salinity effects on wheat plants (Arif and Tomos, 1993; Delane et al., 1982). It differs, however, from one study where salinity inhibited leaf growth of sorghum plants by shortening the length of the leaf elongation zone and, also, by reducing the segmental elongation rate (Bernstein et al., 1993). This difference can be due to the different responses of cell wall properties to salt stress in the elongation zone. Pritchard et al. (1990) reported that if there is no change in the length of the elongation zone, longitudinal changes in wall extensibility are responsible for the local changes in SER, whereas turgor pressure and yield threshold are constant along the elongation zone. When there are changes, both in the length of the elongation zone and SER by environmental factors, longitudinal changes in yield threshold are responsible, but turgor pressure and cell wall extensibility are constant along the elongation zone.

Puncturing reduced the leaf elongation rate by about 45 to 60% when we determined the spatial distribution of leaf growth. Studies on tall fescue leaves have shown that making holes produced a proportional reduction in elongation of segments throughout the growing zone, without changing the spatial distribution of SER (Schnyder et al., 1987; Schnyder et al. 1990). In this study, the results of LER from puncturing measurements, as compared with the results from the measurements by LVDT, show that there were different effects of puncturing on LER in different leaf orders. At 0 mM NaCl, for example, the LER of leaf blades determined by puncturing was about 55, 50, and 40% of that by LVDT for leaves 3, 4, and 5, respectively. A greater effect of puncturing on LER in higher leaf numbers, which had longer elongation zones, is probably due to the greater length of the elongation zone containing more holes within the growing zone.

4.4.3 Kinetics of Leaf Elongation

Leaf growth stages: Elongation rates of leaves vary with the leaf age and diurnally. Therefore, salt effect on leaf growth may be changed with an increase in leaf ages and during the diurnal course. The reduction in leaf elongations for leaves 3 and 4 occurred mainly before the deceasing phase of leaf development (Fig. 4.3), whereas the effect of salinity on LER was less pronounced in the later growth phase. The simplest interpretation for differential inhibition of leaf growth by salinity in different stages of leaf development, is that during the linear phase, the leaf blade has the maximum length of the elongation zone (Skinner and Nelson, 1995). Leaf elongation rate is the product of the cumulative segmental elongation rate within the elongation zone (Figs. 4.5, 4.6). Segmental elongation rate in the elongation zone decreased proportionally under saline conditions (Fig. 4.5). Thus, the largest effect of salinity on the elongation of the leaf must occur when the leaf has its greatest length of the elongation zone. For leaf 5, however, the strong effect of salinity on LER continued after the linear phase of leaf growth. Skinner and Nelson (1995) reported that the decreasing phase of elongation occurs when cell division ceases at the leaf base. As the supply of new cells for leaf growth ceases and older cells reach their final length, the elongation zone shrinks, causing LER to decrease until all cells reach their final length. Schnyder
et al. (1990) observed that the cell elongation rate throughout the elongation zone of ryegrass decreased progressively during the decreasing phase of leaf development. Furthermore, they measured a reduction in the length of the elongating region. For leaf 5, therefore, the longer duration of leaf growth in control treatment as compared with saline conditions suggests that after the linear phase, the length of the elongation zone decreases faster for salinized plants than control plants. A faster shrinking of the length of the elongation zone may result in a greater reduction in leaf growth during the decreasing phase under saline conditions. In addition, the reason for the greater reduction in growth of leaf 5 under saline conditions may also be the longer exposure of plants to salinity as compared with leaves 3 and 4.

Studies on the responses of the growing zone to salt stress for barley (Munns et al., 1982) and sorghum (Bernstein et al., 1993) suggest that the primary location for reduced growth under saline conditions is in the growing tissues, not in the mature photosynthetic tissues. Results from the analysis of the growth trajectory show that the cellular particle initially at 2.5 mm above the ligule required about 1 day more to arrive at the end of the elongation zone for leaf 5, than for leaves 3 and 4 at 120 mM NaCl (data not shown). Generally, effects of salinity on plant growth become more severe with increasing time of exposure to saline conditions. According to the study of Munns and Termaat (1986), short-term responses of growth to salinity may be caused by a water deficit. Long-term limitations are associated with the excessive accumulation of ions which can induce ion toxicity, ion deficiency, ion imbalance, and/or a combination of these factors.

**Diurnal rhythm of leaf elongation**: Kinetic studies on leaf elongation rate show a distinct diurnal rhythm (Fig. 4.4 and Table 4.3). Christ (1978a, b) reported a similar daily oscillation in wheat leaves. He found that during the linear phase of growth, mean LER during the night was about 66-73% of that during the day. LER of leaf 4 averaged over the night was about 70% of the daytime value of LER during the 3-d linear growth period under control conditions (Table 4.3). It seems likely that leaf growth during the night is maintained to a certain degree by consumption of carbohydrates stored from the previous daytime. Possibly there is a process competing with growth for carbohydrate and exhibiting a phase-shifted diurnal rhythm, e.g. turnover of reserve carbohydrates such as starch, perhaps related to the level of sucrose in the tissue (Pongratz and Beck, 1978; Gordon et al, 1980). Another factor which might determine rhythmic variations in growth rate is a light-mediated change in the extensibility of cell walls of expanding leaf tissue. Studies by van Volkenburgh and Cleland (1980, 1981) suggested that cell expansion in bean leaves is controlled in this way. Interestingly, salinity does not limit the supply of carbohydrate (Munns et al., 1982; Chapter 7). This may be one of the reasons why the reduction in growth is less during night than during daytime (Table 4.3). Furthermore, salinity can induce leaf water deficits, especially during the light period. When water stress has been developed during the light period, darkness decreases the stomatal conductance, and the water deficit may be partially recovered.

**Light effect on leaf elongation**: The findings of changes in LER in all treatments by suddenly turning lights on or off in the growth chamber (Fig. 4.4) are in agreement with those reported for wheat (Christ, 1978a, b) and for tall fescue (Parrish and Wolf, 1983; Spollen and Nelson, 1994). This particular pattern of elongation rate for each day induced by light can be interpreted in terms
of stomatal behavior and consequent leaf water status. Christ (1978a, b) explained that the sudden closing of the stomata in darkness and cessation of transpiration affects an increase in the internal water potential in the elongation zone of cells and elongation rate increases for a period of time; the sudden opening of stomata in the morning decreases water potential, and therefore, reduces also the elongation rate for a certain time. The results of the diurnal course of leaf transpiration rates in Figure 4.8 confirm that suddenly switching lights on or off causes a rapidly changed transpiration rate resulting from opening or closing stomata. Volenec and Nelson (1982) have shown a sharp drop in water potential (and LER) when lights are turned on; and Cutler et al. (1980) have shown that turgor pressure drops sharply (along with LER) when rice leaves are illuminated. Figure 4.4 shows that there was a greater reduction in LER at 120 mM than at 0 mM NaCl when the light period started.

In conclusion, these studies suggest that this variety reacts relatively sensitively to salinity. The reason for the high sensitivity of the vegetative stage to salinity is that salt significantly affects the production of leaves and tillers. Salinity spatially affects cell elongation in the leaf elongation zone. The reduction in leaf elongation rate is due to decreasing segmental elongation rate, but not due to shortening the length of the elongation zone.

4.5 References


Spatial Distribution of Water and Dry Weight and their Net Deposition Rates in Elongating Wheat Leaf under Saline Soil Conditions

Abstract Wheat leaf growth is known to have a spatial distribution. Salinity may affect the patterns of spatial distribution of leaf growth. The objective of this study is to quantitatively evaluate the spatial distribution of water and dry weight 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. The experiment was conducted in growth chambers. Plants were grown in 1.5-L pots containing an illitic-chloritic silty loam with 0 and 120 mM NaCl. Three days after emergence of leaf 4, the sampling started at 3 and 13 h into the 16-h photoperiod. Fresh weight (FW) and dry weight (DW) contents (mg mm\(^{-1}\) leaf length) were determined. Water content at 0 mM NaCl slightly increased from the ligule up to the end of the elongation zone for the two harvest times, and then slightly decreased or remained almost constant. At 120 mM NaCl, water content hardly change along the leaf axis up to 50 mm from the ligule and then decreased. The water content was significantly decreased by 120 mM NaCl. DW content linearly decreased from the ligule to reach a minimum near the end of the elongation zone, and then increased to reach a maximum at 60 to 80 mm from the ligule. Beyond about 80 mm, DW content decreased again. The mean DW content in the elongation zone was reduced about 5% by salinity. Beyond this zone, the reduction became greater. Net deposition rate of water and DW in both treatments increased from the base of the leaf to the most actively elongating location at 15 mm from the ligule, and then decreased to near zero near the end of the elongation zone. Water deposition rates in all locations within the elongation zone were greater at 0 mM NaCl than at 120 mM NaCl. Differences in the deposition rate of DW between 0 and 120 mM NaCl were observed from the ligule to 15 mm, but not from 15 to 20 mm. Reduction in leaf cross-sectional area by salinity is attributed to both the initial cross-sectional area and the later decrease in the expansion in the lateral or vertical dimension beyond the elongating zone. The major reduction in the cross-sectional area occurs when the leaf is initiating. Under saline conditions, the slower expansion of leaf growth is reflected in a reduced local net deposition rate of water. The inhibition of salinity to secondary cell wall production may be responsible for the greater reduction in DW content beyond the elongation zone.
5.1 Introduction

Leaf growth of wheat seedlings is severely inhibited by high concentrations of NaCl (Arif and Tomos, 1993; Munns and Tennaat, 1986; Chapter 4). A study of the longitudinal spatial distribution of elongation showed that adverse effects of NaCl in wheat leaf growth were associated with the spatial reduction in the segmental elongation rate (Chapter 4). Although leaf elongation of grasses is largely unidirectional, the leaf expands towards lateral and vertical dimensions as well (Schnyder and Nelson, 1988). However, no information is available on salt effects on the cross-sectional area along the axis of the leaf. Since most of the tissue water is cellular and water is largely non-compressible, water content along the leaf axis can be used to describe the change in cross-sectional area with distance from the ligule. To find the rates at which water is taken up and transported, the continuity equation from hydrodynamics can be used (Silk, 1984).

The spatial change in dry weight (DW) content (mg mm\(^{-1}\) leaf length) of the leaf is closely associated with cell wall production along the leaf axis. As cells elongate in the elongation zone, growth of the primary cell wall accommodates increasing cell volume by the uptake of water and DW content may decrease with distance. But when cell expansion ceases, secondary cell wall deposition occurs and DW content increases with distance (Bailey, 1973; Preston, 1975). The questions then arise: Is the reduced DW content of leaves under saline condition mainly due to a decrease in primary cell wall production in the elongation zone, or in secondary cell wall production beyond the elongation zone, and/or both? Therefore, it is necessary to identify the effects of salinity on the spatial distribution of DW content in growing leaves.

The objective of this study is to quantitatively evaluate the spatial distribution of water and dry weight and their net deposition rates in the elongating and mature zones of leaf 4 of the main stem of spring wheat during its linear growth phase under soil conditions with 0 and 120 mM NaCl.

5.2 Materials and Methods

5.2.1 Growth Conditions

Two days after seeds of spring wheat (Triticum aestivum L. cv. Lona) were pregerminated on filter paper wetted by tap water at 20°C, six seeds were sown in 1.5-L pots (10 cm in diameter and 20 cm high) containing an illitic-chloritic silty loam (fine mixed mesic Aquic Ustifluvent) of which characteristics are shown in Table 4.1 (Schmidhalter et al., 1994). The soil was initially watered to 0.25 g H\(_2\)O g\(^{-1}\) dry soil (soil matric potential: \(\Psi_m = -0.03\) MPa) with 1 strength Hoagland solution for macronutrients, modified by increasing the phosphate concentration by 10 times, and 0.5 strength micronutrients as recommended by Epstein (1972). The composition of 1 strength modified Hoagland nutrient solution was (in mol m\(^{-3}\)): 6.05 K\(^+\), 15.0 NO\(_3^-\), 5.0 Ca\(^{2+}\), 2.0 Mg\(^{2+}\), 10.0 H\(_2\)PO\(_4^-\), and 2.0 SO\(_4^{2-}\). The 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 one week to facilitate equilibration. Thereafter the soil was sieved and placed into pots.
Soil water levels were 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 film, where plants could grow through 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 photon m⁻² s⁻¹ (PPFD) provided by a mixture of 160 watt Sylvania cool white fluorescent and 60 watt Tungsram E27 standard tungsten lamps. The air temperature was 20°C day/night and the relative humidity was maintained at 55-65%.

5.2.2 Tissue Sampling and Determination of Fresh Weight and Dry Weight

Three days after emergence of leaf 4, the sampling started at 3 h (0900 h) and 13 h (1900 h) into the 16-h photoperiod. Replications were harvested successively and harvest time recorded, all sampling being finished within one hour. Elongating leaves were selected for sampling if the blade was equal to and/or more 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 from surrounding leaf sheaths, and then cut at the ligule of leaf blade from the stem. The blade was cut with a razor blade, beginning at the ligule, into six 5 mm long segments followed by three 10 mm and three 20 mm long segments. 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 hours and dry weight was determined. Water content of samples was calculated as the difference between fresh and dry weight. Relative water content (RWC) along the leaf axis in the elongation zone was calculated from the FW and DW of the leaf from the following equation: RWC % = [(FW - DW) x 100] / FW.

5.2.3 Numerical Methods

Local net deposition rates (D, μg mm⁻¹ h⁻¹) of water, DW, and RWC were calculated by the one-dimensional version of the continuity equation as described by Silk (1984):

\[ D = \frac{\partial P}{\partial t} + DV \cdot \frac{\partial P}{\partial x} + (SER \cdot P) \]

where \( P \) is substance density (e.g., μg water mm⁻¹ leaf length); \( t \) is time (h); and \( x \) is distance (mm) from the ligule 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).

On the right side of the continuity equation [1], the first term (I), \( \frac{\partial P}{\partial t} \), represents the local rate of change (time rate change in substance content at a fixed distance from the ligule). It was calculated from data obtained from the tissue sampled at the beginning (\( t_a \)) and end (\( t_b \)):

\[ \frac{\partial P}{\partial t} = \frac{(P_b - P_a)}{(t_b - t_a)} \]

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

$$\frac{\partial P}{\partial x_i} = 0.5 \left[ (P_i - P_{i-1}) \cdot (x_i - x_{i-1})^{-1} + (P_{i+1} - P_i) \cdot (x_{i+1} - x_i)^{-1} \right]$$

where $P_i$ is the substance content of the segment $i$ and $x_i$ is the distance (mm) from the ligule of the leaf. For the first or last segment, $\partial P/\partial x_i$ was calculated by using

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

The third term (III), $(SER \cdot P)$, on the right side of 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). DV and SER were taken from the study on growth analysis (Chapter 4). Since the leaf elongation rate (LER) was decreased by puncturing, the undisturbed DV and SER which were used in Eq. [1] were corrected by the ratio of the LER of leaf 4 of the main stem with punctures to the LER for leaves with a LVDT nondestructive method during the linear phase of growth at daytime (Chapter 4).

5.3 Results

5.3.1 Spatial Distribution of Water, Dry Weight, and Relative Water Content

Spatial distribution of water content (mg mm$^{-1}$ leaf length) in the growing leaf 4 of the main stem of wheat plants with and without 120 mM NaCl, sampled at 0900 and 1900 h of daytime on day 3 after leaf 4 emerged, is shown in Figure 5.1A. During the period of sampling, the growth of leaf 4 occurred in the linear phase of growth and the length of the elongation zone was about 30 mm long for plants in both treatments (Chapter 4). Water content at 0 mM NaCl for the two harvest times slightly increased from the ligule up to the end of the elongation zone, and then slightly decreased or remained almost constant. Beyond about 50 mm from the ligule, water content decreased for both sampling times. At 120 mM NaCl, however, water content hardly change along the leaf axis up to 50 mm from the ligule and then decreased (Fig. 5.1A).

