The effect of NaCl salinity on nitrate and ammonium uptake and kinetics in wheat (Triticum aestivum L. cv. Gamtoos) and the partial amelioration of the salinity stress by supplemental calcium.

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ABSTRACT

A NaCl salinity gradient of 0 to 100 mM inhibited the biomass production, moisture content and water use efficiency of both nitrate- and ammonium-supplied wheat. The biomass production of the nitrate-supplied wheat was more reduced by the salinity gradient than the biomass production of the ammonium-supplied wheat. This was due to, in part, the differential effect of NaCl (50 mM) on NO_3^- and NH_4^+ uptake. Salinity (50 mM NaCl) decreased the $^{15}NO_3^-$ uptake, V_{max} of NO_3^- net uptake and total N content, especially in the shoot, of nitrate-supplied wheat. The kinetic study of NO_3^- uptake in salinized, nitrate-supplied wheat showed that NaCl was acting as a noncompetitive inhibitor which indicated that the effect of NaCl on NO_3^- uptake was at the site of the NO_3^- transporting apparatus. The $^{15}NH_4^+$ uptake of ammonium-supplied wheat was not inhibited by 50 mM NaCl, while the total N content, NH_4^+ depletion of the nutrient medium and V_{max} of NH_4^+ net uptake were reduced. This suggested that the stress of 50 mM NaCl increased NH_4^+ efflux.

An increase in calcium concentration (0.5 mM to 5.0 mM) but not potassium concentration (0.2 mM to 5.0 mM) caused an increase in biomass production, moisture content, plant N content, V_{max} of NO_3^- uptake, $^{15}NO_3^-$ content of the shoot and potassium content in salinized, nitrate-supplied wheat. In the same plants, an increase in calcium concentration partially excluded Na^+ ion entry. Calcium concentration had no effect on the biomass production, moisture content, N content or $^{15}NH_4^+$ uptake in salinized, ammonium-supplied wheat. An increase in potassium concentration (0.2 mM to 5.0 mM) resulted in an increase in the biomass production of salinized, ammonium-supplied wheat. This increase in biomass production was not correlated with any change in the NH_4^+ depletion of the nutrient medium nor with changed values of V_{max} or K_m of NH_4^+ net uptake.

It is suggested that the main effect of NaCl was one of membrane disruption and that high calcium concentrations (5 mM to 8 mM) ameliorated this disruption to the extent that NO_3^- uptake was partially restored and ion selectivity was maintained in nitrate-supplied plants.

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DECLARATION:

I declare that this thesis is my own, unaided work. It is being submitted in fulfilment of the requirements for a Masters of Science Degree (Botany) at the University of Cape Town. It has not previously been submitted for any other degree at any other university.

Signed by candidate

Heidi-Jayne Hawkins
1992:01:03

LIST OF ABBREVIATIONS:

A%E:	Atom % Excess over natural abundance of ^{15}N (0.36%)
a_i:	activity of ion i
ANOVA:	Analysis of Variance
AR:	Analytical Reagent
ATPase:	Adenosine Triphosphatase
$A_{(\lambda)nm}$:	Absorbance at a specific λ (nm)
$[Ca^{2+}]_{cyt}$:	cytosolic Ca^{2+} activity
CCCP:	carbonylcyanide *m*-chorophenylhydrazone
CEC:	Cation Exchange Capacity
CTC:	chlorotetracycline (antibiotic)
cv:	cultivar
$\Delta\mu_{H+}$:	Transmembrane proton electrochemical potential gradient
$\Delta\psi$:	membrane electrical potential
DES:	diethylstilbestrol
DNP:	dinitrophenol
EDTA:	ethylenediaminetetra-acetic acid
EGTA:	ethyleneglycol bis (β-aminoethylether) N,N,N',N', -tetra-acetic acid
dm:	dry mass
E_{max}:	sensitivity to transpiration (E)
ER:	Endoplasmic reticulum
fm:	fresh mass
G_s:	stomatal conductance to H_2O
I:	Ionic strength
$[IP_3]$:	inositol trisphosphate
K_m:	Michealis constant (giving substrate concentration at ½ V_{max})
LCA:	Leaf Chamber Analyzer
LR:	Laboratory Reagent
M:	Molarity or Molar (mol l^{-1})
mM:	Millimolar
nm:	nanometers
PEP:	phosphoenolpyruvate

PEPCase:	phosphoenolpyruvate carboxylase
PLC:	Parkinson Leaf Chamber
P_1:	external mass flow pump of the ADC LCA2
P_2:	internal mass flow pump of the ADC LCA3
P_{max}:	Photosynthetic maximum
ψ_p:	turgor potential
RH:	Relative humidity
r^i:	ionic radius
RuBP:	ribulosebisphosphate
RuBPCase:	ribulose*bis*phosphate carboxylase
RWC:	Relative Water Content
ψ_s:	solute potential
t½:	half time
ψ_w:	water potential
UV:	Ultraviolet irradiance
V_{max}:	maximum enzyme activity where V_o is the initial activity
WUE:	Water Use Efficiency
[X]:	concentration of X (used in the figures and tables)

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1. INTRODUCTION

1.1 Background: The need for research relating to salinity stress physiology in mesophytic plants

Soils afflicted with mineral conditions which are stressful to mesophytic plant growth occupy approximately 30% of the world's land (Epstein and Rains 1987). Naturally occurring areas of salinity include: coastal areas with a geology of oceanic origin, bedrock containing a high mineral content, and arid to semi-arid areas. Saline soils may also occur due to the interference of man (desertification, use of poor quality irrigation water and drainage water) which may initiate a saline soil or exacerbate the naturally occurring tendency towards a saline soil. The climate, geology and overpopulation of South Africa have led to the existence of such saline soils, particularly manifest in parts of the Orange Free State and Eastern Cape. On a global scale, the problem occurs wherever there is a combination of, or any one of a susceptible geology or climate, over exploitation of agricultural land and overpopulation. Since most crops have low to moderate tolerance of saline environments (Flowers and Yeo, 1986), research in the area of mesophyte salinity physiology is particularly needed.

Much of the brackish, marginal soil areas of the world are inherently fertile and thus represent a potential resource (Lahaye and Epstein, 1969) as do the saline aquifers often found in arid areas. If a strategy could be found to ameliorate the effect of high salinity on growth, perhaps these marginal areas could be utilized agriculturally. The available strategies appear to be:

i) improved crop management with respect to the nutrient regime supplied to mesophytic plants,
ii) and/or the development of transgenic salt tolerant crops.

Both of these strategies require further basic knowledge of salinity stress physiology in mesophytic plants especially with respect to the implementation of ii), since salt tolerance is a polygenic trait requiring the interactive study of plant genetics and plant salinity physiology.

Although there exists genetic variation in salt tolerance within and between species, allowing potential for the development of salt tolerant crops, the success of this approach has been confounded by several factors:

i) most agriculturally important crops have moderate to low salt tolerance,

ii) despite advances in plant breeding, somatic hybridization and recombinant DNA technology, present research has been unable to adequately breed, culture or genetically recombine the main crops so that they have halophytic traits while maintaining the agronomic traits of the phenotype,

iii) mechanisms of salt tolerance on both the cellular and organismic level have not been adequately elucidated (Epstein and Rains 1987).

1.2 The nature of saline soils and general plant response

The predominant ions in saline soil water are sodium, calcium, magnesium, bicarbonate, chloride and sulphate. In the review of the literature relating to the effects of salinity on mesophytic growth, mostly NaCl (sodic) salinity will be considered. A saline-sodic soil is defined as having an ionic strength of not less than 50 to 70 mM NaCl (Greenway and Munns, 1980). However, since plant response to sodic salinity is highly variable between species, effects have been reported at as low as 25 mM NaCl (Hoffman *et al.*, 1985).

Most plants respond to the total osmotic effect of these ions, while some herbaceous and most woody species are also susceptible to ion toxicity effects and the nutritional and/or water imbalances and deficiencies that salinity may induce (Maas and Hoffman, 1977). The effect of salt may be direct, affecting physiological functions, or indirect by means of creating an unfavourable cationic soil environment and altering the availability of ions (Rengasamy, 1987).

Adding to the problem of salt tolerance (especially with respect to the genetic approach) is the fact that not only do the quantity and quality of ions vary in saline soils but the amounts vary temporally and spatially as well. The quality of salinity varies geographically in that the ions Na^+ and Cl^- may be replaced in their salinity effect by Ca^{2+}, Mg^{2+} and SO_4^{2-} in a specific saline soil (Epstein and Rains, 1987). Since salinity varies spatially the agronomic practice of leaching salt below the root zone with excess irrigation water is common, especially in clay soils where the residual salt content is higher than for sandy soils. However, leaching leads to the problem of saline drainage water disposal. It is possible that this water could be used if the right nutrient regime for a particular crop were used. Some practices, such as drip irrigation will actually increase saline nonuniformity (Rendig and Broadbent 1985). Other types of irrigation other than drip-irrigation (eg. mist irrigation) can avoid nonuniformity but are very wasteful for a saline area where a linked or causative factor is the shortage of water itself. Although it is possible that the evaporation of water from the soil surface may be decreased by mist irrigation in a cool environment, in an arid to semi-arid environment the water may evaporate on, or before reaching the soil surface. The former could even exacerbate the existing salinity level by concentrating ions in the root zone after salts had been drawn up by capillary action.

Soil salinity varies not only temporally (crops may be successfully planted after rains have leached excess salt from the soil surface) but also the inherent salt tolerance within one plant may vary within a life-cycle. The seedling stage is usually the most sensitive and germination usually requires a nonsaline environment. Plants showing this temporally variable response to salinity are often members of either Poaceae or Fabaceae (Maas and Hoffman, 1977). Clearly the variable physical features of the environment and the variable response of plants to salt, combine to complicate management of crops in the field as well as genetic manipulation.

Interactive research between not only molecular biologists, plant breeders, and biochemists, but equally among plant physiologists and soil scientists is required to further characterize tolerance mechanisms. The only test of a successful genotype would be its proliferation in the field under proper agronomic management.

1.3 Objectives and approach of this study

Many natural and agricultural areas which are saline are also nitrogen poor (Seemann and Sharkey, 1986) and the form of N appears to have an effect on the salinity tolerance of C_3 and C_4 plants (Lewis *et al.*, 1989). Thus, an interactive study, incorporating the effects of NaCl salinity and N form was undertaken on **Triticum aestivum** L. cv. Gamtoos, a C_3 crop plant which has an intermediate to low salinity tolerance. It was confirmed in 1986 by Harvey and Thorpe that **Triticum aestivum** (soft wheat) is unable to compartment ions efficiently within leaf vacuoles, as may be done by halophytes, when under salinity stress; this species is thus suitable for investigating both salinity effects and potential ameliorating strategies.

1.3.1 Objectives of the study

The objectives of the study were as follows:

i) to determine the effects of NaCl salinity stress on the biomass production, moisture content and gaseous exchange in both nitrate and ammonium-supplied plants;

ii) to determine the extent of salinity effects on the uptake, kinetics and translocation of nitrogen (NO_3^- or NH_4^+) in wheat (**Triticum aestivum**), a C_3 plant;

iii) to test the effect of a calcium or potassium supplemented nutrient regime on the biomass production, moisture content, ionic content, gaseous exchange and nitrogen (NO_3^- or NH_4^+) uptake, kinetics and translocation within salt stressed plants;

iv) to establish whether any correlation could be drawn between the applied supplement concentration and the resultant plant biomass production, moisture content, gaseous exchange and uptake, kinetics and translocation of N within salt stressed plants

v) and to propose a mechanism for any amelioration observed.

1.3.2 Scientific approach to the study

The material used for the study was whole wheat plants so as to obtain an indication of the gross effects of a saline nutrient medium on the growth of a C_3 plant. The plant material was cultured hydroponically to exclude confounding effects of a soil matrix on the results obtained. The growth period for each experiment conducted extended over a period of 27 days.

Biomass production, moisture content and gaseous exchange of the plants were all determined by conventional means utilized currently by the Nitrogen Laboratory (UCT), under the direction of Prof. O.A.M. Lewis. The uptake of NO_3^- or NH_4^+ was determined by ^{15}N feeding of the specific isotope, harvest and analysis. The kinetic parameters were determined by nutrient depletion studies using a spectrophotometric technique, and the ion analysis was done using flame photometry.

The thesis was aimed at elucidating the primary effect of salt stress and the implications of NaCl salinity for NO_3^- or NH_4^+ uptake at the whole plant level. The study also tested the potential of Ca^{2+} and K^+ to act as ameliorative agents against salinity stress for the growth of this species of wheat, the application of which may prove useful for crop management in saline and/or arid areas.

2. LITERATURE REVIEW

2.1 The primary and secondary salinity stress events

The primary effect of salinity stress is thought to be mediated via the interaction of NaCl with the plasmalemma and intracellular membranes ie. the sites of ion uptake, exclusion and delimitation (Greenway and Munns, 1980; Cramer *et al.*, 1985; Flowers and Yeo, 1986). In general, the secondary effects of salinity stress in mesophytic plants are manifest in symptoms similar to those seen due to water stress, except that there may be ion toxicity and ion competitive effects as well. It should be noted that the reduction in water potential caused by water stress and salinity stress may be different in nature since in the former the reduction is due to the matrix potential while in the latter there is the additional factor of the solute potential.

2.1.1 The primary salinity stress event

The primary salinity stress event will be discussed with respect to the Na^+ ion and Cl^- ion where the ionic origin of the effect is known, or the NaCl salt where the ionic origin is not known.

2.1.1.1 Primary effects of the Na^+ cation and the NaCl salt

With respect to the membrane porosity, Thomson (1985) found that sodium, which has an ionic radius (r^i) of 9.7×10^{-11} *m* can rapidly and passively enter the cowpea plasmalemma. The passive influx of Na^+ was estimated by Packer *et al.*, (1985) as being $2.3 \times 10^{-13}\, mol\ cm^{-2}\ s^{-1}$, while the active uptake of sodium (competing with K^+ uptake) was first noted by Epstein (1961). Elevated concentrations of Na^+ have been shown to displace Ca^{2+} (Na^+ has a similar r^i to Ca^{2+}) and K^+ ions, due to monovalent ion competition, at the plasmalemma surface and to depolarize the membrane potential (Cramer *et al.*, 1985; Cramer and Läuchli, 1986; Läuchli, 1990). Lynch *et al.*, (1987) found in their work with Zea mays root protoplasts that the Na^+-mediated Ca^{2+} displacement from root plasmalemmas was Na^+ specific. They suggested that the NaCl effect was due to the increase in ionic strength of Na^+ in the external salt solution. Ionic strength, which is a measure of the electric-field intensity of a solution, could affect the association of calcium ions with membranes in several ways:

i) by charge screening of anionic Ca^{2+} exchange sites on the membrane,

ii) by changing the structure or organization of the membrane through alteration of the electrostatic interactions between membrane components,

iii) by changing the activity of Ca^{2+} ions in the treatment solution (Lynch *et al.*, 1987).

If Na^+ displaces Ca^{2+} at a binding site (phospholipid or protein), the conformation of the binding site could be changed since Na^+ has a different charge density, thus preventing access by Ca^{2+}. Hauser *et al.*, (1976) suggested that the binding of a given cation to negatively charged phospholipid surfaces is primarily determined by the charge density and largely independent of the conformation and hydration of the different polar groups. They listed the order of binding of some cations to be:

$$Ca^{2+} > Ba^{2+} >> Li^+ > K^+ \cong Na^+$$

Results by Cramer *et al.*, (1985) do not reflect this trend, so that it is possible that membrane-associated Ca^{2+}/Na^+ are binding to ligands other than phospholipids, for example proteins.

With respect to the depolarizing effect of Na^+; since plasmalemma polarization is a driving force for the transmembranal transport of many ions (Watad *et al.*, 1991), salinity can seriously impair this function. Also, according to Thomson (1985), excess NaCl salt appears to affect the density and the depth of intramembranal proteins on the bilayer, possibly through the hydrophilic/hydrophobic properties of the proteins or by affecting the chemical properties of water at the membrane surface. This may interfere with the efficiency of transporter systems. This has multiple implications for both the ion uptake potential, and thus nutritional status, and metabolic efficiency of the plant.

Lastly, excess NaCl may indirectly affect the plant at the level of the hydroponic nutrient solution and the soil solution. Ion interactions (eg. formation of ion pairs and precipitation) increase with increasing ion concentration so that qualitatively, a charged ion becomes less active in a more concentrated NaCl solution because its movement is diminished by the stronger NaCl electric field present (Cramer and Läuchli, 1986). The concept of ion activities takes into account long range (> 0.5 *nm*) electrostatic interactions among ions while ionic strength effects determine short range (< 0.5 *nm*) interactions. In this way the NaCl concentration of the exogenous medium could limit the uptake of specific nutrient ions, especially if they are present in low concentrations. Cramer and Läuchli (1986), in support of their theory, showed that by increasing the NaCl concentration and thus the NaCl activity in solution, the activity of the Ca^{2+} ions present did indeed decrease.

2.1.1.2 Primary effects of the Cl^- anion

Chlorine, with a $r^i = 1.81 \times 10^{-10}$ m, is larger than Na^+, but Cl^- may still passively enter the plasmalemma (Thomson, 1985). An immediate effect imposed on the plasmalemma by excess Cl^- may be that effect exerted on the hydrophobic bonding of the amphipathic macromolecules of the plasmalemma (phospholipids and proteins), which make a major contribution to membrane stability. The driving force for this stability is the result of the thermodynamically unfavourable interaction of the apolar components of these macromolecules (eg. hydrocarbon tails) with H_2O, as the interaction is associated with an entropy decrease of 10 to 20 *eu mole*$^{-1}$ (Hatefi and Hanstein, 1974). It would be expected that the disordering of water would weaken the hydrophobic bonds and destabilize membranes components as well as other biological macromolecules, including nucleic acids. Such a disordering may be mediated by so called chaotropic ions, such as chlorine. Chaotropic ions are defined as low charge density ions (ie. large radius, single charge) which are, when hydrated, associated with an increase in the entropy (ΔS) component of the free energy (ΔG) of H_2O (Aviram, 1973). Although chlorine is less chaotropic than for instance, Br^- or I^-, due to their larger radii respectively, excess Cl^- would have a destabilizing effect on membrane integrity. Membrane porosity would also be directly affected due to the weakening of hydrophobic bonds between phospholipids, thus exacerbating the influx of Na^+ ions and their destabilizing effect.

Chloride has been shown to compete with NO_3^- for uptake in a limited number of species such as barley and Italian ryegrass (Ullrich, 1987) and Rhodes grass (Kafkafi, 1991).

2.1.1.3 Summary of primary effects of NaCl salinity

In summary, the immediate consequences of the primary NaCl influx are thought to be:

i) a general increase in the membrane porosity (Epstein 1961; 1969; Van Steveninck 1965; Watad *et al.*, 1991) and the destabilization of membranes and proteins (mediated by Cl^-) due to a weakening of hydrophobic bonding (Hatefi and Hanstein, 1974),

ii) the displacement of, and inhibition of Ca^{2+} influx and enhancement of Ca^{2+} efflux (Cramer *et al.*, 1985; Cramer and Läuchli, 1986; Lynch *et al.*, 1987),

iii) increased Na^+ influx and

iv) enhanced K^+ leakage from cells due to membrane depolarization (Läuchli, 1990),

v) possible effects on the efficiency of ion transporters (Ward *et al.* 1986; Lewis *et al.*, 1989),

vi) a lowering of the ion activities within the nutrient medium (Cramer and Läuchli, 1986), further contributing towards possible nutrient deficiencies and exacerbating ion displacement by Na^+.

Membranal displacement of calcium ions at the surface of the plasmalemma cannot account for the entire mechanism of salt toxicity, but it is almost certainly one of the primary events allowing a large influx of NaCl into the cytoplasm (Cramer *et al*, 1985; Läuchli, 1990). The ionic interactions of NaCl as well as ion compartmentations subsequent to this primary Na^+ influx event are likely to be complex. The importance of Ca^{2+} concentration (exogenous and endogenous) in mesophytic salinity tolerance will be discussed in section **2.4**.

2.1.1.4 Salinity tolerance in halophytes compared to mesophytes

Generally, in halophytes the demand for ions in the shoot is not exceeded by that supplied from the root and the concentration of apoplast ions is not greater than the symplast concentration, while the opposite is true for mesophytes (Jennings, 1976; Flowers and Yeo, 1986). Thus, for mesophytes the deleterious effects may be due to either dehydration of the symplast, due to a higher solute potential in the external medium and/or apoplast, or an excessive ion concentration in the symplast causing ion toxicity. Several differences in tolerance strategy account for the success of halophytes over mesophytes.

Halophytes have a high NaCl uptake under saline-sodic conditions with a simultaneous sequestering of the salt into the vacuole (compartmentation). The ionic balance may be maintained by inorganic salts, or by organic solutes derived from photosynthate so that NaCl concentration in the cytoplasm is maintained at a low level (Jennings 1976, Greenway and Munns 1980). It is the very rapidity with which NaCl may enter the symplasm that partially accounts for the success of halophytes since the osmotic effects from a hypertonic medium are avoided. Evidence for the sequestering of excess ions has been found using transmission analytical electron microscopy (TAEM) on Suaeda maritima (Harvey *et al.*, 1981). Some nonhalophytes such as some species of wheat and sorghum, have also shown a positive osmoregulatory response by accumulating Na^+ into the vacuole (Devitt and Stolzy 1985). In general, this sequestering mechanism appears to be much less efficient in mesophytes. It is the compartmentation of the excess ions into the vacuole and the subsequent limiting of the concentration of Na^+ or Cl^- ions reaching the young metabolizing and photosynthetic tissue which prevents major metabolic disruptions. Young actively metabolizing tissue (germinating embryos, expanding leaf tissue, buds and fruit) is especially sensitive to salt stress. Germination is affected since one of the secondary effects of salinity stress is osmotic stress (which causes dehydration) and a certain hydration level is required for germination (Thomson, 1985).

Protection of the mesophyte shoot occurs by exclusion of sodium from the transpirational stream and export via the phloem. In the root a sodium gradient may exist caused by the barrier of the endodermis and an antiport may exist in the root tips of some species (Flowers and Yeo, 1986). In a halophytic cyanobacterium, Na^+ extrusion was proposed to be due to an energy dependent H^+/Na^+ antiporter driven by a trans-cytoplasmic membrane pH gradient (Packer *et al.* 1985). An antiporter was also suggested to exist in Hordeum vulgare (barley) root tips (Ratner and Jacoby, 1976). In Zea mays (maize) this Na^+ extrusion mechanism appeared to be localized within the root cortex (inner cortical cells or xylem parenchyma) thus removing the Na^+ from the transpirational stream, while older root parts retained Na^+ in the vacuoles in a similar manner to halophytes (Läuchli and Epstein 1985). Ionic concentration in nonhalophytes however, tends to differ spatially. This is accomplished by Na^+ exclusion pumps at the plasmalemma, the suberized barrier of the endodermis, exclusion of sodium from the xylem/transpirational stream, sequestration into the vacuole or the hydraulic sealing of leaves to maintain water potential gradients between tissues/organs. Consequently, there is the danger of water deficit in expanding tissue and ion excess in expanded tissue (Greenway and Munns, 1980; Pammenter and Smith, 1983; Terry and Waldron, 1985). This spatial difference in ion concentration is avoided in halophytes.

The ionic balance in the cytoplasm may be maintained by ions (eg. chloride) or nitrogenous compounds (so-called "compatible solutes") in mesophytes and halophytes. Alternatively, some halophytes use the exogenous salt of the medium as an osmoticum (Dawes, 1981). Organic acids or "compatible solutes", being derived from photosynthate, are harmless to enzymes even at high concentrations, although there is little evidence that such solutes actively protect enzymatic reactions.

Unlike nonhalophytes (where the principle cation is K^+), halophytes may utilize Na^+ instead of K^+, thus sparing the latter ion for metabolically important processes such as the transport of NO_3^- and pyruvate kinase synthesis (Jensen and Petterson, 1978) while utilizing some of the excess of Na^+ ions. Sodium ions also appear to important for NO_3^- uptake in C_4 plants (Ohta *et al.*, (1988).

While some halophytes are more effective at obtaining K^+, levels not toxic to nonhalophytes prove to be so for some halophytes (Rush and Epstein 1981). Under saline conditions both halophytes and nonhalophytes tend to maintain a high K^+:Na^+ ratio while a high Ca^{2+}:Na^+ ratio is especially important for nonhalophytes. Nonhalophytes may maintain a high K^+:Na^+ ratio at low ionic strength (5 to 7 mM) of NaCl, after which Ca^{2+} becomes important for the maintenance of ion selectivity.

Another strategy specific to halophytes is that of succulence. This involves the accumulation of solutes solely from the external medium which lowers the internal plant water potential thus maintaining turgor and reducing water loss from the cell (Dawes, 1981). Succulence geometry apparently may also increase CO_2 fixation due to decreased CO_2 resistance in the guard cells (Greenway and Munns 1980).

Lastly, it has been shown that the root plasmalemma lipid composition is an important factor determining the salinity tolerance of different species or cultivars (Kafkafi, 1991). The composition of root cell membranes not only affects cation selectivity, but also the enrichment of some grape plasmalemmae with phospholipid as opposed to monogalactose diglyceride has been positively correlated with chloride exclusion and enhanced salinity tolerance.

2.1.2 The secondary salinity stress events

All secondary salinity stress events are osmotic and/or ionic in nature. Any further metabolic disturbances originate from these two major effects of salinity stress (Greenway and Munns 1980, Terry and Waldron 1985, Kingsbury and Epstein 1986, Lewis *et al.* 1989).

2.1.2.1 The ionic effect

The ionic effect, apart from being a primary event, may also be a secondary event depending on the site of salinity interference. The ionic effect is operative mainly by interfering with ion uptake or causing ion toxicity. NaCl may compete with or displace other ions at the root surface, leading not only to nutrient deficiencies but also to metabolic and structural disruption once the salt has entered the symplasm. *In vitro* measurements of cytoplasmic enzyme activities and protein synthesis have shown the need to maintain NaCl concentrations below about 150 mM in the cytoplasm (Harvey and Thorpe, 1986). Salt concentration affects enzyme activity as well as mRNA activity and polypeptide synthesis (León *et al.*, 1990; Ho and Mishkind, 1991; Schmitt, 1990; Ramagopal and Carrusda-Ars, 1991). Evidence exists for ionic effects on many enzymes, for example, the two carboxylating enzymes (Passera and Albuzio, 1978) and the proton pumping H^+-ATPase (Gronwald *et al.*, 1990). A case for specific ion[1] toxicity was found for wheat (**T. aestivum**) by Kingsbury and Epstein (1986), where this line of wheat was more sensitive to the Na^+ ion than the Cl^- ion.

2.1.2.2 The osmotic effect

The osmotic effect occurs as a result of the high solute potential(ψ_s) of the external medium causing a lowered cellular internal water potential (ψ_w) with a subsequent loss of turgor (ψ_p) within the plant cell. Loss of turgor results in a lack of cell wall extension and subsequently, reduced growth. This reduced growth is due mostly to decreased cell enlargement and not decreased cell division (Greenway and Munns, 1980; Schnapp, 1989). It has been found, however, that chloride ions may reduce cell division in epidermal cells of nonhalophytes (Greenway and Munns, 1980). Although growth reduction is associated with lowered internal plant ψ_w, wilting is less evident in salt stressed plants than in water stressed plants at equivalent water potentials (Terry and Waldron 1985, Hsiao, 1985). This is due to the partial acquisition of solutes directly from the external medium by plants in a saline environment.

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[1] *"Specific ion" refers to the ionic properties of the ion as opposed to the osmotic properties of the ion (Kafkafi, 1991)*

Theoretically, water stress induced by an excess of exogenous ions, as opposed to a deficit of water, allows a more energetically efficient tolerance of water stress, since water stressed plants have to synthesize organic solutes for the purpose of osmoregulation. However, the actual impact of organic solute synthesis would depend somewhat on both the availability of nutrients in the environment and the inherent ability of the plant to acquire nutrients within such an environment as well the ability of the plant to make use of available inorganic solutes for osmoregulation.

It should be mentioned that cell wall extension is not only dependent on turgor pressure and it has been shown that in mung bean roots, cell wall extension was resumed before the turgor pressure had been fully regained during NaCl-adaption (Kafkafi, 1991). This author proposes that the ionic component of the salt stress, by affecting the negative surface charge of the root membrane and the calcium binding capacity of the cell wall, allows the stressed cell wall to yield to lower turgor pressures, thus allowing cell wall extension. If the concentration of solutes in the exogenous medium affect the resumption of turgor and growth in salt stressed plants, then reduction in turgor and growth are not specific indicators of solely an osmotic effect and may involve an ionic component as well.

The most reliable indicator of the growth reducing osmotic effect is that it is offset by increasing the relative humidity, (Jennings 1976). Both ionic and osmotic effects of salinity stress have been noted for clover, barley and wheat (Termaat and Munns, 1986).

2.1.2.3 Secondary salinity stress effects on metabolism and ultrastructure

Most other responses, apart from those caused directly by water deficit and ionic effects, appear to be caused by multiple effects or injury.

Harvey *et al.*, (1986) reported that in wheat mesophyll cells, under a salinity stress of 100 mM NaCl, the Na^+, K^+ and Cl^- concentrations in the cytoplasm were too high to be compatible with biochemical activity. Secondary effects caused by salinity stress include those on photosynthetic rate (Terry and Waldron 1985), enzyme activity, nucleic acid and peptide synthesis, N and Pi uptake and metabolism, enzymes of C metabolism, Na^+:K^+ ratios and Ca^{2+} nutrition (Lauchli and Epstein, 1985).

The influence of salinity on enzymes *in vivo* appears to be variable. Cation stimulated ATPases (necessary for oxidative phosphorylation and ionic transport processes) have been observed only in mangroves and sugarbeet which are salt tolerant plants. There have been suggestions that ATP may be synthesized by reversal of the sodium ion pump at the plasmalemma (Noggle and Fritz, 1983).

The interaction between salinity stress and hormones is also an area of uncertainty. A "brake" on cell expansion has appeared after salinity stress in some halophytes, where abscisic acid may be a stress signal from the shoot to the root in order to prevent toxic/osmotic effects of salt affecting the photosynthetic apparatus (Jennings, 1976; Greenway and Munns, 1980).

Salinity has had observable effects on cell ultrastructure. This has been reported by several researchers cited by Harvey *et al.*, (1986). These effects include swelling of the golgi bodies and the lumen of the endoplasmic reticulum (ER), an increase in rough ER and the numbers of mitochondria and ribosomes in **Zea mays** and **Triticum aestivum**. Damage to root and cortical cell mitochondria, the condensation of nuclear chromatin and a change in chloroplast ultrastructure have been reported in barley. Harvey *et al.*, (1986) reported that in wheat mesophyll cells, 100 mM NaCl caused the volume fractions occupied by the cytoplasm and chloroplasts to increase.

2.1.3 The effect of physical factors on salinity stress

Physical factors affecting plant response to soil salinity include temperature, relative humidity, soil aeration, soil matric potential, water table level and leaching fraction. The osmotic effects of salinity stress are somewhat alleviated by increasing the ambient relative humidity (Terry and Waldron, 1985) as shown in **Beta vulgaris** (sugarbeet), while osmotic effects are aggravated by high temperatures (>28°C to 30°C) (Jennings, 1976).

Physical effects are often additive to the existing nutrient and salinity status of the soil and may be difficult to separate from the effect they might have on plant growth in the absence of salinity. For example, sulphate salinity and poor root aeration severely interfere with Na^+ exclusion from the shoot (Lauchli and Epstein 1985), but poor root aeration may induce other effects on plant metabolism so that the reduced sodium exclusion is only a secondary effect of a restricted metabolism. Devitt and Stolzy (1985) found that the response of wheat and sorghum to saline irrigation water was more favourable in soils having a high Cation-Exchange-Capacity (CEC). This may be due to the fact that clay-loam and montmorrillonite clay show a preference for Ca^{2+} ions which have beneficial effects for both the soil (by coagulating soil colloids) and the plant under saline conditions.

2.1.4 Ameliorating salinity stress with specific nutrients

The K^+ ion, according to Devitt and Stolzy (1985) and Cramer *et al.*, (1985), and the Ca^{2+} ion, according to Epstein (1960), Huffaker and Rains (1985), Ward *et al.*, (1986), Lewis *et al.*, (1989), Nakamura (1990) and Läuchli (1990) are known to ameliorate salinity stress. Although soil fertility interacts with salinity to affect apparent increased tolerance (Maas and Hoffman 1977) it is essential to investigate both the specific nutrients which have ameliorative effects and the mechanism behind such effects. Ultimately, these findings could be applicable to plant physiological responses in the soil-plant-air continuum. The use of saline drainage water from irrigated land, the disposal of which has presented problems, has already been tried on a block rotation basis for cotton (salt tolerant), wheat, (intermediate tolerance), and alfalfa (sensitive) as well as on a successive-crop basis for wheat, sugarbeet and melons (Rhoades and Bingham, 1985). Manipulation of the nutrient regime may further increase the plant biomass produced as a result of such a regime.

The following sections of the **Literature Review** will deal with the effects of salinity on N and C metabolism as a whole, as well as the ameliorating potential of Ca^{2+} and K^+ for salinized plants.

2.2 Nitrogen metabolism and effects of salinity interference.

In terms of quantities, nitrogen is required in the largest amounts of all the nutrients and is secondary only to carbon in its importance for plant growth. Any factor which disrupts N metabolism would have obvious effects on plant growth since nitrogen forms an essential component of nucleic acids, which control structure and behaviour from the molecular to the organismic level, as well as numerous secondary products and amino acids which regulate biological processes (protein, enzymes and coenzymes).

2.2.1 Nitrogen forms and availability

The form of the nitrogen ion itself has multiple and integrated effects on photosynthetic parameters and related structural features of the plant. In the soil, after microbial mineralization of complex organic material; ammonification and nitrification; NH_4^+ and NO_3^- become available for plant utilization. The ionic ratio thereof is dependent on the relative amounts of microbial mineralization, nitrification and plant utilization. Nitrogen is a very mobile element in the soil and the N cycle is sensitive to environmental variables, the availability being tempered both seasonally and spatially. Ultimately, both forms of N may be lost by denitrification. Thus N is often limiting in ecosystems, especially in arid and/or saline ecosystems (Seeman and Sharkey, 1986).

2.2.1.1 Availability, advantages and disadvantages of NO_3^- nutrition

Since NO_3^- is a negatively charged ion, the diffusion thereof is quite unrestricted, making it more accessible for uptake by plant roots. The unrestricted diffusion of NO_3^- also means that NO_3^- is susceptible to being leached out of the rhizosphere. Nitrate is the most common ionic form of nitrogen found except when the soil is acidic or particularly micaceous, where ammonium may predominate. Advantages of NO_3^- nutrition besides availability include:

i) NO_3^- is non-toxic to plants,
ii) NO_3^- assimilation is commonly in close proximity to the photosynthetic machinery which produces the required C-skeletons,
iii) the absorbtion of cations (especially Ca^{2+}, Mg^{2+} and K^+) is favoured under NO_3^- nutrition, possibly due to the exchange of NO_3^- with HCO_3^-, thus raising the soil pH which favours cation uptake,
iv) also it has been shown that NO_3^- nutrition enhances plant productivity under saline conditions (Kirkby and Knight, 1977; Lewis, 1986).

Nitrate nutrition is generally energetically expensive for the plant and potential disadvantages include:

i) NO_3^- must be reduced to NH_4^+ (requires 347 $kJ\ mol^{-1}$),
ii) absorbtion is ATP-requiring, making uptake dependent on oxidative phosphorylation and thus sensitive to anoxia (Lewis, 1986)
iii) also, to maintain electroneutrality once NO_3^- has been taken up, the amounts of organic acid anions are changed, (notably malate and oxalate); these organic acids

may be responsible for complexing Fe and certain trace elements leading to deficiency under NO_3^- nutrition (Lewis, 1986).

2.2.1.2 Availability, advantages and disadvantages of NH_4^+ nutrition

The NH_4^+ ion tends to remain bound to negatively charged soil micelles, so that it is less susceptible to leaching but is also less available to plants. NH_4^+ may be lost by NH_3 volatilization, if the pH >8. Advantages of NH_4^+ nutrition include:

i) NH_4^+ does not require reduction prior to incorporation into organic compounds,
ii) metabolic inhibitors and anoxia have less effect on NH_4^+ uptake since NH_4^+ acquisition may be energy independent;
iii) due to H^+ excretion upon NH_4^+ uptake and the subsequent lowering of the rhizosphere pH, the uptake of phosphate as HPO_4^{2-} (as opposed to the H_2PO_4 form) is more easily facilitated (Noggle and Fritz, 1983; Lewis, 1986).

Disadvantages of NH_4^+ nutrition include:

i) the NH_4^+ ion can be toxic to plants, causing the uncoupling of photophosphorylation at as low as 2 mM, and acidification of the rhizosphere which retards root growth,
ii) NH_4^+ must be immediately assimilated into organic compounds (eg. glutamate and aspartate) in the root, to prevent toxicity. This requires that large amounts of C be immobilized into such compounds, placing a restriction on the amount of C allocated to structural and other requirements of the plant (Lewis, 1986; Lewis, Soares and Lips, 1986),
iii) ammonium nutrition suppresses the uptake of NO_3^-, Ca^{2+}, Mg^{2+} and K^+,
iv) and the shoot-root translocation system must provide the root based assimilation of NH_4^+ with adequate C-skeletons, and this potentially places a strain on the system (Lewis, 1986)

Within the plant, nitrogen is also readily redistributed. The degree to which nitrogen is accumulated and distributed in crops, as well as the form preferentially taken up depends on nitrogen supply, genotype and environmental factors (Hocking *et al.*, 1984). Preferential uptake of nitrogen forms (NO_3^- or NH_4^+) differs depending on the plant type (C_3/C_4) and may be also related to the water status of the rooting medium. Apparently, plants grown in hydroponics are less sensitive to the N ionic form compared to when their growth is in soil

(Noggle and Fritz, 1983). C_4 plants have a much more efficient dry matter production per unit N absorbed due to less N being allocated to RuBPCase (insoluble fraction 1 protein) (Noggle and Fritz, *op cit*). Since C_4 plants may accumulate greater amounts of CO_2 due to the bundle sheath enzyme PEPCase, there are potentially more C-skeletons available for N assimilation into amino acids.

2.2.2 Nitrogen ion uptake

The cytosolic compartment has a negative electrical charge, and depending on the charge of the ion being transported across the plasmalemma, the cell expends energy to maintain the pH of the cytosol and the negative membrane potential of the cell (Ullrich, 1987). Thus, according to the Nernst equation, the transmembranal transport of cations will be thermodynamically passive while the transport of anions will be "uphill" thermodynamically, and will require energy consumption (Noggle and Fritz, 1983). However, accumulation of either cations or anions may require energy input (Ullrich, 1987).

The absorbtion of both the NO_3^- and the NH_4^+ ions may be metabolically dependent on and coupled to H^+ gradients generated by plasma membrane (H^+ pumping) adenosine triphosphatases (ATPases). Fluxes of both the NO_3^- and the NH_4^+ ions are sensitive to feedback regulation (Clarkson, 1986). Since reduction and assimilation of N are apparently not affected by salinity stress (Huffaker and Rains, 1985) only N ion uptake shall be discussed.

2.2.2.1 NO_3^- ion uptake

Nitrate uptake follows Michaelis Menten kinetics, while some controversy exists over the existence of multiphasic NO_3^- carrier activity (Clarkson, 1986). Typically, the K_m of NO_3^- uptake is 17 to 50 μM (Ullrich, 1987; McClure *et al.*, 1990a), but both the K_m and V_{max} of NO_3^- uptake may change under conditions of N starvation (Clarkson, 1986). Two NO_3^- carrier forms are thought to exist, one high affinity constitutive carrier and one labile, low affinity inductive carrier formed by *de nova* protein synthesis (Clarkson, *op cit*). These carriers are thought to be distinct and to have dissimilar K_m. The inducible (low affinity) carrier is synthesized in response to the presence of NO_3^- in the medium, and after a lag period of ≈ 4 to 6*h*, NO_3^- absorbtion is increased by 2 to 5 times compared to that of the constitutive level of absorbtion.

NO_3^- uptake is unique in that it is induced by the presence of NO_3^-, as is the enzyme involved in NO_3^- assimilation, nitrate reductase (NR). The absorbtion of NO_3^- is associated with alkalinization of the growth medium and this has been explained in terms of an OH^-/anion exchange (Eisele and Ullrich, 1977), a NO_3^-/H^+ symport (Ullrich and Novacky, 1981; McClure *et al.*, 1990a and b) and a HCO_3^-/anion exchange (Ben-Zioni *et al.*, (1970). Ben-Zioni *et al.*, (1970) proposed the HCO_3^-/nitrate exchange model for nitrate uptake in which the decarboxylation of malate (from the shoot) yielded HCO_3^- in the root which could be excreted, so that effectively, at the root plasmalemma a HCO_3^-/anion exchange or antiport existed to maintain electroneutrality (Lewis, 1986, Clarkson, 1986). The model was founded on the basic premise that the reduction of NO_3^- in leaves establishes a surplus of cations and that this is neutralized by anionic acids, notably malate and oxalate. This was supported by the evident stoichiometric synthesis of malate with NO_3^- reduction. Potassium was thought to accompany nitrate up the shoot for reduction and malate down to the root for oxidation, thus forming an integral component in the shuttle system between root and shoot.

