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Interaction between salinity and nutrients in cotton

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INTERACTION BETWEEN SALINITY AND NUTRIENTS IN COTTON

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By

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In the Name of

ALLAH

The Most Gracious

The Most Merciful

Whose Help We Solicit

Dedicated to

my Late Parents

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LIST OF ABBREVIATIONS

1 kmol m^{-3}	Equivalent to 1 Molar
ATP	Adenosine triphosphate
cm^3	Equivalent to 1 ml
cv.	cultivar
DW	Dry Weight
dm^3	Equivalent to 1 litre
dS m^{-1}	deci Siemens per metre
E	Transpiration rate
EC	Electrical Conductivity
FW	Fresh Weight
g	gram
GLM	General Linear Model
gs	Stomatal conductance
h	hour
l	litre
mm	millimetre
mol m^{-3}	mole per cubic metre
MPa	Mega Pascal
nm	nanometre
ns	non significant
NUR	Net Uptake Rate
P	Probability
Pn	Photosynthesis (net)
ppm	parts per million
RH	Relative Humidity
SAR	Sodium Absorption Ratio
SE	Standard Error
<	less than

ABSTRACT

This is a study of physiological changes associated with salt stress and the deleterious effects of salinity on cotton. I investigated how these effects may be minimized by the use of additional nutrients such as ammonium nitrate, glycinebetaine and potassium chloride. Differences between ammonium and nitrate as nitrogen sources were also investigated. The effects of the method of NaCl and KCl application (foliar or root) on the morphology of cotton plant, ion concentrations, and K^+ uptake rate by roots have been examined.

Comparison between NH_4^+ and NO_3^- nitrogen sources showed NO_3^- to be more beneficial than NH_4^+. The low growth in a low N treatment, and the similar reduction in relative growth (% of controls) by salinity at two N levels, indicated that N and salinity effects were independent. Increasing levels of salinity did not decrease nitrogen content in the leaves. Exogenous glycinebetaine did not influence the growth of the plant or the ion contents of its tissues.

The two methods of NaCl and KCl application produced distinct results. The leaves of cotton plants were capable of absorbing salt supplied in an aqueous medium. This decreased leaf K^+ concentrations and vegetative growth of cotton. Soil-applied salinity resulted in a lower accumulation of Na^+ in the leaves than foliar-applied salinity and did not significantly alter leaf K^+ concentrations.

Foliar application of KCl reduced the entry of Na^+ into the leaves and substantially increased growth when the leaves were wetted with saline water. Foliar and root-applied salts inhibited K^+ uptake by the roots and its translocation to the shoot in hydroponic culture. It is concluded that foliar application of KCl at 10 mol m^{-3} is enough to reduce the passage of Na^+ into the leaf cell and enhance plant growth under highly saline conditions.

The most important and interesting result was that K^+ concentrations decreased in the leaves in response to salt application via foliage. It is postulated that this occurs as a result of some signal from shoot to root to slow down K^+ uptake which consequently decreased K^+ concentrations in the leaves. A decrease in the rate of transpiration flow due to salinity could also reduce K^+ uptake. In this study, however, the rate of transpiration was not decreased by foliar-applied salt.

CHAPTER ONE

GENERAL INTRODUCTION/ LITERATURE REVIEW

CHAPTER 1

GENERAL INTRODUCTION/ LITERATURE REVIEW

1.1 General description of cotton

The main fibre-producing crop of the world is cotton, and it is an important cash crop. It is widely grown in the tropical and sub-tropical regions of Africa, Asia, Australia and America. It is capable of growing on marginally fertile soils and can tolerate moderate salinity (Ahmad and Abdullah, 1982). It is a warm-season crop requiring a temperature range of 20-30 °C (Reddy *et al.*, 1991). It can be grown as an annual summer crop in more temperate zones. Summer temperatures in the cotton growing regions of Pakistan often reach 48-50 °C (Ashraf, 1994).

Most cotton is used for the manufacture of textiles. Cotton seeds also contain an edible oil, which can be used in cooking and margarine manufacture. The lower grade oils are also utilized in soap manufacture and also as lubricants (Ahmad and Makhdum, 1992). Anthony (1991) has reported that cotton is used in the manufacture of medicinal supplies, tarpaulins, cordage, and belting. Skordilis (1992) mentioned the successful production of lime by using the cotton gin residues as a combustible material in the furnace of a continuous-operation, modern lime kiln. Dried cotton sticks are a very important and cheap source of fuel for domestic use.

Cotton belongs to the genus *Gossypium* of the family Malvaceae. This genus includes more than 50 wild, cultivated and lint-less species (Gorham *et al.*, 1995). The modern cultivated cotton falls into two separate groups consisting of two diploid species, *Gossypium arboreum* L. and *Gossypium herbaceum* L. and two tetraploid species *Gossypium barbadense* L. and *Gossypium hirsutum* L.

1.1.1 Cotton in Pakistan

In Pakistan, the cotton-growing region has a length and breadth of 960 and 320 km respectively (Ahmad and Makhdum, 1992). Eisa *et al.* (1994) have mentioned that Pakistan is currently the world's fifth largest cotton producer, with

a 1991/92 harvested area of 2.881 Mha, about 8.2% of the world total (34.932 Mha), and with 1991/ 92 production of 2.176 million metric tonnes (about 10.5% of the world total 20.793 million metric tonnes). Cotton is cultivated mostly in Sindh and Punjab provinces. Nearly 98% of the cotton areas in Pakistan are planted to *G. hirsutum* and the rest to *G. arboreum* (Munir-ud-Din Khan, personal communication).

1.2 Salinity

Salinity is an important ecological factor that creates serious problems for agricultural productivity in many parts of the world. The economic importance of salinity is strongly substantiated by the dangerous trend of a 10% per year increase of salinized areas all over the world, a substantial loss of actual arable land (Ponnamperuma, 1984). The origins of salinity can be classified as those arising from seawater inundation and salt spray. Na^+,Ca^{2+}, Mg^{2+} and numerous other elements are deposited on soils during irrigation of the lands from rivers. Water and dissolved salts are essential to plant growth, but water reuse and high evaporation rates in arid or semiarid regions concentrate the salts as the general phenomenon of salinization occurs (Shannon *et al.*, 1994). In saline soils, the concentration of salts increases to levels at which crop growth is adversely affected. In these soils, the electrical conductivity of the saturation extracts (Ec_e) is greater than 4 dS m^{-1}, the pH usually ranges between 7.5 and 8.5, and the sodium absorption ratios (SAR) are less than 13 (Qureshi and Barrett-Lennard, 1998). However, salinity also arises as a result of human activities such as mismanagement of irrigation systems (Choukr-Allah, 1995).

High salinity, however, has ion-specific effects in addition to osmotic effects, ion toxicity, nutrient imbalance or a combination of these factors (Läuchli and Epstein, 1990). Imbalances of ions in plants occur when the concentration of Na^+ in the soil reduces the amounts of K^+, Mg^{2+}, and Ca^{2+} taken up by the plant (Epstein, 1972). Sometimes Na^+ has direct toxic effects, as when it interferes with enzyme structure and function. Many of the deleterious effects of Na^+, however,

seem to be related to the structural and functional integrity of membranes (Leopold and Willing, 1984).

Soil salinity is classified into terms of Ec_e (dS m^{-1}).

Salt free	less than 4 dS m^{-1}
Slightly saline	4-8 dS m^{-1}
Moderately saline	8-15 dS m^{-1}
Strongly saline	more than 15 dS m^{-1}

Source: Water and Power Development Authority (1981)

The other problem soils are sodic (black alkali) soils having high exchangeable sodium concentrations, which dissolve the organic matter present in the soil and give it a dark brown or black colour. In these soils, the structure is deteriorated, permeability has decreased, and root growth is restricted. These soils have EC_e values less than 4 dS m^{-1}, pH values greater than 8.5, and sodium absorption ratios greater than 13 (Qureshi and Barrett-Lennard, 1998).

1.2.1 Extent of saline soils

On a world-wide basis, it is estimated that there are between 400-950 Mha of salt-affected soils (Epstein *et al.*, 1980). Australia has the largest saline and sodic area (357.24 Mha) followed by Asia (316.54 Mha), Latin America (131.13 Mha), Africa (80.44 Mha) and North America (15.76 Mha) (Abrol *et al.*, 1988). The most affected are those countries that depend entirely on irrigation water for crop production. About 50-75 % of Iraq (Abdul-Halim *et al.*, 1988) and 30% in Egypt is salt affected (Kovda, 1977). Another estimate shows that about 13 % (Flowers and Yeo, 1988) to 23 % (Tanji, 1990) of land under cultivation or 30 % (Epstein *et al.*, 1980) to 50 % (Flowers and Yeo, 1988) of the land under irrigation are salt affected. At present about 65% of water in the world is being used for agricultural production and much of this is used inadequately resulting in the salinization of land and water resources (Ghassemi *et al.*,1995).

Table 1.1 Irrigated land damaged by salinization in the top five irrigators and the world, estimated for the mid-1980s

Country	Area affected (Mha)	Share of irrigated land affected (percent)
India	20.0	36
China	7.0	15
United States	5.2	27
Pakistan	3.2	20
Former Soviet Union	2.5	12
Subtotal	37.9	24
World	60.2	24

Source: Postel (1990)

1.2.2 Salinity in Pakistan

Land salinization is a major cause of desert formation in Pakistan, and environmental factors are a part of salinization in Pakistan. The Indus Plain is composed of alluvial sediments, which were deposited by rivers into a shallow sea. The receding sea has left behind residues of salt, both in the soil profile and in the groundwater aquifer. In addition, weathering of parent rocks can release significant amounts of salt into the soil. In Pakistan, salt-affected soils cover more than 40% of the irrigated land (Choukr-Allah, 1995). Pakistan occupies a total geographical area of 80.5 Mha, out of which 33 Mha are considered suitable for cultivation while only 20.4 Mha are actually under cultivation. An area of 10.23 Mha is irrigated through canals and tube wells and the remaining 4.13 Mha is independent of rain (Rafiq, 1990). Salt-affected lands in Pakistan are estimated to be about 6.3 Mha (Qureshi and Barrett-Lennard, 1998), while temporary or permanent waterlogging has affected an area of 6.17 Mha (Rafiq, 1990).

Salinization occurs both naturally (primary salinity) and as a result of human activities (secondary salinity). Primary salinity on the Indus Plain occurs around the margins of natural depressions in the landscape where rain and flood water accumulate. These soils become loaded with salts because of the movement

of water via capillaries to the soil surface. Low rainfall and high temperatures are also the main causes of saline problems in Pakistan. The annual rainfall ranges from >1000 mm in the northern to 90 mm in the southern parts of Pakistan (Khan, 1996). Generally, summers in Pakistan are very hot. The temperatures begin to rise rapidly from April onward, reaching a maximum in June. At this time, daily maximum temperature normally remains 40-46 °C (Qureshi and Barrett-Lennard, 1998). Soil salinity and sodicity are natural agricultural problems under these climatic conditions.

Secondary salinity on the Indus Plain is related to the development of the modern irrigation system in Pakistan. There are four causes of secondary salinity, 1) Seepage from irrigation canals, 2) high salt concentrations in the irrigation water, 3) insufficient leaching of salt and 4) types of salts in the irrigation water. Many soils have become saline-sodic because of the use of saline groundwater for irrigation. Moreover, the salt concentration in canal water in Pakistan is generally between 150-200 mg l^{-1} (Ghassemi *et al.*, 1995). The application of 500 mm of water with a salinity of 200 mg l^{-1} would add 1 tonne of salt per hectare (Qureshi and Barrett-Lennard, 1998). Of course the scale of the problem of salt accumulation in the root-zone is even greater if saline groundwater is used for irrigation.

1.3 Salt tolerant crops

Salt tolerance is a complex phenomenon, because different plants respond to saline conditions in different ways, but also because of the great variation in the stress itself. Gorham (1992) reported that salt tolerance of a plant depends on a balance between allowing sufficient salt to enter the shoot for osmotic adjustment, and preventing the accumulation of toxic levels within the plant. Plants can be broadly divided into two groups: halophytes, the plants which like or tolerate saline water and can survive at about 44 dS m^{-1} or 500 mol m^{-3} NaCl and secondly non-halophytes, the plants that like sweet water and grow better on non-saline soils (Gorham, 1992). Qureshi and Barrett-Lennard (1998) further subdivided non-halophytes into two groups: salt-tolerant non-halophytes,

the plants grow at moderate salt concentrations (8-15 dS m^{-1}) and salt-sensitive non-halophytes (sensitive to even low salt concentrations). They further reported that about 150 agriculturally important species have been ranked for salt tolerance.

There are, however, various statements by different authors about salt tolerance of crops. Gorham (1993) and Wyn Jones (1981) reported that the adjustment of K^+/Na^+ ratio in the plant cell is a good indicator of salt tolerance. Jeschke and Wolf (1988) described that phloem translocation of K^+ is an important component of salinity tolerance of plants. According to Maas (1990), salt tolerance can be defined as the plant's capacity to endure the effects of excess salt in the medium of root growth. This is not something easily measured, because it depends on many factors, e.g. the type of salts involved, the growth stage of the plant, and the growing conditions. Kent and Läuchli (1985) reported that salt tolerance in cotton was related to K^+/Na^+ selectivity. Yeo (1983) emphasised the difference between salt tolerance, where a plant is adapted to live in an adverse environment and salt resistance, where a plant is able to grow and maintain normal metabolic functions in non-optimal conditions. Shannon (1997) suggested that plants that limit uptake of toxic ions and maintain normal ranges of nutrient ions could be more salt tolerant than those do not restrict ion accumulation and lose nutrient balance. Limited Na^+ uptake is a trait related to salinity tolerance in several crop plants (Fortmeier and Schubert, 1995 and Gorham *et al.*, 1985). Shannon *et al.* (1994) suggested that when the volume of water transpired is orders of magnitude greater than the volume of the plant, the relative permeability of roots to water and ions is a significant factor relating to salt tolerance. Allen *et al.* (1995) reported that the maintenance of a low Na^+ concentration in the cytoplasm is an important factor in the tolerance of crop plants to salinity. Benlloch *et al.* (1994) suggested that the characteristics of K^+ and Na^+ transports are determinant of the NaCl tolerance in plants.

Thus, all these factors should be specified when giving an estimation of the salt tolerance of a crop. However, for comparative purposes, general values are commonly used for different species. Rice for example, is sensitive during the early seedling stages and at flowering (Akbar and Yabuno, 1977), sugar beet is

tolerant during later growth stages but is sensitive during germination (Beatty and Ehlig, 1993), and corn is tolerant at germination but is more sensitive at seedling growth than for ear and grain yield (Maas *et al.*, 1983). In cotton, flowering occurs earlier under salt stress, but salinity delays flowering of tomato (Pasternak *et al.*, 1979).

1.3.1 Measurement of salt tolerance

Maas and Hoffman (1977) reported that the growth response of a plant species to increasing salinity could be in terms of a growth curve (Fig. 1.1). They suggested that the comparisons were easily made between species if growth was expressed as relative yield (i.e. yield as a percentage of what it would be with zero salt). The response of relative yield can be defined in terms of the 'threshold' and the slope of the relative yield response to salinities higher than the threshold. In Fig. 1.1, the relationship between the yield of cotton and soil salinity (Ec_e dS m^{-1}) shows that cotton has threshold of 7.7 dS m^{-1}, and a slope of 5.2% per dS m^{-1}.

Using this kind of analysis, Maas and Hoffman defined categories of relative yield response curve (Fig. 1.2). Based on their relative yield response curves, plant species were categorised as being 'sensitive', 'moderately sensitive', 'moderately tolerant' or 'tolerant' to salinity. Fig. 1.2 shows that the curve for cotton (reproduced from Fig. 1.1) actually falls into the 'tolerant' region of Fig. 1.2. Cotton was therefore classified by Maas and Hoffman as being 'tolerant' to salinity.

If a more accurate of the response to salinity is needed some non-linear models can be used (see Van Genuchten and Hoffman, 1984). For example, one of the models proposed by Van Genuchten (1983) takes the form:

$$Y_r = \frac{YM}{[1+ (EC/EC_{50})^P]}$$

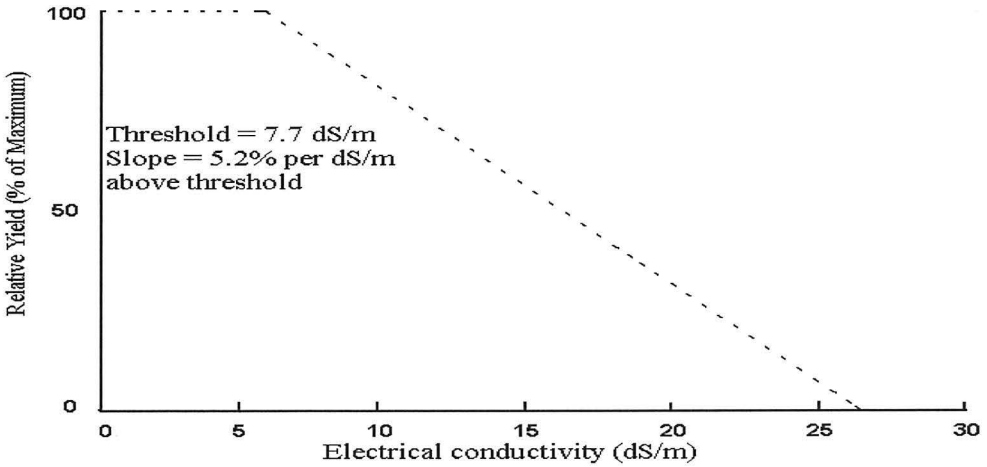

Fig.1.1. Response of the relative yield of cotton to increasing soil salinity (Maas and Hoffman, 1977)

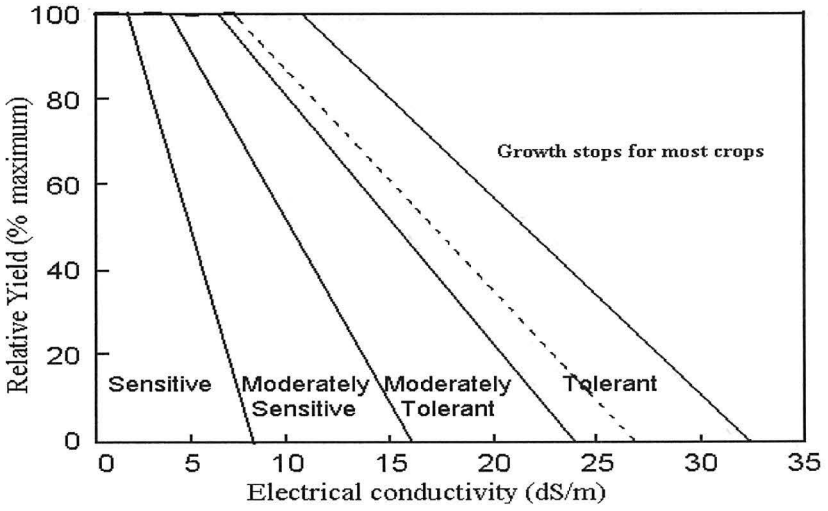

Fig. 1.2. Divisions for classifying crop tolerance to salinity (Ec_e) along with the relative yield response for cotton (dotted line from Fig. 1.1 Maas and Hoffman, 1977)

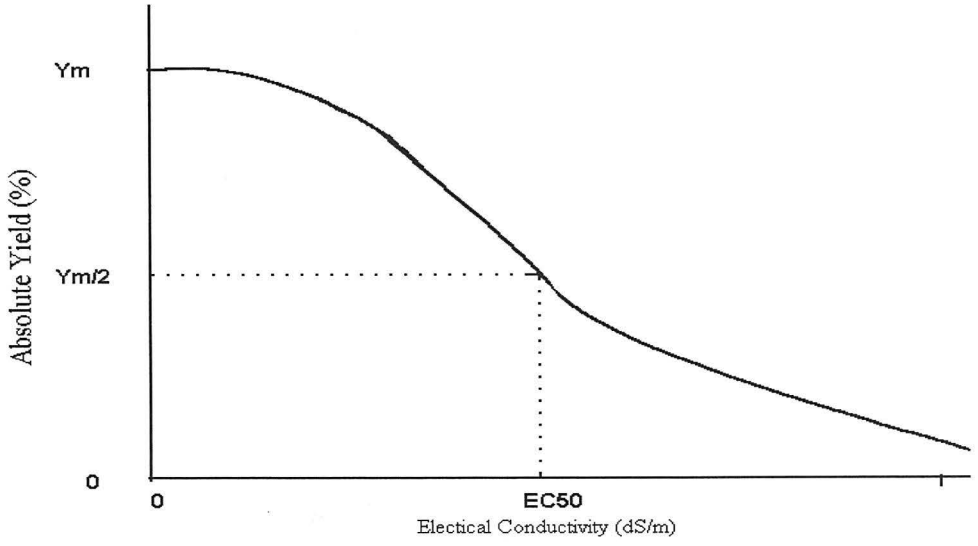

Fig. 1.3. Sigmoidal model (Van Gencuhten, 1983)

Where EC is the soil salinity (Ec_e), EC_{50} is the soil salinity that reduces yield (Y_m) by 50%, and P is an empirical constant related to the slope of the curve. The graph model can be seen in Fig 1.3.

In all these models, and even in the definition of salt tolerance, there is implicit the idea that a plant can withstand a certain amount of salt without adverse effects. This is generally true, although if not enough data are available at low salinities (below the threshold), a simple linear regression may sometimes give a better fit.

1.3.2 Salt tolerance of root system

It is well known that tolerance to salt applied as salt spray or in the soil are different characteristics. Plants are most often exposed to salinity through their root system. Plant roots have two important roles, firstly for providing water and secondly supplying nutrients from the soil. The roots and shoots of the plants are separate in their function, but mutually dependent. Thus the shoot receives inorganic nutrients from the root, and metabolites from the shoot are translocated to the root (Kannan, 1986). It is well documented that the large heterogeneity, both spatial and temporal, of salinity in soil precludes the use of natural saline soils for testing crop tolerance (Shannon and Nobel, 1990). Soil conditions also influence the apparent salt tolerance of many crops. Plants grown on fertile soils may seem relatively more salt tolerant than those grown with inadequate fertility because fertility, not salinity, is the primary factor limiting growth. Water stress is also an important factor under conditions of soil salinity. As plants extract water from the soil, the remaining soil water becomes more concentrated, consequently, plants experience increased salt stress as well as water stress when the soil water is depleted (Maas, 1987). He reported that bean yield is inhibited almost entirely at 50 mol m^{-3} NaCl.

1.3.3 Salt tolerance of foliage system

When plants are irrigated with saline water, they initially experience salinity problems when the roots encounter excess salts in the soil water. This is

not the case with sprinkler irrigation when foliage is wetted by saline irrigation waters. The tolerance of crops to soil salinity may not be the same as their tolerance to saline sprinkling waters (Maas *et al.*, 1982). The inorganic nutrient elements given in the form of aqueous sprays have to be first absorbed by the leaf before translocation to other parts. The absorption of these nutrients by the leaf is a multistep process, involving surface absorption, passive penetration through the cuticle, and active absorption by the leaf cells beneath the cuticle (Kannan and Wittwer, 1967). Many crops accumulate salts through the leaves when they are wetted by saline waters. This accumulation may cause foliar injury and decrease crop yield (Maas *et al.*, 1982). Leaf damage by high nutrient concentrations is a serious problem encountered in the foliar application of mineral nutrients. The damage is mainly the result of local nutrient imbalance in the leaf tissue rather than osmotic effects (Marschner, 1995). Generally, sensitivity to foliar injury by sprinkling with saline water depends more on leaf surface properties (foliar uptake through epidermis) than on crop tolerance to salinity (Maas, 1985). Leaves of fruit tress (almonds, apricots, plums, etc.) absorb Na^+ and Cl^- readily and are more severely damaged. Avocado and strawberry, which are very sensitive to soil salinity, absorb salt so slowly into the leaves that foliar absorption is a negligible factor in sprinkling (see Maas, 1987).

1.3.4 Salt tolerance in cotton

Cotton is regarded as one of the salt tolerant crop species (Maas, 1984). There are, however, conflicting statements about the effects of salinity on cotton. Dean (1981) found that irrigation using brackish water, containing 6000 ppm salt, gave an adequate yield without effects on fibre or seed quality. On the other hand, Razzouk and Whittington (1991) reported that cotton yield was lowered by 86 mol m^{-3} NaCl. Thomas (1980) confirmed the negative relationship between salt and cotton growth, stating that this was due to an osmotically induced water-deficit and imbalance in nutritional status. In most cases increasing salinity reduces vegetative growth of cotton, but there are instances of increased yield by low concentrations of salt (Gorham 1995a, Pessarakli, 1995). Other

authors have also noted the effect of salt on the growth of cotton (Ahmad and Abdullah, 1982 and El-Sharkawi *et al.*, 1986).

It is reported that the rate of cell production in cotton roots was affected by treatment of high concentrations of NaCl and $CaCl_2$ (Kurth *et al.*, 1986). Na^+ also inhibits K^+ and Ca^{2+} uptake in salt-stressed cotton roots (Cramer *et al.*, 1987). Brugnoli and Björkman (1992) reported that at moderate salinity and with adequate nutrition, the growth of cotton was not affected, but at higher levels of salinity it was affected. Leaf senescence, leaf shedding and increasing leaf thickness were also observed. Gossett *et al.* (1994) found that at higher salinities, plant height, leaf expansion and development of nodes of cotton plants were suppressed.

1.4 Physiological effects of salt on plants

Plant response to salinity is one of the most widely researched subjects in plant physiology. The adverse effects of salinity on plant growth have been broadly characterised as osmotic effects (water deficit), specific ion effects or toxicity and ion imbalance or induced nutrient deficiency.

1.4.1 Osmotic effects

Salts present in the soil solution reduce the availability of water to plants and thus become responsible for the so-called 'physiological drought' due to low water potential. When water is removed from the soil by transpiration or evaporation, the salt concentration of the soil solution in the root zone rises, thereby osmotic potential of the soil water decreases and toxic ion concentration increases. This decrease of osmotic potential of the medium causes the adverse salinity effect on the plant growth. To avoid this, the intracellular water potential must be decreased by increasing the osmotic pressure of the cell sap (Greenway and Munns, 1980). Their view is supported by Gorham (1992) who reported that the immediate problem faced by a plant in a saline soil is the osmotic effect. He suggested that a salt-tolerant plant must be able to adjust its internal osmotic potential, by increasing its internal salt content, to overcome this problem. Wyn

Jones (1985) also suggested that any plant cell must adjust osmotically to a hyperosmotic external medium or it will rapidly lose turgor pressure and ultimately suffer a lethal dehydration. Blum and Johnson (1992) reported that under extreme conditions of salt stress not only may roots fail to absorb water from the soil but, as observed in wheat, a reverse situation may arise, i.e. roots may lose their water to the soil.

Plant cells suddenly exposed to salinity must experience initially some decline in turgor because of the low water potential of the root medium. To avoid dehydration and regain turgor, the cell must increase its internal solute concentration sufficiently to lower its osmotic potential below that of the medium. This maintenance of cell turgor by an increase in cell solutes to compensate for the external osmotic stress is called osmotic adjustment (Levitt, 1980). Munns (1988) defined osmotic adjustment as a net increase in the quantity of osmotically active solutes, may be regulated as an adaptive process for the survival of plant tissues exposed to salinity stress.

There are two factors that control the flux of water between a plant cell and the environment, 1) the hydraulic conductance of the plant tissue (a measure of the resistance to water flow), and 2) the difference of water potential between the cell and the medium (the driving force for water movement).

The water potential of a cell can be defined as: $\Psi = P-\pi$

where P is the turgor pressure and π is the osmotic pressure (Nobel, 1983).

1.4.2 Specific ion effects

The effects of specific salts on plant growth is an extremely complex subject involving many fundamental principles of plant nutrition. Plant growth is adversely affected when specific ion concentrations exceed their thresholds and become toxic. Specific toxicities could arise from the high concentrations of ions such as Na^+, Cl^-, SO_4^{2-} and Mg^{2+} etc. Salt injury arises due to excessive uptake of salts by plants. Grattan and Maas (1984) found that salinity caused leaf injury to soybean plants. In non-halophytes, the reasons for toxicity under saline conditions have been assigned to 'ion excess' (Greenway and Munns, 1980) which affects

membrane permeability or enzyme activity. Salt damage to non-halophytes is due to mainly Na^+ and Cl^- accumulation in the plant tissues and/or water deficiency in the plant (Silberbush and Lips, 1991). Shannon *et al.* (1994) reported that specific major ions associated with high salinity may also damage metabolic processes directly. For instance, high Na^+ concentrations relative to other salts disrupt root permeability to ions by displacing Ca^{2+} in the plasma membrane. NaCl suppressed NH_4^+ and NO_3^- uptake by cotton and interfered with nitrogen metabolism (Pessarakli and Tucker, 1985). Na^+ interacts with uptake of Ca^{2+} and K^+, while Cl^- interferes with uptake of NO_3^- (Kafkafi *et al.*, 1982). According to Lewis *et al.* (1989), ionic effects include interference with nitrogen uptake, dislocation of nitrogen assimilation and protein assembly, interference with the transport of essential ions within the plant and a lowering of net photosynthetic rates in the salt affected plants.

1.4.3 Nutrient imbalance

Salts reduce plant growth by interfering with nutrient uptake. High ionic imbalances could be caused by the high levels of Na^+, Cl^-, SO_4^{2-} and Mg^{2+} on K^+ uptake. Excess Na^+ or high Cl^- could also influence NO_3^- or $H_2PO_4^-$ uptake and utilization (Wyn Jones, 1985). According to Mikkelsen *et al.* (1988), the yields of alfalfa shoots and roots were reduced by addition of salinity either as Cl^- or SO_4^{2-} salts. Inhibition of ion uptake by salinity has also been reported for Ca^{2+} (Cramer *et al.*, 1985) and NO_3^- (Klobus *et al.*, 1988). They suggest that NaCl directly affects transport of ion across the plasmalemma of root cells. High concentration of Na^+ and Cl^- can cause ionic imbalance within the plant as well as contribute to its osmotic adjustment (Rather,1983). Ion imbalances in plants can occur when high concentrations of Na^+ in the soil reduce the amounts of available K^+, Mg^{2+} and Ca^{2+} (Epstein, 1972) or when Na^+ displaces membrane-bound Ca^{2+} and alters K^+ and Ca^{2+} transport in cotton roots (Cramer *et al.*, 1985).

1.5 Responses to salinity

1.5.1 Effects of salt on germination

Saline irrigation water may affect germination and emergence either by increasing the osmotic pressure of the soil solution which will retard or prevent the intake of water or by causing toxicity to the embryo and seedling. Salinity reduced final emergence and delayed seedling emergence of citrus (Zekri, 1993). Salinity generally delays and reduces the percentage germination of cotton seeds (Ahmad and Makhdum, 1992, Kent and Läuchli, 1985 and Varghese *et al.*, 1995). Two processes of importance in the establishment of seedlings in a saline environment are cell elongation and maintenance of balanced nutrient ion uptake, both of which require Ca^{2+} (Mengel and Kirkby, 1982). The soil microenvironment in which a seed is expected to germinate and become established as a seedling is likely to have a higher salt concentration than the bulk of the soil because of evaporation and capillary rise of saline water to the soil surface (Pasternak *et al.*, 1979).