The water content was significantly decreased by 120 mM NaCl. Salt effects on water content became greater in the two sampling times beyond 60 mm from the ligule. For example, 120 mM NaCl reduced the mean water content from the ligule to 60 mm by 36 and 39.8% at 0900 and 1900 h, respectively, and from 60 to 120 mm by 47.8 and 55.9% at 0900 and 1900 h, respectively.

Time significantly affected the water content in the control treatment and the effect became greater beyond the elongation zone. For example, mean water content was higher by 8.5% in the region from 0 to 30 mm, 14% from 30 to 60 mm, and 16.4% from 60 to 120 mm at 1900 h than at 0900 h. At 120 mM NaCl, the water content was about 7% higher at 1900 h than
at 0900 h in the elongation zone, whereas no time effect on the water content was observed beyond the elongation zone (Fig. 5.1A).

The pattern of change in DW content (mg mm\(^{-1}\) leaf length) along the leaf axis was similar in both treatments at the two harvest times, which may be characterized by three regions along the leaf axis (Fig. 5.1B). DW content linearly decreased from the ligule to reach a minimum at 25 to 30 mm which was near the end of the elongation zone, and then increased from about 30 mm to reach a maximum at 60 to 80 mm from the ligule. Beyond about 80 mm, DW content decreased again. In the first region (from the ligule to 30 mm), the mean DW content was about 5% higher in the control treatment than that at 120 mM NaCl for the two sampling times. From 30 to 60 mm above the ligule, mean DW content in the control treatment was not different at 0900 h from that at 120 mM NaCl, whereas at 1900 h, DW content was 16.4% greater at 0 mM NaCl than at 120 mM NaCl. Beyond 60 mm from the ligule, mean DW content was reduced 25.9 and 36.7% by 120 mM NaCl at 0900 and 1900 h, respectively (Fig. 5.1B).

Time significantly affected DW content in the control treatment and the effect became greater beyond the elongation zone. For example, mean DW content was 7.6% higher from 0 to 30 mm, 16% from 30 to 60 mm, and 20.6% from 60 to 120 mm at 1900 h than at 0900 h. At 120 mM NaCl, no time effect on the mean DW content was observed from the elongation zone to about 60 mm from the ligule, but mean DW content was about 7% higher at 1900 h than at 0900 h from 60 to 120 mm above the ligule.

The pattern of change in RWC along the leaf axis was similar in both treatments at the two harvest times (Fig. 5.1C). RWC linearly increased from the ligule to reach a maximum at the end of the elongation zone and then decreased. The RWC at 0 mM NaCl was consistently higher than
at 120 mM NaCl. Mean RWC in the whole leaf was approximately 5% higher at 0 mM NaCl for both harvest times than at 120 mM NaCl. There was no difference between the two harvest times for either treatment.

5.3.2 Net Deposition Rate of Water and DW

Net deposition rates of water and DW were obtained from the average of the two harvest times. Net deposition rate of water content in both treatments increased from the base of the leaf to the most actively elongating location at 15 mm from the ligule, and then decreased to near zero at the end of the elongation zone (about 30 mm from the ligule) (Fig. 5.2). Water deposition rate at all locations within the elongation zone was greater at 0 mM NaCl as compared with 120 mM NaCl. The cumulative rate of water deposited into the elongation zone at 0 mM NaCl, calculated by integrating the rate from 0 to 30 mm above the ligule, was twice as high as that at 120 mM NaCl (Fig. 5.3). Net deposition rate of water in both treatments slightly decreased beyond the elongation zone, but there was no difference in the water deposition rate between the two treatments.

Net deposition rate of DW in both treatments increased from the base to the
most actively elongating location at 15 mm from the ligule, and then sharply decreased from 15 to 25 mm. The difference in the rate of DW deposition between 0 and 120 mM NaCl was observed from the ligule to 15 mm, but not in the remaining part of the elongation zone (15 to 30 mm from the ligule). Beyond the end of the elongation zone, water deposition rate slightly decreased. The rate of deposition became negative at 80 and 60 mm from the ligule for the control and salt treatments, respectively (Fig. 5.4A). Deposition rate of DW was consistently higher in the control treatment as compared with the saline treatment. In contrast to the deposition rate of water, the cumulative rate of DW deposited into the elongation zone at 0 mM NaCl was only 1.3-fold as high as that at 120 mM NaCl (Fig. 5.4B) and the differences between the two treatments increased with distance from the ligule.

5.4 Discussion

5.4.1 Spatial Distribution of Leaf Expansion

Since most of the tissue water is cellular and water is largely non-compressible, the change in the water content along the leaf axis can be used to describe the change in volume per mm leaf length or cross-sectional area with distance from the ligule. Results in Figure 5.1A show that in the control treatment, leaf cross-sectional area appears to increase slightly during a 10-h period of growth in the region from the ligule up to the end of the elongation zone (at 30 mm) between the two harvest times of 0900 and 1900 h. After 10 h growth, the mean cross-sectional area in the elongation zone and beyond the elongation zone was increased by 8 and 15%, respectively. This indicates that the leaf is growing toward three dimensions, but changes in vertical or lateral dimensions are small. There exists a further expansion beyond elongation zone (Fig. 5.1A). The further expansion of the cross-sectional area after longitudinal growth ceases implies (1) that the expansion in lateral or vertical dimensions in the non-elongating regions is not synchronized with the longitudinal expansion, and/or (2) due to a larger cellular particle from the elongation zone (MacAdam and Nelson, 1987). An increase in the width of grass leaves with distance from the

![Fig. 5.4 Spatial distribution of dry weight deposition rates and cumulative dry weight deposition rates of the growing leaf 4 of wheat plants with 0 and 120 mM NaCl.](image-url)
ligule within the elongation zone and then a decrease with the distance from the ligule has been reported for the tall fescue leaf blade (MacAdam and Nelson, 1987). A similar observation of the spatial distribution of water content in mg per unit mm leaf length in the control treatment in this study suggests, that if changes in the vertical dimension are not significant, water content in mg per unit mm leaf length provides a useful means to estimate the change in the width of leaves as well. At 120 mM NaCl, however, no change in the cross-sectional area occurs up to 50 mm from the ligule with distance (Fig. 5.1A).

Data in Figure 5.2, together with data in the inset of Figure 5.2, show that the spatial distribution of the rates of water deposition in the elongation zone is related to the distribution of the segmental elongation rate. The relationship between water deposition rate and SER can be explained by the component analysis as shown in Figure 5.5. The local rate of change in water content (I), \( \partial P/\partial t \), contributed only about 7% to the total net rate of water deposition in the elongation zone for both treatments (Table 5.1), confirming that the spatial distribution of water content within the elongation zone was relatively steady (time invariable). The convective rate of change term (II), \( DV \cdot (\partial P/\partial x) \), is the change due to the movement of cellular particles away from the leaf base occurring through a spatial gradient in water content (Fig. 5.1A). Throughout the elongation zone, 10.6% of the net rate of water deposition was attributed to convective rate of change in the control treatment, suggesting that expansion in the lateral and vertical dimensions contributed only a small portion to overall growth of cells in the elongating tissue (Schnyder and Nelson, 1988). The convective rate of change was only 4.5% of the entire rate of water deposition under salinity, further confirming that besides the reduction in longitudinal expansion, salt inhibition also occurs in lateral and vertical dimensions, but is quite small (Table 5.1).

The stretch rate (III), \( (SER \cdot P) \), i.e. the change due to elongation of the segment, is the largest proportion of water net deposition rate, accounting for 82.3 and 88% of the water deposition into the elongation zone at 0 and 120 mM NaCl, respectively (Table 5.1). Figure 5.1A shows that there is a small gradient in water content within the elongation zone as compared with the SER in the elongation zone (Fig. 5.2 inset). Thus, it is clear that the spatial distribution of the

![Fig. 5.5 Components (local rate of change, convective rate of change, and stretch rate) of net rate of water deposition of the growing leaf 4 of wheat plants with 0 mM NaCl (A) and 120 mM NaCl (B).](image-url)
stretch rate of change, SER · P, is mainly dependent on the spatial change in the SER. Results from plants with 120 mM NaCl show a similar behaviour as compared with 0 mM NaCl (Fig. 5.5).

Table 5.1 Contributions of growth components to the net water deposition rate in the elongation zone (0 to 30 mm from ligule) in control and salt treatments.

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<thead>
<tr>
<th>Growth components</th>
<th>NaCl (mM)</th>
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<tr>
<td></td>
<td>0</td>
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<tr>
<td></td>
<td>µg (growing zone)⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Local rate change (I)</td>
<td>245.5</td>
</tr>
<tr>
<td>Convective rate of change (II)</td>
<td>366.6</td>
</tr>
<tr>
<td>Stretch rate (III)</td>
<td>2861.6</td>
</tr>
<tr>
<td>Net rate of deposition (total)</td>
<td>3473.7</td>
</tr>
</tbody>
</table>

The difference in the rate of water deposition between 0 and 120 mM NaCl was greater within the elongation zone than that in SER, resulting from the reduction in water net deposition rate which may reflect the reduction in leaf growth in three dimensions (Fig. 5.2).

5.4.2 Effects of Salinity on the Spatial Distribution of Leaf Expansion

Salinity effects on the cross-sectional area can occur during leaf initiation near the ligule and during later leaf expansion. Figure 5.1A clearly shows that salinity induced reductions in the cross-sectional area occur near the ligule. For example, the cross-sectional area was reduced 32% by 120 mM NaCl at 5 mm from the ligule and 36% between 5 and 30 mm from the ligule. In contrast to the control treatment, there is only a small increase in water content during a 10-h period of leaf growth (Fig. 5.1A). Thus, although the reduction in the cross-sectional area of the leaf under saline soil conditions is probably attributable to both the initial cross-sectional area and the further decrease in the expansion in the lateral or vertical dimension beyond the elongation zone, the major reduction in the cross-sectional area occurs when the leaf is initiated (Fig. 5.1A). In addition, since the major effect of salinity on the cross-sectional area occurs during leaf initiation, the effects on leaf expansion will be mainly one-dimensional only and become manifest.

5.4.3 Spatial Distribution of Dry Weight Content

Results in Figure 5.1B demonstrate that DW content mg per unit mm leaf length is relatively high at the base, where cells are closely packed and actively divide (MacAdam and Nelson, 1987). The DW content decreases at the location of the most active elongation. Since data in Figure 5.1B, C show a negative relationship between DW content and relative water content, the decrease in DW content with distance within the elongation zone is due to water dilution. After elongation ceases at 30 mm from the ligule, an increase in DW content up to 50-60 mm is probably due to the increase in secondary cell wall deposition (MacAdam and Nelson, 1987; Schnyder and Nelson,
1988). The decrease in DW content per unit mm leaf length beyond 60 mm from the ligule is mainly due to the decrease in the cross-sectional area (Fig. 5.1A). Interestingly, the DW content mg per mm³ water is consistently higher at 120 mM NaCl as compared with the control treatment in contrast to DW content mg per mm leaf length (Fig. 5.6). This may indicate that cell size in the saline treatments is smaller, resulting in higher density of cells at 120 mM NaCl than at 0 mM NaCl. Furthermore, Figure 5.6 also demonstrates a different pattern of DW content along the leaf axis, especially after the elongation ceases at 30 mm (DW content mg per mm³ water continues to increase). There was no time effect on DW content in both treatments during a 10-h period of growth. The consistently higher DW content mg per mm³ water at 120 mM NaCl may imply that under salinity, the osmotic adjustment is obtained by reducing the volumetric growth, which is in agreement with a report of root growth under water stress conditions (Sharp et al., 1990).

A comparison of data in Figures 5.2 and 5.4A shows a positive relationship between net rate of water and DW deposition, suggesting that rapid volumetric growth is accompanied by high rates of assimilate import and use for synthesis of cellular components.

5.4.4 Can Negative or Positive Net Deposition Rates Be Used to Estimate the Import or Export Rates of Substances in the Maturation Zone?

The change in the leaf dimensions causes the negative or positive net deposition rates of water and DW. Beyond the elongation zone of the leaf, for instance, there is a slight decrease in the rates of water deposition in both treatments, and water deposition rate becomes negative at 50 and 60 mm from the ligule for the plants with and without salt, respectively (Fig. 5.2). Since the SER is zero, i.e. SER P = 0, only two components, local rate and convective rate of change, contribute to total net deposition rate. Negative convective rate of change for both treatments (40 mm above the ligule) shows that the cross-sectional area decreases with distance in the mature region of the leaf (Fig. 5.2).

Similarly, the fact that the rate of DW deposition with distance in the leaf becomes positive or negative may be mainly due to the positive or negative change in gradient of cross-section with distance. Therefore, if the change in leaf morphology causes the positive or negative DW deposition rate, the results can not be used to estimate the import or export of DW at a given
location, which is not in agreement with a report of the leaf of tall fescue (Schnyder and Nelson, 1988). This can be further confirmed by rates of DW deposition per mm$^3$ water as shown in Figure 5.7. No negative deposition rate for DW occurs in the whole leaf for either treatment. In the most actively elongating region, the rate of DW deposition is even higher at 120 mM than at 0 mM NaCl. Under saline conditions, the location of the most active cell elongation may require more solutes to meet the demand for sufficient osmotic adjustment. There is no difference in the rates of DW deposition in the mature part between the treatments.

In conclusion, this study suggests that the reduction in the cross-sectional area of the leaf is attributable to both the decrease in the initial cross-sectional area and in the expansion in the lateral or vertical dimension under saline conditions. Probably the major reduction in the cross-sectional area occurs when the leaf is initiated near the ligule. To confirm this suggestion, however, further information on salt effect on cell initiation near the ligule are required. Under saline conditions, the slower expansion of leaf growth is reflected in reduced local net deposition rate of water. The inhibition of secondary cell wall production by salinity may be responsible for the greater reduction in DW content beyond the elongation zone.

5.5 References


Spatial Distribution of Mineral Elements and their Net Deposition Rates in the Elongating Wheat Leaf in Saline Soil Conditions

Abstract Wheat leaf growth is known to be spatially affected by salinity. Altered spatial distribution of leaf growth under saline conditions may be associated with spatial changes in tissue mineral elements. The objective of this study is to quantitatively evaluate the spatial distribution of ion concentrations 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. The experiment was conducted in growth chambers. Plants were grown in 1.5-L pots containing an illitic-chloritic silty loam with 0 and 120 mM NaCl. Three days after emergence of leaf 4, the sampling started at 3 and 13 h into the 16-h photoperiod. Spatial distribution of fresh weight, dry weight, and Na⁺, K⁺, Cl⁻, NO₃⁻, Ca²⁺, Mg²⁺, P, and total N in the elongating and mature tissues were determined on a millimetre scale. The patterns of spatial distribution of ion concentrations were affected by 120 mM NaCl. Sodium, K⁺, Cl⁻, Ca²⁺, Mg²⁺, 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 ligule, whereas NO₃⁻ concentration was lower at 120 mM NaCl. The elongation zone is the strongest sink for mineral elements in the leaf tissues. Local net deposition rates of Na⁺, Cl⁻, Ca²⁺, and Mg²⁺ (mmol kg⁻¹ FW h⁻¹) in the most actively elongating region were enhanced by 120 mM NaCl, whereas for nitrate this was depressed. The limitation of leaf growth by NaCl may be due to Cl⁻ toxicity in both elongating and mature regions. Higher Cl⁻ accumulation in the elongation zone for the plants with 120 mM NaCl is due to the greater net deposition rate of Cl⁻. The great decrease in the net deposition rate of NO₃⁻ in the elongation zone and low nitrate concentration in the more mature region under saline conditions may also be responsible for the inhibition of leaf growth by salinity. Higher tissue Na⁺ may cause ion imbalance, but does not result in ion toxicity in the growing leaves. Potassium, Ca²⁺, Mg²⁺, P, and total nitrogen are less plausibly responsible for the reduction in leaf growth in this study. Higher tissue K⁺ and Ca²⁺ concentrations at 120 mM NaCl are probably due to the presence of high Ca²⁺ in the soil of this study.
6.1 Introduction

Leaf growth of wheat seedlings is severely inhibited by high concentrations of NaCl (Munns and Termaat, 1986; Arif and Tomos, 1993; Chapter 4). The inhibition of growth under saline soil conditions may be due to either 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). In the long-term, for plants exposed to salinity, the reduction of plant growth is most probably due to the specific ion toxicity such as Na⁺ and Cl⁻ and/or ion imbalance in plant tissue (Munns and Termaat, 1986).