In a model by Kirkby (1974) it was suggested that after the oxidation of malate, carbon would be retained and the OH^- ions excreted instead of bicarbonate in exchange for nitrate ions taken up from the exogenous medium. To date a lack of correlation between NO_3^- uptake and OH^- efflux exists (Glass, 1988).

Ullrich and Novacky (1981) suggested that the free energy required for NO_3^- transport is derived from the $\Delta\mu_H{}^+$ and that a $2H^+:1NO_3^-$ symport is brought about by a NO_3^--dependent depolarization of the $\Delta\psi$ in Lemna gibba cells. Depolarization would only occur if more than one proton or hydroxyl entered or left respectively with NO_3^- uptake. Since the depolarization is greatest in mildly acidic solution and thus dependent on the number of protons, the $2H^+:1NO_3^-$ symport is favoured (Clarkson, 1986). The membrane depolarization or cytoplasmic pH changes associated with NO_3^- uptake possibly initiate increased proton translocation and thus membrane repolarization, through the membrane H^+-ATPase. There was doubt as to the existence of a symport, in view of the lack of inhibition of NO_3^- uptake by diethylstilbestrol (DES), an inhibitor of the H^+-translocating ATPase (Thibaud and Grignon, 1981). However, McClure *et al.*, (1990a and b) supported the model of Ullrich and Novacky (1981) by affirming the existence of a nitrate-inducible NO_3^-/H^+ symport in the roots of whole maize seedlings. McClure *et al.*, (1990a) also showed that a transient depolarization occurred with NO_3^- uptake due to a H^+/NO_3^- stoichiometry greater than one, and that the subsequent net hyperpolarization was due to stimulated proton pumping by the plasma membrane H^+-ATPase. The lack of inhibition by DES noted by Thibaud and Grignon (1981) was repudiated by the claim of McClure *et al.*, (1990a) that the time period of exposure to DES was insufficient to allow complete inhibition of NO_3^- uptake.

Hyperpolarization of the root plasmalemma has been found in Zea mays, due to NO_3^- entry (Thibaud and Grignon, 1981). Hyperpolarization suggests that not all the incoming negative charge is being balanced, so that the apparent stoichiometry is either an OH^-:2 NO_3^- antiport or a H^+:2 NO_3^- symport. The discrepancy between the Lemna and the Zea studies may be due to the difference in the NO_3^- concentration used for induction and uptake; the former study used a concentration where the influx would be via the high affinity constitutive system whereas the latter used a concentration (5 mM) where appreciable influx may have occurred via the low affinity system (Clarkson, 1986). Ullrich (1987) maintains that the same NO_3^-/H^+ cotransport mechanism is functional in several species but that different recovery kinetics occur for different species. At present, the NO_3^-/H^+ cotransport with excess protons seems to be the best explanation of NO_3^- transport.

The work of Deane-Drummond (1984a, 1984b, 1985) contrasts with both of the above proposals since in Chara corallina, NO_3^- influx was electrically silent. However, poor correlation was found between nutrient medium pH and NO_3^- uptake. Deane-Drummond (1984b) postulated a flux sensitive NO_3^-:NO_3^- exchange where the energetically "downhill" NO_3^- efflux would drive the energetically "uphill" NO_3^- influx. According to Granstedt and Huffaker (1982) the vacuole is the major storage pool of NO_3^-. Transport of the NO_3^- ion from the relatively dilute cytosol ($<$20 mM) across the tonoplast into the concentrated sap of the vacuole ($<$ 100 mM) would also necessitate active kinetics and in all likelihood, occurs in series with the plasmalemma influx.

Thus it seems likely that H^+-pumping ATPases and pyrophosphatases are required for the nitrate anion accumulation (plasmalemma and tonoplast) driven by $\Delta\psi$ or $\Delta\mu_H^+$. Release of NO_3^- is thought to be passive, both from the plasmalemma and the tonoplast. Nevertheless, Blumwald and Poole (1985) suggest efflux from the vacuole may be H^+-coupled, while Clarkson (1986) suggests efflux from the plasmalemma is H^+-coupled. The former evidence comes from the dissipation of an artificially imposed ΔpH by NO_3^- (and Cl^-) so that a H^+:anion antiport is proposed for uptake and a H^+:anion symport for the release. The saturable nature of the NO_3^- kinetics responsible for the dissipation of the $\Delta\psi$ is in agreement with the idea of specific ion channels or porters. Efflux of NO_3^- from the root may represent as much as 70% of the influx, depending on the concentration of the previous nitrate nutrition. The rapid rate of exchange of the cytoplasmic NO_3^- pool ($t\frac{1}{2}$ = ≈4 to 7 *min*) means that to measure influx alone, measurements must occur within ≈3 to 10 *min* (Clarkson, 1986).

Empirical kinetic evidence has defined transport systems for both nitrogen ion forms kinetically (Glass, 1988), but biochemical elucidation of the systems is yet to be achieved.

2.2.2.2 NH_4^+ ion uptake

The uptake of NH_4^+ is thermodynamically passive and is associated with a transient or even permanent depolarization of the plasmalemma and acidification of the nutrient medium. This suggests the existence of an ammonium uniport, where repolarization of the plasmalemma occurs via the proton extrusion pump, or a NH_4^+/H^+ antiport (Ullrich, 1987). Unlike NO_3^- uptake, the uptake of NH_4^+ is thermodynamically passive although accumulation may still be energetically dependant (Glass, 1988). Ammonium uptake is, however, limited by light and ammonium metabolism. In general, ammonium uptake kinetics resembles that of K^+ uptake in roots and has a K_m of ≈ 17 μM (Ullrich, 1987). Morgan and Jackson (1989) showed that in both wheat and oats, significant efflux of recently absorbed ammonium/ammonia occurs if ammonium is present in the exogenous medium.

In most plants the absorbtion of NH_4^+ ions is more rapid than NO_3^- (Lewis, 1986) and NO_3^- uptake is suppressed by the presence of ammonium. There are three ways in which ammonium may become inhibitory: NH_4^+ may inhibit the formation or activity of the nitrate carrier and NR or it may severely reduce the $\Delta\mu_H{}^+$ required for NO_3^- uptake by reducing the membrane potential (Ullrich, 1987).

It was shown for the green alga, **Ankistrodesmus falcatus** that simple Michaelis-Menten kinetics did not hold for NH_4^+ uptake. Ower and Cresswell (1986) proposed a multiphasic system of discontinuous kinetics (reported frequently in ion uptake of higher plants) for NH_4^+

influx. Like NO_3^- uptake, NH_4^+ uptake is sensitive to temperature but is less sensitive to cold temperatures than is NO_3^- absorbtion. Ammonium ion influx is also sensitive to pH (pH = 8 being the optimum) and C supply (Lewis, 1986), since C is required for the incorporation of NH_4^+ into amino compounds.

2.2.3 Salinity interference with nitrogen uptake and metabolism

Since the form of inorganic nitrogen appears to modulate plant response to salinity (Lewis *et al.*, 1989), the form of nitrogen in soil or hydroponics is pertinent to the salinity tolerance of the plant. Although cations at the cell surface should theoretically stimulate NO_3^- uptake, this is not the case for excess of the Na^+ ion due to the dispersive effect that monovalent cations have on the plasmalemma (section **2.1.1.1**) and presumably, the ion permeases. Adding to the weakening of membrane integrity would be the chaotropic effect of excess Cl^- (section **2.1.1.2**).

2.2.3.1 Salinity interference with N uptake

The limiting effect of salinity on nitrogen assimilation and thus growth, has been shown to be due to decreased uptake of NO_3^- and NH_4^+ and not due to decreased nitrogen reduction (Passera and Albuzio, 1978; Huffaker and Rains, 1985, Lewis *et al.* 1989). Considering the proteinaceous nature of the porters within the lipid bilayer of the membrane, and the disruption of this organization due to the presence of chaotropic ions and Na^+, it is likely that the effect of salt is at the site of NO_3^- and NH_4^+ transport. Since nitrogen uptake is decreased in the presence of salt (Huffaker and Rains, 1985), it might be surmised that an increase in supply would increase net uptake and growth. However, this would only be the case if Na^+ ions were competing with N for uptake, ie. by increasing the K_m of N uptake. Also, according to Terry and Waldron (1985) a lower nitrogen supply facilitates turgor maintenance, which is important for growth. This is probably since, at a lowered growth rate due to a decreased N supply, the accumulation of solutes relative to the plant size is greater than if a elevated growth rate had occurred. However, if organic solutes (so-called compatible solutes) play a role in the specific osmoregulation of the plant, an increased N supply would be potentially advantageous. The protection of at least the NO_3^- transporter by Ca^{2+} has been proposed by Ward *et al.*, (1986) and Lewis *et al.*, (1989).

2.2.3.2 Salinity interferences with N metabolism

In studies by Huffaker and Rains, (1985) the uptake of N by barley was severely inhibited by NaCl, while more than 90% of the absorbed nitrogen compounds were in fact reduced/assimilated whether the nitrogen supply was ammonium or nitrate, although NO_3^- assimilation was less affected than the NH_4^+ assimilation. One explanation for this difference could be the compartmentation of assimilation which occurs (Lewis *et al.*, 1989), as mentioned in section **2.2.1.2**. It is plausible that ammonium nitrogen assimilation could be more susceptible owing to its proximity, in the root, to the saline environment. In the same way, assimilation in the shoot may remain less affected. Lewis *et al.*, (1989) hypothesized that the ionic effect dislocates nitrogen assimilation and protein assembly while lowering the net photosynthetic rate, although details appear to vary with plant species. Passera and Albuzio (1978) reported an increase in the protein content of **Triticum durum** with an increase in salinity below 50 mM NaCl, but not in **Triticum aestivum**. NR activity measured by Huffaker and Rains (1985) was only slightly reduced and they did not regard the reduction as being rate limiting. However, according to Helal and Mengel (1979) accumulation of free amino acids and protein in barley was reduced while only inorganic nitrogen accumulated, suggesting that NO_3^- reduction was impaired. A study by Barber *et al.*, (1989) has shown that **Spinacea oleracea** nitrate reductase was inhibited by the Cl^- anion at 157 mM for the native enzyme and at 176 mM for the enzyme-substrate-[NO_3^-] complex. Presumably the halide, acting as mixed competitive and noncompetitive type inhibitor, prevented the reversible protonation and deprotonation (ie. donation of reducing equivalents [NADH] to NO_3^-) of the enzyme Mo(V) centre by direct ligation.

In a study of wheat (C_3) and maize (C_4) the effects of salinity did not appear to be dependent on the form of nitrogen. For both nitrogen forms the plant moisture content, shoot:root ratio and the water use efficiency was decreased while the photosynthetic rate only decreased at 80 mM in wheat (Lewis *et al* 1989). However, the extent of the effect was different for the different N forms. In contrast to maize, wheat had greater biomass production when grown on NO_3^- as opposed to NH_4^+ under nonsaline conditions. Under saline conditions both the C_3 and the C_4 plant studied showed a greater reduction in biomass production when NH_4^+ was the nitrogen source (Soares and Lewis 1986, Lewis *et al.* 1989). In addition, amelioration by Ca^{2+} only ensued for the nitrate-supplied wheat. This is possibly due to protection by calcium only at the site of NO_3^- transport, and not NH_4^+ transport. This is supported by

Huffaker and Rains (1985) in studies on barley. The reason for the greater salt sensitivity in the NH_4^+ fed plants is not known but the following has been suggested by Lewis *et al.* (1989):

i) ammonium assimilation, since it occurs in the root, may be dislocated due to the proximity of the saline environment, while the leaf based assimilation would not be so readily affected;

ii) for the same reason, carbon metabolism in the root may be disturbed which would have indirect effect on N assimilation since C-skeletons are required for the end products of assimilation

iii) and in NH_4^+-supplied plants the absence of the malate-nitrate shuttle operating between the root and shoot may bring about ion translocating problems, further complicated by the presence and uptake of NaCl.

2.3 Effects of salinity stress on carbon metabolism.

2.3.1 The effect of saline conditions on the photosynthetic pathways of C_3 and C_4 plants

Survival and growth in a saline environment imposes additional energy requirements on plant cells, requiring the diversion of carbon for adaptive processes such as ion transport and compartmentation, osmotic solute synthesis and protein turnover for cellular repair. It is well known that, under salinity stress, there is a diversion of carbon to the storage pool (Cheeseman, 1988). While the latter diversion does not represent a C loss to the plant, C allocated to the storage pool is not immediately available for metabolism and growth. Salinity stress, even in NaCl adapted plants, was shown to limited growth even after turgor was regained (Binzel, 1985). This was suggested by Schnapp *et al.*, (1990) to be related to a reduced C availability. A reduced C availability could be due to stomatal effects on C assimilation (Sen Gupta, 1989) since it is well known that photosynthetic rates are reduced under water stress, predominantly due to stomatal closure (Sharkey and Seemann, 1988). Nonstomatal effects, such as effects on the carbon use efficiencies and the activity of the carboxylating enzymes, possibly have their origin in ionic interference by NaCl (Passera and Albuzio, 1978; Greenway and Munns, 1980).

Conversely, it is known that in barley and wheat and in a number of halophytes, the chloroplast has a greater potential for tolerating high ionic concentrations than other organelles. This would be especially important to species such as **Triticum aestivum** which are unable to effectively compartment high ionic concentrations into the vacuole. The chloroplast represents a relatively high proportion of the cell volume (Harvey and Thorpe, 1986) and may serve to protect the cytoplasmic enzymes from NaCl concentrations below 150 mM.

2.3.1.1 Stomatal effects on C assimilation

Due to water deficit, especially in unadapted or adapting cells, the main effect of salinity is osmotic, causing stomatal closure. In the salt tolerant sugarbeet, Terry and Waldron (1985) reported that at external salinities below 200 mM NaCl, although stomatal conductances were reduced, thus lowering the intercellular CO_2 concentration, the rates of photosynthetic CO_2 uptake per unit area were not significantly affected. While the total dry matter produced was reduced at as low as 25 mM NaCl they concluded that the primary effect of salinity was due to a reduction in the expansion of photosynthetic area, and not rate. The reason for the constant photosynthetic rate was that, in parallel with the salinity increase, there was an increase in the total chlorophyll content and other components of the photosynthetic apparatus (Terry and Waldron, *op cit*; Passera and Albuzio, 1978 {wheat}). This meant that even though the photosynthetic rate per chloroplast had decreased the total photosynthetic rate remained similar to the controls. However, this evidence cannot account for the reduction in growth and dry matter. The compensation continued until approximately 350 mM NaCl was reached in the exogenous nutrient medium. At higher exogenous NaCl concentrations, stomatal conductances decreased as did subsequently, the transpiration and photosynthetic rates.

Prunus salicina, a salt sensitive tree also showed long term adjustment of the photosynthetic rate as well as osmotic adjustment after being subject to a range of salinity treatments from 0.2 $dS\ m^{-1}$ NaCl to 6.0 $dS\ m^{-1}$ NaCl (2 mM to 60 mM NaCl) over a period of 3 years in the field (Hoffman *et al.*, 1985; Ziska *et al.*, 1989). There was evidently no difference between the leaf ψ_w, RWC or leaf ψ_p of the treated and control plants. Maintenance of the leaf water status under saline conditions was as a consequence of increased stomatal closure with a subsequent reduction in transpiration rate. Leaf water deficits were only evident during the period of fruit expansion and ripening, possibly since the latter is associated with greater assimilate demand. Salinity (43 to 173 mM NaCl) was also shown to decrease stomatal diffusive conductance to water vapour, thus decreasing both the transpiration and photosynthetic rate in **Vigna unguiculata** (Plaut *et al.*, 1990). Photosynthetic and transpiration rates of the plants decreased during the first few days after being salinized, where this effect was attributed to osmotic stress. Thereafter a partial recovery occurred and a new steady-state, lower than the controls, was reached. The new steady-state was attributed to a specific ionic effect of the sodium ions as well as to water status since a linear relationship between sodium ion concentration in the plant and CO_2 assimilation was noted. That a homeostasis of metabolic efficiency is established after cells have adapted to NaCl is also supported by Schnapp *et al.*, (1990) in C use efficiency studies using **Nicotiana tabacum** cells. From long term seasonal studies on red algae by Dawes *et al.*, (1976) it was shown that salinity influenced photosynthetic rates differentially over the season.

2.3.1.2 The effect of NaCl on C use efficiency and the activity of the carboxylating enzymes (nonstomatal effects)

Although salinized cells are able to regain turgor by osmotic adjustment, the inhibition of cell expansion which results in reduced photosynthetic area still persists (Binzel *et al.*, 1985). Schnapp *et al.*, (1990) investigated a hypothesis which suggests that reduced C use efficiency is the cause of reduced growth in osmotically stressed plants. Although reduced available C can be explained in terms of stomatal closure mediated by water deficit (Ziska *et al.*, 1989), reduction in C assimilation, mediated by NaCl, can also have nonstomatal origins (Ziska *et al.*, 1990). A reduction in photosynthetic rate as a consequence of nonstomatal inhibition has been observed, but the biochemical basis for such alterations by salt stress are not yet understood. Nonetheless, such alterations must be the result of either a change in the leaf content of photosynthetic machinery and/or an alteration in the efficiency with which the machinery operates (Seemann and Sharkey, 1986).

In studies on NaCl-adapted and unadapted **Nicotiana tabacum** L. cells it was found that no net increase in C costs were associated with growth and survival of the NaCl-adapted cells in a saline environment (Schnapp *et al.*, 1990). Carbon use efficiencies (gram cell organic dry mass accumulated per gram sugar assimilated from the medium) at high NaCl concentration (428 mM NaCl) in the smaller, NaCl-adapted cells were appreciably higher than in the unadapted cells. This indicated that a homeostasis was obtained after salt adaption, but that altered C availability cannot account for the reduced cell volume which occurred as a result of NaCl adaption. This did not preclude the possibility that NaCl caused an altered intracellular partitioning of C and that this affected cell expansion. Seemann and Sharkey (1986) found in their studies of Phaseolus vulgaris L. that, when N was limiting, photosynthetic capacity was reduced independent of stomatal closure, due to the effect of salinity (100 mM NaCl) on the efficiency of RuBPCase. The effect on the enzyme efficiency could not be explained in terms of deactivation or inhibitor activity but was due to a reduction in both the RuBP (ribulose 1,5-bisphosphate) pool size and the RuBPCase/oxegenase (ribulose 1,5-bisphosphate carboxylase/oxygenase) efficiency, by an unknown mechanism.

Ziska *et al.*, (1990) found that limitations on the photosynthetic CO_2 assimilation capacity in mature prune trees were nonstomatal, as determined by CO_2 assimilation *versus* sub-stomatal CO_2 concentration (A/Ci) curves and biochemical assay. The nonstomatal limitations were related to a decline in the activity of RuBPCase, the pool size of RuBP, phosphoglycerate, and Pi, and a reduced leaf chlorophyll content. All these effects were correlated with increasing Cl^- salinity in the leaf. Excess salt ions, notably Cl^- have been shown to be preferentially accumulated in leaf sheaths, away from the photosynthetic apparatus (Huang and Steveninck 1989). Passera and Albuzio (1978) found that RuBPCase/oxygenase and PEPCase (phosphoenolpyruvate carboxylase) in wheat (Triticum aestivum and T. durum) had different maximal activities depending on the degree of salinity. RuBPCase activity actually reached a maximum at 25 mM NaCl, whereas PEPCase showed a constant increase in activity with increasing salt concentration up to 50 mM NaCl, at which point $^{14}CO_2$ fixation began to decrease. Since water stress alone apparently has no effect on the activity of RuBPCase in Phaseolus vulgaris (Sharkey and Seeman, 1988), the effects on this CO_2 fixing enzyme were conceivably due to some ionic effect, as was the case for prune trees (Ziska *et al.*, 1990). When the photosynthetic rate, in the same wheat plants, was evaluated by O_2 evolution the effect of salinity on photosynthesis was inhibitory. This implied that the O_2-evolving, energy generating mechanisms were more sensitive to salinity than were the CO_2 assimilation reactions in wheat.

Plants having the C_3 carboxylating pathway exhibit photorespiration and customarily give a light-dark $^{14}CO_2$ evolution ratio of 3.0 (Kennedy, 1976). Passera and Albuzio (*op cit*) found that increasing NaCl concentrations (0 mM to 50 mM NaCl) increased the light-dark $^{14}CO_2$

evolution ratios from ≈ 2.50 (0 mM NaCl) to ≈ 4.20 (50 mM NaCl) in durum wheat. These findings indicate that in salt treated C_3 plants, photosynthetic rate was reduced by increased photorespiration.

The results of Passera and Albuzio show that the ratio between RuBPCase and PEPCase activities decreased with increasing NaCl in both soft and durum wheat, concomitantly causing a shift in the carbon fixation pathway from C_3 to C_4. In the C_4 pathway RuBPCase is required for the actual reduction of CO_2 and if only the PEPCase enzyme is functioning no net accumulation of carbohydrate can occur. Accordingly, there can be no apparent beneficial consequence of the extended salt tolerance of PEPCase for the C_3/C_4 plant. Shitole and Joshi (1984) reported the same trends using **Chlorella**, where the optimal activity of RuBPCase was 1 to 5 mM NaCl and that of PEPCase was 1 to 100 mM NaCl. Thus, for algal growth, a mixed C_3/C_4 pathway, at about 5 mM NaCl, may be an important response to environmental stress but at higher salt concentrations the functioning of the PEPCase enzyme alone would be useless in terms of carbon assimilation. Such a functional mixed C_3/C_4 pathway, even at intermediate salinities, has not been observed for higher plants.

Terry and Waldron (1985) found that salinity did not induce any adaptive change in the structure or function of sugarbeet or mangrove thylakoid membranes, as had been suggested, nor did they find that salinity ($<$ 250 mM) directly affected enzymes of the Calvin cycle *in vivo*. Thus, it would appear that the thylakoid is largely protected from salinity effects. However, if salt were completely excluded, the effects on RuBPCase and PEPCase reported by Passera and Albuzio, (1978) would be hard to explain, except in terms of genotype (mangrove and sugarbeet are salt tolerant while wheat is moderately tolerant). In the halophytic alga **Dunaliella viridis**, RuBPCase was only inhibited at an external NaCl concentration of 375 mM (Dawes, 1981). This alga accumulates the solute glycerol in response to increasing salt concentration. Since it is unlikely that the actual enzyme conformation between species differs drastically, it is possible that the so-called "compatible solutes" do play a role in osmoregulation.

It would appear that together with stomatal closure, the main effects of salinity on photosynthetic efficiency in higher plants are those on the RuBPCase carboxylating enzyme and the O_2 evolving mechanism of photolytic hydrolysis.

2.3.2 The role of organic solutes in salinity tolerance

The role of "compatible" organic solutes (glycine betaine, glycerol, sucrose, proline) which appear to increase within plant cells experiencing salinity stress, has been an area of debate. It is not always clear, especially when they occur in nonhalophytes, whether they are significantly contributing to the maintenance of turgor or whether they are simply a symptom of salt stress (Greenway and Munns, 1980).

Osmoregulatory solutes may, depending on the species, be amino acids or carbohydrate. The amino acids most commonly associated with osmoregulation (eg. proline and glycine) seem to be the ones associated with glycolysis and the Krebs cycle enzymes (Dawes, 1981). The use of various sugars in osmoregulation occurs in unicellular algae such as the diatom **Cylindrotheca** which utilizes mannose (Paul, 1976) and the flagellate green alga **Dunaliella** which synthesizes glycerol (Wegmann, 1971). Various other organic solutes may contribute to osmotic balance, including sorbitol and proline in **Klebsormidium marinum**, sucrose and glutamic acid in **Ulothrix fimbriata** and mannitol in two eustigmatophytes (Brown and Hellebust, 1980). Thus, organic solutes are found to be functional in both vacuolated and nonvacuolated algae. Bisson and Kirst (1979) noticed in studies of red, green and brown algae that the initial maintenance of turgor pressure over a wide range of osmotic concentrations (470 to 1860 $mOs\ kg^{-1}$) was achieved by changing the concentrations of Na^+, K^+ and Cl^-. They proposed that the transient change in the ion concentrations was a temporary ion imbalance to cover the time period required to synthesize the relevant organic solute.

These organic solutes have also been discovered in several nonhalophytic species. It has been shown that glycine betaine accumulated in both salt tolerant and more salt sensitive cultivars of barley at the onset of salinity stress. It was evident that a threshold of adaption was reached at 175 to 200 mM NaCl, since at these NaCl concentrations the rate of glycine betaine accumulation abated. The glycine betaine accumulation was no different in the sensitive and the tolerant variety (Huffaker and Rains 1985). However, a difference would not be detected if, with increasing NaCl concentration the proportion of betaine allocated to the cytoplasm increased, to protect enzymes, while the total betaine concentrations, vacuolar and cytoplasmic, remained the same. Glycine accumulation occurred in the barley leaves but not roots.

Whether these organic compounds are a symptom of stress or have an active role, they represent a potential drain on the carbon metabolism due to the use of photosynthate. Whether or not this is uneconomical for the plant depends on the extent of the osmoregulation and "protective effect" of such solutes and whether the plant can afford C loss as the incorporation into such solutes.

Certain soluble organic acids such as malates and citrates, decrease with increasing salinity. The latter have been implicated in nitrogen metabolism and are associated with the alkaline earth metals, functioning in their translocation; they provide substrates for cellular energy needs, and by decarboxylating to HCO_3^- ions can exchange for NO_3^- entry into the root cells (Rendig and Broadbent 1985; Clarkson, 1986; Lewis, 1986). Possibly, their decrease is correlated with both ionic transport and increased cellular energy requirements due to metabolically dependent extrusion mechanisms.

The use of nitrogenous compounds for osmoregulation would represent a drain on the C pool as well as on the N pool. The limiting effect of this on survival and growth of the plant in a saline environment would again depend on the supply of N in the soil and the genetic capacity of the plant to assimilate N and C.

Before the osmotic role of these compounds can be determined, further cellular compartmentation studies are needed as well as characterization of their specific metabolic role.

The presence of increasing concentrations of organic solutes in the cytoplasm of vacuolated plant organisms placed in an osmotically stressful environment, may be taken at least as a symptom and indication of osmotic stress and of possible osmoregulatory measures taken by the plant cell. The possibility of other repercussions for the plant exist but are not as yet clear (Greenway and Munns, 1980).

2.4 The role of Ca^{2+} in ameliorating the ionic effect of NaCl on the plasma membrane

2.4.1 The role of calcium in maintaining membrane integrity, ion selectivity, and regulating plant responses to salinity

Although little is known about transmembranal signal transduction in higher plant cells, calcium has become recognized as a key regulator of plant metabolism, usually as a second messenger between external stimuli and cytosolic responses (Eklund and Eliasson, 1990).

Calcium is important for maintaining ion selectivity under both saline and nonsaline conditions; it increases the uptake of Br^-, Cl^-, SO_4^{2-} and NO_3^- under nonsaline conditions (in several grass species) and has been reported to increase the uptake of NO_3^- in barley seedlings under saline conditions (Ward *et al.*, 1986). According to Cramer *et al.*, (1985) and Kafkafi (1991), the main effect of exogenous Ca^{2+} on the salinity stressed root membrane is to maintain membrane integrity, thus preventing the loss of the negative surface charge of the plasmalemma and the leakage of potassium. Calcium activates protein kinases and directly affects the electrical transmembrane potential at the plasma membrane which is generated by the electrogenic proton ATPase. It plays a role in cell division, secretion (Noggle and Fritz, 1983; Lew, 1989), protoplast fusion and tropic responses (Rincon and Boss, 1987) and decreases cell permeability under saline conditions (Epstein, 1961; Rengasamy, 1987; Lynch *et al.*, 1989; Lewis *et al.*, 1989; and Lew, 1989).

Under saline conditions (50 mM to 200 mM NaCl) calcium, by virtue of its colloidal chemical effect, has not only been implicated in maintaining membrane integrity but also in ameliorating alkaline metal toxicity, maintaining selective ion transporters, inducing high affinity K^+ uptake, reducing Na^+ influx and K^+ efflux while enhancing Na^+ extrusion. (Frost *et al.* 1978, Ward *et al.* 1986). It has been shown by Hedrich and Neher (1987) that calcium regulates ion channels in the plant vacuolar membranes and that the release of calcium from internal vacuolar stores is induced by 1,4,5,-inositoltriphosphate. There is also evidence for the intracellular calcium-calmodulin complex having a direct effect on electrogenic transport at the plasmalemma (Rincon and Boss, 1987). All of these physiological responses appear to be preceded by an increase in cytosolic free Ca^{2+} concentration, which has been shown to be elevated by high salinity (Cramer *et al.*, 1985). It is however uncertain as to whether this is due to an influx into the cell or due to release of Ca^{2+} from internal stores (ER, vacuole mitochondrion), or both.

In animal cells, inositol trisphosphate, [IP_3], acts as a second messenger during the process of signal transduction, mobilizing calcium from intracellular stores upon being hydrolysed from phosphatidylinositol 4,5-bisphosphate by a specific phosphodiesterase (Lenter, 1986). There is evidence that polyphosphoinositides are biosynthesized and metabolized in plant cells (Wheeler and Boss, 1987). Furthermore it was shown by Grimes and Boss (1985) that [$^3H^+$]-inositol incorporation into phosphatidylinositol 4,5-bisphosphate and other phosphoglycerides closely regulates the mobilization of Ca^{2+} stores, and more specifically, [IP_3] stimulates calcium efflux in the presence of calmodulin (Rincon and Boss, 1987).

Thus calcium is a regulator of plant growth and development, usually as a secondary messenger. Calcium interacts with membrane phospholipids or phosphoglycerides and possibly protein ligands which possess anionic headgroups, leading to the high rigidity and surface tension of the plasma membrane. It has already been mentioned that NaCl has a dispersive effect on membranes and that multivalents have an opposite effect. It has been shown by Ben-Hayyim and Kochba (1982) that a quantitative relationship exists between the nutritional calcium requirement and the degree of salt stress in plants. The growth and functioning of nonhalophyte membranes are sensitive to high Na^+:Ca^{2+} ratios and the mitigating effect of high calcium concentrations (5 mM to 10 mM) on salt stress may be due to:

i) a calcium mediated "protective effect" on the plasma membrane,
ii) restoration of the normal levels of calcium in the plant once high Na^+ concentrations have resulted in calcium deficiency or displacement (Läuchli, 1990),
iii) maintenance of normal K^+ transport and increasing K^+/Na^+ selectivity under NaCl stress
iv) and restoration of normal cell permeability

The above proposals are probably all dependent on the undefined protective effect of calcium on the plasma membrane. A mechanism for this is yet to be biochemically elucidated, although a cellular model for the role of calcium in regulating the responses of nonhalophytes to salinity, suggested by Läuchli (1990) is shown in **Figure 1** (section **2.4.1.4**).

2.4.1.1 The importance of the $Na^+:Ca^{2+}$ ratio

It has been found that at the cellular level, high concentrations of Na^+ ions displace Ca^{2+} ions, supposedly from the low-affinity phospholipid anionic headgroups of plasma membranes and possibly from high-affinity protein binding sites, and that these effects may be ameliorated by increasing the calcium concentration. This was found to be the case in corn root protoplasts (Lynch *et al.*, 1987) and cotton root hairs (Cramer *et al.*, 1985) using the fluorescent probe for membrane bound Ca^{2+}, CTC (chlorotetracycline). It was shown that increasing EGTA (a Ca^{2+} specific chelator which may not enter cell membranes) and NaCl both led to quenching of CTC fluorescence. This was taken as evidence that both compounds were acting on the same pool of Ca^{2+}, ie. that pool bound to the cell plasmalemma. It is important to note that in the case of the root protoplasts, it was the ionic component of salt stress which was ameliorated by the raised calcium, since all the salt solutions were isosmotic. With regards to the specificity of the effect of the NaCl to either the Na^+ or the Cl^- ion, this appears to differ depending on the species (Lynch *et al.*, 1987).

It was proposed by Lynch *et al.*, (*op cit*) that the NaCl mediated displacement of calcium ions from the plasmalemma was the primary source of salt stress. However, as mentioned by these authors, this explanation cannot itself account for the specific toxicities of certain other ions (eg. Li and Cs) in maize seedlings. It is plausible that the displacement is a primary effect of salt stress and that specific ion toxicities, depending on the identity of the ion, are secondary responses occurring once the membrane selectivity and permeability have been damaged. Also, NaCl may displaced Ca^{2+} from intracellular membranes and internal pools once a massive influx of NaCl has occurred (Lynch and Läuchli, 1988). Lynch *et al.*, (1989) showed that NaCl did indeed elevate cytosolic Ca^{2+} activity, presumably due to displacement of calcium from internal sites into the cytosol. The level of cytosolic Ca^{2+} could also be raised in response to a phosphoinositide system mediated response to the NaCl stimulus at the plasmalemma surface. Either way, NaCl could trigger metabolic and growth responses by way of stimulating the Ca^{2+} second-messenger system in the cytosol (see **Figure 1**).

2.4.1.2 The effect of the $Na^+:Ca^{2+}$ ratio on leaf expansion

It has been widely observed that leaf expansion in the young shoot of salinized plants is severely affected . A correlation between low Ca^{2+} status and high Na^+ concentration in the shoot apical meristem has been found for salinized lettuce using quantitative x-ray microanalysis (Läuchli, 1990). It has been found by several researchers that a high Ca^{2+} concentration (Ca^{2+} activity) in the nutrient medium alleviates the low Ca^{2+} status in the shoot and improves the shoot growth (LaHaye and Epstein, 1969; Ward *et al.*, 1986; Lewis *et al.*, 1989; Läuchli, 1990). Possibly, the protective role of the calcium is mostly due to the cellular displacement of sodium ions, firstly at the root membrane surface and later at internal calcium pool sites. This would alleviate not only Na^+ induced displacement of Ca^{2+}, but also the dispersive effect of sodium ions on the membrane which lead to loss of membrane rigidity and surface tension (section **2.1.1.3**). It is even possible that salt tolerant variants of species may avoid overt calcium displacement by virtue of having highly stable Ca^{2+} binding sites or very negative membrane surface charge. This could explain the dissimilar salt tolerances of different species or cultivars; for example, the salt sensitive barley cv "Maraini", and the salt tolerant, "California Mariout" (Bittisnich *et al.*, (1989), cited by Läuchli (1990).

Calcium not only decreases membrane permeability and offsets the deleterious ionic/osmotic effect of NaCl but also offsets ionic imbalances which induce nutrient deficiencies, by actively functioning to maintain the correct univalent:divalent ratio, as well as the correct $Na^+:K^+$ ratio (Epstein 1961, Huffaker and Rains 1985, Ward *et al.* 1986, Nakamura *et al.*, 1990). The univalent:divalent ratio is especially important in the meristematic regions due to the role K^+ has in maintaining turgor in the plant cell (Huffaker and Rains 1985, Ward *et al.* 1986). Maintenance of a low $Na^+:Ca^{2+}$ ratio in the shoot appears especially important for salt sensitive plants. In Phaseolus vulgaris growth was reduced when the $Na^+:Ca^{2+}$ ratio reached 17 (Rengasamy 1987). High $Na^+:Ca^{2+}$ ratios would be due to either a high Na^+ or a low Ca^{2+} concentration and if plants are to survive, as the salinity increases, the Ca^{2+} concentration must be increased proportionately (Greenway and Munns 1980).

2.4.1.3 The effect of the $Na^+:Ca^{2+}$ ratio on root membrane potential

As a consequence of a high $Na^+:Ca^{2+}$ ratio and subsequent Ca^{2+} displacement from the root plasmalemma, a secondary effect would be the depolarization of the root plasmalemma, ie. a reduction in the membrane potential (Cramer *et al.*, 1985). Depolarization severely undermines the nutrient ion uptake efficiency of the root. High cytoplasmic Ca^{2+} concentrations, stimulated by exogenous Na^+, would further the depolarization of the root plasmalemma. Williamson and Ashley (1982), established that increases in intracellular Ca^{2+} inhibit cyclosis. A decrease in cyclosis has been positively correlated with K^+ leakage

(Cramer *et al.*, 1985).

At low Ca^{2+} concentration (0.4 mM $CaSO_4$), mild salt stress (25 mM NaCl) depolarized the root membrane potential of intact corn seedlings within 2 minutes of salinization, followed by a slow recovery after ≈20 minutes (Läuchli, 1990). The root membrane was depolarized less by Na^+ when the calcium concentration was 10 times greater (4.0 mM $CaSO_4$) and the potential was recovered after ≈12 minutes. Under conditions of mild salt stress the membrane potentials recover completely upon the removal of NaCl. Under severe salt stress (50 mM to 200 mM) depolarization may be irreversible. Work cited in the review by Läuchli (1990) confirmed that one of the consequences of membrane depolarization was increased K^+ loss from the root cells, and indeed the K^+ efflux of root cells is often taken as an index of membrane integrity (Cramer *et al.*, 1985). Potassium ion efflux may also occur as a direct result Na^+ displacing Ca^{2+} from membrane potassium channels.

According to Läuchli (1990) the depolarization is related to Na^+ influx and recovery may be due to increased H^+ efflux ascribed to stimulation of the H^+-ATPase activity. If H^+ efflux is correlated with the recovery of the salt stressed root then the effect of additional Ca^{2+} in the exogenous medium may be explained in terms of the following: direct evidence for the intracellular activation of the electrogenic proton pump by Ca^{2+} in Neurospora crassa was given by Lew (1989) and in this way it is possible that, by inducing an electrical transmembranal potential, Ca^{2+} may indirectly regulate the transport of ions in higher plants. Recovery of the membrane polarization may also be facilitated by a Ca^{2+}/H^+ antiport at the plasmalemma (Cramer *et al.*, 1985).

Nakamura *et al.*, (1990) reported that external Ca^{2+} prevented the leakage of intracellular K^+ in salinized mung bean roots thereby supporting the root elongation. Potassium is thought to be important for osmoregulation and in this way it is hypothesized by Nakamura *et al.*, that Ca^{2+} may indirectly play a role in osmoregulation within the root. This was found to be the case and only where the $[Ca^{2+}]/[Na^+]^2$ ratio was above 5.0×10^{-4} mM^{-1}.

2.4.1.4 A cellular model for the role of Ca^{2+} in the regulation of plant responses to high NaCl concentrations

The model shown in **Figure 1** is after Läuchli (1990) and is based on results obtained from root protoplasts. The figure summarizes the processes that are thought to be elicited by sodic salinity stress on the root plasmalemma. This model is incomplete in that specific effects on membrane structure and ion transporters (permeases) are as yet unidentified.

With reference to **Figure 1**, Ca^{2+} is displaced by Na^+ at the outer surface of the plasma membrane. This may be sensed by a receptor complex at the membrane which activates the signal transduction chain consisting of the $[IP_3]$ system and cytosolic Ca^{2+} activity ($[Ca^{2+}]_{cyt}$). The latter is proposed to be elevated by the displacement by Na^+ of Ca^{2+} from intracellular pools, predominantly the ER.

Proteins that bind Ca^{2+} have dissociation constants in the micromolar range, while membranes have dissociation constants in the millimolar range (Wimmers *et al.*, 1990). It is generally considered that intracellular Ca^{2+} is regulated at the level of 1.0 μM and that transient increases in the micromolar level may initiate changes in gene expression and altered metabolism, growth and development (Wimmers *et al.*, 1990) and other cellular processes, often in response to environmental stimuli (Lew 1989). Elevated intracellular Ca^{2+} levels affect the electrogenic pump H^+-ATPase known to play a central role in plant growth and development. This activation requires cytosolic factors and may be due to a Ca^{2+} stimulated phosphorylation of the pump (Hedrich and Neher 1987; Lew 1989). Elevated levels of calcium in the cytosol may be lowered to the resting level by Ca^{2+} pumps at the plasmalemma, ER and tonoplast. In the case where the salt stress is severe enough to inhibit the restoration of the resting level Ca^{2+} concentration, the increased Na^+ activity in the cytosol may also interfere with metabolic regulation and processes in the cytosol and organelles. The increase cytosolic Ca^{2+} would also regulate cellular metabolism by activating enzymes (eg. protein kinases) and other Ca^{2+}/calmodulin-regulated responses. As can be seen from the cellular model in **Figure 1**, the mitigating effect of supplemental Ca^{2+} takes affect at the plasma membrane, presumably allowing the cell to avoid response to the external stimulus of high NaCl concentrations.