1.5.2 Effects of salt on plant growth

There is a reduction in growth of many crop plants when they are exposed to saline stress. Munns and Termaat (1986) suggested that the reduction in growth is a consequence of several physiological responses including modifications of ion balance, water status, mineral nutrition, stomatal behaviour, photosynthetic machinery, carbon allocation and utilization. Growth of most salt-sensitive glycophytes is severely affected by even 50 mol m^{-3} NaCl (Gorham, 1995a). In cotton 50% growth reduction occurs at about EC_e 17 dS m^{-1} [about 180 mol m^{-3} NaCl] (Mass and Hoffman, 1977). Flowering and fruit set of tomatoes were adversely affected by NaCl stress. In tomatoes, reduction of flower number was 44% relative to the control plants, and fresh fruit yield decreased by 78% when plants received 50 mol m^{-3} NaCl (Satti *et al.*, 1994). Salinity affects the growth and production of cotton by raising the osmotic pressure of the soil solution and thus reducing the uptake of water by the roots (Lashin and Atanasiu, 1972, Levitt, 1980, Rathert, 1982).

Munns (1993) reported that the growth of plants is limited by a reduced turgor, reduced photosynthesis or the changed rate of another important metabolic process under saline conditions. She reported that there is no evidence that stomatal conductance or cell expansion is regulated by turgor, or that osmotic adjustment has any direct effect on these processes. Furthermore, turgor of salt-affected plants is not always reduced. Turgor is sometimes lower in expanded leaves of salt-affected plants than controls (Thiel *et al.*, 1988), but often turgor is similar to controls (see Yang *et al.*, 1990). Termaat *et al.* (1985) reported that turgor does not control growth of plants growing in saline soils. Cerdá *et al.* (1995) reported that the maintenance of internal positive turgor potential of plants exposed to saline conditions is an important factor for maintaining growth.

Reductions in the rate of cell expansion can result from changes in turgor and/or wall extensibility. If excess ions do not accumulate inside the cell (with proper compartmentation), they may remain in the cell wall (apoplast). Ion concentrations in the apoplast would rise very fast (because of the small volume of this compartment) and become unbalanced with the rest of the cell. In this situation, the cell wall would tend to extract water from inside the cell causing loss of turgor and dehydration. This may happen in some halophytes, e.g. *Suaeda maritima* (Clipson *et al.*, 1985). Neumann (1993) found that primary leaf elongation in salinized (100 mol m^{-3} NaCl) maize seedlings was associated with reduction in cell wall extensibility. He further reported that reduction was not caused by hydraulic or turgor differences between the growing tissues. He concluded that reductions in the extensibility of expanding cell walls make an important contribution to the inhibition of leaf growth by salinity stress. Soil salinity influences both chemical and physical properties of the cell wall. For instance, high sodium concentration relative to other salts can disrupt root permeability to ions by displacing calcium in the plasma membrane. Secondary effects may be caused by upsetting calcium metabolism and uptake of essential nutrients such as potassium (Läuchli and Epstein, 1990).

Munns (1993) reported that excessive accumulation of salts into cells directly affects the production of a particular metabolite that directly affects

growth. Her idea is hard to prove or disprove, since it is unclear what metabolite this would be, what enzyme would be controlling its synthesis, or whether it would be located in growing or fully expanding tissues. Munns and Termaat (1986) suggested that the effects of salinity might arise from osmotic effects in roots, via a messenger that regulates metabolic processes in the growing leaves. That messenger would probably be a hormone or growth regulators (gibberellic acid, abscisic acid, cytokinins or ethylene).

Gorham and Wyn Jones (1993) reported that there are two major causes of salt damage in plants, the first of which is decrease in soil water potential. This is important when a stress is imposed suddenly and the second cause is the toxicity of the salts entering the plant. Primarily this is due to high concentrations of Na^+ and Cl^-, but a secondary effect is the suppression of uptake of essential nutrients such as K^+. Shalhevet (1993) also reported that under water stress conditions, cell dehydration is the primary cause of the growth-damaging processes. However, both factors affect growth. The question is whether the growth reduction in saline conditions is due more to osmotic stress or to salt toxicity.

In very salt-sensitive species the growth response to salinity is obviously associated with the salt's toxic effect within the plant. Salt-sensitive plants have a poor ability to exclude salt from the transpiration stream. In lupin, for example, salt quickly accumulates in the leaves, causing injury and abscission within a few days of exposure of 100 mol m^{-3} NaCl (Munns, 1988). In that case, the salt within the plant appears to affect growth as quickly as the salt outside the plant. However, salt-tolerant plants can grow for some time without visible injury, but at a reduced rate. Barley for instance, can grow for weeks in 100 mol m^{-3} NaCl with little visible injury. In contrast to lupin, barley efficiently excludes salts: the transpiration stream contains only 5 mol m^{-3} NaCl in plants growing in 100 mol m^{-3} NaCl, and the concentration increases little with higher salinity (Munns, 1985). She reported that it is not clear in barley how much growth reduction is due to salt outside the roots, i.e. to the osmotic effect of the salt, and how much is due to the effect of salt within the plant i.e. to ion excess.

In order to confirm this two-phase hypothesis, Munns *et al.* (1995) have tested by using 17 cereals cultivars known to differ in salt tolerance, and measured growth rate for up to 7 weeks of exposure to 250 mol m^{-3} NaCl in increments over 10 days. They found that in the first phase there was a large decrease in growth caused by the salt outside the roots, i.e. an osmotic response. In the second phase there was an additional decline in growth caused by salt having built up to toxic levels. From the results of their experiment, they strongly support the hypothesis that the growth response to salinity has two phases, and indicate that most changes in metabolism or gene expression leading to growth reduction during the first phase relate to the osmotic effect of salinity, not to any salt specific effect. They also indicate that the salt within the plant reduces growth by causing premature senescence of old leaves and hence a reduced supply of assimilates to the growing regions.

1.5.3 Effects of salt on photosynthesis

Growth is limited by the rate of photosynthesis under saline conditions. This view is based on the observations that photosynthesis in salt-affected plants is reduced (Ungar, 1991 and Yeo *et al.*, 1991). This inhibition may be either directly through stomatal closures or as a result of non-stomatal inhibition of photosynthesis (Khan *et al.*, 1994b). However, some reports (Kaiser *et al.*, 1983 and Lewis *et al.*, 1989) show little or no effect of salinity on photosynthesis, whereas in the same conditions plant growth was greatly reduced. Seemann and Critchley (1985) found that in *Phaseolus vulgaris* the photosynthetic rate was reduced with increasing leaf chloride concentration. Salinity stress imposes additional energy requirements on plant cells (e.g. for osmotic synthesis and ion extrusion), moreover, salinity stress also results in the diversion of metabolic carbon to storage pools (Cheeseman, 1988). Photosynthetic rate, as expressed on a unit leaf basis, declined when plants were subjected to increasing levels of NaCl (Cordovilla *et al.*, 1995, Khan *et al.*, 1994b and Wyn Jones and Gorham, 1989). Stomatal conductance is significantly decreased even under mild salt stress (Robinson *et al.*, 1983). A simultaneous determination of water vapour

conductance and CO_2 fixation by intact cowpea indicated that stomatal conductance was much more sensitive than the capacity of CO_2 fixation to salinity (Plaut and Littan, 1974).

Leaf expansion is the most sensitive physiological process under saline conditions. Salinity causes early leaf senescence and sometimes leaf death. Reduced vegetative growth in saline conditions is mainly observed as reduced leaf area, whereas root expansion is much less affected (Munns and Sharp, 1993). If the root water potential decreases suddenly, the response of leaf expansion is so rapid and large that it must be due to a change in the rate of expansion of existing cells, rather than to a change in the rate of production of new cells (Cramer and Bowman, 1991). However, when plants have grown for some time in soils of low water potential, smaller leaves with fewer cells are formed (Lecoeur *et al.*, 1995). Salinity directly affects rate of cell division, to a slower rate of expansion, or a decrease in the duration of expansion. If cell division affected, even if cell growth potential does not effect, final leaf size will be limited due to reduced cell number (Volkmar *et al.*, 1998). These observations suggest that reduced cell formation during water stress may limit leaf size.

1.5.4 Effects of salt on potassium uptake

Salinity can interfere with root uptake capacity for essential ions such as K^+ and NO_3^- (Criddle *et al.*, 1989). Kochian *et al.* (1985) also reported that NaCl inhibited the linear component for K^+ uptake, while saturable uptake was unchanged. Also, excess of Na^+ in the root media may result in a passive accumulation of this ion in root and shoot, and a high Na^+/K^+ ratio will lead to metabolic disorders such as reduction in protein synthesis and enzymatic activities (Brady *et al.*, 1984; Runge, 1983). K^+ deficiency can be induced under saline conditions because high concentration of Na^+ can interfere with K^+ uptake (Chow *et al.*, 1990). Zidan *et al.* (1992) reported that K^+ concentrations were decreased in maize by salinization. They suggested that an alternative explanation is that rates of ion delivery from the root may have been internally regulated to match a decreased demand for ions in growth inhibited leaves. Nakamura *et al.* (1990)

suggest that maintenance of adequate K^+ concentration and K^+/Na^+ ratios in the cells is essential for normal cellular function under saline conditions.

1.6 Responses to ionic and toxic effects

1.6.1 Ion exclusion versus ion inclusion

Plants seem to depend upon physiological, morphological and anatomical mechanisms, which enable them to survive in saline habitats. Among the physiological mechanisms, two contrasting types of salt tolerance can be recognised (Munns *et al.*, 1983). These are 1) substantial salt uptake, accompanied by efficient compartmentation of salts into large vacuoles and 2) salt exclusion from the shoots, with accumulation of sugars to reduce leaf water potentials (Gorham and Wyn Jones, 1993). If the plant is growing in saline soil then the amount of salt present in the transpiration stream must be severely limited if an excess of salt is not to accumulate in the leaves. If the plant did not take up water from the soil at the same rate that it was lost through transpiration stream, it would rapidly dehydrate (Gorham, 1992a). He further suggested that there are two solutions to the problem: strict control of the influx of salt (salt exclusion) and high volume growth (succulence) to accommodate the salt that enters the plant (salt inclusion). The latter cannot, however, operate in the absence of the former, and the terms 'includer' and 'excluder' are misleading as all plants are excluders, but there are differences in the extent to which exclusion occurs.

Halophytes have increased growth at low salt concentrations (compared to no salt), with decreased growth at much higher concentrations. River saltbush *Atriplex amnicola* has a 10% increase in growth at salinities 5 dS m^{-1}, a 50% decrease in growth at 40 dS m^{-1}, and is still alive at 75 dS m^{-1}. Other plants in this group include: *A. lentiformis*, *Suaeda fruticosa* and *Salicormia bigelovii* (Qureshi and Barrett-Lennard, 1998). The most salt-tolerant higher plants include succulent members of the Chenopodiaceae such as *Salicornia*, *Suaeda* and *Atriplex spp*. Other halophytes are the tropical trees and shrubs known as mangroves, some of which survive in seawater levels (Gorham, 1995a).

In glycophytes, salinity tolerance is related to exclusion of Na^+ and Cl^- from plant shoots (Greenway and Munns, 1980, Gorham *et al.*, 1990, Munns, 1990 and Qureshi *et al.*, 1990). But salt exclusion may increase water stress while on the other hand accumulation of salt is helpful for osmotic adjustment but may cause nutrient imbalance (Wyn Jones, 1981) and cause the leaf injury (Munns and Termaat, 1986). Cramer *et al.* (1994) suggested that Na^+ exclusion has often been implicated as one of the mechanism of salt tolerance in non-halophytes. Some tolerance to salinity has been reported as the result of the enhanced ability of particular genotypes to actively or passively exclude ions (Shannon *et al.*, 1994). Schubert and Läuchli (1990) attributed high salt tolerance in a maize genotype to the ability of the plasmalemma in the root epidermis and cortex to passively exclude Na^+ more effectively than the sensitive genotypes. In glycophytes, the inability of the leaves to utilize the salt transported from the root with delivery leads to a slow leaf growth rate and eventually leaf death. There is evidence linking exclusion of salt from the leaf with salt tolerance (Volkmar *et al.*, 1998). This is true for wheat and barley (Gorham, 1993), corn (Alberico and Cramer, 1993) and beans (Awada *et al.*, 1995). Generally, glycophytes exclude salts or sequester salts within roots and stems. Thus, osmotic adjustment is more dependent on organic solute synthesis. Under salinity stress, the dry weight to fresh weight ratio of many glycophytes will increase as a result of osmotic adjustment (Shannon *et al.*, 1994).

1.6.2 Cellular ion compartmentation

Plant cells can be divided into three major compartments, cell wall, cytoplasm and vacuole (Hsaio and Läuchli, 1986). Flowers and Yeo (1992) suggested that the plant cell wall is a tough barrier modulating the access of external solutes to the plasma membrane, because the cell wall has a large ion-exchange capacity and because it is a substantial unstirred layer, it intervenes both chemically and physically between the outside solution and the surface of the plasma membrane. In plants the plasma membrane is usually pressed tightly against the cell wall by positive hydrostatic pressure.

In cytoplasm, a 'metabolic pool' is assumed to be located in which solutes are metabolised rapidly and in the vacuole, a 'storage pool' which is only slowly utilized (Leigh and Storey, 1991). A large (sometimes 90% or more) proportion of the volume of a plant cell is the internal vacuole. As well as providing size and shape and being a store for resources, the vacuole can also store toxic material or waste products (Yeo, 1998). The compartmentation of ions between vacuole and cytoplasm is an important determinant of the response of plants to salinity. Leigh and Storey (1991) suggest that the enzymes and salt are separated within the cells and it is proposed that the NaCl is compartmented in the vacuole where it has no effect on the activity of salt-sensitive enzymes which are located in the cytoplasm where a relatively constant ionic environment dominated by K^+ is maintained. When salt loads in the vacuole are high it is proposed that osmotic balance between the cytoplasm and vacuole is maintained by the accumulation of compatible solutes, such as glycinebetaine and proline, in the cytoplasm (Wyn Jones *et al.*, 1977). Gorham *et al.* (1995a) suggested that K^+ is almost as inhibitory to enzyme activity as Na^+ at high concentrations, it was further suggested that, at high salinities, the osmotic pressure of the cytoplasm was maintained by the accumulation of organic solutes that were compatible with enzyme activity.

In particular, studies with the technique of X-ray microanalysis have shown that the cytoplasm of meristematic cells is rich in K^+ even in conditions where the vacuole of mature cells is loaded with NaCl (e.g. Storey *et al.*, 1983, Gorham and Wyn Jones, 1983). However, some studies have shown that Na^+ can be accumulated to relatively high concentrations in the cytoplasm of mature halophyte cells (Harvey *et al.*, 1981). There is a general acceptance that mechanisms operate to prevent excessive salt loads in the cytoplasm and allow the accumulation in the cytoplasm only of those ions and solutes that will be not adversely affect the operation of metabolism. In the vacuole, Na^+ is compartmentalized in the preference to K^+, resulting in the maintenance of a high K^+/Na^+ ratio in the cytosol of shoot cells (Gorham *et al.*, 1985). Most investigations on ion compartmentation have concentrated on Na^+, Cl^- and the

major cations (essentially K^+ and to a lower extent Ca^{2+}) (Yeo, 1983). Flowers and Läuchli (1983) reported that K^+ is preferentially concentrated in the symplasm while Na^+ ions are largely but not exclusively occluded in vacuoles.

A model of ion compartmentation is shown in Fig. 1.4 (see Gorham 1995a). The ions are unequally distributed between different compartments within plant cells and this intracellular compartmentation is important in the responses of plant to excesses or deficiencies of nutrients (Leigh and Wyn Jones, 1986). For example, salinity tolerance is related, in part, to the ability of plants to compartment NaCl in the vacuole thus protecting salt-sensitive enzymes in the cytoplasm (Wyn Jones and Pollard, 1983).

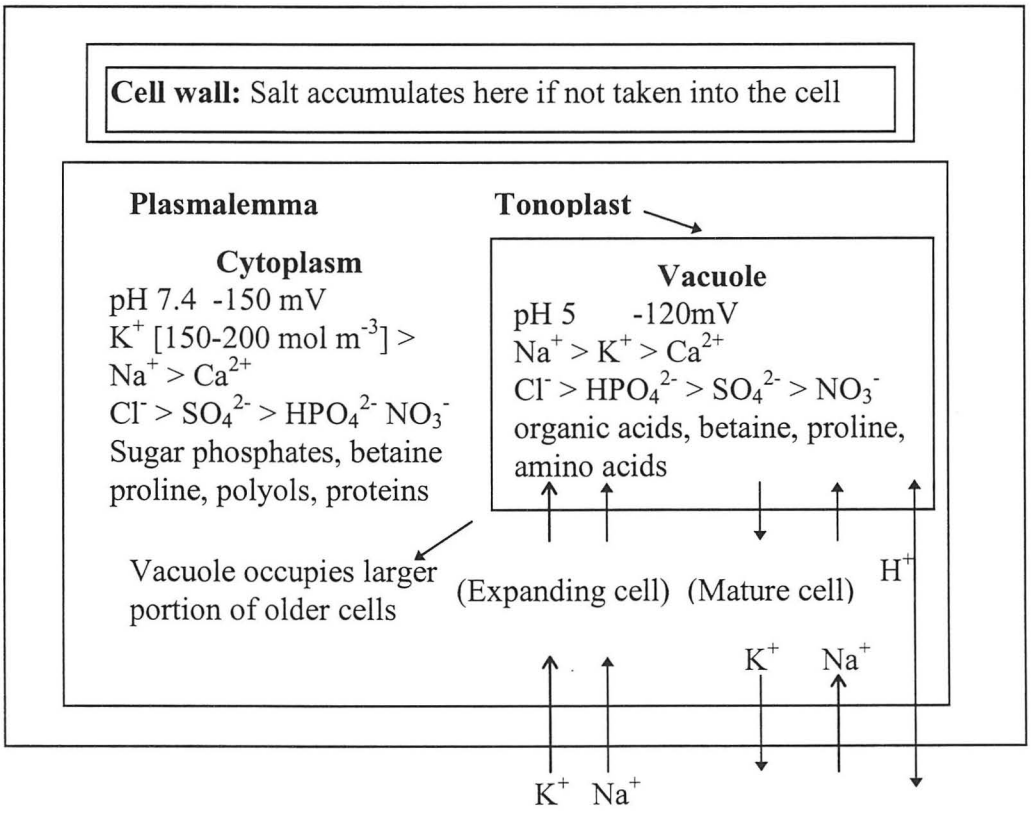

Fig 1.4 Intracellular compartmentation of solutes in plants subjected to salinity. In an expanding cell there are net influxes of Na^+ and K^+; in a mature cell there is a net exchange of Na^+ for K^+, and the K^+ is available for recirculation to sink tissues. Na^+ and Cl^- are mainly accumulated in the vacuole, the size of which increases with cell age. Compatible organic solutes (proline, glycinebetaine, polyols etc.) are thought to accumulate in the cytoplasm to maintain its osmotic pressure and volume. If the salt delivered to the cell is not taken up into the symplasm it may accumulate in the cell walls and intercellular space, where it may compete with solutes within the cell for water and reduce the turgor pressure (From: Gorham, 1995a).

Flowers and Yeo (1986) noted that salt damage in leaves of sensitive species may be the result of excess apoplastic ion concentration or ion toxicity effects on metabolic processes in the symplast. Speer and Kaiser (1994) suggest that the development of damage symptoms begins with wilting of leaflet margins. It seems possible that salt accumulates preferentially in the apoplast to cause protoplast dehydration.

1.6.2.1 Intercellular

There are relatively few detailed studies of the distribution of ions in between cells. Generally it is accepted that the compartmentation of nutrients and other solutes between vacuole and cytoplasm of higher-plant cell is important in the response of plants to change in nutrient availability and to their ability to withstand stresses such as salinity (Leigh and Wyn Jones, 1986). However, it is now becoming clear that solutes are also differentially distributed between different cell types in leaves and these intercellular solute distributions may also respond to changes in nutrient availability (Leigh and Tomos, 1993).

Leigh and Storey (1993) suggest that the distribution of nutrients between epidermal and mesophyll cells of barley leaves show that there is strict compartmentation of P in the mesophyll, and of Cl^- and Ca^{2+} in the epidermis while in leaves of salt-stressed barley, Na^+ and Cl^- are differentially distributed between the mesophyll and epidermis. Dietz *et al.* (1992) reported that when barley plants were grown on a standard nutrient solution, epidermal protoplasts contained 2.8 mol m^{-3} P compared with a concentration of 76 mol m^{-3} in mesophyll protoplasts. In contrast, Cl^- was present at 23 mol m^{-3} in mesophyll protoplasts from the same plants, but at 62 mol m^{-3} in epidermal protoplasts. Leigh and Storey (1993) suggest that the accumulation of Na^+ in mesophyll is not necessarily deleterious for growth.

Fricke *et al.* (1994) reported that in plants subjected to various levels of salinity (up to 200 mol m^{-3} NaCl), epidermal concentrations of Cl^- always exceeded those of the bulk extract, while Na^+ concentrations were similar. Epidermal cells osmotically adjusted to the increase in the external salt

concentration. Dietz *et al.* (1992a) found that in barley leaves, epidermal cells occupy about 27% of the total leaf symplastic volume and contain a large central vacuole. This renders the epidermis suitable for the accumulation, storage and release of leaf solutes during periods of changing nutrient availability and external salinity. For example, Leigh and Storey (1993) observed for NaCl grown barley that leaf Cl^- was preferentially accumulated in the epidermis, while in K^+-deficient plants, K^+ levels in the mesophyll were maintained at the expense of epidermal K^+.

From the studies of X-ray techniques, Yeo (1981) found that leaf solutes are compartmentalized not only at the subcellular level (i.e. between vacuole and cytoplasm), but also at the intercellular level (i.e. between the epidermis and the remaining leaf symplast). Recently modified techniques have been developed by Tomos *et al.* (1994) that allow the extraction of sap from individual leaf cells of cereals and its analysis for osmolality and inorganic and organic solute concentrations. Using these techniques, it has been shown for barley leaves that the concentrations of leaf solutes may vary significantly between mesophyll, bundle sheath and epidermal cells (Fricke *et al.*, 1994a). They further found that saps extracted from mesophyll cells represented a mixture of both vacuolar and cytoplasmic constituents.

1.6.2.2 Retranslocation of solutes during ageing

A non-uniform distribution of K^+ and Na^+ between leaves of plants in saline environments, with high concentrations of K^+ and low concentrations of Na^+ in young, and the opposite trend for old leaves is usually observed. The pattern of unequal ion partitioning has been attributed to a co-operation of phloem and xylem transport involving preferred retranslocation of K^+ from older to younger leaves (Jeschke and Wolf, 1985) as well as restricted entry of Na^+ into the phloem and retention of Na^+ in mature tissues due to exchange of K^+ for Na^+ in leaf cell vacuoles (Jeschke, 1984). Potassium may be replaced by sodium as the tissue ages, but the contribution of this process to salt exclusion from the shoots

can only be significant in plants exposed to low salinities, or where K^+/Na^+ selectivity is high at the point of entry into the root (Gorham, 1992a).

Potassium is retranslocated from the leaves of plants as the leaf ages (Jeschke and Wolf 1985). Retranslocation of K^+ is not restricted to plants of low K^+ status nor to plants under salinity stress and is sufficient to lead to net decrease in the K^+ contents of leaves of both wheat and barley (Greenway *et al.*, 1965). This decrease shows that retranslocation of K^+ is not solely a phenomenon restricted to transfer of K^+ from the xylem to the phloem, and is consistent with leaf cells functioning as nutrient stores. Salinity may increase the quantity of K^+ exported. Wolf *et al.* (1990, 1991) demonstrated increased losses of K^+ from the oldest leaf and stem of barley grown with 100 mol m^{-3} sodium despite a nutrient K^+ concentration of 6.5 mol m^{-3}. In old leaves, phloem export of K^+ greatly exceeded xylem import whereas sodium export was restricted and sodium was retained within the leaf. It was found that the older leaves towards the base of the plant tended to export K^+ directly towards the apex. The net effect of retranslocation and nutrient cycling was that a good supply of nutrients was available for the growth of expanding tissue, whilst the import of deleterious ions such as sodium was restricted.

1.6.2.3 Recirculation of potassium in plant

Phloem translocation of K^+ is an important component of salinity tolerance of plants (Jeschke and Wolf, 1988). Young leaves are supplied with a K^+- rich inorganic solute supply via the phloem, while Na^+ accumulates in older leaves, often replacing K^+ previously accumulated there. The K^+ in older leaves is thus available for recirculation via the phloem to sink tissue (Gorham *et al.*, 1995a). Retranslocation of K^+ from leaves via the phloem has been observed in the presence of external salt, e.g. in barley (Jeschke and Wolf, 1985), *Atriplex amnicola* (Aslam *et al.*, 1986). High K^+ and low Na^+ concentrations were found for the salt tolerant barley (Munns *et al.*, 1986). In these species phloem loading showed high selectivity for K^+ relative to Na^+ (Wolf and Jeschke, 1987). Jeschke

(1984) suggested that poor recirculation of K^+ may also contribute to insufficient turgor.

1.6.3 Long distance transport

The long distance transport of water and solutes, major and minor nutrients takes place in the vascular system of xylem and phloem. During long distance transport, mineral nutrients and organic solutes are transferred to and from xylem and phloem by exchange processes, referred to as loading and unloading (Marschner, 1995). Ion uptake by a leaf involves long distance transport of both water and ions. Rapidly transpiring leaves are predominantly supplied by xylem transport. In young dividing and expanding tissues there is great demand for solutes for synthesis of essential metabolites, and to maintain turgor. Since transpiration and hence xylem transport is low, these solutes are predominantly delivered by the phloem (Wolf *et al.*, 1991). Ions taken up by the root and transported within the xylem to the shoot have to be transferred to the phloem to meet the requirements of growing leaves (Pate, 1984). Such transfer processes have been proposed in leaves for N compounds (Pate, 1984) and K^+ and Na^+ (Wolf and Jeschke, 1987). Wolterbeek and De Bruin (1986) pointed out that the transfer of nutrients from xylem to phloem is inversely related to the speed of the water volume flow in the xylem. K^+ is quite mobile both in xylem and in the phloem, and a high proportion of the K^+ taken up in root zones is translocated via the phloem toward the root tip, which acts as a sink for this mineral nutrient (Marschner, 1995).

1.6.3.1 Xylem transport

The primary driven force of xylem transport is evapotranspiration. The concentrations of mineral elements and organic solutes in the xylem sap depend on factors such as plant species, mineral element supply to the roots, assimilation of mineral nutrients in the roots and nutrient recycling (Marschner, 1995). With nitrate as the N-source, nitrate is the major inorganic anion in the xylem of *Ricinus* (Peuke and Jeschke, 1995) and this requires cotransport of cations. In addition to

its functions as N-source and negative charge, nitrate may also present an important intracellular osmoticum as pointed out by Smirnoff and Stewart (1985). If the root is a major site of N-assimilation more organic nitrogen than nitrate will be transported in the xylem. The latter is true if the xylem transport of nitrate is inhibited under the conditions of salinity. Allen and Raven (1987) found that in *Ricinus* nitrate was largely transported in the xylem, and the ratio of nitrate to total nitrogen in xylem sap increased with nitrate supply in the nutrient solution. In contrast in the case of *Phaseolus vulgaris*, under ammonium nutrition, very little ammonium was found in the xylem sap, indicating that most of it was retained and assimilated in the root (Allen *et al.*, 1988). Both inorganic N-forms were found in *Ricinus* to be low in phloem (Peuke *et al.*, 1996).

1.6.3.2 Phloem transport

Phloem transport is an important process for cycling of mineral nutrients between shoots and roots. The phloem is the means by which the products of photosynthesis are transported to non-photosynthetic parts of the plant, such as the roots, and to developing leaves, fruits and seeds (Baker and Milburn, 1989). Jeschke *et al.* (1986) reported that phloem mobility of Na^+ and Cl^- and the ability of phloem loading mechanisms to discriminate between K^+ and Na^+ are issues in understanding how species accommodate and redistribute salt which has already entered the shoots from the xylem. High K^+ and low Na^+ concentrations were found in salt-tolerant barley (Munns *et al.*, 1986). In this species phloem loading showed high selectivity for K^+ relative to Na^+ (Wolf and Jeschke, 1987). However, in a highly salt-sensitive species, *Lupinus albus,* the reverse was true, even under conditions of low NaCl (40 mol m^{-3}).

1.6.4 Membrane transport

Membranes are effective barriers to the passage of ions and uncharged molecules. On the other hand, they are also the sites of selectivity and transport against the concentration gradient of solutes (Marschner, 1995). Membranes play a significant role in separating toxic ion concentrations within the plants from the

sensitive metabolic machinery responsible for growth and reproduction (Shannon *et al.*, 1994).

There are two ways for a solute to move from the outside solution into the root. The extracellular (apoplastic) pathway is a free diffusional continuity of the external solution with its properties modified by the fixed negative charges of the cell walls. The intracellular or symplastic, route may be viewed as the cytoplasm of all the living cells in the plant linked by the plasmadesmata (Shannon *et al.*, 1994). The apoplastic and symplastic systems appear to merge, at least for a short distance, at the endodermis with its heavily suberized Casparian strip. However, solutes must, in principal, pass through the endodermal cells as they move from the root cortex to the stele. The Casparian strip would provide a barrier that minimizes free ion influx through the apoplast to the root xylem and finally into the transpirational stream (Shannon *et al.*, 1994).

McKersie and Leshem (1994) suggested that in a higher plant three membrane barriers are especially relevant for the regulation of Na^+ and Cl^- transport: a) the plasmalemma of the root cortical and epidermal cells, where the ions and water actually enter the living plant, b) the tonoplast of the vacuoles in root and shoot, wherein Na^+ and Cl^- can be sequestered, and c) the plasmalemma of the xylem-parenchyma cells that control the division between root and shoot.

1.6.4.1 Ion channels

Channels conduct ions across membranes according to the difference in electrochemical potential. An ion channel is a selective pore in a membrane, formed by a protein, that can be in an open or closed state (Yeo, 1998). Passage of an ion through the channels is driven by the electrochemical gradient of that ion across the membrane. Such transport is often referred to as being passive or downhill. Because of the negative membrane potential and the typically high external Na^+ concentration the electrochemical gradient for Na^+ is in the direction of the cell, i.e. Na^+ enters the cell passively. The opposite is true for Cl^- that is taken up via some active transport system, while it can leave the cell passively (McKersie and Leshem, 1994). They further reported that the precise pathway for

Na^+ entry is still unknown, it is assumed that Na^+ crosses the plasmalemma through ion channels. Until now no Na^+ selective channels have been found in plant cell membranes, but K^+ channels often show a large enough conductance for Na^+ to account for the observed Na^+ influxes. The K^+/Na^+ permeability ratio of a major plasmalemma K^+ channel in wheat was 30 (Schachtman *et al.*, 1991). In other plant cells K^+ channels are found that are far less specific and thus more conductive for Na^+ (Schroeder *et al.*, 1987). In *Vicia faba*, guard cells and corn suspension cell cytoplasmic Ca^{2+} is part of the regulatory system of the K^+ channels (Ketchum and Poole, 1991).

1.7 Nitrogen

Among the many nutrients required for the growth and development of plant cells the ions of inorganic nitrogen are of special importance (Ullrich, 1992). Nitrogen metabolism is directly related to yield potential of crop plants. The evidence available suggests that the metabolism of nitrogen compounds plays a key role in the ability of plants to tolerate salinity (Botella *et al.*, 1993). Garg *et al.* (1993) supported their evidence that improved soil fertility may increase nutrient uptake, particularly that for nitrogen, which may in turn enhance N metabolism which augments the growth and yield despite the salt stress. Limiting N availability during plant growth causes a reduction in the rate of photosynthesis. Additional nitrogen reduced accumulation of Na^+ and increased growth of barley (Shen *et al.*, 1994), alfalfa (Khan *et al.*, 1994a) and Indian mustard (Garg *et al.*, 1993). The two major forms of nitrogen taken up by plants are NO_3^- and NH_4^+ ions, although there are great differences in the utilization of these sources by plants (Pilbeam and Kirkby, 1992).