Leaf elongation in grasses is restricted to a small region at the base of the blade enclosed by older leaves (Kemp, 1980). Although the elongation zone is enclosed, grass leaves present a good opportunity to study leaf growth processes because the growing zone is so distinct and relatively simply organized. Leaf elongation is largely unidirectional and a cellular particle is displaced away from the leaf base as a result of production of younger tissue and longitudinal growth. A study of longitudinal spatial distribution of elongation showed that the adverse effects of NaCl on wheat leaf growth were associated with the reduction of the relative elemental growth rate (Chapter 4). The spatial decrease in growth along the leaf axis may be associated with the spatial distribution of ion uptake and accumulation in the growing leaves. Therefore, an understanding of physiological processes of leaf growth in saline soils requires the 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 that may be viewed as a quantitative picture of sink and source relationships can be calculated according to the continuum equation. Data on the spatial distribution of both growth velocity and concentration of ions are required for this calculation. Kinetic analyses for ion content have been used for maize leaves (Meiri et al., 1992) and growing roots of cotton (Zhong and Lauchli, 1994). However, there is no information available on the spatial distribution and net deposition rates of ions in the elongation and mature zones of wheat leaves under saline soil conditions.

The objective of this study is to quantitatively evaluate the spatial distribution of ions, e.g. Na⁺, Cl⁻, K⁺, Ca²⁺, Mg²⁺, NO₃⁻, 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 conditions with 0 and 120 mM NaCl.

6.2 Materials and Methods

6.2.1 Growth Conditions

Two days after seeds of spring wheat (Triticum aestivum L. cv. Lona) were pregerminated on filter paper wetted by tap water at 20°C, six seeds were sown in 1.5-L pots (10 cm in diameter and 20 cm high) containing an illitic-chloritic silty loam (fine mixed mesic Aquic Ustifluvent) of which characteristics are shown in Table 4.1 (Schmidhalter et al., 1994). The soil was initially watered to 0.25 g H₂O g⁻¹ dry soil (soil matric potential: \(\Psi_m = -0.03\) MPa) with 1 strength...
Hoagland solution for macronutrients, modified by increasing the phosphate concentration tenfold, and 0.5 strength micronutrients as recommended by Epstein (1972). The composition of 1 strength modified Hoagland nutrient solution was (in mol m\(^{-3}\)): 6.05 K\(^+\), 15.0 NO\(_3^-\), 5.0 Ca\(^{2+}\), 2.0 Mg\(^{2+}\), 10.0 H\(_2\)P\(_4\)\(^-\), and 2.0 SO\(_4^{2-}\). The 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 one week to facilitate equilibration. Thereafter the soil was sieved and placed into pots. Soil water levels were 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 film, where plants could 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 \(\mu\)mol photon m\(^{-2}\) s\(^{-1}\) (PPFD) provided by a mixture of 160 watt Sylvania cool white fluorescent and 60 watt Tungsram E27 standard tungsten lamps. The air temperature was 20°C day/night and the relative humidity was maintained at 55-65%.

### 6.2.2 Tissue Sampling

Three days after emergence of leaf 4, the sampling started at 3 h (0900 h) and 13 h (1900 h) into the 16-h photoperiod. Replications were harvested successively and harvest time recorded, all sampling being finished within one hour. Elongating leaves were selected for sampling if the blade was equal to and/or more 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 from surrounding leaf sheaths, and then cut at the ligule of leaf blade from the stem. The blade was cut with a razor blade, beginning at the ligule, into six 5 mm long segments followed by three 10 mm and three 20 mm long segments. 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 hours and dry weight was determined. Dry plant material was stored for the analysis of ion concentration.

### 6.2.3 Analysis of Ion Concentration

Dry samples from different position in the leaf 4 of the main stem were ground by hand with a glass rod in test tubes. The concentration of ions was measured by the following methods:

- **Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), and P**: Approximately 25 mg plant samples were ashed at 560°C for 6 hours and digested with 1 ml of 20% HCl at 65°C for 5 min and then diluted to 25 ml. The concentration of Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), and P was determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty. Ltd., Mulgrave Victoria, Australia).

- **Cl\(^-\) and NO\(_3^-\)**: 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 Ltd., Halstead, Essex, England) and NO\(_3^-\) with a HPLC detector (LC 75, Perkin-Elmer Co., Norwalk, Connecticut, USA).

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

6.2.4 Numerical Methods

Local net deposition rates \( D, \text{ mmol kg}^{-1} \text{ FW h}^{-1} \) of mineral elements like \( \text{Na}^+, \text{Cl}^-, \text{K}^+, \text{Ca}^{2+}, \text{Mg}^{2+}, \text{NO}_3^-, \text{P} \), etc. were calculated by the one-dimensional version of the continuity equation as described by Silk (1984):

\[
D = \left( \frac{\partial P}{\partial t} \right) + DV \cdot \left( \frac{\partial P}{\partial x} \right) + (\text{SER} \cdot P) \tag{1}
\]

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

On the right side of the continuity equation [1], the first term (I), \( \partial P/\partial t \), represents the local rate of change (time rate change in substance content at a fixed distance from the ligule). It was calculated from data obtained from the tissue sampled at the beginning \( (t_a) \) and end \( (t_b) \):

\[
\frac{\partial P}{\partial t} = \frac{(P_b - P_a)}{(t_b - t_a)}
\]

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

The second term (II), \( DV \cdot \left( \frac{\partial P}{\partial x} \right) \), in Eq. [1] is called "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 from the following equation (Schnyder and Nelson, 1987):

\[
\frac{\partial P}{\partial x} = 0.5 \left[ (P_i - P_{i+1}) (x_i - x_{i+1})^{-1} + (P_{i+1} - P_i) (x_{i+1} - x_i)^{-1} \right]
\]

where \( P_i \) is the substance content in the segment \( i \) and \( x_i \) is the distance (mm) from the ligule of the leaf. For the first or last segment, \( \frac{\partial P}{\partial x} \) was calculated by using

\[
(P_{i+1} - P_i) (x_{i+1} - x_i)^{-1} \text{ or } (P_1 - P_{i+1}) (x_i - x_{i+1})^{-1}
\]

The third term (III), (\( \text{SER} \cdot P \)), on the right side of 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). \( DV \) and \( \text{SER} \) were taken from the study on growth analysis (Chapter 4). Since the leaf elongation rate (LER) was decreased by puncturing, the undisturbed \( DV \) and \( \text{SER} \) which were used in Eq. [1] were corrected by the ratio of the LER of leaf 4 of the main stem with punctures to the LER for leaves with a LVDT nondestructive method during the linear phase of growth at daytime (Chapter 4).
6.3 Results

6.3.1 Spatial Distribution of the Concentration of Mineral Elements

A consideration of the importance of mineral elements in plant tissue requires an interpretation based on dry weight (DW) or fresh weight (FW). For practical purposes such as the calculation of the total nutrient uptake of a crop or the use of plant analysis as a tool for diagnosing nutrient availability of the soil, mineral contents expressed on dry weight basis are appropriate. For physiological considerations, however, it is often more convenient to express nutrient concentrations in the plant on a fresh weight basis. This can give a more realistic impression of the actual mineral concentration in plant cells (Mengel and Kirby, 1987). In this study, both DW and FW bases were used for the different purposes. On a fresh weight basis, Figures 6.1 and 6.2 show that different elements in the growing leaf of the main stem differ in patterns of spatial distribution. In general, Na⁺, K⁺, Cl⁻, Ca²⁺, Mg²⁺, 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 ligule, whereas NO₃⁻ concentration was lower at 120 mM NaCl.

Data from Figure 6.1A show that in the control treatment, Na⁺ concentration (mmol kg⁻¹ FW) was comparable along the leaf axis, while under saline soil conditions, the spatial distribution pattern of Na⁺ concentration in the elongation zone appeared to be similar to the segmental elongation rate (Fig. 4.5) for both harvest times. There was a continuous increase in Na⁺ concentration with distance beyond the elongation zone. Na⁺ concentration did not change markedly with time up to 50 mm from the ligule in the two treatments. Beyond 50 mm, however, Na⁺ concentration was higher at 0900 h than at 1900 h under saline conditions (Fig. 6.1A), whereas in the control treatment, this was reversed.

Potassium concentration in the control treatment was higher in the fully expanded region than in the elongation zone for both harvest times. There was no difference between the two harvest times (Fig. 6.1B). Under saline conditions, however, there was an increase in K⁺ concentration in the elongation zone and a decrease in the fully expanded region with distance, especially at 0900 h. There was a different effect of time on K⁺ concentration along the leaf axis, for example, K⁺ concentration in the region between 0 to 60 mm from the ligule was higher at 0900 h than at 1900 h and lower in the region between 60 to 120 mm from the ligule.

Chloride concentration in the elongation zone increased slightly with distance in the control treatment, and then remained constant in the mature part of the leaf for the control treatment, whereas at 120 mM NaCl, 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. 6.1C). Thus, the difference in Cl⁻ concentration between 0 and 120 mM NaCl became bigger as the distance increased from the ligule. The mean Cl⁻ concentration in the elongation zone was 21% higher at 0900 h than at 1900 h under saline conditions. In contrast, no difference in Cl⁻ concentration between the two harvest times was found in the fully expanded region. In the control treatment, for any location of the leaf, Cl⁻ concentration did not change with time.

Nitrate concentration increased sharply with distance up to 40 mm from the ligule for all treatments (Fig. 6.1D). NO₃⁻ concentration was at a maximum about 40 mm from the ligule and
Fig. 6.1 Spatial distribution of sodium (A), potassium (B), chloride (C), and nitrate (D) concentrations (mmol kg\(^{-1}\) FW) in the growing leaf 4 of main stem of wheat plants grown in soil with 0 and 120 mM NaCl and at the two harvest times (at 0900 and 1900 h). Error bars represent standard deviations. Error bars fit within the plot symbol if not otherwise shown. Arrows indicate the length of elongation zone and position of the end of leaf sheath.
then decreased with distance. NO$_3^-$ concentration in the elongation zone was higher in the control treatment as compared with the salinized treatment. In the more mature region, the mean NO$_3^-$ concentration (from 30 to 120 mm from the ligule) at 0900 and 1900 h was about twice and threefold higher, respectively, for plants grown in soil with 0 mM NaCl than with 120 mM NaCl.

Calcium concentration decreased with distance from the ligule to reach a minimum in the region between 25 to 50 mm from the ligule and then increased with distance, but the increase and/or decrease in Ca$^{2+}$ concentration was sharper at 0 mM NaCl than at 120 mM NaCl (Fig. 6.2A). In the elongation zone, the mean Ca$^{2+}$ concentration in the elongation zone was 30% and 14% higher for plants with and without salt at 1900 h than at 0900 h, while there was no difference between the two harvest times for the mature part of leaf.

The patterns of spatial distribution of Mg$^{2+}$, P, and total N concentration were similar and comparable to the spatial distribution of DW content (mg mg$^{-1}$ H$_2$O) (Figs. 5.2B, C, D, 5.7). Their concentration decreased with distance from the ligule to reach a minimum at the end of the elongation zone and then slightly increased or remained constant with distance. No time effect on Mg$^{2+}$, P, and total N concentrations was observed along the leaf axis.

On the DW basis, the patterns of spatial distribution for all elements were similar to those on the FW basis in the two treatments and two harvest times (Fig. 6.3). In contrast to the FW basis, the K$^+$ concentration was higher at 0 mM NaCl than at 120 mM NaCl on the DW basis (Figs. 6.1B, 6.3B). Furthermore, the pattern of spatial distribution of K$^+$ concentration was similar to that of the relative water content (Fig. 5.1C). Mg$^{2+}$, P, and total N concentrations (mmol kg$^{-1}$ DW) in the control treatment did not differ from those in the saline treatment on the DW basis (Fig. 6.4).

6.3.2 Net Deposition Rate of Mineral Elements

The net deposition rate curves may be described as the quantitative result of sink and source relationships. Positive net deposition rate of substance can be viewed as substance sink, while negative net deposition rate of substance is regarded as source of substance (Silk et al., 1986). Net deposition rate of mineral elements as shown in Figures 6.5 to 6.9 were obtained from the average of the two harvest times. Results in Figures 6.5 to 6.9 show that the spatial distribution of net deposition rates for all elements in leaf 4 of the main stem had a similar pattern in the elongation zone for both treatments, while the distribution pattern varied with different elements in the more mature region of the leaf.

The elongation zone is the strongest sink for water (Chapter 5). This is also true for the mineral elements in the elongation zone. There was a large deposition rate of K$^+$ in the elongation zone. In contrast, net deposition rate of K$^+$ (mmol kg$^{-1}$ FW h$^{-1}$) was minimal in the fully expanded zone for both treatments (Fig. 6.5A). There was no difference between 0 and 120 mM NaCl. Although the pattern of spatial distribution of net deposition rate of K$^+$ on the DW basis was similar to that on the FW basis, net deposition rate of K$^+$ in the elongation zone was higher at 0 mM NaCl on the DW basis as compared with 120 mM NaCl. The mean K$^+$ deposition rate (mmol kg$^{-1}$ DW h$^{-1}$) in the elongation zone was decreased 33% by 120 mM NaCl. Net deposition rate of K$^+$ on the dry weight basis was negative in the mature region in both treatments (Fig. 6.5B).

The pattern of the spatial distribution of net deposition rate of Cl$^-$, like K$^+$, was
Fig. 6.2 Spatial distribution of calcium (A), magnesium (B), phosphorus (C), and total nitrogen (D) concentrations (mmol kg\(^{-1}\) FW) in the growing leaf 4 of main stem of wheat plants grown in soil with 0 and 120 mM NaCl and at the two harvest times (at 0900 and 1900 h). Error bars represent standard deviations. Error bars fit within the plot symbol if not otherwise shown.
Fig. 6.3 Spatial distribution of sodium (A), potassium (B), chloride (C), and nitrate (D) concentrations (mmol kg\(^{-1}\) DW) in the growing leaf 4 of main stem of wheat plants grown in soil with 0 and 120 mM NaCl and at the two harvest times (at 0900 and 1900 h). Error bars represent standard deviations. Error bars fit within the plot symbol if not otherwise shown.
Fig. 6.4 Spatial distribution of calcium (A), magnesium (B), phosphorus (C), and total nitrogen (D) concentrations (mmol kg\(^{-1}\) DW) in the growing leaf 4 of main stem of wheat plants grown in soil with 0 and 120 mM NaCl and at the two harvest times (at 0900 and 1900 h). Error bars represent standard deviations. Error bars fit within the plot symbol if not otherwise shown.
Fig. 6.5 Spatial distribution of potassium net deposition rates based on mmol kg$^{-1}$ FW h$^{-1}$ (A) and mmol kg$^{-1}$ DW h$^{-1}$ (B) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl.