It should be stressed that the model in **Figure 1** has only been proposed thus far for root

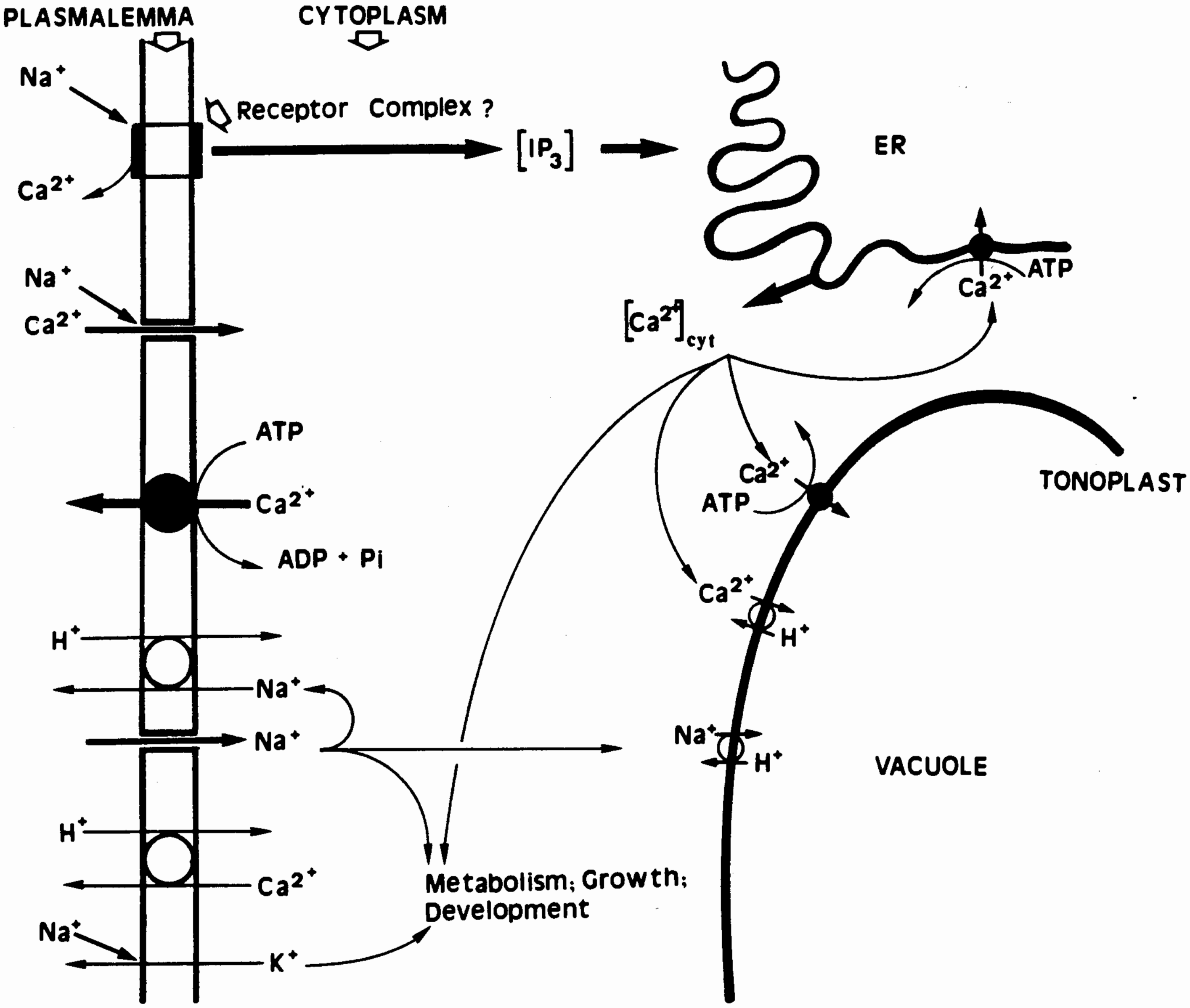

Figure 1: Cellular model for the role of Ca^{2+} in the regulation of plant responses to salinity stress (high Na^+ concentrations). Reproduced from Läuchli, (1990).

protoplasts and needs to be tested for intact roots in addition to shoot apical meristem and developing leaves. The correlation between the extent of NaCl mediated Ca^{2+} displacement and salt tolerance of diverse species or genotypes may in future be useful for evaluating the physiological significance of the salt tolerance phenomenon and in developing salt resistant strains of species. However, as yet a close correlation between Ca^{2+} displacement from membranes and reduction in seedling growth has not been found, indicating that the membranal displacement of calcium ions at the surface of the plasmalemma cannot account for the entire mechanism of salt toxicity.

2.4.1.5 The effect of additional Ca^{2+} on the activity of the nitrate transporter under saline conditions

Ca^{2+} has been shown to increase the activity of the NO_3^- transporter under saline, and not nonsaline conditions, in barley seedlings, wheat, sorghum (Huffaker and Rains 1985, Lewis *et al.* 1989) and maize (Ward *et al.* 1986). It was established that for the transport of NO_3^-, NH_4^+ and Pi, the V_{max} (maximal activity of the transporter) was increased while the K_m (Michaelis constant) was not affected (Ward *et al.*, *op cit*). This implied that the ion carrier affinity for the substrate ion (indicated by K_m) was unchanged while the V_{max} increased, indicating an increase in the substrate made available to the transporter, or else an increase in the total number of transporters (Lehninger, 1987). Ca^{2+} also decreased the transporter induction time resulting in a long term increase in NO_3^- assimilation and thus growth. Using Lineweaver-Burk analysis the optimum Ca^{2+} concentration for NO_3^- uptake was established at 3 to 5 mM where NO_3^- uptake in the stressed seedlings was increased from 31% to 55% of the unstressed controls (Ward *et al.*, *op cit*). The protection of the transporter may be as a result of Ca^{2+}-mediated displacement of Na^+ ions.

2.4.2 Ca^{2+} and K^+ requirements under saline conditions

The effects of Ca^{2+} and K^+ on the plant under saline conditions appear to be interdependent. Since Ca^{2+} is important for the selection of K^+ over Na^+, this, together with the effect on the NO_3^- transport apparatus, contributes to growth. NO_3^- uptake and translocation are stimulated by the pretreatment with K^+ (Frost *et al.* 1978, Ward *et al.* 1986). Helal and Mengel (1979) reported that as NaCl increased (0 to 80 mM) so the internal Ca^{2+} concentration and K^+ concentration in barley decreased, due to enhanced efflux and other possible factors. This was supported by Devitt and Stolzy (1985) and Nakamura *et al.*, (1990). Since the ability to transport Ca^{2+} from the root to the shoot is especially inhibited in salt sensitive species due to inhibited release into the xylem, it is important that an excess of both K^+ and Ca^{2+} are present under saline conditions. In experiments by Ward *et al.*, (1986) where pretreatment did not include K^+, but only Ca^{2+} or sodium, malate (produced stoichiometrically with NO_3^- reduction) accumulated in the root, due to K^+ shortage, and this put a metabolic strain on the root.

2.5 The effect of the K^+ ion on plant response to saline conditions

Potassium, a macronutrient, is the only monovalent ion essential for all higher plants. Whole plant autoradiography (using Rb), of both halotolerant and salt sensitive plants showed an equal distribution of Rb between the plants and throughout the plants showing the importance of K^+ for metabolic processes (Rush and Epstein, 1981). The maintenance of high levels of K^+ and low levels of Na^+ in the cytoplasm is essential for the activities of many enzymes (Greenway and Munns, 1980) and K^+ is indispensable for the growth of plants. In glycophytes especially, transport of potassium to meristematic regions is important as potassium reputedly plays a role in the maintenance of turgor, as well as being required for pyruvate kinase and nitrate translocation (Frost *et al.* 1978). Even halotolerant plants experience optimal growth in an 1:1 ratio of potassium and sodium as opposed to sodium only, showing that sodium may not completely substitute for the potassium ion in halophytes (Jennings, 1976; Rush and Epstein, 1981).

2.5.1 The Na^+:K^+ ratio under saline conditions

Oxygen deficient soil, which often occurs due to the high water tables in saline irrigated areas, and sulphate/sodium salinity simultaneously inhibits both the uptake of potassium as well as the Na^+ exclusion process. The overall effect is that the ratio of Na^+:K^+ ions entering the root and thus the shoot is greatly increased (Lauchli and Epstein, 1985). According to Ward *et al.*, (1986), the K^+ concentration needs to be increased in parallel to the increasing salinity, as is the case for Ca^{2+} when salinity is present. The reason an increase in potassium is required is due to the ion competition between Na^+ and K^+ at the potassium uptake sites, as first suggested by Epstein (1961). This would be true if, as is widely suggested, potassium uptake is analogous to enzyme kinetics and thus the effect of a competitive inhibitor may be overcome by an increase in the substrate concentration

(Ferdinand, 1976). According to Läuchli and Epstein (1985), media salinization (15 to 45 mM NaCl) led not only to the inhibition of potassium uptake but also to inhibition of K^+ release into the xylem in excised barley roots. Leakage of K^+ from the root cells in mung beans has also been reported by Nakamura *et al.*, (1990) and Läuchli (1990).

Thus, under saline conditions potassium plays a role in osmoregulation, potentially increasing nitrate uptake, stimulating sodium efflux (Nakamura *et al.*, 1990; Frost *et al.* 1978; Ratner and Jacoby, 1976, respectively). Also, K^+ may have a protective action on photosynthesis (Sen Gupta *et al.*, 1988). Potassium pretreatment favours, in the presence of salt, the synthesis of protein and nucleic acids by reversing the $Na^+:K^+$ imbalance (Wrona and Epstein, 1985).

2.5.2 The role of K^+ in NO_3^- uptake under saline conditions

In experiments on salinized Triticum vulgare seedlings, Wrona and Epstein (1985) pretreated seedlings with K_2SO_4, Na_2SO_4 or $CaSO_4$ and thereafter transferred all treatments to $Na_2NO_3^-$, to test the effect of the three cations on nitrate uptake. Both Ca^{2+} and Na^+ pretreatment resulted in significantly less nitrate in the xylem exudate than for the potassium pretreated samples. From this they surmised that a K^+ shortage would have a reducing effect on the uptake and translocation of nitrate. Since small amounts of nitrate were found in the exudates of the other treatments they concluded that K^+ is not an obligative counterion in the translocation of NO_3^-, as was hypothesized by Ben-Zioni *et al.*, (1971). However, they did not state whether the seedling were analyzed for their residual K^+ content at the time of the exudate studies. Malate was shown to parallel K^+ distribution in the exudates of the K_2SO_4 pretreated seedlings suggesting the possibility that malate may be a counterion in the transport of potassium from root to the shoot. Since salinity stress induces the leakage of potassium ions from the root membrane (section **2.4.1.3**), it is presumed that Ca^{2+} and the K^+ concentration must be increased under saline conditions. This is not only in view of the role of K^+ for NO_3^- uptake but also in view of the other metabolic roles of the K^+ ion.

2.5.3 Na^+ competes with K^+ for plasmalemma uptake sites

It was first hypothesized by Epstein (1961) that Na^+ ions compete with K^+ for uptake sites on the root plasmalemma and possibly at other internal pool sites. In order to discuss this it will be necessary to outline the K^+ uptake mechanism thought to occur in higher plants.

2.5.3.1 K^+ uptake kinetics

The uptake of potassium has been suggested to follow (like many other ions) kinetics analogous to typical Michaelis-Menten saturation kinetics. Some authors have observed sigmoidal potassium kinetics indicating allosteric regulation of potassium uptake. Epstein (1961) suggested that both Na^+ and K^+ have two specific transporters each, one of high affinity and one of low affinity while Glass (1976) suggested that an ion carrier exists having one active site for the external substrate (K^+) and four highly cooperative allosteric binding sites located on four subunits for the internal K^+. According to the hypothesis, when the allosteric sites become saturated, the conformation of the carrier changes and K^+ influx is inhibited due to a negative feedback response to potassium influx, thus controlling the intracellular potassium concentration. Several other authors have confirmed the negative cooperativity of the as of yet uncharacterized allosteric sites.

Jensen and Petterson (1978), in studies on **Triticum aestivum** suggested the following relationship to describe the negative feedback response:

$$[K^{+internal}]^2 \propto \frac{1}{[K^{+external}]} \qquad (1)$$

Similarly, Sediqi and Glass (1982) found that uptake kinetics depended on both tissue and the external solution concentration of potassium, where the V_{max} and K_m were not constants but depended exponentially on the internal potassium concentration. Wrona and Epstein (1985) also reported feedback inhibition by potassium for its transporter since a decrease in K^+ uptake occurred in two tomato genotypes after K^+ pretreatment.

The allosteric model of Jensen and Petterson (1978) was tested for ecologically different species. Using the linear form of the Hill equation they determined the number of allosteric sites to be between 14 and 16. However, the number is not known with any certainty since studies by Laszlo *et al.*, (1984) determined the number to be between 2 and 16. The former authors reported that V_{max} and n (Hill coefficient/number of allosteric sites) depended on the external K^+ concentration. They also found different efficiencies of uptake (V_{max}) for the different species and suggested that the carrier synthesis was under genetic control.

Laszlo *et al.* (1984) proposed potassium uptake to be a biphasic allosterically controlled process having an induction and a repression phase. In the first phase (0 to 0.1 mM K^+) uptake increased while from 0.1 to 1.0 mM K^+, potassium uptake decreased. Since these processes were inhibited by 2,4,-dinitrophenol (DNP) they were proposed to require metabolic energy. Above 1 mM both the uptake and release (largely offsetting the uptake) were high and not inhibited by DNP. The uptake at low potassium concentrations was supposedly due to an increase in the number of available sites, while the decrease at 0.1 to 1.0 mM was due to negative feedback. Several authors have suggested that the uptake of potassium at high concentrations of the ion may be directly inhibited by negative feedback and indirectly by a decrease in the carrier synthesis (Laszlo *et al.* 1984, Greenway and Munns, 1980). The passive process occurring at high potassium concentrations is possibly due to free diffusion or K^+/K^+ exchange.

In some salt sensitive plants sodium may be removed from the xylem during upward transport. If saturation kinetics for sodium removal occurs, as is suggested by Greenway and Munns (1980), then this mechanism would only be effective at low NaCl concentrations. However, according to Epstein (1961) due to the specificity of the potassium ion carrier, the carrier sites tend to preferentially take up potassium when the sodium ion concentration is high. In this way the plant may be protected from the saline environment at the level of the root plasmalemma.

2.5.3.2 Na^+ ion competition

Two carrier sites for K^+ uptake were hypothesized by Epstein (1961). Firstly, a low affinity site operating at high concentrations of K^+ and inhibited by Na^+, especially at high concentrations. This effect was mitigated by Ca^{2+} but not reversed. The inhibition by Na^+ was possibly due to the low affinity site for potassium being similar to that of a low affinity site for sodium. Secondly, a high affinity site for potassium was operative and was largely indifferent to sodium, if Ca^{2+} was present. The significance of the hypothesis is that in the absence of Ca^{2+}, potassium interferes with sodium absorption and *visa versa*, and in the presence of Ca^{2+}, low sodium concentrations interfere slightly with potassium absorption but at higher concentrations of sodium there is little or no effect. As mentioned in section **2.4**,

Ca^{2+} is required for the carrier selectivities.

Apart from Na^+:K^+ competition occurring at the potassium uptake sites, there is a high selectivity for potassium over sodium in phloem exudate. This may indicate ion selectivity during transport and/or a higher K^+:Na^+ ratio *versus* the vacuole, ie. due to the vacuole sequestering Na^+ (Greenway and Munns, 1980). The latter is supported by Pitman *et al.*, (1981) who, using electron probe x-ray analysis on freeze fractured barley seedling cells, found potassium and sodium to be partitioned mostly in the cytoplasm and vacuole respectively. In addition, evidence has been found for a lower tonoplast permeability for sodium versus potassium (Greenway and Munns, 1980). According to Greenway and Munns (*op cit*), the main barrier preventing high NaCl concentrations from entering the root inner cortex and stele (and thus shoot), is the suberized endodermis.

2.5.4 Effect of potassium on photosynthetic rates under saline conditions

Sen Gupta *et al.* (1989) showed from *in vivo* experiments on water stressed wheat that the addition of 6 mM KCl (supraoptimal under nonstress conditions) reduced the decrease in chloroplast volume and thus relative water content (RWC). Another effect shown by *in vitro* experiments on chloroplasts and protoplasts established that at 6 mM KCl, potassium efflux from the chloroplast which normally occurring at low leaf potentials, was reduced. One or both of these effects apparently resulted in a photosynthetic protective effect by potassium.

Although active transport for both sodium and potassium may occur and direct stimulation of sodium efflux at the endodermis plasmalemma by K_2SO_4 has been found in barley roots, such direct stimulation has not been reported in other plants. The most important roles of K^+, under saline conditions, appear to be that on the nitrate uptake and translocation, and the ionic competitive effect with Na^+.

3. MATERIALS AND METHODS

3.1 General growth conditions

Wheat plants, **Triticum aestivum** L. cv. Gamtoos, were germinated (section **3.2**) and after *7d* transferred to 16 light impermeable tanks with a *23l* capacity each. These plants were grown hydroponically using N-free modified Long Ashton nutrient solutions (Hewitt, 1966), where the appropriate N form (NO_3^- or NH_4^+) and NaCl concentration was added depending on the experiment. The nutrient solutions were modified only with respect to K^+, Ca^{2+}, and NaCl concentrations and the N forms used. The respective experiments to which these modifications apply are summarized in **Tables 1, 2** and **3**. A full description of the separate nutrient regimes and procedures applied to the plants is given in section **3.10**, **(Experimental Detail)**. During the period of growth some or all of the following were measured: biomass production, nitrogen uptake and kinetics, gaseous exchange and ionic content of the plants.

The plants were grown in a phytotron for a *27d* period. Conditions were set to give a day temperature of 25 °C and a night temperature of 18 °C and a day/night relative humidity (RH) of 50% / 65% respectively. One high temperature experiment (30 °C maximum during the photoperiod and 18 °C during the dark period) was performed. This was done in order to note any differences in salinized plant growth under different nitrogen regimes when the added stress of a higher temperature was present. The day/night RH was kept the same as for the other experiments. The photosynthetic photon flux density (PPFD) in the phytotron chamber was varied to simulate the diurnal variation in solar spectral irradiance quantity and quality normally experienced under moderate conditions.

The approximate PPFD reaching the earth's surface from solar irradiation is 1800 $\mu E\ m^{-2}\ s^{-1}$ (Hall and Rao, 1987). During the photoperiod the irradiance/photon flux density varies sinusoidally with a period of *24h* and a maximum at 12*h*00 and declines to zero photon flux density between 20*h*00 and 04*h*00. This gives a 16*h* day. Therefore, the phytotron irradiance was set to gradually increase to a maximum of 500 $\mu E\ m^{-2}\ s^{-1}$ over the first 3*h*; to maintain this irradiance for a further 6*h* and decline over 4*h*. The phytotron irradiance was provided by twenty-one 150 *W* high pressure sodium vapour lamps (Wotan, South Africa),

Table 1: Nutrients in N-free Long Ashton nutrient solution supplied to wheat. Ca^{2+} and K^{+} concentrations include all those used in the various experiments (see Experimental Detail).

Nutrient	**Concentration**	
	(mg ml^{-1})	**(mM)**
Macronutrients		
$MgSO_4$	**368**	**1.5**
K_2SO_4	**348**	**2.0**
	87	**0.5**
	174	**1.0**
	870	**5.0**
$CaCl_2$	**588**	**4.0**
	73.5	**0.5**
	147	**1.0**
	367.5	**2.5**
	735	**5.0**
	1176	**8.0**
	1764	**12.0**
$H_2PO_4.2H_2O$	**104**	**0.67**
$Na_2HPO_4.7H_2O$	**239**	**1.5**
Micronutrients		
H_3BO_3	**8.58**	**0.138**
$MnSO_4.4H_2O$	**4.64**	**0.020**
$ZnSO_4.7H_2O$	**0.66**	**0.002**
$CuSO_4.5H_2O$	**0.24**	**0.003**
$Na_2Mo_4.2H_2O$	**0.06**	**0.00025**
FeEDTA	**33.0**	**0.089**

Table 2: Nitrogen sources used in the indicated experiments.

Nitrogen	Concentration (mg ml^{-1})	(mM)	Experiment
KNO_3^-	0.40	4.0	1,2,6,8,9
$NaNO_3^-$	0.34	4.0	4
NH_4^+Cl	0.43	4.0	1,3,5,7,8,9,

Table 3: Concentrations of NaCl added to Long Ashton nutrient solutions for the indicated experiments.

Salinity	Concentration (mg ml^{-1})	(mM)	Experiment
NaCl	2337	40	1
	2922	50	2-9
	4090	70	1
	5844	100	1

fourteen 250 *W* metal-halide lamps (Wotan, South Africa) and twenty-four 150 *W* incandescent lamps (Osram, South Africa). The incandescent lamps were also used alone to simulate dawn and dusk in the growth chamber.

There existed a slight dissimilarity between the irradiance incident on the plant canopy in the middle of the phytotron chamber and that at the ends of the phytotron chamber. This variation ranged from a minimum of 420 $\mu E\ m^{-2}\ s^{-1}$ to a maximum of 500 $\mu E\ m^{-2}\ s^{-1}$. For this reason the hydroponic tanks containing the plants were randomly arranged within the phytotron chamber. Where tanks were used, more than one tank was used per treatment and where bottles were used these were positioned in the middle of the growth chamber.

3.2 Hydroponic culture

Wheat seeds were pre-germinated in aerated water for 24*h* and subsequently germinated in trays filled with expanded mica which had been sprayed with the fungicide, Benelate (1.0 *g* l^{-1}). Once a week until their harvest, the plants were also sprayed with a solution of this fungicide and an insecticide (Melathion 2 *ml* l^{-1}). The seedlings were germinated in the phytotron chamber under the same physical conditions as they were to be grown under during the 27*d* hydroponic culture period. After 7*d* the seedlings were transferred into 16 plastic light-impermeable tanks containing 23*l* of de-ionized water and holding eight plants each. The eight plants per tank were inserted into holes bored in the flat plastic covers of the tanks and secured by a strip of foam wrapped around the hypocotyl, which also supported the plants. For most experiments a total of 16 tanks per experiment were used, this amounted to 128 plants in total and 16 plants per treatment. If, for specific experiments, the number of tanks, ie. treatments, was reduced, the replication per treatment remained the same.

After one day in de-ionized water, nutrient solution (N-free Long Ashton plus the relevant N form, without NaCl) was added in accordance with the particular nutrient experiment under investigation (see **Tables 1** to **3** and **Experimental Detail**). In the case of graded NaCl solutions, incremental addition to the nutrient media, of the NaCl solution occurred over a 3*d* period, 1*d* after the transferral of the seedlings to hydroponic culture. Addition occurred over a 2*d* period where the salt concentration was constant (50 mM NaCl). Since all additions were done over a period of 2 to 3*d*, no more than a 35 mM NaCl addition per day occurred. This was necessary considering that the seedling stage of growth in wheat, as with most plants, is the most salt sensitive and susceptible to osmotic shock (Jennings, 1976). Where saline solutions were graded, additions were administered in such a way that all plants were exposed to their respective saline treatments for an equal amount of time. The pH of the nutrient solutions was monitored daily and maintained between 6.2 and 6.5 pH units by adjustment with HCl or NaOH as required.

In the first two weeks of hydroponic culture the solutions were changed every 5*d* while in the last week of growth solutions were changed every 2*d* to allow for the increased plant volume with respect to the tank volume.

3.2.1 Nutrient solution osmolality determinations

Two main effects of salt stress have been identified, the osmotic and the ionic effects. It was necessary to separate these effects if any statements about salt stress and the amelioration thereof were to be made. It was also necessary to determine whether different mineral treatments significantly changed the nutrient solution osmolality.

The osmolality (*mOs* kg^{-1}) measurements were taken using the 5100 Series Westcor Vapour Pressure Osmometer (Logan, Utah) and were based on the principle that the vapour pressure decreases with the addition of a solute to a solvent. This vapour pressure decrease is mathematically interrelated with other colligative properties; the one of interest being the osmotic pressure increase which occurs after the addition of a solute to a solvent.

The osmometer was calibrated with each new set of measurements, using standard NaCl solutions purchased from the osmometer manufacturers. The standard NaCl concentrations used were: 100 *mOs* kg^{-1}, 260 *mOs* kg^{-1} and 1000 *mOs* kg^{-1}. After calibration, each sample solution was in turn applied with a 5 *ml* syringe to a 4 mm^2 disc of ashless filter paper laid on the sample holder, until the filter paper was saturated. The sample holder containing the sample fluid was then inserted into the osmometer chamber. The chamber contains a sensitive thermocouple hygrometer which operates on a thermal energy balancing principle and is controlled by the electronic circuitry of the instrument. The junction is cooled via the Peltier effect to below dew point. The heat of condensation from the solution being tested raises the junction temperature asymptotically towards dew point with the result that the temperature converges towards the dew point. The output meter is proportional to this temperature depression of the thermocouple junction and thus to the dew point temperature depression in the sample chamber, which will differ depending on the osmolality of the sample, ie. the degree of evaporation/condensation.

Each sample was measured in duplicate, however, the precision of the instrument was such that the standard error bars were too small to be represented graphically (**Results and Discussion**, section **4.1**).

3.3 Biomass determinations

The wheat was harvested after 27*d* just before the flowering stage. Plants were separated into root and shoot; the roots were blotted dry and the fresh mass of the shoot and root determined. Fresh material was dried at 80 °C for 48*h* to determine the dry mass and moisture content of the plants. The moisture contents were measured both on a wet and dry mass basis:

(wet mass basis)

$$M_c = \frac{fm - dm}{fm} \qquad (2)$$

(dry mass basis)

$$M_c = \frac{fm - dm}{dm} \qquad (3)$$

M_c: moisture content
fm: fresh mass
dm: dry mass

All moisture contents values presented were expressed using **(3)** since this allowed a better separation of the results, probably due to the smaller variability of the denominator (dm).

3.4 Gas exchange analysis

Gas exchange and stomatal diffusive resistance was measured using infra red gas analysis and porometry respectively.

3.4.1 Infra red gas analysis

Measurements were taken along the mid-lamina portion of the youngest fully expanded leaf of ten plants in each treatment. Gas exchange was measured at the end of the 27*d* growing period. The measurements were taken at midday; this being the P_{max} of the plants. According to Plaut *et al.* (1990) midday is the time when the maximum effect of salinity is found to occur on carbon assimilation and leaf diffusive conductance. Changes in leaf diffusive conductance appear to be the major response of water stressed plants, as shown in a model of water stressed plant response by McCree *et al.*, (1990) and thus stomatal conductance/resistance measurements are relevant to this investigation.

All the measured leaves were orientated so that the PAR at the leaf surface was ≈ 400 *μmol m⁻² s⁻¹*. Four gaseous exchange measurements were taken simultaneously: CO_2 assimilation, transpiration rate (and thus WUE from the ratio of $mmol\ CO_2/mol\ H_2O$) and stomatal conductance to H_2O. These measurements were taken using the plant CO_2 assimilation rate as measured by an Infra Red Gas Analyser (IRGA), the ADC LCA2 (CO_2 Leaf Chamber Analyser) connected to a DL-2 data logger and independent ADC pump unit. The IRGA was used in conjunction with a narrow leaf Parkinson Leaf Chamber 2 (PLC-2) having a window area of 13 cm^2. All these units were supplied by The Analytical Development Co. (ADC), Ltd., Hoddesdon, England. From experiment **3.10.3 (Experimental Detail)** onwards an ADC LCA3, containing an internally controlled mass flow pump (P_2) and a data logger, was used. With the ADC3 a narrow cereal leaf chamber, the PLC-3 was used which had a window area of 11 cm^2.

These gas exchange measurements as well as PPFD ($\mu E\ m^{-2}\ s^{-1}$), temperature of the ambient air (°C), rH, and reference/analysis CO_2 concentration were collected by a data logger at the time of measurement. These values were required for the calculation of the gaseous exchange values from the raw data. The formulae used by the LCA-2 and LCA-3 were taken from Parkinson (1983) and von Caemmerer and Farquhar (1981) and are given in **Appendix 1.** Since the wheat leaf does not entirely fill the width of the leaf chambers used, the photosynthetic rates, transpiration rates and leaf conductance values were later corrected for each individual leaf area, measured at the time of recording. This was done by taking two width measurements at the two lamina edges enclosed in the leaf cuvette and then using the known value of the leaf cuvette length to calculate the leaf area enclosed.

For both models of the ADC LCA the air supply was taken from the ambient air by means of a diaphragm pump (P_1). This air was first passed through a 23*l* "buffer volume" tank in order to minimize fluctuations in both the P_1 flow delivery and ambient CO_2 concentration. The "buffer tank" air outlet was split between a line going to the reference cell of the IRGA and a bleed line which prevented pressurization of the mass flow pump. In the LCA3 the same procedure was followed although a 2*l* "buffer volume" tank was already included by ADC in the reference line. The mass flow pump was set to deliver 300 $ml\ min^{-1}$ whether this was the internally controlled second pump of the LCA3 or the external pump of the LCA2. The LCA2 and LCA3 were both calibrated with 400 *ppm* CO_2 standard calibration gas

(CryoService, Ltd, Worcester). The air entering the cuvette was of a RH = 0%, dried by means of $Si/CoCl_2$ desiccant columns. The E_{max} value was set to correct the CO_2 measurement for H_2O input from the transpiring plant, ie. to correct for the dilution of the CO_2 by H_2O vapour and for the foreign gas broadening of the CO_2 infra-red absorbtion band by the H_2O vapour. The CO_2 free air was obtained by allowing the reference air to pass over a column of "carbosorb" (soda lime, 4 to 10 mesh). The boundary layer resistance was determined using the method given by Parkinson (1984) and subsequently was set to 0.15 $m^2\ s^{-1}\ mol^{-1}$. The leaf temperature used in the calculations was calculated from the energy balance of the leaf, ie. the energy incident on the leaf between 0.4 μm and 0.7 μm was detected by the PAR (photosynthetically active radiation) sensor and converted by a factor (0.175) involved in the conversion to energy units absorbed by the leaf. Since the energy per quantum varies with the wavelength, the constants for this equation needed to be adjusted since the light source (lamps) had a different spectral quality and quantity to solar irradiation. The constants were thus set accordingly for a high pressure sodium source/metalarc source. These values, including the factor mentioned above were obtained from the ADC instruction manual, Appendix VI.2.

3.4.2 Porometry

Measurements of stomatal diffusive resistance ($s\ cm^{-1}$) were made on a Licor Li 6000 steady-state porometer (Licor, Nebraska, U.S.A). Ten measurements (5 adaxial and 5 abaxial) were taken on ten different plants within each treatment.

3.5 ^{15}N isotope ratio analysis

After feeding plants with nitrate or ammonium, containing the heavy, stable ^{15}N isotope as $K^{15}NO_3$ or $^{15}NH_4Cl$ respectively (99 atom % - Amersham, UK), the $^{14}N/^{15}N$ isotope ratio was analyzed by optical spectroscopy using the Jasco 150 ^{15}N emission spectrometer (J.A.S.C.O. Japan Spectroscopic Co., Ltd.).

An increase in the ^{15}N in the plant tissue is positive evidence that N assimilation has occurred and this amount was quantitatively determined to ascertain the extent to which different treatments were affecting nitrogen uptake and thus assimilation. The isotope ratio analysis of the sample N was a three step process:

i) conversion of sample N to NH_4^+ by acid digestion

ii) oxidation of NH_4^+-N to $N_{2(g)}$

iii) determination of the $N_{2(g)}$ isotopic ratio

The N isotope ratio was calculated from the relative intensities of emitted light in the ultraviolet range occurring when sample $N_{2(g)}$ is excited at low pressures ($<10^{-1}$ *kPa*) by high-frequency radiation (13-100 *MHz*). The vacuum system consisted of a high vacuum pump, which created a pre-vacuum of 0.1 *kPa*, connected in series with a mercury diffusion pump giving the system a final pressure of 1.0 *kPa*. Two liquid nitrogen traps were used to reduce the water vapour pressure of the system. The measurement of the isotope intensity ratios was facilitated since $^{14}N^{14}N$ and $^{15}N^{15}N$ result in band spectra at 297.7 *nm* and 298.3 *nm* respectively, which can be detected by the photomultiplier and converted to electric current. This enables small amounts of N ($< 10\ \mu g$) to be detected. The $^{29}N/^{30}N$ signal, since it was small compared to the ^{28}N signal, could be amplified up to 32 times by a gain selector. Normally the gain used was either x8 or x16.

3.5.1 Conversion of sample N to NH_4^+-N by acid digestion. (The Kjeldahl method)

The Kjeldahl method was used according to Deignan and Lewis (1988), with certain modifications, to determine both total plant N content and to prepare the samples for isotopic ratio analysis.

Dried root and shoot material from each plant which had been fed with ^{15}N was milled and weighed out into Kjeldahl digestion flasks in known duplicate quantities (between 0.1 and 0.05 *g*). To each sample 3 *ml* conc. H_2SO_4 (AR, BDH Chemicals Ltd. England) plus 34% (w/v) salicylic acid and a catalyst tablet (0.05 *g* Se + 1 *g* Na_2SO_4) was added. The selenium sulphate served to expediate and improve the quantitative conversion of sample organic nitrogen to ammonium sulphate. The salicylic acid was present in order to reduce oxidized forms of nitrogen (NO_3^-, NO_2^- and NO) since there is a risk of losing these as dinitrogen or oxides during the digestion. The salicylic acid combines with nitrate and nitrite to form nitro compounds, which are then reduced to their corresponding amino compounds before the digestion. With each batch of digests 3 to 5 blanks were prepared (acid + catalyst) and 6 standards: 0.01 *g* to 0.06 *g* Titriplex III® ($C_{10}H_{14}N_2O_8.2H_2O$), where this range included the estimated sample N content. The standard curve constructed from the results allowed the relative recovery of N to be estimated. A representative standard curve is shown in **Figure 2**. The percentage recovery of the Titriplex III® was not less than 96% in all cases.

Contents of the flasks were digested in a preheated digestion block at 375 °C for 3*h* or until 1*h* after the digest had cleared from a brown-reddish colouration, which indicated that the organic C in the sample had been oxidized. The resultant acid digest solutions, in which the nitrogenous compounds were all converted to $(NH_4)_2SO_4$, were then prepared for steam distillation in order to determine the total N content of the samples. The digest solution was made up to 25 *ml* volume with distilled water, and a 2 *ml* aliquot of this was taken for each distillation on a Büchi micro-distillation unit (Lab Techniques, Ltd, Switzerland). Once the sample N had been converted to $(NH_4)_2SO_4$ by acid digestion, the N could be released as NH_3(g) during the micro-distillation procedure, captured in a weak acid solution and back titrated against a weak alkali. The molarity of the alkali was such that 1 mol alkali = 1 mol N so that the mass of total N in the distillation sample could be determined simply by multiplication of the distillation value, after subtracting the blank, with the M_w of N.

Each sample distillation was done in duplicate or with greater replication if large variation occurred. The ammonium sulphate was rendered alkaline inside the reaction vessel by the addition of 15 *ml* (excess) 25% (w/v) NaOH and 2.5% (w/v) $Na_2S_2O_3$. The $NH_{3(g)}$ liberated into the steam distillation unit was condensed and the distillate (30 *ml*) was collected into 2 *ml* of acid (0.2 M HCl, Titrisol®, Merck). The amount of nitrogen was then determined by back titration, using a Schott Geräte automatic titrator (Germany), against a weak alkali (0.005 M NaOH - Titrisol®, Merck) to pH 5.2. The sample was immediately afterwards re-acidified with 2 *ml* 0.04 M H_2SO_4 (AR, BDH) to prevent the loss of N as $NH_{3(g)}$. The automatic titrator was calibrated to a titration end point of pH = 5.2 by adding 2 drops of Tashiro's indicator to 2 *ml* 0.2 M HCl and 30 *ml* distilled water and titrating the alkali manually until the solution turned from light pink to pale grey (pH 5.2).

The samples then underwent a concentration-evaporation procedure. The distillate samples were heated to ≈80 °C (not above 90 °C) while being "blown down" under a light airstream facilitated by an overhead manifold. This was done in order to concentrate each sample to at least 25 *μg* N per 0.2 *ml* sample, which is the concentration required for the isotopic ratio analysis, each sample requiring 0.2 *ml*. Since these samples were to be used for isotope ratio analysis the following precautions were taken:

i) samples were re-acidified in order to prevent the loss of $NH_{3(g)}$ to the atmosphere; the acid used was H_2SO_4 as opposed to HCl since at temperatures above 90 °C N may be lost as NH_4Cl (Hauck, 1982);

ii) all reagents were N free; although additional N could be accounted for by blanks w.r.t. total N determinations, additional ^{14}N would disturb the true isotopic ratio of the sample;

iii) and acidification did not exceed 2 *ml* of 0.04 M H_2SO_4 since excess acid reacts with hypobromite to release $Br_{2(g)}$ which could interfere with the band spectra absorbtion of the ^{15}N or ^{14}N.

3.5.2 Oxidation of NH_4^+-N to $N_{2(g)}$

The alkaline hypobromite method of Faust (1967) enables the use of molecular nitrogen for isotopic ratio analysis. For the correction of the final percentage enrichment values of the samples, a calibration curve was constructed (**Figure 3**). The oxidation of the sample NH_4^+ (acidic) was done by reacting it under vacuum with alkaline sodium hypobromite (NaOBr) which acted as the oxidant, allowing the release of the sample N as $N_{2(g)}$ according to the reaction equation:

$$2NH_3 + 3NaBr \rightarrow 3NaBr + 3H_2O + N_{2(g)} \qquad (4)$$

3.5.3 Determination of the $N_{2(g)}$ isotopic ratio

Optical emission spectroscopy was used to determine the isotopic composition of the sample N. An emission spectrum for nitrogen molecules contains vibration spectra for each of $^{14}N_2$, $^{14}N^{15}N$, and $^{15}N_2$. The band heads of these molecules occur at 297.7 *nm*, 298.3 *nm* and 298.9 *nm* respectively and can be used for quantitative compositional analysis, since the intensities of the band heads at 297.7 *nm* (I^{28}), 298.3 *nm* (I^{29}) and 298.9 *nm* (I^{30}) are proportional to the concentrations of the corresponding molecules. The A%E (^{15}N atom % excess) was calculated as:

$$[^{15}N(atom\,\%)] = \frac{100}{\frac{2([^{14}N^{14}N]}{[^{14}N^{15}N]}+1} \qquad (5)$$

The calibration curve in **Figure 3** was used to correct the A%E values. The final corrected A%E values were thus calculated as follows:

$$A\,\%\,E = ((\frac{100}{2R+1})\,x\,1.015 - 0.192) - 0.3663 \qquad (6)$$

R = $[^{14}N^{14}N]/[^{14}N^{15}N]$
1.015 = m (slope)
0.192 = c (y intercept constant)
0.3663 = natural abundance (%) of ^{15}N

The complete set of formulae for the derivation of A%E appear in **Appendix 1**. From the A%E the ^{15}N content ($mg\ ^{15}N\ g^{-1}\ dm$) could be determined since the total N content had been determined from the micro-distillation procedure. The results presented are expressed as $\mu g\ ^{15}N\ g^{-1}\ dm\ min^{-1}$ taken up from the nutrient solution over 8*h*.

3.6 Determination of nitrogen uptake rate from nutrient solutions

3.6.1 NO_3^- uptake measurement

In order to monitor any plant - nutrient interactions with respect to NaCl and Ca^{2+}, the disappearance of NO_3^- from the nutrient solutions was followed over 8*h*. Samples (2 *ml*) were taken every 2*h* from the nutrient solutions. Three plants per treatment were used. These were grown under the same nutrient and other conditions as given in section **3.1** except that 2*d* prior to the experiment the lights were kept on continuously at an irradiance of 500 $\mu E\ m^{-2}\ s^{-1}$. This was in order to eliminate any diurnal effects on NO_3^- uptake. Such effects were reported and modelled by Sutherland (1989). During the uptake experiments the plants were contained in well aerated 1*l* vessels which were impermeable to light. The light

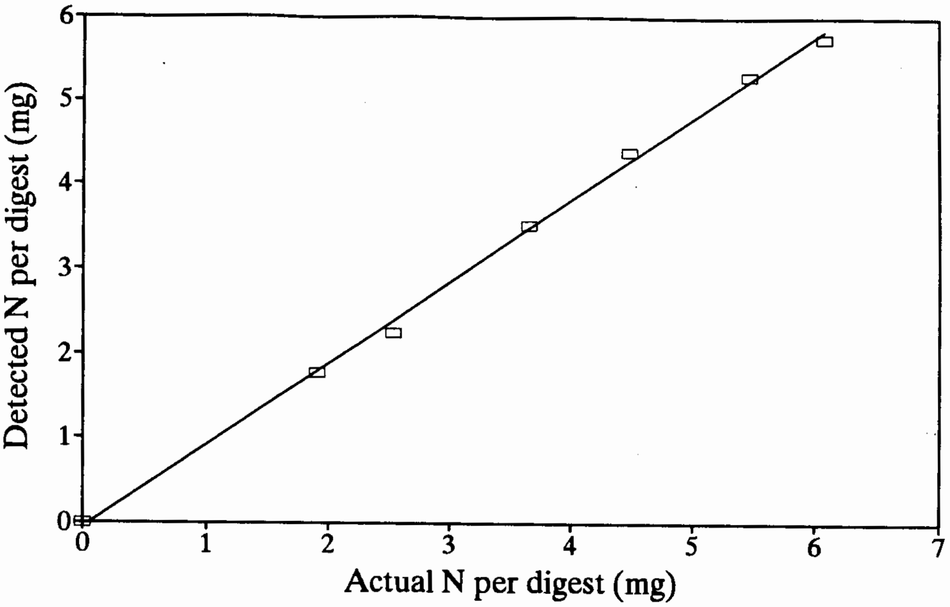

Figure 2: Standard curve for determining sample recovery (post-Kjeldahl digestion) using Titriplex III. $r^2 = 1.00$

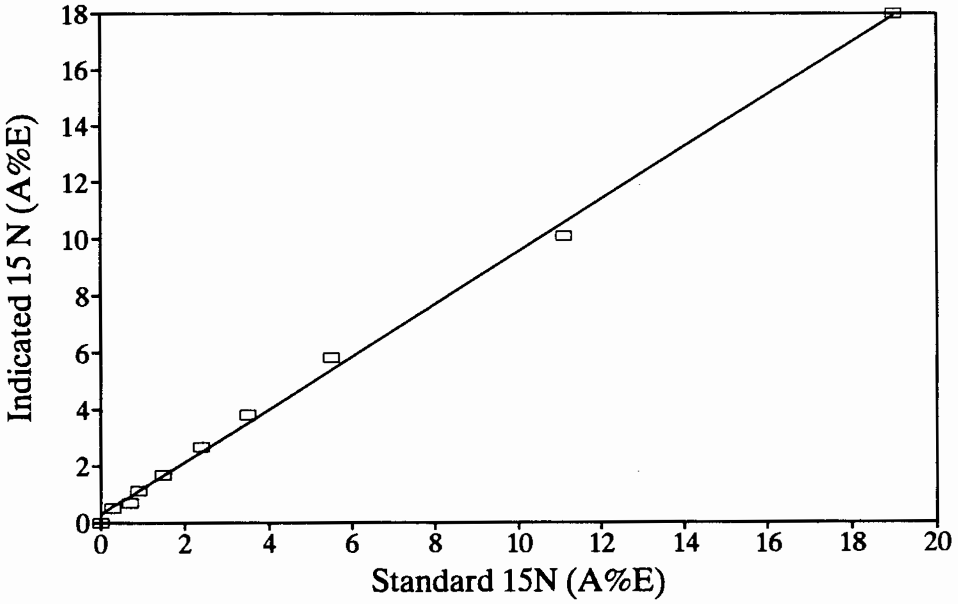

Figure 3: Calibration curve for the correction of sample ^{15}N enrichment using the JASCO emmission spectrophotometer. $r^2 = 0.99$

intensity was maintained at 500 $\mu E\ m^{-2}\ s^{-1}$ for the length of the experiment.