1.7.1 Ammonium versus nitrate

A fundamental difference between nitrate and ammonium is that one is an anion and the other is a cation. Plants take up both NH_4^+ or NO_3^-, but crop growth and yield are affected by these N sources, and by the NH_4/NO_3 ratio when both ions are present (Feigin, 1990). Lips *et al.* (1990) suggested the actual

assimilation of nitrogen into organic compounds is carried out as ammonium, a product of nitrate reduction. The uptake of NO_3^- needs energy and so does the reduction of NO_3^- to NH_4^+ making nitrate use by the plants far more demanding energetically. According to Cox and Reisenauer (1973), ammonium increases anion uptake, whereas nitrate increases cation concentration in wheat plants. Lewis *et al.* (1989) reported that in maize and wheat, NH_4^+-fed plants show a considerably greater sensitivity to salinity than do NO_3^--fed plants. He suggested the cause of this effect could be that in wheat and maize, nitrate assimilation takes place primarily in the shoot and ammonium assimilation in the root. As the roots are in immediate contact with the saline medium it is possible that nitrogen assimilation in ammonium-fed plants is dislocated by ionic effects that would not interfere with leaf-based nitrogen assimilation. Leidi *et al.* (1992) found that root dry weight in peanut and cotton was more affected by salinity in NH_4^+-fed than NO_3^--fed solution.

1.7.2 Salinity and nitrogen interactions

Nitrogen, a substrate required for cell growth, is severely affected under salt stress (Luque and Bingham, 1981). The interaction between nitrogen and salinity has been studied using several plant species, such as peanut, wheat and maize (Lewis *et al.* 1989, Silberbush and Lips, 1988). NH_4^+ was a suitable nitrogen source under non saline conditions for some species, whereas for other species (e.g. cotton), NH_4^+ caused much less growth than did NO_3^-. Furthermore, detrimental effects of salinity were more pronounced in NH_4^+-fed plants than in NO_3^--fed plants (Lewis *et al.*, 1989). Plants grown under saline conditions absorb excessive amounts of inorganic cations and anions, with frequently Cl^- and Na^+ being the predominant ions absorbed (Martinez and Cerdá, 1989). The presence of Cl^- inhibits the absorption of NO_3^-, while increasing NO_3^- reduces Cl^- concentration in the leaves. (Deane-Drummond, 1986). Under such conditions, the competing effect between Cl^- and NO_3^- uptake has implications for crop production (Kafkafi *et al.*, 1982). Leaf nitrogen concentrations were unaffected or increased by salinity (Bhivare and Nimbolkar, 1984, Seemann and Critchley,

1985). Contradictory results in response to salinity have also been found in cotton. Leaf nitrogen content decreased in tomato (Brugnoli and Björkman, 1992). Total N contents have been reported to increase (Pessarakli and Tucker, 1988), to remain the same (Hernando *et al.*, 1967) or to decrease (Papadopoules *et al.*, 1985). Thus, effects of salt stress on nitrogen contents in the plant are inconsistent and unpredictable.

1.8 Organic solutes

Plants growing in saline media may diminish internal water deficits by the absorption of inorganic ions and the synthesis of organic solutes, both of which contribute to osmotic adjustment (Epstein, 1980). Balancing of osmotic pressure within the cell is a necessary function of plants exposed to high salt. Osmotic adjustment involves both uptake and accumulation of ions and synthesis of organic solutes. Organic compounds are involved in osmotic and ionic balance in a range of organisms. Some of these noninhibitory metabolites may aid osmotic adjustment between the cytoplasm and the vacuole (Venkatesalu *et al.*, 1994). Sugars, proline, glycinebetaine and some other organic solutes are believed to improve salt tolerance by contributing to osmotic balance and preserving enzyme activity in the presence of toxic ions (Greenway and Munns, 1980). Inorganic ions may also be stored in vacuoles. As a consequence non-toxic organic solutes accumulate in the cytoplasm. These solutes are called compatible solutes and may play the role of balancing cytoplasmic and vacuolar water potential (Koheil *et al.*, 1992).

1.8.1 Glycinebetaine

Glycinebetaine is a compound that some plants accumulate as a defence against stress conditions. There is good evidence to suggest that it acts as a non-toxic cytoplasmic osmoticum and plays a central role in adaptation to stress (Yancey *et al.*, 1982). Glycinebetaine is preferentially located in the cytoplasm and chloroplasts of the cells of green leaves of barley (Wyn Jones *et al.*, 1977 and Leigh *et al.*, 1981). It is then translocated to young leaves and roots by phloem

tissues (Robinson and Jones, 1986 and Wyn Jones, 1981). Gorham (1996) has suggested that due to very high concentrations of betaine found in several genera of Malvaceae, it is also located in cell vacuoles. He further suggested that it is a major nitrogen-containing compound in this family. As a cytoplasmic osmoticum, betaine enables the plant to maintain photosynthetic activity in osmotic stress conditions by stabilizing enzymes and maintaining turgor pressure. Betaine is a compatible solute and can carry out its function even at leaf concentrations of up to 500 mol m^{-3} (Borowitzka, 1981). Glycinebetaine is metabolically inert and readily translocated from its site of synthesis in the leaves to other parts of the plant (Hanson and Wyse, 1982). However, it is readily degraded by soil microbes (Wyn Jones *et al.*, 1973). The young actively growing tissues contain much more betaine than mature tissues (Nakamura *et al.*, 1996).

1.8.2 Salinity and betaine interactions

Accumulation of betaine in salinized plants, presumably for osmotic adjustment of the cytoplasm (Matoh *et al.*, 1987), is considered to be an important physiological trait contributing to the maintenance of growth under salinity (Rhodes and Hanson, 1993). Synthesis and accumulation in response to salinity stress are found in many cereals, grasses and other higher plants (more than 20 genera) and have been implicated as an adaptive metabolic response of some plant species (Gorham *et al.*, 1985). In a greenhouse study of drought-stressed tobacco, (a non-betaine accumulating crop), the foliar application of betaine significantly increased leaf area and leaf dry weight (Agboma *et al.*, 1997a). Also, externally supplied betaine has been reported to stimulate the growth of salt-stressed barley embryos (Lone *et al.*,1987) and rice tissue cultures (Kavi-Kishor, 1988).

1.9 Potassium

Potassium is an essential element in plant nutrition with many physiological roles in plant growth. Hsiao and Läuchli (1986) reported that next to N, K^+ is the inorganic nutrient required in the largest amount by plants for metabolic functions and growth. Potassium is the dominant cation and the K^+/Na^+

ratio is relatively high even when the tissue contains a preponderance of Na^+ (Gorham and Wyn Jones, 1983, Storey *et al.*, 1983). K^+ salts of organic acids and sugars are the major solutes of glycophytes (Hellebust, 1976 and Wyn Jones *et al.*, 1979). High level of Na^+ in root tissues often leads to a dramatic drop in K^+ levels in leaves of various glycophytes (Wyn Jones and Storey, 1978). K^+ accumulation is closely related to metabolic activity in the plant and is highly selective. It is highly mobile in plants at all levels, within cells, tissues and in long distance transport via the xylem and phloem (Marschner, 1995). K^+ nutrition is specially important to salt-stressed glycophytes since evidence indicates that metabolic damage results from high cytoplasmic Na^+:K^+ ratios (Wyn Jones *et al.*, 1979) and high Na^+ concentrations may interfere with K^+ ions (Orton, 1980).

In the cytoplasm, K^+ has an important role in providing the correct ionic circumstance for metabolic processes and, in the vacuole, K^+ is involve in the generation of turgor. When K^+ is not available it can be replaced by other cations, i.e. Na^+ and Mg^{2+} (Leigh and Wyn Jones, 1984). When K^+ is with held, its vacuolar concentration decreases and other substances are accumulated to replace it. K^+ can be replaced to varying extents by Na^+, Ca^{2+}, Mg^{2+} or sugars depending upon the treatment (Leigh *et al.*, 1986, Mott and Steward, 1972, Mengel and Arneke, 1982, Pitman *et al.*, 1971). However, the type and mix of vacuolar solutes that may contribute to turgor appears not to be greatly restricted (Mott and Steward, 1972).

It was proposed by Leigh and Wyn Jones (1984, 1986) and Memon *et al.* (1985) that K^+ was maintained constant within the cytoplasm despite changes in its concentration in the vacuole. Growth was predicted to be adversely affected only when the K^+ concentration in the cytoplasm had declined to a critical level that would occur only after the vacuolar K^+ concentration had declined to a critical minimum. Thus the accumulation/retention of K^+ by the cytoplasm would take precedence over the use of K^+ as an osmoticum by the vacuole. Meanwhile, other solutes would accumulate in the vacuole to replace K^+ in its osmotic role.

The effect of Na^+ on K^+, as a result of the antagonism between these two ions, is well documented (Kawasaki *et al.*, 1983, Patil and Patil, 1983).

Sodium and potassium when present in sufficient amounts, are involved in the fairly general antagonism between monovalent (K^+ and Na^+) and divalent (Ca^{2+} and Mg^{2+}) cations, and this affects the cationic balance of the plant (Garcia and Morard, 1976). High Na^+ concentrations may disrupt K^+ and Ca^{2+} transport and interfere with growth of many plant species, cotton (*Gossypium hirsutum* L.) included (Cramer *et al.*, 1987). An increase in K^+ over Na^+, i.e. improved K^+/Na^+ selectivity, appears to be related to salt tolerance in cotton (Kent and Läuchli, 1985). In addition, K^+/Na^+ selectivity may play a role, since metabolism is adversely affected by low K^+/Na^+ ratios (Greenway and Munns, 1980). Plants showing tolerance to salinity stress have developed mechanism to maintain K^+ uptake in the presence of high Na^+. It has also been suggested (Gorham *et al.*, 1991) that all plants discriminate to some extent in favour of K^+ and against Na^+ in ion uptake into the roots and in translocation of absorbed cations to the shoots.

Potassium is essential for photosynthesis and for starch formation and the translocation of sugars. The inhibition of CO_2 assimilation under K^+ deficiency has been attributed to smaller stomatal opening as well as to reduce ability of the photosynthetic activity (Peaslee and Moss, 1966). In the case of cotton grown in solution under a relatively low light level, stomatal resistance was found to remain constant whereas mesophyll resistance increased with decreases in K^+ supply (Longstreth and Nobel, 1980). It is necessary in the development of chlorophyll, although it does not, like magnesium, enter prominently into its molecular structure.

The accumulation of K^+ may be important for osmotic adjustment in leaves of salt-tolerant nonhalophytes (Colmer *et al.*, 1995). Hsiao and Läuchli (1986) reported that K^+ is the major cation in plants, and it contributes significantly to osmotic adjustment in the cytoplasm and turgor generation in the vacuole of the cells, except under conditions of K^+ deficiency. An increase in the K^+ concentration in the guard cells increases their osmotic potential and results in the uptake of water from the adjacent cells and a corresponding increase in turgor in the guard cells and thus stomatal opening (Hsiao and Läuchli, 1986, Marschner, 1995).

The accumulation of K^+ in the vacuoles has to be balanced by a counteranion, mainly Cl^- or malate, depending on the plant species. In plant species that do not use Cl^- as an accompanying anion for K^+ in guard cells, malate serves as the accompanying anion for K^+ (Marschner, 1995). According to the Ben-Zioni *et al.* (1971) model, NO_3^- would migrate from the root to the leaves via the xylem, accompanied by K^+. In the leaves NO_3^- is reduced to amines as a by-product of photosynthesis, which in turn would produce malate. The malate would then migrate back to the roots accompanied by K^+. This model suggests that once Na^+ and Cl^- entered the vascular system of a plant instead of K^+ and NO_3^-, respectively, they should migrate to the leaves and accumulate like NO_3^-, and Na^+ can replace K^+ as a co-ion to malate. Na^+ and Cl^- are therefore, undesirable ions to non-halophytes.

1.10 General objectives of the present work

In the present study an attempt has been made to answer the following questions.

* Does additional nitrogen affect the growth of cotton and leaf sap ion contents under various levels of salinity? (see chapter 3.)
* Is there any difference in vegetative growth of cotton and ion concentrations under saline and non-saline conditions if ammonium or nitrate is supplied as a nitrogen source? (see chapter 3.)
* Does glycinebetaine ameliorate saline stress and enhance the growth of cotton? (see chapter 4.)
* What are the physiological responses of cotton to foliar and soil-applied KCl and NaCl? (see chapter 5.)
* What is the optimum foliar requirement of potassium to ameliorate sodium toxicity in cotton? (see chapter 5.)
* Which is the best accompanying anion of potassium to decrease the effects of NaCl salinity? How are K^+ and Na^+ partitioned between the petiole and the leaf? (see chapter 5.)
* What are the effects of low and high concentrations of NaCl applied to roots and shoots on the net uptake rate and transportation of potassium? (chapter 6.)
* How do NaCl and Na_2SO_4 salinities affect the net uptake rate of potassium when applied to shoots and roots? (see chapter 6.)
* Does cotton shoot transport Na^+ to root when shoots are sprayed with saline water? (see chapter 6)

CHAPTER TWO

GENERAL MATERIALS AND METHODS

CHAPTER 2

GENERAL MATERIALS AND METHODS

This chapter describes the materials used and the procedures followed throughout the thesis. Other methods that were occasionally used are described in the relevant experimental chapters.

2.1 Plant material

Seeds of *Gossypium hirsutum* L., Acala SJ2 were used throughout the research work, and were obtained from Dr. John Gorham, School of Biological Sciences, University of Wales, Bangor.

Acala types of cotton were derived in the early 1900s in USA and their development has been well documented. The initial stock was collected by G.N. Collins and C.B. Doyle in 1906 near the village of Acala, Chiapas, Mexico (Kohel and Lewis, 1984). Selected seeds were then sent to California, where the first commercial planting of Acala was made near Bakersfield in 1919 (Turner, 1974). Acala SJ2 has maintained the high reputation of San Joaquin Valley cotton and also established improved levels of performance for yield, with salt resistance, and earliness (Kohel and Lewis, 1984).

2.2 Seed treatment

Cotton seeds were delinted by the procedure described by Akhtar, (1996). The seeds (20 g) were placed in a 200 cm^3 beaker and concentrated sulphuric acid added dropwise in a ratio of 1 cm^3 acid to 5 g seeds. The acid and seeds were stirred thoroughly with a glass-rod for 3 minutes and left for 5 minutes. They were then transferred to a Buchner funnel where they were washed with running tap water for 3 minutes. The seeds were then placed in 200 cm^3 1% sodium bicarbonate solution for 10 minutes to neutralise remaining acid and washed thoroughly with distilled water. The delinted seeds were soaked for an hour in 1 litre of distilled water. Finally the seeds were washed many times with distilled water and blotted dry with tissue paper.

2.3 Germination of seeds (hydroponic culture)

The method adopted to germinate the seeds has been described by Chachar, (1996). Delinted seeds were placed in 10 rows, with 5 seeds in each row, on a sheet of Whatman No. 1 filter paper (23 x 57 cm). The filter paper was moistened with distilled water. It was rolled up round a wooden stick (32 x 0.5 cm) and placed in a polythene bag (35 x 23 cm). The top of the bag was folded over and tied lightly to maintain sterile conditions and allow air exchange. Finally, the bag and its contents were stood upright in a 500 cm^3 beaker in a way that the roots go down in straight. The beaker was placed in an incubator in the dark at 30 °C. After 48 hours, the seeds that germinated were used for the experiment and ungerminated seeds were discarded.

2.4 Transfer of seedlings to hydroponic culture

Transparent 'Magenta' vessels (Sigma Chemical Co. USA) were used, containing approximately 300 cm^3 of water. The vessels were covered with black pots to prevent light encouraging algal growth in the nutrient solution. A waterproof closed-cell foam sheet was placed on top as a lid of the vessel. The lid was tightened to prevent water loss by evaporation and care was taken that solution being sprayed did not leak into the nutrient solution in the pot. For air supply, a hole was made in the lid and one end of capillary tubing (0.58 mm inner and 0,96 mm outer diameter, Portex Ltd. Hythe Kent, England) was passed through the hole, and the other end was fixed to a needle (23G x 1" Terumo Europe N.V. Belgium). The needle was then connected with a main air supply silicone tube that was further connected to an aquarium air pump (Model Whisper 600). For adding to or draining away the nutrient solution, a hole was made in the lid and a polythene tube (2 mm inner and 4 mm outer diameter Portex Ltd. Hythe Kent England) was passed through the hole. In the centre of the lid, a 1 mm hole was also made and seedling root was inserted through this hole such that it was not damaged.

2.5 Plant Nutrition

All the experiments conducted in hydroponic culture were given macronutrients as; 1 mol m^{-3} $NH_4H_2PO_4$, 4 mol m^{-3} Ca $(NO_3)_2$, and 2 mol m^{-3} $MgSO_4$ (Hoagland's No. 1) with 0.5 cm^3 micronutrients (Hoagland and Arnon, 1950). The different concentrations of KCl were used in the various experiments (for details see Chapter 6). These nutrients were applied from transferring the seedlings into hydroponic culture until harvesting the experiments. The nutrient solution was changed every alternate day, but when the treatments were begun after 20-30 days from sowing, nutrient solution was changed daily.

The experiments in Chapters 4 and 5 conducted in soil culture were given Phostrogen-based (Phostrogen Ltd, Corwen, Clwyd) nutrient solution at 1g l^{-1} with 0.5 cm^3 micronutrient solution (for details see Gorham, 1987). Gorham and Bridges (1995) reported that Phostrogen-based solution gave better growth of cotton than either of the Hoagland and Arnon solutions.

2.6 Growing conditions

The sand culture and flood bench system experiments were conducted in a heated glasshouse at Pen-y-Ffridd field station, University of Wales, Bangor. The minimum temperatures were 25/20 °C day/night with a photoperiod of 16 h d^{-1} (Natural daylight supplemented with 400 W Son-T high pressure sodium lamps Osram, UK). Some panes of the glasshouse were broken due to wind, hence, the temperature was not controlled at some times.

Hydroponic culture experiments were conducted in a growth cabinet (Fisons 600THTL, 28 °C day/20 °C night, 16 h photoperiod, with 350 µmol photons m^{-2} s^{-1} photosynthetically active radiation (400-700 nm) supplied by warm white fluorescent tubes plus tungsten light bulbs). Some experiments were also carried out in a Vindon growth room in the basement of the Thoday Building, University of Wales, Bangor with the same temperature and photoperiod.

2.6.1 **Flood bench system** (Fig. 2.1)

Experiment 3.1 was conducted in flood bench culture (see Gorham 1996). The flood-bench facility was made from plastic containers 35 x 80 x 60 cm fitted with sections of 15 mm hosepipe connected to a pump that was immersed in 300 dm^3 storage tanks of treatment solution. The amount of different treatments was calculated for 200 dm^3 and well mixed in the reservoirs. The reservoirs were placed underneath the bench. The solutions from these reservoirs were pumped into flood benches at 8.00 am every day for 20 minutes to a level 5 mm below the surface of the soil, after which solution flowed back into the reservoir. Evaporation losses were replaced by adding water after every couple of days. Flooding started at the time of sowing and continued until plants were harvested.

2.6.2 **Soil culture** (Fig. 2.2)

Most of the experiments were performed in soil culture in the glasshouse mentioned in section 2.6. Soil culture provides natural aeration to the roots more effectively than hydroponic culture as drainage from the pot accelerates air penetration into the pores vacated by the solution. The pots were placed over metal mesh benches for free drainage. 5 litre plastic pots were used in soil culture experiments. The plants were covered approximately after 30 days from sowing with waterproof foam sheets as lids to ensure that there would not be any chance of leakage of the solution being sprayed into the root medium (Fig. 2.2). The root-treated plants were also covered with the same foam sheet to give a uniform environment to all the treatments. Before starting the treatments, the plants were marked at the youngest fully expanded leaf with plastic-coated wire. The application of salt in soil and spray treatments was done in the morning because plants are more sensitive to shock at the beginning of the dark period (Blum, 1988).

Fig. 2.1 Flood bench system

Fig. 2.2 Soil culture

Fig. 2.3 Hydroponic culture

2.6.3 Hydroponic culture (Fig. 2.3)

Hydroponic medium tends to facilitate availability and uptake of elements. Aeration causes sufficient stirring to make additional stirring unnecessary and aerated hydroponic culture is considered better than soil culture in terms of control over salinity and nutrient levels. The solution in each pot was aerated constantly via an air regulator. Hydroponic culture systems avoid potential confounding effects due to salinity on soil structure. Electrical conductivity in solution culture is relatively constant, whereas in soil it fluctuates in response to transpiration and irrigation. The experiments described in chapter 6 were performed in hydroponic culture.

2.7 Growth measurements

The following agronomic parameters were recorded at the time of harvesting the experiments. Height of the plant was measured by using a metre rule. After recording fresh weight, the samples were dried in an oven at 70 °C for 48 hours to determine dry weight. Leaf area was measured using an automatic leaf area meter (Model AA-7, Hayashi Denkoh Co. Ltd. Tokyo). Leaf thickness was measured by using a micrometer.

Observations that were taken at the time of harvesting the experiments were:

- Total height of plant (mm)
- Height of plant above the marked leaf (mm)
- Total fresh weight of plant (g)
- Fresh weight of plant above the marked leaf (g)
- Total number of nodes on main stem
- Number of nodes above the marked leaf
- Total number of leaves per plant
- Leaf area per plant (cm^2)
- Leaf thickness
- Fresh and dry weight of the leaf
- Fresh and dry weight of shoots and roots

2.8 Measurements of photosynthesis, transpiration and stomatal conductance

Carbon dioxide exchange rates, transpiration and stomatal conductance were measured with an Infra-Red Gas Analyser (IRGA) operating in differential mode and coupled to a DL1 data logger and an ASU/MF mass air flow controller. This method is the most widespread of determining photosynthetic CO_2 exchange in plants. The ADC LCA2 system (Analytical Development Co. Ltd., Hoddesdon, Herts., UK) contains four basic units.

a. An IRGA.

b. A Parkinson broad leaf chamber.

c. An air supply unit (ASU) with mass flowmeter.

d. Data logger.

A Parkinson leaf chamber and IRGA were connected to a portable mast and air supply unit. The leaf being measured was adaxial side up and supplementary light was used to take measurements under saturating light intensity > 1000 μmol m^{-2} s^{-1} PAR. Air from outside the greenhouse was used through the mast and a 10 litre bottle that acted as buffer to minimise variations in CO_2 supply. The air was dried by passing it through two columns of blue silica gel (dry air is supplied and the RH of the air leaving the chamber is measured). Leaves were immediately placed in the chamber and measurements were taken after 60 seconds. Initially when a leaf is clamped in the chamber, RH will change rapidly due to the establishment of an initial equilibrium. When the reading of RH is changing slowly, readings of CO_2 depletion may be taken. (From Parkinson leaf chamber manual, Analytical Development Company). Photosynthesis is calculated from the difference in the mole fraction of CO_2 between the chamber entrance (reference) and the outlet (sample).

Net Photosynthesis (Pn) = CO_2 reference - CO_2 sample/leaf area

Stomatal conductance and transpiration can also be determined from the data logger information using the equations of von Caemmerer and Farquhar (1981).

Mass flow of air can be converted to mole flow of air:

$f=f_v/1000*1/22.4*273.15/(273.15+T)*p/101.3*1/60$

where

f = mole flow of air (mol s^{-1})

f_v = volumetric flow of air (cm^3 min^{-1})

T = temperature recorded during measurement (°C)

p = atmospheric pressure during measurement (kPa)

22.4 = volume in dm^3 of one mole of air at S.T.P.

Transpiration rate, E:

$E = f/s* (x_0 - x_e)/(1-x_0)$

where

E = transpiration rate (mol m^{-2} s^{-1})

x_0 = mole fraction of water vapour at leaf chamber outlet (mol mol^{-1})

x_e = mole fraction of water vapour at leaf inlet (mol mol^{-1})

x_0 and x_e are calculated from saturated vapour pressure (xs)

At the measured leaf temperature and the given RH:

$x_0 = x_s * RH/100$

Assimilation rate, A:

$A = f/s* \Delta c$

where

Δc = CO_2 differential between reference and analysis streams (mol mol^{-1})

A correction for the increase in water vapour by transpiration of the leaf is necessary.

$A = f/s* \Delta c*(1-x_e)/(1-x_0)$

Stomatal conductance, gs

$gs = E/x_s, T1-x_0$

where

gs = stomatal conductance (mol m^{-2} s^{-1})

x_s = mole fraction of water vapour at saturation. It is assumed here that the leaf is saturated with water at the actual leaf temperature T1.

Internal CO_2 concentration, ci:

$$ci = c_0\text{-}A*1.6/g_s$$

where

c_0 = mole fraction of CO_2 in outlet air from leaf chamber given by (c_e-Δ_c) from reference and differential measurements (μmol mol^{-1}), and 1.6 = ratio of diffusivity of CO_2 and water in air.

A Qbasic programme on a Viglen PC was used to download the data from the LCA2 data logger as an ASCll file into a spreadsheet (Quattro).

2.9 Chlorophyll measurements (SPAD)

Chlorophyll estimations were made with a Minolta (SPAD) chlorophyll meter. An average of ten observations were used to determine chlorophyll SPAD readings at the time of harvesting the plants. SPAD readings were obtained from the most fully extended leaves in the plant. The principal and measurement and operation of this device have been described by Inada (1963) and Wood *et al.*, (1992a). Briefly, the chlorophyll meter detects the difference in leaf-blade light attenuation at 430 and 750 nm, and displays a numerical SPAD unit, ranging from 0-80. Chlorophyll meter readings are instantaneous and involve no tissue collection.

2.10 Extraction of cell sap from leaves, petioles and stems

The procedure of sap extraction from the samples was the same as described by Gorham *et al.*, (1984). Replicated samples of the youngest fully-expanded leaf, its petiole and the stem below the petiole of each treatment were cut, blotted dry with tissue paper and individually stored in 1.5 cm^3 labelled microcentrifuge tubes and sealed for chemical analysis. The samples were frozen in a commercial freezer (at -18 °C) for a minimum 24 h. The tubes were taken out of the freezer, thawed and crushed with a metal rod with a tapered end. Two holes

were bored using a pin, one at the base and another at the top in the cap of the tube. Each tube was placed in another empty microcentrifuge tube and centrifuged at approximately 6000 x g for 10 minutes. This allowed the sap released from the crushing action to be collected in the second empty tube, leaving the tissue residue in the upper tube. The sap in the tubes was analysed or stored frozen for subsequent analysis.

2.11 Extraction from dry shoots and roots

After oven drying, the shoot and root samples were ground in an electric dry grinder mill (BL 350T, Kenwood Ltd. UK). A measured amount of ground shoot and root samples were put in labelled screw top test tubes and known amount of deionized water was added in the tubes, the lids were put on the tubes and placed in a heating block (Techne, Cambridge UK) at 105 °C for extraction for about 2 hours. The samples were filtered using Whatman No.1 (9.0 cm) filter paper and the extract of samples was diluted with deionized water (Dr. John Gorham, personal communication). The determination of Na^+ and K^+ was done using a Jenway PFP7 flame photometer (Jenway Ltd. UK).

2.12 Chemical Analysis

2.12.1 Na^+ and K^+ analysis by flame photometer

Extracted sap of plant parts was diluted with deionized water and standard solutions for calibration curve were prepared using NaCl and KCl for the determination of Na^+ and K^+ ions using a Jenway PFP7 flame photometer. Flame photometry is frequently adequate for routine analysis of sodium, potassium, lithium, calcium, particularly in biological tissues and fluids. It is a direct reading digital instrument and relies upon the fact that the compounds of alkali and alkaline metals can be thoroughly degraded in a flame. As a consequence, the spectra are simple, and interference filters can be used to isolate the desired emission line. The outputs can then be measured separately. Calibrations were performed after every ten samples.

2.12.2 Ion-exchange Chromatography

For cation analysis (Na^+, K^+, Ca^{2+} and Mg^{2+}) 20 mm^3 of sap was diluted in an autoinjector vial with 1.4 cm^3 of cation eluant (20 mol m^{-3} methanesulphonic acid in deionized water, > 18 Mohm cm^{-1}) and analysed by ion-exchange HPLC (Dionex 2000i, Dionex (UK) Ltd, Camberley, Surrey) fitted with a CS12 cation exchange column and a Self-Regenerating Cation Suppresser operated in autoregeneration model. The column was operated at 40 °C. The system was automated by coupling to a Spark-Holland 'Marathon' autosampler fitted with a 5 mm^3 PEEK sample loop, and a Shimadzu CR5A plotting integrator linked to an Atari 1040 computer.

For anion (Cl^-, NO_3^-, $Malate^{2-}$ SO_4^{2-}) analysis, 20 mm^3 of sap samples were diluted in an autoinjector vial with 1.4 cm^3 of anion eluant (2.5 mol m^{-3} Na_2CO_3 + 2.4 mol m^{-3} $NaHCO_3$ in 2.5% propan-2-ol) and analysed with the Dionex ion chromatograph fitted with an AS4A anion exchange column and an Anion Micro-Membrane Suppresser (see Gorham and Bridges, 1995).

Ion-exchange chromatography relies on the attraction between particles of opposite charge. Ion-exchange separations are carried out mainly in columns packed with an ion exchanger. There are two types of ion-exchanger, namely cation and anion exchanger. Cation exchangers possess negatively charged groups and these will attract positively charged cations. Anion exchangers have positively charged groups that will attract negatively charged anions.

2.13 Nitrogen determination

For nitrogen determination, the dry leaves of four replicates in experiment 3.1 and two replicated plants in experiment 3.2 were pooled together to make one sample. Total nitrogen content of leaf samples was analysed by the Kjeldahl method. It is a two stage process, consisting of (a) acid digestion and (b) distillation (Association of Official Agriculture Chemists, 1955).

(a) Acid digestion.

200 mg of milled leaf sample was transferred into micro digestion tubes. Half a kjeltablet was added, and this acted as a catalyst. Then 5 cm^3 of

H_2SO_4 was added to each tube and the tubes were put in a rack and placed in a heated fume cupboard. Digestion is complete when the acid mixture turns to the characteristic green colour. The tubes were then cooled in a fume cupboard.

b) Distillation.

Each of three 20 cm^3 distilled water (blanks) were titrated prior to running the samples. The blank values entered as a constant and the results adjusted accordingly. Each tube was inserted into the auto analyser. The NH_4^+ produced from the break-down of nitrogenous components during the acid digestion stage was released by an automated addition of 5 cm^3 of NaOH (1M) through the resulting mixture. The NH_4^+ was distilled off and collected in the receiver solution.

The results were calculated from the amount of titrant (HCl) required to reach the end point of the receiver solution. An inbuilt calculator displayed the result as N%. Total N% was then calculated in mmol kg^{-1} dw. The equipment used for this analysis was Kjeltec Auto 1030 Analyser.

2.14 Betaine Assay

Betaine was measured by the periodide method (see Gorham 1996). The saps of the four replicated plants (experiment 3.1) per tub were pooled together to make one sample.

100, 200, 300, 400, or 500 µl of 5 mol m^{-3} betaine were added as standards in microcentrifuge tubes. 20 mm^3 of sap was taken for sample and 750 mm^3 of 1 kmol m^{-3} H_2SO_4 was added to each tube and then they were placed on ice.

When the mixture was cold, 20 mm^3 of cold I/KI solution (15.7g I_2 + 20g KI in 100 cm^3 water) mixed and stored at -4 °C overnight. The mixture was centrifuged for 10 minutes in a cold room and the supernatant carefully aspirated off without disturbing the periodide crystals. The periodide pellet was dissolved in 1 cm^3 methanol. The solution was then diluted with100 mm^3 of methanol and a 3 cm^3 of alcohol in a cuvette, and the absorbance was measured in a spectrophotometer at 360 nm.