Fig. 6.6 Spatial distribution of chloride (A) and nitrate (B) net deposition rates (mmol kg$^{-1}$FW h$^{-1}$) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl.

Comparable with that of the spatial distribution of segmental elongation rate (Fig. 6.6A). Net deposition rate of Cl$^-$ and the significant effect of NaCl were found in the elongation zone. Mean value of Cl$^-$ net deposition rate in the elongation zone was 43% higher at 120 mM NaCl than at 0 mM NaCl. In contrast, net deposition rate of Cl$^-$ was minimal in the mature region and remained nearly constant. The maximum NO$_3^-$ deposition rate occurred in the region between 15 to 20 mm from the ligule in both treatments (Fig. 6.6B). Zero net deposition rate was reached at about 35 and 45 mm from the ligule for the control and salt treatments. The greatest difference in net deposition rate of NO$_3^-$ between the two treatments occurred in the elongation zone. For example, mean deposition rate of NO$_3^-$ was 52% greater at 0 mM NaCl than at 120 mM NaCl. In the more mature region, NO$_3^-$ deposition rate became negative for both treatments.

Changes in the net deposition rates of Na$^+$, Ca$^{2+}$, Mg$^{2+}$, P, and total N were slightly different from K$^+$, Cl$^-$, and NO$_3^-$ deposition rates in the elongation zone (Figs. 6.7, 6.8A, B, 6.9A, B). Maximum net deposition rate occurred at the middle of the elongation zone (at about 15 mm) and followed a sharp decrease to reach zero value before the elongation zone ended (at about 20
to 25 mm), but not at the end of the elongation zone. The net deposition rate of Na⁺ in the elongation zone was significantly enhanced by 120 mM NaCl (Fig. 6.7). Under saline conditions, negative rates of Na⁺ deposition occurred in two regions, between 25 and 40 mm and between 80 and 120 mm from the ligule. The net deposition rate of Na⁺ between 0 and 120 mM NaCl became different beyond 60 mm from the ligule. There were differences in the net deposition rates of Ca²⁺, Mg²⁺, and total N in the elongation zone between

Fig. 6.7 Spatial distribution of sodium deposition rates (mmol kg⁻¹ FW h⁻¹) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl.

Fig. 6.8 Spatial distribution of calcium (A) and magnesium (B) deposition rates (mmol kg⁻¹ FW h⁻¹) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl.

Fig. 6.9 Spatial distribution of phosphorus (A) and total nitrogen (B) deposition rates in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl.
the two treatments as well. Mean net deposition rates of Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), and total N were 42, 31, 27, and 12\%, respectively, higher at 120 mM NaCl than at 0 mM NaCl (Figs. 6.7, 6.8A, B, 6.9B). Ca\(^{2+}\) net deposition rate in the elongation zone was higher at 120 mM NaCl than at 0 mM NaCl (Fig. 8A). Mean Ca\(^{2+}\) net deposition rate in the mature part of the leaf was 53\% greater at 0 mM NaCl than at 120 mM NaCl. In contrast, net deposition rate of Mg\(^{2+}\), P, and total N remained constant and there were no differences between the two treatments in the fully expanded zone (Figs. 6.8B, 6.9A, B).

6.4 Discussion

Salinity stress is generally recognized as injurious to plants by disturbing the electrolyte balance, resulting in toxicity of specific ions, in deficiency of some essential nutrient elements, and in ionic imbalance in plant tissues. In this study, the results from the spatial distributions of concentrations of ions and their net deposition rates in the growing leaf under saline conditions show that adverse effects of Na\(^+\) and Cl\(^-\) on the nutrient ion acquisition and translocation within the leaf of wheat plants are spatially affected (Figs. 6.1 to 6.9). This suggests that the reductions in leaf growth may be dependent upon spatially varying mineral elements which may cause specific ion toxicity, ion deficiency, and/or ionic imbalance depending on the location of the growing leaf under saline conditions. For instance, high Na\(^+\) accumulation in the elongation zone may be responsible for the osmoregulation, while high Na\(^+\) in mature tissues may result in ion imbalance or specific toxicity; limitation of NO\(_3^-\) concentration in leaf tissues may be due to its low transport rate in the elongating tissues, and its deficiency and/or an ionic imbalance in the mature tissue. Thus, the discussion in this section is restricted to interpreting the implications of the results shown in Figures 6.1 to 6.9 for the nutrient allocation and transport in the elongation and mature regions of leaf 4 in wheat plants.

Sodium: Under saline conditions, high Na\(^+\) content in leaf tissues disturbs the nutrient balance and osmotic regulation and causes specific ion toxicity (Saad Eddin and Dodema, 1986). In this study, Na\(^+\) accumulation in the leaf was higher under saline conditions, but the difference between the two treatments depended on the location along the leaf axis (Fig. 6.1A). In the elongation zone, the pattern of spatial distribution of Na\(^+\) accumulation for plants with 120 mM NaCl was closely related to that of the segmental elongation rate, reflecting that the maximum Na\(^+\) accumulation at the site of most active elongation is probably needed to meet the demand for osmotic adjustment under salt stress conditions (Fig. 6.1A). The explanation for the high Na\(^+\) accumulation in the elongation zone is that Na\(^+\) replaced K\(^+\) since the K\(^+\) concentration was relatively low in the elongation zone (Fig. 6.1B) and that there was a large influx of Na\(^+\) in the elongation zone from the mature tissues as compared with the control treatment (Fig. 6.7). Although leaf Na\(^+\) concentration in this study was consistently high at 120 mM NaCl, it was only approximately 1/10 of that in our previous study on wheat plants grown in hydroponics with a similar level of NaCl (Chapter 3). This indicates that wheat plants in this study were restricting the transport of Na\(^+\) to the leaves. Since Ca\(^{2+}\) concentration in this soil was very high (Table 4.1). Therefore, this exclusion mechanism probably relies on relatively high external Ca\(^{2+}\) concentration.
and Ca²⁺/Na⁺ ratios, respectively (Marschner, 1995). High Na⁺ concentration under saline conditions may cause ion imbalance.

**Chloride:** Chloride concentration in the leaf is about fourfold higher than Na⁺ under saline conditions (Fig. 6.1A, C). The higher Cl⁻ concentration than Na⁺ for the plants with 120 mM NaCl suggests that Cl⁻ is probably a more sensitive indicator of salt damage than Na⁺ (Fig. 6.1A, C). Since transport of Na⁺ is restricted to the leaf as compared with Cl⁻, Cl⁻ toxicity may be largely responsible for growth inhibition in this species. Marschner (1995) reviewed that the Cl⁻ levels of soybean cultivars to about 1% of the leaf dry weight caused leaf scorch and a severe reduction in grain yield and that in sensitive woody species such as *Picea omorika*, Cl⁻ levels of 0.2 and 0.3% of needle dry weight are toxic and lead to chlorosis and necrosis. In this study, however, Cl⁻ concentration with 120 mM NaCl reached a maximum of 1.7% of leaf dry weight at 30 mm from the ligule at 0900 h, which is almost twice as high as that for plants in the control treatment (Fig. 6.1C). Therefore, the similarly high Cl⁻ accumulation occurring in the elongating and the mature tissues under saline conditions may reflect that there is a primary limitation of leaf growth by Cl⁻ toxicity in leaf tissues in both regions. In addition, chloride concentration in the elongation zone with 120 mM NaCl was lower in the evening than in the morning, indicating a higher Cl⁻ concentration in the morning may be needed to meet the requirement for osmotic adjustment (Chapter 8).

Chloride net deposition rate in the elongation zone (Figs. 6.6A, 6.7) was 74% higher at 120 mM NaCl than at 0 mM NaCl. Higher Cl⁻ influx rate in the elongation zone results in greater Cl⁻ accumulation. Furthermore, results from Chapter 5 showed that approximately 90% of the reduction in the cross-sectional area at 120 mM NaCl occurred near the leaf base. Large differences found in Cl⁻ and Na⁺ accumulation (Fig. 6.1A, C) suggesting high Cl⁻ and Na⁺ contents in the leaf base may also be responsible for the reduction in cross-sectional area by 120 mM NaCl.

**Nitrate:** Nitrate accumulates mainly in vacuoles, where its concentration has been estimated to be up to 100 times higher than in the cytoplasm (Granstedt and Huffaker, 1982). Therefore, the low NO₃⁻ concentration near the ligule for both treatments indicates that the cells located at the base of the leaf with relatively small vacuoles may limit nitrate accumulation (Fig. 6.1D). Barnal et al. (1974) proposed that the relatively greater uptake of Cl⁻ than Na⁺ in salt-stressed plants could be responsible for the growth reduction by depressing uptake of other anions as well, such as nitrate. In this study, however, no difference in NO₃⁻ accumulation between the two treatments were found in the region between 0 and 15 mm from the ligule. Although nitrate concentration was significantly depressed in the more mature tissues under saline conditions (Fig. 6.1D), especially at 1900 h. A low nitrate concentration in plants grown in salinized media does not necessarily reflect ion competition (Oertli, 1991). Figure 6.2D shows that total nitrogen concentration was even higher in the saline treatment than in the control treatment. One explanation may be that the reduction of nitrate to ammonium in plants was accelerated by salinity. Saad Eddin and Doddema (1995) and Schoppel-Meier and Kaiser (1988) observed a decrease in nitrate from approximately 11 to 2 mM in the leaf sap of spinach treated with 300 mM NaCl. At the same time, soluble
quaternary ammonium compounds increased from 21 to 47 mM. Thus, quartenary ammonium compounds more than compensated for the lower nitrate concentrations in salinized plants. Net deposition rates or influx rate of \( \text{NO}_3^- \) was large in the elongation zone in both treatments as compared with the net deposition rate in the mature zone. Unlike Cl\(^-\), nitrate had a negative net deposition rate in the mature part of the leaf for both treatments. The negative deposition rate may reflect the conversion of nitrate into ammonium, but not the export of \( \text{NO}_3^- \) from this region to other parts of the leaf (Fig. 6.6B).

Since nitrate is not reduced in the elongation zone, the net deposition rate probably represents the rate of nitrate import into the elongation zone (Gastal and Nelson, 1994). Under saline conditions, therefore, the great decrease in net deposition rate of \( \text{NO}_3^- \) in the elongation zone suggests that the reduction in leaf growth may be due to the limitation of \( \text{NO}_3^- \) import from other organs of wheat plants into the elongation zone of the leaf (Fig. 6.6B).

Time significantly affected the concentration of nitrate during the 10-h period in the mature zone. The reason for this may be that light stimulates \( \text{NO}_3^- \) reduction in leaf tissues (Marschner, 1995), resulting in the decreased \( \text{NO}_3^- \) content in leaf tissues during the light period. The results in Figure 6.1D show that \( \text{NO}_3^- \) rapidly decreases under saline conditions during the light period, implying that \( \text{NO}_3^- \) reduction in the leaf is accelerated by 120 mM NaCl. On the whole, the greater decrease in influx of \( \text{NO}_3^- \) into the elongation zone and low \( \text{NO}_3^- \) concentration in the mature tissues may be responsible for the salt inhibition of leaf growth as well.

**Potassium:** Potassium, which is an essential cytoplasmic element because of its involvements in osmotic regulation, is frequently considered important under saline conditions. Increased Na\(^+\) in growth medium generally decreases the K\(^+\) content (Chapter 3). In this study, however, K\(^+\) concentration on a fresh weight basis was increased by 120 mM NaCl, especially in the elongation zone, suggesting that this was most likely the result of high soil Ca\(^{2+}\) concentration (Fig. 6.1B). It has been shown that Ca\(^{2+}\) enhanced the uptake of K\(^+\) in pigeonpea resulting in higher K\(^+\) in the plants grown under saline conditions (Subbarao et al., 1990). Cramer et al. (1990) reported higher concentration of K\(^+\) in the root tips of two corn cultivars in the presence of calcium. This can be further confirmed by no differences in the K\(^+\) net deposition rate between the two treatments, especially in the elongation zone (Fig. 6.5A).

It has been reported that K\(^+\) concentrations of 100 to 150 mM in the cytoplasm, where K\(^+\) helps to stabilize pH, are required for the protein synthesis and for regulating the rates of important enzymatic reactions (Leigh and Wyn Jones, 1986; Meiri et al., 1992; Marschner, 1995). In our study, the K\(^+\) concentration in the less vacuolated cells, i.e. in the region near the ligule, exceeded the requirement for optimal growth and was approximately 170 mM at 0 mM NaCl and 190 mM at 120 mM NaCl. At the end of the elongation zone, K\(^+\) concentration reached more than 200 mM under saline conditions. As a consequence, ion excess of K\(^+\) in this study is also possible. 200 mM K\(^+\) inhibited in vitro protein synthesis in E. Coli by 15%, and this inhibition increased to 60% at 100 mM Na\(^+\) and 100 mM K\(^+\) (Lubin and Ennis, 1964; Munns et al., 1982).

**Calcium:** On the DW basis, Ca\(^{2+}\) concentration in the leaf tissues was higher at 120 mM than at 0 mM NaCl in the region between the ligule and about 60 mm, where cells are actively elongating and/or secondary walls of cells are increasing, but not beyond (Fig. 6.4A). In both treatments,
Ca\(^{2+}\) concentration was higher at 1900 h than at 0900 h. Although high external Ca\(^{2+}\) may enhance Ca\(^{2+}\) accumulation in the leaf tissues under saline conditions, this could be due to high NO\(_3^-\) reduction in the plants with 120 mM NaCl during the light period. Ca\(^{2+}\) plays a vital role in the regulation of ionic relations in plants. In vacuolated cells of leaves in particular, a large proportion of Ca\(^{2+}\) is located in the vacuoles, where it contributes to the cation-anion balance by acting as a counterion for the inorganic and organic anions (Marschner, 1995). In plant species which preferentially synthesize oxalate in response to nitrate reduction, the formation of calcium oxalate in the vacuoles helps to maintain a low level of free Ca\(^{2+}\) in the cytosol and also in the chloroplast (Mix and Marschner, 1974). The formation of sparingly soluble calcium oxalate is also important for the osmoregulation of cells and provides a means of salt accumulation in the vacuoles of nitrate-fed plants without increasing the osmotic pressure in the vacuoles themselves (Osmond, 1967).

Calcium differs from other cations because it is much less mobile in the phloem, transport of Ca\(^{2+}\) is mostly apoplastic and strongly related to xylem flow (Marschner, 1995). Thus, Ca\(^{2+}\) deposition rate is facilitated by transpiration of the sink tissue. The higher net deposition rate at 0 mM NaCl than at 120 mM NaCl in the mature tissue where Ca\(^{2+}\) transports through xylem by transpiration stream is probably due to the greater transpiration rate in the control plants (Fig. 6.8A; Chapter 4). This may further explain the high Ca\(^{2+}\) accumulation in the leaf tip. In contrast, the higher Ca\(^{2+}\) content in meristematic tissue in the two treatments (Figs. 6.2A, 6.4A) could be readily explained if it is true that younger cells contain more pectins in their wall, since the polyuronides of pectins are major Ca\(^{2+}\)-binding sites of the cell wall (Zhong and Läuchli, 1994).