3.6.1.1 NO_3^- determination

The ultraviolet spectroscopy method of Cawse (1967) was used with slight modifications. The determination was based on the measurement of nitrate absorbtion in the ultraviolet range of 202 *nm* and 250 *nm* (maximum absorbtion occurs at 202 *nm* where the trough of the peak at 250 *nm* was measured and subtracted from the peak at 202 *nm*). All determinations were done on the Pye Unicam SP1800 UV Spectrophotometer after the removal of interfering ions, mostly nitrite. It is highly probable that this precaution was unnecessary since the concentration of NO_2^- in normal nutrient solutions was likely to approximate zero. Nevertheless, any NO_2^- ions were removed by the addition of 20% (v/v) perchloric acid and 10% (w/v) sulphamic acid (amidosulphuric acid) to the samples and standards. $A_{202\ nm}$ and $A_{250\ nm}$ were measured in quartz cuvettes and the final nitrate concentration was determined after reading ($A_{202\ nm}$ - $A_{250\ nm}$) from a standard curve (**Figure 4**).

It was found that the nutrient solutions plus the perchloric acid absorbed light only in the visible range of 390 *nm* to 700 *nm*, due mostly to the perchloric acid. Thus it was considered that the nutrient medium would not interfere with the ultraviolet based determination of nitrate. The results were expressed as cumulative nitrate uptake ($\mu mol\ NO_3^-\ g^{-1}$ *root fm*). The NO_3^- uptake rates were expressed in *nmol* $NO_3^-\ g^{-1}$ *root fm* s^{-1}. The cumulative NO_3^- uptake was determined from the regression of the calibration curve. The latter was set up using standard solutions of KNO_3^- ranging in concentration from 0 $\mu mol\ l^{-1}$ to 400 $\mu mol\ l^{-1}$. This method, unlike ^{15}N isotope ratio analysis did not give an indication of the net influx of NO_3^- but the final NO_3^- concentration measured gave an indication of the net uptake due to the processes of both influx and efflux.

3.6.2 NH_4^+ uptake measurement

N uptake at different K^+ concentrations was tested in salinized and control plants. The K^+ concentrations were: 0.5 mM, 1.0 mM and 5.0 mM. The conditions of the experiment were identical to those described in section **3.6.1** except that the N source supplied to the plants was NH_4^+ and the variable was the potassium concentration.

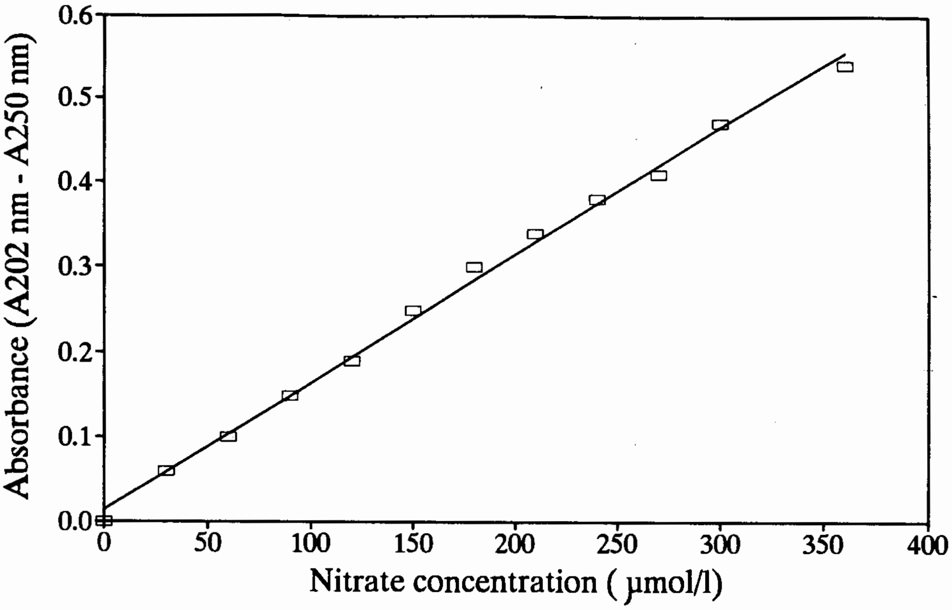

Figure 4:* *Standard curve for the spectrophotometric determination of nitrate at A_{202nm} - A_{250nm}. $r^2 = 0.99$

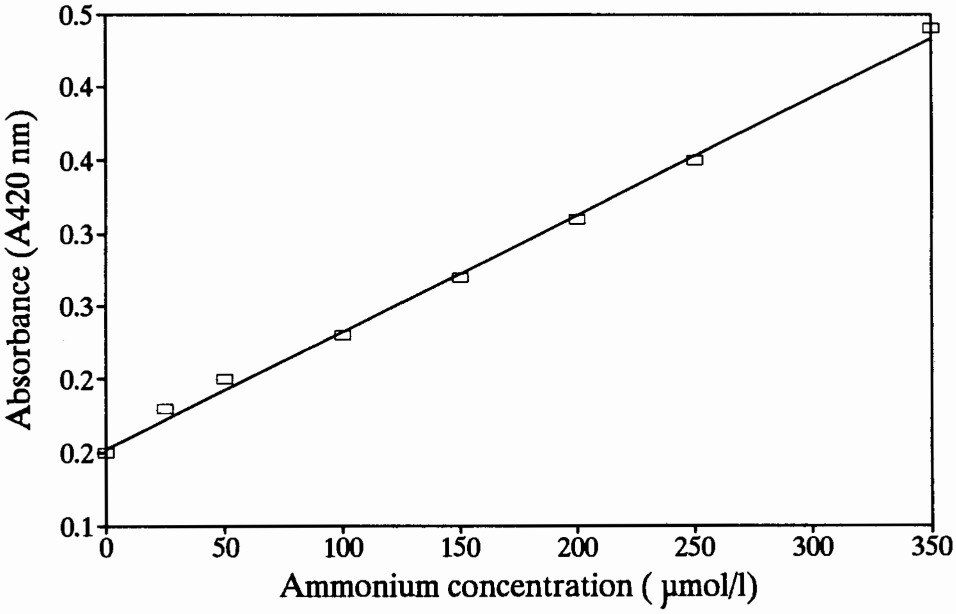

Figure 5:* *Standard curve for the spectrophotometric determination of ammonium at A_{420nm}. $r^2 = 0.99$

3.6.2.1 NH_4^+ determination

Ammonium concentration in the nutrient medium was determined by reacting the NH_4^+ in the samples and standards with Nessler's reagent (3.6% w/v $HgI_2.KI.1½\ H_2O$ and 9% (w/v) Na_2O) and reading the absorbance of the yellow reaction product at $\lambda = 420$ *nm*. The samples/standards were added in a ratio of 1:5 to the reagent and read immediately after mixing. This was done to standardize measurements since the progression of the reaction proceeds for at least a minute, the time depending on the concentration of NH_4^+ in the cuvette. More dependable readings were obtained by observing the absorbance immediately than by taking readings after a selected time interval. A calibration curve was prepared each day that a sample batch was measured in order to eliminate differences in absorbance due to temperature, humidity or reagent batch. The calibration curve was set up using NH_4Cl solutions with concentrations ranging from 25 $\mu mol\ l^{-1}$ to 600 $\mu mol\ l^{-1}$. The samples were diluted appropriately to fall within the range of the standard curve. A representative standard curve is shown in **Figure 5**. The cumulative NH_4^+ uptake and NH_4^+ uptake rates were determined in the same way as for the NO_3^- uptake studies and the results were expressed using the same units.

3.7 Kinetics of nitrogen uptake in salinized wheat plants

The kinetics of NO_3^- and NH_4^+ uptake was measured in salinized (50 mM NaCl) and control (0 mM NaCl) plants in order to determine the effect of high NaCl concentrations on the kinetic parameters, V_{max} and K_m.

3.7.1 Kinetics of NO_3^- uptake in control and salinized wheat

Control and salinized wheat plants were exposed for 12*h* to a range of NO_3^- concentrations in fresh N-free Long Ashton medium. These concentrations were 1 mM, 2 mM, 4 mM, 6 mM, 8 mM and 10 mM NO_3^-. The plants to be used for the experiment were placed in the respective nutrient solutions containing the correct NO_3^- concentration 2*d* prior to the experiment. Two *ml* samples from the nutrient media were taken after 12*h* and analyzed for their NO_3^- content according to the method described in section **3.6.1.1**. The data was then fitted to the Michaelis Menten kinetics rate equation:

$$V_o = \frac{V_{\max}[A_o]}{K_m + [A_o]} \qquad (5)$$

V_o: initial reaction rate
V_{max}: maximal rate of the reaction
$[A_o]$: initial concentration of the substrate $[NO_3^-$ or $NH_4^+]$
K_m: Michaelis constant

The program Enzfitter®, version 1.03 (1987, Elsevier Biosoft, Cambridge, UK) was used for this purpose. The kinetic parameters V_{max} and K_m were determined using Lineweaver-Burke plots constructed by this program which employed the inversion of equation 5:

$$\frac{1}{v_o} = \frac{K_m}{V_m} . \frac{1}{[A_o]} + \frac{1}{V_m} \qquad (6)$$

3.7.2 Kinetics of NH_4^+ uptake in control and salinized wheat

The procedures were as described in the previous section, except that the N source was NH_4^+ and the samples were analyzed as described in section **3.6.2.1**.

3.7.3 Kinetics of NO_3^- uptake in the presence of NaCl and different Ca^{2+} concentrations.

The effect of calcium concentration (0.5 mM, 1.0 mM and 5.0 mM) on the NO_3^- uptake kinetic characteristics was tested in salinized (50 mM NaCl) plants. The plants were pre-treated in the experimental solution for 2*d* prior to the experiment. The NO_3^- concentrations used were: 1 mM, 2 mM, 4 mM, 6 mM, 8 mM and 10 mM. After a 12*h*, 2 *ml* samples of the nutrient media were taken and analyzed as described in section **3.6.1.1.**

3.7.4 Kinetics of NH_4^+ uptake in the presence of NaCl and different K^+ concentrations.

The procedures were as for **3.7.3** except for that the plants used were ammonium-supplied. Salinized plants (50 mM NaCl) were fed 4 mM NH_4^+ and the following concentrations of

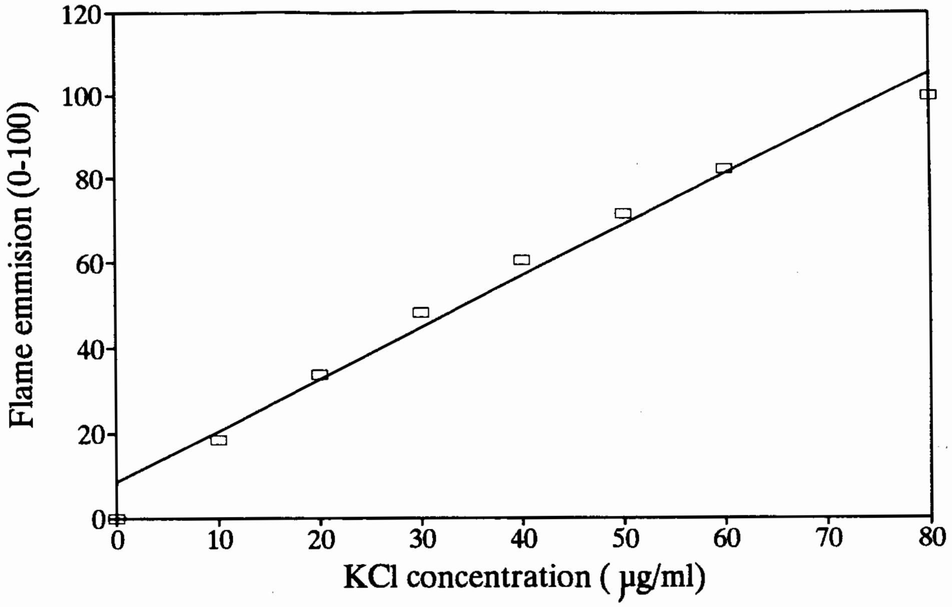

Figure 6: Standard curve for the flame emission specroscopic determination of potassium. $r^2 = 0.99$

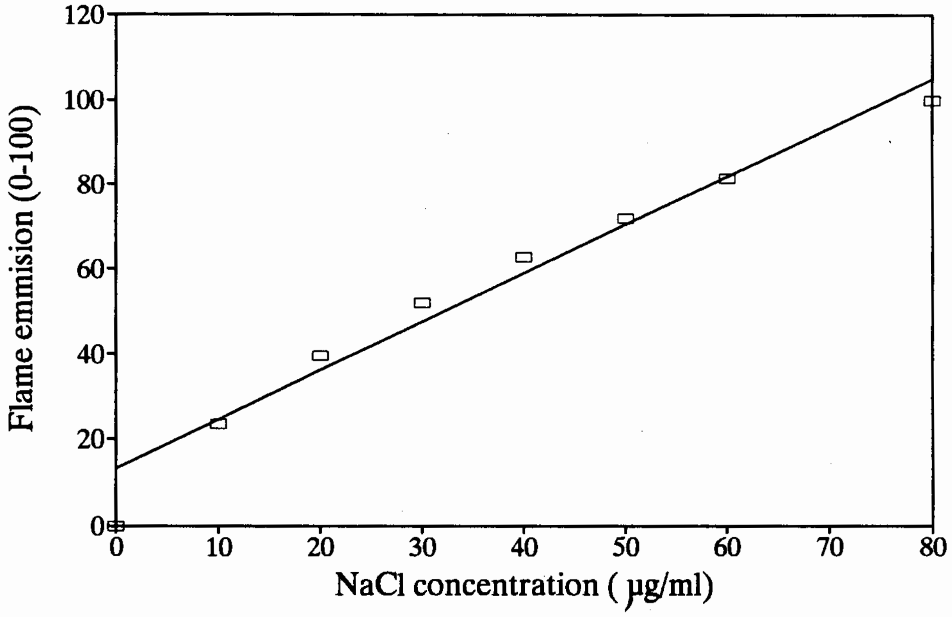

Figure 7: Standard curve for the flame emission spectroscopic determination of sodium. $r^2 = 0.99$

potassium: 0.5 mM, 1.0 mM and 5.0 mM. The samples were analyzed as described in section **3.6.2.1.**

3.8 Inorganic ion analysis

The sodium and potassium ionic contents from selected plants were determined by flame emission spectroscopy. The method used for the sample preparation was modified from Devitt *et al.*, (1984).

3.8.1 Sample digestion and preparation

Wet oxidation of the plant material was chosen over a dry ashing procedure, due to the potential volatilization of K^+ at the usual ignition temperatures of 550 °C to 600 °C (Jackson, 1958) and the possibility of cross contamination between samples during dry ashing procedures. All reagent used in the digestion procedures were of analytical grade. Plant shoot and root material were digested for 80 *min* in a binary acid mixture of HNO_3 and $HClO_4$ in a 2:1 ratio, after a pre-digestion in HNO_3.

3.8.1.1 Pre-digestion in HNO_3

Duplicate 200 *mg* samples of milled shoot and root material from eight plants of each treatment were weighed out into 300 *mm* long digestion tubes. Two *ml* of conc. HNO_3 (AR) and 1 Selenium catalyst tablet (0.05 *g* Se + 1.00 *g* Na_2SO_4) were added to each tube. Blank tubes contained only the conc. HNO_3 and the catalyst. The digestion tubes were then transferred to a block digester pre-heated to 150 °C.

3.8.1.2 HNO_3:1 $HClO_4$ digest

One *ml* of perchloric acid ($HClO_4$) acid was added to the digestion mixture once the brown fumes from the pre-digestion had dissipated (this required an additional 2 *ml* increment of HNO_3 to be added). The digestion was then carried out as indicated by the programme in **Table 4**. After the samples had cleared to a colourless - pale yellow liquid, they were filtered using Whatman, no. 5, and distilled water was added to make the samples up to 100 *ml* volume. The samples were then diluted 1:10 for K^+ analysis and 1:150 for Na^+ analysis. The distilled water used to dilute the samples contained an acid + flame suppressant mixture (1.0 $mg\ ml^{-1}$ CsCl, 5.0 $mg\ ml^{-1}$ $SrCl_2$ and 10% conc. $HClO_4$). Prepared, digested and diluted in parallel with the samples were 3 blanks and 6 working standards.

Table 4: Pre-ion analysis digestion programme for plant material.

Time (min)	Temperature (°C)
20	180
20	200
20	220
20	240

Calibration for flame emission was done using NaCl and KCl standards in the 10% $HNO_3/CsCl/SrCl_2$ mixture mentioned above and had the concentration range 0 to 200 *mg* l^{-1}.. The calibration curves for KCl and NaCl appear in **Figures 6** and **7**.

3.8.2 Flame photometry

The diluted samples were analyzed for Na^+ and K^+ content by measuring the flame emission, after aspiration of the sample, on a Varian Techtron (1000) Atomic Absorbtion Spectrophotometer (Varian Techtron Pty. Ltd., Melbourne, Australia). Potassium may be detected at two lines (λ = 404.4 *nm* and 776,5 *nm*). Since spectral interference between the emission peak of K^+ (λ = 404.4 *nm*) and that of Mn^{2+} (λ = 403.3 *nm*) occurs and detection at 404.4 *nm* is less sensitive than at 766,5 *nm* (10 $\mu l\ ml^{-1}$ versus 0.1 $\mu l\ ml^{-1}$ in an air-acetylene flame respectively), the emission line at 766,5 *nm* was used (Alkemade *et al.*, 1970). Sodium was measured at 589 *nm*. Flame photometry was carried out in an air-acetylene flame.

All samples were measured in duplicate. The ion contents were expressed on the basis of tissue water (*mmol* l^{-1}) to approximate physiological concentrations (Blits and Gallagher, 1990). This was done by making use of the known moisture contents of the plants. Potassium versus sodium selectivity ($S_{[K,Na]}$) of the plants was determined by:

$$S_{[K,Na]} = \frac{[K]_i \times [Na]_o}{[Na]_i \times [K]_o} \qquad (7)$$

[ ]$_i$: concentration in tissue water
[ ]$_o$: concentration in exogenous nutrient solution

3.9 Statistical analysis

The data obtained was subjected to one-way analysis of variance (ANOVA) using Statsgraphics® (Statistical Graphics System Inc. (4.0), USA) and multiple range tests (95% Confidence Limits) to establish the significance of the treatment variable (NaCl, $CaCl_2$ or K_2SO_4 concentration) within each group (nitrate and ammonium-supplied plants or control and salinized plants), for the shoot, root and plant measurements. To demonstrate interaction between factors (NaCl presence and the treatment variable) as well as the difference between the stressed and control plants, the whole data set was in each experiment subject to two-way ANOVAs using Statsgraphics®. For the sake of clarity, only the results of the multiple range tests from the one-way ANOVAs are shown in the figures, where significant differences between treatments of the same group are indicated by letter. This means that letters indicating significance for the control group (or nitrate-supplied group, in Experiment 1) are not comparable to the letters shown for the salinized group (or ammonium-supplied, in Experiment 1). Where an important correlation between control and stressed groups existed this was indicated in the text. A full list of statistical results is presented in **Appendix 2**.

The Lineweaver-Burk equations were compared using the method of Zar (1984) for equating two regression coefficients (slopes) of linear equations. Nitrate and ammonium uptake over time was also compared in this way. Where more than two linear regression lines were compared, analysis of covariance was used to compared the regression coefficients (Zar, 1984).

3.10 Experimental detail

The overall experimental designs of the separate experiments are described here in their logical sequence.

3.10.1 The effect of a salinity gradient on the biomass production and gaseous exchange of nitrate- and ammonium-supplied wheat (Experiment 1)

The aim of this experiment was to determine the effects of a salinity gradient on wheat biomass production and photosynthetic rate as well as to determine a suitable NaCl concentration to use for ensuing experiments.

Tables 2 and **3** shows respectively that the plants were grown separately in either NO_3^- (K^+ salt) or NH_4^+ (Cl^- salt) and in a salinity gradient of 0 mM to 100 mM NaCl. The salinity gradient was 0 mM, 40 mM, 70 mM and 100 mM NaCl. The nitrate and ammonium-supplied plants were grown together and 64 plants were used for each group having a different nitrogen source. At the time of harvest, the biomass production of the shoot, root and plant were determined as well as the root:shoot ratios and moisture contents of the plants within the different treatments (section **3.3**).

The photosynthetic rates, transpiration rates and stomatal conductances of the plants were measured one day prior to the harvest (section **3.4**). Biomass production and gaseous exchange determinations were carried out at 25 °C and 30 °C.

From the experiment described in **3.10.1** it was decided that 50 mM NaCl was a suitable NaCl to use to induce salt-stress while ensuring that the plants would be healthy enough to reach maturity.

3.10.2 Effect of calcium addition on the biomass production (including plant morphology, shoot:root ratios and moisture contents), N content, ^{15}N uptake, ionic content and gaseous exchange of salinized, nitrate-supplied wheat (Experiment 2)

Firstly, the effect of a fairly broad range of $CaCl_2$ concentration was tested: 2.5 mM, 5.0 mM, 8.0 mM and 12.0 mM $CaCl_2$. For this initial experiment the factors considered were biomass production, moisture content, gaseous exchange and ionic content of the plants.

Dried material from eight plants grown at 2.5 mM, 5.0 mM and 8.0 mM $CaCl_2$ were prepared for ion analysis as described in section **3.8**.

Using the lower range to which the salinized plants appeared sensitive: 0.5 mM, 1.0 mM, and 5.0 mM $CaCl_2$, the biomass production, gaseous exchange and N content and $^{15}NO_3^-$ uptake of the plants was measured. The steady state N content as well as the NO_3^- uptake and was determined by feeding the plant with 4 mM $K^{15}NO_3^-$ (99 atom % enriched) as described in section **3.5**. All other nutrient concentrations and forms remained unchanged. ^{15}N uptake was measured in four nutrient treatments: the control and salinized plants, where each group was grown on 2.5 mM and 8.0 mM $CaCl_2$.

For each of the treatments four replicates were used, where each plant was inserted into a 500 *ml* light impermeable, well aerated container which was large enough to exclude both possible damage to the root system and depletion of the nutrient solution within the 8*h* period of the feed. The feed commenced at 8*h*00 and after 8*h* was terminated by harvesting the plant sequentially. The roots and shoots of the plants were immediately weighed and dried at 80 °C for 48*h*, weighed again to obtain their dry mass and milled using a Bodine Electrical Co. mill (Chicago, U.S.A.) with a pore size of 1 *mm*². The samples were then prepared for Kjeldahl digestion and micro-distillation to determine the total N content and ^{15}N content.

3.10.3 Effect of calcium addition on biomass production (including shoot:root ratios and moisture contents), total N content, ^{15}N uptake and gaseous exchange of salinized, ammonium-supplied wheat (Experiment 3)

The calcium concentrations used in the experiment were: 1 mM, 2 mM, 4 mM, and 6 mM. Biomass production, photosynthetic rates, transpiration rates and stomatal conductances were determined. The total N content and uptake of $^{15}NH_4Cl$ (99 atom % enrichment) after an 8*h* $^{15}NH_4^+$ feed was also determined. The references for the above procedures are as for those in section **3.10.2.**

3.10.4 Effect of potassium addition on the biomass production (including shoot:root ratios and moisture contents) and gaseous exchange of salinized, nitrate-supplied wheat (Experiment 4)

It is possible that there exists some interaction between the Ca^{2+} ion and the K^+ ion with respect to salt-stress amelioration. In the experiments under this section the Ca^{2+} concentration was maintained at the normal Long Ashton concentration of the Ca^{2+} salt (4.0 mM). The plants were subject to the following range of K_2SO_4 concentrations: 0.2 mM, 1.0 mM, 2.5 mM and 5.0 mM. The biomass production and gaseous exchange of the plants was determined.

3.10.5 Effect of potassium addition on the biomass production (including plant morphology, shoot:root ratios and moisture contents) and gaseous exchange of salinized, ammonium-supplied wheat (Experiment 5)

The experimental design was identical to Experiment 4, except that the N source was ammonium.

3.10.6 The effect of NaCl plus $CaCl_2$ on the rate of NO_3^- uptake in salinized wheat (Experiment 6)

Nitrate-supplied, salinized (50 mM NaCl) and control (0 mM NaCl) plants were exposed to fresh nutrient solution containing three concentrations of $CaCl_2$: 0.5 mM, 1.0 mM and 5.0 mM. Three replicates per treatment were used. Samples (2 *ml*) of the nutrient solutions were taken every two hours for 8*h*. The plants used were 14*d* old.

3.10.7 The effect of NaCl plus K_2SO_4 on the rate of NH_4^+ uptake in salinized wheat (Experiment 7)

The experimental design was as described in Experiment 6, except that the N source was NH_4^+ and the variable nutrient was K^+. The concentrations of K^+ used were 0.5 mM, 1.0 mM and 5.0 mM. The plants used were 14*d* old.

3.10.8 Kinetics of NO_3^- and NH_4^+ uptake in the presence of NaCl (Experiment 8)

Salinized (50 mM NaCl) and control (0 mM NaCl) plants (between 14 and 27*d* old) were exposed to nitrate concentrations between 1 mM and 10 mM in order to determine the effect of the NaCl on the V_{max} and K_m of the purported NO_3^- transporter. All other nutrient concentrations were kept as for unmodified Long Ashton medium. For the experiment, the plants were transferred into 1*l* light impermeable, well aerated containers and the light and temperature conditions were maintained at the midday condition for the duration of the kinetic experiment. The nitrate concentrations used were 1 mM, 2 mM, 4 mM, 6 mM, 8 mM and 10 mM with four replicates at each concentration within the salinized or control group. After 12*h* samples of the nutrient media were taken and analyzed as described in sections **3.6.1** and **3.6.1.1** respectively.

For the determination of kinetic parameters of NH_4^+ uptake, the experimental design was as described above, except that the N source was ammonium and the samples were taken and analyzed as described in sections **3.6.2** and **3.6.2.1** respectively.

3.10.9 The respective effects of $CaCl_2$ and K_2SO_4 on the NO_3^- and NH_4^+ uptake kinetics of salinized wheat (Experiment 9)

Nitrate-supplied, salinized plants which had been growing on Ca^{2+} concentrations of 0.5 mM, 1.0 mM and 5.0 mM were transferred into 1*l* containers holding the respective modified Long Ashton solutions. The nitrate concentration used were: 1 mM, 2 mM, 4 mM, 6 mM and 10 mM. Four replicates per treatment were used. The feeding time was 12*h* and the samples were analyzed to determine V_{max} and K_m as described in section **3.6.1.1**.

For the determination of the kinetic parameters of NH_4^+ uptake under different potassium regimes (0.5 mM, 1.0 mM and 5.0 mM), the experimental design was as described above, except that the N source was ammonium, the variable nutrient was K^+ and the sample analysis was as described in section **3.6.2.1**.

4. RESULTS AND DISCUSSION

The results of the research described in Chapter 3 are presented and discussed in this chapter. The procedure for the interpretation of the letters appearing in the figures, which indicate results of multiple range tests from one-way ANOVAs, is explained in section **3.9** of **Materials and Methods**, and is consistent for all the figures where letters appear. A comprehensive account of the statistical tests appears in **Appendix 2**.

4.1 Osmolality of the various nutrient media

Figures 8 to **10** show the osmolality (mOs kg^{-1}) of the nutrient solutions used in the experiments, thus indicating where an osmotic/ionic effect of the nutrient mediums used in the ensuing investigations may or may not be expected.

From **Figure 8** it can be seen that above 40 mM NaCl the osmolality differed significantly[2] from the controls (0 mM NaCl) and these nutrient solutions (40 to 100 mM NaCl) would exert an osmotic stress, besides any ionic effects, on hydroponically grown plants. The addition of increasing calcium concentrations (up to 12 mM $CaCl_2$) or potassium concentrations (up to 5 mM K_2SO_4) to a N-free nutrient solution (**Figures 9** and **10** respectively) did not result in a significant change in osmolality of the N-free nutrient solutions. Thus, the influences exerted by Ca^{2+} or K^+ ions on plant roots, within the ranges shown in **Figures 9** and **10**, would be ionic in nature.

4.2 The effect of a salinity gradient the biomass production (including plant morphology, shoot:root ratios and moisture contents) and gaseous exchange of nitrate- and ammonium-supplied wheat (Experiment 1)

The results of this experiment (described in section **3.10.1**) are shown in **Figures 11** to **20**. These results show the effect of (i) increasing NaCl concentration and (ii) nitrogen form, on wheat biomass production, water relations and photosynthetic rates. The effects of (i) and (ii) were investigated at two temperatures (25 °C and 30 °C) in order to establish the effect of combined heat and salinity stress on the biomass production and water relations as well as

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[2] *"Significantly" has been used in the statistical sense throughout the thesis*

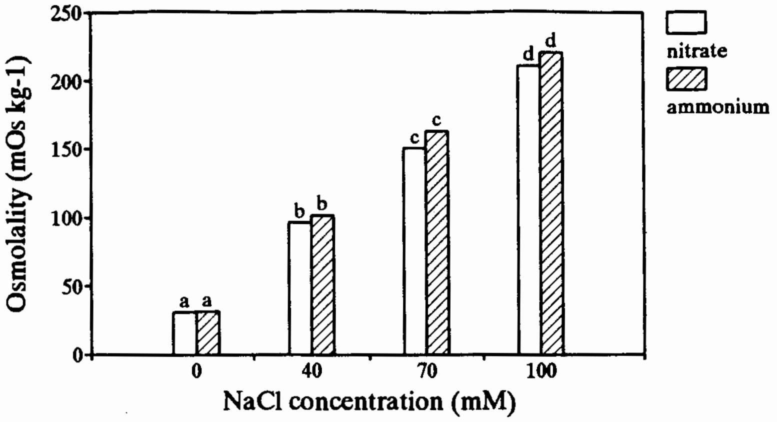

Figure 8: Osmolality of modified Long Ashton nutrient solution with increasing NaCl salinity. The number of replicates (n) = 3. Each series of letters represents a one-way anova.

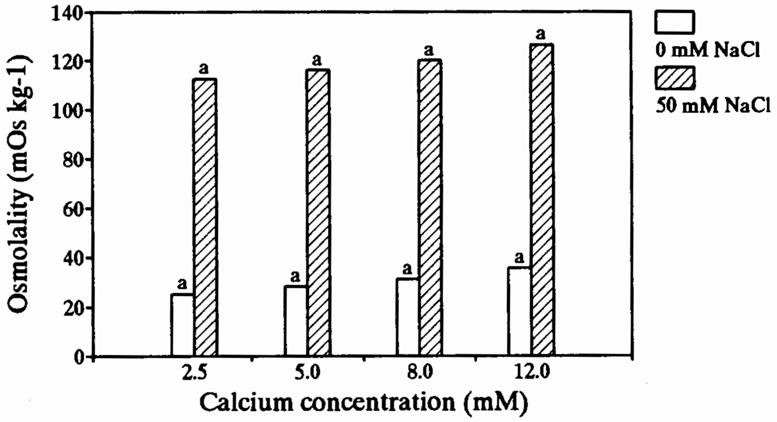

Figure 9: Osmolality of modified, N-free Long Ashton nutrient solution with increasing $CaCl_2$ concentration. (n) = 3. Each series of letters represents a one-way anova.

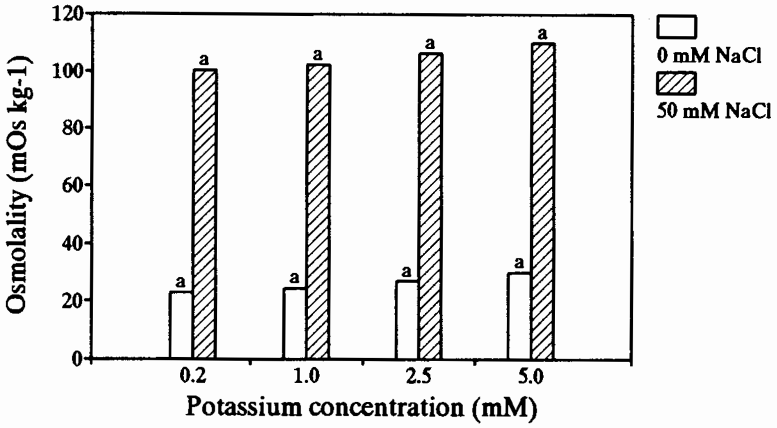

Figure 10: Osmolality of modified, N-free Long Ashton nutrient solutions with increasing K_2SO_4 concentration. (n) = 3. Each series of letters represents a one-way anova.

photosynthetic rates of the plants.

4.2.1 Biomass production of nitrate- and ammonium-supplied wheat grown at 25 °C

It is evident from **Figure 11**, that in both nitrate- and ammonium-supplied wheat, NaCl concentration was a significant determinant of the biomass production of the plant, shoot and root ($P < 0.0001$ for shoot, root and plant, one-way ANOVAs). For plants fed on either nitrogen source, a decrease of approximately 77% to 80% in plant biomass production accompanied the increase in NaCl concentration from 0 mM to 100 mM. Also, it was apparent from the difference between the biomass production of the nitrate- and ammonium-supplied plants that the nitrogen form (NO_3^- or NH_4^+) had a significant effect on plant biomass, predominantly due to the effect of N form on shoot biomass production ($P = 0.0004$, two-way ANOVA). At intermediate salinities (40 mM to 70 mM NaCl) the ammonium-supplied plants had a higher shoot and root biomass production and shoot:root ratio (**Figure 12**) compared to the nitrate-supplied wheat. The effect of nitrogen form on biomass production was not evident at 0 mM NaCl or 100 mM NaCl. While the mean mass of the control (0 mM NaCl) ammonium-supplied root was slightly reduced compared to the nitrate-supplied root, the shoot of the control ammonium-supplied wheat was larger than that of the nitrate-supplied wheat so that there was no significant difference in plant biomass production between the controls of the ammonium and the nitrate grown plants.

Where problematic soil salinities are encountered in crop agriculture, salinities are generally of intermediate concentration (50 mM to 70 mM NaCl) and thus it is plant survival at these levels that is important. Also, arid and saline areas are often deficient in nitrogen, so that salinized plants may become nitrogen starved (Seeman and Sharkey, 1986). *In vivo* assays by Aslam *et al.*, (1984) showed that NaCl salinity had very little effect on the enzymes of NO_3^- and NH_4^+ assimilation. Thus it seems that it is at the site of nitrogen uptake that salinity is taking effect and it has been shown by several authors that N uptake under saline conditions is inhibited (Ward *et al.*, 1986).

The difference observed between the nitrate and ammonium-supplied plants (at intermediate salinities) was possibly due to a separate effect of NaCl on the uptake systems of NO_3^- and NH_4^+. It is possible that due to (i) the disruptive effect of NaCl on the plasmalemma transmembranal proton gradient and (ii) the displacement of Ca^{2+} by Na^+ at the plasmalemma, the effect of increased NaCl may be greater on nitrate-supplied than ammonium-supplied wheat. This may be due to several factors: The uptake of both NO_3^- and NH_4^+ may be passive or active but evidence suggests that NO_3^- influx is strongly dependent on and coupled to the proton motive force across the plasmalemma. Although doubt exists about the role of plasmalemma ATPases in NO_3^- transport, evidence for the existence of permeases across the plasmalemma has been given by Fuggi (1985). It has been shown by Gronwald *et al.*, (1990) and Matoh *et al.*, (1989) respectively that sodic salinity decreases plasmalemma ATPase activity and collapses the ATP-induced pH gradient in Atriplex microsomal membrane vesicles. Thus a NaCl-mediated reduction of the ATPase capacity at the plasmalemma may have a greater influence on NO_3^- influx than on NH_4^+ influx.

In addition, it would appear that protons have a direct effect on the NO_3^- transporter, especially at low NO_3^- concentrations (Deane-Drummond, 1984). There is substantial evidence for NO_3^- uptake being a $2H^+/NO_3^-$ co-transport system (Novacky *et al.*, 1978; Fuggi, 1985; Clarkson, 1986) and that NO_3^- influx is pH dependent (Clarkson, 1986). It has been suggested by Matoh *et al.*, (1989) that Na^+ ions may collapse the pH gradient as a result of a Na^+-specific uptake system, possibly a Na^+/H^+ antiport (see **Figure 1**, section **2.4.1.4**). Thus, if the transmembrane proton electrochemical gradient, at either the plasmalemma or the tonoplast, is disturbed by high NaCl concentrations, such concentrations may inhibit the influx of NO_3^- and/or NH_4^+ as well as other ions (eg. K^+, reported by Läuchli, 1990) and lead to a marked decrease in biomass. Together with the NaCl mediated displacement of Ca^{2+} from the plasmalemma (Läuchli, 1990) and the potentially disruptive effect of NaCl on the orientation and activity of membrane proteins, the former concepts may account for nitrate-supplied wheat biomass production being lower than ammonium-supplied plants at intermediate salinities. Other factors are, however, likely to play a role in the reduced biomass of nitrate-supplied wheat. It is possible that the higher biomass production of the ammonium grown plants was linked to the hydroponic culture medium and would not occur with soil as the culture medium due to a greater resistance to water acquisition in soil media.

For both nitrate- and ammonium-supplied wheat, the shoot and root morphology of the salinized plants (40 to 100 mM NaCl) was visibly different from the controls. The shoot was smaller and the stem was less fibrous compared to the controls. This could be as a result of inadequate turgor potential (see discussion of moisture contents within this section), reduced Ca^{2+} content in the cell walls due to displacement by Na^+ (Läuchli, 1990) and/or potassium leakage (and loss of osmotic potential) caused by NaCl-mediated root plasmalemma depolarization. Some evidence of massive Na^+ influx and K^+ loss from salinized plants was found (section **4.3.4**). The root elongation of the salinized plants was severely reduced compared to the controls and the branches had necrotic tips.

Both NaCl concentration and nitrogen form were significant determinants of the shoot:root ratio ($P < 0.0001$, one-way and two-way ANOVA respectively). The shoot:root ratios in **Figure 12** demonstrate that in plants supplied with either nitrogen form, the reducing effect of salinity on growth was more pronounced in the shoots than in the roots, resulting in a decrease in the shoot:root ratio with increasing salinity. The decrease in shoot:root ratio in the nitrate-supplied plants was less significant ($P = 0.0131$, one-way ANOVA) than in the ammonium-supplied plants ($P = 0.0002$, one-way ANOVA). This decrease in shoot:root ratio in nonhalophytes exposed to salinity has been previously observed by several authors (Greenway and Munns, 1980, Termaat and Munns, 1986; Lewis *et al.*, 1989, Läuchli, 1990).

It is possible that reduced leaf expansion may be correlated with the NaCl mediated decrease in leaf calcium status as well as water deficit. As noted by Läuchli (1990), the leaf apical meristem may be affected by the local ion balance in the expanding shoot and it was proposed by Termaat and Munns (1986) that the reduction in leaf expansion (in wheat, barley and clover) was due to a nutrient deficiency mediated by the root. Although this claim was not accompanied by any empirical evidence, it is feasible considering the observed leakage of the macronutrient K^+ due to NaCl mediated plasma membrane depolarization (Cramer *et al.*, 1985), the displacement of calcium ions by NaCl from the plasmalemma and, possibly, internal pools (Cramer *et al.*, 1984, 1987, 1988; Läuchli, 1990) and the reduced nitrogen uptake evident in most nonhalophytes (Ward *et al.*, 1986).