2.15 Statistical analyses

Data were analysed using the DESCRIBE and ANOVA and General Linear Model routines of the Minitab 11 statistical software package. The data are presented with standard errors of the means and were tested for significant differences between means at $P>0.05$ using Tukey's test.

CHAPTER THREE

SALINITY AND NITROGEN INTERACTIONS

CHAPTER 3

SALINITY AND NITROGEN INTERACTIONS

3.1 Introduction

Nitrogen is essential for plant growth, as it is one of the essential constituents of plant cells. Nitrogen is of special important to the agriculture industry (Kirkby, 1981). Nitrogen is used by plants to manufacture proteins and nucleic acids that are present in all plant tissues (Tisdale *et al.*, 1993).

Nitrogen is present in the soil in organic and inorganic forms. The inorganic nitrogen is present as ammonium and nitrate, which are major sources taken up by the roots of higher plants. Most of the ammonium has to be incorporated into organic compounds in the roots, whereas nitrate is readily mobile in the xylem and can also be stored in the vacuoles of roots, shoots, and storage organs. Nitrate accumulation in vacuoles is important for cation-anion balance and for generation of turgor (Marschner, 1995). Plants generally grow better in the presence of nitrate than ammonium and, even better, in a mixture of ammonium and nitrate (Hageman, 1984). Within the plant, nitrate must first be reduced to ammonium before being assimilated into amino acids (Beevers and Hageman, 1983).

Nowadays, there are faster-fruiting and higher-yielding cotton cultivars and increased use of nitrogenous fertilizers occurs in cotton production. Cotton that is subjected to nitrogen stress may not photosynthesise efficiently, have reduced growth and utilize water poorly (Radin and Mauney, 1984). Bondada *et al.* (1996) reported that vegetative growth, lint yield and photosynthetic capacity in cotton increased with increasing N rate (0, 55, 82 or 110 kg N/ha).

Salinity decreases plant growth depending upon the plant species, salinity levels and ionic composition of the salts (Cordovilla *et al.*, 1994, Delgado *et al.*, 1993, Flowers *et al.*, 1977, Gorham, 1995a, Greenway and Munns, 1980, Kent and Läuchli, 1985, Läuchli, 1984). In cotton 50% yield reduction occurs at about 180 mol m^{-3} NaCl treatment (Maas and Hoffman, 1977). Cotton is classified

as a salt tolerant crop (Maas and Hoffman, 1977, Maas, 1984 and Shimose and Sekiya, 1991), but is quite salt sensitive in the seedling stage (Abul-Naas and Omaran, 1974, Kent and Läuchli, 1985).

Nitrogen fertilization has a decisive role to play in the adequate development of the plants, and the correct level of nitrogen could help to correct nutritional imbalances due to saline water (Al-Rawahy *et al.*, 1992). Several attempts have been made to minimize or limit the salinity effects on plants by a supplementary nitrogen supply (Feigin, 1985, Kafkafi, 1984, Lewis *et al.*, 1989 and Leidi *et al.*, 1992). Torres and Bingham (1973) found that excessive NaCl in the growth medium appeared to reduce growth in wheat by restricting nitrate uptake to a point where it became deficient.

Uptake of nitrogen by cotton was inhibited under high concentration of NaCl and Na_2SO_4 in the root medium, and the excess amount of absorbed Na^+ depressed NH_4 absorption (Pessarakli, 1995). The absorption and metabolism of NH_4^+ and NO_3^- in red kidney beans (*Phaseolus vulgaris* L.) were reduced significantly under salt or water stress (Frota and Tucker, 1978).

The relationship between photosynthesis and nitrogen has been widely studied because of the importance of photosynthesis to plant productivity and the status of nitrogen as a limiting element (Chapin, 1980, Novoa and Loomis, 1981). The photosynthetic capacity of many plant species is reduced in the presence of NaCl. This inhibition may be either directly through stomatal closures or as a consequence of non-stomatal inhibition of photosynthesis (Downton, 1977, Gale *et al.*, 1967, Klein Kopf *et al.*, 1976). Salinity affects yield by limiting the leaf area available for photosynthesis (Gorham *et al.*, 1985a and Wyn Jones and Gorham, 1989).

The objective of these experiments is to investigate the effect of supplemental nitrogen on the growth of cotton and ion contents under saline conditions. It was also examined which form of nitrogen, i.e. NH_4^+ or NO_3^-, is more effective under control and saline conditions.

3.2 Materials and Methods

3.2.1 Experiment 3.1 (Salinity and N interactions)

A preliminary investigation was carried out to examine the effects of the interaction between NaCl and NH_4NO_3 on germination, vegetative growth and internal ion contents of cotton plants and to understand the relationship between the above parameters and salt tolerance.

The experiment was conducted in a heated greenhouse at Pen-y-Ffridd field station, University of Wales, Bangor from October to November,1995. The minimum temperatures were 25/20 °C day/night with a photoperiod of 16 h d^{-1} (Natural daylight supplemented with 400 W Son-T high pressure sodium lamps; Osram, UK).

Three seeds of cotton *(Gossypium hirsutum* L cv. Acala SJ2) were sown in a mixture of equal parts of loam-based John Innes No. 1 compost, fine grade horticultural vermiculite and seramis in 2 litre pots. The experiment was conducted in a flood bench (see Chapter 2.6.1) with 200 litre nutrient reservoirs. Macronutrient concentrations were, 1 mol m^{-3} KNO_3, 1 mol m^{-3} KH_2PO_4, 2 mol m^{-3} $MgSO_4$, and 2 mol m^{-3} K_2SO_4 and micronutrients were 0.5 ml per litre (Hoagland and Arnon, 1950). $CaCl_2$ was added at 10 mol m^{-3} to all the treatments.

The experiment comprised 10 treatments with 16 replicates in a factorial design. There were two levels of NH_4NO_3 (0 and 7 mol m^{-3}) giving a total of 1 and 15 mol m^{-3} N respectively and five concentrations of NaCl (0,50,100,150, and 200 mol m^{-3}). The salt was dissolved in the nutrient reservoirs at the time of sowing.

Emergence was counted daily for 13 days starting from first emergence of the seed. The first sign of unfolding by the cotyledon was considered seedling emergence. After recording the emergence, the plants were thinned to one uniform plant per pot. The plants were harvested 52 days after sowing. Shoot height, shoot fresh weight, number of nodes on main stem, fresh weight of a young expanded leaf and dry weight of the same leaf after oven drying at 70 °C for 48 hours were recorded.

For ion analysis, the youngest fully-expanded leaf, its petiole and the stem below the petiole were collected in micro-centrifuge tubes for chemical analysis. For sap extraction see Chapter 2.10. For Na^+ and K^+, the sap samples were diluted with deionised water and determined on a flame photometer (see Chapter 2.12). For anions, the saps of the four replicated plants were pooled together to make one sample. The samples were analysed by ion exchange HPLC (Dionex 2000i, Dionex (UK) Ltd, see section 2.12.2).

Chlorophyll estimations were made with a Minolta (SPAD) chlorophyll meter (see Chapter 2.9). For nitrogen determination, the dry leaves of four replicated plants were pooled together to make one sample. Total nitrogen contents of leaf samples were analysed by Kjeldahl method (see Chapter 2.13). Betaine was measured by the periodide method as mentioned in section 2.14.

3.2.2 Experiment 3.2 (NH_4^+ versus NO_3^-)

The purpose of this experiment was to study the effects of salinity and nitrogen interaction in cotton during its vegetative growth. The main objective of this research was to evaluate the relative efficiency of two forms of nitrogen at the same concentration.

In this experiment, seeds of *Gossypium hirsutum* cv. Acala SJ2 were sown in pots containing 5 litre of John Innes No.1 compost. The pots were placed over a metal mesh bench for free drainage at Pen-y-Ffidd field station. The experiment was conducted under the same light and temperature conditions as experiment 3.1.

The experiment consisted of 4 treatments. Nutrient concentrations in all the treatments were, 2 mol m^{-3} KH_2PO_4, 3 mol m^{-3} $MgSO_4$, 6 mol m^{-3}, K_2SO_4, 10 mol m^{-3} $CaCl_2$ and micronutrients 1 ml per litre. In non-saline treatment with NH_4^+, 8 mol m^{-3} NH_4Cl and in salt treatment 150 mol m^{-3} NaCl and 8 mol m^{-3} NH_4Cl were used. In NO_3^- treatment, 8 mol m^{-3} $NaNO_3$ in non-saline treatment and 150 mol m^{-3} NaCl and 8 mol m^{-3} $NaNO_3$ in salt treatment were added. The treatments were started 12 days after sowing and applied daily.

The experiment was harvested 76 days after sowing. Measurements of photosynthesis, transpiration and stomatal conductance were made using an IRGA (see details in Chapter 2.8). Other physical observations were the same as in experiment 3.1. Leaf, petiole, and stem samples were collected as in experiment 3.1 for ion analysis, glycinebetaine and total N%. The methods for these determinations were the same as in experiment 3.1.

3.3 Results

3.3.1 Experiment 3.1 (Salinity and N interactions)

3.3.1.1 Percentage and number of days to emergence (Table 3.1)

Salinity significantly ($p \leq 0.000$) decreased the percentage of seed emergence, whereas there was no significant effect of nitrogen and its interaction with salinity on the % of seed emergence. A consistent decrease in % of seed emergence was found both in low and high nitrogen treatments with increasing concentrations of salinity. Seed emergence at 50 mol m^{-3} NaCl in low and high nitrogen treatments was the same, but at other salt levels, emergence % was slightly higher (not significant) in high nitrogen than in low nitrogen treatments.

Table 3.1 Effect of various concentrations of NaCl and NH_4NO_3 on percentage seed emergence and days to first emergence of *Gossypium hirsutum* L. Acala SJ2. The values are means of 16 replicates ± standard errors. (Experiment 3.1)

NaCl (mol m^{-3})	NH_4NO_3 (mol m^{-3})			
	0		7	
	% Emergence	Days to emergence	% Emergence	Days to emergence
0	98 ± 2	3.0 ± 0.1	100 ± 0	2.7 ± 0.1
50	94 ± 3	3.3 ± 0.1	94 ± 3	3.4 ± 0.2
100	89 ± 4	4.5 ± 0.3	92 ± 4	4.6 ± 0.2
150	79 ± 6	7.6 ± 0.4	85 ± 5	8.8 ± 0.2
200	74 ± 7	8.9 ± 0.3	77 ± 7	9.8 ± 0.4

Significant effects of salinity, nitrogen and their interaction ($p \leq 0.000$, $p \leq 0.012$ and $p \leq 0.012$ respectively) were observed on the number of days to emergence. The number of days to emergence significantly and consistently

increased with increasing concentrations of NaCl in both nitrogen treatments. The number of days to germinate at the control was less with high nitrogen compared with low nitrogen treatments, whereas it was the same at 50 and 100 mol m^{-3} NaCl. At 150 and 200 mol m^{-3} NaCl, the number of days to germinate was greater in high nitrogen than in low nitrogen treatments.

3.3.1.2 Growth parameters

Shoot fresh height, shoot fresh weight and number of nodes on the main stem. (Table 3.2)

Salinity significantly ($p \leq 0.000$) and consistently decreased the shoot height, shoot fresh weight and number of nodes on the main stem. Supplementary N significantly ($p \leq 0.000$) increased the above growth parameters at most concentrations of salt compared with low N treatments. There were significant ($p \leq 0.000$) effects of salinity x nitrogen interactions (inverse) on shoot height and shoot fresh weight, whereas no significant effect on the number of nodes was found. When additional N was supplied, it was found that the percentage of growth in comparision to the control (%) was in fact lower at most of the salinity levels. Although, this result is somewhat irregular, it showed that additional nitrogen does not interact with negative effects of salinity on growth of cotton. This suggests that nitrogen and salinity effects were independent.

FW/DW ratio (Table 3.2)

The analysis of variance showed significant effects of salinity and its interaction with nitrogen ($p \leq 0.000$) on the FW/DW ratio, also a significant effect of nitrogen ($p \leq 0.005$) on FW/DW was found. The results show that FW/DW ratio was significantly higher at high nitrogen than low nitrogen in the control. However, at 150 and 200 mol m^{-3} NaCl, FW/DW ratio was significantly lower in high N compared with low N treatments.

Table 3.2 Effect of various concentrations of NaCl and NH_4NO_3 on growth parameters of *Gossypium hirsutum* L. Acala SJ2. The values are means of 16 replicates ± SE. (Experiment 3.1).

NaCl	NH_4NO_3 (mol m^{-3})	Shoot height (mm)	Percent	Shoot fresh weight (g)	Percent	Number of nodes	FW/DW ratio
0	0	470 ± 27	100	21 ± 2	100	9 ± 1	6 ± 0
50	0	347 ± 11	74	15 ± 0	71	8 ± 0	8 ± 0
100	0	262 ± 8	56	12 ± 0	57	7 ± 0	7 ± 0
150	0	218 ± 7	46	9 ± 1	43	7 ± 1	9 ± 0
200	0	137 ± 5	29	4 ± 0	19	4 ± 0	10 ± 0
0	7	721 ± 19	100	60 ± 5	100	10 ± 1	8 ± 0
50	7	522 ± 21	72	36 ± 2	60	9 ± 0	7 ± 0
100	7	505 ± 13	70	39 ± 2	65	9 ± 0	7 ± 0
150	7	276 ± 11	38	17 ± 1	28	7 ± 0	8 ± 0
200	7	157 ± 8	22	8 ± 1	13	5 ± 0	8 ± 1

3.3.1.3 Leaf Chlorophyll (SPAD) (Fig. 3.1a)

Additional nitrogen significantly increased chlorophyll measurements at all salinity levels. The chlorophyll measurements increased in high nitrogen treatments with increasing concentration of NaCl up to 150 mol m^{-3} compared with the control. In low nitrogen treatments, chlorophyll did not show any clear difference at various salinity levels.

3.3.1.4 Leaf Nitrogen Content (Fig. 3.1b)

Analysis of variance shows that salinity, nitrogen and their interaction produced significant ($p \leq 0.000$) effects on leaf nitrogen concentrations. The results show that nitrogen concentrations were significantly higher at high nitrogen than low nitrogen treatments at all concentrations of salt. Generally, nitrogen concentrations were not decreased by salinity. Chlorophyll meter readings were significantly positively correlated (0.56**) with leaf N concentration.

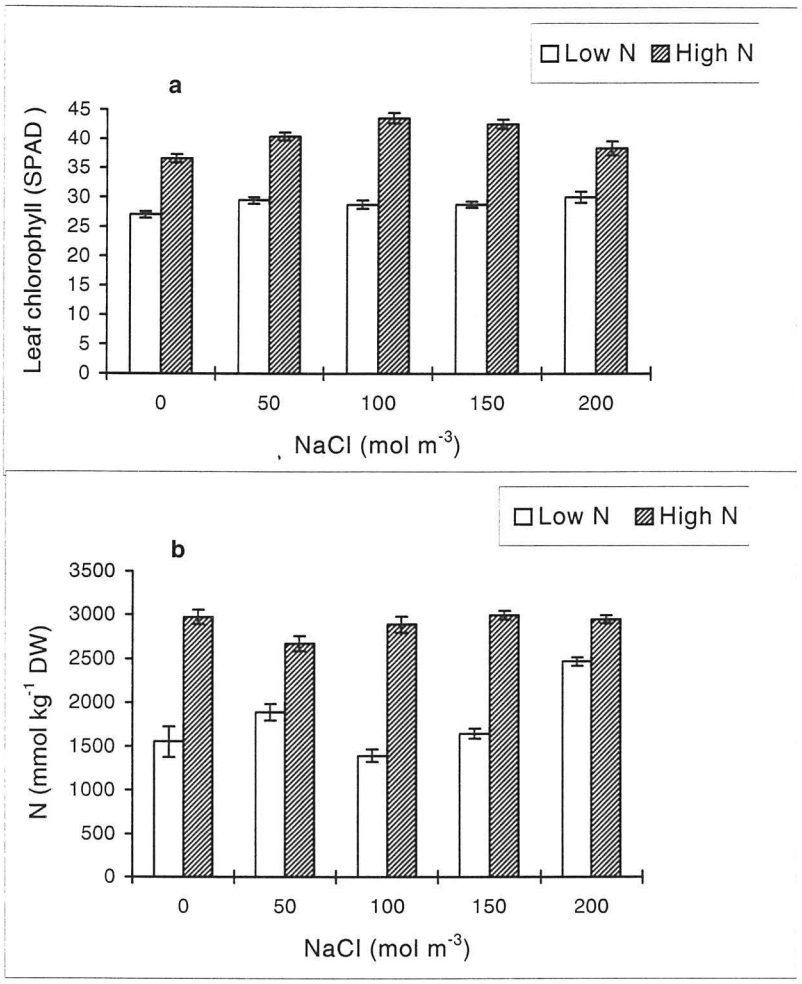

Fig. 3.1 Interactive effects of various concentrations of NaCl and NH_4NO_3 on **(a)** leaf chlorophyll (SPAD), **(b)** leaf nitrogen concentration. Each column is the mean of 16 replicates and vertical bars indicate standard errors of the means. (Experiment 3.1).

3.3.1.5 Glycinebetaine Concentrations (Table 3.3)

Salinity, nitrogen, different tissues and interactions between salinity and nitrogen produced significant ($p \leq 0.000$) effects on betaine concentrations Generally, betaine concentrations were significantly higher in high N than in low N treatments at most salt levels in all tissues. In the leaf, petiole and stem tissues, with high nitrogen, betaine concentrations were significantly greater with increasing salt concentrations compared with the control. Betaine concentrations were also increased in petioles and stems to some extent in low N treatments at most salt levels compared with the control.

Table 3.3 Effects of various concentrations of NaCl and NH_4NO_3 on betaine concentrations (mol m^{-3} expressed sap) in the leaves, petioles and stems of *Gossypium hirsutum* L. Acala SJ2. The values are means of 16 replicates ± standard errors. (Experiment 3.1)

NaCl (mol m^{-3})	NH_4NO_3 (mol m^{-3})					
	0			7		
	Leaf	Petiole	Stem	Leaf	Petiole	Stem
0	35 ± 2	31 ± 1	46 ± 4	35 ± 7	37 ± 6	35 ± 5
50	28 ± 3	25 ± 3	31 ± 2	48 ± 2	45 ± 2	45 ± 2
100	29 ± 4	38 ± 4	55 ± 3	57 ± 7	55 ± 3	52 ± 4
150	19 ± 4	37 ± 4	47 ± 5	49 ± 2	61 ± 4	60 ± 9
200	33 ± 4	42 ± 2	40 ± 3	45 ± 4	53 ± 4	56 ± 2

3.3.1.6 Cation Analysis

Na^+ Concentrations (Table 3.4)

Statistical analysis (3 way) shows that salinity significantly ($p \leq 0.000$) and consistently increased Na^+ concentrations in the leaf, petiole and stem tissues whereas, supplementary nitrogen significantly ($p \leq 0.000$) decreased Na^+ concentrations in the same tissues. Different tissues also produced significant effect on Na^+ concentrations. In low nitrogen treatments, Na^+ concentrations were in the order stem >> leaf > petiole, whereas in high N treatments, Na^+ concentrations were generally in the order stem >> petiole > leaf. Interactions between salinity and nitrogen produced significant ($p \leq 0.000$) effects on Na^+ concentrations. It is clear from the results that Na^+ concentration was lower when high nitrogen was applied compared with low nitrogen at all concentrations of salt.

K^+ Concentrations (Table 3.4)

Statistical analysis (3 way) showed that salinity, nitrogen and various tissues produced significant ($p \leq 0.000$) effects on K^+ concentrations. The interaction between these factors was also significant on K^+ concentrations. The results show that applied nitrogen significantly increased K^+ concentrations in all tissues compared with low nitrogen treatments under saline conditions. External salinity significantly decreased K^+ concentrations in all tissues in low nitrogen treatments compared with the control. However, with high nitrogen, increasing

salinity did not decrease K^+ concentrations in the leaves and stems, while those of petioles were significantly higher with increasing salt levels than the control. K^+ concentrations in both nitrogen treatments were in the order petiole >> stem > leaf.

Table 3.4 Effect of various concentrations of NaCl and NH_4NO_3 on Na^+ and K^+ concentrations (mol m^{-3} expressed sap) in the leaves, petioles and stems of *Gossypium hirsutum* L. Acala SJ2. The values are means of 16 replicates ± standard errors. (Experiment 3.1).

NaCl (mol m^{-3})		NH_4NO_3 (mol m^{-3})			
		0		7	
	Tissue	Sodium	Potassium	Sodium	Potassium
0	Leaf	1 ± 0	143 ± 9	1 ± 0	155 ± 7
	Petiole	1 ± 0	264 ± 6	1 ± 0	228 ± 5
	Stem	1 ± 0	252 ± 6	1 ± 0	228 ± 5
50	Leaf	35 ± 5	133 ± 6	23 ± 5	169 ± 5
	Petiole	29 ± 2	229 ± 7	22 ± 5	262 ± 6
	Stem	50 ± 5	210 ± 7	32 ± 8	251 ± 8
100	Leaf	114 ± 10	110 ± 10	25 ± 4	168 ± 5
	Petiole	81 ± 6	254 ± 7	20 ± 3	282 ± 6
	Stem	143 ± 8	182 ± 12	26 ± 4	273 ± 4
150	Leaf	153 ± 10	78 ± 7	37 ± 3	159 ± 11
	Petiole	110 ± 7	232 ± 7	44 ± 5	299 ± 5
	Stem	155 ± 9	164 ± 13	66 ± 7	276 ± 7
200	Leaf	103 ± 11	121 ± 12	83 ± 10	154 ± 13
	Petiole	103 ± 11	223 ± 11	93 ± 10	273 ± 13
	Stem	180 ± 10	172 ± 12	146 ± 10	219 ± 19

3.3.1.7 Anion Analysis

Cl⁻ concentrations (Table 3.5)

Analysis of variance (3 way) revealed significant ($p \leq 0.000$) effects of salinity, nitrogen and different tissues on Cl^- concentrations. The interactions between these factors were significant on Cl^- concentrations. In the leaves, petioles and stems, Cl^- concentrations were significantly increased in response to increasing concentration of NaCl in both nitrogen treatments. Generally, Cl^- concentrations were lower in high nitrogen than in low nitrogen treatments at most

concentrations of salinity. Generally, Cl^- were in the order petioles>>stems> leaves at most of salt concentrations in low and high nitrogen treatments.

Table 3.5 Effect of various concentrations of NaCl and NH_4NO_3 on Cl^-, malate, NO_3^- and SO_4^{2-} concentrations (mol m^{-3} expressed sap) in the leaves, petioles and stems of *Gossypium hirsutum* L. Acala SJ2. The values are means of 16 replicates ± standard errors. (Experiment 3.1).

Treatments NaCl (mol m^{-3})	NH_4NO_3	Tissue	Chloride	Malate	Nitrate	Sulphate
0	0	Leaf	131 ± 6	36 ± 5	1 ± 0	32 ± 2
		Petiole	270 ± 14	78 ± 5	2 ± 2	10 ± 1
		Stem	197 ± 8	55 ± 3	1 ± 1	13 ± 1
50	0	Leaf	181 ± 11	24 ± 2	5 ± 3	35 ± 3
		Petiole	315 ± 13	38 ± 3	16 ± 11	11 ± 2
		Stem	235 ± 12	23 ± 2	11 ± 6	12 ± 1
100	0	Leaf	225 ± 4	15 ± 4	0 ± 0	17 ± 3
		Petiole	349 ± 5	31 ± 4	0 ± 0	7 ± 0
		Stem	334 ± 55	45 ± 6	0 ± 0	14 ± 1
150	0	Leaf	261 ± 16	14 ± 2	1 ± 1	17 ± 1
		Petiole	324 ± 10	26 ± 1	3 ± 2	7 ± 0
		Stem	307 ± 9	31 ± 2	3 ± 1	12 ± 0
200	0	Leaf	259 ± 18	13 ± 1	30 ± 7	22 ± 2
		Petiole	240 ± 40	13 ± 6	43 ± 15	6 ± 1
		Stem	243 ± 23	11 ± 1	51 ± 5	12 ± 1
Treatments NaCl	NH_4NO_3	Tissue	Chloride	Malate	Nitrate	Sulphate
0	7	Leaf	62 ± 7	34 ± 1	42 ± 2	68 ± 4
		Petiole	104 ± 20	14 ± 2	133 ± 5	10 ± 1
		Stem	124 ± 15	18 ± 1	109 ± 5	13 ± 0
50	7	Leaf	146 ± 7	26 ± 3	30 ± 5	57 ± 7
		Petiole	239 ± 19	14 ± 2	94 ± 13	11 ± 1
		Stem	228 ± 17	17 ± 4	73 ± 8	14 ± 1
100	7	Leaf	181 ± 13	28 ± 5	22 ± 3	54 ± 4
		Petiole	272 ± 3	18 ± 2	101 ± 15	12 ± 1
		Stem	260 ± 6	24 ± 2	74 ± 7	16 ± 1
150	7	Leaf	166 ± 23	18 ± 4	18 ± 3	30 ± 5
		Petiole	322 ± 17	14 ± 1	71 ± 5	12 ± 1
		Stem	336 ± 11	18 ± 2	59 ± 4	16 ± 1
200	7	Leaf	246 ± 16	13 ± 4	23 ± 3	23 ± 4
		Petiole	304 ± 33	6 ± 2	58 ± 8	8 ± 1
		Stem	337 ± 8	17 ± 1	52 ± 4	16 ± 1

Malate^{2-} Concentrations (Table 3.5)

Analysis of variance (3 way) revealed significant effects of salinity ($p \leq 0.000$), nitrogen ($p \leq 0.000$) and various tissues ($p \leq 0.018$) on malate concentration. Malate was significantly higher in the petioles and stems than in leaves in low N treatments at the control and also up to 150 mol m^{-3} NaCl. The interactions between these factors were also significant ($p \leq 0.000$) for malate. Malate concentrations decreased consistently in the leaves with increasing concentrations of salt in both nitrogen treatments. Significant negative correlations (-0.65**) were found between leaf malate and leaf Cl^- concentrations. In the petioles and stems, in low nitrogen treatments, malate concentrations decreased with increasing salinity. However, when high nitrogen was in the medium, malate concentrations in petiole and stem tissues did not show any clear differences at the various concentrations of salt. Generally, malate concentrations were higher in low nitrogen than in high nitrogen treatments with an exception in leaves.

NO_3^- Concentrations (Table 3.5)

Analysis of variance (3 way) showed that salinity, nitrogen and various tissues produced significant ($p \leq 0.000$) effects on NO_3^- concentrations. The interaction between salinity and nitrogen was significant. NO_3^- concentrations were reduced in all tissues with increasing concentrations of salinity in high N treatments. In low nitrogen treatments, NO_3^- was present at 200 mol m^{-3} and also low NO_3^- was found at 50 mol m^{-3} NaCl in all tissues while NO_3^- concentrations were negligible at other salt levels. When nitrogen was applied, NO_3^- concentrations were in the order petiole >> stem > leaf.

SO_4^{2-} Concentrations (Table 3.5)

Significant effects ($p \leq 0.000$) of salinity, nitrogen, and various tissues and also their interactions on SO_4^{2-} concentrations were found. SO_4^{2-} concentrations consistently decreased in the leaves with increasing salinity in high nitrogen treatments. SO_4^{2-} concentrations in the leaves were significantly higher with high nitrogen than low nitrogen at all salt levels. In the petioles, SO_4^{2-}

concentrations decreased as salt levels increased in low nitrogen treatments. No clear difference in stems for SO_4^{2-} concentrations either with additional nitrogen or salt application was observed. SO_4^{2-} concentrations were in the order in both nitrogen treatments leaf >> stem > petiole.

3.3.2 Experiment 3.2 (NH_4^+ versus NO_3^-)

3.3.2.1 Growth parameters

Fresh shoot height, fresh shoot weight, and number of nodes on the main stem (Table 3.6)

150 mol m^{-3} NaCl significantly ($p \leq 0.000$) decreased shoot height, shoot fresh weight and number of nodes on main stem compared with the control. Interaction between salinity and form of nitrogen was significant ($p \leq 0.001$) on only shoot fresh weight. In the control, shoot fresh weight was significantly ($p \leq 0.001$) higher in NO_3^- than in NH_4^+ treatments, while the same shoot fresh weight was found in salinity treatment with both forms of nitrogen. Shoot height was higher with NO_3^- compared to NH_4^+-fed plants in the controls, whereas the opposite results were observed in salinity treatments.

Table 3.6 Effect of various concentrations of NaCl on growth parameters of Acala SJ2 (*Gossypium hirsutum* L.) grown under NH_4^+ or NO_3^- nutrient. The values are means of 10 replicates ± standard errors. (Experiment 3.2).

Treatments NaCl (mol m^{-3})	NH_4^+	Shoot height (mm)	Shoot fresh weight (g)	No. of nodes	FW/DW ratio
0	8	650 ± 23	109 ± 4	11 ± 0	6.8 ± 0.2
150	8	397 ± 27	50 ± 8	9 ± 0	7.2 ± 0.2
Treatments NaCl (mol m^{-3})	NO_3^-	Shoot height (mm)	Shoot fresh weight (g)	No. of nodes	FW/DW ratio
0	8	727 ± 33	150 ± 6	12 ± 1	7.3 ± 0.2
150	8	373 ± 16	50 ± 3	9 ± 0	7.0 ± 0.2

FW/DW ratio (Table 3.6)

Salinity, form of nitrogen and their interaction did not significantly affect FW/DW ratio. In the control, FW/DW was slightly greater in NO_3^- than in NH_4^+ treatments, whereas, the opposite was true in salinity treatments.

3.3.2.2 Photosynthesis, Transpiration and Stomatal conductance (Table 3.7)

Salinity significantly ($p \leq 0.009$) reduced net photosynthetic rate, whereas form of nitrogen and its interaction with salinity did not produce significant effects on photosynthesis. There was no significant effect of salinity, form of nitrogen, and their interaction on the rate of transpiration. Salinity significantly ($p \leq 0.006$) decreased stomatal conductance in NH_4^+-fed plants compared with the controls. At salinity, stomatal conductance was higher in NO_3^- than in NH_4^+-fed plants, but the difference was not significant.

Table 3.7 Effect of various concentrations of NaCl on photosynthesis, transpiration, stomatal conductance, leaf chlorophyll (SPAD) and N content of Acala SJ2 (*Gossypium hirsutum* L.) grown under NH_4^+ or NO_3^- nutrient. The values are means of 10 replicates ± standard errors. (Experiment 3.2).

Treatments NaCl (mol m^{-3})	NH_4^+	Photosynthesis (μmol m^{-2} s^{-1}) CER	Transpiration (mmol m^{-2} s^{-1}) E	S. Conductance (mmol m^{-2} s^{-1}) gs	SPAD	Nitrogen (mmol kg^{-1} DW)
0	8	11 ± 1	5 ± 1	125 ± 10	37 ± 1	2571 ± 49
150	8	8 ± 1	4 ± 1	86 ± 13	37 ± 1	2663 ± 56
Treatments NaCl (mol m^{-3})	**NO_3^-**	**Photosynthesis (μmol m^{-2} s^{-1}) CER**	**Transpiration (mmol m^{-2} s^{-1}) E**	**S. Conductance (mmol m^{-2} s^{-1}) gs**	**SPAD**	**Nitrogen (mmol kg^{-1} DW)**
0	8	11 ± 1	5 ± 0	123 ± 10	38 ± 1	2517 ± 102
150	8	9 ± 1	4 ± 0	98 ± 10	38 ± 1	2812 ± 45

3.3.2.3 Leaf Chlorophyll (SPAD) (Table 3.7)

The data presented show that salinity, form of nitrogen and their interaction did not produce any significant effect on chlorophyll levels.