**Magnesium:** A rapid decrease in magnesium concentration in the elongation zone was found in both treatments (Fig. 6.2B), indicating that Mg\(^{2+}\) is accumulated less rapidly than water into growing tissue. The high Mg\(^{2+}\) concentration in the dividing cells may be responsible for high rates of protein synthesis and RNA polymerization (Marschner, 1995). As compared with the Mg\(^{2+}\) concentration on DW basis which showed no difference between the two treatments (Fig. 6.4B), higher Mg\(^{2+}\) concentration on FW basis under saline conditions results from the lower water content for plants in 120 mM NaCl. However, magnesium in the leaf tissue under saline conditions is less plausibly responsible for the reduction in leaf growth.

**Phosphorus and Total Nitrogen:** Throughout the elongation zone, P and total N concentrations, like Mg\(^{2+}\), rapidly declined with distance in the two treatments (Fig. 6.2B, C, D). This may be partly explained by a decrease in DW content (mg mg\(^{-1}\) H\(_2\)O) (Chapter 5) in the elongation zone which may indicate that water is rapidly deposited into the elongation zone. Under saline conditions, a comparison of concentrations of P and total N on the DW and FW basis indicates that the higher concentration of P and total N at 120 mM NaCl on the FW basis as compared with 0 mM NaCl resulted from the lower water content of the plants under saline conditions (Figs. 6.2C, D, 6.4C, D). Nevertheless, P and total N contents, like Ca\(^{2+}\) and Mg\(^{2+}\) in the leaf tissue, probably do not limit leaf growth under saline conditions.
6.5 Conclusion

We suggest that under saline conditions, the pattern of ion concentration along the leaf axis is altered. The elongation zone is the strongest sink for mineral elements in the leaf tissues. The limitation of leaf growth by NaCl may be due to Cl⁻ toxicity in both elongating and mature regions. The greater net deposition rate of Cl⁻ causes the higher Cl⁻ accumulation in the elongation zone for plants with 120 mM NaCl. Low nitrate concentration in the more mature region, which may be caused by a low transport rate and the great decrease in the net deposition rate of NO₃⁻ in the elongating zone, may also be responsible for the salt inhibition of leaf growth. Higher tissue Na⁺ may cause ion imbalance, but did not result in ion toxicity in this study. Under saline conditions, a dramatic reduction in the cross-sectional area near the ligule might be due to high Na⁺ and Cl⁻ concentrations in the region near the ligule. Potassium, Ca²⁺, Mg²⁺, P, and total nitrogen are less plausibly responsible for the reduction in leaf growth. Higher tissue K⁺ and Ca²⁺ concentrations (mmol kg⁻¹ FW) at 120 mM NaCl may be due to the presence of high content of Ca²⁺ available in the soil of this study.

6.6 References


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Spatial Distribution of Sucrose, Glucose and Fructose and their Net Deposition Rates in the Elongating Wheat Leaf under Saline Soil Conditions

Abstract Spatial distribution of carbohydrates which has been found in grass leaves is expected to be changed under saline conditions. Changes in carbohydrates are of particular importance because of their direct relationship with physiological processes such as photosynthesis, translocation, respiration, and/or biosynthesis. The objective of this study is to quantitatively evaluate the spatial distribution of glucose, fructose, sucrose, fructan, and total carbon 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. The experiment was conducted in growth chambers. Plants were grown in 1.5-L pots containing an illitic-chloritic silty loam with 0 and 120 mM NaCl. Three days after emergence of leaf 4, the sampling started at 3 and 13 h into the 16-h photoperiod. Spatial distributions of glucose, fructose, sucrose, fructan, and total carbon concentrations in the elongating and mature tissues were determined on a millimeter scale and spatial distributions of their net deposition rates were calculated. Glucose and fructose concentrations were low at the base of the elongation zone and greatly increased up to the end of the elongation zone in the two treatments at both harvest times. The increase in hexoses with distance may correspond with an increase in the demand for osmotic adjustment and high biosynthesis in the elongation zone. In contrast, sucrose concentration in the elongation zone was high at the leaf base and sharply decreased with distance from the ligule up to the end of the elongation zone for both treatments, which is probably due to dilution with water, the rate of hydrolysis into glucose and fructose, and the rate of sucrose transport. Sucrose concentration was consistently higher at 120 mM NaCl as compared with 0 mM NaCl in the elongation zone, showing that the limitation of leaf growth may be due to the utilization rather than supply of sucrose. The elongation zone is the strongest sink for carbohydrates. Local net deposition rates of sucrose and fructan in the elongation were enhanced by 120 mM NaCl, whereas glucose and fructose were not affected. At 120 mM NaCl, more negative deposition rates of glucose, fructose, sucrose, and fructan were observed in the zone of most active secondary cell wall production in the two treatments. This suggests that the supply of carbohydrates in this region may contribute to salinity induced inhibition of leaf growth.
7.1 Introduction

Leaf growth of wheat seedlings is severely inhibited by high concentration of NaCl (Arif and Tomos, 1993; Munns and Termaat, 1986; Chapter 4). Generally, the carbohydrate status in leaves is changed with increasing salinity (Greenway and Munns, 1980; Munns et al., 1982). Changes in carbohydrate in the study of salt stress are of particular importance because of their direct relationship with physiological processes such as photosynthesis, translocation, respiration, and/or biosynthesis (Kameli and Lösel, 1993).

Spatial distribution of carbohydrates in the elongation zone has been shown for tall fescue (Schnyder and Nelson, 1987; Schnyder et al., 1988). Salinity may affect the pattern of spatial distribution of carbohydrates in the growing leaves, which in turn induces the change in rates of their partitioning and transport at a given location. Therefore, an understanding of physiological processes of leaf growth under saline conditions requires knowledge of the spatial distribution and translocation of carbohydrates in growing leaves.

Gandar (1980) and Silk (1984) have discussed and demonstrated that the local net deposition rate, which may be viewed as quantitative pictures of sink and source relationships, can be calculated according to the continuum equation. Leaf elongation in grasses is restricted to a small region at the base of the blade enclosed by older leaves (Kemp, 1980). Although the elongation zone is enclosed, grass leaves present a good opportunity to study leaf growth processes because the growing zone is so distinct and relatively simply organized (Schnyder and Nelson, 1988; Chapter 4). Leaf elongation is largely unidirectional, and a cellular particle is displaced away from the leaf base as a result of production of younger tissue and longitudinal growth (Chapter 4). Kinetic analyses for carbohydrates have been used in growing leaves of tall fescue (Schnyder and Nelson, 1987; Schnyder et al., 1988). However, few experimental data have been reported on the spatial distribution and net deposition rates of sugar concentrations in the elongating and maturation zones of wheat leaves under saline conditions. In this study, net deposition rates of carbohydrates were used for the description of their translocation within the leaf.

The objective of this study is to quantitatively evaluate the spatial distribution of carbohydrates, e.g. sucrose, glucose, fructose, fructan, and total carbon, 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.

7.2 Materials and Methods

7.2.1 Growth Conditions

Two days after seeds of spring wheat (Triticum aestivum L. cv. Lona) were pregerminated on filter paper wetted by tap water at 20°C, six seeds were sown in 1.5-L pots (10 cm in diameter and 20 cm high) containing an illitic-chloritic silty loam (fine mixed mesic Aquic Ustifluvent) of which characteristics are shown in Table 4.1 (Schmidhalter et al., 1994). The soil was initially watered to 0.25 g H₂O g⁻¹ dry soil (soil matric potential: \( \Psi_m = -0.03 \) MPa) with 1 strength
Hoagland solution for macronutrients, modified by increasing the phosphate concentration tenfold, and 0.5 strength micronutrients as recommended by Epstein (1972). The composition of 1 strength modified Hoagland nutrient solution was (in mol m\(^{-3}\)): 6.05 K\(^+\), 15.0 NO\(_3^-\), 5.0 Ca\(^{2+}\), 2.0 Mg\(^{2+}\), 10.0 H\(_2\)PO\(_4^-\), and 2.0 SO\(_4^{2-}\). The 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 one week to facilitate equilibration. Thereafter the soil was sieved and placed into pots. Soil water levels were 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 film, where plants could grow through 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 \(\mu\)mol photon m\(^{-2}\) s\(^{-1}\) (PPFD) provided by a mixture of 160 watt Sylvania cool white fluorescent and 60 watt Tungsram E27 standard tungsten lamps. The air temperature was 20°C day/night and the relative humidity was maintained at 55-65%.

### 7.2.2 Tissue Sampling and Analysis of Carbohydrate Concentration

Three days after leaf 4 emerged, sampling started at 3 h (0900 h) and 13 h (1900 h) into the 16-h photoperiod. Replications were harvested successively and harvest time recorded, all sampling being finished within one hour. Elongating leaves were selected for sampling if the blade was equal to and/or more 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 from surrounding leaf sheaths, then cut at the ligule of leaf blade from the stem. The blade was cut with a razor blade, beginning at the ligule, into six 5 mm long segments followed by three 10 mm and three 20 mm long segments. Segments from 20 blades of leaf 4 from the main stem for control plants and from 30 blades for the stressed plants within one replication were combined according to position and quickly placed in preweighed 15 ml test tubes, capped tightly and held in ice. Each treatment consisted of two replications. After fresh weight was determined, tissue samples were extracted with 92% ethanol at 60°C for 20 min. Sucrose, glucose and fructose were measured using enzymatic methods from a kit from Boehringer Mannheim with a Kontron spectrophotometer (UVIKON 810, Tegimenta AG, Rotkreuz, Switzerland). Thereafter, the sediment was twice extracted with water at 60°C for the determination of fructans. 0.5 ml 1 N H\(_2\)SO\(_4\) was used to hydrolyze fructans into fructose and glucose at 100°C for 15 min, and then the sample was neutralized with 0.5 ml 1 N KOH. Fructose and glucose content was determined with enzymatic methods from a kit from Boehringer Mannheim with a Kontron spectrophotometer.

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

### 7.2.3 Numerical Methods

Local net deposition rates (\(D, \text{ g kg}^{-1} \text{ FW h}^{-1}\)) of carbohydrates like sucrose, glucose, fructose, fructan, and total carbon were calculated using the one-dimensional version of the continuity...
equation as described by Silk (1984):

\[
D = \left( \frac{\partial P}{\partial t} \right) + DV \cdot \left( \frac{\partial P}{\partial x} \right) + (SER \cdot P)
\]

where \( P \) is substance density (e.g., g kg\(^{-1}\) FW); \( t \) is time (h); and \( x \) is distance (mm) from the ligule 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\(^{-1}\)).

On the right side of the continuity equation [1], the first term (I), \( \frac{\partial P}{\partial t} \), represents the local rate of change (time rate change in substance content at a fixed distance from the ligule). It was calculated from data obtained from the tissue sampled at the beginning \( (t_a) \) and end \( (t_b) \):

\[
\frac{\partial P}{\partial t} = \frac{(P_b - P_a)}{(t_b - t_a)}
\]

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

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

\[
\frac{\partial P}{\partial x_i} = 0.5 \left[ (P_i - P_{i+1})(x_{i+1} - x_i)^{-1} + (P_{i+1} - P_i)(x_{i+1} - x_i)^{-1} \right]
\]

where \( P_i \) is the substance content in the segment \( i \) and \( x_i \) is the distance (mm) from the ligule of the leaf. For the first or last segment, \( \frac{\partial P}{\partial x_i} \) was calculated by using

\[
(P_{i+1} - P_i)(x_{i+1} - x_i)^{-1} \text{ or } (P_1 - P_{i-1})(x_1 - x_{i-1})^{-1}
\]

The third term (III), \( (SER \cdot P) \), on the right side of 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). \( DV \) and \( SER \) were taken from the study on growth analysis (Chapter 4). Since the leaf elongation rate (LER) was decreased by puncturing, the undisturbed \( DV \) and \( SER \) which were used in Eq. [1] were corrected by the ratio of the LER of leaf 4 of the main stem with punctures to the LER for leaves with a LVDT nondestructive method during the linear phase of growth at daytime (Chapter 4).

7.3 Results

7.3.1 Spatial Distribution of Carbohydrate Concentrations

Glucose and fructose concentrations were low at the base of the elongation zone and greatly increased up to the end of the elongation zone where a maximum was reached in the two
treatments at both harvest times (Fig. 7.1A, B). Beyond the elongation zone, a sharp decrease occurred up to 50 to 60 mm from the ligule, and then concentration remained constant with distance. High glucose and fructose concentrations at 120 mM NaCl were observed in the region between 20 and 50 mm from the ligule at 0900 h. An increase in glucose concentration with time occurred in the region between 20 and 60 mm from the ligule for either treatment. In contrast, fructose concentration at 0 mM NaCl was greater at 1900 h than at 0900 h between 20 and 50 mm from the ligule, while at 120 mM NaCl, fructose concentration was lower at 1900 h than at 0900 h.

In contrast to the spatial distribution of glucose and fructose concentration, sucrose concentration (g kg\(^{-1}\) FW) in the elongation zone was high at the leaf base and sharply decreased with distance from the ligule up to the end of the elongation zone for both treatments (Fig. 7.1C). Sucrose concentration at 120 mM NaCl was consistently high in the elongation zone. The mean value in the elongation zone at 120 mM NaCl was approximately 2.5-fold as high as that at 0 mM NaCl. No difference between the two harvest times was observed for both treatments in this region. Beyond the elongation zone, however, different patterns of spatial distribution of sucrose concentration were observed (Fig. 7.1C).

At 0900 h, for example, sucrose concentration at 120 mM NaCl continuously decreased up to about 60 to 80 mm from the ligule and then remained almost constant and was close to that in the plants with 0 mM NaCl, whereas sucrose concentration at 0 mM NaCl did not change with distance beyond the elongation zone. Conversely, sucrose concentration at 1900 h sharply increased with distance beyond the region between 30 and 80 mm from the ligule in both treatments, where sucrose concentration remained almost unchanged. Sucrose concentration was consistently high at 120 mM NaCl in the mature tissues.

The pattern of spatial distribution of fructan concentration was similar to that of sucrose
concentration except in the control treatment at 0900 h (Fig. 7.2A). The fructan concentration was consistently higher at 120 mM NaCl than at 0 mM NaCl in the elongating tissue for the two harvest times and in the mature tissues at 1900 h. Mean values of fructan concentration in the elongation zone at 1900 h were 3.5-fold greater at 120 mM than at 0 mM NaCl. The fructan concentration in the control treatment at 0900 h was near zero at each location in the leaf. After the 10-h light period, however, fructan concentration was considerably increased in the elongation zone and reached a maximum of 1.21 g kg\(^{-1}\) FW at the leaf base (Fig. 7.2A). At 120 mM NaCl, however, the concentration of fructan at 1900 h was comparably higher along the leaf axis in the elongating and mature tissues as compared with control conditions.

The pattern of change in carbon concentration (g kg\(^{-1}\) FW) along the leaf axis was similar to that of the dry weight content (mg per mg H\(_2\)O) in both treatments at the two harvest times (Fig. 7.2B; Chapter 5), indicating that dry weight is closely correlated with the carbon content in the leaf. Carbon concentration decreased linearly from the ligule to reach a minimum at 25 to 30 mm, and then increased linearly up to 120 mm from the ligule. Carbon concentration was consistently higher at 120 mM NaCl than at 0 mM NaCl. No time effect on the carbon concentration was observed in the two treatments.

### 7.3.2 Net Deposition Rates of Carbohydrate

Net deposition rates of glucose, fructose, sucrose, and fructan as shown in Figures 7.3 to 7.5 were obtained from the average of the two harvest times. The profiles shown in Figures 7.3 to 7.5 were the results of transport and metabolism which occurred during tissue elongation and cell displacement away from the leaf ligule.