Figure 13 shows the moisture contents (g g^{-1} dry mass (dm)) of both nitrate- and ammonium-supplied plants subject to a salinity gradient. Regardless of the nitrogen form, NaCl concentration had a significant reducing effect on the moisture content of the plant ($P < 0.0001$ for both nitrate and ammonium-supplied plants, two-way ANOVAs). The results are shown for the whole plant and shoot only since the moisture content values of the root

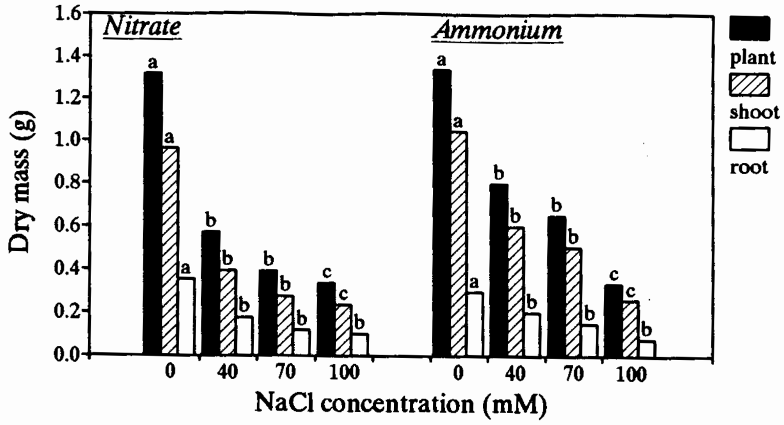

***Figure 11:** The effect of a salinity gradient on biomass production in nitrate or ammonium-supplied wheat at 25 °C. (n) = 16. Each series of letters represents a one-way anova.*

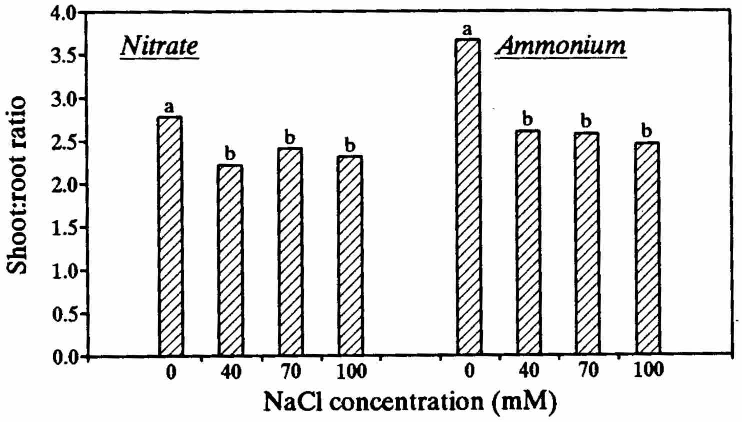

***Figure 12:** The effect of a salinity gradient on the shoot:root ratio of nitrate or ammonium-supplied wheat at 25 °C. (n) = 16. Each series of letters represents a one-way anova.*

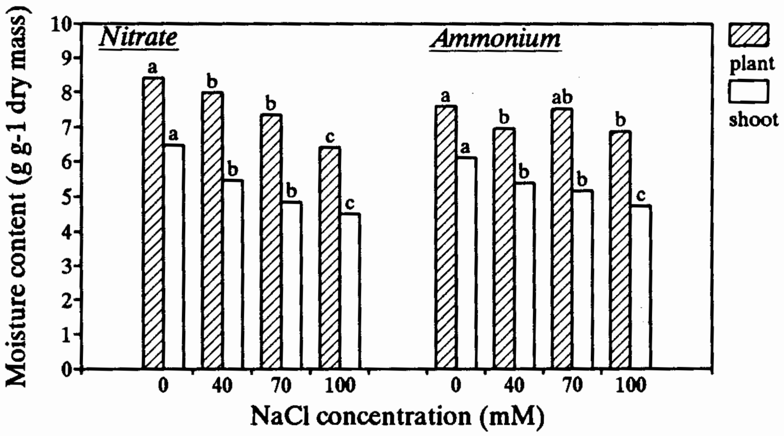

***Figure 13:** The effect of a salinity gradient on the moisture content of nitrate or ammonium-supplied wheat at 25 °C. (n) = 16. Each series of letters represents a one-way anova.*

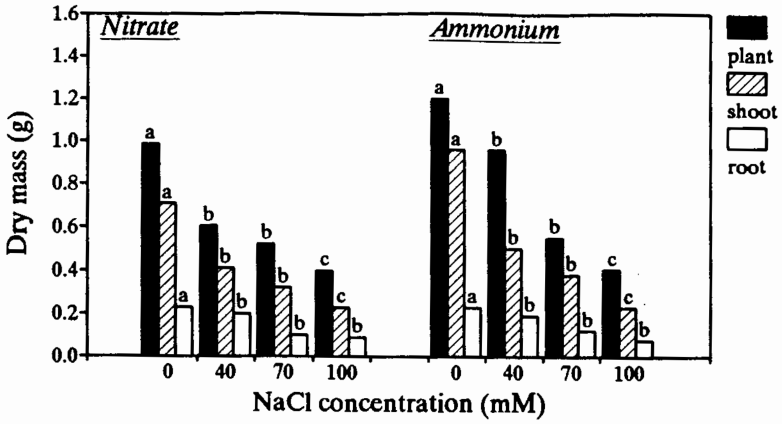

Figure 14: The effect of a salinity gradient on the biomass production of nitrate or ammonium-supplied wheat at 30°C. (n) = 16. Each series of letters represents a one-way anova.

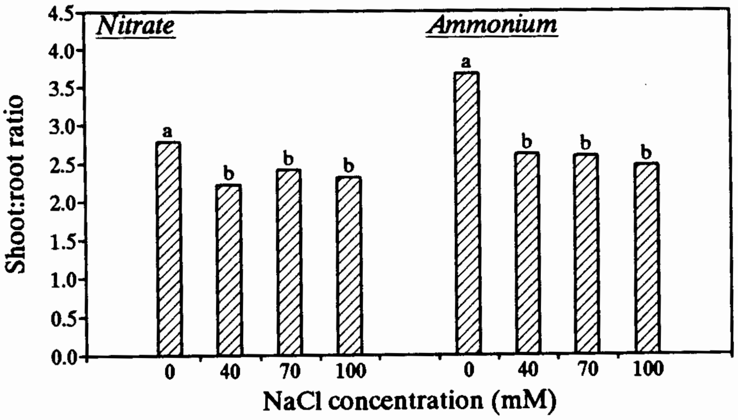

Figure 15: The effect of a salinity gradient on the shoot:root ratio of nitrate or ammonium-supplied wheat at 30 °C. (n) = 16. Each series of letters represents a one-way anova.

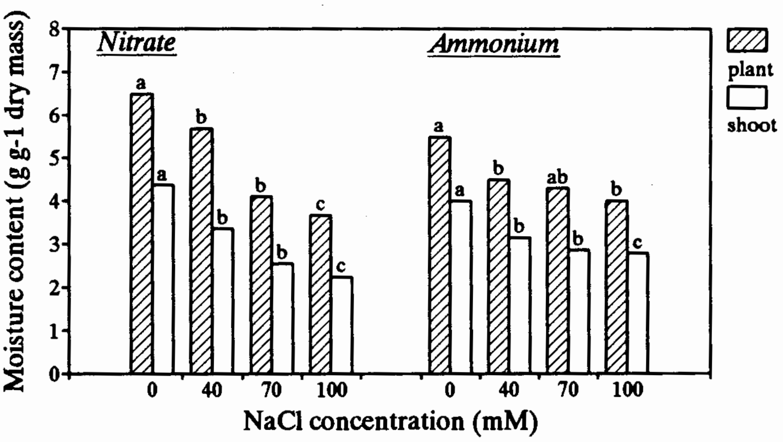

Figure 16: The effect of a salinity gradient on the moisture contents of nitrate or ammonium-supplied wheat at 30 °C. (n) = 16. Each series of letters represents a one-way anova.

were variable. This was probably due to the necessity of blotting the roots dry before weighing. The decrease in the moisture content value of the plants could be ascribed to the non-specific osmotic effect of the NaCl lowering the ψ_w of the plant.

4.2.2 Biomass production of nitrate- and ammonium-supplied wheat grown at 30 °C

Figures **14**, **15** and **16** illustrate the biomass production, shoot:root ratios and moisture contents respectively, of control plants and plants grown under a salinity gradient at 30°C. The same trends were reflected at 30°C as at 25°C, except that the plant biomass production at 30 °C was slightly reduced and at the higher salinities (70 mM and 100 mM NaCl) a few individual plants did not survive the 27 day period. The reduced biomass was most obvious in the controls, while the effect of salt stress mostly concealed any temperature induced effects. Since the few individual plants which died were all salinized plants, it can be concluded that the temperature increase did exacerbated the effect of salinity, even in hydroponic culture.

From the results described under the section **4.2**, 50 mM NaCl was selected as the constant NaCl concentration to be used for ensuing experiments. At this NaCl concentration the plants were reduced in size while not being in a state of wilt.

4.2.3 Gaseous exchange (including photosynthetic and transpiration rates, stomatal conductances and WUE) of nitrate- and ammonium-supplied wheat grown at 25 °C and 30 °C.

The photosynthetic rates, transpiration rates, stomatal conductances and WUE (water use efficiency) of salinized, nitrate- or ammonium-supplied wheat grown at 25 °C are shown in **Figures 17, 18, 19** and **20** respectively. The corresponding data at 30 °C was similar in trend and is not shown.

It should be noted that the stomatal conductance measurements were obtained from the ADC2 and ADC3 IRGAs which rely on a calculated variable (leaf temperature) to determine stomatal conductance. This makes the stomatal conductance measurements somewhat less sensitive than the stomatal diffusive resistance measurements obtained using the Licor porometer.

It is evident from **Figure 17**, that there was no significant difference between the photosynthetic rates of the nitrate and the ammonium grown wheat regardless of the NaCl concentration at which the plants were grown. Below 70 mM NaCl, as the salinity increased the photosynthetic rates also increased in both the nitrate- and the ammonium-supplied plants. The photosynthetic rates of the nitrate and the ammonium grown plants at 70 mM NaCl were almost double the rates of the respective controls. It is likely that this increase under mild salt stress was a consequence of the stomatal conductances (**Figure 19**) having increased at these salt concentrations. Despite the fact that the stomatal conductances continued to rise above 70 mM NaCl, it is likely that at 100 mM NaCl the plants were suffering from osmotic stress since the nitrate- and ammonium-supplied plants appeared wilted and the transpiration rates (**Figure 18**) had dropped. Under the osmotic stress imposed upon the plants at 100 mM NaCl a reduction in photosynthetic rates also occurred.

Although those plants grown at 100 mM NaCl appeared wilted, **Figure 19** clearly indicates that the stomatal conductances increased with increasing NaCl concentration. NaCl had a visually (observable macroscopically and microscopically) damaging effect on the root cells. Considering the alleged "chaotropic" effect of Cl^- ions on the root cell membranes (section **2.1.1.2**) it is possible that the increased NaCl concentration mediated a decrease in resistance to mass inflow at the root level and hence the increased stomatal conductances. It is also evident from **Figure 19** that the ammonium grown plants, as a whole, had significantly lower stomatal conductances than those receiving nitrate nutrition. This is further illustrated by the transpiration rates (**Figure 18**) and most noticeably by the WUE (**Figure 20**) of the nitrate and ammonium grown plants. The WUE (*mmol* CO_2 *gained per mol* H_2O *lost*) of the ammonium-supplied plants was higher at comparable salinities (except for 100 mM NaCl) than the WUE of the nitrate-supplied plants. As expected, with increasing NaCl concentration the WUE of the plants decreased, indicating the osmotic effect of the saline nutrient solution on the ψ_w of the plants.

Increasing salinity had little to no deleterious effect on the photosynthetic and transpiration rates of the plants. Therefore it is unlikely that the salinity had any ionic effect on the photosynthetic apparatus. This is supported by the fact that the internal CO_2 concentrations of the control and the salinized plants were similar and unaffected by the NaCl gradient. An

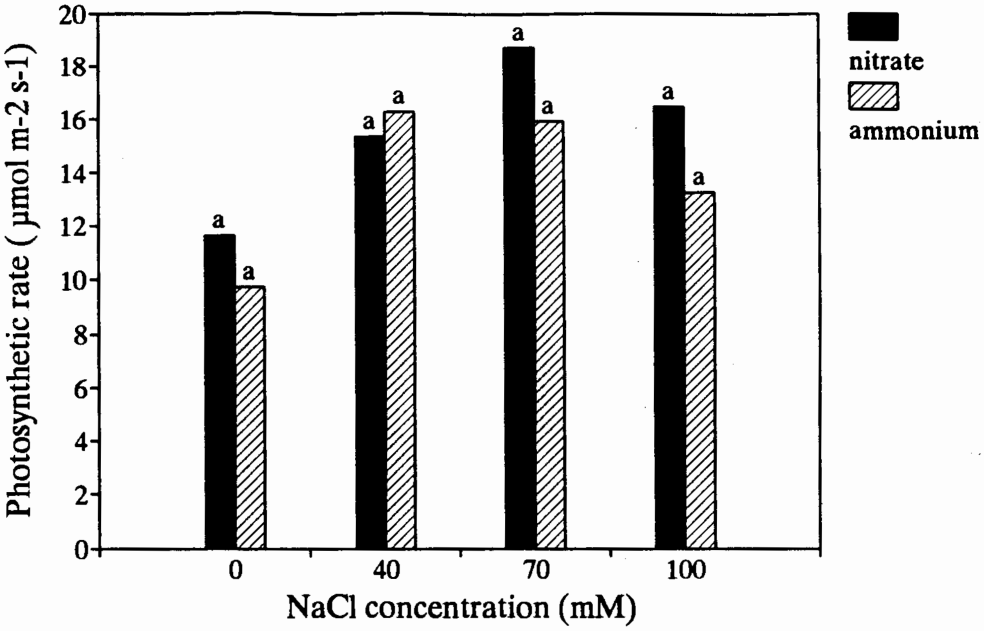

Figure 17: The effect of a salinity gradient on the photosynthetic rate in nitrate or ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

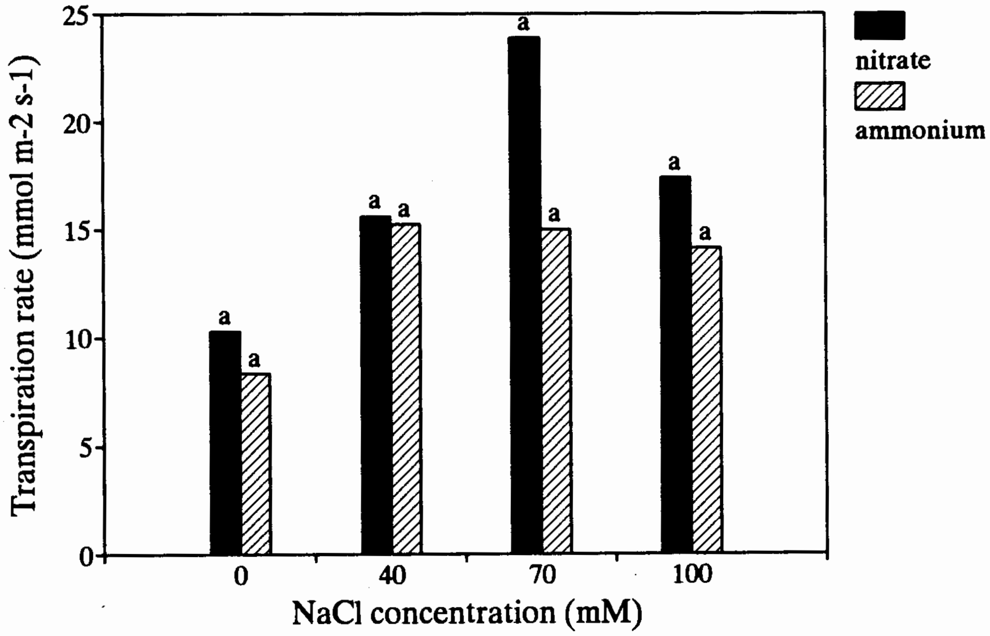

Figure 18: The effect of a salinity gradient on the transpiration rate of nitrate or ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

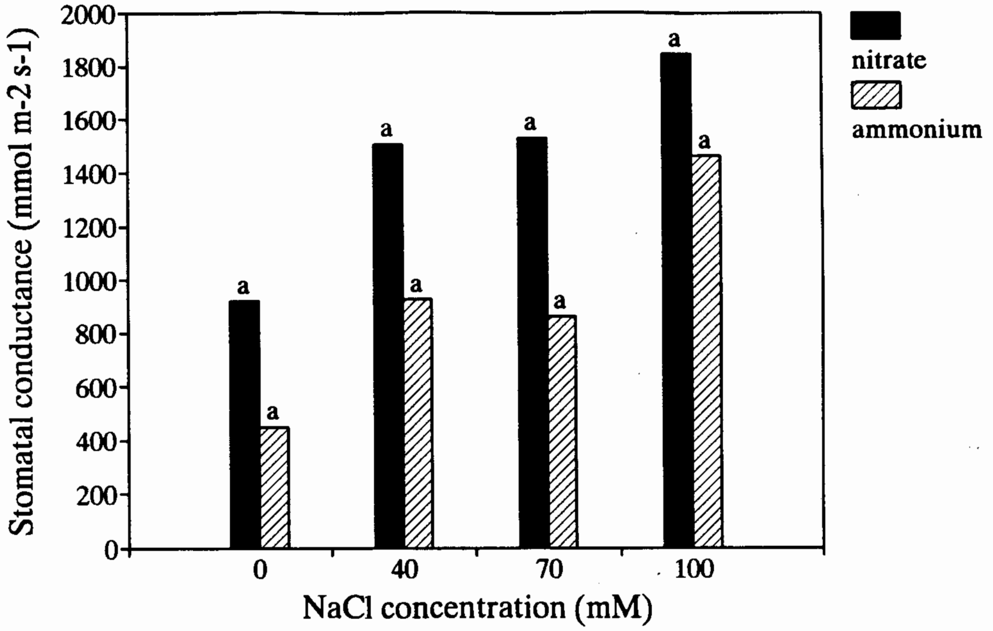

Figure 19: The effect of a salinity gradient on the stomatal conductance to water of nitrate or ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

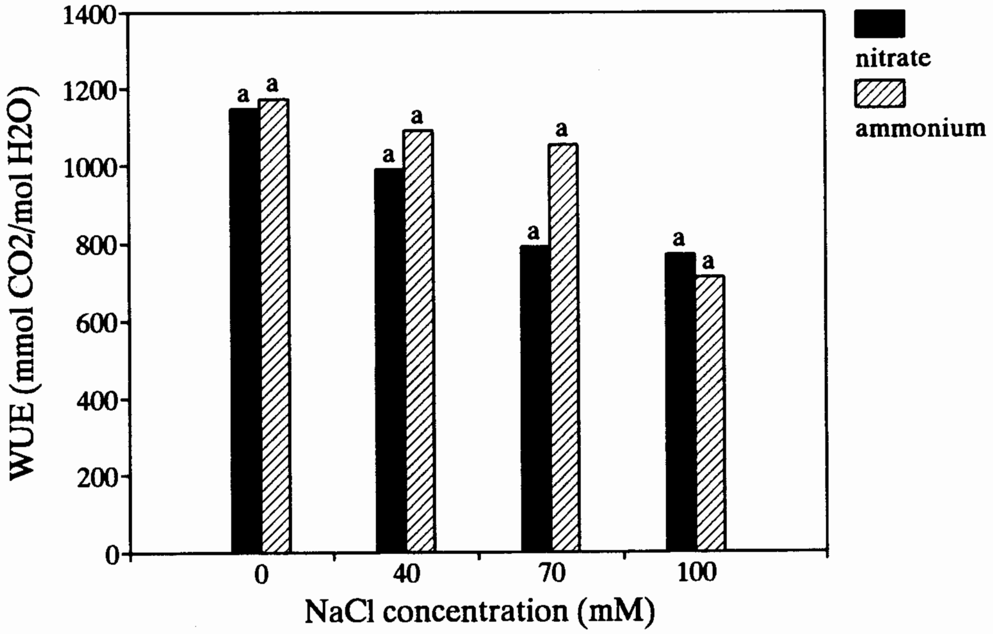

Figure 20: The effect of a salinity gradient on the WUE of nitrate or ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

increase in internal CO_2 concentration in the salinized wheat would have indicated a decrease in the CO_2 assimilation efficiency. The steady decrease in WUE of the plants as the salinity was raised indicates that the primary stress imposed on the gaseous exchange capacity of the nitrate and ammonium grown plants was osmotic in nature.

4.3 Effect of calcium addition on the biomass production (including plant morphology, shoot:root ratios and moisture contents), N content, ^{15}N uptake, ionic content and gaseous exchange of salinized, nitrate-supplied wheat (Experiment 2)

This experiment (described in section **3.10.2**) investigated the potential of exogenous $CaCl_2$ to ameliorate the effects of 50 mM NaCl on biomass production, gaseous exchange, $^{15}NO_3^-$ uptake and ionic content of Na^+ and K^+ in nitrate-supplied wheat. These results are illustrated in **Figures 21** to **38**.

4.3.1 Effect of calcium addition on the biomass production of salinized, nitrate-supplied wheat

The biomass production of salinized (50 mM NaCl) and control plants (0 mM NaCl) supplied with nitrate and ranges of $CaCl_2$ concentrations is shown in **Figures 21** and **22**. **Figure 21** shows biomass production over a range of calcium concentrations in the nutrient medium from 2.5 mM to 12 mM $CaCl_2$, while **Figure 22** shows the biomass production over the smaller range to which the salinized plants appeared sensitive: 0.5 mM to 5.0 mM $CaCl_2$.

With reference to **Figure 21**, the presence of NaCl had a significant reducing effect on the biomass production of the plants ($P < 0.0001$, two-way ANOVA) while calcium concentration significantly determined the biomass production of both the control and stressed plants. In the case of the controls, however, calcium concentration had a reducing effect at 12 mM $CaCl_2$. It was evident that the biomass production of both control and the salinized plants passed through an optimum: at 5.0 to 8.0 mM $CaCl_2$ for the control plants and 5.0 mM $CaCl_2$ for the salinized plants. For both the control and the salinized plants, at low calcium concentration (2.5 mM $CaCl_2$) and high calcium concentration (12.0 mM $CaCl_2$) biomass production was reduced, while at intermediate calcium concentrations the biomass production of the stressed plants was increased. In the salinized plants the increase in biomass production due to the exogenous calcium concentration was most evident in the shoot component. Increasing the calcium concentration from low to higher concentrations (2.5 mM to 5.0 mM $CaCl_2$) resulted in a $\approx$50% increase in the salinized shoot biomass production while there was only an $\approx$18% increase in the control. It has been reported by several authors that the amelioration by calcium of NaCl-mediated biomass reduction was most evident in the shoot. This was also found to be so in this experimentation.

The condition of shorter and necrotic root tips in the salinized roots (section **4.2.1**) was alleviated by the higher Ca^{2+} concentrations (5 mM to 12 mM $CaCl_2$). Cramer *et al.*, (1991a) also found that additional exogenous calcium in the nutrient medium (10 mM $CaCl_2$) had a beneficial effect on the growth of salinized, hydroponically grown barley, where the salt stress was 125 mM NaCl.

When the lower range of calcium concentrations to which the salinity stressed plants appeared sensitive, was expanded (**Figure 22**), the same trends were obtained as in **Figure 21**, except that the increase in calcium concentration had no significant effect on the biomass production of the controls. This was to be expected considering that only a calcium concentration of 12.0 mM $CaCl_2$ had a significant effect on the biomass production of the control plants. Evidently, for salinized plants, there was some significant, beneficial $CaCl_2$/NaCl or $CaCl_2$/plasmalemma interaction occurring at approximately 5.0 mM $CaCl_2$ concentration which was not evident for the controls. Which one of these interactions was occurring is elucidated in section **4.10.1**. **Figure 22** shows that increasing calcium concentration significantly elevated the biomass production of the salinized shoot, root and plant ($P < 0.0001$, $P = 0.0015$ and $P < 0.0001$ respectively, one-way ANOVAs). Possible reasons for this Ca^{2+}-mediated increase in biomass production are suggested from the data presented in sections **4.3.2, 4.3.4** and **4.10.**

Calcium concentrations between 0.5 mM and 5.0 mM $CaCl_2$ influenced the shoot:root ratio of the salinized plants (**Figure 23**) but not of the control plants ($P < 0.0001$ and $P = 0.3918$ respectively, one-way ANOVAs). The shoot:root ratio of the stressed plants reached a maximum at 5.0 mM corresponding to the increased biomass production and moisture content (**Figure 24**) at this calcium concentration. The exogenous calcium was to some extent counteracting the NaCl-mediated decrease in shoot expansion observed in section **4.2.1**.

The addition of increasing calcium concentration from 0.5 mM to 5.0 mM significantly

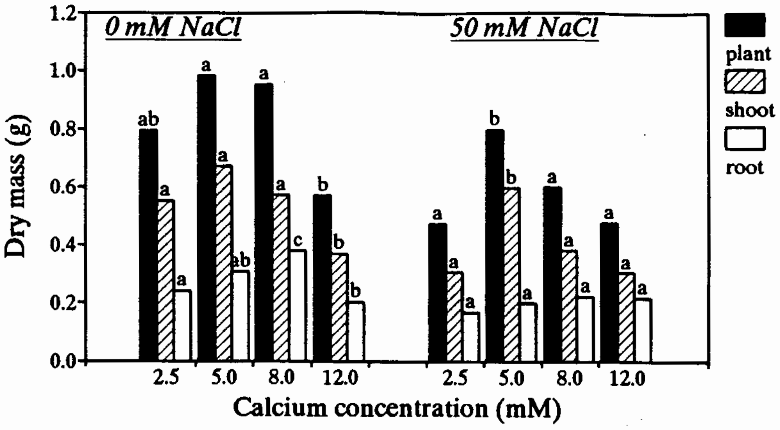

***Figure 21:** The effect of calcium concentration on the biomass production of salinized, nitrate-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.*

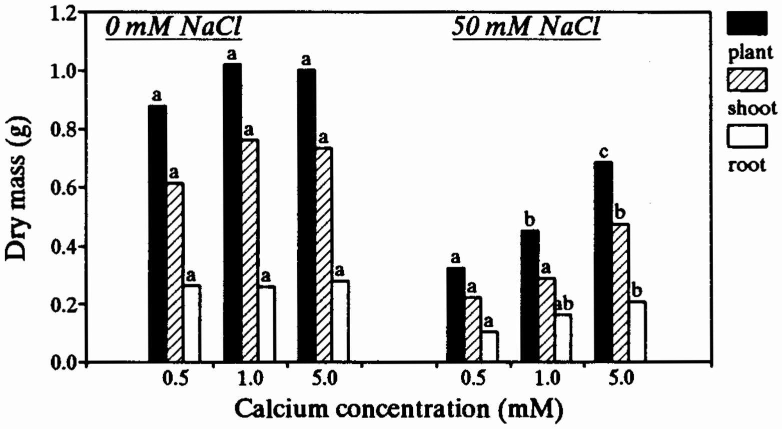

***Figure 22:** The effect of calcium concentration (0.5 mM - 5.0 mM) on the biomass production of salinized, nitrate-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.*

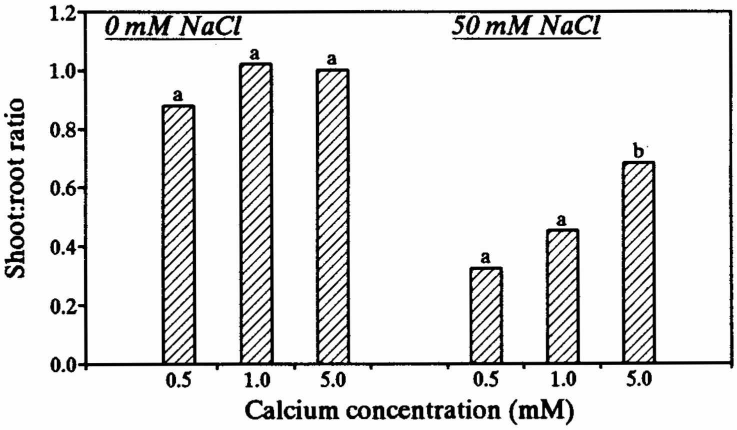

***Figure 23:** The effect of calcium concentration on the shoot:root ratio in salinized, nitrate-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.*

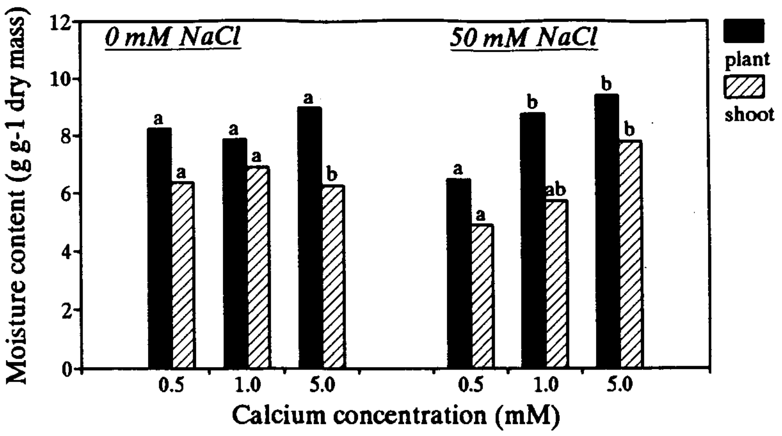

Figure 24: The effect of calcium concentration on the moisture content of salinized, nitrate-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.

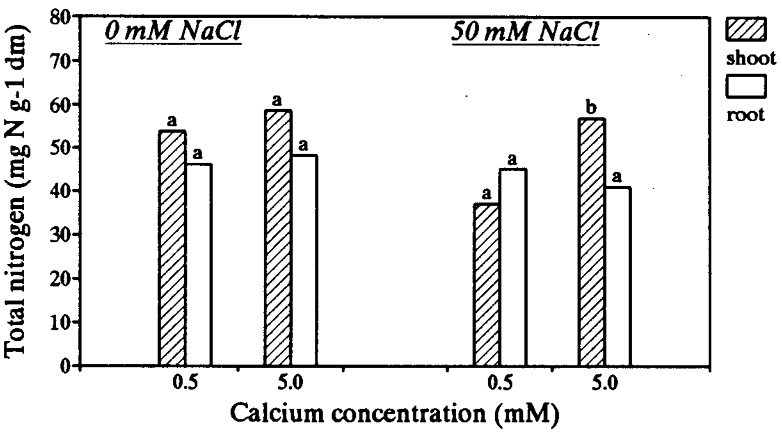

Figure 25: The effect of calcium concentration on the total nitrogen content of salinized, nitrate-supplied wheat. (n) = 4. Each series of letters represents a one-way anova.

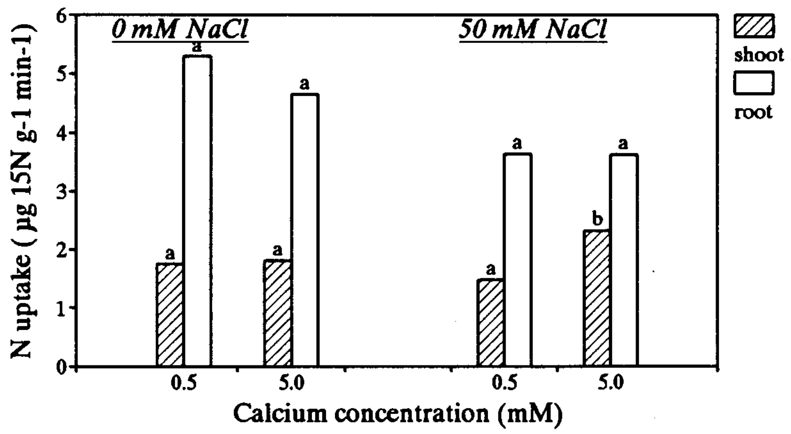

Figure 26: The effect of calcium concentration on $^{15}NO_3^-$ uptake in salinized, nitrate-supplied wheat. (n) = 4. Each series of letters represents a one-way anova.

elevated the moisture content of the stressed plants (P = 0.0007, one-way ANOVA) so that the moisture contents (**Figure 24**) of the stressed plants at 5.0 mM $CaCl_2$ approximated those of the controls at the same $CaCl_2$ concentration. Calcium concentration had no effect on the moisture content of the control plants. As will be discussed in section **4.3.4**, calcium may have exerted an effect on the moisture contents of the plants by protection of the root from K^+ leakage or Na^+/K^+ competition.

4.3.2 Effect of calcium addition on the total N content and $^{15}NO_3^-$ uptake in salinized, nitrate-supplied wheat

The steady-state total N content (*mg N g⁻¹ dm*) of control and salinized plants at two calcium concentrations (0.5 mM and 5.0 mM $CaCl_2$) is shown in **Figure 25**. Regardless of the calcium concentration there was a reduction in the total N of the salinized plants relative to the controls. While there was no effect of calcium on the total N content of the control plants, there was a significant increase in total N content in the shoot of the salinized plants with an increase in the calcium level of the nutrient medium. This increase in nitrogen content in the stressed shoots may account for the increased biomass production of the shoots of the same plants (**Figures 21** and **22**) and the increase in the shoot:root ratio (**Figure 23**). The increase in N content of the shoot with an increase in calcium concentration from 0.5 mM to 5.0 mM resulted in N levels equivalent to those levels found in the controls at the same calcium concentration. Calcium concentration was found to have no significant effect on the N content of either the control or stressed root.

It has already been shown that Na^+ has little effect on the enzymes of nitrate assimilation (Passera and Albuzio, 1978; Huffaker and Rains, 1985) and it is believed that the main effect of salinity toxicity on N metabolism is by reducing N uptake. The ^{15}N uptake ($\mu g\ ^{15}N\ g^{-1}\ dm\ min^{-1}$) of the same plants is shown in **Figure 26**. Both the control and the salinized plants showed a greater ^{15}N content in the roots than in the shoots since the time scale of the experiment (8*h*) excluded relatively higher shoot ^{15}N contents. At 0.5 mM $CaCl_2$, a salinity stress of 50 mM NaCl did decrease $^{15}NO_3^-$ uptake into the root and the ^{15}N content of the shoot. An increase in calcium concentration from 0.5 mM to 5.0 mM $CaCl_2$ resulted in more $^{15}NO_3^-$ being taken up into the shoot, so that at 5.0 mM $CaCl_2$ the ^{15}N content of the salinized plants approximated that of the control plants.

The influence of calcium concentration on N content and $^{15}NO_3^-$ uptake may be due to the effect of calcium at several sites. The higher calcium concentration may be operative in a general membrane effect, protecting it from the electrostatic interference effect Na^+ has on membrane components. The effect of calcium may alternatively have a direct effect on the NO_3^- transporter at the root membrane, allowing improved NO_3^- uptake compared to that of the "low" calcium plants.

One way in which Ca^{2+} ions may facilitate the "protection" of the NO_3^- transporter is by the fact that calcium has been shown to activate an electrogenic proton pump at the plasma membrane (Lew, 1989) and it has been shown that NO_3^- uptake is electrogenic in nature, ie. dependent on pH (Deane-Drummond, 1984a, 1984b) and stimulated by lipophilic carriers facilitating transport of cations (K^+-valinomycin and H^+-CCCP (Garland et al., 1975). If such a mechanism existed then it may act in conjunction with the general membrane protective effect first proposed by Epstein (1961).

4.3.3 Effect of calcium addition on the gaseous exchange (including photosynthetic and transpiration rate, stomatal conductances and WUE) of salinized, nitrate-supplied wheat

Photosynthetic rates (**Figure 27**) increased with an increase in calcium concentration from 2.5 to 12.0 mM $CaCl_2$ in both control and salinized wheat plants. However, similar experiments performed under the same conditions as prevailed for the plants in **Figure 27** contradicted the apparent increase in photosynthetic rate with an increase in calcium concentration. It must be concluded that either calcium had no measurable effect on the photosynthetic rates of salinized or control wheat supplied with nitrate, or that such effects were not measurable within the experimental period. The other gaseous exchange measurements were reproducible.

Transpiration rates (**Figure 28**), like photosynthetic rates, followed the pattern of stomatal conductances to H_2O (**Figure 29**). There appeared to be no effect of calcium concentration on the transpiration rates of the salinized plants while an increase in transpiration was observed for the control plants at 8.0 mM $CaCl_2$. The transpiration rates of the salinized wheat was higher than those of the controls contrary to what might be expected from water stressed plants, where an increased WUE is often observed. The increase in transpiration rates was due to the higher overall stomatal conductances of the stressed plants (section

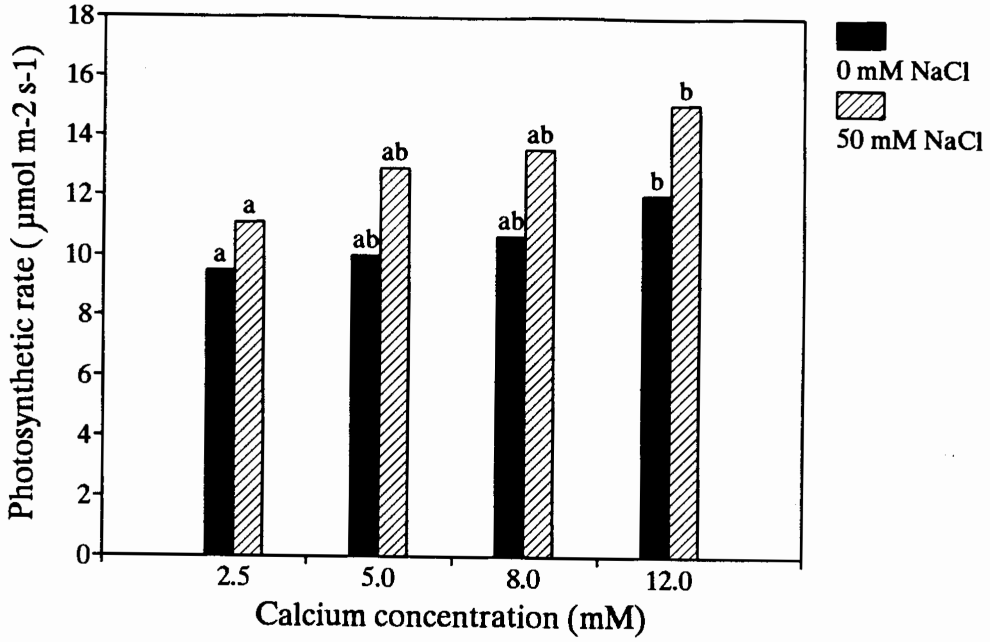

Figure 27: The effect of calcium concentration on the photosynthetic rate of salinized, nitrate-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

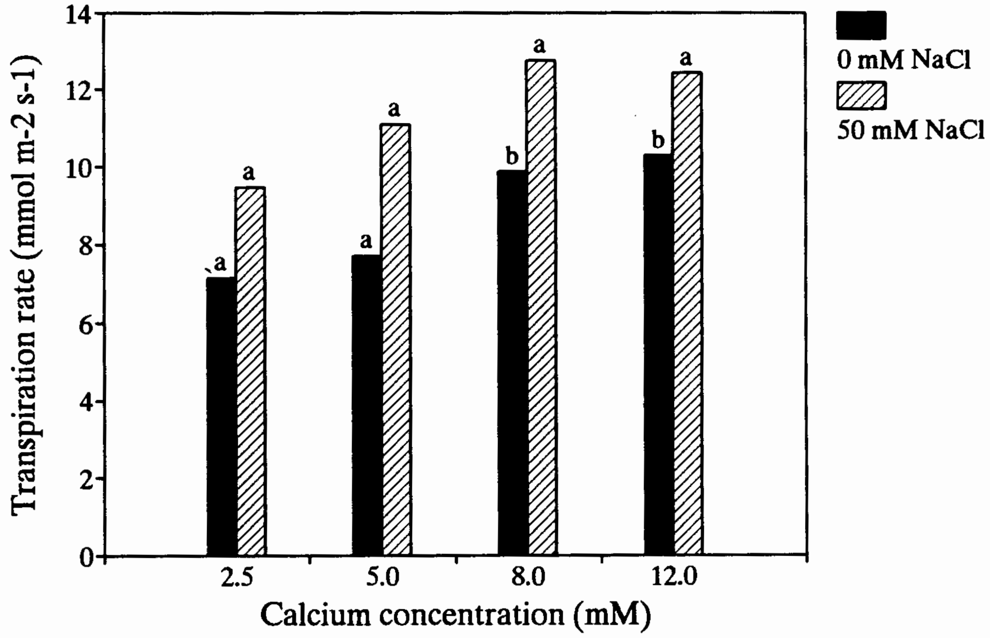

Figure 28: The effect of calcium concentration on the transpiration rate in salinized, nitrate-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

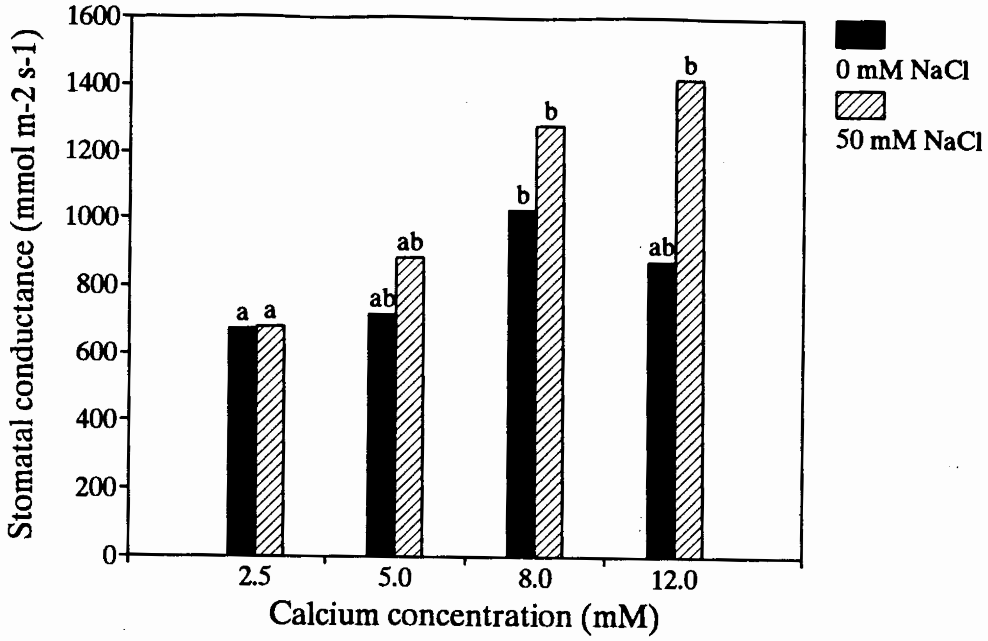

Figure 29: The effect of calcium concentration on the stomatal conductance to water of salinized, nitrate-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

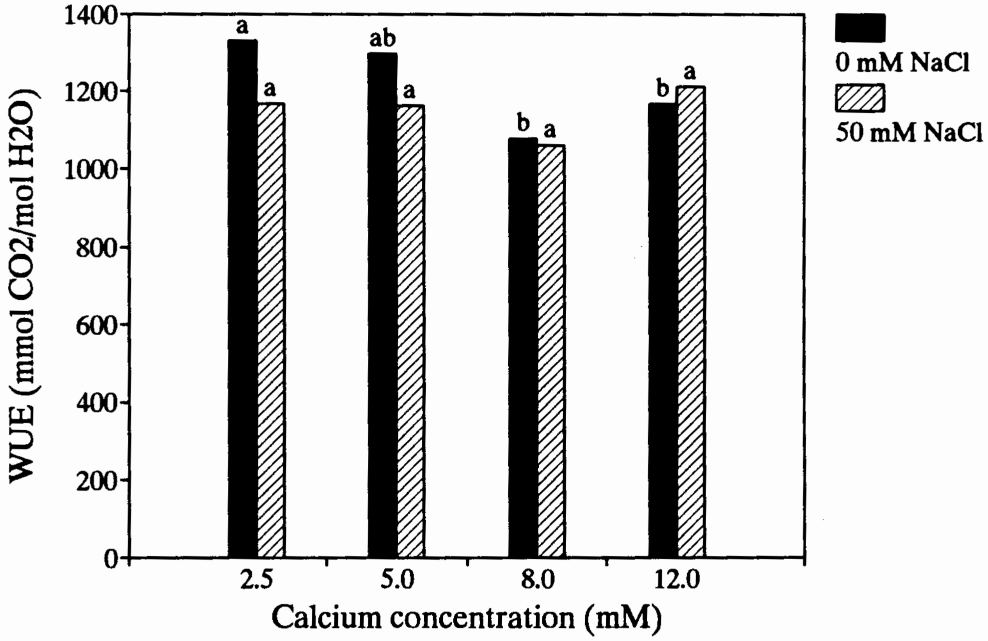

Figure 30: The effect of calcium concentration on the WUE of salinized, nitrate-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

4.2.3).