3.3.2.4 Leaf Nitrogen Content (Table 3.7)

Salinity significantly ($p \leq 0.011$) increased nitrogen content in the leaves in NO_3^- treatment compared with the controls. Interaction was not significant between both forms of nitrogen and salinity.

3.3.2.5 Glycinebetaine Concentrations (Table 3.8)

Analysis of variance (3 way) revealed that salinity did not produce significant effects on betaine concentrations. However, forms of nitrogen and various tissues showed significant ($p \leq 0.001$) effects on betaine concentrations. Interaction was not significant between salinity and forms of nitrogen for betaine concentrations. In the leaves, betaine was significantly higher with NO_3^- than NH_4^+-fed plants in the control. Betaine concentrations were in the order leaf>>stem>petiole with both forms of nitrogen in the control and salinity treatments.

Table 3.8 Effect of various concentrations of NaCl on betaine concentrations (mol m^{-3} expressed sap) of Acala SJ2 (*Gossypium hirsutum* L.) grown under NH_4^+ or NO_3^- nutrient (8 mol m^{-3}). The values are means of 10 replicates ± standard errors. (Experiment 3.2).

NaCl	$NH4^+$			$NO3^-$		
(mol m^{-3})	Leaf	Petiole	Stem	Leaf	Petiole	Stem
0	55 ± 4	41 ± 1	50 ± 2	68 ± 3	42 ± 2	49 ± 2
150	57 ± 5	41 ± 2	46 ± 2	69 ± 4	45 ± 3	51 ± 2

3.3.2.6 Cation Analysis

Na^+ Concentrations (Table 3.9)

Analysis of variance (3 way) showed that salinity significantly ($p \leq 0.000$) increased Na^+ concentrations, whereas nitrate significantly ($p \leq 0.004$) decreased Na^+ concentrations in the leaves and petioles compared with NH_4^+-fed plants under salinity. Various tissues produced significant ($p \leq 0.000$) differences in Na^+ concentrations. Stem Na^+ concentrations were significantly higher than leaves and petioles at 150 mol m^{-3} NaCl. The interaction was significant between salinity x nitrogen at ($p \leq 0.020$) level.

Table 3.9 Effect of various concentrations of NaCl on Na^+ and K^+ concentrations (mol m^{-3} expressed sap) of Acala SJ2 (*G. hirsutum* L.) grown under NH_4^+ or NO_3^- nutrient. The values are means of 10 replicates ± standard errors. (Experiment 3.2).

Treatments NaCl (mol m^{-3})	NH_4^+	Sodium Leaf	Sodium Petiole	Sodium Stem	Potassium Leaf	Potassium Petiole	Potassium Stem
0	8	5 ± 3	2 ± 1	4 ± 2	172 ± 14	292 ± 12	238 ± 12
150	8	42 ± 8	36 ± 7	84 ± 13	158 ± 15	265 ± 8	230 ± 11
Treatments NaCl (mol m^{-3})	NO_3^-	Sodium Leaf	Sodium Petiole	Sodium Stem	Potassium Leaf	Potassium Petiole	Potassium Stem
0	8	2 ± 0	1 ± 0	2 ± 0	185 ± 9	282 ± 10	263 ± 5
150	8	23 ± 3	22 ± 2	64 ± 9	152 ± 14	276 ± 9	243 ± 12

K^+ Concentrations (Table 3.9)

Various tissues produced significant ($p \leq 0.000$) effect, whereas other factors did not produce significant effects on K^+ concentrations. K^+ concentrations in the petioles and stems were significantly higher than leaves at the control and salinity with both forms of nitrogen. Petiole K^+ was greater than stem K^+ concentrations in all treatments. Applied salinity decreased K^+ concentrations in all tissues with both forms of nitrogen, but the difference was not significant.

3.3.2.7 Anion Analysis

Cl^- Concentrations (Table 3.10)

Analysis of variance (3 way) revealed that salinity significantly ($p \leq 0.000$) increased Cl^- concentrations in all tissues, whereas form of nitrogen significantly ($p \leq 0.000$) decreased Cl^- concentrations from the same tissues. Cl^- concentrations were higher in NO_3^- than in NH_4^+ treatments at the control and salinity. There were significant differences between the tissues. Cl^- concentrations were higher in the petioles and stems than in leaves. Generally, petiole Cl^- was higher than stem Cl^-, but the difference was not significant. All interactions were not significant for Cl^- concentrations.

Table 3.10 Effect of various concentrations of NaCl on Cl^-, malate, NO_3^- and SO_4^{2-} concentrations (mol m^{-3} expressed sap) of Acala SJ2 (*Gossypium hirsutum* L.) grown under NH_4^+ or NO_3^- nutrient. The values are means of 10 replicates ± standard errors. (Experiment 3.2).

Treatments NaCl (mol m^{-3})	NH_4^+	Tissue	Chloride	Malate	Nitrate	Sulphate
0	8	Leaf	81 ± 5	29 ± 5	27 ± 4	47 ± 5
		Petiole	125 ± 8	15 ± 3	123 ± 10	7 ± 1
		Stem	116 ± 9	14 ± 3	82 ± 10	11 ± 1
150	8	Leaf	155 ± 15	27 ± 6	28 ± 4	47 ± 4
		Petiole	202 ± 10	12 ± 3	124 ± 8	6 ± 0
		Stem	199 ± 25	12 ± 3	83 ± 8	10 ± 0
Treatments NaCl (mol m^{-3})	NO_3^-	Tissue	Chloride	Malate	Nitrate	Sulphate
0	8	Leaf	114 ± 11	26 ± 7	20 ± 6	40 ± 5
		Petiole	146 ± 19	15 ± 5	113 ± 10	6 ± 1
		Stem	140 ± 10	15 ± 6	82 ± 9	9 ± 1
150	8	Leaf	177 ± 17	22 ± 6	24 ± 3	38 ± 5
		Petiole	220 ± 13	13 ± 3	111 ± 10	8 ± 3
		Stem	255 ± 27	16 ± 4	91 ± 7	9 ± 1

Malate^{2-} Concentrations (Table 3.10)

There were no significant effects of salinity, forms of nitrogen, whereas significant ($p \leq 0.000$) effects of tissues were observed for malate concentrations. Leaf malate was significantly higher than petiole and stem tissues at all the cases, while petiole and stem malate was more or less the same. All interactions were not significant for malate concentrations.

NO_3^- Concentrations (Table 3.10)

No significant effects of salinity and form of nitrogen were found between the treatments on NO_3^- concentrations, whereas various tissues have significant ($p \leq 0.000$) effect on NO_3^- concentrations. NO_3^- concentrations were significantly in the order petiole>>stem>leaf at the control and salinity with both forms of nitrogen. All interactions were not significant for NO_3^- concentrations. There were no great differences in NO_3^- concentrations in all tissues at control and

salinity with both forms of nitrogen. It was surprising that NO_3^- application did not increase NO_3^- concentrations in all tissues.

SO_4^{2-} Concentrations (Table 3.10)

No significant differences with salt or form of nitrogen on SO_4^{2-} concentrations were found. Leaf SO_4^{2-} was significantly ($p \leq 0.000$) higher than petiole and stem tissues. All interactions between salinity, nitrogen and tissues were not significant.

3.4 Discussion

The objectives of these experiments were to study the growth and ion accumulation of cotton in nitrogenous nutrient under continuous salinity stress and also to examine the effect of NaCl salinity on growth, photosynthesis, transpiration and mineral composition of cotton plants grown under NH_4^+ or NO_3^- nutrients.

The results show (Table3.1) that increasing concentrations of NaCl reduced and delayed seed emergence. They also illustrate the beneficial effects of additional nitrogen on the germination of cotton under NaCl stress. However additional nitrogen did not show a response in the number of days to emerge. The emergence data supports the findings of Kent and Läuchli (1985), who studied the effects of NaCl salinity on germination and early seedling growth of cotton. They reported that germination was delayed and reduced by 200 mol m^{-3} NaCl in the presence of a complete nutrient medium. Khan (1996) also reported that NaCl at 100-200 mol m^{-3} significantly decreased the germination percentage of different genotypes of wheat. Qada (1994) reported that soil salinity of EC 8.0 dS/m lowered the percentage of germination as well as delayed it in wheat.

Cotton plants exhibited a sharp decline in fresh shoot height, fresh shoot weight and the number of nodes on main stem when subjected to increasing salinity levels. On the other hand, high nitrogen treatments brought about a significant increase in biomass in stressed and non-stressed plants compared with low N treatments (Table 3.3). Delane *et al.* (1982) suggested that the growth of the

shoots of barley at high NaCl was limited by an insufficient supply of ions or other solutes to the growing region, resulting in insufficient osmotic solutes to generate turgor. Garg *et al.* (1993) reported that Indian mustard was grown under low and high nutrient solutions and irrigated with saline water of different concentrations (0,50,100, and 150 mol m^{-3} NaCl). They found that vegetative growth and seed yield were consistently and significantly greater for high nitrogen than low nitrogen nutrient. The results were in accordance with experiment conducted by Pessarakli and Tucker (1985) and Rathert (1982 and 1983) on cotton, they reported that when salinity levels were boosted, plant growth was decreased.

In Experiment 3.2, shoot height, shoot fresh weight and number of nodes were greater with NO_3^- than NH_4^+-fed plants at the control while, no great difference was found in salinity treatments (Table 3.6). Lewis *et al.* (1989) reported that in cotton, detrimental effects of salinity were greater in NH_4^+-fed plants than in NO_3^-. 150 mol m^{-3} NaCl significantly decreased the growth of cotton with both forms of nitrogen but, the effect was more with NO_3^- than NH_4^+-fed plants for shoot height. Leidi *et al.* (1991) found that salinity at 100 mol m^{-3} affected the growth of cotton plants cultivated with NH_4^+ or NO_3^- nutrients. NH_4^+ grown plants were smaller than those grown with NO_3^- solution.

Additional NH_4NO_3 brought about a significant increase in chlorophyll measurements (Fig. 3.1a). Chlorophyll meter readings were significantly positively correlated (0.56**) with leaf N concentration. These findings are in agreement with an experiment conducted by Baker *et al.* (1993) on cotton and Schepers *et al.* (1992) on maize. They reported that SPAD readings increased with an increase in the leaf N concentration. Chlorophyll measurements did not decrease due to salinity in both experiments. Leidi *et al.* (1992) found that chlorophyll content in cotton remained remarkably unaffected by salinity of the nutrient solutions.

Photosynthetic activity was significantly reduced by NaCl salinity in experiment 3.2 (Table 3.7) and the effect was greater in NH_4^+ than in NO_3^--fed plants. Other authors (Kaiser *et al.*, 1983, Lewis *et al.*, 1989 and Leidi *et al.*, 1991) have found that salinity has no effect on photosynthesis. Gorham and Bridges

(1995) reported that leaf growth rate in cotton reduced due to salinity stress resulting in reduced leaf area available for photosynthesis. Khan *et al.* (1994b) reported that when salinity interacted with nitrogen in alfalfa, there was a greater reduction in photosynthesis in NH_4^+ than in NO_3^--fed plants at 100 mol m^{-3} NaCl. Jeffrey and Thomas (1986) reported that salinity at 100 mol m^{-3} NaCl was found to reduce photosynthetic capacity in *Phaseolus vulgaris* L. In the leaves of barley, the photosynthetic rate was reduced by NaCl (1 and 2 g per kg soil), whereas it was increased significantly with added N increasing from 0-100 mg N/kg soil (Shen *et al.*, 1994).

Salinity also affected the rate of transpiration and stomatal conductance (Table 3.7). In NH_4^+-fed plants, the rate of transpiration and stomatal conductance were lower than in NO_3^--fed plants. Leidi *et al.* (1991) found that salinity reduced the rate of transpiration and stomatal conductance only in NH_4^+-fed plants. These results also agree with the findings of El-Saidi and Kortam (1974), who observed a salt-induced decrease in transpiration rate of cotton plants. Khan *et al.* (1994b) found that salinity at 100 mol m^{-3} NaCl caused a substantial reduction in the rate of stomatal conductance, while transpiration rate was least affected in alfalfa plants. They further reported that transpiration rate was increased more by ammonium-N than nitrate-N.

As illustrated in Table 3.4, Na^+ concentrations in various parts of the plant increased significantly by salinity, and this increase of Na^+ became significantly lowered with additional NH_4NO_3. The results are accordance with the experiment conducted by Shen *et al.* (1994), who reported that Na^+ content in barley increased when NaCl was applied at the rate of 1 and 2 g per kg soil and decreased significantly by additional N from 50 to 200 mg N/kg soil. NaCl at 100 mol m^{-3} in the nutrient medium caused an increase of Na^+ and Cl^- concentrations in peanut and cotton (Leidi *et al.*, 1992). In this study, NH_4^+ grown plants accumulated much higher Na^+ than NO_3^- grown plants when exposed to 150 mol m^{-3} NaCl. Lewis *et al.* (1989) reported that in both hydroponically grown maize and wheat NH_4^+-fed plants showed a considerably greater sensitivity to salinity than NO_3^--fed plants.

In Experiment 3.1, K^+ levels were significantly affected by NaCl. K^+ concentrations decreased with increasing salt levels in low nitrogen treatment (Table 3.4). Many salt-tolerant plants show a decrease in K^+ when exposed to salinity (Greenway and Munns, 1980, Kingsburry *et al.*, 1984, Kumar and Yadav, 1983 and Leidi *et al.*, 1992). In this study, it was found that K^+ levels were greater with high nitrogen than low nitrogen treatments at all different concentrations of salinity. The results are in accordance with the findings of Shen *et al.* (1994) that K^+ content of shoots of barley increased with increasing N. In Experiment 3.2, K^+ levels were lower in all tissues with salinity compared with the control but, the difference was not significant. K^+ did not show a clear difference between NH_4^+ and NO_3^--fed solutions. Leidi *et al.* (1991) reported that K^+ concentrations were not changed significantly with NH_4^+ or NO_3^- nutrients in cotton under stressed and non-stressed conditions.

Nitrogen concentration in the leaves was not significantly affected by salinity at all levels, indicating that cotton plants had adjusted somewhat to salinity and its effect on N-uptake (Figure 3.1b and Table 3.7). Pessarakli (1995) studied the effects on cotton plants grown in control and NaCl-treated Hoagland solution. He reported that low and medium levels of salinity did not significantly effect on N-uptake, but high salt level caused a substantial reduction in N-uptake rate. Frota and Tucker (1978) reported that NaCl drastically reduced the uptake rate of nitrogen in red kidney beans. Leidi *et al.* (1992) reported that increasing salinity resulted in a decrease of total N content in the shoots. Findenegg *et al.* (1989) found that the increase of Cl^- concentration in the medium did not change the level of total N in sugarbeet leaves.

Concentrations of the anions, malate and sulphate were generally decreased by salinity in both the experiments. Cramer *et al.* (1995) also reported that low contents of malate in the leaf of *Lycopersion escutentum* L. were found in salinized compared to the control plants.

In Experiment 3.1, applied nitrogen increased betaine concentrations. Colmer *et al.* (1996) found that betaine was the major organic solute accumulated in leaf blades of *Spartina alterniflora* grown at 500 mol m^{-3} NaCl. Glycinebetaine,

though formed in rather large amounts, only increased with increasing salinity to a modest extent. Gorham (1996) reported that glycinebetaine concentrations increased in expressed sap of young, fully expanded leaves of four cotton varieties at 125 and 200 mol m^{-3} NaCl under complete nutrient solution. In experiment 3.1 (Table 3.3), betaine concentrations were not increased in the various tissues with increasing salt levels in low nitrogen treatments. However, betaine concentrations were increased in all tissues with increasing concentrations of salinity in high nitrogen treatments.

3.5 Conclusions

- Increasing salinity lowered the percentage of emergence as well as delayed it in cotton compared with the control. Additional nitrogen did not increase seed emergence.
- Increasing concentration of NaCl consistently decreased growth parameters in low and high nitrogen treatments.
- Additional nitrogen significantly increased growth of cotton under control and saline conditions compared with low nitrogen treatments.
- Additional NH_4NO_3 (7 mol m^{-3}) reduced Na^+ concentration and increased K^+ concentrations in the leaf, petiole and stem tissues.
- Nitrate-N significantly increased growth compared with ammonium-N at the control. Na^+ concentration was lower with NO_3^- than NH_4^+-fed solution while, the opposite was true for Cl^- concentrations.
- Salinity at 150 mol m^{-3} NaCl decreased net photosynthesis in NH_4^+ or NO_3^--fed plants. No difference in net photosynthesis was observed between NH_4^+ or NO_3^- nutrients under stress and non-stress conditions.
- Increasing concentration of salinity did not decrease nitrogen concentration of the leaves.
- Additional nitrogen did not compensate for the negative effects of salinity on growth of cotton.

CHAPTER FOUR

NaCl AND GLYCINEBETAINE INTERACTIONS

CHAPTER 4

NaCl AND GLYCINEBETAINE INTERACTIONS

4.1 Introduction

Glycinebetaine is the simplest member of the group of fully N-methylated amino acids (Wyn Jones and Storey, 1981). Wyn Jones (1980) reported that the concentration of glycinebetaine in leaf tissues of plants grown in the presence of salt varies from 10 to 100 μmol g^{-1} fresh weight. The young tissues of barley plants contain much more betaine than mature tissues (Storey and Wyn Jones, 1977).

Wyn Jones *et al.* (1977) proposed that glycinebetaine acts as a compatible solute (i.e. an organic solute that does not inhibit enzyme activity or other metabolic processes and is accumulated in the cytoplasm in stressed plants). Robinson and Jones (1986) reported that the physiological role of glycinebetaine has not been fully established, but that it has a role in osmotic adjustment. Betaine stabilises cell membranes (Jolivet *et al.*, 1982) and certain photosynthetic reactions under stress conditions (Mamedow *et al.*, 1991, Papageorgiou *et al.*, 1991).

There is much evidence for betaine accumulation in various halophytes exposed to increasing salt NaCl stress, especially members of the *Chenopodiaceae* (Wyn Jones and Storey, 1981), *Limonium* spp. (Hanson *et al.*, 1991), *Atriplex semibaccata* and *A. halimus* (Koheil et *al.*, 1992). In these halophytic species, glycinebetaine probably acts as a compatible cytoplasmic solute, and is involved in the osmotic adjustment responses of the plants to hypersaline stress (Wyn Jones *et al.*, 1977). In glycophytes, its accumulation is reported in *Triticum aestivum* (Gorham *et al.*, 1985a, Gorham 1995 and Wyn Jones *et al.*, 1984) and in 35 species of *Gossypium* and many other genera of *Malvaceae* (Gorham, 1995 and 1996).

Foliar application of betaine to field grown plants has resulted in yield increases of 10 to 50% in a variety of crop and pasture plants (Campbell *et al.*, 1996, Naidu *et al.*, 1992). These studies indicated that plants are able to

translocate foliar-applied glycinebetaine from their leaves to other organs (Makela *et al.*, 1996). Foliar application of betaine to potatoes and tomatoes indicated its possibilities for reducing crop failures under drought and salinity stress (El-Amin, personal communication). Field trials with betaine on cotton in Pakistan have shown that it can increase yields (Gorham, 1999 in press).

Plants growing in saline media may diminish internal water deficits by the absorption of inorganic ions and the synthesis of organic solutes, both of which contribute to osmotic adjustment (Epstein, 1980). In addition, salinity tolerance of cereals may be related to the accumulation of Na^+ in old leaves and the continued transport of K^+ to young leaves (Wolf *et al.*, 1991, Yeo and Flower, 1986). High K^+ and low Na^+ concentrations in young leaves and increasing Na^+ concentrations in older leaves were found in *Hordeum vulgare* (Jeschke and Wolf, 1985) and *Atriplex spp.*, (Aslam *et al.*, 1986).

Gorham *et al.* (1986) postulated that distribution of salt and glycinebetaine between young and old leaves of salt-stressed plants (i.e. an increase in the level of salt in old leaves, and an accumulation of glycinebetaine in young leaves) would allow the younger leaves to support sufficient metabolic and physiological activity for survival under high salinity conditions. Accumulation of betaine in salinized plants, presumably for osmotic adjustment of the cytoplasm (Matoh *et al.*, 1987), is considered to be an important physiological trait contributing to the maintenance of growth under salinity (Rhodes and Hanson, 1993).

The purpose of the experiments reported here was to obtain a better understanding of the action of betaine in cotton, and its distribution within the plant when applied with and without NaCl to the soil and leaves. The experiment was designed to examine the effects of betaine and its interaction with NaCl salinity on growth, photosynthesis, transpiration, stomatal conductance, chlorophyll contents, glycinebetaine and ion concentrations.

4.2 Materials and Methods

4.2.1 Experiment 4.1 (Root-applied NaCl, $CaCl_2$ and betaine)

Seeds of *Gossypium hirsutum* cv. Acala SJ2 were sown on March 20, 1996, in pots containing 5 litres of John Innes No. 1 compost. The experiment was conducted at Pen-y-Ffridd field station under the same conditions as described in section 2 6. The nutrient regime was 1 g phostrogen and 0.5 cm^3 micronutrients solution per litre. 10 mol m^{-3} $CaCl_2$ was used in all the treatments including the control. The effect of $CaCl_2$ applied to the soil and leaves showed great differences in the results, hence soil and leaf treatments were separated.

The experiment comprised eight treatments with five replicates, containing four levels of betaine (0, 0.1, 1.0, and 10 mol m^{-3}) and two levels of NaCl (0 and 200 mol m^{-3}). Phostrogen, micronutrients, NaCl and $CaCl_2$ were mixed and dissolved in a container and applied 37 days after sowing. A stock solution (1 kmol m^{-3}) of betaine was prepared and stored in a cold room. Betaine was applied with nutrient solution to the roots (approx. 500 cm^3). The treatments were started 37 days after sowing and were applied daily for a further 37 days. Before treatments began, the plants were marked at the youngest fully expanded leaf.

Before harvesting, measurements of photosynthesis, transpiration and stomatal conductance were made on the youngest fully-expanded and old leaf using an IRGA (see section 2.8). SPAD readings were made by chlorophyll meter (details in section 2.9). Physical observations including total shoot height, total shoot fresh weight, number of nodes on main stem and also height, weight and nodes from the marked leaf were recorded. The thickness of young and old leaves was measured with a micrometer. Fresh weight of an uppermost expanded leaf and dry weight of the same leaf after oven drying at 70 °C for 48 hours were recorded. For ion analysis, the youngest fully-expanded leaf, its petiole and the stem below the node and an old leaf (one node above the marked leaf), petiole and stem samples were collected in micro-centrifuge tubes. For cations and anions the samples were analysed by ion exchange HPLC (for details see section 2.12.2). For betaine analysis, the same procedure described in section 2.14 was adopted.

4.2.2 Experiment 4.2 (Foliar-applied NaCl, $CaCl_2$ and betaine)

The material used in this experiment was the same as described in experiment 4.1. In this experiment, nutrient solution was applied to the root medium by watering and betaine and/or NaCl and $CaCl_2$ were applied to the leaves. Each leaf treatment was separated by plastic curtains during spraying. A 1.25 litre capacity hand sprayer was used to spray the plants. The height of the nozzle and the delivery pressure were adjusted so that all the plants in a treatment were thoroughly wetted on abaxial and adaxial sides of the leaves. The treatments and nutrients were applied 37 days after sowing for a further 37 days. The plants were sprayed daily in the morning. Before treatments, the soil surface within the pots was covered with a waterproof foam sheet to prevent foliar treatments from entering the substrate.

After treatments, the plants were washed thoroughly with tap water and surface dried. The wash would remove Na^+ and betaine from the surface without removing a large amount of any substance present in the tissues. Tap water contained 0.189 mol m^{-3} Na^+, 0.006 mol m^{-3} K^+, 0.055 mol m^{-3} Mg^{2+}, 0.097 mol m^{-3} Ca^{2+}, 0.204 mol m^{-3} Cl^-, 0.024 mol m^{-3} NO_3^-, 0.031 mol m^{-3} HPO_4^{2-} and 0.038 mol m^{-3} SO_4^{2-} (see Gorham *et al.* 1994). Physical and chemical observations were the same as in experiment 4.1.

Statistical Analysis:

Data were analysed using Describe and Anova (GLM) routines of the Minitab statistical package by using 4 levels of betaine x 2 NaCl with 5 replicates. The data are presented with standard error of the means and were tested for significant differences between means at $P>0.05$ using Tukey's test.

4.3 Results

4.3.1 Experiment 4.1 (Root-applied NaCl, $CaCl_2$ and betaine)

4.3.1.1 Growth Parameters

In general, additional betaine and its interaction with salinity did not significantly influence all growth parameters. However, total shoot fresh weight

increased significantly ($p \leq 0.005$) at 0 and 200 mol m^{-3} NaCl when betaine was applied at 10 mol m^{-3} compared with no additional betaine, whereas both heights consistently decreased with increasing concentrations of betaine. Salinity significantly ($p \leq 0.000$) decreased total shoot height, height above the marked leaf, shoot fresh weight, weight above the marked leaf, total nodes, nodes above the marked leaf. However, salinity significantly ($p \leq 0.000$) increased FW/DW ratio and thickness of young and old leaf (see Table 4.1 and Appendix 1).

Table 4.1 Growth parameters of *Gossypium hirsutum* L. treated with various concentrations of glycinebetaine (GB) with and without NaCl (0 and 200 mol m^{-3}) to the soil. The values are means of 5 replicates ± standard errors. (Experiment 4.1).

GB (mol m^{-3})	Height above marked leaf (mm)		Total shoot fresh weight (g)		Weight above marked leaf (g)		No. of nodes above marked leaf	
	0	200	0	200	0	200	0	200
0	722 ± 47	422 ± 11	322 ± 16	212 ± 17	180 ± 5	96 ± 8	8 ± 0	6 ± 0
0.1	714 ± 48	416 ± 28	320 ± 9	235 ± 12	191 ± 6	110 ± 6	8 ± 0	6 ± 0
1	666 ± 26	396 ± 5	312 ± 22	240 ± 6	164 ± 9	100 ± 2	7 ± 0	6 ± 0
10	598 ± 36	392 ± 27	368 ± 6	261 ± 9	172 ± 5	110 ± 9	7 ± 0	6 ± 0

4.3.1.2 Photosynthesis, Transpiration and Stomatal conductance

Initial Anova showed that applied betaine and its interaction with salinity did not produce significant effects on photosynthesis, transpiration and stomatal conductance. Two way anova (salinity x leaf age) was then used to analyse the data. Analysis of variance revealed that salinity had no significant effect on photosynthesis. Generally, photosynthesis was significantly ($p \leq 0.006$) higher in the young than in old leaves in the control and saline treatments. Salinity significantly decreased transpiration ($p \leq 0.003$) and stomatal conductance ($p \leq 0.000$). There were no significant differences between young and old leaves for transpiration, whereas stomatal conductance was significantly ($p \leq 0.025$) higher in the young than in old leaves. (see Table 4.2 and Appendix 1).

Table 4.2 Mean values (combining four concentrations of betaine) showing the effect of 0 and 200 mol m^{-3} NaCl applied to the soil on photosynthesis (μmol m^{-2} s^{-1}), transpiration (mmol m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) in young (Y) and old (O) leaves of *Gossypium hirsutum* L. . The values are means of 5 replicates (Experiment 4.1). P>0.05 (*), P>0.01(**), P>0.001 (***)

Treatments	Photosynthesis (CER)		Transpiration (E)		Stomatal Conductance (gs)	
NaCl (mol m^{-3})	Y. leaf	O. leaf	Y. leaf	O. leaf	Y. leaf	O. leaf
0	19	16	13	12	556	453
200	17	16	11	11	393	378

	Pn	E	gs
Age	***	ns	*
Salinity	ns	***	***

4.3.1.3 Betaine concentrations in young and old leaves, petioles and stems

Initial Anova revealed that exogenous betaine and its interaction with salinity had no significant effects on betaine concentrations in all tissues. Three way anova (salinity x tissues x age) was subsequently used to analyse the data. Generally, salinity significantly ($p \leq 0.000$) increased betaine concentrations in all the tissues. Leaf betaine concentrations were significantly ($p \leq 0.000$) higher than petiole and stem concentrations which were more or less the same. Betaine concentrations were significantly ($p \leq 0.000$) higher in the young than in the old leaves, whereas in the petioles and stems the difference between young and old tissue was not clear (see Table 4.3 and Appendix 1).

Table 4.3 Mean values (combining four concentrations of betaine) showing the effect of 0 and 200 mol m^{-3} NaCl applied to the soil on betaine concentrations (mol m^{-3} expressed sap) in young (Y) and old (O) leaves, petioles and stems of *Gossypium hirsutum* L. The values are means of 5 replicates (Experiment 4.1). P>0.001 (***)

NaCl (mol m^{-3})	Y. leaf	O. leaf	Y. petiole	O. petiole	Y. stem	O. stem
0	98	83	57	51	58	57
200	123	109	67	68	73	66

Tissue	***
Age	***
Salinity	***

4.3.1.4 Leaf Chlorophyll (SPAD)

Applied salinity and betaine significantly ($p \leq 0.000$) increased leaf chlorophyll measurements. Leaf chlorophyll was higher with 10 mol m^{-3} betaine compared to other lower levels of betaine in the control and saline treatments. Interaction between applied salinity and betaine was not significant for leaf chlorophyll (see mean values in Appendix 1).

4.3.1.5 Cation Analysis

Initial Anova showed that applied betaine and its interaction with salinity had no significant effects on cation and anion concentrations. Three way anova (salinity x tissues x age) was used to further analyse the data.

Salinity significantly increased Na^+ concentrations in all the tissues. Na^+ concentrations were significantly ($p \leq 0.000$) higher in the leaf than in petiole and stem tissues. There was no clear difference in Na^+ concentrations between petiole and stem tissues. Na^+ concentrations were significantly ($p \leq 0.000$) greater in old than in young tissues of leaf, petiole and stem.

Table 4.4 Mean values (combining four concentrations of betaine) showing the effect of 0 and 200 mol m^{-3} NaCl applied to the soil on Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations (mol m^{-3} expressed sap) in various tissues of *Gossypium hirsutum* L. The values are means of 5 replicates (Experiment 4.1). P>0.001 (***) Non-significant (ns)

	Young leaf		Old leaf		Young petiole		Old petiole		Young stem		Old stem	
	0	200	0	200	0	200	0	200	0	200	0	200
Na^+	6	82	7	119	3	50	4	76	3	56	3	71
K^+	129	128	95	85	218	234	274	287	190	199	164	120
Mg^{2+}	40	28	57	49	29	25	38	36	31	28	21	18
Ca^{2+}	84	55	173	113	48	30	62	52	32	21	20	15

	Na^+	K^+	Mg^{2+}	Ca^{2+}
Tissue	***	***	***	***
Age	***	***	***	***
Salinity	***	ns	***	***

200 mol m^{-3} NaCl did not produce significant effects on K^+ concentrations in the leaf, petiole and stem tissues. Petiole K^+ concentrations were significantly ($p \leq 0.000$) higher than leaf and stem K^+ concentrations. Stem K^+

concentrations were significantly higher than leaf K^+ concentrations. In the young leaf and stem, K^+ concentrations were significantly ($p \leq 0.000$) high compared with the old leaf and stem, whereas the opposite was true for petioles, where K^+ concentrations were significantly ($p \leq 0.000$) higher in the old than in young tissues. K^+ concentrations decreased in the old stems with salinity at all concentrations of betaine.