Net deposition rates of glucose and fructose in both treatments increased from the ligule to 20 and 25 mm, respectively, and then sharply decreased in the regions between 25 and 50 mm and between 20 and 30 mm (Fig. 7.3A, B). Negative deposition rates in the two treatments occurred in the regions between about 40 and 80 mm and between about 30 and 60 mm from the ligule for glucose and fructose, respectively. Thereafter, the net deposition rates of glucose and
Fig. 7.3 Spatial distribution of glucose (A) and fructose (B) deposition rates (g kg⁻¹ FW h⁻¹) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl.

Fructose remained near zero from 80 mm up to 120 mm from the ligule in the two treatments. Differences in glucose and fructose deposition rates between the two treatments were observed only in the region between 30 and 60 mm from the ligule, where secondary cell wall formation occurs.

Net rate of sucrose deposition was relatively high at the leaf base in both treatments and increased from the leaf ligule to the most actively elongating location at 15 mm, then sharply decreased to negative net deposition rates at approximately 25 mm from the ligule (Fig. 7.4A).

Fig. 7.4 Spatial distribution of sucrose (A) and fructan (B) deposition rate (g kg⁻¹ FW h⁻¹) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl.

The net deposition rate of sucrose in both treatments reached a minimum at about 25 mm from the ligule, and then increased again. Beyond 60 mm from the ligule, a rapid increase was observed in both treatments (Fig. 7.4A). In the region between 0 to 20 mm from the ligule, sucrose deposition rate was higher at 120 mM NaCl than at 0 mM NaCl. In the region between 25 to 50 mm, however, the rate of sucrose deposition was slightly lower at 120 mM NaCl than at 0 mM NaCl, while it was greater beyond 50 mm from the ligule.
Net rate of fructan deposition was closely associated with the rate of sucrose deposition in the region from the leaf base up to 40 mm (Fig. 7.4B). In this region, sigmoid change in the rate of fructan net deposition with distance was observed in the two treatments. Thereafter, however, the rate of fructan deposition remained constant in the two treatments. Furthermore, fructan deposition rate was higher at 120 mM NaCl in the regions between 0 and 20 mm and between 40 and 120 mm from the ligule, whereas it was lower at 120 mM NaCl in the region between 20 and 40 mm.

Net rate of carbon deposition sharply increased in the region between the leaf ligule and about 15 mm, and was then followed by a sharp decrease to reach a minimum in the deposition rate at 25 mm from the ligule (Fig. 7.5). After an increase within a short distance, net rate carbon deposition remained almost constant in the mature tissues.

7.4 Discussion

7.4.1 Causes of Change in Carbohydrate Contents along the Leaf Axis

Carbohydrates are the major substrates for leaf growth. Glucose and fructose in the leaves are the prime substrates for the biochemical processes leading to leaf growth. Sucrose is used for transport, for storage in the vacuole, or for conversion to fructan. Fructans have roles for removing sucrose to facilitate sucrose transport and for serving as a reserve substance (Nelson and Smith, 1986). Furthermore, sucrose and fructans need to be hydrolyzed into their hexose components before they are used for further metabolism. For a better understanding of these processes, it is necessary to investigate the sugar uses, transport, and storage along the leaf axis.

**Hexoses:** The increase in hexoses with distance along the leaf axis may correspond to an increase in the demand for osmotic adjustment in the elongation zone (Fig. 7.1A, B). Furthermore, the growing zone is the region of the highest biosynthetic activity which requires large amounts of glucose and fructose as substances. Therefore, the increase in glucose and fructose with distance from the ligule to the end of the elongation zone is probably due to the increase in the hydrolysis of sucrose and fructan (Fig. 7.1A, B). A large deposition of secondary cell wall occurred in the zone between 30 and 60 mm from the ligule, which is in agreement with the reports for tall fescue (Schnyder and Nelson, 1988; Nelson and MacAdam, 1989; Chapter 5). The marked decrease in glucose and fructose in this zone reflects the large requirement of hexoses for the synthesis of
secondary cell walls. The low rate of hydrolysis of sucrose may also result in a decrease in hexoses between 30 and 60 mm. In addition, negative net rates of glucose and fructose deposition in the two treatments were found in this region (Fig. 7.3A, B), indicating that the rate of synthesis of the secondary cell wall is higher than import or hydrolysis rate of sucrose, since the negative net deposition rate describes the excess rate of utilization over import.

**Sucrose:** In both treatments, a decrease in sucrose concentration in the elongation zone with distance (Fig. 7.1C) may be related to: i) an increase in the rate of conversion to fructan, ii) the rate of sucrose transport, iii) the rate of hydrolysis into glucose and fructose for biosynthetic processes and osmotic adjustment, and iv) a dilution with water. The decrease in fructan concentration with distance (Fig. 7.2A) coincides with the sucrose concentration, implying that the decrease in sucrose concentration with distance is probably not regulated by the change in fructan concentration in this study. After the 10-h light period, however, the increase in the fructan concentration in the elongation zone and no change in the sucrose concentration in the two treatments may show that at a given location, fructan content could be responsible for the regulation of the sucrose concentration with time (Figs. 7.1C, 2A).

There are at least two pool types of sucrose: cytosol and vacuole. Sucrose in the cytosol is readily available for translocation and in the vacuole for storage (Farrar and Farrar, 1985a, b). Generally, the proportion of vacuole volume increases with distance from the ligule in the elongation zone. Thus, this may cause an increase in the proportion of the storage sucrose, resulting in a decrease in the translocation rate of sucrose with distance. In this case, we assumed the main direction of sucrose flux in the elongation zone is from the leaf base, since sucrose may mainly come from the old leaves, especially at 0900 h when sucrose concentration is low in the mature zone. The decrease in the sucrose concentration was more rapid at 120 mM NaCl than at 0 mM NaCl in the elongation zone, indicating that a greater decrease in cytosol sucrose and/or a greater increase in the rate of hydrolysis of sucrose occurred with distance in the elongation zone at 120 mM NaCl as compared with 0 mM NaCl.

The increase in glucose and fructose concentrations in the elongation zone may cause the decrease in sucrose concentration with distance, resulting from an increase in the rate of hydrolysis of sucrose with distance.

High water deposition in the zone of the most active elongation is most likely responsible for the dilution of sucrose concentration with distance (Chapter 5). The net rate of sucrose deposition was relatively high at the leaf base, whereas little water deposition occurred (Chapter 5). As a consequence, the sucrose concentration was high in this region. The net deposition rate of water increased about fourfold in both treatments between the base and the location of the most active elongation (15 mm from the ligule), whereas the net rate of sucrose deposition increased only about 1.5-fold in the two treatments, resulting in significantly decreased sucrose content. Conversely, the decrease in net deposition rate of sucrose in the region between 15 and 30 mm from the ligule occurred more rapidly than decreases in water deposition, resulting in rapidly declining sucrose concentration with distance. After the cell elongation zone, the slight decrease and/or no change in sucrose concentration in the region between 30 and 60 mm from the ligule (Fig. 7.1C) may be due to a small water deposition (Chapter 5), and/or a decrease in the rate of hydrolysis of sucrose to glucose and fructose. Beyond this region where the leaf was
exposed, an increase in sucrose concentration in mature tissues with distance in the two treatments at 1900 h indicates that high sucrose concentration was due to increased production from photosynthesis during the light period. In the same location at 0900 h sucrose concentration was almost unchanged with distance, indicating that the sucrose in this region is stored for translocation during darkness.

### 7.4.2 Time Effect on the Pattern of Spatial Distribution of Carbohydrates

Results at 1900 h show that sucrose concentration was high at the leaf base and leaf tip. At 0900 h, however, sucrose concentration in the photosynthetic part of the leaf was near zero and the value was similar for the two treatments. Interestingly, sucrose concentration in the elongation zone in the two treatments was almost unchanged after the 10-h light period, while sucrose concentrations were higher at 1900 h than at 0900 h in the maturation zone (Fig. 7.1C). There are probably several reasons for this. One reason may be that in a linear phase, local growth rates, respiration rates, and concentrations of carbohydrates and of other substances do not vary with time. The second one is that the rapid increase in sucrose concentration occurs during the first 2 to 3 h in the light period (Sicher et al., 1984). The third reason is that sucrose concentration unaffected by time in the elongation zone may be regulated by fructan, since fructan concentration increased parallely after the 10-h light period. For instance, fructan at 0 mM NaCl could not be detected in the elongation zone at 0900 h. Ten hours after the beginning of the light period, the fructan concentration increased. Furthermore, high storage fructan in tissues under saline conditions may be responsible for the smaller decrease in leaf elongation rate in the darkness (Chapter 4). Many studies have shown that fructan plays a very important role in the regulation or storage of sucrose, especially under stress conditions (Pollock, 1984; 1986; Spollen and Nelson, 1994).

### 7.4.3 Limitation of Salinity to Leaf Growth: Utilization or Supply of Carbohydrates?

Previous reports for the growing leaf of wheat plants under temperature stress (Kemp, 1981), water stress (Munns and Weir, 1981), and salt stress (Munns et al., 1982) have shown that high concentration of sucrose indicated that growth was limited by the utilization of sucrose rather than by the supply of sucrose. In this study, however, although the higher concentration of sucrose in the growing leaf occurred under saline conditions, we suggest that the supply of sucrose probably limited leaf growth as well, mainly depending on the location in the leaf. Farrar and Farrar (1985a, b) reported that growth may be controlled by the carbon flux in the growing leaf, since there are few simple relationships driving carbon fluxes in sources and sinks. The rate of sucrose synthesis in leaves does not drive the rate of translocation. Sucrose storage in the leaf probably has priority over translocation (Farrar and Farrar, 1985a, b). The net deposition rate can be partly used to explain the mechanism of sugar translocation in the growing leaf. The positive net rate of sucrose deposition in the region between 0 and about 20 mm from the ligule was higher at 120 mM NaCl than at 0 mM NaCl, indicating an excess rates of import of sucrose over utilization in this
location. In the region between about 20 and 50 mm, the net rate of sucrose deposition at 0 mM NaCl was close to zero, whereas a negative rate (-0.1 g kg\(^{-1}\) FW h\(^{-1}\)) occurred at 120 mM NaCl (Fig. 7.4A). The pattern of spatial distribution of fructan deposition in this region was similar to that of sucrose in the two treatments and a negative rate of fructan deposition in this region was found (Fig. 7.4B). Net rates of glucose and fructose deposition at 120 mM NaCl were more negative than in the control treatment (Fig. 7.3A, B). In general, the changes of glucose and fructose with distance in the leaf occurred in the zones of the most active cell elongation and the highest deposition of secondary cell wall (Fig. 7.1A, B). Salt effects on glucose and fructose occurred in the region of large deposition of secondary cell wall at 0900 h. Supply of glucose and fructose to this region may inhibit leaf growth under saline conditions. As a consequence, the difference in total carbon content (μg mm\(^{-1}\)) between the two treatments in this region at 1900 h became greater as compared with that in the region from the leaf base to the end of the elongation zone (Fig. 7.6). These findings indicate that the rate of import of sugar may limit the synthesis of secondary cell wall at 120 mM NaCl.

### 7.5 Conclusions

The results in this study confirm that there are distinct patterns of spatial distribution of carbohydrates along the leaf axis for both treatments and the patterns are affected by salinity. Spatial distribution of hexoses may be associated with an increase in the demand for osmotic adjustment, the highest activity of biosynthesis in the elongation zone, and large deposition of secondary cell wall in the zone located between 30 and 60 mm from the ligule. The spatial distribution of sucrose concentration may be related to the water dilution, the rate of conversion to fructan, the rate of sucrose transport, the rate of hydrolysis into glucose and fructose, and photosynthetic production in the exposed part of leaves. Our data show that salt limitation to growth may be due to the utilization of carbohydrates in the elongation zone and their supply in the zone of large deposition of secondary cell walls. However, further study may be required to identify whether under saline conditions, limitations to growth are due to the utilization, or supply of carbohydrates, and/or both.
7.6 References


Leer - Vide - Empty
Spatial Distribution of Ions and Sugars Contributing to Osmotic Adjustment in the Elongating Wheat Leaf under Saline Soil Conditions

Abstract Osmotic adjustment is generally regarded as an important adaptation to salt stress. In particular, growing cells must produce or import solutes for osmotic adjustment, since the solutes in the elongation zone are greatly diluted by water uptake. The objective of this study is to quantitatively evaluate the spatial distribution of total osmotica, inorganic ions, and sugars, inorganic ions and sugars contributing to osmotic adjustment, 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 1.5-L pots containing an illitic-chloritic silty loam with 0 and 120 mM NaCl in growth chambers. Three days after emergence of leaf 4, the sampling started at 3 and 13 h into the 16-h photoperiod. Spatial distributions of fresh weight, dry weight, total osmotica, inorganic ions (Na⁺, K⁺, Cl⁻, NO₃⁻, Ca²⁺, Mg²⁺, and H₂PO₄⁻), and sugars (glucose, fructose, sucrose, and fructan) in the elongating and mature tissues were determined on a millimetre scale. Leaf osmotic potentials tended to decrease slightly with distance in the two treatments irrespective of time. There was a significant decrease in the osmotic potential at 120 mM NaCl as compared with 0 mM NaCl. Net deposition rates of total osmoticum, cations, anions, and sugars in both treatments increased from the base of the leaf to the most actively elongating location, and then decreased near the end of the elongation zone. Total osmoticum, cation, and anion deposition rates in the most actively elongating location were greater at 120 mM NaCl than at 0 mM NaCl. Ions accounted for the major contribution to the osmotic adjustment. Cations, anions, and sugars contributing to osmotic adjustment were about 21-30%, 15-21%, and 13%, respectively, in the elongation zone and about 28-39%, 22-24%, and 3-8%, respectively, in the mature tissue. Ions contributing to osmotic adjustment is mainly due to the net accumulation of K⁺ and Cl⁻ which accounted for 36-47% and 34-43% of the total ions in the elongation zone. In this study, the limitation to plant growth was probably not due to a water deficit caused by salinity.
8.1 Introduction

Plants exposed to salt stress often have higher tissue solute concentrations, i.e. lower osmotic potentials, than in control conditions in order to adjust osmotically. Osmotic adjustment is generally regarded as an important adaptation to salt stress (Greenway and Munns, 1980). In particular, since the solutes in the elongation zone are greatly diluted by water uptake, growing cells must produce or import solutes to maintain osmotic potential. Our studies (Chapters 4, 5) have shown that under saline conditions, leaf growth is spatially affected along the leaf axis as compared with the control treatment. In order to identify mechanisms of salt inhibition to leaf growth, therefore, it is very important to understand: How do solutes build up along the leaf axis in growing leaves under saline conditions? How much do solutes spatially contribute to osmotic adjustment at a given location?

Gandar (1980) and Silk (1984) have discussed and demonstrated that the local net deposition rate, which may be viewed as quantitative pictures of sink and source relationships, can be calculated according to the continuity equation. Leaf elongation of grasses is largely unidirectional, and a cellular particle is displaced away from the leaf base as a result of production of younger tissue and longitudinal growth. Thus, grass leaves present a good opportunity to study leaf growth processes and to use the continuity equation because the growing zone is so distinct and relatively simply organized (Schnyder and Nelson, 1988; Chapter 4). The relationships between the net deposition rates of solutes and water in the elongation zone may be used to analyze the mechanisms of the build-up of solutes for osmotic adjustment (Sharp et al., 1992). However, there is no information available on this in the elongating leaves of wheat plants under saline conditions.

The objective of this study is to quantitatively evaluate the spatial distribution of total osmotica, inorganic ions, and sugars, inorganic ions and sugars contributing to osmotic adjustment, 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.