Stomatal conductances to H_2O are shown in **Figure 29**. Below a $CaCl_2$ concentration of 5.0 mM the stomatal conductances of the control and stressed plants were comparable while the exogenous calcium concentration had no effect on either the control or the salinized plants. As the calcium concentration was raised above 5.0 mM the stomatal conductances of the stressed wheat increased to $\approx 1400\ mmol\ m^{-2}\ s^{-1}$. It is possible that the stressed plants subject to these higher concentrations of $CaCl_2$ suffered from the additional osmotic stress imposed by the $CaCl_2$ while for the control plants the additional osmoticum was not significant.

The WUE (**Figure 30**) of the salinized plants was lower overall than that of the control plants. Calcium concentration had no significant or observable effect on the salinized plants while the WUE of the control plants decreased with an increase of $CaCl_2$ concentration above 2.5 mM $CaCl_2$. This was due to the increase in transpiration rates being proportionally larger than the increase in photosynthetic rate with the increase in calcium concentration (**Figures 27** and **28**).

4.3.4 Effect of calcium addition on the K^+ and Na^+ content, K^+:Na^+ ratio and K^+ *versus* Na^+ selectivity of salinized, nitrate-supplied wheat

Inorganic ion analysis (described in section **3.8**) enabled the determination of the extent of variation in sodium and potassium concentrations of the different plant components (shoot tip, shoot and root). Ion content was observed as a function of presence or absence of 50 mM NaCl and the concentration of exogenous calcium fed to nitrate-supplied wheat. "Shoot tip" is used here to denote that part of the shoot composed of unexpanded leaf material. The ionic contents of the plant components were expressed as concentration of ions in the tissue water ($mmol\ l^{-1}$) and these values are given in the tables while the figures show the results as percentages of the controls. The K^+:Na^+ ratio and K^+:Na^+ Selectivity ($S_{[K+,Na+]}$) values are also shown in the figures.

4.3.4.1 Effect of calcium addition on the K^+ content of salinized, nitrate-supplied wheat

Potassium content of plant tissue, especially young expanding tissue, is important for turgor pressure maintenance (Noggle and Fritz, 1983), while the maintenance of a favourable

K^+:Na^+ ratio during salinity stress is especially important for growth and many metabolic processes (Greenway and Munns, 1980).

It was found that a salt stress of 50 mM NaCl significantly influenced the K^+ content of the shoot tip (unexpanded leaves), shoot and root ($P < 0.0001$, $P = 0.0002$ and $P < 0.0001$ respectively, two-way ANOVAs). The actual K^+ and Na^+ contents of the plant components in control and salinized plants are given in **Table 5**. This table shows that the presence of 50 mM NaCl in the root medium caused the net potassium content of the plants to increase. It should be noted that the net K^+ content does not account for K^+ efflux or mobilization from internal pools, thus the apparent stimulation does not necessarily indicate increased influx. The K^+ content of the shoot was higher than that of the root at all calcium concentrations. Increasing the calcium concentration from 2.5 mM to 8.0 mM increased the K^+ content in both the control and salinized plants. Thus, a clearer indication of the effect that calcium concentration had on the ionic contents of the salinized plants is given when the results are expressed as percentages of the controls.

Figure 31 shows the potassium content as a percentage of control plants, for the salinized shoot tip, shoot and root. It is evident from this figure that the relative K^+ content of the shoot tip increased significantly with increasing exogenous calcium concentration, while the K^+ content of the rest of the shoot decreased with an increase in calcium concentration. The K^+ content of the root (lower than that of the entire shoot) did not change with an increase in calcium concentration.

High calcium concentrations (5.0 to 8.0 mM) caused an increase in the K^+ content of the salinized shoot, but not the root of nitrate-supplied plants (**Table 5** and **Figure 31**). By some mechanism likely to occur primarily at the root plasmalemma, the available K^+ may have been redirected to the expanding shoot from (i) older leaves and/or (ii) selective uptake from the root. This would benefit the expanding shoot which would require that adequate turgor pressure be maintained in the meristematic region for the continuance of growth. The trends shown in **Figure 31** are indicative of the highly significant trends found in the K^+:Na^+ ratios and K^+ versus Na^+ selectivity ($S_{[K,Na]}$) of these plants (**Figures 33** to **38**).

Often it has been found that it is the ratios of different cations that is important for plant growth and function, and not the actual concentrations (Cramer *et al.*, 1991b). This is due to the potential for ion "antagonisms", such as ion competition at the root medium-root interface and the influencing of ion activities by other ions.

4.3.4.2 Effect of calcium addition on the Na^+ content of salinized, nitrate-supplied wheat

A salt stress of 50 mM NaCl significantly increased the Na^+ content of the shoot tip, shoot and root ($P < 0.0001$ respectively, two-way ANOVAs) while the increasing exogenous Ca^{2+} concentration had a significant reducing effect on the sodium content of the salinized plants only (**Table 5** and **Figure 32**). With an increase in Ca^{2+} concentration in the external medium, there was a significant decrease in the Na^+ content of stressed shoot tips, shoots and root (**Table 5**) and these actual decreases were reflected in the data shown as percentages of the controls in **Figure 32**. The relative Na^+ content in the shoot tips decreased from 406% to 133% of the control value with an increase in Ca^{2+} from 2.5 mM to 8.0 mM. The same trends were evident for the shoot and root components but were less obvious. Nonetheless, the relative Na^+ content of the entire shoot and root were reduced to values approaching those of the controls. The lowest Na^+ content was found in the root (**Table 5**) despite the proximity of the organ to the salinized exogenous medium. However, the values for the root should be interpreted with care since it is possible that ions were removed during the washing procedure, prior to analysis.

4.3.4.3 Effect of calcium addition on the K^+:Na^+ ratio and selectivity for K^+ *versus* Na^+ ($S_{[K,Na]}$) of salinized, nitrate-supplied wheat

It was found that NaCl and $CaCl_2$ had a significant effect on the K^+:Na^+ ratio of the shoot tip component ($P = 0.0406$, one-way ANOVA). **Figure 33** shows that the K^+:Na^+ ratio of the stressed shoot tip and root increased with increasing Ca^{2+} concentration while Ca^{2+} concentration had no significant effect on the ratio of the shoot component.

Both the control and stressed shoot tip components showed an increase in the ratio with an increase in the Ca^{2+} concentration. In the control shoot tips this was due mostly to the apparent increase in K^+ content with an increase in calcium concentration, an effect also observed in the stressed shoot tips. The salinized shoot tip showed an increase in K^+ content (**Figure 31**) as well as a significant decrease in Na^+ content (**Figure 32**) which made the effect of Ca^{2+} on the K^+:Na^+ ratio highly significant ($P < 0.0001$, one-way ANOVA) while

Table 5: Potassium and sodium ion contents of salinized, NO_3^--supplied, Ca^{2+} supplemented wheat plants.

Plant part	Treatment	Ionic content (mmol ion l^{-1} tissue H_2O)	
	Calcium (mM)	K^+	Na^+
<u>0mM NaCl</u>			
Shoot tip	2.5	372 ± 5	2158 ± 35
Shoot	2.5	358 ± 8	1277 ± 36
Root	2.5	153 ± 2	866 ± 21
Shoot tip	5.0	417 ± 4	1140 ± 29
Shoot	5.0	410 ± 3	1661 ± 52
Root	5.0	132 ± 5	440 ± 43
Shoot tip	8.0	410 ± 0.2	1569 ± 148
Shoot	8.0	532 ± 4	1681 ± 191
Root	8.0	238 ± 3	848 ± 126
<u>50mM NaCl</u>			
Shoot tip	2.5	457 ± 6	8768 ± 348
Shoot	2.5	468 ± 2	4567 ± 273
Root	2.5	131 ± 3	1906 ± 55
Shoot tip	5.0	590 ± 4	3462 ± 131
Shoot	5.0	485 ± 1	3062 ± 306
Root	5.0	102 ± 3	1094 ± 66
Shoot tip	8.0	627 ± 8	2094 ± 202
Shoot	8.0	495 ± 4	2834 ± 262
Root	8.0	173 ± 2	1062 ± 150

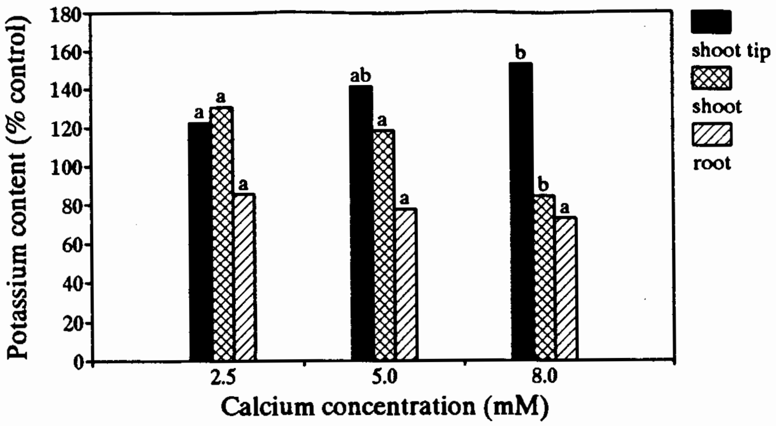

Figure 31: Effect of calcium concentration on the potassium content of salinized, nitrate-supplied wheat (as % of controls). (n) = 8. Each series of letters represents a one-way anova.

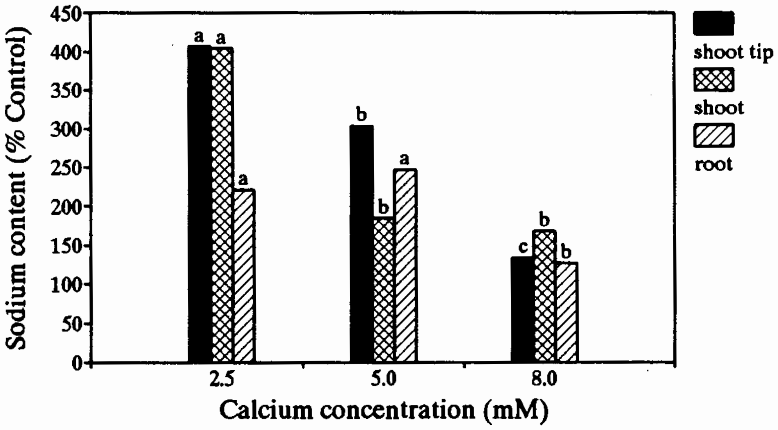

Figure 32: Effect of calcium concentration on the sodium content of salinized, nitrate-supplied wheat (as % of controls). (n) = 8. Each series of letters represents a one-way anova.

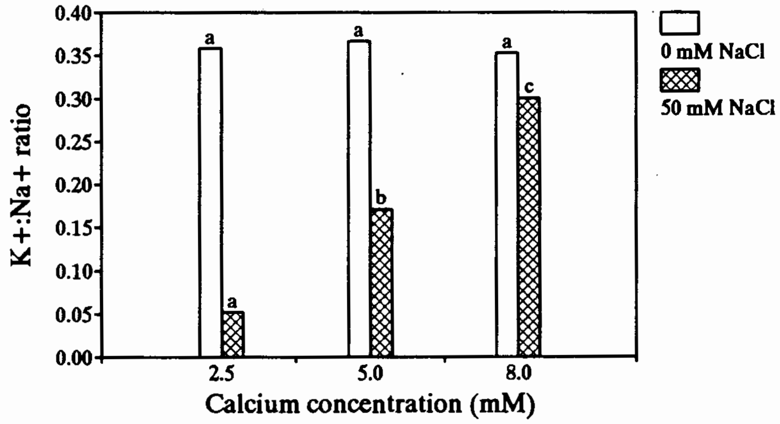

Figure 33: Effect of calcium concentration on the K^+:Na^+ ratio of salinized, nitrate-supplied shoot tips. (n) = 8. Each series of letters represents a one-way anova.

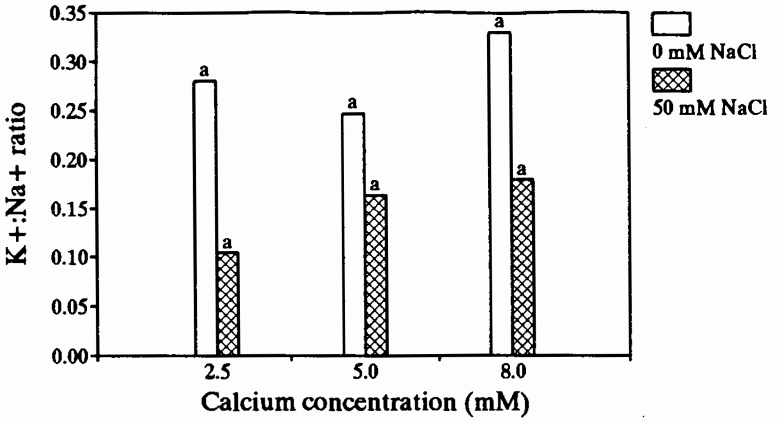

Figure 34: Effect of calcium concentration on the K^+:Na^+ ratio of salinized, nitrate-supplied shoots. (n) = 8. Each series of letters represents a one-way anova.

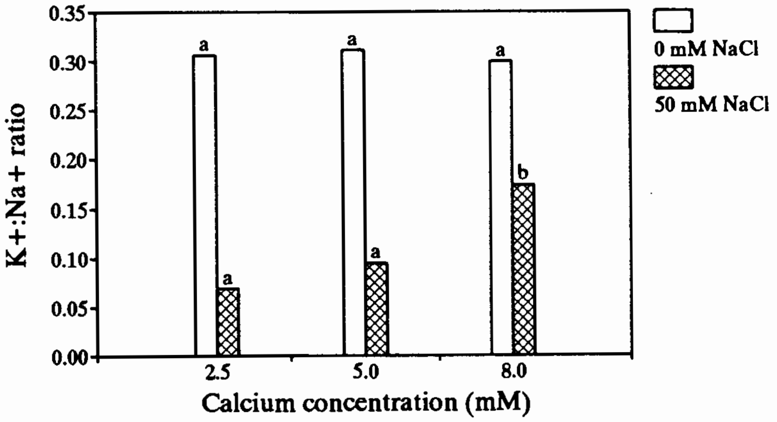

Figure 35: Effect of calcium concentration on the K^+:Na^+ ratio in salinized, nitrate-supplied roots. (n) = 8. Each series of letters represents a one-way anova.

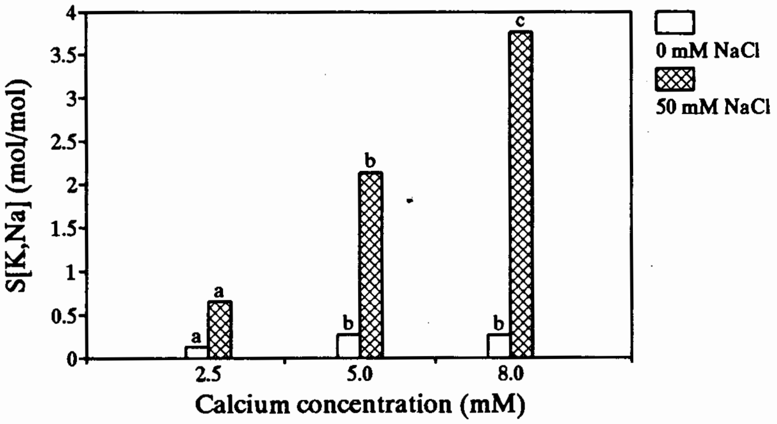

Figure 36: Effect of calcium concentration on the $S_{[Na,K]}$ of salinized, nitrate-supplied shoot tips. (n) = 8. Each series of letters represents a one-way anova.

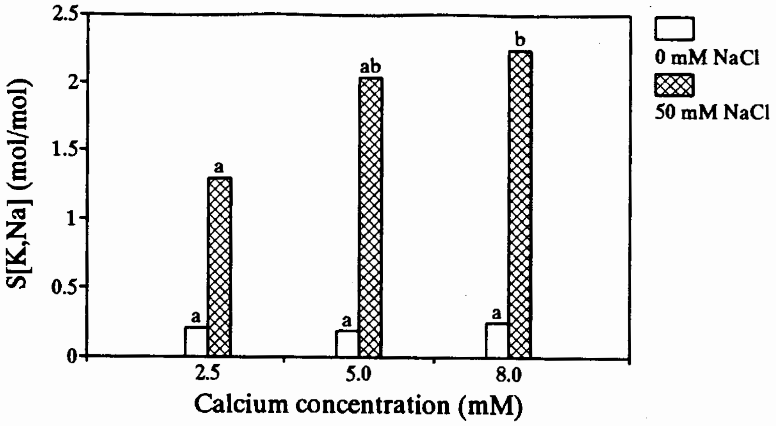

Figure 37: Effect of calcium concentration on the $S_{[Na,K]}$ of salinized, nitrate-supplied shoots. (n) = 8. Each series of letters represents a one-way anova.

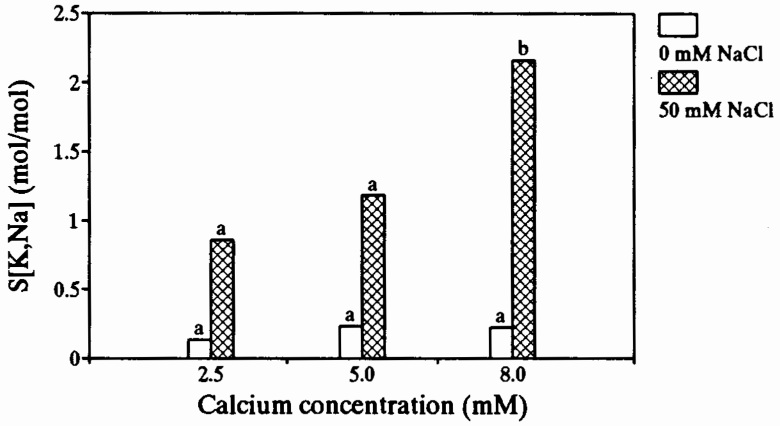

Figure 38: Effect of calcium concentration on the $S_{[Na,K]}$ of salinized, nitrate-supplied roots. (n) = 8. Each series of letters represents a one-way anova.

the effect of Ca^{2+} on this ratio in the controls was not significant. **Figure 34** illustrates that the same trend is evident in the K^+:Na^+ ratio of the stressed shoot and root components although in the case of the shoot the effect of Ca^{2+} was not significant. **Figure 35** shows that there was a significant increase in the ratio for the stressed root component where this was mostly due to a decrease in the sodium ion content of the root with an increase in the Ca^{2+} concentration. It is evident from **Figures 33** to **35** that the K^+:Na^+ ratio in the control plants was much higher than that of the salinized plants. However, in the salinized shoot tips, the K^+:Na^+ ratio approached that of the controls when the calcium concentration was raised to 8.0 mM. The lowered K^+:Na^+ ratio of the salinized plants indicates that the presence of 50 mM NaCl caused a loss of potassium, which was accompanied by an large influx of Na^+ ions.

These results suggest that the salinized plants, when supplied with sufficient exogenous Ca^{2+}, are able to exclude sodium ions from entering the plant to some extent. Since there were gradients in Na^+ and K^+ between different plant components, the plants were to some degree partitioning the leaf salt loads to abate the toxic build-up of Na^+ and to establish a favourable K^+:Na^+ ratio, especially in the growing regions. It is well known that calcium is required for the maintenance of membrane integrity and selective ion uptake under saline conditions. These properties of calcium responsible for the protection of membrane integrity may account for the Na^+ exclusion and increased K^+ content evident from these results.

Under saline conditions and high calcium concentrations (8.0 mM $CaCl_2$), selective uptake, transport and foliar compartmentation of the K^+ and Na^+ ions occurred within both the shoot and the root. In the stressed, foliar component, with an increase in calcium concentration, there were definite gradients of K^+ and Na^+ concentration in opposite directions (**Table 5**). Also, between the expanding and mature portions of the shoot there were clear gradients in K^+ content and a slight gradient in Na^+ content. K^+ and Na^+ gradients have been observed in some halophytes and have been taken to be indicative of part of a salt tolerance strategy (Blits and Gallagher, 1990). There was also a gradient between the root K^+ and Na^+ contents and the shoot K^+ and Na^+ contents.

The selectivity for K^+ versus Na^+ ($S_{[K,Na]}$) ratio gives a value indicating the selectivity (or exclusion potential) of the plant for certain ions in relation to the external concentrations of those ions (see **Eq. 7**, section **3.8.2**). This means that if a control and a salinized plant take up the same amount of K^+, the $S_{[K,Na]}$ of the stressed plant will be much higher due to the presence of high exogenous sodium concentrations compared to exogenous potassium concentrations. The salt tolerance of various species of the Triticeae was recently found to partially reside in the D genome, where this genome is thought to be responsible for increased selectivity for K^+ over Na^+ in the root plasmalemma under saline conditions (Kafkafi, 1991).

The $S_{[K,Na]}$ for the shoot tip, shoot and the root components are shown in **Figures 36, 37** and **38**. With reference to these figures, it is evident that both the presence of 50 mM NaCl and increasing concentrations of $CaCl_2$ increased this selectivity within the salinized plants. Although increases in K^+ content were observed (**Figure 31** and **Table 5**) the selectivity results reflect sodium exclusion by calcium. The most highly significant effect of $CaCl_2$ on the $S_{[K,Na]}$ was evident for the stressed shoot tip shown in **Figure 36** ($P < 0.0001$, one-way ANOVA). The $S_{[K,Na]}$ of the rest of the stressed shoot (**Figure 37**) also increased with increasing $CaCl_2$ concentration ($P < 0.0227$, one-way ANOVA). The $S_{[K,Na]}$ of the root (**Figure 38**) increased in the same manner ($P < 0.0028$, one-way ANOVA).

Sodium ions depolarises the membrane, disrupting ion uptake and promoting leakage of ions. Exogenous calcium maintains membrane integrity and selectivity (Ward *et al.*, 1986; Lewis *et al.*, 1989) and competes directly with Na^+ ions (Läuchli, 1990). According to Frost *et al.*, (1978), Ca^{2+} may compete with K^+ to some degree for K^+ channels and thereby prevent excess K^+ leakage. These factors together with the importance of the $Na^+:Ca^{2+}$ ratio for the expanding shoot can explain the trends seen in the stressed wheat plants with respect to their potassium and sodium ion contents.

4.4 Effect of calcium addition on biomass production (including shoot:root ratios and moisture contents), total N content, ^{15}N uptake and gaseous exchange of salinized, ammonium-supplied wheat (Experiment 3)

This experiment (described in section **3.10.3**) investigated the effect of NaCl on biomass production, gaseous exchange, total N content and $^{15}NH_4^+$ uptake in ammonium-supplied wheat, as well as the potential of $CaCl_2$, as an ameliorative agent, to mitigate any adverse effects of NaCl. These results are illustrated in **Figures 39** to **46**.

4.4.1 Effect of calcium addition on the biomass production of salinized, ammonium-supplied wheat

While NaCl concentration decreased the biomass production of the ammonium-supplied plants, calcium chloride concentration had no significant effect on the biomass production, shoot:root ratios or moisture content of salinized (50 mM NaCl), ammonium-supplied wheat. Increasing calcium concentration had some promoting effect on the biomass production of the control plants (**Figure 39**). The data did imply that there may be some $NaCl/NH_4^+$ interaction independent of the Ca^{2+} concentration, since the plants responded differently to salt stress compared to the nitrate-supplied plants in the previous experiment. The possibility of a $NaCl/NH_4^+$ interaction will be discussed under section **4.9**. At low calcium concentrations the ammonium-supplied plants appeared to be less affected by the salt stress than were the nitrate-supplied plants at low calcium concentrations. Shoot:root ratios (**Figure 40**) also showed that calcium had no significant effect on biomass production in salinized, ammonium-supplied wheat.

The moisture contents of both the control and the stressed plants were also independent of the Ca^{2+} concentration as shown in **Figure 41**. It is of interest that the moisture contents of the salinized and control plants were comparable, unlike what was found for the nitrate-supplied plants, where NaCl had a significant influence on the moisture content of the plants. The influence of NaCl on the moisture content of both nitrate- and ammonium-supplied plants is shown under section **4.2** where it is evident that both the moisture content and WUE of ammonium-supplied plants at comparable levels of salinity stress were higher than in nitrate-supplied plants.

4.4.2 Effect of calcium addition on the total N content and $^{15}NH_4^+$ uptake in salinized, ammonium-supplied wheat

The total N content (*mg N g^{-1} dm*) of control and salinized ammonium-supplied wheat is shown at two Ca^{2+} concentrations, (0.5 mM and 5.0 mM $CaCl_2$) in **Figure 42**. A salt stress of 50 mM NaCl was not found to be a significant factor determining the total N content of the ammonium-supplied plants, as opposed to what was found for the nitrate-supplied plants (section **4.3.2**). There was no significant or observable effect of calcium concentration on the total N of either the stressed or control plants.

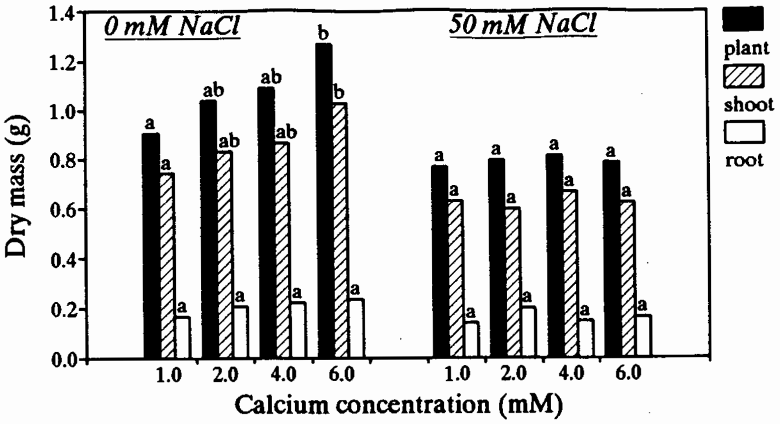

Figure 39: Effect of calcium concentration on the biomass production of salinized, ammonium-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.

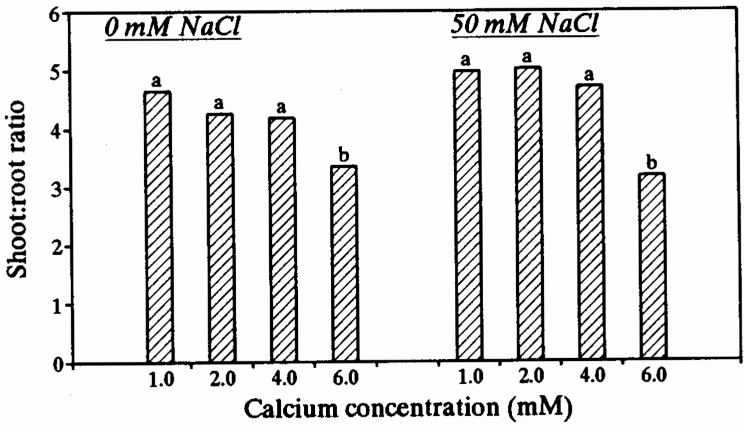

Figure 40: Effect of calcium concentration on the shoot:root ratio of salinized, ammonium-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.

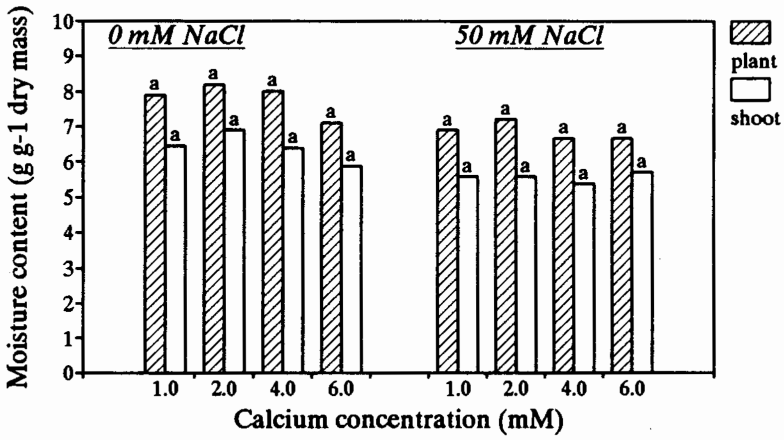

Figure 41: Effect of calcium concentration on the moisture content of salinized, ammonium-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.

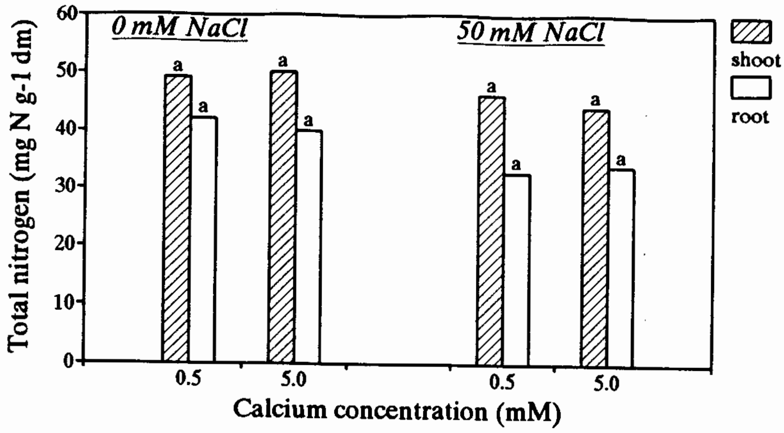

Figure 42:* *Effect of calcium concentration on the total nitrogen content of salinized, ammonium-supplied wheat. (n) = 4. Each series of letters represents a one-way anova.

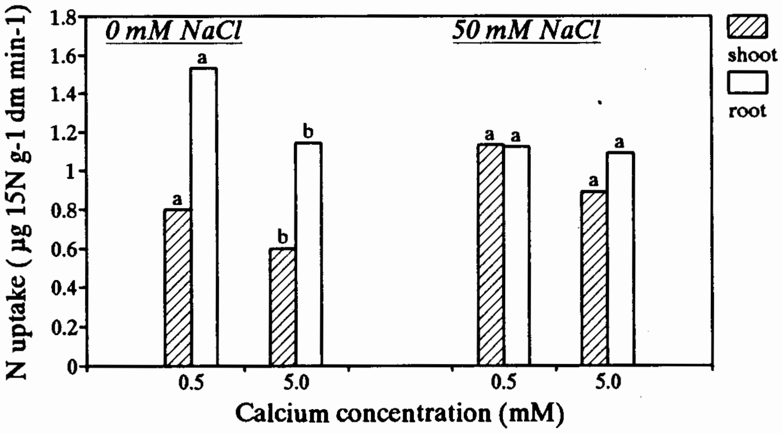

Figure 43:* *Effect of calcium concentration on the $^{15}NH_4^+$ uptake in salinized, ammonium-supplied wheat. (n) = 4. Each series of letters represents a one-way anova.

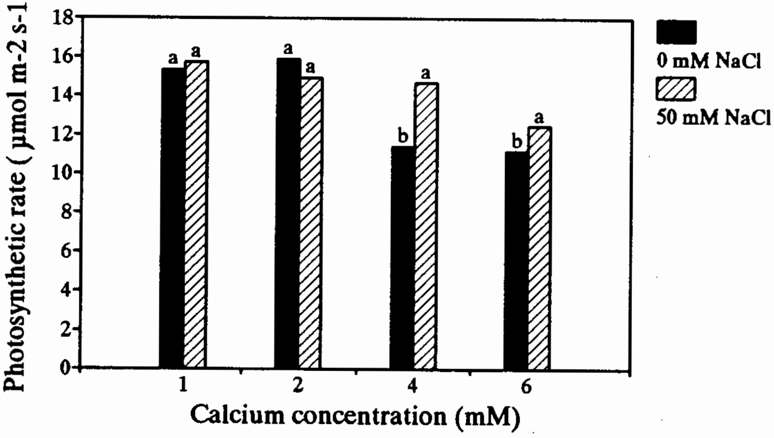

Figure 44:* *Effect of calcium concentration on the photosynthetic rate of salinized, ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

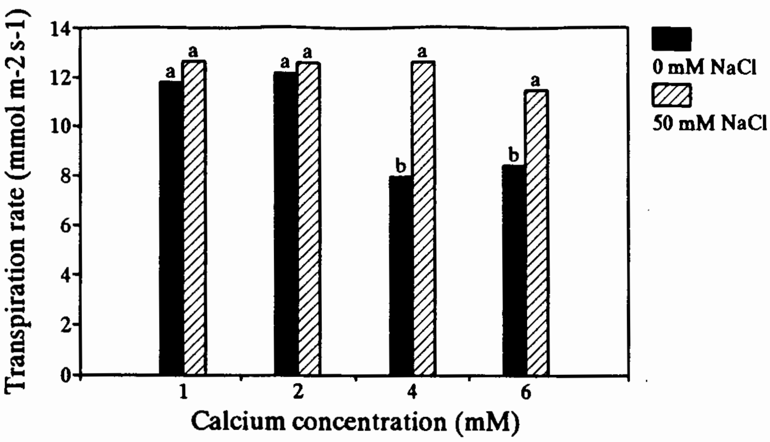

Figure 45: Effect of calcium concentration on the transpiration rate of salinized, ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

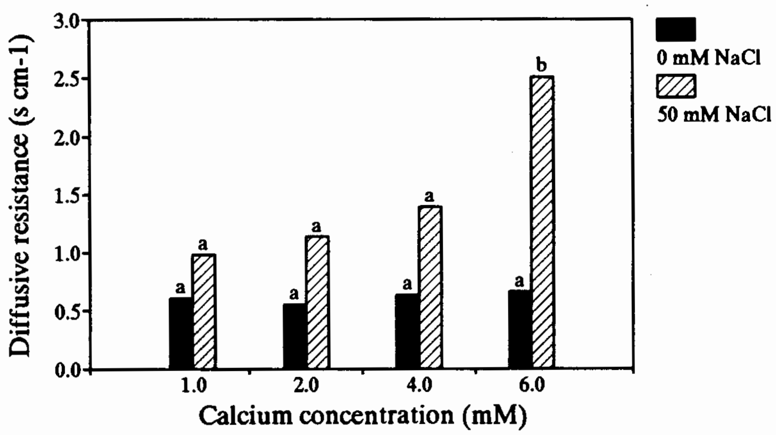

Figure 46: Effect of calcium concentration on the stomatal diffusive resistance of salinized, ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

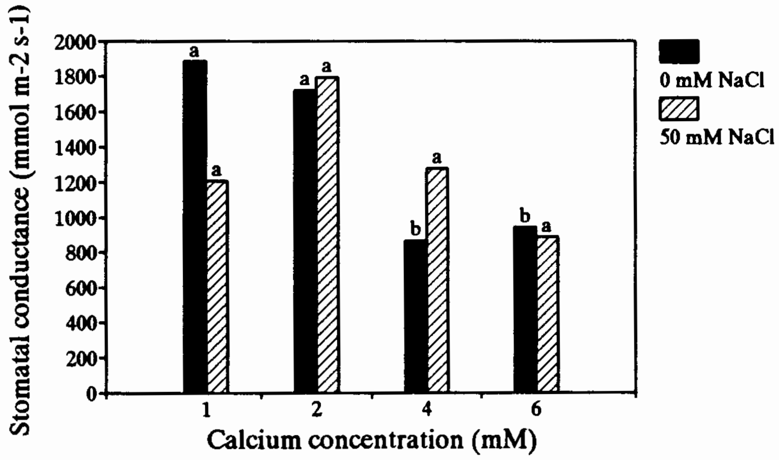

Figure 47: Effect of calcium concentration on the stomatal conductance to water of salinized, ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

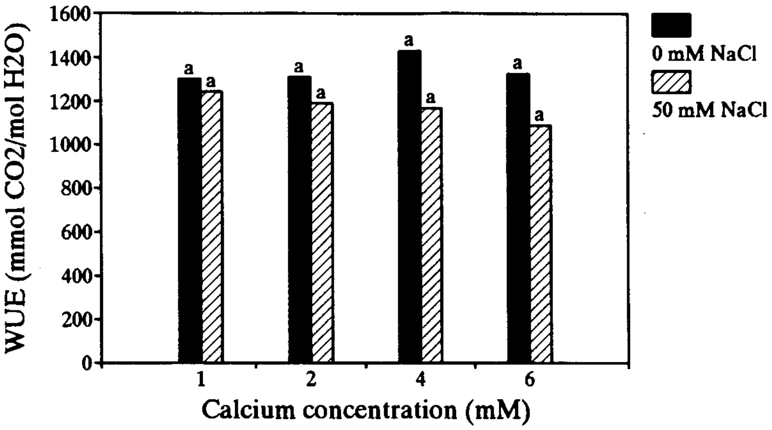

Figure 48: Effect of calcium concentration on the WUE of salinized, ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

Salinity stress did decrease biomass production in ammonium-supplied wheat and it is possible that no effect of NaCl on N content was noted because part of the N taken up was allocated to so-called stress compounds (eg. proline, glycinebetaine). Alternatively, NH_4^+ uptake was not as susceptible to disruption as was NO_3^- uptake.

The ^{15}N uptake rate (*μg ^{15}N g^{-1} dm min^{-1}*) over an 8*h* period for the same plants is shown in **Figure 43**. It was found that with an increase in Ca^{2+} concentration the control plants actually decreased their $^{15}NH_4^+$ uptake rate. The $^{15}NH_4^+$ uptake of the stressed plants was not adversely affected by the stress.

4.4.3 Effect of calcium addition on the gaseous exchange (including photosynthetic and transpiration rate, stomatal diffusive resistance, stomatal conductances and WUE) of salinized, ammonium-supplied wheat

No significant trends could be discerned from the photosynthetic rates or transpiration rates of ammonium-supplied wheat supplemented with calcium (**Figures 44** and **45**).

The stomatal diffusive resistance data (**Figure 46**) and stomatal conductances (**Figure 47**) were derived separately (see **Materials and Methods**, section **3.4**). It is evident that there was no significant effect of calcium concentration on the stomatal conductance/resistance of the control plants, while there was an increase in the stomatal diffusive resistance of the salinized plants as the calcium concentration in the nutrient medium was raised. This suggests that the salinized plants had reduced stomatal openings under salt stress and ammonium nutrition, which in turn may explain why the moisture contents of the salinized, ammonium-supplied plants did not appear to be affected by the saline conditions (**Figure 41**).

Calcium concentration had no effect on the WUE of salinized plants (**Figure 48**), nor was the effect of salinity on WUE significant.

4.5 Effect of potassium addition on the biomass production (including shoot:root ratios and moisture contents) and gaseous exchange of salinized, nitrate-supplied wheat (Experiment 4)

This experiment (described in section **3.10.4**) investigated the potential of supplemental, exogenous K^+ to ameliorate the effects of NaCl on biomass production and gaseous exchange

exchange in nitrate-supplied wheat. The results are illustrated in **Figures 49** to **56**.