Salinity significantly ($p \leq 0.000$) decreased Mg^{2+} concentrations. Leaf Mg^{2+} was significantly ($p \leq 0.000$) higher than petiole and stem tissues. Mg^{2+} concentrations in young tissues of petiole and stem were more or less the same, whereas Mg^{2+} concentrations were significantly higher in the old petiole than in old stems. Mg^{2+} concentrations were higher in old leaf and petiole than in young leaf and petiole respectively, whereas the opposite results were found in stems, i.e. young stem Mg^{2+} was greater than old stem Mg^{2+} concentrations.

200 mol m^{-3} NaCl significantly ($p \leq 0.000$) decreased Ca^{2+} concentration in the leaf, petiole and stem tissues. Leaf Ca^{2+} was significantly ($p \leq 0.000$) higher than petiole and stem Ca^{2+} and petiole Ca^{2+} was significantly greater than stem Ca^{2+} concentrations. Ca^{2+} concentrations were significantly lower ($p \leq 0.000$) in the young tissues of leaf and petiole than old tissues, whereas the opposite was true for stem tissues (see Table 4.4 and Appendix 3 for cation concentrations).

4.3.1.6 Anion Analysis

Cl^- concentrations were significantly ($p \leq 0.000$) higher at 200 mol m^{-3} NaCl than at 0 mol m^{-3} NaCl in the leaves, petioles and stems. Cl^- concentrations were significantly ($p \leq 0.000$) higher in the leaves than in petioles and old stems. Cl^- concentrations in young petiole and stem were significantly ($p \leq 0.000$) greater than in old petiole and stem respectively, whereas the opposite was true for leaves.

Salinity significantly ($p \leq 0.000$) decreased malate concentrations in the leaf, petiole and stem tissues. Malate concentrations in young tissues were in the order stem>>petiole>leaf in the control, whereas in old tissues, they were significantly higher in leaf than in petiole and stem tissues. Malate concentrations

were significantly ($p \leq 0.016$) higher in old than in young leaves, whereas in stems, malate was greater in young than in old tissues. There was no clear cut difference in malate concentrations between young and old petioles. Negative correlations in old leaf (-0.46**) and in old petiole (-0.42**) were observed between malate and Cl^- concentrations.

Table 4.5 Mean values (combining four concentrations of betaine) showing the effect of 0 and 200 mol m^{-3} NaCl applied to the soil on Cl^-, malate and NO_3^- concentrations (mol m^{-3} expressed sap) in various tissues of *Gossypium hirsutum* L. The values are means of 5 replicates (Experiment 4.1). P>0.05 (*), P>0.01(**), P>0.001 (***), Non-significant (ns).

	Young leaf		Old leaf		Young petiole		Old petiole		Young stem		Old stem	
	0	200	0	200	0	200	0	200	0	200	0	200
Cl^-	202	263	224	376	159	223	88	148	226	309	114	130
malate	42	22	68	38	33	27	37	23	91	44	36	25
NO_3^-	19	28	52	40	15	23	8	13	1	0	0	0

	Cl^-	malate	NO_3
Tissue	***	***	***
Age	***	*	ns
Salinity	***	***	ns

There were no significant effects of salinity and tissue's age on NO_3^- concentrations. Leaf NO_3^- was significantly ($p \leq 0.000$) higher than petiole and stem NO_3^- concentrations. NO_3^- concentrations were higher in old than in young leaves, whereas the opposite was true for petioles. Negligible NO_3^- was present in stems. Mean values of Cl^-, malate and NO_3^- concentrations are shown in Table 4.5 and Appendix 3.

4.3.2 Experiment 4.2 (Foliar-applied NaCl, $CaCl_2$ and betaine)

4.3.2.1 Growth Parameters

200 mol m^{-3} NaCl significantly increased shoot height, shoot fresh weight, number of nodes and also the same measurements above the marked leaf. FW/DW ratio and old leaf thickness were significantly ($p \leq 0.000$) higher at 200 mol m^{-3} NaCl compared with the control. Betaine at 0.1 mol m^{-3} significantly increased total shoot height, height above the marked leaf, total number of nodes and number of nodes above the marked leaf ($p \leq 0.007$, $p \leq 0.029$, $p \leq 0.018$ and

$p \leq 0.029$ respectively) compared with no added betaine at 0 and 200 mol m^{-3} NaCl. However, additional betaine did not produce significant effects for other growth parameters. Interaction between salinity and betaine was not significant for all growth parameters (see mean values in Table 4.6 and Appendix 2).

Table 4.6 Growth parameters of *Gossypium hirsutum* L. treated with various concentrations of glycinebetaine (GB) with and without NaCl (0 and 200 mol m^{-3}) applied on the leaves. The values are means of 5 replicates ± standard errors. (Experiment 4.2).

GB (mol m^{-3})	Height above marked leaf (mm)		Total shoot fresh weight (g)		Weight above marked leaf (g)		No. of nodes ab. marked leaf	
	0	200	0	200	0	200	0	200
0	470 ± 24	568 ± 38	378 ± 8	461 ± 24	146 ± 10	223 ± 47	5 ± 1	7 ± 0
0.1	602 ± 49	690 ± 50	372 ± 10	481 ± 17	173 ± 16	244 ± 24	7 ± 0	8 ± 0
1	486 ± 17	648 ± 26	379 ± 19	468 ± 13	118 ± 19	252 ± 11	6 ± 0	7 ± 0
10	530 ± 38	602 ± 58	369 ± 15	433 ± 19	164 ± 25	232 ± 19	7 ± 1	7 ± 0

4.3.2.2 Photosynthesis, Transpiration and Stomatal conductance

Initial Anova showed that applied betaine did not produce significant effects, 2 way anova (salinity x leaf age) was then used. Salinity significantly decreased net photosynthesis ($p \leq 0.027$), transpiration ($p \leq 0.000$) and stomatal conductance ($p \leq 0.003$) in the old leaves, whereas the young leaves were not significantly affected by salinity for the same measurements. Photosynthesis, transpiration and stomatal conductance were significantly ($p \leq 0.000$) higher in young than in old leaves under saline conditions, whereas there was no clear difference at the control (see Table 4.7 and Appendix 2).

Table 4.7 Mean values (combining of 4 concentrations of betaine) showing the effect of 200 mol m^{-3} NaCl applied on the leaves on photosynthesis (μmol m^{-2} s^{-1}), transpiration (mmol m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) in young (Y) and old (O) leaves of *Gossypium hirsutum* L. The values are means of 5 replicates (Experiment 4.2). P>0.05 (*), P>0.01(**), P>0.001 (***).

Treatments NaCl (mol m^{-3})	Photosynthesis (CER)		Transpiration (E)		Stomatal Conductance (gs)	
	Y. leaf	O. leaf	Y. leaf	O. leaf	Y. leaf	O. leaf
0	18	18	12	13	467	517
200	19	11	11	8	512	264
	Pn		E		gs	
Age	***		*		***	
Salinity	*		***		***	

4.3.2.3 Betaine concentrations in young and old leaves, petioles and stems

Initial Anova revealed the additional betaine did not show significant effects for betaine concentrations in all the tissues, hence a 3 way anova (salinity + tissue + age) was used.

Foliar application of 200 mol m^{-3} NaCl significantly ($p \leq 0.002$) decreased betaine concentrations in all tissues. Leaf betaine was significantly ($p \leq 0.000$) higher than petiole and stem betaine concentrations, whereas no clear cut difference in betaine concentrations between petiole and stem tissues was observed. Betaine concentrations in the young leaves were significantly ($p \leq 0.000$) higher than in old leaves, while in the young and old petioles and stems, betaine concentrations were more or less the same (see mean values in Table 4.8 and Appendix 2).

Table 4.8 Mean values (combining of 4 concentrations of betaine) showing the effect of 0 and 200 mol m^{-3} NaCl applied on the leaves on betaine concentrations (mol m^{-3} expressed sap) in young (Y) and old (O) leaves, petioles and stems of *Gossypium hirsutum* L. The values are means of 5 replicates (Experiment 4.2). P>0.001 (***).

NaCl (mol m^{-3})		Y. leaf	O. leaf	Y. petiole	O. petiole	Y. stem	O. stem
0		102	93	73	74	66	66
200		101	70	61	56	54	54
Tissue	***						
Age	***						
Salinity	***						

4.3.2.4 Leaf Chlorophyll (SPAD)

Salinity significantly ($p \leq 0.000$) lowered leaf chlorophyll (SPAD). Leaf chlorophyll was higher when betaine was applied at 10 mol m^{-3} at 0 and 200 mol m^{-3} NaCl, but the difference was not significant (Appendix 2).

4.3.2.5 Cation Analysis

Initial Anova showed that additional betaine with various levels did not show significant effects on all cations and anions, hence a 3 way anova (salinity + tissue + age) was used.

Salinity significantly ($p \leq 0.000$) increased Na^+ concentrations in all tissues. Leaf Na^+ concentrations were significantly ($p \leq 0.000$) greater than petiole and stem Na^+ concentrations. Na^+ concentrations were higher in petioles than in stems. All old tissues had significant ($p \leq 0.000$) higher concentrations of Na^+ compared with young tissues.

K^+ concentrations were significantly ($p \leq 0.000$) lower with NaCl (200 mol m^{-3}) in leaf and stem tissues, whereas petioles were less affected by salinity. K^+ concentrations were significantly ($p \leq 0.000$) greater in petioles than in leaf and stem tissues in the control and salinity treatment. Stem K^+ was higher than leaf K^+ concentrations. Young tissues of leaf and stem were significantly ($p \leq 0.000$) higher in K^+ concentration than old tissues, whereas the opposite was true for petiole tissues.

Table 4.9 Mean values (combining four concentrations of betaine) showing the effect of 0 and 200 mol m^{-3} NaCl applied on the leaves of *Gossypium hirsutum* L. on Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations (mol m^{-3} expressed sap) in various tissues. The values are means of 5 replicates (Experiment 4.2). P>0.05 (*), P>0.01(**), P>0.001 (***), Non-significant (ns).

	Young leaf		Old leaf		Young petiole		Old petiole		Young stem		Old stem	
	0	200	0	200	0	200	0	200	0	200	0	200
Na^+	7	151	20	346	2	21	4	64	2	18	4	38
K^+	148	68	130	24	217	188	268	263	173	146	154	134
Mg^{2+}	50	31	77	31	32	30	43	45	33	27	25	23
Ca^{2+}	86	52	175	59	33	34	46	53	21	21	16	15

	Na^+	K^+	Mg^{2+}	Ca^{2+}
Tissue	***	***	***	***
Age	***	ns	***	***
Salinity	***	***	***	***

Significant ($p \leq 0.000$) decreases in Mg^{2+} concentrations were observed in leaves under salinity, whereas petiole and stem tissues were less affected. In the control, leaf Mg^{2+} concentrations were significantly ($p \leq 0.000$) higher than in petioles and stems and old stem Mg^{2+} concentrations were greater than in old petioles. Under salinity, Mg^{2+} concentrations in old petioles were significantly higher than in leaves and stems. Mg^{2+} concentrations were significantly ($p \leq 0.000$) greater in old than in young leaves in the control, while little difference was

observed with salinity. Mg^{2+} concentrations of old petioles were also significantly higher than in young petiole in both saline and non-saline treatments.

Ca^{2+} concentrations were significantly ($p \leq 0.000$) lower in the leaves with salinity, while petioles and stems were not affected as for Mg^{2+}. Leaf Ca^{2+} was significantly ($p \leq 0.000$) higher than petiole and stem Ca^{2+} and petiole Ca^{2+} was also significantly higher than stem Ca^{2+} concentrations in the control and salinity treatments. Ca^{2+} concentrations in the old leaves and petioles were significantly ($p \leq 0.000$) greater than in young leaves and petioles, whereas the opposite results were observed for stems. Mean values of Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} concentrations are shown in Table 4.9 and Appendix 4.

4.3.2.6 Anion Analysis

200 mol m^{-3} NaCl significantly ($p \leq 0.000$) increased Cl^- concentrations in all tissues. Generally, young stem Cl^- concentrations were significantly ($p \leq 0.000$) higher than petiole and leaf Cl^- concentrations in saline and non-saline treatments. At 200 mol m^{-3} NaCl, Cl^- concentrations were significantly higher in the old leaves than in both petioles and old stems. The lowest Cl^- concentrations were observed in the old petioles compared with leaf and stem tissues in the control and salinity. Cl^- concentrations in the young petioles and stems were significantly ($p \leq 0.000$) greater than in old petioles and stems respectively, whereas the opposite was true for the leaf tissues.

Table 4.10 Mean values (combining four concentration of betaine) showing the effect of 0 and 200 mol m^{-3} NaCl applied on the leaves of *Gossypium hirsutum* L. on Cl^-, NO_3^- and malate concentrations (mol m^{-3} expressed sap) in various tissues. The values are means of 5 replicates (Experiment 4.2). P>0.05 (*), P>0.01(**), P>0.001 (***), Non-significant (ns).

	Young leaf		Old leaf		Young petiole		Old petiole		Young stem		Old stem	
	0	200	0	200	0	200	0	200	0	200	0	200
Cl^-	69	143	78	236	67	117	26	71	92	358	84	136
malate	62	41	94	66	45	32	43	36	120	16	47	26
NO_3^-	37	30	68	70	25	24	13	15	3	0	1	0

	Cl^-	malate	NO_3^-
Tissue	***	***	***
Age	***	ns	*
Salinity	***	***	ns

Salinity significantly ($p \leq 0.000$) decreased malate concentration in all tissues. Malate concentrations were significantly ($p \leq 0.000$) higher in the leaves than in petioles and stems with an exception in young stems in the control. Analysis of variance revealed non-significant effect of tissue age for malate concentrations. However, malate was higher in the old than in young leaves. Significant negative correlations in the young (-0.59**) and in the old (-0.41**) leaves were observed between Cl^- and malate concentrations.

Analysis of variance showed no significant effect of salinity, whereas significant effects of various tissues ($p \leq 0.000$) and their ages ($p \leq 0.014$) on NO_3^- concentrations were found. Leaf NO_3^- was higher than petiole and stem NO_3^- concentrations. NO_3^- concentrations were greater in old than in young leaves, whereas the opposite results were occurred in petioles. Negligible NO_3^- was found in stems. Mean value of Cl^-, malate and NO_3^- concentrations are given in Table 4.10 and Appendix 4.

4.4 Discussion

The objectives of experiments described in this chapter were to determine the effects of NaCl salinity and glycinebetaine 1) applied to the soil, 2) sprayed on the leaves, on the growth and mineral ions in cotton.

$CaCl_2$ (10 mol m^{-3}) was applied to the soil and leaves in the controls (0 mol m^{-3} NaCl). This altered the results of growth at both modes of application. If there had been a treatment with no $CaCl_2$, it could be possible to compare the differences in the treatments. With combined four concentrations of betaine,. height and shoot fresh weight above the marked leaf were greater in the soil than in the leaf treatment i.e. the height was 675 (mm) in the soil and 522 (mm) in the leaf treatment whereas, weight was 177 (g) in the soil and 150 (g) in the leaf treatment. The absorption rates of Ca^{2+} are different in roots and leaves. These results suggest that uptake of Ca^{2+} is more efficiently by the roots than by the leaves. The petiole and stem tissues showed higher Ca^{2+} concentrations in the soil than leaf treatment. It is possible that an increase of growth in the soil treatment could be because, Ca^{2+} may activate some hormones (e.g. gibberellin or cytokinin)

to stimulate the growth in the soil-treated $CaCl_2$. The maintenance or stimulation of growth by Ca^{2+} is dependent upon an increase in cell length and cell division (Kurth *et al.*, 1986). The transport of Ca^{2+} into the root may be significant in these growth responses.

The reduction in shoot heights, shoot fresh weights and number of nodes on main stem was significantly lower with 200 mol m^{-3} NaCl applied to the soil compared with 0 mol m^{-3} NaCl. However, the same parameters were higher when the same concentration of salt was applied on the leaves of cotton compared with non-saline treatments. Generally, no differences in the growth parameters were observed with additional betaine either applied alone or with NaCl to the soil and leaves. It might be that the opposite trends for these parameters with 200 mol m^{-3} NaCl applied to the leaves were due to additional 10 mol m^{-3} $CaCl_2$ when applied to the leaves. Epstein (1961) reported that the addition of Ca^{2+} would neutralise the harmful effects of Na^+ in various plants. Adding supplementary Ca^{2+} to saline media increased shoot growth and decreased Na^+ uptake in cotton (Cramer *et al.*, 1987, Kent and Läuchli, 1985), barley (Cramer *et al.*, 1989 and Suhayda *et al.*, 1992), maize (Maas and Grieve, 1987). The role of Ca^{2+} in maintaining membrane integrity and selective ion transport is well known (Colmer *et al.*, 1994). They further described the essential role of Ca^{2+} especially in K^+ transport in the cell and functioning of Ca^{2+} in plant nutrition.

The same concentrations of $CaCl_2$ was also given to soil-treated plants, where the results showed a significant decrease in growth parameters at 200 mol m^{-3} NaCl salinity. It seems that high external Na^+ strongly reduces the chemical activity of Ca^{2+} ions in the root medium, and thus decreases the amount of Ca^{2+} that is available for uptake by the plant (Cramer and Läuchli, 1986, Cramer *et al.*, 1986). Adding Ca^{2+} at 2 and 10 mol m^{-3} to the saline treatment (100 mol m^{-3} NaCl) did not significantly reverse the inhibitory effect of NaCl on the growth of cotton plant (Leidi *et al.*, 1991). The presence of 50 to 100 mol m^{-3} NaCl in the nutrient medium decreased the growth rate of cotton cv. Acala (Gouia *et al.*, 1994). Salinity affects the growth of cotton by raising the osmotic pressure

of the soil solution and thus reducing the uptake of water by plant roots (Abd-Ella and Shalaby, 1993).

In experiment 4.2, in the old leaves, rate of photosynthesis, transpiration, stomatal conductance and chlorophyll measurements were lower at all concentrations of betaine when 200 mol m^{-3} NaCl was applied on the leaves while, young leaves were not significantly affected by salinity. The results show (Table 4.9) that Na^+ concentrations were highest in the old leaves when salt was applied on the leaves. High Na^+ concentrations in the leaves probably decreased photosynthesis, transpiration and stomatal conductance. Reduction in net photosynthesis rate caused in the mature leaves due to excessive accumulation of ions is reported by Cramer and Bowman (1991), Greenway and Munns (1980) and Munns and Termaat (1986). Benes *et al.* (1996) reported that saline sprinkling or saline soil (47 mol m^{-3} and 23.5 mol m^{-3} $CaCl_2$) significantly reduced leaf stomatal conductance in barley. K^+ concentrations were significantly reduced in the old leaves with foliar application of salt. K^+ deficiency in the leaves could cause decreasing photosynthesis and stomatal conductance.

In general, betaine concentrations were not increased with increasing concentrations of external betaine in all tissues of different ages when applied to the leaves or soil. Exogenous betaine did not increase vegetative growth of cotton. This may have been due to the breakdown and metabolism of betaine by micro-organisms in the soil, thus preventing its uptake and action in the plants. Another reason could be that, the external concentration of betaine might be low, therefore, the internal concentration of betaine did not change. Makela *et al.* (1996) reported that the optimum betaine concentration producing advantageous effects on growth and crop physiology in turnip rape and wheat was 100 and 300 mol m^{-3} respectively.

Generally, betaine concentrations were higher in young than in old tissues of leaf, petiole and stem under the control and saline conditions in both experiments. Nakamura *et al.* (1996) found that the level of glycinebetaine in young leaf blades of *Hordeum vulgare* was approximately three times that in old leaf blades. That betaine concentrations were much higher in young than in old

leaves is also reported by Gorham (1996) in cotton and Colmer *et al.* (1995) in wheat. In young and old leaves, petiole and stems, betaine concentrations were generally higher when 200 mol m^{-3} NaCl was applied to the soil compared with the control. Gorham (1996) found that with increasing salt stress in cotton, betaine concentrations also increased. When 200 mol m^{-3} NaCl was applied to the leaves, betaine concentrations decreased especially in old leaves. This could be explained in that these leaves were overloaded with Na^+, which may have resulted in low concentrations of betaine.

Na^+ concentrations in all tissues increased strongly with age in both experiments. In contrast to Na^+, K^+ concentrations were higher in young leaves and stems, but K^+ concentrations were higher in old than in young petioles. Aslam *et al.*, (1986) found that leaves of *Atriplex amnicola* grown at 25-750 mol m^{-3} NaCl, had low Na^+ concentrations when young, but very high Na^+ concentrations when old. They also found that K^+ concentrations were higher in young rapidly expanding leaves rather than in the old leaves. Wolf *et al.* (1990 and 1991) demonstrated increased losses of K^+ from the oldest leaf and stem of barley grown with 100 mol m^{-3} NaCl. The findings are in agreement with the results of Bhatti *et al.* (1993), who found that in *Leptochloa fusca* leaves, Na^+ concentrations increased strongly with leaf age, while K^+ concentrations were highest in young leaves and decreased with leaf age. At the whole plant level this reflected an efficient partitioning of K^+ and Na^+ between growing and mature tissues, which has been attributed to a preference of phloem translocation for K^+ (Jeschke, 1984). Regarding this distribution of Na^+ and K^+, cotton resembled *Hordeum vulgare* (Jeschke and Wolf, 1985), species of *Atriplex* (Jeschke and Stelter, 1983) as well as *Ricinus communis* (Jeschke and Wolf, 1988). K^+ concentrations in the petioles were high, this indicated that K^+ may have been derived by phloem imported from their corresponding leaves and stem.

Foliar application of saline water increased Na^+ concentrations in leaf tissues compared with soil application, whereas the opposite was true for the petiole and stem tissues. Benes *et al.* (1996) reported that saline spraying increased leaf sap Na^+ concentrations much more than did soil salinity in maize,

even though the saline spray was given only two or three times per week for 30 minutes, whereas the roots of plants grown in soil saline were continuously exposed to salinity. The results of these experiments suggest that cotton roots effectively restricted Na^+ uptake when NaCl was applied to the soil, while leaves could include Na^+ when NaCl was applied on the leaves. It is, therefore, concluded that cotton leaves lack the ability to maintain K^+ in the presence of high Na^+ as K^+ was replaced by Na^+ when leaves were sprayed with saline water. This is not the case when saline water was applied to the soil.

In experiment 4.1, 200 mol m^{-3} NaCl applied to the soil did not decrease K^+ concentration in various tissues at both age stages with an exception in old stems. There were significant negative correlations (-0.49**) between Na^+ and K^+ concentrations in old stems, whereas positive correlations in young petioles (0.59**) and stems (0.46**) were found in soil treatment. Salt-tolerant glycophytes also possess the ability to maintain adequate K^+ in the presence of high Na^+ concentrations in the soil, thus maintaining favourable K^+:Na^+ ratios in the foliage (Benes *et al.*, 1996). From the results of these experiments, it was found that cotton could tolerate high salinity and establish favourable K^+-Na^+ relations under soil salinity. However, in foliar salinity, cotton could not maintain high potassium as K^+ was replaced by Na^+. Significant inverse correlation between Na^+ and K^+ concentrations in young (-0.67**) and in old leaves (-0.91**) and also in old stems (-0.44**) was observed in the leaf treatment. The results are in agreement with the statement of Benes *et al.* (1996), who reported that in both maize and barley, K^+ concentrations in leaf sap were decreased in saline sprinkled plants when leaves were given 30 mol m^{-3} NaCl and 2.8 mol m^{-3} $CaCl_2$. To enhance K^+:Na^+ discrimination is considered to be an important selection criterion in cereals for the development of genotypes with improved salt tolerance (Gorham and Wyn Jones, 1993).

The results presented in Table 4.4 and 4.9 show that Mg^{2+} and Ca^{2+} concentrations were low in young leaves and petioles and increased with age. The distribution of Mg^{2+} and Ca^{2+} within the shoot of *Leptochloa fusca* as affected by external salinity was reported by Jeschke *et al.* (1995) who found that Mg^{2+} and

Ca^{2+} concentrations increased with leaf age. Bhatti *et al.* (1993) suggested that both of these cations had been imported via the transpiration stream in excess of phloem export and accumulated with time.

Foliar application of 200 mol m^{-3} NaCl significantly decreased Mg^{2+} and Ca^{2+} concentrations in leaves, as it did for K^+, whereas little effect on petioles and stems was observed. Soil salinity significantly decreased Ca^{2+} concentrations in leaves, whereas petiole and stems were less affected. Ca^{2+} concentrations in both leaves decreased much more with foliar saline water rather than soil salinity. The effect was more in old than in young leaves since the highest concentrations of Na^+ were found in old leaves. High Na^+ reduces Ca^{2+} concentrations and causes Ca^{2+} deficiency in barley plants (Lynch and Läuchli, 1985). The results for Ca^{2+} agree with the statement of Benes *et al.* (1996), who found that in maize, saline sprinkling water and saline soil decreased leaf sap Ca^{2+} compared with the control. The effect was more pronounced in saline sprinkling rather than in saline soil treatments. Gorham *et al.* (1994) also found that Ca^{2+} concentrations were lower in flag leaves of barley cv. CM67, when 150 mol m^{-3} NaCl was applied on leaves rather than soil-applied. Generally, Mg^{2+} concentrations were low in all the tissues under soil salinity treatments.

Cl^- concentrations increased with increasing concentrations of external salinity. Cl^- concentrations were lower when salt was applied on the leaves rather than when applied to the soil in all tissues. However, in young and old stems under highly saline treatments, the effect was not clear. The data serve to support the evidence of Gorham *et al.* (1994), who found that when 150 mol m^{-3} NaCl was given to barley, Cl^- concentrations were significantly higher in soil salinity rather than when salt was applied on the leaves. Under highly saline treatments, negative correlation between Cl^- and Na^+ in young and old leaves in soil and foliar treatments was found i.e. an increase of Na^+ and a decrease of Cl^- in foliar treatments, while a decrease of Na^+ and an increase of Cl^- in soil treatments was observed.

Cl^- concentrations were higher in old than in young leaves, whereas the opposite was true for petiole and stems. These findings are in agreement with

the results of Jeschke and Pate (1991) and Lleonart (1994), who found that Cl^- concentrations increased with age of leaves of *Ricinus* and barley respectively. Cl^- concentrations were higher than Na^+ concentrations in petiole and stem tissues. Petioles and stems may be able to absorb Cl^- from the transpiration stream, thereby considerably protecting leaves from salt damage.

Malate concentrations in the leaves rose with leaf age. Malate decreased in both experiments. Klagges *et al.* (1993) found that malate concentrations in *Leptochloa fusca* rose with leaf age. Negative correlations between malate and chloride were observed in various tissues (see section 4.3.1.6 and 4.3.2.6). In the soil treatments, Cl^- concentrations were higher than leaf treatments and malate was lowered, whereas the opposite was true for leaf treatments.

The effect of soil and foliar salinity showed major alterations to increase leaf thickness. The thickness was more in young and old leaves when salt was applied on the leaves rather than when applied to the soil, but the effect was more pronounced in the old leaves. These findings support the evidence of Alpha *et al.* (1996) who reported that leaf thickness of *Scaevola sericea* increased under high salinity in substrate and salt spray treatments, however the effect was less in substrate rather than salt spray treatments. Zekri and Parsons (1990) found that the leaf thickness of *Citrus aurantium* under NaCl stress increased due to the development of larger cells in the spongy mesophyll.

4.5 Conclusions

- Additional betaine neither increased vegetative growth and photosynthesis of cotton nor decreased Na^+ concentrations of all tissues.
- Betaine concentrations were higher in the young than in old leaf, petiole and stem tissues under saline and non-saline conditions.
- Betaine concentrations were higher in the leaves than in petioles and stems.
- K^+ concentrations in young and old leaves were not decreased with NaCl (200 mol m^{-3}) applied to the soil while, K^+ concentrations were severely decreased

with the same concentrations of NaCl applied on the leaves. The effect was greater in old than in young leaves.

- K^+ concentration in the petiole and stem tissues were less affected by 200 mol m^{-3} NaCl either applied to the soil or leaves.
- Na^+ concentrations were higher in the old than in young leaves, whereas the opposite was true for K^+ concentrations.
- Na^+, Mg^{2+} and Ca^{2+} concentrations were greater in the leaves than petioles and stems, while K^+ concentrations were in the order petiole>>stem>leaf.
- Cl^- concentrations were higher in stems than in leaves and petioles, while NO_3^- concentrations were greater in leaves than in petioles and stems.

CHAPTER FIVE

SODIUM AND POTASSIUM INTERACTIONS

CHAPTER 5

SODIUM AND POTASSIUM INTERACTIONS

5.1 Introduction

After nitrogen, K^+ is the mineral nutrient required in the largest amount by plants. Keino *et al.* (1995) reported that K^+ is second only to nitrogen as the most limiting nutrient for cotton production. Cotton appears to be more sensitive to low K^+ availability than other field crops (Cassman *et al.*, 1989). There have been numerous research reports involving soil-applied K^+ (e.g. Kerby and Adams, 1985), but only a few on the usefulness of foliar-applied K^+ (Oosterhuis, 1993). Oosterhuis and Janes (1993) found that foliar-applied K^+ in Arkansas increased lint yield of cotton by 51 and 14 lb/acre compared to the low and high soil-applied K^+ treatments respectively. Oosterhuis and Janes (1993) suggested that foliar application of K^+ has the advantage of rapid absorption into the leaf and efficient movement to the developing cotton bolls. Foliar application of cotton with different potassium sources has been researched in Arkansas and other southern states. Foliar KNO_3 has been the preferred K^+ source for cotton (Snyder *et al.*, 1994). Foliar KNO_3 increased the yield of two cultivars of cotton in 1992 at the trials in Arkansas (Janes *et al.*, 1993).

Salinity generally decreases plant growth at low concentration and is lethal at higher concentration. Salt-affected plants appear darker green and are stunted, have shorter and fewer internodes, or may develop succulence or have their growth inhibited (Shannon *et al.*, 1994). Pessarakli and Tucker (1985) reported that low levels of salinity (-0.4 MPa osmotic potential) slightly enhanced the dry matter production and increased the yield of cotton. Leaf growth is an important process in crop production system, characterised by the production rate of new leaves, the total number of leaves produced, the rate of emergence and duration of area of each leaf (Warrington and Kanemasu, 1983). Increase in leaf area was found to be more sensitive to salinity than either leaf emergence rate or dry matter accumulation in *Hibiscus cannabinus* (Curtis and Läuchli, 1987). In bean plants, reduced leaf growth and thicker and smaller leaves were observed

under NaCl salinity (Meiri, 1984). A decline in total leaf area is often the first detectable response of salt or water stress in crop plants (Bradford and Hsiao, 1983).

Addition of K^+ to a saline culture solution has been found to increase the dry weight and K^+ content of the shoots with a corresponding decrease in Na^+ content in rice (Shah Muhammad *et al.*, 1987). The higher potassium requirement in the leaves of spinach plants exposed to salinity stress is primarily caused by the necessity to maintain high guard cell K^+ concentrations (Chow *et al.*, 1990). They further reported that K^+ demand for optimal photosynthesis in the leaf tissue of spinach plants is at least twice as high as in non-saline substrate.

Sprinkler irrigation tends to increase Na^+ and Cl^- concentrations in the leaves, indicating substantial direct leaf uptake from the irrigation water. The levels of these two mineral elements in the leaves therefore become toxic rapidly when saline water is used for sprinkler irrigation (Maas, 1985). With sprinkler irrigation, the K^+ content of the leaves is lower and declines with increasing concentration of salt. This indicates export of K^+ from the leaves, and enhancement of this process by Na^+/K^+ replacement within the leaf tissue (Marschner, 1995).