8.2 Materials and Methods

8.2.1 Growth Conditions

Two days after seeds of spring wheat (Triticum aestivum L. cv. Lona) were pregerminated on filter paper wetted by tap water at 20°C, six seeds were sown in 1.5-L pots (10 cm in diameter and 20 cm high) containing an illitic-chloritic silty loam (fine mixed mesic Aquic Ustifluvent) of which characteristics are shown in Table 4.1 (Schmidhalter et al., 1994). The soil was initially watered to 0.25 g H₂O g⁻¹ dry soil (soil matric potential: Ψₘ = -0.03 MPa) with 1 strength Hoagland solution for macronutrients, modified by increasing the phosphate concentration tenfold, and 0.5 strength micronutrients as recommended by Epstein (1972). The composition of 1 strength modified Hoagland nutrient solution was (in mol m⁻³): 6.05 K⁺, 15.0 NO₃⁻, 5.0 Ca²⁺, 2.0 Mg²⁺, 10.0 H₂PO₄⁻, and 2.0 SO₄²⁻. The 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 one week to facilitate equilibrium. Thereafter the soil was sieved and filled into pots. Soil water levels were maintained at the initial water content by watering with tap water. In order to avoid water loss by evaporation, the pots were covered with a perforated plastic film, where plants could grow through 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 light period per day. The light intensity was approximately 550 μmol photon m$^{-2}$ s$^{-1}$ (PPFD) provided by a mixture of 160 watt Sylvania cool white fluorescent and 60 watt Tungsram E27 standard tungsten lamps. The air temperature was 20°C day/night and the relative humidity was maintained at 55-65%.

8.2.2 Tissue Sampling

Three days after emergence of leaf 4, the sampling started at 3 h (0900 h) and 13 h (1900 h) into the 16-h photoperiod. Replications were harvested successively and harvest time recorded, all sampling being finished within one hour. Elongating leaves were selected for sampling if the blade was equal to and/or more 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 from surrounding leaf sheaths, and then cut at the ligule of leaf blade from the stem. The blade was cut with a razor blade, beginning at the ligule, into six 5 mm long segments followed by three 10 mm and three 20 mm long segments.

About 120 leaf segments from the same position were combined into a sample for analysis of ion concentration. After fresh weight was determined, the samples were dried at 65°C for 48 hours and dry weight was determined. Dry plant material was stored for the analysis of ion concentration.

For the analysis of sugar concentration, segments from 20 blades of control plants and from 30 blades of the stressed plants within one replication were combined according to position and quickly placed in preweighed 15 ml test tubes, capped tightly and held in ice. Each treatment consisted of two replications.

8.2.3 Analysis of Ion and Sugar Concentration

**Ion concentration:** Dry segmental samples from different positions on leaf 4 of the main stem were ground by hand with a glass rod in test tubes. The concentration of ions was measured using the following methods:

- Na$^+$, K$^+$, Ca$^+$, Mg$^{2+}$, and H$_2$PO$_4^-$: approximately 20 mg of ground sample was extracted with 15 ml distilled water at 100°C for 5 min, shaken for about 1 min, and then filtered.

The concentration of Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, and P was determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia).

Chloride, NO$_3^-$, and total osmotica: 20 mg ground samples were extracted with 2 ml distilled water at 100°C for 5 min, shaken with a Vortex for about 1 min, and then filtered with a Millex-HV$_{13}$ filter unit (Millipore Corporation, Bedford, MA 01730, USA). Chloride was determined with a chloride-selective electrode (Chloride analyzer 926, Corning Ltd., Halstead,
Essex, England) and NO$_3^-$ with a HPLC detector (LC 75, Perkin-Elmer Co., Norwalk, Connecticut, USA). Total osmotica was determined with a micro-osmometer (Roebbling, Medizinische Technik und Elektronik, Vogel, 6300 Giessen, Germany).

**Carbohydrate concentration:** Sugars were extracted from the fresh tissue samples with 92% ethanol at 60°C for 20 min. Sucrose, glucose, and fructose were measured according to enzymatic methods from a kit from Boehringer Mannheim with a Kontron spectrophotometer (UVIKON 810, Tegimenta AG, Rotkreuz, Switzerland).

Thereafter, the sediment was twice extracted with water at 60°C for the determination of fructans. 0.5 ml 1 N H$_2$SO$_4$ was used to hydrolyze fructans into fructose and glucose at 100°C for 15 min, and then the sample was neutralized with 0.5 ml 1 N KOH. Then fructose and glucose content was determined according to enzymatic methods from a kit from Boehringer Mannheim with a Kontron spectrophotometer.

The osmotic potential ($\Psi_p$) in MPa was estimated according to the conventional formula:

$$\Psi_p = \frac{(R \cdot T \cdot n)}{V}$$

where $R$ is the gas constant, $T$ is the absolute temperature ($T = 293$ K), $V$ is the volume of water, and $n$ is the number of moles of solutes.

### 8.2.4 Numerical Methods

Local net deposition rates ($D$, mmol kg$^{-1}$ H$_2$O or nmol mm$^{-1}$ h$^{-1}$) of total osmotica, cations, anions, sugars, and total osmotica were calculated by the one-dimensional version of the continuity equation as described by Silk (1984):

$$D = (\frac{\partial P}{\partial t}) +DV \cdot (\frac{\partial P}{\partial x}) + (SER \cdot P)$$

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

On the right side of the continuity equation [1], the first term (I), $\frac{\partial P}{\partial t}$, represents the local rate of change (time rate change in substance content at a fixed distance from the ligule). It was calculated from data obtained from the tissue sampled at the beginning ($t_a$) and end ($t_b$):

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

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

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

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

where $P_i$ is the substance content in the segment $i$ and $x_i$ is the distance (mm) from the ligule of the leaf. For the first or last segment, $\frac{\partial P_i}{\partial x_i}$ was calculated by using

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

The third term (III), (SER - P), on the right side of 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). DV and SER were taken from the study on growth analysis (Chapter 4). Since the leaf elongation rate (LER) was decreased by puncturing, the undisturbed DV and SER which were used in Eq. [1] were corrected by the ratio of the LER of leaf 4 of the main stem with punctures to the LER for leaves with a LVDT nondestructive method during the linear phase of growth at daytime.

8.3 Results and Discussion

8.3.1 Spatial Distribution of the Osmotic Potential

Spatial distribution of the osmotic potential and total osmoticum content is presented in Figure 8.1A, B. The osmotic potential tended to decrease slightly with distance in the two treatments regardless of time. The osmotic potential from 0 to 120 mm from the ligule ranged from about -0.88 to -1.2 MPa and -0.88 to -1.23 MPa with 0 mM NaCl at 0900 and 1900 h, respectively, and -1.38 to -1.55 MPa and -1.25 to -1.58 MPa with 120 mM NaCl at 0900 and 1900 h, respectively. There was a significant difference in the osmotic potential between 0 and 120 mM NaCl. For instance, the mean osmotic potential in the elongation zone (from 0 to 30 mm from the ligule) was decreased by -0.55 MPa at 0900 h and -0.43 MPa at 1900 h, while these values were -0.42 and -0.37 MPa, respectively, in the mature tissues. A time effect on the

Fig. 8.1 Spatial distribution of osmotic potential (MPa) (A) and total osmoticum content (mOsm kg⁻¹) (B) 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 (0900 and 1900 h). Error bars represent standard deviations. Error bars fit within the plot symbol if not otherwise shown. Arrows indicate the length of elongation zone and the position of the end of leaf sheath.
osmotic potential was observed only for plants with 120 mM NaCl in the region between 0 and 60 mm from the ligule (Fig. 8.1).

### 8.3.2 Spatial Distribution of Solute Concentration

Cation, anion, and sugar concentrations were calculated by summing Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\), Cl\(^-\), NO\(_3\)^-, and H\(_2\)PO\(_4\)^-, and sucrose, glucose, fructose, and fructan (assuming the average degree of polymerization is 5) (Fig. 8.2). The pattern of spatial distribution of cation concentration (Fig. 8.2A) was similar to that of the total osmoticum content. Furthermore, the cation concentration was slightly higher in the mature tissue than in the elongating tissue.

Different patterns of spatial distribution of the anion concentration between the two treatments are shown in Figure 8.2B. In the control treatment, anion concentration slightly increased with distance, ranging from about 70 to 95 mmol kg\(^{-1}\) H\(_2\)O for the two harvest times. In contrast, under saline conditions a sharp increase in the anion concentration occurred from 0 to 30-40 mm from the ligule, and then beyond this location the anion concentration remained almost constant. Under saline conditions, the mean anion concentration was increased by 61% at 0900 h and 35% at 1900 h, respectively, in the elongation zone, whereas these values were increased by 42% at 0900 h and 37% at 1900 h, respectively, in the mature tissue. Time did not affect the anion concentration at 0 mM NaCl, whereas at 120 mM NaCl the anion concentration was consistently higher at 0900 h than at 1900 h.

A marked increase in the sugar concentration in both treatments at 1900 h was observed between 15-20 and 30 mm and beyond 60 mm from the ligule as compared with that harvested at 0900 h (Fig. 8.2C). Sugar accumulation was enhanced by 120 mM NaCl.
8.3.3 Net Deposition Rate of Total Osmoticum and Solutes

Net deposition rate of total osmoticum, cations, anions, and sugars as shown in Figures 8.3 to 5 were obtained from the average data of the two harvest times. Net deposition rates of total osmoticum, cations, and anions (mOsm kg⁻¹ h⁻¹ or mmol kg⁻¹ H₂O h⁻¹) in both treatments increased from the base of the leaf to the most actively elongating location at 15 mm from the ligule, and then decreased to near zero at the end of the elongation zone (about 30 mm from the ligule) (Figs. 8.3, 8.4A, B). Total osmoticum, cation, and anion deposition rates in the region between 10-15 and 25 mm from the ligule were greater at 120 mM NaCl as compared with those at 0 mM NaCl. Net deposition rates of total osmoticum, cations, and anions in both treatments slightly decreased or remained almost unchanged with distance beyond the elongation zone, but there was no difference in osmoticum, cation, and anion deposition rates between the two treatments.

![Fig. 8.3 Spatial distribution of total osmoticum deposition rate (mOsm kg⁻¹ h⁻¹) in the growing leaf 4 of wheat plants grown in soil with 0 and 120 mM NaCl.](image)

The net rate of sugar deposition (mmol kg⁻¹ H₂O h⁻¹) increased from the leaf base to the most actively elongating location at 15-20 mm from the ligule, then sharply decreased, and even became negative at approximately 30-40 mm from the ligule in the salinized treatment (Fig. 8.5). Thereafter the net rate of sugar deposition in both treatments reached a minimum at about 40-50 mm from the ligule.
mm from the ligule and then increased again. Beyond 60 mm from the ligule, the increase was greater in both treatments. Between 0 to 15-20 mm from the ligule, the sucrose deposition rate was higher at 120 mM NaCl. Between 30 to 60 mm, however, the rate of sugar deposition was lower at 120 mM NaCl, whereas it was greater beyond 60 mm from the ligule.

8.3.4 Spatial Distribution of Cations, Anions, and Sugars contributing to Osmotic Adjustment

Osmotic adjustment was calculated as the difference (ΔΨ_s) in the osmoticum content between control and stressed plants at a given segmental location for the same time (Fig. 8.6). Mean relative water content from 0 to 120 mm above the ligule was parallelly about 4-4.5% greater at 0 mM NaCl than at 120 mM NaCl (Chapter 5). Therefore, the contribution of dehydration to changes in osmoticum content under saline conditions was minor, and this contribution was not considered in this study. The osmotic adjustment rapidly increased in the region between 5 and 10 mm from the ligule and was greater in the elongation zone than in the maturation zone for the two harvest times. A high water deposition in the elongation zone may be associated with a lower water potential. Osmotic adjustment which was smaller at 1900 h than at 0900 h in the elongation zone may be associated with the greater decrease in the osmotic potential for plants with 0 mM NaCl than with 120 mM NaCl.

To estimate the inorganic ions contributing to osmotic adjustment, the ionization of solutes (i.e., cations and anions) and other deviations from perfect solutions were not considered in this study. Different solutes contributing to osmotic adjustment was expressed as the percentage of ΔΨ_s,i (i = cation, anion or sugar) to ΔΨ_s total (total osmoticum). The results presented in Figure 8.7A, B show that at 0900 h cations, anions, and sugars contributing to osmotic adjustment were about 30, 21, and 13%, respectively, in the elongation zone and about 28,
Fig. 8.7 Spatial distribution of the solutes contributing to osmotic adjustment (%) at 0900 (A) and at 1900 h (B) in the growing leaf 4 of wheat plants grown in soil with 120 mM NaCl.

24, and 3%, respectively, in the mature tissue, whereas these values were about 21, 15, and 13%, respectively, in the elongation zone and about 39, 22, and 8%, respectively, in the mature leaf tissues at 1900 h. Contribution of the total ions (cations and anions) in the elongation zone accounted for 51% at 0900 h and 36% at 1900 h, and was 52% at 0900 h and 62% at 1900 h in the mature leaf tissue. Ions accounted for the major contribution to osmotic adjustment as compared with 8-10% from sugars at the two harvest times. The ions contributing to osmotic adjustment was mainly due to the net accumulation of Na⁺, K⁺, and Cl⁻. In this study, for instance, K⁺ and Cl⁻ in the elongation zone accounted for 47 and 34% of the total ions at 0900 h, and 36 and 43% at 1900 h, whereas these values were 32 and 44% at 0900 h and 52 and 39% at 1900 h in the mature tissues (data not shown). Sodium accounted for 8% of the total ions at 0900 h and 15% at 1900 h in the elongation zone, and 17% of the total ions at 0900 h and 8% at 1900 h in the mature tissues. The low proportion of Na⁺ in the leaf tissues may be due to the characteristics of Na⁺ exclusion by roots for this species (Marschner, 1995; Chapter 6).

Together, Sugars + ions contributed 64% at 0900 h and 49% at 1900 h to osmotic adjustment under saline conditions in the elongation zone and 55% at 0900 h and 70% at 1900 h in the mature leaf tissues. Solutes contributing to the remaining osmotic adjustment (about 30-50%) are unknown. The organic acids like proline, betaine, etc. may be responsible for the remaining osmotic adjustment (Greenway and Munns, 1980; Delane et al., 1982; Bachmann, 1990).

8.3.5 How Do Solutes Build up for Osmotic Adjustment under Saline Conditions?

Under saline conditions, osmotic adjustment generally results from the increase in either the rate of solute supply to the cells, the rate of solute uptake by the cells, or the decrease in the utilization of organic substances. The growing zone is the location of the most active cell elongation (Chapter 5; Schnyder et al., 1988). Furthermore, it has been observed that a higher osmotic
adjustment occurred in the elongation zone as compared with the maturation zone of the leaf (Fig. 8.6). Therefore, the mechanism of osmotic adjustment should first be considered in the elongation zone. In the elongation zone, osmotic adjustment could occur by two basic mechanisms: increase in the net rate of osmoticum deposition and/or reduction in the rate of tissue volume expansion (Sharp et al., 1990). In this study, we suggest that osmotic adjustment is probably due to both the increase in the net rate of osmoticum deposition and the reduction in growth under saline conditions. For instance, mean net deposition rate of total osmoticum in the elongation zone was 20% higher at 120 mM NaCl than at 0 mM NaCl (Fig. 8.3), reflecting that solute accumulation was probably more rapid at 120 mM NaCl than at 0 mM NaCl. In addition, the mean net deposition rate of total osmoticum in the mature tissue was -1.32 mOsm kg\(^{-1}\) h\(^{-1}\), which implies that part of the higher rate of solute accumulation may represent imports from the mature region, since the negative net deposition rates probably mean the export of solutes (Chapter 5).