4.5.1 Effect of potassium addition on the biomass production of salinized, nitrate-supplied wheat

The addition of K^+ to the nutrient medium in the range 0.2 mM to 5.0 mM K_2SO_4 had no significant or observable effect on the biomass production, shoot:root ratio or moisture content of the control (0 mM NaCl) or salinized (50 mM NaCl), nitrate-supplied plants (**Figures 49, 50** and **51** respectively). The only factor determining the biomass production and moisture content of the plants was the presence or absence of salinity in the nutrient medium which was significant to $P < 0.0001$ (two-way ANOVA). Neither the NaCl concentration nor the K^+ concentration significantly affected the shoot:root ratio (**Figure 50**).

Potassium ions leak out of the root plasmalemma due to the depolarization of the membrane by Na^+ ions (section **2.1.1.1**, Literature Review), and it is suggested from the data presented here that additional exogenous K^+ was not sufficient to compensate for the leakage caused by the excess NaCl, in the absence of sufficient calcium. This can be explained in terms of the requirement for calcium ions to maintain membrane integrity (Rengasamy, 1987; Lynch *et al.*, 1989; Lew, 1989) as well as K^+ over Na^+ ion selectivity Ward, 1986; Hedrich and Neher, 1987). It would appear that the concentration of exogenous Ca^{2+} is more important with respect to the K^+ status of the salinized wheat plant than the concentration of exogenous K^+. This is further supported by the significant increase in the K^+:Na^+ ratio and $S_{[K+:Na+]}$ shown to exist in the shoot tips and roots of calcium supplemented (5.0 to 8.0 mM $CaCl_2$), salinized plants (section **4.3.4.3**).

A high K^+:Na^+ ratio has significance for osmoregulation in mesophytes (Frost, 1978) and generally, a high K^+:Na^+ ratio in the cytoplasm is essential for the activity of many enzymes (Greenway and Munns, 1980; Harvey and Thorpe, 1986).

4.5.2 Effect of potassium addition on the gaseous exchange (including photosynthetic and transpiration rates, stomatal diffusive resistance, stomatal conductances and WUE) of salinized, nitrate-supplied wheat

The gaseous exchange results are illustrated in **Figures 52** to **56**. All results from the gaseous exchange measurements made on these plants were nonsignificant with respect to K^+

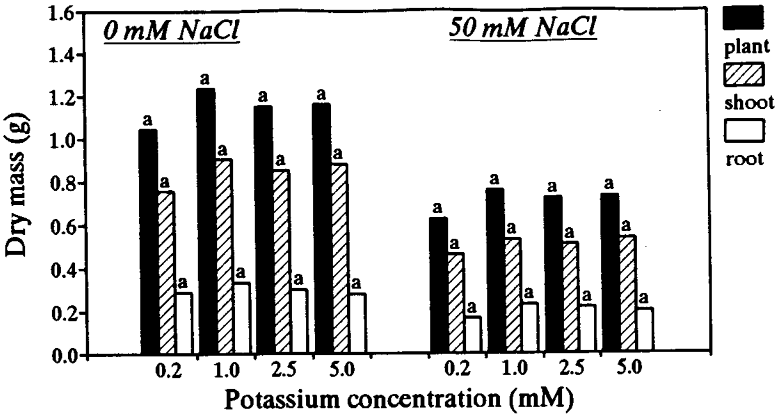

Figure 49: Effect of potassium concentration on the biomass production of salinized, nitrate-supplied wheat. (n) = 16.

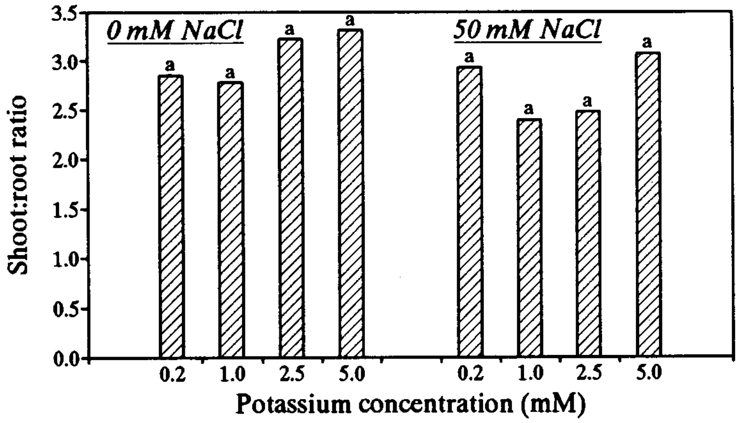

Figure 50: Effect of potassium concentration on the shoot:root ratio of salinized, nitrate-supplied wheat. (n) = 16.

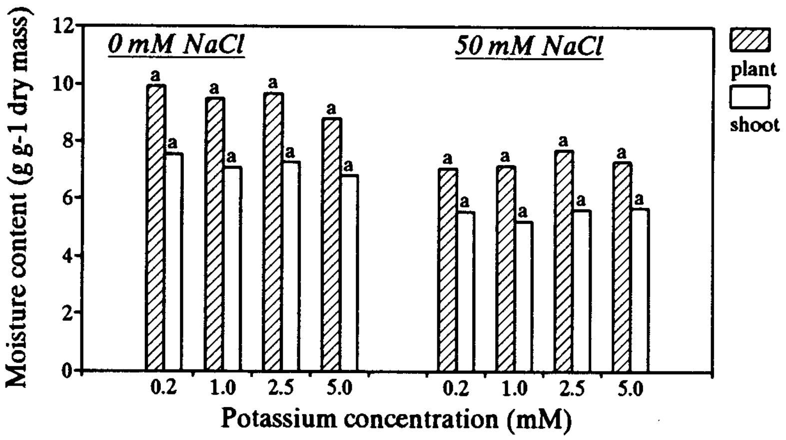

Figure 51: Effect of potassium concentration on the moisture content of salinized, nitrate-supplied wheat. (n) = 16.

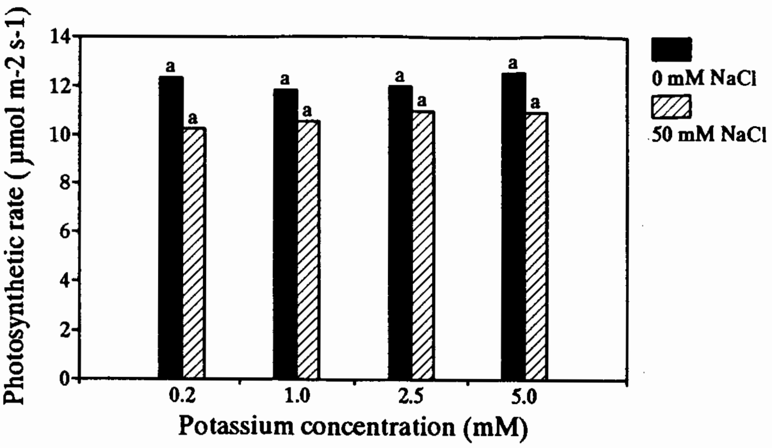

Figure 52: Effect of potassium concentration on the photosynthetic rate of salinized, nitrate-supplied wheat. (n) = 10.

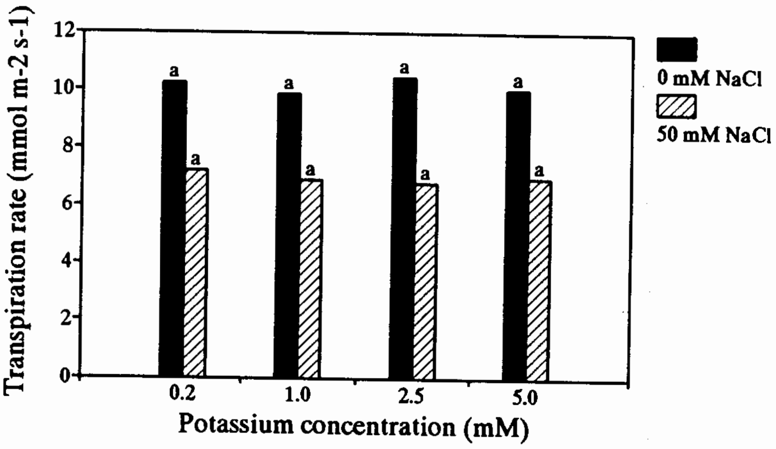

Figure 53: Effect of potassium on the transpiration rate of salinized, nitrate-supplied wheat. (n) = 10.

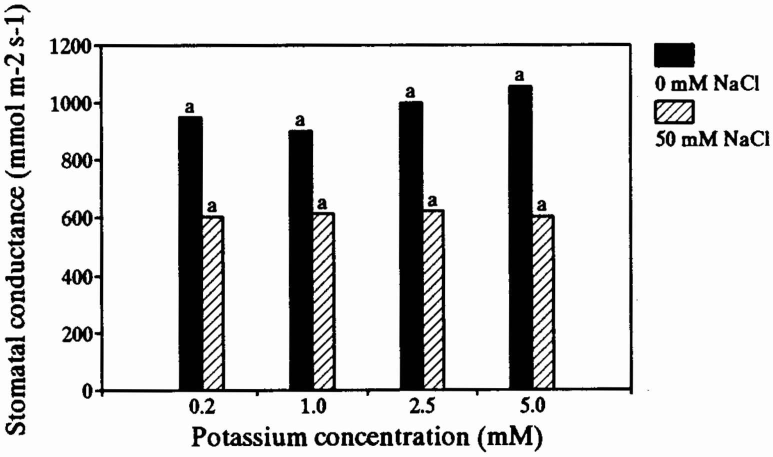

Figure 54: Effect of potassium concentration on the stomatal conductance of salinized, nitrate-supplied wheat. (n) = 10.

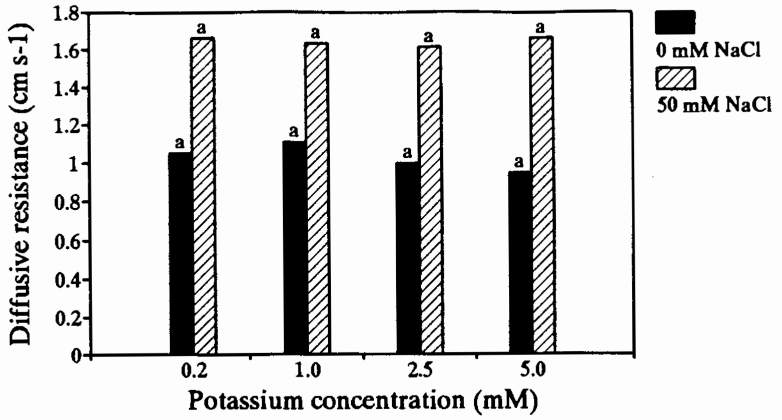

Figure 55: Effect of potassium on the stomatal diffusive resistance of salinized, nitrate-supplied wheat. (n) = 10.

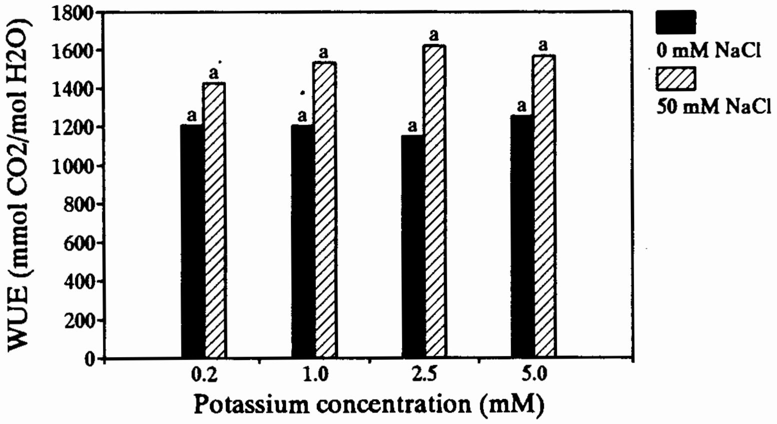

Figure 56: Effect of potassium concentration on the WUE of salinized, nitrate-supplied wheat. (n) = 10.

concentration but significant with respect to the presence or absence of a NaCl concentration of 50 mM.

The presence of NaCl had a significant reducing effect on stomatal conductance($P < 0.0001$), two-way ANOVA); this was confirmed by a reverse effect on stomatal diffusive resistance ($P < 0.0001$, two-way ANOVA) and the reducing effect on the transpiration rates ($P = 0.0002$, two-way ANOVA) of these plants (**Figures 54**, **55** and **53** respectively). Clearly the salinized plants had a higher stomatal diffusive resistance relative to the control plants (**Figure 55**) and this in turn resulted in the lowered transpiration rates (**Figure 53**) and as a consequence of this, an increased WUE in the same plants (**Figure 56**) since the photosynthetic rate (**Figure 52**) was less affected ($P = 0.0414$, two-way ANOVA) by the stress. An increased WUE would be imperative for the survival of salinized plants undergoing water deficit. Lewis *et al.*, (1989) also reported lowered transpiration rates and increased WUE in salinized wheat (80 mM NaCl). The reason for the increase in stomatal diffusive resistance may be either a decrease in the turgidity of the guard cells due to osmotic stress, or an unknown signal response which affects stomatal closure. However, the influence of potassium concentration on gaseous exchange neither affected the biomass production nor the moisture contents of the salinized, nitrate-supplied plants (section **4.5.1**).

This effect of salt stress on stomatal resistance was not seen in nitrate-supplied plants which had been provided with supplemental $CaCl_2$ (section **4.3.3**) although it was shown that increased exogenous Ca^{2+} elevated the moisture content of salinized, nitrate-supplied plants (section **4.3.1**).

4.6 Effect of potassium addition on the biomass production (including plant morphology, shoot:root ratios and moisture contents) and gaseous exchange of salinized, ammonium-supplied wheat (Experiment 5)

This experiment (described in section **3.10.5**) investigated the potential of exogenous K^+ to ameliorate the effects of NaCl on biomass production and gaseous exchange in ammonium-supplied wheat. The results are illustrated in **Figures 57** to **63**.

4.6.1 Effect of potassium addition on the biomass production of salinized, ammonium-supplied wheat

The biomass production of the salinized plants were significantly affected by the presence of 50 mM NaCl ($P < 0.0001$, two-way ANOVA, for shoot and root), while potassium concentration significantly influenced the shoot, root and plant dry mass production (**Figure 57**) of the salinized plants only ($P = 0.0001$, 0.0010, and 0.0001 respectively, two-way ANOVAs). Both the shoot and root biomass production of the stressed plants passed through an optimum at 1.0 mM K^+ and declined thereafter, but not to a lower value than was produced by plants grown on 0.2 mM K^+ (the K^+ concentration in unmodified Long Ashton nutrient solution is 2.0 mM K_2SO_4).

Morphologically, the salinized plants grown on low potassium were severely affected; the roots were necrotic, the stems delicate and the leaves chlorotic. While the reason for the apparent decline in biomass after 1.0 mM K^+ remains unexplained, it was evident that the salinized wheat required a critical quantity of K^+ in the exogenous nutrient medium, while this quantity was not critical for the controls. This is contrary to what was found for nitrate-supplied wheat (section **4.5**) where a critical level of exogenous K^+ did not appear to be an important factor determining the biomass production of the salinized plants.

The moisture content of the stressed shoot (**Figure 59**) was negatively influenced by the presence of salt ($P = 0.0003$, two-way ANOVA) but was not influenced by the concentration of K^+. The overall plant moisture content was not influenced by the presence of NaCl or the concentration of K^+. Thus the increase in biomass production of the stressed plants at 1.0 mM K^+ was not correlated with either an increase in moisture content or an increase in CO_2 assimilation (**Figure 60**, in the following sub-section) at this K^+ concentration. Potassium concentration had no effect on either the control or stressed shoot:root ratio (**Figure 58**).

4.6.2 Effect of potassium addition on the gaseous exchange (including photosynthetic and transpiration rates, stomatal diffusive resistance, stomatal conductances and WUE) of salinized, ammonium-supplied wheat

There was a significant influence exerted by the presence of 50 mM NaCl on the photosynthetic, transpiration rates and WUE of the salinized plants (**Figures 60, 61** and **63**

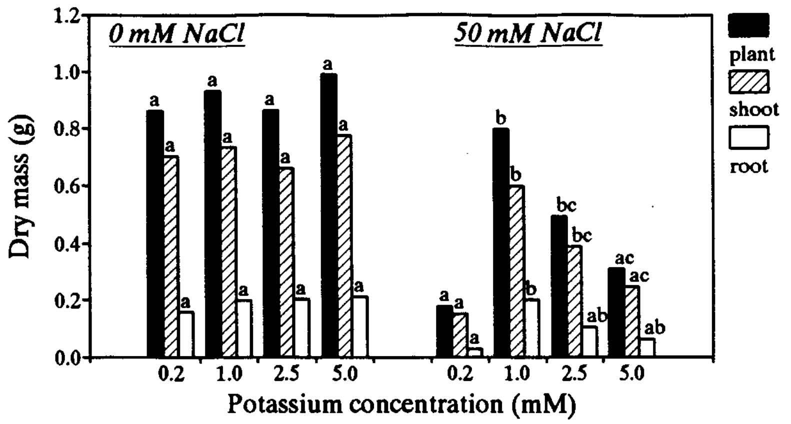

Figure 57: Effect of potassium concentration on the biomass production of salinized, ammonium-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.

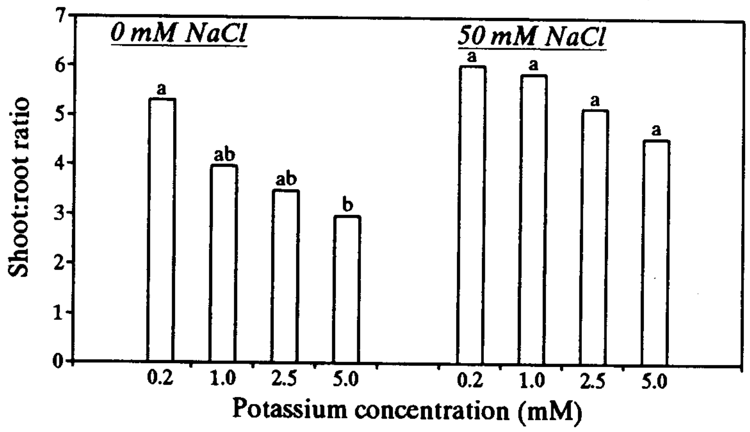

Figure 58: Effect of potassium concentration on the shoot:root ratio of salinized, ammonium-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.

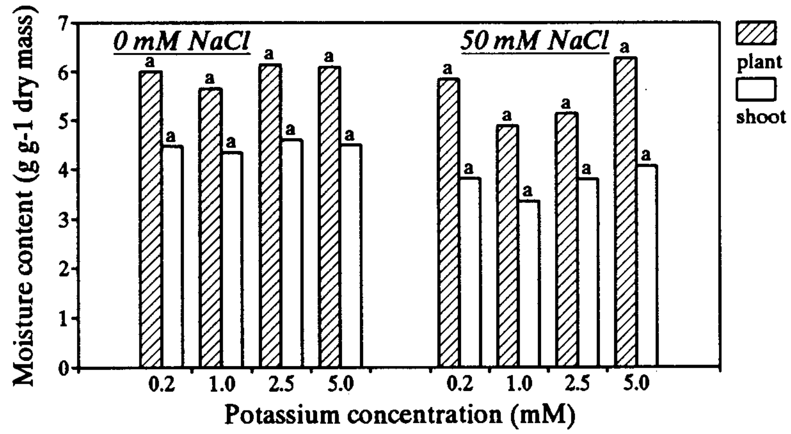

Figure 59: Effect of potassium concentration on the moisture content of salinized, ammonium-supplied wheat. (n) = 16. Each series of letters represents a one-way anova.

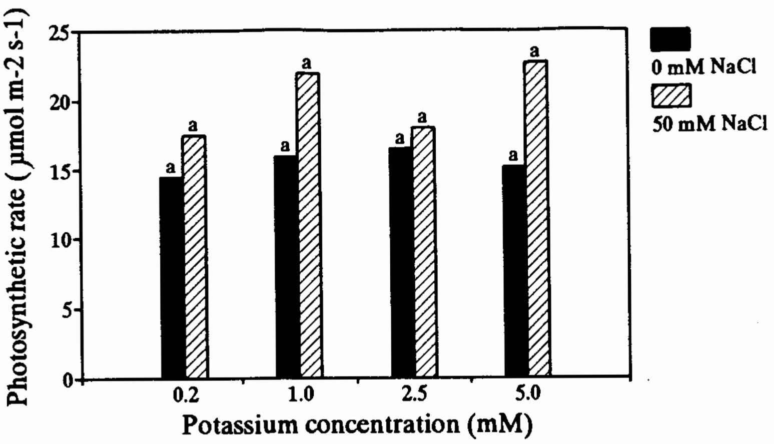

Figure 60: Effect of potassium concentration on the photosynthetic rate of salinized, ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

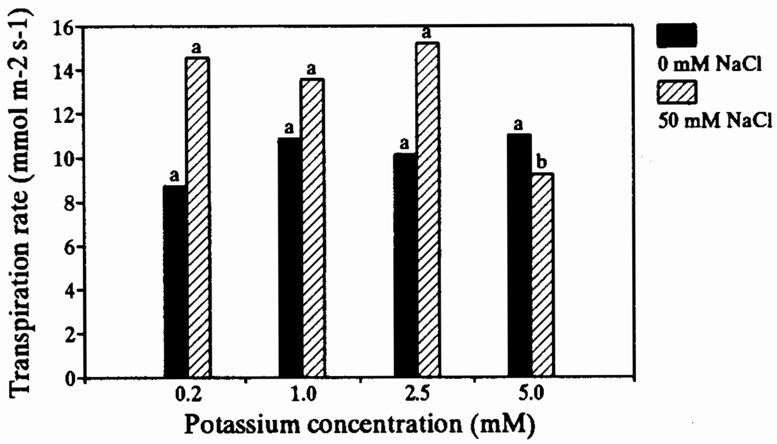

Figure 61: Effect of potassium concentration on the transpiration rate of salinized, ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

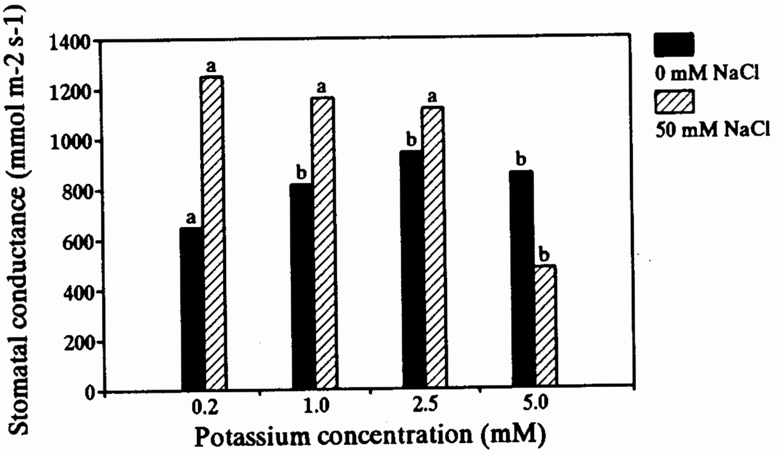

Figure 62: Effect of potassium on the stomatal conductance of salinized, nitrate-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

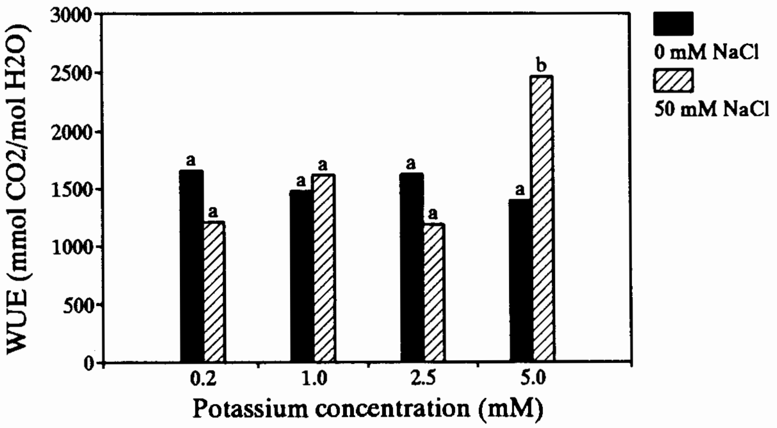

Figure 63: Effect of potassium concentration on the WUE of salinized, ammonium-supplied wheat. (n) = 10. Each series of letters represents a one-way anova.

respectively) where P = 0.0009, 0.0001 and 0.0001 respectively (two-way ANOVAs). Although the stomatal conductances to H_2O were not significantly affected by the presence of NaCl, the same trends were evident as for transpiration rate of the salinized plants (**Figure 61**).

Only the WUE was significantly influenced by potassium concentration since the WUE increased at 5.0 mM K^+. The K^+ treatment within the stressed plants group had no significant effect on the photosynthetic rate, while at 5.0 mM K^+ the transpiration rates were significantly lowered within the stressed group (P = 0.0119, one-way ANOVA). This same trend is reflected by the stomatal conductances of the stressed group (P = 0.0319, one-way ANOVA). It would appear that the raised stomatal conductances in the stressed plants as a group, could be a factor contributing to the lower moisture contents (section **4.6.1**, **Figure 59**) of these plants.

4.7 The effect of NaCl plus $CaCl_2$ on the rate of NO_3^- uptake in salinized wheat (Experiment 6)

This experiment (described in section **3.10.6**) investigated both the effects of NaCl (0 mM and 50 mM) and $CaCl_2$ concentration (0.5 mM to 5.0 mM) on the rate of NO_3^- uptake in control and stressed plants over an 8*h* period. The results are illustrated in **Figures 64, 65** and **66** as NO_3^- uptake given in *µmol* g^{-1} *root fresh mass (fm)* and in **Table 6** as uptake rates given in *nmol* g^{-1} *root fm* s^{-1}.

After a 2*h* period the cumulative uptake of the control and stressed plants (**Figures 64** to **66**) started to diverge from each other at all three of the calcium concentrations supplied. After 8*h* the cumulative NO_3^- uptake in the salinized plants was approximately half that of the control plants except at 5.0 mM Ca^{2+} (**Figure 66**), where the reduced NO_3^- uptake by the stressed plants was somewhat ameliorated. It was determined from an analysis of the regression coefficients of the stressed and control uptake curves (Zar, 1984), that the stressed and control curves, at all the calcium concentrations, were significantly distinct ($P < 0.0001$). The uptake rates (**Table 6**) reflect the trends seen in the figures. It was apparent that calcium had little effect on the uptake rates, except with respect to the salinized plants which had been fed 5.0 mM $CaCl_2$.

Table 6: Average NO_3^- uptake rates after 8h feeding. Rates refer to control and salt stressed wheat which had been supplied with different calcium concentrations.

[Calcium] (mM)	Control root uptake rate (nmol g^{-1} root fm s^{-1})	Stressed root uptake rate (nmol g^{-1} root fm s^{-1})
[0.5]	12.00 ± 1.35	5.20 ± 1.80
[1.0]	12.90 ± 3.50	6.10 ± 1.90
[5.0]	11.80 ± 3.70	7.10 ± 2.30

Table 7: Average NH_4^+ uptake rates after 8h feeding. Rates refer to control and salt stressed wheat which had been fed different potassium concentrations.

[Potassium] (mM)	Control root uptake rate (nmol g^{-1} root fm s^{-1})	Stressed root uptake rate (nmol g^{-1} root fm s^{-1})
[0.5]	25.80 ± 2.61	12.73 ± 2.93
[1.0]	23.04 ± 7.49	11.45 ± 2.71
[5.0]	14.85 ± 0.91	15.96 ± 2.68

4.8 The effect of NaCl plus K_2SO_4 on the rate of NH_4^+ uptake in salinized wheat (Experiment 7)

This experiment (described in section **3.10.7**) investigated both the effects of NaCl (0 mM and 50 mM) and K_2SO_4 concentration (0.5 mM to 5.0 mM) on the uptake of NH_4^+ and the rate of uptake over an 8*h* period. The results are illustrated by **Figures 67, 68** and **69** and **Table 7**.

The cumulative ammonium uptake after 8*h* for the stressed plants was approximately half the

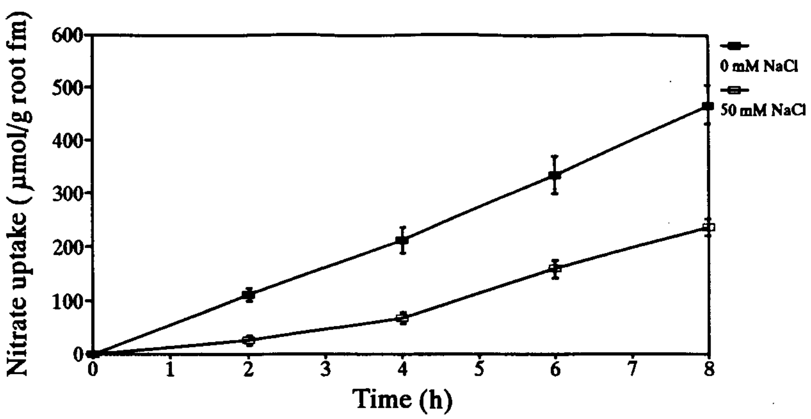

***Figure 64:** Nitrate uptake over 8h in control (0 mM NaCl) and salinized (50 mM NaCl) wheat at 0.5 mM* $CaCl_2$ *concentration. (n) = 3.*

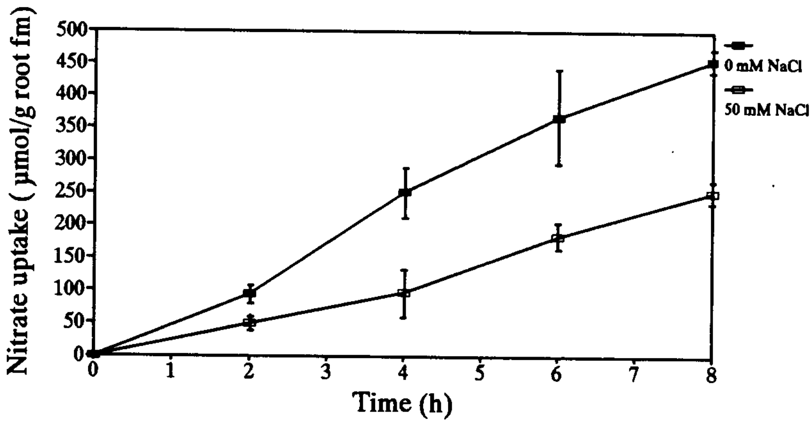

***Figure 65:** Nitrate uptake over 8h in control (0 mM NaCl) and salinized (50 mM NaCl) wheat at 1.0 mM* $CaCl_2$ *concentration. (n) = 3.*

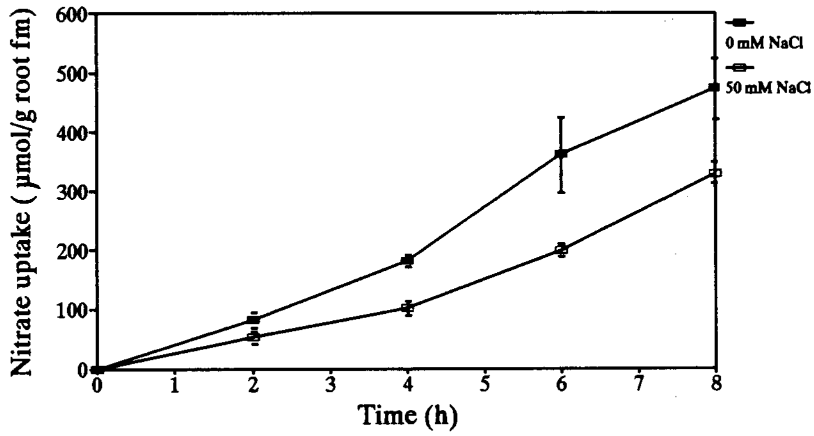

***Figure 66:** Nitrate uptake over 8h in control (0 mM NaCl) and salinized (50 mM NaCl) wheat at 5.0 mM* $CaCl_2$ *concentration. (n) = 3.*

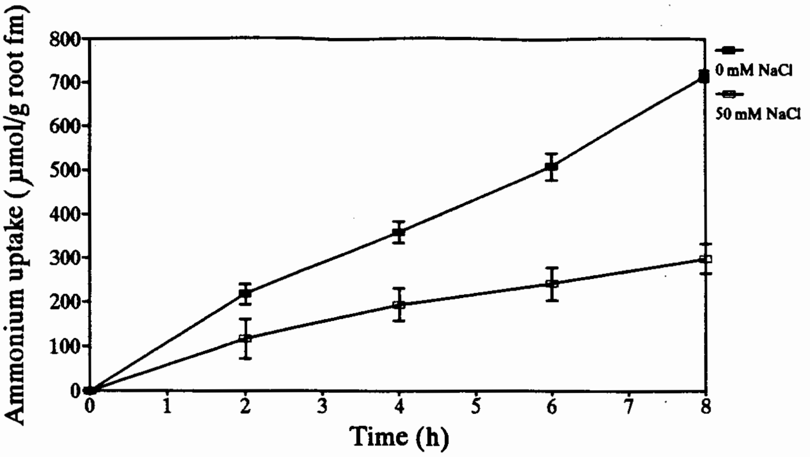

Figure 67: Ammonium uptake over 8h in control (0 mM NaCl) and salinized (50 mM NaCl) wheat at 0.5 mM K_2SO_4 concentration. (n) = 3.

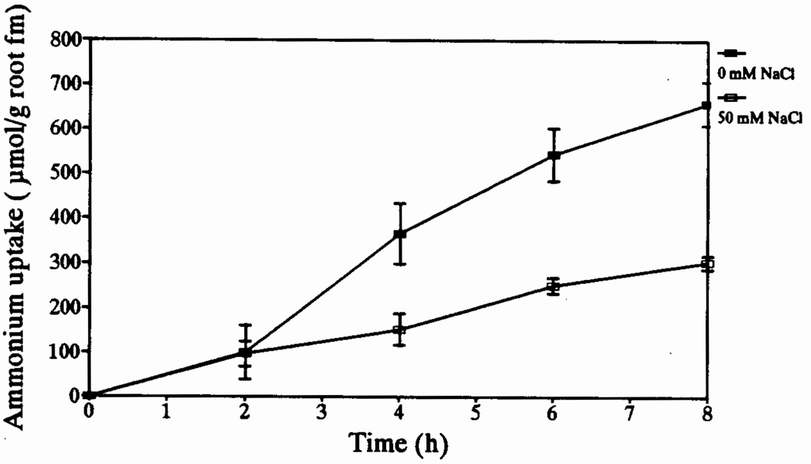

Figure 68: Ammonium uptake over 8h in control (0 mM NaCl) and salinized (50 mM NaCl) wheat at 1.0 mM K_2SO_4 concentration. (n) = 3.

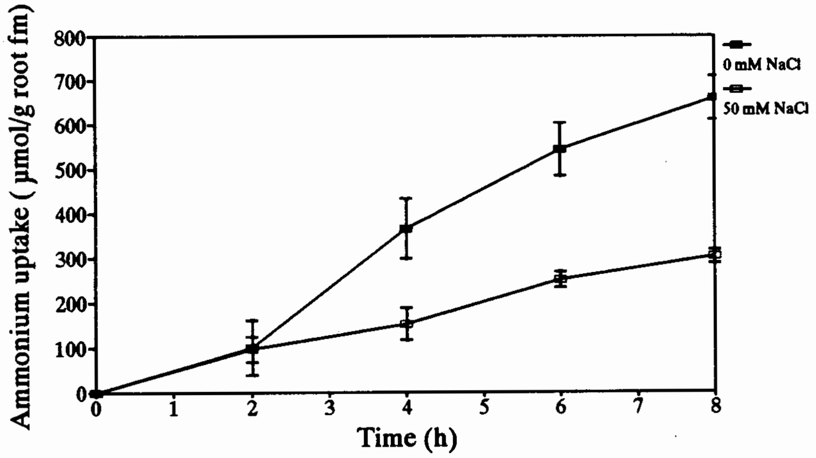

Figure 69: Ammonium uptake over 8h in control (0 mM NaCl) and salinized (50 mM NaCl) wheat at 5.0 mM K_2SO_4 concentration. (n) = 3.

uptake observed for the control plants at all three of the potassium concentrations (**Figures 67** to **69**). From **Table 7** it can be seen that this is reflected in the NH_4^+ uptake rates. In both the controls and the stressed plants the uptake rate was not significantly affected by the exogenous K^+ concentration. An analysis of the regression coefficients of the stressed and control uptake curves showed that the stressed and control curves were significantly distinct ($P < 0.0001$).

It can be noted from **Table 6** and **7** that in the case of both the controls and the stressed plants, NH_4^+ uptake was approximately double that of NO_3^- uptake over the same period.

4.9 Kinetics of NO_3^- and NH_4^+ uptake in the presence of NaCl (Experiment 8)

This analysis (described in section **3.10.8**) investigated the effect of NaCl (50 mM) on the kinetic parameters of N uptake. The kinetics of NO_3^- or NH_4^+ uptake by plants grown in control and saline media are illustrated by Michaelis-Menten plots and the reciprocal Lineweaver-Burk plots (**Figures 70** to **73**). The kinetic parameters V_{max} and K_m (estimated from Lineweaver-Burk plots by Enzfitter®) are given in **Tables 8** and **9**. It should be noted that the kinetic data was derived from nutrient depletion studies carried out over 12*h* and will thus be a measure of both the influx and efflux kinetics. For this reason, the observed K_m will not be similar to those expected for influx alone.

4.9.1 NO_3^- kinetics in salinized wheat

Figures 70 and **71** show the Michaelis-Menten and Lineweaver-Burk plots respectively for the kinetics of NO_3^- uptake in the presence and absence of 50 mM NaCl.

It was evident from these plots that salinity had a significant effect on the V_{max} of NO_3^- uptake but a small and nonsignificant effect on the K_m. The regression coefficients (slopes) of the control and stressed Lineweaver-Burk plots were shown to be statistically distinct ($P < 0.0001$) upon comparison of the two regression equations. **Table 8** shows that the presence of NaCl in the nutrient medium resulted in a decrease of the V_{max} of NO_3^- uptake and a small increase in K_m (decrease in "affinity" for NO_3^- uptake) relative to the controls. The pattern of inhibition by NaCl (**Figure 71**), if NaCl can be seen as an kinetic "inhibitor", is one of noncompetitive inhibition (ie. only the V_{max} was changed). Since the presence of NaCl effectively did not change the K_m, the NaCl must exert its effect other than by direct

Table 8: Vmax and Km of NO_3^- uptake by control and salinized wheat. The kinetic parameters were obtained from Lineweaver-Burk plots.

Kinetic parameters	Control plants	Stressed plants
V_{max} (μmol g^{-1} root fm)	305 ± 23	189 ± 27
K_m (mM)	2.14 ± 0.51	3.19 ± 1.28

Table 9: Vmax and Km of NH_4^+ uptake by control and salinized wheat. The kinetic parameters were obtained from Lineweaver-Burk plots.

Kinetic parameters	Control plants	Stressed plants
V_{max} (μmol g^{-1} root fm)	4490 ± 199	1884 ± 70
K_m (mM)	3.93 ± 0.43	1.38 ± 0.21

competition with the NO_3^- ion. This suggests that the effect must be associated with the membrane and/or membranal proteins (including the postulated NO_3^- transporter) as suggested by several authors (Aslam *et al.*, 1984; Cramer *et al.*, 1985, 1988; Ward *et al.*, 1986; Lynch *et al.*, 1987). The reduced V_{max} infers that in salinized, nitrate-supplied plants the net NO_3^- sequestered per gram root fresh mass was less than in unsalinized plants. Consequently, salinized plants would be expected to have a lower total N content, as was shown to be the case (section 4.3.2).

4.9.2 NH_4^+ kinetics in salinized wheat

Figures 72 and 73 show the Michaelis-Menten and Lineweaver-Burk plots respectively for

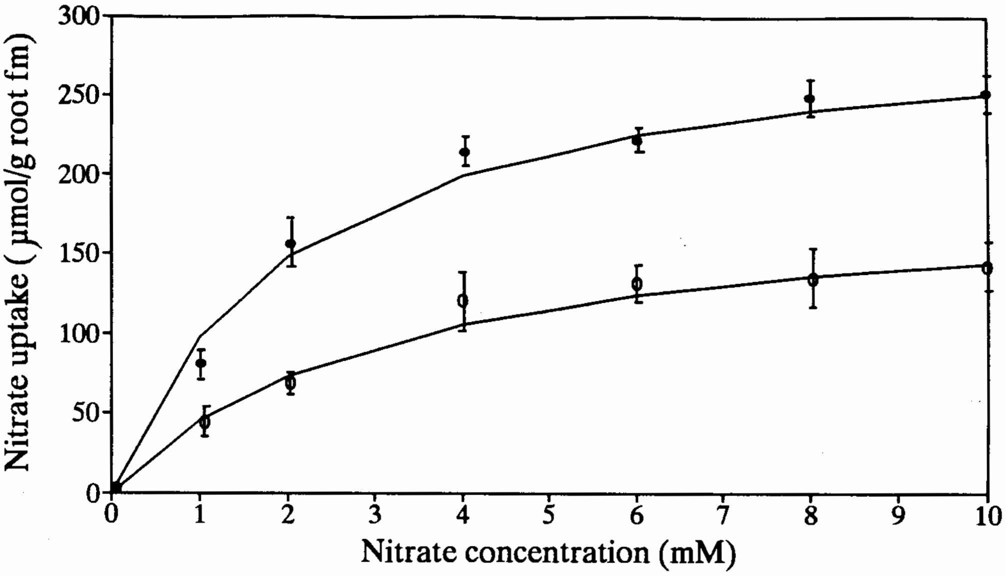

Figure 70: Michaelis-Menten plot of nitrate uptake in control (0 mM NaCl) and salinized (50 mM NaCl) wheat. ● = control, ○ = salinized. (n) = 4.