The two forms of salt application, spray on the leaves and salt in the rooting zone show different results. Accordingly, the level of crop tolerance to saline spray may differ from that for tolerance to soil salinity (Maas, 1985). Barley varieties are different in the accumulation of Na^+, K^+ and Cl^- when salt is applied to soil or leaves (Gorham *et al.*, 1994). Many crops accumulate salts through the leaves when they are wetted by saline waters. This accumulation may cause foliar injury and decrease crop yield (Maas *et al.*, 1982). The injury varies among crop species and depends more on leaf characteristics and rate of foliar salt absorption than on tolerance to soil salinity (Maas, 1990).

The results obtained in experiment 4.2 showed that K^+ concentrations were lower when NaCl was applied to the leaves compared with soil-applied salinity. To increase K^+ concentrations, KCl was applied to the soil

and leaves with and without NaCl to see its effects on growth and ion concentrations.

5.2 Materials and Methods

5.2.1 Experiment 5.1 (Method of NaCl and KCl application)

The objective of this experiment was to investigate physiological responses of cotton to foliar-and soil-applied KCl and NaCl. The possible enhancement of growth of cotton plant in response to the application of potassium under saline and non-saline conditions as examined.

The experiment was conducted in a heated greenhouse at Pen-y-Ffridd field station as described in section 2.6. Seeds of cotton (*Gossypium hirsutum* L. cv. Acala SJ2) were sown in pots containing 5 l of compost. The nutrient regime was 1g phostrogen and 0.5 cm^3 micronutrients solution per litre and started 32 days after sowing and applied daily. Additional $CaCl_2$ was not given to the plants as applied in experiments of Chapter 4. In those experiments, $CaCl_2$ (10 mol m^{-3}) applied to the soil and foliar showed different results of growth and ion concentrations. As the aims of this experiment were to assess the effects of KCl, therefore extra $CaCl_2$ was not applied. There were eighteen treatments containing three levels of KCl (0, 1 and 10 mol m^{-3}), two levels of NaCl (0 and 200 mol m^{-3}) and three ways of application of KCl and NaCl i.e. NaCl applied to the soil and KCl to the leaves and NaCl applied to the leaves and KCl to the soil.

Thirty two days after sowing, the pots were covered with foam sheet with a hole for the stem to prevent foliar treatments from entering the soil. The soil-treated pots were also covered to provide the same environmental conditions to all the treatments. The treatments were started 32 days after sowing and applied daily in the morning for a further 30 days. Before starting the treatments, the youngest fully-expanded leaf was marked. After the final treatment, the plants were washed thoroughly with tap water and surface-dried leaf samples (young and old) were taken for ion analysis (for details see section 2.12.2). Growth measurements, photosynthesis, transpiration, leaf chlorophyll estimations were taken at harvesting the plants.

Statistical Analysis

Data were analysed using Describe and Anova (GLM) routines of the Minitab statistical package by using 3 levels of KCl x 2 NaCl x 2 modes of NaCl and KCl application with 5 replicates. The data are presented with standard error of the means and were tested for significant differences between means at $P>0.05$.

5.2.2 Experiment 5.2 (Optimum level of KCl)

This experiment was designed on the basis of the results of experiment 5.1, from which it could be concluded that additional K^+ increased vegetative growth of cotton plants and reduce the effects of salinity, when both KCl and NaCl were applied to the leaves. The objectives of this experiment were to ameliorate sodium hazards in cotton by foliar application of potassium and find the optimum dosage of potassium under foliar salinity.

Identical materials and environmental conditions were used in this experiment as in experiment 5.1. In experiment 5.2, all the treatments were applied to the leaves. The experiment comprised 12 treatments with 5 replications. A range of six KCl concentrations was chosen (0, 10, 25, 50, 100, and 150 mol m^{-3}) with two concentrations of NaCl (10 and 150 mol m^{-3}).

Approximately one month from the date of sowing, the plants were covered with foam sheet 'lids' to prevent spray dripping in the soil. Before starting the treatments, the youngest fully expanded leaves were marked with a plastic coated wire. The plants were treated daily in the morning for 32 days. After the final treatments, the plants were washed thoroughly and dried. The youngest fully-expanded leaf and old leaf (above the marked leaf) were chosen as standard leaves because of the steep gradient in ion concentrations from young to old leaves. Sap was extracted from frozen/thawed and crushed samples by centrifugation (see section 2.10). Inorganic ion analyses were performed on a Dionex ion chromotograph (details in section 2.12.2). Plants were harvested after taking the same observations as described in experiment 5.1. Total number of leaves per plant was counted and their leaf area (cm^2) was measured using an automatic leaf area meter.

Statistical Analysis: as experiment 5.1

5.2.3 Experiment 5.3 (Accompanying anions of potassium)

The purpose of this experiment was to examine the best accompanying anion of K^+ to decrease the effect of NaCl salinity. Furthermore, the relationship between petiole and leaf ions was examined.

The experiment was conducted at Pen-y-Ffridd field station under the same greenhouse conditions as experiments 5.1 and 5.2. Seeds of *Gossypium hirsutum* L. cv. Acala SJ2 were used. There were six treatments with 5 replicates. The various accompanying anions of K^+ were used, i.e. sulphate, chloride, phosphate, nitrate and acetate in this experiment. Solutions of these K^+ sources were prepared at the rate of concentrations of 10 mol m^{-3} (5 mol m^{-3} K_2SO_4) with 150 mol m^{-3} NaCl.

After approximately 40 days, the pots were covered with foam sheets, the youngest fully expanded leaf was marked and the treatments started and continued for a further 35 days. All treatments were carried out via foliar application. After the final treatment, the plants were washed thoroughly with tap water and surface-dried leaf and petiole samples (young and old) were taken for ion analysis (for details see section 2.12.2). Photosynthesis, transpiration, stomatal conductance and growth measurements were taken at the time of harvesting the plants as for experiments 5.1 and 5.2.

Statistical Analysis: Data were analysed using one way Anova.

5.3 Results

5.3.1 Experiment 5.1 (Method of NaCl and KCl application)

5.3.1.1 Growth parameters

Total shoot height and height above the marked leaf (Appendix 5)

The growth data were split into saline and non-saline treatments to see the effect of KCl under both conditions. Generally, additional KCl did not produce significant effects on all growth parameters in non-saline treatments shown in Appendix 5 with an exception that total height and height above the marked leaf increased significantly with 10 mol m^{-3} KCl compared with not adding KCl.

However, under saline conditions, additional KCl either applied to the soil or leaves, both heights increased significantly compared with 0 mol m^{-3} KCl, but the effect of foliar application of KCl was more than soil application. Shoot heights were higher with 10 mol m^{-3} than 1 mol m^{-3} KCl in both control and salinity treatments. 200 mol m^{-3} NaCl either applied to the soil or leaves without adding KCl decreased both heights significantly, but the effect of NaCl was more pronounced in foliar rather than soil application. No significant interactions between NaCl and KCl were observed for both heights. Method of application of salt and KCl was not significant for both heights, but interaction of salt and KCl application method was significant ($p \leq 0.000$) for both heights. Both heights were higher when NaCl and KCl applied on the leaves (data set 6) than when NaCl and KCl applied to the soil (data set 3) and/or NaCl applied to the leaves and KCl to the soil (data set 5).

Total shoot fresh weight and weight above the marked leaf (Appendix 5)

Salinity significantly ($p \leq 0.000$) decreased total shoot fresh weight and weight above the marked leaf. Additional KCl significantly ($p \leq 0.000$) increased both weights under saline conditions. Interaction between NaCl and KCl was not significant. Mode of salt application was not significant for total shoot fresh weight, whereas significant ($p \leq 0.012$) for weight above the marked leaf. Method of KCl application was significant ($p \leq 0.001$) for total shoot weight and ($p \leq 0.012$) for weight above the marked leaf. Interaction of salt and KCl application had significant ($p \leq 0.000$) effects on both shoot fresh weights. The same trends of results were found for both shoot weights as for shoot heights. In saline treatments, shoot fresh weights were greater when KCl was applied to the leaves rather than when KCl was applied to the soil. Both shoot fresh weights were higher with 10 mol m^{-3} than 1 mol m^{-3} KCl in the control and salinity treatments. Substrate and salt spray salinities without adding KCl decreased both shoot weights significantly, but salt applied to the leaves decreased both weights much more compared with soil salinity treatments.

FW/DW ratio (Appendix 5)

Salinity significantly ($p \leq 0.000$) increased FW/DW ratio. KCl significantly ($p \leq 0.000$) reduced FW/DW in saline treatments. Method of application of salt, KCl and their interaction showed significant ($p \leq 0.000$) effects on FW/DW. The FW/DW ratio was significantly greater in foliar salinity treatments when only salt was applied rather than with KCl.

Thickness of young and old leaves (Appendix 5)

Salinity significantly ($p \leq 0.000$) increased the leaf thickness of young and old leaves. Mode of application of salt, KCl and their interaction produced significant effects ($p \leq 0.000$) on the thickness of both leaves. The thickness of old leaves was higher than young leaves in the control and saline treatments. In foliar salinity treatments, external KCl either applied to the soil or leaves decreased leaf thickness of both leaves. Foliar salinity with and without KCl increased leaf thickness of both leaves more than soil salinity.

5.3.1.2 Photosynthesis and transpiration rate (Table 5.1)

Salinity produced a significant ($p \leq 0.000$) effect on net photosynthesis and transpiration. Additional KCl did not have significant effects on photosynthesis, but had significant ($p \leq 0.001$) effects on transpiration. Interaction between salinity and KCl did not produce significant effects on photosynthesis and transpiration. Method of salt application did not show significant effects on transpiration, while produced significant ($p \leq 0.015$) effects on photosynthesis. Method of application of salt x potassium interaction had significant ($p \leq 0.007$) effects on photosynthesis and transpiration. Photosynthesis and transpiration were higher with 1 and 10 mol m^{-3} KCl in the soil and foliar salinity treatments compared with not adding KCl.

5.3.1.3 Leaf Chlorophyll (SPAD) (Table 5.1)

Salinity significantly ($p \leq 0.000$) decreased chlorophyll measurements, while KCl and its interaction with salinity did not produce significant effects on

leaf chlorophyll. Method of application of salt, KCl and their interaction showed significant effects on chlorophyll measurements. Additional KCl either applied to the soil or leaves increased chlorophyll measurements in the control and salinity treatments. Chlorophyll measurements were higher when KCl was applied to the leaves rather than soil-applied.

Table 5.1 Net photosynthesis, transpiration rate and leaf chlorophyll (SPAD) of *Gossypium hirsutum* L. treated with various concentrations of NaCl and KCl either sprayed on the leaves (L) or applied to the soil (S). The values are means of 5 replicates ± standard errors. (Experiment 5.1).

Data set	Treatments NaCl (mol m^{-3})	KCl	Photosynthesis (μmol m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})	SPAD
1	0 S	0 S	21 ± 1	15 ± 1	42 ± 0
	0 S	1 S	19 ± 1	16 ± 0	43 ± 1
	0 S	10 S	19 ± 1	17 ± 0	42 ± 1
2	0 L	0 L	19 ± 1	15 ± 1	42 ± 0
	0 L	1 L	20 ± 1	16 ± 0	44 ± 1
	0 L	10 L	21 ± 1	17 ± 0	44 ± 1
3	200 S	0 S	18 ± 1	15 ± 1	41 ± 0
	200 S	1 S	17 ± 1	15 ± 1	40 ± 1
	200 S	10 S	18 ± 1	17 ± 0	43 ± 1
4	200 S	0 L	14 ± 2	13 ± 1	42 ± 2
	200 S	1 L	19 ± 1	16 ± 1	45 ± 2
	200 S	10 L	20 ± 1	17 ± 0	45 ± 1
5	200 L	0 S	13 ± 3	13 ± 1	29 ± 2
	200 L	1 S	13 ± 2	13 ± 1	34 ± 1
	200 L	10 S	15 ± 1	15 ± 0	33 ± 1
6	200 L	0 L	13 ± 0	13 ± 1	32 ± 4
	200 L	1 L	19 ± 0	17 ± 0	35 ± 1
	200 L	10 L	17 ± 2	16 ± 1	37 ± 1

5.3.1.4 Cation Analysis

Na^+ Concentrations (Table 5.2)

Na^+ concentrations in the young and old leaves increased significantly ($p \leq 0.000$) with 200 mol m^{-3} NaCl. Na^+ concentrations were significantly ($p \leq 0.000$) higher in the old than in young leaves in accordance with the results described in Chapter 4. Overall KCl and its interaction with salinity had no significant effect on Na^+ concentration. Method of salt, KCl application and their interaction showed significant effects on Na^+ concentration. Soil salinity increased

Table 5.2 Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations (mol m^{-3} expressed sap) in young (Y) and old (O) leaves of *Gossypium hirsutum* L. treated with various concentrations of NaCl and KCl either sprayed on the leaves (L) or applied to the soil (S). The values are means of 5 replicates ± standard errors. (Experiment 5.1).

Data set	Treatments NaCl (mol m^{-3})	Treatments KCl (mol m^{-3})	Sodium Y. leaf	Sodium O. leaf	Potassium Y. leaf	Potassium O. leaf
1	0 S	0 S	11 ± 0	9 ± 1	87 ± 15	97 ± 19
	0 S	1 S	7 ± 1	11 ± 1	122 ± 8	112 ± 7
	0 S	10 S	7 ± 1	23 ± 9	134 ± 23	86 ± 7
2	0 L	0 L	11 ± 2	15 ± 2	118 ± 26	98 ± 8
	0 L	1 L	21 ± 7	17 ± 3	115 ± 6	95 ± 12
	0 L	10 L	10 ± 2	12 ± 2	122 ± 6	97 ± 10
3	200 S	0 S	28 ± 3	59 ± 5	77 ± 25	71 ± 2
	200 S	1 S	18 ± 4	57 ± 22	42 ± 7	38 ± 5
	200 S	10 S	19 ± 3	44 ± 6	99 ± 9	102 ± 12
4	200 S	0 L	27 ± 9	62 ± 18	82 ± 25	70 ± 12
	200 S	1 L	24 ± 4	71 ± 7	101 ± 18	50 ± 8
	200 S	10 L	31 ± 3	71 ± 10	87 ± 7	65 ± 6
5	200 L	0 S	101 ± 27	312 ± 6	70 ± 8	21 ± 5
	200 L	1 S	79 ± 15	309 ± 42	81 ± 6	20 ± 1
	200 L	10 S	60 ± 16	291 ± 26	84 ± 12	24 ± 2
6	200 L	0 L	111 ± 17	286 ± 11	51 ± 11	25 ± 10
	200 L	1 L	93 ± 31	250 ± 32	59 ± 29	19 ± 2
	200 L	10 L	83 ± 12	183 ± 6	59 ± 6	28 ± 2

Data set	Treatments NaCl (mol m^{-3})	Treatments KCl (mol m^{-3})	Magnesium Y. leaf	Magnesium O. leaf	Calcium Y. leaf	Calcium O. leaf
1	0 S	0 S	36 ± 4	45 ± 5	96 ± 4	183 ± 9
	0 S	1 S	42 ± 3	46 ± 3	125 ± 9	172 ± 8
	0 S	10 S	41 ± 6	36 ± 3	124 ± 33	146 ± 13
2	0 L	0 L	38 ± 2	42 ± 2	105 ± 0	153 ± 3
	0 L	1 L	36 ± 3	45 ± 4	105 ± 9	187 ± 11
	0 L	10 L	38 ± 3	44 ± 2	108 ± 15	190 ± 7
3	200 S	0 S	31 ± 9	42 ± 4	86 ± 18	155 ± 25
	200 S	1 S	15 ± 2	22 ± 5	43 ± 10	71 ± 16
	200 S	10 S	34 ± 3	37 ± 3	81 ± 7	117 ± 7
4	200 S	0 L	26 ± 5	34 ± 3	83 ± 22	84 ± 20
	200 S	1 L	29 ± 5	32 ± 9	54 ± 8	98 ± 24
	200 S	10 L	32 ± 2	32 ± 2	66 ± 4	111 ± 4
5	200 L	0 S	36 ± 3	25 ± 1	95 ± 21	64 ± 1
	200 L	1 S	35 ± 2	18 ± 2	124 ± 20	55 ± 1
	200 L	10 S	39 ± 5	20 ± 1	90 ± 14	56 ± 4
6	200 L	0 L	30 ± 6	25 ± 2	72 ± 6	56 ± 14
	200 L	1 L	21 ± 9	19 ± 2	54 ± 10	70 ± 8
	200 L	10 L	28 ± 3	23 ± 2	75 ± 9	74 ± 2

Na^+ concentrations slightly while, foliar application of salt substantially increased Na^+ concentrations in the young and old leaves. Na^+ concentrations in both leaves were decreased in foliar salinity treatments when 1 and 10 mol m^{-3} KCl was applied to the soil and leaves and the effect of 10 mol m^{-3} KCl was more than 1 mol m^{-3} KCl (data set 5 and 6). Potassium reduced Na^+ concentrations most when 200 mol m^{-3} NaCl + 10 mol m^{-3} KCl were applied to the leaves.

K^+ Concentrations (Table 5.2)

K^+ concentrations were significantly ($p \leq 0.000$) decreased with salinity, while no consistent effects of KCl and its interaction with salinity on K^+ concentrations were found in leaf tissues. Young leaf K^+ concentrations were significantly ($p \leq 0.000$) higher than old leaves. Method of salt application was significant while, mode of KCl application was not significant. Interaction of NaCl x KCl for method of application was significant ($p \leq 0.009$) on K^+ concentrations. In the control, young leaves showed an increase of K^+ concentrations with increasing concentrations of soil-applied KCl. In saline treatments, K^+ concentrations in the young leaves were slightly higher with KCl. In the old leaves, generally, K^+ concentrations were not increased with increasing concentrations of KCl with an exception when salt and 10 mol m^{-3} KCl were applied to the soil (data set 3). Foliar-applied NaCl decreased K^+ concentrations in the young and old leaves much more than soil salinity treatments but, the effect was more pronounced in the old leaves. Significant negative correlations between Na^+ and K^+ concentrations in young (-0.4**) and in old (-0.78**) leaves were observed.

Mg^{2+} Concentrations (Table 5.2)

Salinity significantly ($p \leq 0.000$) reduced Mg^{2+} concentrations, while KCl did not show significant effects on Mg^{2+} concentrations. Interaction between NaCl x KCl were significant ($p \leq 0.025$) on Mg^{2+} concentrations. Mode of applied salt and KCl were not significant but, their interaction was significant ($p \leq 0.039$) for Mg^{2+} concentrations. In the old leaves, foliar application of salt decreased Mg^{2+} concentrations more than in soil salinity treatments.

Ca^{2+} Concentrations (Table 5.2)

Applied salinity significantly ($p \leq 0.000$) lowered Ca^{2+} concentrations whereas, KCl and its interaction with salinity did not produce significant effects on Ca^{2+} concentrations. Generally, Ca^{2+} concentrations were significantly ($p \leq 0.000$) higher in the old than in young leaves. Method of salt and KCl application was not significant but, their interaction was significant ($p \leq 0.000$) for Ca^{2+} concentrations. In substrate and salt spray treatments, an increase in Ca^{2+} concentrations in the old leaves was observed with foliar-applied KCl. Foliar-applied salinity decreased Ca^{2+} concentrations much more in the old leaves than in soil salinity treatments.

5.3.1.5 Anion Analysis

Cl^- Concentrations (Table 5.3)

200 mol m^{-3} NaCl significantly ($p \leq 0.000$) increased Cl^- concentrations in the young and old leaves. Applied KCl produced significant ($p \leq 0.010$) effects while, KCl x NaCl interaction did not show significant effects on Cl^- concentrations. In saline treatments, Cl^- concentrations were greater in the old than in young leaves. Method of salt and KCl application was not significant but, their interaction was significant for Cl^- concentrations. In the control, Cl^- concentrations increased in both leaves with increasing concentrations of KCl applied to the soil, and they also increased in the old leaves with 10 mol m^{-3} KCl applied to the leaves. In saline treatments, external KCl either applied to the soil or leaves decreased Cl^- concentrations consistently in young leaves except when salt and 10 mol m^{-3} KCl both were applied to the leaves. Cl^- concentrations in the old leaves decreased when salt and KCl were applied to the soil (data set 3), but Cl^- concentrations increased in substrate salinity when KCl was applied to the leaves (data set 4). No clear differences of Cl^- concentrations in young leaf tissues were observed between substrate and salt spray salinity treatments. However, in old leaves, Cl^- concentrations were substantially higher in foliar-applied salinity compared with soil salinity treatments as for Na^+ concentrations.

Table 5.3 Cl^- and malate concentrations (mol m^{-3} expressed sap) in young (Y) and old (O) leaves of *Gossypium hirsutum* L. treated with various concentrations of NaCl and KCl either sprayed on the leaves (L) or applied to the soil (S). The values are means of 5 replicates ± standard errors. (Experiment 5.1).

Data set	Treatments NaCl (mol m^{-3})	Treatments KCl (mol m^{-3})	Chloride Y. leaf	Chloride O. leaf	Malate Y. leaf	Malate O. leaf
1	0 S	0 S	57 ± 2	27 ± 6	61 ± 2	137 ± 7
	0 S	1 S	83 ± 12	68 ± 9	51 ± 6	119 ± 13
	0 S	10 S	103 ± 3	117 ± 23	50 ± 6	112 ± 12
2	0 F	0 F	56 ± 19	50 ± 4	67 ± 14	88 ± 6
	0 F	1 F	36 ± 12	24 ± 4	46 ± 1	81 ± 17
	0 F	10 F	49 ± 3	82 ± 6	38 ± 4	120 ± 7
3	200 S	0 S	171 ± 25	205 ± 10	57 ± 13	93 ± 5
	200 S	1 S	89 ± 12	146 ± 31	20 ± 2	66 ± 16
	200 S	10 S	134 ± 16	166 ± 13	22 ± 4	82 ± 8
4	200 S	0 F	158 ± 14	165 ± 16	64 ± 13	82 ± 4
	200 S	1 F	128 ± 3	185 ± 7	35 ± 4	60 ± 4
	200 S	10 F	83 ± 5	241 ± 9	14 ± 1	47 ± 3
5	200 F	0 S	160 ± 7	256 ± 1	66 ± 13	19 ± 6
	200 F	1 S	138 ± 36	257 ± 1	42 ± 12	25 ± 10
	200 F	10 S	136 ± 16	255 ± 1	38 ± 6	17 ± 3
6	200 F	0 F	120 ± 16	256 ± 1	70 ± 13	23 ± 6
	200 F	1 F	110 ± 14	247 ± 6	51 ± 7	26 ± 3
	200 F	10 F	155 ± 9	253 ± 1	64 ± 3	25 ± 3

Malate Concentrations (Table 5.3)

Applied salinity and KCl significantly ($p \leq 0.009$) decreased malate concentrations while, their interaction was not significant. In the controls and soil salinity treatments, malate concentrations were significantly ($p \leq 0.000$) higher in the old than in young leaves whereas the opposite was true for foliar-applied salinity. Method of application of salt and KCl was not significant while, their interaction was significant for malate concentrations. In the old leaves, malate concentrations were lower when salt was applied to the leaves than soil-applied salinity. Negative correlation (-0.73**) was found in the old leaves between malate and Cl^- concentrations.

5.3.2 Experiment 5.2 (Optimum level of KCl)

5.3.2.1 Growth parameters

Total shoot height and height above the marked leaf (Table 5.4)

10 and 150 mol m^{-3} NaCl treatments were split for growth parameters shown in Table 5.4 to see the effect of KCl under both low and highly saline conditions separately.

Analysis of variance revealed that various levels of KCl did not produce significant effects between the treatments on all growth measurements with 10 mol m^{-3} NaCl. However, with 150 mol m^{-3} NaCl, additional KCl significantly ($p \leq 0.003$) increased total shoot height and height above the marked leaf. Both heights were lower at 150 mol m^{-3} KCl compared with other levels of KCl, probably due to high concentrations of Cl^- in the solution. 150 mol m^{-3} NaCl significantly ($p \leq 0.000$) decreased both heights. Interaction between NaCl x KCl was significant for both heights at $p \leq 0.006$ level. At 10 mol m^{-3} NaCl, the highest total shoot height and height above the marked leaf were recorded when KCl was applied with 150 and 10 mol m^{-3} respectively. However, at 150 mol m^{-3} NaCl, both heights were highest when KCl was applied at 50 mol m^{-3}.

Total shoot fresh weight and weight above the marked leaf (Table 5.4)

150 mol m^{-3} NaCl significantly decreased ($p \leq 0.000$) total shoot fresh weight and weight above the marked leaf. Applied KCl significantly ($p \leq 0.003$) increased both weights in high salinity treatments. Interaction between salinity and KCl had significant effects ($p \leq 0.000$) on both weights. The same effects of KCl in shoot fresh weights as for shoot heights were observed in low and high salinity treatments. Under low and high saline conditions, the highest shoot fresh weights were found with 10 and 25 mol m^{-3} KCl respectively.

Total number of nodes and nodes above the marked leaf (Table 5.4)

Analysis of variance revealed significant decrease ($p \leq 0.000$) in both nodes with increasing salinity. Applied KCl significantly increased both nodes at 150 mol m^{-3} NaCl compared with no adding KCl. Interaction between salinity and

KCl was significant ($p \leq 0.001$) for total number of nodes and ($p \leq 0.020$) nodes above the marked leaf. Both number of nodes decreased at 150 mol m^{-3} KCl compared with lower levels of KCl in high salinity treatments.

Table 5.4 growth parameters of *Gossypium hirsutum* L. treated with various concentrations of NaCl and KCl both sprayed on the leaves (foliar). The values are means of 5 replicates ± standard errors (Experiment 5.2).

Treatments NaCl (mol m^{-3})	KCl	Total shoot height (mm)	Ht. above mark leaf (mm)	Total shoot fresh wt. (g)	Wt. above mark leaf (g)	Total No. of nodes	Nodes above mark leaf
10	0	960 ± 23	736 ± 13	357 ± 22	294 ± 23	16 ± 0	11 ± 1
10	10	1070 ± 10	828 ± 21	386 ± 12	308 ± 10	17 ± 0	11 ± 0
10	25	976 ± 45	728 ± 44	351 ± 28	254 ± 15	16 ± 1	10 ± 1
10	50	1046 ± 28	792 ± 39	376 ± 34	296 ± 39	15 ± 0	10 ± 1
10	75	1052 ± 39	788 ± 28	327 ± 21	248 ± 20	16 ± 0	10 ± 0
10	150	1094 ± 44	824 ± 32	349 ± 30	270 ± 25	16 ± 0	10 ± 0
150	0	538 ± 41	354 ± 35	86 ± 20	73 ± 17	11 ± 0	7 ± 0
150	10	834 ± 58	630 ± 41	279 ± 26	213 ± 16	14 ± 1	10 ± 0
150	25	852 ± 54	644 ± 53	325 ± 24	259 ± 23	15 ± 1	10 ± 1
150	50	900 ± 71	678 ± 65	299 ± 14	245 ± 17	15 ± 1	10 ± 0
150	75	844 ± 67	564 ± 56	280 ± 34	211 ± 26	15 ± 1	9 ± 0
150	150	626 ± 95	412 ± 93	160 ± 41	140 ± 38	13 ± 1	8 ± 1

Treatments NaCl (mol m^{-3})	KCl	Number of leaves	leaf area (cm^2)	FW/DW ratio	Thickness of young leaf	Thickness of old leaf
10	0	79 ± 3	8147 ± 667	7.4 ± 0.3	12 ± 1	16 ± 1
10	10	80 ± 5	8555 ± 348	6.7 ± 0.3	13 ± 1	15 ± 0
10	25	78 ± 4	8029 ± 624	8.0 ± 0.4	12 ± 0	16 ± 0
10	50	75 ± 5	7889 ± 482	7.7 ± 0.1	12 ± 1	16 ± 1
10	75	72 ± 5	7149 ± 594	7.2 ± 0.6	13 ± 1	15 ± 0
10	150	76 ± 4	7356 ± 578	8.1 ± 0.3	11 ± 1	16 ± 1
150	0	12 ± 3	1124 ± 271	12.0 ± 1.5	16 ± 1	21 ± 1
150	10	48 ± 4	5734 ± 546	9.3 ± 0.2	12 ± 1	19 ± 0
150	25	55 ± 5	6675 ± 512	8.8 ± 0.6	12 ± 0	20 ± 0
150	50	52 ± 5	5689 ± 190	7.6 ± 0.9	12 ± 0	20 ± 1
150	75	46 ± 3	5116 ± 543	8.9 ± 0.5	13 ± 1	21 ± 1
150	150	26 ± 6	2590 ± 685	7.6 ± 1.6	16 ± 1	22 ± 1

Total number of leaves and leaf area (Table 5.4)

Application of high salinity significantly ($p \leq 0.000$) decreased the number of leaves and leaf area. Additional KCl substantially increased the number of leaves and leaf area under highly saline conditions. At 150 mol m^{-3} NaCl, the

number of leaves and leaf area significantly decreased when KCl was applied at 150 mol m^{-3} compared with other lower levels of KCl, possibly due to high concentrations of Cl^- in the solution. Interaction of salt and KCl was significant ($p \leq 0.000$) for the number of leaves and leaf area. The highest number of leaves and also leaf area was recorded when KCl was applied with 10 mol m^{-3} in low salinity and with 25 mol m^{-3} in high salinity treatments.

FW/DW ratio (Table 5.4)

High salinity significantly ($p \leq 0.001$) increased FW/DW ratio. Applied KCl did not show significant effects on FW/DW ratio, while its interaction with salinity produced significant effects ($p \leq 0.018$) on FW/DW. The highest FW/DW was recorded when 150 mol m^{-3} NaCl was applied alone.

Thickness of young and old leaves (Table 5.4)

150 mol m^{-3} NaCl significantly ($p \leq 0.000$) increased the thickness of young and old leaves. Under highly saline conditions, applied KCl (except 150 mol m^{-3}) significantly ($p \leq 0.000$) decreased the young leaf thickness compared with not adding KCl while no effect of KCl was found on the thickness of old leaf. Interaction between salinity and KCl showed significant ($p \leq 0.000$) effects on only the thickness of young leaf. The thickness of old leaves was greater than young ones. The maximum leaf thickness of both leaves was observed when both NaCl and KCl were applied with 150 mol m^{-3}.

5.3.2.2 Photosynthesis, Transpiration and Stomatal conductance (Table 5.5)

High salinity significantly ($p \leq 0.014$) reduced net photosynthesis, while external KCl significantly ($p \leq 0.008$) increased net photosynthesis. Salt and KCl interaction had no effect on photosynthesis. Overall, the rate of transpiration and stomatal conductance were not significantly affected by salinity and KCl. In low salinity treatments, the highest photosynthesis and stomatal conductance were recorded when KCl was applied at 10 mol m^{-3}. However, in highly saline treatments, the highest rate of photosynthesis and transpiration were recorded with

75 mol m^{-3} KCl. K^+ deficiency could cause significant decrease in photosynthesis, rate of transpiration and stomatal conductance, as K^+ concentrations were lower at 150 mol m^{-3} NaCl alone (see Table 5.6). Since K^+ is the major solute in guard cells, it is suggested that K^+ deficiency may result in stomatal closure, thereby decreasing carbon exchange rate.

Table 5.5 Photosynthesis (CER), transpiration (E), stomatal conductance (gs) and leaf chlorophyll (SPAD) of *Gossypium hirsutum* L. treated with various concentrations of NaCl and KCl both sprayed on the leaves (foliar). The values are means of 5 replicates ± standard errors. (Exp. 5.2).