Furthermore, net deposition rate of total osmoticum per unit water deposition rate was consistently higher at 120 mM NaCl than at 0 mM NaCl in all locations of the elongation zone (Fig. 8.8). Because water net deposition rate was closely related to cell elongation (Chapter 5), this may reflect that the reduction in cell elongation may be greater than the reduction in net deposition rate of total osmotica under saline conditions as compared with that in the control treatment. The ratios of solute to water deposition rates varied with distance as well. At 0 mM NaCl, the ratio of total osmotica to water deposition rate increased slightly with distance, indicating that the net deposition rate of water was similar to that of total osmotica deposition along the leaf axis. At 120 mM NaCl, however, this ratio increased in the region between 5 and 25 mm from the ligule and then decreased to the location at 30 mm. Ratios of cation and anion deposition rates to water deposition rates at 120 mM NaCl were almost the same as that at 0 mM NaCl at the location of 5 mm from the ligule, whereas beyond this location these ratios at 120 mM NaCl increased faster with distance (Fig. 8.9A, B). Ratios of sugar deposition rates to water deposition rates in the leaf base were much higher at 120 mM NaCl than at 0 mM NaCl, and became more similar in the region between 15 and 20 mm from the ligule (Fig. 8.9C). Beyond this location, the difference between the two treatments became greater again up to 25 mm and then a sharp decrease occurred at 120 mM NaCl.
8.3.6 The Role of Osmotic Adjustment

Osmotic adjustment is regarded as an important adaptation of plants to salinity because it helps to maintain turgor and cell volume. According to the biphasic model of growth responses to salinity proposed by Munns (1993), osmotic adjustment might be an adaptation for surviving stress during the first phase or in the short term of salt stress. Later, the reduction in leaf growth may be due to toxicity, ion deficiency, and/or other processes such as hardening of cell walls which limit cell expansion (Neumann, 1994; Nabil and Coudret, 1995). It is difficult to know when the first phase ends or the second phase starts. Reports from our previous study (Chapter 4) showed that at the same growth stage, turgor in the mature tissue of leaf 4 was not affected by 120 mM NaCl. In this study, data cannot be interpreted in terms of turgor pressure within the elongation zone since total water potential was not measured. However, the increase in osmolality of the total solutes between 0 and 120 mM was higher in the elongating tissues than in the growth media (data not shown). Since this study was conducted during the period of linear leaf growth for both treatments, the effective turgor in the elongation zone must be maintained for a constant rate of leaf elongation. Arif (1990) reported that in contrast to the mature cells of wheat plants, no decrease in turgor pressure (measured with a pressure probe) occurred in the elongating cells despite the decrease in growth of plants with 25-150 mM NaCl. This may indicate that plants probably do not suffer from water deficit in the elongation zone at this stage.

Independence of growth from turgor has been found during a study on the influence of temperature on the leaf growth for Lolium temulentum (Thomas et al., 1989) and for barley (Pollock et al. 1990). According to the biophysical model of leaf elongation (Lockhart, 1965;
Cosgrove, 1986; Boyer, 1987), the rate of leaf elongation (r) is regulated or controlled by alterations in any of several parameters: cell wall extensibility (ϕ), turgor pressure (P), and yield threshold (Y). This relationship among the parameters may be expressed as: 

\[ r = \phi \frac{P - Y}{(P - Y)} \]  

(Lockhart, 1965). From the equation, the limitation of salinity to growth is probably due to either the decrease in wall extensibility, the increase in yield threshold, and/or both. The decrease in cell wall extensibility (ϕ) has been reported for both maize roots (Neumann et al., 1994) and leaves (Cramer and Bowman, 1992; Neumann, 1993) under a long-term salt stress. Several previous reports have suggested that Y of growing root and leaf tissues may increase in response to osmotic stress or water stress (Hsiao and Jing, 1987; Randall et al., 1989; Pritchard et al., 1991; Cramer and Bowman, 1992; Neumann et al., 1994). Our preceding study (Chapter 6) has shown salt induced ion toxicity, deficiency, and/or ion imbalance in the growing leaf of wheat plants. Thus, the limitation of leaf growth by salinity during this stage is probably due to either mechanical properties of cell walls and/or ion toxicity, ion deficiency, or ion imbalance rather than due to the water deficit.

8.4 Conclusions

In conclusion, greater osmotic adjustment occurs in the elongation zone in the morning. Ions accounted for the major contribution to osmotic adjustment. The cations, anions, and sugars contributing to osmotic adjustment was about 21-30%, 15-21%, and 13%, respectively, in the elongation zone and about 28-39%, 22-24%, and 3-8%, respectively, in the mature tissue. The ions contributing to osmotic adjustment was mainly due to the net accumulation of K⁺ and Cl⁻ which in the elongation zone accounted for 36-47% and 34-43% of the total ions. Solutes build up by increasing net rate of osmoticum deposition and the reduction in growth under saline conditions. In this study, the limitation to plant growth probably was not due to water deficit caused by salinity.

8.5 References


9

General Discussion

9.1 Growth of Wheat in Response to Salinity

9.1.1 Effect of Salinity on Yield Parameters of Wheat

There is a close relationship between vegetative growth parameters, such as the number of leaves and tillers, and the dry weight of leaves, stems, and grains, indicating that salinity exerts significant effects during the early growth stages (Chapter 2). The reason for the highest sensitivity of the vegetative stage is that salt significantly affects the production of leaves and tillers (Chapter 4). In later growth stages higher salt levels in the root zone can be tolerated by wheat plants (Maas and Poss, 1989).

Grain yield of wheat is highly dependent upon the number of fertile tillers produced by each plant (Power and Alessi, 1978; Nerson, 1980). The data in Chapter 2 showed that salinity significantly reduced the number of spikes, which is in agreement with the report by Maas et al. (1994). Grain yield per spike was much less affected by salinity as compared with the number of spikes per plant. Under low and medium salinity, the reduction in the final grain yield was mainly due to the effect of salinity on the spike number per plant, whereas under high salinity, the grain yield was partly reduced by decreasing the spike number as well as by an effect on the differentiation of the spikelet and development of the spike during the period of spike emergence to anthesis and maturity (Chapter 2). These findings suggest that under saline conditions, a higher grain yield could be obtained by increasing the number of spikes per plant and/or the seeding density.

9.1.2 Effect of Salinity on Leaf Growth

Leaf growth of wheat is one of the most sensitive processes to salinity during the vegetative stage. Data in Chapter 4 demonstrated that salinity significantly delayed leaf appearance. Leaves 3, 4, and 5 of the main stem emerged about 3 to 4 days later at 120 mM NaCl than at 0 mM NaCl. Leaf appearance rate determines the production rate of potential tiller sites, i.e. axillary buds, while site
filling decreases the actual usage of those sites (Davies, 1974). Thus, the significantly reduced number of tillers per plant under saline conditions may be associated with delayed leaf appearance.

The leaf production of the main stem was significantly reduced by increasing salinity (Chapters 4, 5). At 120 mM NaCl, the final length of leaves 3, 4, and 5 in the main stem was reduced by about 14%, 22%, and 31%, respectively, as compared with the control treatment (Chapter 4). The leaf width significantly decreased as well with increasing salinity (e.g., about 30% reduced by 120 mM NaCl).

Leaf elongation of wheat is restricted to a small region at the base of the blade (Kemp, 1980), which is called the leaf elongation zone. The reduction in final leaf length can result from a reduced leaf elongation rate. The elongation rate is a function of the length of the elongation zone and the segmental elongation rate in the elongation zone. Thus, the reduction in leaf growth of wheat under saline conditions may be due to a reduced elongating rate and/or a shortened elongation zone. Salinity spatially affects cell elongation in the leaf elongation zone (Chapters 4, 5). Data of Chapters 4 and 5 show that the reduction in leaf elongation rate is due to decreasing segmental elongation rate, but not due to shortening the length of the elongation zone. The effect of salinity on the leaf growth became severe with increasing leaf number, which corresponded with the initiation of new tillers during the same period.

The study of the spatial distribution of water content in leaf 4 (Chapter 5) showed that the reduction in cross-sectional area mainly occurred near the ligule of wheat leaves. Under saline conditions, the slower expansion of leaf growth may be due to the limitation of the local net deposition rate of water. The inhibition of the secondary cell wall production by salinity may also be responsible for the greater reduction in DW content beyond the elongation zone.

9.2 The Effect of Macronutrients on Wheat Growth under Saline Conditions

The salt tolerance of wheat was significantly increased by increasing the macronutrient concentration under low fertility level and by using a high macronutrient level as compared with a medium macronutrient level at high salinity levels (100-150 mM NaCl) (Chapter 2). Growth or yield in salinized wheat plants seemed not to be limited at 0.2 and 1 x strength Hoagland nutrient solution by nutrients. Several other studies also showed only little improvement in yields in saline soils by increasing the nutrient level to the optimal nutrient levels (Grattan and Grieve, 1992; Feigin, 1985).

9.3 Mechanism of Salt Limitation to Wheat

Leaf growth was significantly affected by salinity in the dimensions of longitude and width. What causes the reduced leaf growth under saline conditions? Under saline conditions, soils contain extreme ratios of Na+/Ca2+, Na+/K+, Ca2+/Mg2+, and Cl−/NO3−. The growth inhibition of salinity could primarily be caused by either the osmotic or ionic components of salinity, acting either on biophysical or on metabolic components of expansive growth, and/or on both (Thiel et al., 1988).
9.3.1 Ionic Effect

Although a close relationship between yield components and mineral contents in leaves and stems were found at grain maturity (Chapter 3), it is still difficult to interpret the evidence of ionic toxicity and deficiency, since growth is mainly affected at early stages (Chapter 2) and reductions in growth may be related to expansive growth in the leaf growing zone.

The elongation zone is the strongest sink for mineral elements in the leaf tissue. Under saline conditions, the pattern of ion concentration along the leaf axis is altered (Chapter 6). The limitation of NaCl to leaf growth is mostly due to Cl\(^-\) toxicity in both elongating and mature regions. The greater net deposition rate of Cl\(^-\) causes higher Cl\(^-\) accumulation in the elongation zone for the plants with 120 mM NaCl. Low nitrate concentration and net deposition rate that may be caused by a low transport rate in the more mature region under saline conditions could also be responsible for the inhibition of leaf growth by salinity. Higher tissue Na\(^+\) may cause ion imbalance, but not direct ion toxicity in this study. A dramatic reduction in cross-sectional area near the ligule by increasing NaCl is probably due to high Cl\(^-\) and Na\(^+\) concentrations. Potassium, Ca\(^{2+}\), Mg\(^{2+}\), P, and total nitrogen are less plausibly responsible for the reduction in leaf growth in this study. Higher tissue K\(^+\) and Ca\(^{2+}\) concentrations (mmol kg\(^{-1}\) FW) with 120 mM NaCl than with 0 mM NaCl may be due to the presence of a high amount of Ca\(^{2+}\) available in the soil of this study.

9.3.2 Salt Effects on Carbohydrate Status in Leaves

The results in Chapter 7 demonstrate that there are distinct patterns in spatial distribution of carbohydrates along the leaf axis. Those patterns are affected by salinity. We suggest that the spatial distribution of hexoses may be associated with an increase in the demand for the osmotic adjustment and the highest activity in biosynthesis in the elongation zone and for the synthesis of nonstructural material in the zone located between 30 and 60 mm from the ligule. The change in spatial distribution of sucrose concentration may be related to water dilution, rate of conversion to fructan, the rate of sucrose transport, the rate of hydrolysis into glucose and fructose, and photosynthetic production in the exposed part of the leaf.

Decreased growth is primarily associated with a reduction in photosynthetic area rather than a reduction in photosynthesis per unit leaf area (Munns, 1993). In addition, the transport of photosynthesis in the phloem may be inhibited (Iyengar and Reddy, 1994). Thus, the amount of assimilates reaching the growing region may decrease with increasing salinity. The tolerance of photosynthetic systems to salinity is associated with a capacity to control ion distribution into the vacuole, away from cytoplasm and chloroplasts. In salt-sensitive species, this mechanism may break down at high salinity levels if the membrane function is affected (Noble and Rogers, 1994). Data show that salt limitation to growth is due to the utilization of carbohydrates in the elongation zone and their supply in the zone of a large deposition of secondary cell walls.
9.3.3 Osmotic Adjustment

The leaf turgor is not affected by increasing NaCl in this study (Chapter 4). In Chapter 8, data showed the increase in the osmolality between 0 and 120 mM was higher in the elongating tissue than in the growth media. Since the study was conducted during the period of linear leaf growth for both treatments, maintenance of effective turgor must be postulated for a constant rate of leaf elongation. Arif (1990) reported that in contrast to the mature cells of wheat plants, no decrease in turgor pressure (measured with a pressure probe) occurred in the elongating cells despite the decrease in growth of plants stressed with NaCl from 25-150 mM. This may indicate that plants probably do not suffer from water deficit in the elongation zone at this stage.

The maintenance of leaf turgor is probably due to osmotic adjustment by means of taking up solutes under saline conditions. Osmotic adjustment is regarded as an important adaptation of plants to salinity because it helps to maintain turgor and cell volume. Ions accounted for the major contribution to osmotic adjustment. The contributions of cations, anions, and sugars to osmotic adjustment were about 21-30%, 15-21%, and 13%, respectively, in the elongation zone and about 28-39%, 22-24%, and 3-8%, respectively, in the mature tissue. The contribution of ions to osmotic adjustment was mainly due to net accumulation of K⁺ and Cl⁻ which in the elongation zone accounted for 36-47% and 34-43% of the total ions. Solutes were built up by the increase in the net rate of osmoticum deposition and the reduction in growth under saline conditions (Chapter 8). A greater osmotic adjustment occurred in the elongation zone in the morning.

9.4 Conclusions and Outlook

In summary, grain yield of wheat plants was significantly reduced by increasing salinity, while increasing the macronutrient supply significantly increased salt tolerance at low macronutrient levels. It appears that leaf growth is one of the most sensitive processes to salinity during the vegetative stages. Inhibition of leaf growth by salinity is mainly due to the toxicity of Cl⁻ and deficiency of NO₃⁻. A low supply of carbohydrates to the region of secondary cell wall deposition beyond the elongation zone and lower utilization in the elongation zone may be also responsible for the reduction in leaf growth under saline soil condition. However, further studies may be required to identify whether under saline conditions, the limitation of growth is due to the utilization, or supply of carbohydrates, and/or both. Na⁺ toxicity and water deficit seem to play a lesser role in this study. Higher tissue K⁺ and Ca²⁺ concentrations (mmol kg⁻¹ FW) at 120 mM NaCl are probably due to the presence of high Ca²⁺ in the soil of this study.

Since leaf growth of wheat plants reacts very sensitively to salinity and the decrease in grain yield by salinity is closely related to the reduction in tiller and spike number, we suggest that under saline conditions the relationships between leaf growth and fertile tiller number need to be further investigated. Salinity significantly reduced not only leaf length, but also leaf width and mainly affected leaf width near the ligule. Are the salt effects on leaf width due to the reduced cell number in the growing leaf? In this study, the salt inhibition on leaf growth is probably due to the significantly increased Cl⁻ concentration and deceased NO₃⁻ concentration in the elongating leaf. Does high Cl⁻ (or low NO₃⁻) concentration in the elongating leaves lead to toxicity (or deficiency)
or affect other physiological processes of leaf growth? The reduction in leaf elongation probably is not due to the turgor affected by salinity. Therefore, it is necessary to investigate salt effects on cell wall properties in the elongating leaves in future studies. Since the interactive effects of salinity and macronutrient level on the wheat growth were conducted in hydroponics in growth chamber, the conclusions from this study should be verified in future field experiments.

9.5 References


Curriculum Vitae

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