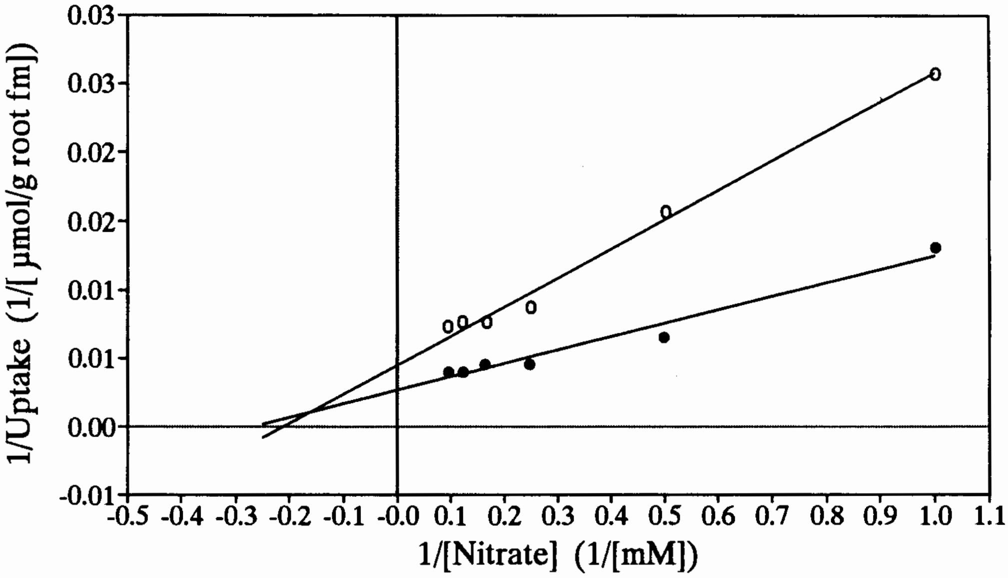

Figure 71: Lineweaver-Burk plot of nitrate uptake in salinized and control plants. ● = control, ○ = salinized. (n) = 4.

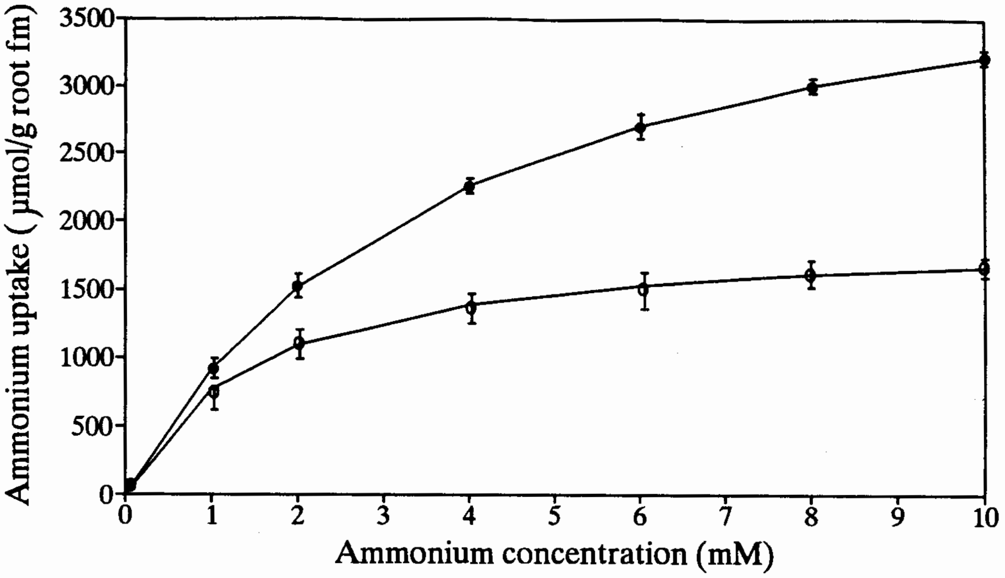

Figure 72: Michaelis-Menten plot of ammonium uptake in control (0 mM NaCl) and salinized (50 mM NaCl) wheat. ● = control, ○ = salinized. (n) = 4.

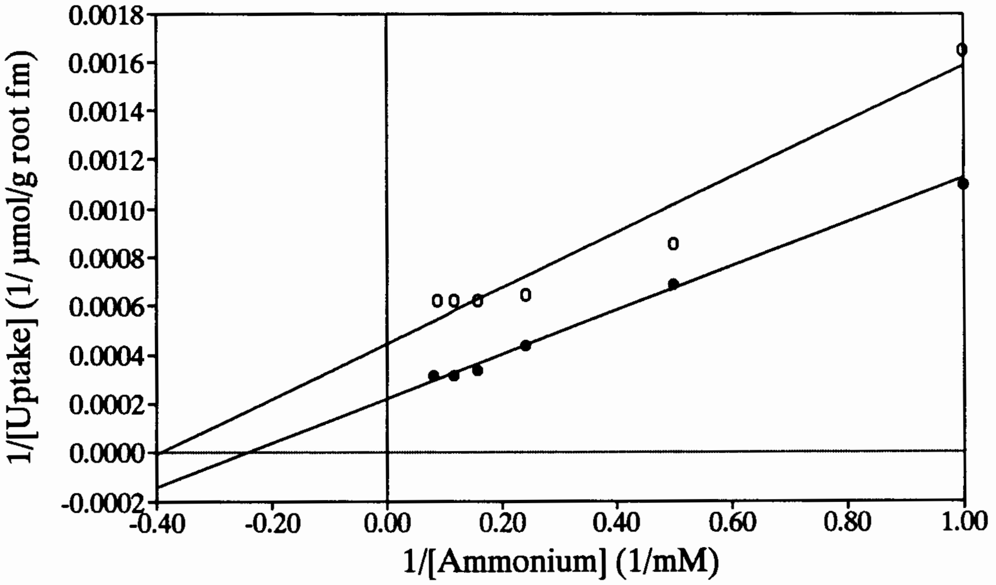

Figure 73: Lineweaver-Burk plot of ammonium uptake in salinized and control plants. ● = control, ○ = salinized. (n) = 4.

the kinetics of NH_4^+ uptake in the presence and absence of salinity. It was evident from these plots that salinity had a significant effect not only on the V_{max} of NH_4^+ uptake but also on the K_m. Similar to the nitrate-supplied plants, the regression coefficients of the control and stressed Lineweaver-Burk plots were shown to be statistically distinct ($P < 0.0001$) upon comparison of the two regression equations.

Table 9 shows that the presence of NaCl in the nutrient medium resulted in a decrease of the V_{max} of NH_4^+ uptake and a decrease in K_m (increase in "affinity" for NH_4^+ uptake) relative to the controls. However, it was not that the K_m of the stressed plants decreased but rather that the K_m of the controls was very large. The reason for this is easily observed from the Michaelis-Menten curve (**Figure 72**), from which it can be seen that the ammonium uptake in the controls did not reach zero order kinetics (ie. did not "level off"). This is not surprising considering that, while ammonium uptake may be energy dependent, it is thermodynamically passive (Glass, 1988) and would not be limited by the saturation of specific "carriers". The uptake in the stressed plants does, however, show zero order kinetics. This may suggest that uptake in the stressed plants was limited to active uptake, perhaps due to ion imbalances caused by the excess NaCl. Nonetheless the pattern of inhibition by NaCl is one of so-called uncompetitive inhibition, and all that may be deduced from this is that the effect of NaCl was not by direct interaction with the NH_4^+ ion.

The reduced V_{max} of the salinized plants infers that salinized, ammonium-supplied plants sequestered less NH_4^+ per gram root mass than the controls, so that, as for the nitrate-supplied plants, nitrogen may become limiting under saline conditions.

4.10 Effect of $CaCl_2$ plus K_2SO_4 on the NO_3^- and NH_4^+ uptake kinetics respectively in salinized wheat (Experiment 9)

This experiment (described in section **3.10.9**) investigated (i) the purported ability of Ca^{2+} to ameliorate decreased NO_3^- uptake in salinized plants and (ii) whether the increased biomass production of K^+-fed, ammonium-supplied salinized plants (section **4.6**) was correlated with increased NH_4^+ uptake. (i) and (ii) were tested by determining the kinetic parameters of N uptake under the conditions described in section **3.10.9**. The kinetics of NO_3^- or NH_4^+ uptake by salinized plants are illustrated by Michaelis-Menten plots and the reciprocal Lineweaver-Burk plots (**Figures 74** to **77**). The kinetic parameters V_{max} and K_m are given in **Tables 10** and 11.

4.10.1 Effect of $CaCl_2$ on the kinetics of NO_3^- uptake in salinized wheat

The Michaelis-Menten and Lineweaver-Burk plots for salinized, nitrate-supplied plants grown at three calcium concentrations (0.5 mM, 1.0 mM and 5.0 mM $CaCl_2$) are shown in **Figures 74** and **75**. It is evident from both figures that calcium concentration had a significant effect on the V_{max} of NO_3^- uptake under saline conditions, although the K_m remained unaffected. The V_{max} values are given in **Table 10**. The three slopes of the linear regression equations of Lineweaver-Burk plots corresponding to the three calcium treatments (**Figure 75**), were significantly distinct ($P < 0.0001$) as determined by analysis of covariance (Zar, 1984).

As the calcium concentration was increased, the V_{max} of the salinized plants increased so that at 5.0 mM $CaCl_2$ the V_{max} (287 ± 27 *µmol g-1 root fm*) approximated that of the control nitrate-supplied plants of the previous experiment (305 ± 23 *µmol g-1 root fm*). If calcium may be seen as an "activator" of NO_3^- uptake in salinized plants, then the pattern of activation was one which directly counteracted the noncompetitive inhibition by NaCl (section **4.9.1**). This implies that the "activation" by calcium was not due to direct interaction between the NO_3^- and Ca^{2+} ions, but rather due to some effect on the transporting apparatus on the plasmalemma, where increasing the calcium concentration decreased NaCl inhibition of NO_3^- uptake in stressed plants. This implies that the site of NaCl and Ca^{2+} interaction with NO_3^- uptake is the same, ie. the plasmalemma.

At least one of the beneficial effects of exogenous Ca^{2+} with respect to salinized plants was the stimulation of NO_3^- uptake. The latter may partially account for the elevated biomass production observed in salinized, nitrate-supplied plants when supplemented with 5.0 mM exogenous Ca^{2+} (section **4.3.1**). The partial restoration of NO_3^- uptake by exogenous calcium may be due to

i) a direct, undefined "protective" effect on the NO_3^- transporting system

ii) replacement of Ca^{2+} ions displaced by Na^+ ions (Läuchli, 1990) so that calcium activities in solution are maintained at near normal levels, thus maintaining membrane integrity

iii) increased K^+/Na^+ selectivity in salinized plants after calcium addition (section

4.3.4.3), mainly due to Na^+ exclusion

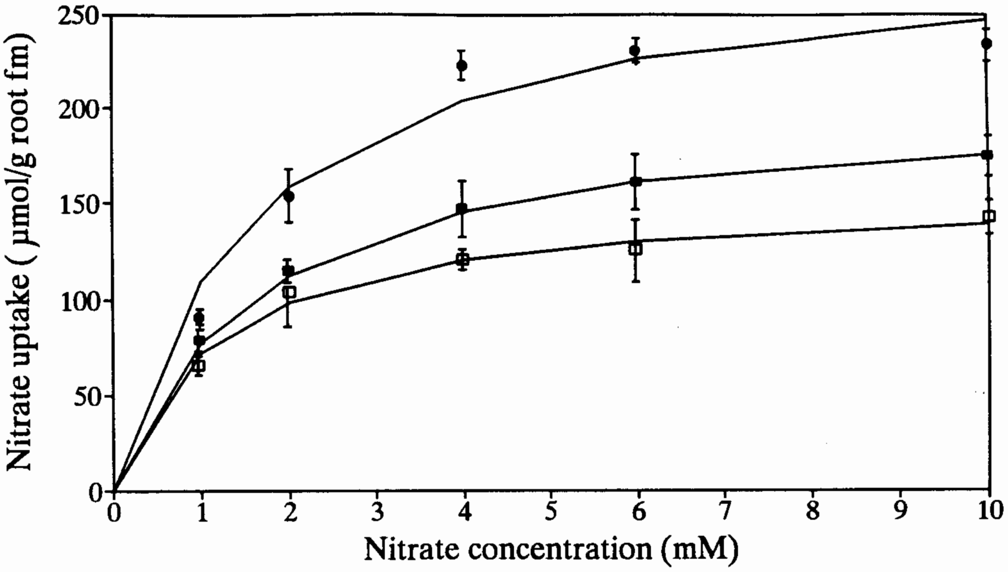

Figure 74: Michaelis-Menten plot of nitrate uptake in salinized, calcium supplemented wheat. ☐ = *0.5 mM* Ca^{2+}, ■ = *1.0 mM* Ca^{2+}, ● = *5.0 mM* Ca^{2+}. *(n) = 4.*

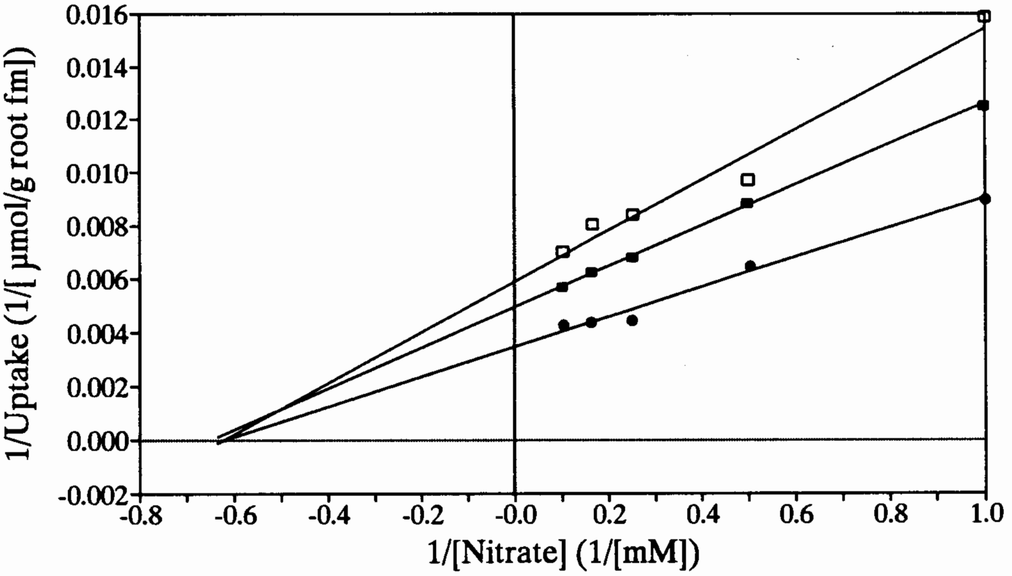

Figure 75: Lineweaver-Burk plot of nitrate uptake in salinized, calcium-supplemented wheat. ☐ = *0.5 mM* Ca^{2+}, ■ = *1.0 mM* Ca^{2+}, ● = *5.0 mM* Ca^{2+}. *(n) = 4.*

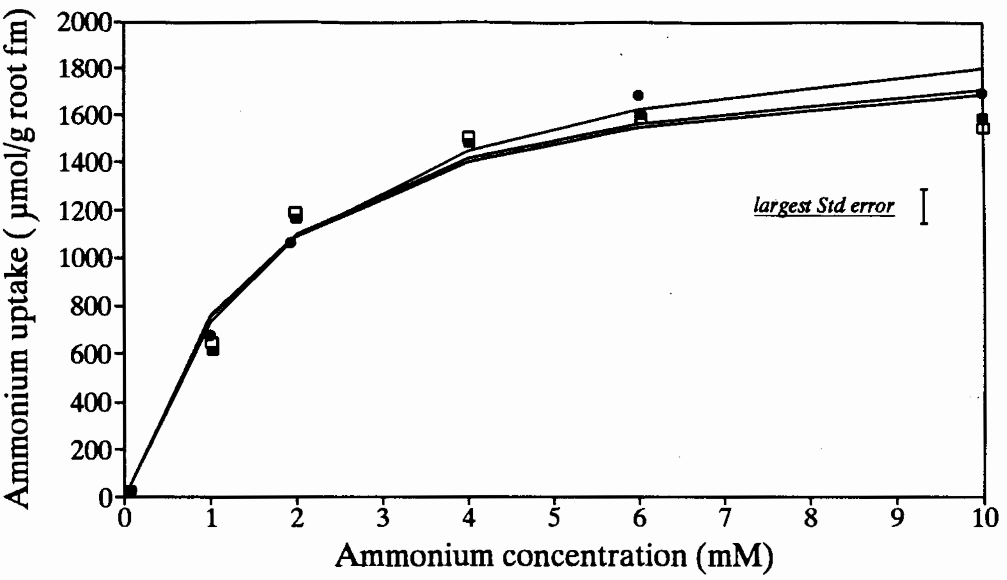

Figure 76: Michaelis-Menten plot of ammonium uptake in salinized, potassium-supplemented wheat. ☐ *= 0.5 mM K^+,* ■ *= 1.0 mM K^+,* ● *= 5.0 mM K^+. (n) = 4.*

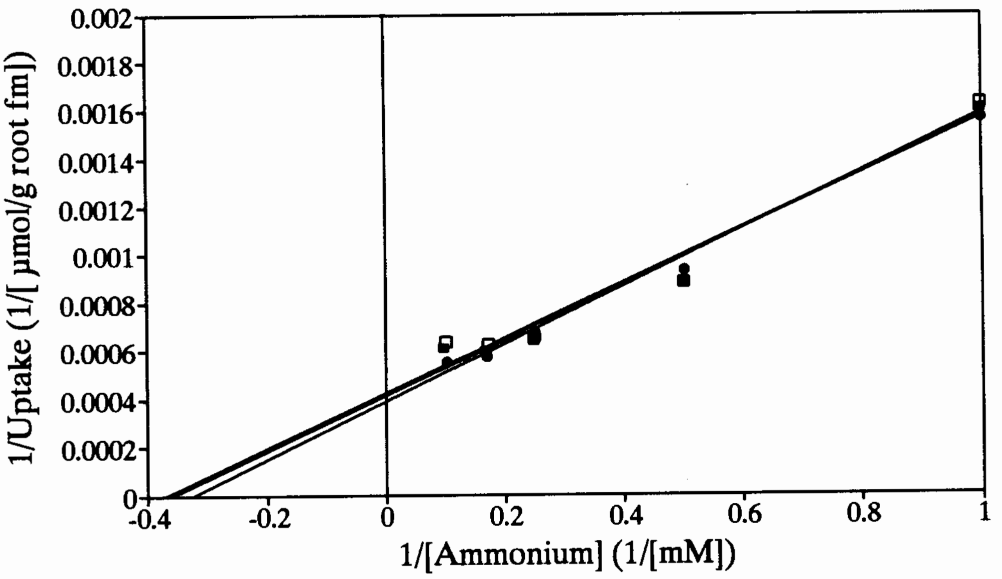

Figure 77: Lineweaver-Burk plot of ammonium uptake in salinized, potassium-supplemented wheat. ☐ *= 0.5 mM K^+,* ■ *= 1.0 mM K^+,* ● *= 5.0 mM K^+. (n) = 4.*

Table 10:* *Vmax and Km of NO_3^- uptake by salinized wheat supplied with different concentrations of calcium. The kinetic parameters were obtained from Lineweaver-Burk plots.

[Calcium] (mM)	Stressed plant kinetic parameters	
	V_{max} (μmol g^{-1} root fm)	K_m (mM)
[0.5]	154 ± 8	1.14 ± 0.25
[1.0]	204 ± 32	1.64 ± 0.09
[5.0]	287 ± 27	1.63 ± 0.50

Table 11:* *Vmax and Km of NH_4^+ uptake by salinized wheat supplied with different concentrations of potassium. The kinetic parameters were obtained from Lineweaver-Burk plots.

[Potassium] (mM)	Stressed plant kinetic parameters	
	V_{max} (μmol g^{-1} root fm)	K_m (mM)
[0.5]	1962 ± 105	1.61 ± 0.29
[1.0]	1965 ± 110	1.53 ± 0.31
[5.0]	2137 ± 120	1.88 ± 0.35

4.10.2 Effect of K_2SO_4 on NH_4^+ kinetics in salinized wheat

The Michaelis-Menten and Lineweaver-Burk plots for salinized ammonium-supplied plants grown at three potassium concentrations (0.5 mM, 1.0 mM and 5.0 mM K_2SO_4) are shown in **Figures 76** and **77**.

It is obvious from both figures that potassium concentration had no effect on either the V_{max} or K_m of NH_4^+ uptake under saline conditions. The V_{max} and K_m values are given in **Table 11**. The three slopes of the Lineweaver-Burk plots (**Figure 77**) were not analyzed for covariance since it was clear that the regression lines were not distinct. The increase in biomass observed for salinized, ammonium-supplied plants which had been supplied with exogenous K^+ (section **4.6.1**) thus cannot be accounted for by any effect of K^+ on NH_4^+ uptake, and must be due to some other effect(s).

5. CONCLUSIONS

5.1 A salinity gradient of 0 to 100 mM NaCl severely inhibited biomass production and moisture content in both nitrate and ammonium-supplied wheat, where the inhibition of biomass production at intermediate salinities (40 to 70 mM NaCl) was greater in the nitrate-supplied plants. The difference was partially due to ~~an~~ a differential effect of NaCl on NO_3^- and NH_4^+ uptake, where NaCl inhibited NO_3^- uptake more than NH_4^+ uptake.

5.2 The salinity gradient had no effect on photosynthetic rates in either nitrate or ammonium-supplied plants, while stomatal conductance and transpiration were slightly promoted at 40 mM NaCl in nitrate supplied plants. Clearly associated with the decrease in moisture content in nitrate and ammonium-supplied plants was the decrease in WUE with increasing salinity.

5.3 Salinity (50 mM NaCl) decreased $^{15}NO_3^-$ uptake, net NO_3^- depletion of the nutrient medium, V_{max} of NO_3^- uptake and N content (especially in the shoot) in nitrate supplied wheat. From the kinetic data it was discerned that NaCl was acting as a noncompetitive inhibitor of NO_3^- uptake, and thus not interacting with the NO_3^- ion but presumably affecting the NO_3^- transporting apparatus. $^{15}NH_4^+$ uptake was not severely inhibited by 50 mM NaCl, but when measured as NH_4^+ depletion of the nutrient medium, net uptake and uptakes rate were decreased. This suggests that the salinity increased the NH_4^+ efflux while the influx remained unaffected. Both the V_{max} and K_m of NH_4^+ uptake were decreased by salinity. Evidence of ammonium efflux has been shown to occur by Morgan and Jackson (1989).

5.4 Calcium concentration (5 to 8 mM $CaCl_2$) increased biomass production, moisture content, plant N content, V_{max} of NO_3^- uptake, $^{15}NO_3^-$ content of the shoot as well as K^+ content and $S_{[Na,K]}$ in salinized, nitrate-supplied plants. Calcium notably decreased the Na^+ content of salinized, nitrate-supplied plants. In ammonium-supplied wheat calcium concentration had no influence on biomass production, moisture content, N content nor $^{15}NH_4^+$ uptake.

5.5 Potassium concentration (0.2 to 5 mM) had no effect on biomass production, moisture

No K effect in nitrate plants
but present in ammon plants.

content or gaseous exchange in salinized and control nitrate-supplied plants. It was determined that the K^+ status of the plants was more dependent on the exogenous calcium concentration than the exogenous potassium concentration. At 2.5 mM K^+, biomass production was enhanced in salinized, ammonium-supplied plants. This increase in biomass production was not correlated with an increase in the net NH_4^+ depletion of the nutrient medium or with changed values of V_{max} or K_m of NH_4^+ uptake.

Due to the specific effect which NaCl had on NO_3^- uptake and content, as well as K^+ loss (K^+ leakage being an indicator of membrane integrity), it is suggested that the main NaCl effect is one of membrane disruption. This is supported by (i) the massive influx of Na^+ which occurred under salinity stress and (ii) the evidently noncompetitive inhibition that NaCl had on N kinetics. The latter implies that the disruption by NaCl on N uptake was at the site of the transporting apparati (passive or active) and thus at the membrane.

High calcium concentrations somewhat ameliorated this apparent disruption, but only in nitrate-supplied wheat. One of the reasons for the amelioration was revealed by this research ie. the counteraction of the noncompetitive inhibition of NO_3^- uptake by NaCl. This inferred that the action of calcium was at the same site as that of NaCl ie. the plasmalemma.

APPENDIX 1: FORMULAE USED IN THE DATA ANALYSIS

Gaseous Exchange Equations used by the LCA-2 and LCA-3.

$$A = (Cr - (\frac{Ca(P - ei)}{(P - eo)}) \times W \qquad (8)$$

$$E = (\frac{(eo - ei)}{(P - eo)}) \times W \qquad (9)$$

$$Gs = \frac{(e_l - eo)}{(eo - ei)} \times \frac{(\frac{P - eo}{P})}{(W - B)} \qquad (10)$$

A = CO_2 Assimilation (μmol m^{-2} s^{-1})
Cr = corrected reference CO_2 concentration (μbar)
Ca = corrected analysis CO_2 concentration (μbar)
P = atm pressure (bar)
el = saturated vapour pressure at leaf temperature (bar)
ei = vapour pressure of the air at the inlet of the cuvette (bar)
eo = vapour pressure of the air at the outlet of the cuvette (bar)
W = mass flow of dry air per unit leaf area (mol m^{-2} s^{-1})
B = boundry layer resistance to H_2O vapour (m^{2} s^{-1} mol^{-1})

Formulae for the derivation of A%E

The intensities of the band heads at 297.7nm (I^{28}), 298.3nm (I^{29}) and 298.9nm (I^{30}) are proportional to the concentrations of the corresponding molecules:

$$\frac{I^{28}}{I^{29}} = \frac{[^{14}N_2]}{[^{14}N^{15}N]} \qquad (11)$$

$$\frac{I^{28}}{I^{30}} = \frac{[^{14}N_2]}{[^{15}N_2]} \qquad (12)$$

Thus the ^{15}N content is given by:

$$[^{15}N(atom\%)] = \frac{2(I^{30}) + I^{29}}{2(I^{28} + I^{29} + I^{30})} \qquad (13)$$

Where the occurrence of ^{30}N is low compared to ^{28}N with respect to quantitative determination the following perfect statistical equilibrium may be assumed:

$$[^{14}N^{14}N] + [^{15}N^{15}N] = 2[^{14}N^{15}N] \qquad (14)$$

For this equilibrium:

$$K = \frac{[^{14}N^{15}N]}{[^{14}N^{14}N]\ [^{15}N^{15}N]} = 4 \qquad (15)$$

and

$$[^{15}N^{15}N] = \frac{[^{14}N^{15}N]^2}{4[^{14}N^{14}N]} \qquad (16)$$

Then substitution of equation **(16)** for **(13)** gives:

$$[^{15}N(atom\,\%)] = \frac{100}{\frac{2([^{14}N^{14}N]}{[^{14}N^{15}N]}+1} \qquad (17)$$

The final corrected A%E values were thus calculated as follows:

$$A\,\%\,E = ((\frac{100}{2R+1})\,x\,1.015-0.192)-0.3663 \qquad (18)$$

$R = [^{14}N^{14}N]/[^{14}N^{15}N]$
1.015 = m (slope)
0.192 = c (y intercept constant)
0.3663 = natural abundance (%) of ^{15}N

APPENDIX 2: STATISTICAL TESTS

Table 12: Multifactor analysis of variance in biomass production and moisture content as affected by sodium chloride, calcium chloride and potassium sulphate concentration.

EFFECT		SHOOT	ROOT	PLANT	S:R	H2O CONT.	
						shoot	root
NaCl	F-ratio	85.92	52.51	79.32	831.08	443.63	556.523
	P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	d.f.	3	3	3	3	3	3
N Form	F-ratio	13.40	0.01	7.17	7.73	843.79	1000
	P	0.0004	0.9469	0.0085	0.0002	0.0001	0.0001
	d.f.	1	1	1	1	1	1
NaCl (n)	F-ratio	55.96	35.77	60.99	0.47	21.85	4.328
	P	0.0001	0.0001	0.0001	0.5001	0.0001	0.0399
	d.f.	1	1	1	1	1	1
Ca	F-ratio	9.58	8.62	9.31	14.70	3.29	17.8
	P	0.0001	0.0001	0.0001	0.0001	0.0236	0.0001
	d.f.	3	3	3	3	3	3
NaCl (a)	F-ratio	29.35	23.04	29.82	3.11	1000	1000
	P	0.0001	0.0001	0.0001	0.0803	0.0001	0.0001
	d.f.	1	1	1	1	1	1
Ca	F-ratio	1.89	2.57	2.17	2.44	415.50	500.748
	P	0.1347	0.0576	0.0952	0.0674	0.0001	0.0001
	d.f.	3	3	3	3	3	3
NaCl (n)	F-ratio	29.35	23.04	29.82	2.57	21.20	3.563
	P	0.0001	0.0001	0.0001	0.0576	0.0001	0.0165
	d.f.	1	1	1	1	1	1
K	F-ratio	1.89	0.81	1.75	0.50	0.03	0.192
	P	0.1347	0.4889	0.1601	0.6845	0.8623	0.6685
	d.f.	3	3	3	3	3	3
NaCl (a)	F-ratio	80.31	33.98	74.48	4.39	14.19	0.001
	P	0.0001	0.0001	0.0001	0.0385	0.0003	0.9780
	d.f.	1	1	1	1	1	1
K	F-ratio	2.45	4.12	3.26	3.56	0.76	9.212
	P	0.0672	0.0082	0.0243	0.0165	0.5180	0.0001
	d.f.	3	3	3	3	3	3

ABBREVIATIONS:

(n) nitrate-supplied
(a) ammonium-supplied
(cn) control, n-supplied
(sn) salinized, n-supplied
(ca) control, a-supplied
(sa) salinized, a-supplied
(Ca) Calcium chloride
(K) Potassium sulphate

Table 13: Multivariate analysis of total nitrogen and 15N uptake in salinized, calcium supplemented, nitrate-supplied wheat.

EFFECT		SHOOT	ROOT	PLANT	SHOOT	ROOT	PLANT
		Total N (n)			Total N (a)		
NaCl	F-ratio	39.31	21.36	49.14	128.23	133.81	117.014
	P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	d.f.	1	1	1	1	1	1
Ca	F-ratio	9.53	118.82	28.95	0.10	0.03	0.192
	P	0.0040	0.0001	0.0001	0.5985	0.8623	0.6685
	d.f.	1	1	1	1	1	1
		SHOOT	ROOT	PLANT	SHOOT	ROOT	PLANT
		15N uptake (n)			15N uptake (a)		
NaCl	F-ratio	1000	872.54	1000	500.13	108.81	26.983
	P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	d.f.	1	1	1	1	1	1
Ca	F-ratio	716.78	798.61	1000	43.85	63.97	91.685
	P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	d.f.	1	1	1	1	1	1

Table 14: Multivariate analysis of the effect of calcium chloride on the ionic content of salinized, nitrate-supplied wheat.

EFFECT		(St)	(S)	(R)	(St)	(S)	(R)
		K+ Content (% control)			Na+ Content (% control)		
NaCl (n)	F-ratio	127.86	20.84	58.66	1000	98.22	77.66
	P	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001
	d.f.	1	1	1	1	1	1
CaCl2	F-ratio	102.50	30.04	102.58	860.16	4.38	20.12
	P	0.0001	0.0001	0.0001	0.0001	0.0281	0.0001
	d.f.	2	2	2	2	2	2
		(St)	(S)	(R)	(St)	(S)	(R)
		K+:Na+ ratio			S[Na,K]		
NaCl (n)	F-ratio	1.51	3.95	19.00	1000	192.69	112.123
	P	0.2656	0.0625	0.0004	0.0001	0.0001	0.0001
	d.f.	1	1	1	1	1	1
CaCl2	F-ratio	35.29	3.01	5.44	865.53	6.14	13.218
	P	0.0005	0.0745	0.0142	0.0001	0.0093	0.0003
	d.f.	2	2	2	2	2	2

ABBREVIATIONS
(St) shoot tip
(S) shoot
(R) root

Table 15: Comparison of regression equations and analysis of covariance of Lineweaver-Burk plots.

Comparison of regression equations		
	NO3- uptake	NH4+ uptake
t0.06(2)	2.31	2.31
t	1.5E+08	1.3E+09
P	0.0001	0.0001
Ho	reject	reject
Analysis of covariance		
	NO3- uptake	NH4+ uptake
F0.05(1),k-1,9	5.71	5.71
F	7.7E+15	1.2E+13
P	0.0001	0.0001
Ho	reject	reject

(Ho = lines are of equal slope)

ONE-WAY ANALYSIS

Table 16: One-way analysis of variance in biomass production and moisture content as affected by sodium chloride, calcium chloride and potassium sulphate concentration.

EFFECT		SHOOT	ROOT	PLANT	S:R	H2O CONT.	
						shoot	root
NaCl(n)	F-ratio	61.97	33.19	54.23	831.08	424.63	546.793
	P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	d.f.	3	3	3	3	3	3
NaCl(a)	F-ratio	32.58	22.00	30.66	7.73	32.58	22.002
	P	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001
	d.f.	3	3	3	3	3	3
Ca (cn)	F-ratio	2.86	0.73	0.40	2.94	13.96	7.435
	P	0.0681	0.4857	0.6758	0.0630	0.0001	0.0016
	d.f.	3	3	3	3	3	3
Ca (sn)	F-ratio	14.76	7.56	7.56	17.89	7.89	16.641
	P	0.0001	0.0015	0.0015	0.0001	0.0012	0.0001
	d.f.	3	3	3	3	3	3
Ca (ca)	F-ratio	3.04	2.37	2.98	0.13	461.73	501.464
	P	0.0361	0.0793	0.0387	0.9434	0.0001	0.0001
	d.f.	3	3	3	3	3	3
Ca (sa)	F-ratio	0.13	0.54	0.09	2.80	0.66	0.1
	P	0.9434	0.6549	0.9647	0.0474	0.5816	0.9598
	d.f.	3	3	3	3	3	3
K (cn)	F-ratio	19.56	31.26	59.00	0.76	19.23	0.806
	P	0.0001	0.0001	0.0001	0.5180	0.0001	0.4966
	d.f.	3	3	3	3	3	3
K (sn)	F-ratio	0.03	0.54	0.32	0.50	3.29	0.492
	P	0.8623	0.6515	0.8085	0.6845	0.0236	0.6897
	d.f.	3	3	3	3	3	3
K (ca)	F-ratio	0.44	0.88	0.42	4.22	0.95	2.066
	P	0.7250	0.4578	0.7370	0.0091	0.4213	0.1144
	d.f.	3	3	3	3	3	3
K (sa)	F-ratio	11.23	6.34	11.75	0.49	0.48	8.44
	P	0.0001	0.0010	0.0001	0.6897	0.7009	0.0001
	d.f.	3	3	3	3	3	3

ABBREVIATIONS:

(n) nitrate-supplied
(a) ammonium-supplied
(cn) control, n-supplied
(sn) salinized, n-supplied
(ca) control, a-supplied
(sa) salinized, a-supplied
(Ca) Calcium chloride
(K) Potassium sulphate

Table 17: One-way analysis of variance in total nitrogen and 15N uptake in salinized, calcium supplemented, nitrate and ammonium-supplied wheat.

EFFECT		SHOOT	ROOT	PLANT	SHOOT	ROOT	PLANT
		Total N (n)			Total N (a)		
Ca (c)	F-ratio	3.56	39.60	8.65	0.56	2.61	0.269
	P	0.0775	0.0001	0.0096	0.4737	0.1216	0.6163
	d.f.	1	1	1	1	1	1
Ca (s)	F-ratio	6.14	86.80	22.10	3.57	5.66	0.009
	P	0.0233	0.0001	0.0002	0.0733	0.0230	0.9280
	d.f.	1	1	1	1	1	1
		SHOOT	ROOT	PLANT	SHOOT	ROOT	PLANT
		15N uptake (n)			15N uptake (a)		
Ca (c)	F-ratio	764.91	773.19	1000	3.57	2.20	2.391
	P	0.0001	0.0001	0.0001	0.0733	0.1467	0.1377
	d.f.	1	1	1	1	1	1
Ca (s)	F-ratio	18.91	90.09	95.06	43.85	63.97	91.685
	P	0.0004	0.0001	0.0001	0.0001	0.0001	0.0001
	d.f.	1	1	1	1	1	1

Table 18: One-way analysis of variance of the effect of calcium chloride on the ionic content of salinized, nitrate-supplied wheat.

EFFECT		(St)	(S)	(R)	(St)	(S)	(R)
		K+ Content (% control)			Na+ Content (% control)		
Ca	F-ratio	9.80	25.60	1.40	103.70	36.43	6.519
	P	0.0055	0.0002	0.2952	0.0001	0.0001	0.0178
	d.f.	2	2	2	2	2	2
		K+:Na+ ratio			S[Na,K]		
Ca (c)	F-ratio	7.34	0.89	2.64	103:24	2.57	5.493
	P	0.0699	0.4447	0.1257	0.0001	0.1307	0.0276
	d.f.	2	2	2	2	2	2
Ca (s)	F-ratio	60.28	3.18	4.98	807.69	5.94	12.082
	P	0.0038	0.0901	0.0349	0.0001	0.0227	0.0028
	d.f.	2	2	2	2	2	2

ABBREVIATION
(St) shoot tip
(S) shoot
(R) root

GASEOUS EXCHANGE:

Two-way Anovas

Table 19: Multifactor analysis of variance of gaseous exchange in salinized wheat as affected by sodium chloride, calcium chloride and potassium sulphate concentration.

EFFECT		A	E	Gs	WUE	EFFECT		A	E	Gs	WUE	SDR
NaCl	F-ratio	4.31	10.28	1.69	3.19	NaCl (n)	F-ratio	4.47	16.52	19.07	7.46	33.95
	P	0.0084	0.0001	0.179	0.0796		P	0.0414	0.0002	0.0001	0.0097	0.0001
	d.f.	3	3	3	3		d.f.	1	1	1	1	1
N form	F-ratio	0.27	3.19	1.98	2.11	K	F-ratio	0.05	0.15	0.06	1.47	1.18
	P	0.6131	0.0796	0.1648	0.1291		P	0.9867	0.9316	0.979	0.2401	0.3309
	d.f.	1	1	1	1		d.f.	3	3	3	3	3
NaCl (n)	F-ratio	12.36	18.00	4.25	7.99	NaCl (a)	F-ratio	12.36	17.88	3.69	8.44	
	P	0.0009	0.0001	0.0597	0.0001		P	0.0009	0.0001	0.0597	0.0001	
	d.f.	1	1	1	1		d.f.	1	1	1	1	
Ca	F-ratio	1.30	2.39	2.49	4.53	K	F-ratio	1.31	2.40	2.54	4.22	
	P	0.2807	0.0774	0.0651	0.0090		P	0.2807	0.0774	0.0651	0.0090	
	d.f.	3	3	3	3		d.f.	3	3	3	3	
NaCl (a)	F-ratio	1.51	7.26	0.21	2.20							
	P	0.2229	0.0087	0.6569	0.0952							
	d.f.	1	1	1	1							
Ca	F-ratio	3.27	1.14	5.91	1.82							
	P	0.0259	0.3376	0.0011	0.1517							
	d.f.	3	3	3	3							

A Assimilation
E Transpiration
Gs Stomatal conductance
WUE Water use efficiency
SDR Stomatal diffusive resistance

One-way Anova

Table 20: One-way analysis of variance of gaseous exchange in salinized wheat as affected by sodium chloride, calcium chloride and potassium sulphate concentration.

EFFECT		A	E	Gs	WUE	EFFECT		A	E	Gs	WUE	SDR
NaCl (n)	F-ratio	3.01	15.34	4.85	3.19	K (cn)	F-ratio	0.64	0.42	0.18	1.32	0.66
	P	0.0464	0.0001	0.0075	0.0796		P	0.6010	0.7406	0.9086	0.2962	0.4774
	d.f.	3	3	3	3		d.f.	3	3	3	3	3
NaCl (a)	F-ratio	1.95	2.71	1.05	2.11	K (sn)	F-ratio	1.18	1.06	0.33	3.01	1.10
	P	0.1450	0.0650	0.3887	0.1291		P	0.3485	0.3930	0.8009	0.0609	0.3773
	d.f.	3	3	3	3		d.f.	3	3	3	3	3
Ca (cn)	F-ratio	3.38	8.66	4.29	6.66	K (ca)	F-ratio	0.58	2.81	3.353	3.12	
	P	0.0289	0.0002	0.0112	0.0015		P	0.6329	0.0701	0.0324	0.0700	
	d.f.	3	3	3	3		d.f.	1	1	1	1	
Ca (sn)	F-ratio	4.21	2.92	5.42	1.97	K (sa)	F-ratio	0.26	4.38	3.385	3.42	
	P	0.0119	0.0471	0.0035	0.1402		P	0.2807	0.0119	0.0319	0.0120	
	d.f.	3	3	3	3		d.f.	3	3	3	3	
Ca (ca)	F-ratio	3.19	3.19	5.64	9.79							
	P	0.0349	0.0347	0.0028	0.0001							
	d.f.	3	3	3	3							
Ca (sa)	F-ratio	1.65	0.31	2.87	1.63							
	P	0.1943	0.8209	0.0489	0.1983							
	d.f.	3	3	3	3							

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