Treatments NaCl (mol m^{-3})	KCl	Photosynthesis (μmol m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})	Stomatal cond. (mmol m^{-2} s^{-1})	SPAD
10	0	15 ± 1	11 ± 1	479 ± 87	39 ± 1
10	10	21 ± 1	14 ± 2	717 ± 95	38 ± 1
10	25	16 ± 1	13 ± 1	467 ± 77	37 ± 1
10	50	20 ± 2	14 ± 1	580 ± 158	37 ± 1
10	75	19 ± 2	14 ± 1	646 ± 96	36 ± 1
10	150	16 ± 2	12 ± 1	503 ± 128	36 ± 0
150	0	8 ± 1	8 ± 1	224 ± 45	28 ± 2
150	10	17 ± 3	13 ± 2	517 ± 166	33 ± 1
150	25	15 ± 1	13 ± 2	603 ± 117	31 ± 1
150	50	14 ± 2	12 ± 2	486 ± 91	33 ± 0
150	75	20 ± 3	14 ± 1	599 ± 93	33 ± 1
150	150	17 ± 3	13 ± 2	485 ± 143	31 ± 2

5.3.2.3 Leaf Chlorophyll (SPAD) (Table 5.5)

Leaf chlorophyll measurements decreased significantly ($p \leq 0.000$) with increasing salinity. KCl did not show significant effects on chlorophyll. Interaction between salinity and potassium had significant effects ($p \leq 0.007$) on chlorophyll measurements. Chlorophyll measurements were slightly decreased with KCl in low salinity, while increased with KCl in high salinity treatments.

5.3.2.4 Cation Analysis

Na^+ Concentrations (Table 5.6)

Three way Anova (NaCl x KCl x leaf age) revealed that high external salinity resulted in significantly ($p \leq 0.000$) higher Na^+ concentrations in the young and old leaves, whereas KCl significantly ($p \leq 0.000$) decreased Na^+ concentrations

in both leaves. Interaction between salinity and KCl had significant effects ($p \leq 0.000$) on Na^+ concentrations. A gradient was observed between leaves of different ages, with older leaves showing significantly ($p \leq 0.000$) higher Na^+ concentrations than younger leaves. Na^+ concentrations were dramatically reduced in high salinity treatments in both leaves when any external KCl was applied. However, with increasing concentrations of KCl, there were no significant differences observed in Na^+ concentrations in both salinity treatments in the young and old leaves.

Table 5.6 Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations (mol m^{-3} expressed sap) in young (Y) and old (O) leaves of *Gossypium hirsutum* L. treated with various concentrations of NaCl and KCl both sprayed on the leaves (foliar). The values are means of 5 replicates ± standard errors. (Exp. 5.2).

Treatments NaCl (mol m^{-3})	KCl	Sodium Y. leaf	Sodium O. leaf	Potassium Y. leaf	Potassium O. leaf
10	0	10 ± 1	62 ± 6	112 ± 14	51 ± 12
10	10	7 ± 1	42 ± 3	179 ± 13	52 ± 4
10	25	7 ± 1	35 ± 2	164 ± 13	76 ± 4
10	50	7 ± 0	38 ± 3	149 ± 18	63 ± 6
10	75	7 ± 1	41 ± 7	154 ± 15	102 ± 18
10	150	5 ± 1	24 ± 4	129 ± 5	110 ± 11
150	0	134 ± 19	283 ± 17	51 ± 7	25 ± 5
150	10	20 ± 2	187 ± 23	124 ± 20	20 ± 3
150	25	17 ± 2	171 ± 16	118 ± 5	22 ± 2
150	50	17 ± 4	184 ± 12	109 ± 15	27 ± 5
150	75	18 ± 8	168 ± 13	111 ± 17	30 ± 4
150	150	18 ± 6	144 ± 14	118 ± 18	65 ± 11

Treatments NaCl (mol m^{-3})	KCl	Magnesium Y. leaf	Magnesium O. leaf	Calcium Y. leaf	Calcium O. leaf
10	0	47 ± 2	47 ± 4	80 ± 7	177 ± 16
10	10	63 ± 2	38 ± 2	118 ± 5	205 ± 5
10	25	37 ± 2	32 ± 6	95 ± 6	174 ± 5
10	50	45 ± 4	27 ± 2	110 ± 9	168 ± 7
10	75	42 ± 6	24 ± 3	97 ± 9	133 ± 13
10	150	28 ± 2	16 ± 1	59 ± 5	94 ± 5
150	0	23 ± 4	12 ± 1	68 ± 6	62 ± 9
150	10	31 ± 1	17 ± 1	80 ± 6	102 ± 4
150	25	37 ± 2	18 ± 1	93 ± 4	109 ± 5
150	50	33 ± 4	21 ± 1	76 ± 6	100 ± 3
150	75	38 ± 3	18 ± 2	90 ± 7	88 ± 2
150	150	39 ± 2	17 ± 2	89 ± 4	67 ± 8

K^+ Concentrations (Table 5.6)

150 mol m^{-3} NaCl significantly ($p \leq 0.000$) decreased K^+ concentrations in the young and old leaves compared with 10 mol m^{-3} NaCl, while they significantly ($p \leq 0.000$) increased in both leaves with additional KCl. Salinity and KCl interaction did not show significant effects on K^+ concentrations. K^+ concentrations were significantly ($p \leq 0.000$) higher in the young than in old leaves at both salinities. Negative correlations between Na^+ and K^+ concentrations in young (-0.61**) and in old leaves (-0.66**) were observed. The highest K^+ concentrations were found in the young and in old leaves at both salinities when KCl was applied with 10 and 150 mol m^{-3} respectively. In low salinity treatments, K^+ concentrations were lower in the young leaves at 150 mol m^{-3} KCl. Generally, K^+ concentrations were not increased in the young leaves at both salinities with increasing concentrations of KCl. However, in the old leaves, K^+ concentrations significantly increased with 75 and 150 mol m^{-3} KCl in low salinity and with 150 mol m^{-3} KCl in high salinity treatments compared with other treatments.

Mg^{2+} Concentrations (Table 5.6)

High salinity significantly ($p \leq 0.000$) reduced Mg^{2+} concentrations in the young and old leaf tissues. KCl and its interaction with salinity showed significant ($p \leq 0.000$) effects on Mg^{2+} concentrations. Young leaf Mg^{2+} concentrations were significantly ($p \leq 0.000$) higher than old leaves in both salinity treatments. Generally, Mg^{2+} concentrations decreased consistently in the young and old leaves with increasing concentrations of KCl in low salinity treatments, whereas the opposite was true for high salinity treatments. There were significant negative correlations in young (-0.5**) and in old (-0.54**) leaves between Na^+ and Mg^{2+} concentrations.

Ca^{2+} Concentrations (Table 5.6)

High salinity significantly ($p \leq 0.000$) reduced Ca^{2+} concentrations, whereas applied KCl significantly ($p \leq 0.000$) increased Ca^{2+} concentrations compared with no KCl. Interaction between salinity and KCl was significant

($p \leq 0.000$) for Ca^{2+} concentrations. Generally, Ca^{2+} concentrations were significantly ($p \leq 0.000$) higher in the old than in young leaves at both salinities. The highest Ca^{2+} concentrations were recorded in both leaves in low and high salinity treatments with 10 and 25 mol m^{-3} KCl respectively. At both salinities, KCl increased Ca^{2+} concentrations in both leaves. However, when 150 mol m^{-3} KCl was applied in low salinity treatments, Ca^{2+} concentrations decreased in both leaves compared with not adding KCl and also compared with lower concentrations of KCl. A consistent decrease of Ca^{2+} concentrations in the old leaves was found in low salinity treatments with increasing concentrations of KCl.

5.3.2.5 Anion Analysis

Cl^- Concentrations (Table 5.7)

150 mol m^{-3} NaCl significantly ($p \leq 0.000$) increased Cl^- concentrations in both young and old leaves compared with 10 mol m^{-3} NaCl. Applied KCl consistently and significantly ($p \leq 0.000$) increased Cl^- concentrations at low salinity, whereas, at high salinity, they generally decreased with KCl. Cl^- concentrations were significantly ($p \leq 0.000$) higher in the old than in young leaves. Interaction between NaCl x KCl was significant ($p \leq 0.000$) for Cl^- concentrations. The lowest Cl^- concentrations were observed in the young and old leaves when KCl was applied with 50 and 25 mol m^{-3} respectively under highly saline conditions.

Malate Concentrations (Table 5.7)

High salinity significantly ($p \leq 0.000$) decreased malate concentrations while, additional KCl significantly ($p \leq 0.000$) increased malate concentrations in both leaves compared with no external KCl. Salinity and its interaction with KCl produced significant ($p \leq 0.000$) effects on malate concentrations. Generally, malate concentrations were significantly ($p \leq 0.000$) higher in the young than in old leaves. In low salinity treatments, malate concentrations in the old leaves reduced consistently with increasing concentrations of KCl. However, in high salinity treatments, they increased with additional KCl in both leaves. The highest malate

concentrations were found in the young leaves when KCl was applied with 10 mol m^{-3} in low and high salinity treatments. Negative correlations between Cl^- and malate concentrations in young (-0.44**) and in old leaves (-0.87**) were found. Also negative correlations in young (-0.66**) and in old (-0.52**) leaves were observed between Na^+ and malate concentrations

Table 5.7 Cl^- and malate concentrations (mol m^{-3} expressed sap) in young (Y) and old (O) leaves of *Gossypium hirsutum* L. treated with various concentrations of NaCl and KCl both sprayed on the leaves (foliar). The values are means of 5 replicates ± standard errors. (Experiment 5.2).

Treatments NaCl (mol m^{-3})	KCl	Chloride		Malate	
		Y. leaf	O. leaf	Y. leaf	O. leaf
10	0	30 ± 5	91 ± 8	67 ± 4	94 ± 19
10	10	29 ± 2	99 ± 7	84 ± 8	87 ± 6
10	25	43 ± 8	108 ± 8	67 ± 6	67 ± 4
10	50	51 ± 8	185 ± 8	79 ± 3	42 ± 1
10	75	53 ± 3	232 ± 10	75 ± 4	25 ± 4
10	150	61 ± 4	254 ± 19	59 ± 6	11 ± 5
150	0	168 ± 12	301 ± 16	32 ± 6	9 ± 1
150	10	80 ± 5	256 ± 22	79 ± 2	24 ± 3
150	25	83 ± 2	238 ± 14	77 ± 7	27 ± 3
150	50	66 ± 9	265 ± 15	67 ± 6	25 ± 2
150	75	88 ± 8	285 ± 14	78 ± 8	18 ± 5
150	150	110 ± 11	313 ± 30	72 ± 5	12 ± 3

5.3.3 Experiment 5.3 (Accompanying anions of K^+)

5.3.3.1 Growth parameters

Total shoot height and height above the marked leaf (Table 5.8)

NaCl + various K^+ treatments significantly ($p \leq 0.000$) increased total shoot height and height above the marked leaf compared with NaCl (alone). Total shoot height with NaCl + K-phosphate was significantly lower compared with NaCl + K-sulphate and K-chloride treatments. Generally, no large difference in both heights was observed between varying K^+ sources, with an exception in K-phosphate treatment for total shoot height. Both heights were in the order K-chloride>K-sulphate>K-acetate>K-nitrate>K-phosphate>NaCl (alone).

Table 5.8 Growth parameters of *Gossypium hirsutum* L. treated with various accompanying anions of K^+ (10 mol m^{-3}) and NaCl (150 mol m^{-3}) sprayed on the leaves (foliar). The values are means of 5 replicates ± standard errors. (Experiment 5.3).

Treatments	Total shoot height (mm)	Height above mark leaf (mm)	Total shoot fresh wt. (g)	Weight above mark leaf (g)
NaCl	715 ± 66	335 ± 39	76 ± 16	59 ± 12
NaCl + K_2SO_4 (5mM)	1028 ± 42	558 ± 40	208 ± 28	142 ± 18
NaCl + KCl	1050 ± 29	566 ± 46	243 ± 34	148 ± 10
NaCl + KH_2PO_4	892 ± 25	480 ± 33	174 ± 31	121 ± 19
NaCl + KNO_3	922 ± 12	466 ± 30	238 ± 19	160 ± 21
NaCl + KCH_3COO	970 ± 38	538 ± 26	265 ± 36	162 ± 12

Treatments	Total No. of nodes	No. of nodes ab. mark leaf	FW/DW ratio	Thickness young leaf	Thickness old leaf
NaCl	17 ± 1	7 ± 1	13 ± 2	14 ± 0	17 ± 0
NaCl + K_2SO_4 (5mM)	18 ± 0	7 ± 0	7 ± 1	14 ± 1	17 ± 1
NaCl + KCl	20 ± 1	8 ± 0	8 ± 0	13 ± 0	17 ± 1
NaCl + KH_2PO_4	20 ± 0	8 ± 0	8 ± 1	14 ± 1	18 ± 1
NaCl + KNO_3	19 ± 1	7 ± 1	7 ± 0	14 ± 1	17 ± 0
NaCl + KCH_3COO	19 ± 0	7 ± 0	8 ± 1	13 ± 0	18 ± 1

Total shoot fresh weight and weight above the marked leaf (Table 5.8)

NaCl + various K^+ sources significantly ($p \leq 0.003$) increased both shoot fresh weights compared with NaCl (alone). Generally, no great difference in shoot fresh weight among the different K^+ treatments was detected except with K-phosphate treatment, which produced less shoot fresh weight than other K^+ treatments. Both fresh weights were highest in K-acetate treatment.

Total number of nodes and nodes above the marked leaf (Table 5.8)

Total number of nodes on the main stem in NaCl + K-chloride and NaCl + K-phosphate treatments significantly increased from NaCl (alone) treatment. No large difference in both number of nodes among various K^+ treatments was found.

FW/DW ratio (Table 5.8)

Significant ($p \leq 0.001$) increase in FW:DW was observed in NaCl (alone) treatment compared with NaCl + K sources treatments. Among the various K^+ sources, the low FW:DW ratio at NaCl + KNO_3 was found.

Thickness of young and old leaves (Table 5.8)

No significant differences were found in the thickness of young and old leaves between the treatments. The thickness of old leaves was greater than the thickness of young leaves.

5.3.3.2 Photosynthesis, Transpiration and Stomatal conductance (Table 5.9)

Analysis of variance revealed significant ($p \leq 0.041$) increase in net photosynthesis in the young leaves with various K^+ sources (except K-phosphate) compared with NaCl (alone). No significant differences of photosynthesis were observed in the old leaves between the treatments. However, photosynthesis was higher in all K^+ sources compared with NaCl (alone). Photosynthesis was higher in the young than in old leaves.

No significant difference of transpiration in developing and mature leaves was observed. However, the rate of transpiration was lower when NaCl (alone) and K-phosphate were applied compared with other K^+ treatments.

Table 5.9 Photosynthesis (μmol m^{-2} s^{-1}), transpiration (mmol m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) in young (Y) and old (O) leaves of *Gossypium hirsutum* L. treated with various accompanying anions of K^+ (10 mol m^{-3}) and NaCl (150 mol m^{-3}) both sprayed on the leaves (foliar). The values are means of 5 replicates ± standard errors. (Experiment 5.3).

Treatments	Photosynthesis (CER)		Transpiration (E)		Stomatal conductance (gs)	
	Y. leaf	O. leaf	Y. leaf	O. leaf	Y. leaf	O. leaf
NaCl	5 ± 3	5 ± 2	7 ± 2	7 ± 2	330 ± 108	276 ± 87
NaCl + K_2SO_4 (5mM)	11 ± 2	7 ± 1	10 ± 1	9 ± 0	560 ± 87	434 ± 58
NaCl + KCl	10 ± 1	7 ± 1	9 ± 1	9 ± 1	454 ± 76	389 ± 54
NaCl + KH_2PO_4	7 ± 2	6 ± 1	7 ± 2	7 ± 2	345 ± 104	306 ± 95
NaCl + KNO_3	11 ± 1	8 ± 2	9 ± 1	8 ± 1	446 ± 65	337 ± 82
NaCl + KCH_3COO	14 ± 1	8 ± 1	10 ± 0	9 ± 1	559 ± 83	394 ± 86

There were no significant differences in stomatal conductance in the young and old leaves between the treatments. However, stomatal conductance in the young and old leaves was higher in NaCl + various K^+ compared with NaCl (alone). Stomatal conductance in both young and old leaves was in the order K-sulphate>K-acetate>K-chloride>K-nitrate>K-phosphate>NaCl (alone).

5.3.3.3 Leaf Chlorophyll (SPAD)

NaCl + various K^+ sources significantly ($p \leq 0.000$) increased leaf chlorophyll measurements (32-36) units in comparison with NaCl (alone) (25 units). No large differences of chlorophyll measurements among the different K^+ sources were detected. The highest chlorophyll content was found in K-nitrate and K-acetate treatments (36 units).

5.3.3.4 Cation Analysis

Na^+ Concentrations (Table 5.10)

Na^+ concentrations were significantly ($p \leq 0.000$) lower in the young leaves with NaCl + various K^+ compared with 150 mol m^{-3} NaCl (alone). The lowest Na^+ concentrations in the young leaves were found in K-acetate followed by K-chloride treatments. Na^+ concentrations in the young leaves in K-sulphate, K-phosphate and K-nitrate treatments were more or less the same. In the old leaves, K-chloride and K-acetate significantly ($p \leq 0.019$) lowered Na^+ concentrations compared with NaCl (alone), whereas, no significant differences between NaCl (alone) versus NaCl + K-sulphate, K-phosphate and K-nitrate treatments were detected. In the old leaves, Na^+ concentrations decreased in the order K-chloride<K-accetate<K-sulphate<K-nitrate<K-phosphate<NaCl alone. Na^+ concentrations were significantly ($p \leq 0.000$) greater in the old than in young leaves. In the young and old petioles, Na^+ concentrations decreased significantly ($p \leq 0.001$) at NaCl + various K^+ compared with NaCl (alone). Na^+ concentrations were higher in the old than in young petioles as for leaf tissues. No large differences in Na^+ concentrations in both young and old petioles with varying K^+ sources were observed. Na^+ concentrations in the leaves were significantly ($p \leq 0.000$) higher than in petioles.

K^+ Concentrations (Table 5.10)

There were no significant differences in K^+ concentrations of young leaves between the various treatments. However, in the old leaves, significant ($p \leq 0.017$) increase in K^+ concentrations at K-acetate compared with K-sulphate treatment

was found. K^+ concentrations were higher in the young than in old leaves. In the young leaves, various K^+ sources had little effect to raise K^+ concentrations compared with NaCl (alone). It could be possible that these leaves were high in Na^+ concentrations (87-117 mol m^{-3}) therefore showed low K^+ concentrations. In

Table 5.10 Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations (mol m^{-3} expressed sap) in young (Y) and old (O) leaves and petioles of *Gossypium hirsutum* L. treated with various accompanying anions of K^+ (10 mol m^{-3}) and NaCl (150 mol m^{-3}) both sprayed on the leaves (foliar). The values are means of 5 replicates ± standard errors. (Experiment 5.3).

Treatments	Sodium			
	Y. leaf	O. leaf	Y. Petiole	O. Petiole
NaCl	269 ± 54	303 ± 25	67 ± 11	98 ± 13
NaCl + K_2SO_4 (5mM)	117 ± 17	196 ± 18	24 ± 5	57 ± 4
NaCl + KCl	91 ± 5	171 ± 13	22 ± 2	51 ± 6
NaCl + KH_2PO_4	104 ± 15	267 ± 51	30 ± 3	67 ± 3
NaCl + KNO_3	115 ± 17	224 ± 15	24 ± 8	64 ± 9
NaCl + KCH_3COO	87 ± 4	177 ± 25	23 ± 2	52 ± 3
Treatments	Potassium			
	Y. leaf	O. leaf	Y. Petiole	O. Petiole
NaCl	26 ± 2	23 ± 1	124 ± 7	111 ± 8
NaCl + K_2SO_4 (5mM)	30 ± 5	19 ± 1	138 ± 7	156 ± 8
NaCl + KCl	38 ± 2	21 ± 1	139 ± 6	146 ± 9
NaCl + KH_2PO_4	30 ± 4	27 ± 3	130 ± 2	135 ± 7
NaCl + KNO_3	40 ± 9	21 ± 2	154 ± 9	150 ± 8
NaCl + KCH_3COO	40 ± 2	27 ± 2	141 ± 4	157 ± 4
Treatments	Magnesium			
	Y. leaf	O. leaf	Y. Petiole	O. Petiole
NaCl	13 ± 1	11 ± 1	12 ± 1	12 ± 1
NaCl + K_2SO_4 (5mM)	12 ± 3	10 ± 1	14 ± 1	16 ± 2
NaCl + KCl	17 ± 3	10 ± 1	13 ± 1	18 ± 2
NaCl + KH_2PO_4	13 ± 1	11 ± 1	12 ± 1	18 ± 3
NaCl + KNO_3	18 ± 5	11 ± 1	15 ± 2	20 ± 3
NaCl + KCH_3COO	22 ± 5	16 ± 2	15 ± 1	20 ± 2
Treatments	Calcium			
	Y. leaf	O. leaf	Y. Petiole	O. Petiole
NaCl	60 ± 5	59 ± 4	41 ± 2	36 ± 5
NaCl + K_2SO_4 (5mM)	69 ± 13	80 ± 7	43 ± 2	43 ± 2
NaCl + KCl	84 ± 16	72 ± 3	39 ± 2	46 ± 3
NaCl + KH_2PO_4	62 ± 11	67 ± 4	39 ± 3	47 ± 4
NaCl + KNO_3	80 ± 15	67 ± 4	42 ± 2	41 ± 6
NaCl + KCH_3COO	93 ± 18	83 ± 5	43 ± 2	50 ± 5

experiment 5.2 (Table 5.6), young leaf Na^+ concentrations in the treatment (150 mol m^{-3} NaCl + 10 mol m^{-3} KCl) were 20 mol m^{-3}, thereby, those young leaves showed higher K^+ concentrations. Different K^+ treatments had no significant effect to increase K^+ concentrations in the young petioles. However, in old petioles, significant ($p \leq 0.004$) increase in K^+ concentrations with various K^+ sources (except K-phosphate) compared with NaCl (alone) was observed. K^+ concentrations were substantially higher in the petioles than in the leaves.

Mg^{2+} Concentrations (Table 5.10)

Mg^{2+} concentrations in the old leaves significantly ($p \leq 0.023$) increased in K-acetate compared with K-sulphate and K-chloride treatments. In the young leaves, no significant effects on Mg^{2+} concentrations between the various treatments were detected. Generally, various K^+ treatments did not effect Mg^{2+} concentrations in both young and old leaves. No significant differences on Mg^{2+} concentrations in the young and old petioles among the various treatments were observed. However, Mg^{2+} concentrations were higher in both petioles in different K^+ treatments in comparison with NaCl (alone).

Ca^{2+} Concentrations (Table 5.10)

K-acetate significantly ($p \leq 0.023$) increased Ca^{2+} concentrations in the old leaves compared with NaCl (alone). In the young leaves, no significant differences in Ca^{2+} concentrations among the various treatments were observed. Ca^{2+} concentrations in both leaves were higher with various K^+ sources than NaCl (alone). Ca^{2+} concentrations in the young leaves were in the order K-acetate>K-chloride>K-nitrate>K-sulphate>K-phosphate>NaCl (alone). There were no significant differences in Ca^{2+} concentrations of young and old petioles between the various treatments. Ca^{2+} concentrations in the old petioles were higher at various K^+ than NaCl (alone). The highest Ca^{2+} concentrations in the old petioles were recorded with K-acetate followed by K-sulphate.

5.3.3.5 Anions Analysis

Cl^- concentrations (Table 5.11)

Young leaf Cl^- concentrations were significantly ($p \leq 0.007$) lower in NaCl + various K^+ treatments compared with NaCl (alone). The lowest Cl^- concentrations were found in K-acetate treatment while Cl^- concentrations were more or less the same in other K^+ treatments. In the old leaves, no significant differences in Cl^- concentrations among the various treatments were observed. In the old leaves, Cl^- concentrations were in the order K-phosphate>NaCl (alone)>K-nitrate>K-sulphate>K-acetate>K-chloride. Cl^- concentrations in the young petioles were significantly ($p \leq 0.001$) lower in various K^+ treatments (except K-phosphate) compared with NaCl (alone). Cl^- concentrations in the old petioles were significantly ($p \leq 0.036$) lower in K-acetate than in NaCl (alone). Cl^- concentrations in the old petioles were in the order NaCl (alone)<K-phosphate<K-chloride<K-nitrate<K-sulphate<K-acetate. A surprising result was found in the KCl treatment i.e. Cl^- concentration in the leaves and petioles were lower in this treatment compared with other K^+ treatments.

Malate Concentrations (Table 5.11)

No significant differences in malate concentrations in young and old leaves and petioles between different treatments were observed. Malate concentrations were higher in the young leaves, young and old petioles in different K^+ treatments than in NaCl (alone). Negative correlations between Na^+ and malate concentrations in young (-0.41*) and in old (-0.44**) leaves were observed.

NO_3^- Concentrations (Table 5.11)

Analysis of variance revealed no significant difference in NO_3^- concentrations in young and old leaves and in young petioles between the different treatments. NO_3^- concentrations did not increase with an application of KNO_3 in all the tissues, but decreased in the young petioles with KNO_3 compared with NaCl (alone). NO_3^- concentrations were significantly ($p \leq 0.000$) higher in the petioles than in leaves. Negligible NO_3^- concentrations were found in the leaves.

Significant negative correlations between Na^+ and NO_3^- concentrations in old petioles (-0.63**) was found, whereas they were positively correlated (0.56**) with K^+ concentrations in the same tissues.

Table 5.11 Cl^-, Malate, NO_3^- and SO_4^{2-} concentrations (mol m^{-3} expressed sap) in young (Y) and old (O) leaves and petioles of *Gossypium hirsutum* L. treated with various accompanying anions of potassium (10 mol m^{-3}) and NaCl (150 mol m^{-3}) both sprayed on the leaves (foliar). The values are means of 5 replicates ± standard errors. (Experiment 5.3).

Treatments	Chloride			
	Y. leaf	O. leaf	Y. Petiole	O. Petiole
NaCl	334 ± 79	397 ± 40	156 ± 18	166 ± 10
NaCl + K_2SO_4 (5 mM)	155 ± 20	302 ± 17	77 ± 8	123 ± 5
NaCl + KCl	143 ± 10	221 ± 56	70 ± 8	139 ± 8
NaCl + KH_2PO_4	167 ± 17	416 ± 86	109 ± 7	152 ± 12
NaCl + KNO_3	155 ± 45	327 ± 27	79 ± 22	124 ± 17
NaCl + KCH_3COO	108 ± 26	246 ± 42	82 ± 6	120 ± 8
Treatments	Malate			
	Y. leaf	O. leaf	Y. Petiole	O. Petiole
NaCl	8 ± 3	2 ± 2	11 ± 2	7 ± 2
NaCl + K_2SO_4 (5 mM)	26 ± 13	2 ± 2	21 ± 7	21 ± 7
NaCl + KCl	29 ± 12	4 ± 2	19 ± 6	25 ± 7
NaCl + KH_2PO_4	17 ± 11	0 ± 0	19 ± 9	15 ± 7
NaCl + KNO_3	18 ± 10	6 ± 4	24 ± 9	24 ± 11
NaCl + KCH_3COO	24 ± 13	9 ± 4	21 ± 9	24 ± 10
Treatments	Nitrate			
	Y. leaf	O. leaf	Y. Petiole	O. Petiole
NaCl	11 ± 5	2 ± 2	140 ± 4	140 ± 6
NaCl + K_2SO_4 (5 mM)	5 ± 3	0 ± 0	167 ± 6	187 ± 3
NaCl + KCl	8 ± 4	4 ± 3	163 ± 10	168 ± 6
NaCl + KH_2PO_4	6 ± 3	2 ± 2	155 ± 14	174 ± 2
NaCl + KNO_3	5 ± 2	5 ± 2	120 ± 35	167 ± 15
NaCl + KCH_3COO	7 ± 5	3 ± 2	165 ± 8	170 ± 10
Treatments	Sulphate			
	Y. leaf	O. leaf	Y. Petiole	O. Petiole
NaCl	13 ± 8	21 ± 8	2 ± 1	3 ± 3
NaCl + K_2SO_4 (5 mM)	19 ± 8	17 ± 6	2 ± 1	3 ± 2
NaCl + KCl	13 ± 5	12 ± 4	2 ± 1	7 ± 3
NaCl + KH_2PO_4	16 ± 5	13 ± 4	3 ± 2	4 ± 2
NaCl + KNO_3	15 ± 8	11 ± 6	16 ± 15	6 ± 4
NaCl + KCH_3COO	11 ± 7	12 ± 6	7 ± 4	2 ± 1

SO_4^{2-} Concentrations (Table 5.11)

There were no significant differences in SO_4^{2-} concentrations of young and old leaves and petioles between the treatments. SO_4^{2-} concentrations were significantly ($p \leq 0.000$) higher in the leaves than in petioles. SO_4^{2-} concentrations were slightly higher in the young leaves with an application of K-sulphate than other treatments.

5.4 Discussion

The objectives of experiments described in this Chapter were to reduce the effect of NaCl salinity by addition of KCl. In experiment 4.2, K^+ concentrations were substantially decreased with 200 mol m^{-3} NaCl applied to the leaves. It could be possible that K^+ may interact with Na^+ and reduce the effect of salinity. Furthermore, the optimum level of KCl to ameliorate sodium hazards and the best accompanying anion of K^+ was evaluated. In Experiment 5.1, increased soil and foliar salinity caused a significant reduction in growth, but the effect of foliar salinity was more serious than soil salinity. Aragüés *et al.* (1994) reported that shoot biomass of barley was reduced more in plants exposed to foliar wetting with saline sprinkler water than in plants in which the leaves were covered during sprinkling. This drastic inhibitory effects by salinity was effectively counteracted with additions of low and high amounts of KCl (1 and 10 mol m^{-3}). This positive response of cotton plants to the addition of KCl was more pronounced via foliar rather than soil application. When KCl was applied to the leaves under soil and foliar salinities, vegetative growth substantially increased, but increased more when both KCl and NaCl were applied to the leaves. Growth was enhanced at 10 mol m^{-3} KCl compared with 1 mol m^{-3} KCl under saline and non-saline conditions. Nair *et al.* (1993) reported that foliar spray of KCl applied at one week before and after panicle initiation of rice in coastal saline soils produced a high yield (88 g/plant) which was double that of its untreated control. Abd-Ella and Shalaby (1993) found that total dry weight of cotton increased when K^+:Na^+ ratios were 1:9 and 1:4 in two levels of saline irrigation water (3200 and 6400 mg/l).

External KCl did not significantly increase vegetative growth of cotton in the control and also little increase in growth was found with external soil-KCl (1 mol m^{-3}) in soil salinity treatments, provided that the K^+ was enough in the soil to meet the K^+ demand of the plant. Further, a high dosage of phostrogen that contains sufficient amounts of K^+ could meet the requirement of the plant for K^+. This was applied daily at the rate of 1g per litre after starting the treatments. Bednarz and Oosterhuis (1994) reported that the cotton plant can store excess K^+ in luxury amounts, which can serve as a reservoir during times of K^+ shortage.

In Experiment 5.2, most dramatic vegetative growth increases were associated with 10 and 25 mol m^{-3} KCl treatments under highly saline conditions. K^+ at 20 ppm significantly improved the growth and nodulation of chick pea at two levels of salinity (4.34 and 8.3 dS m^{-1}), but plant performance was not equal to that of the non-saline control even at 40 ppm K^+ (Saxena and Rawari, 1993). No visual differences of growth were produced by the addition of foliar and nonfoliar-KCl treated plants in low saline treatments. The response of plant growth to K^+ application in low saline treatments indicates that K^+ was not a limiting factor, while this was not true under highly saline conditions.

The results presented in Table 5.4 show that salinity effectively decreased the total number of leaves on the plant and also decreased leaf area. In crop plants, a decline in leaf expansion is often the first distinct response to salt or water stress (Terry *et al.*, 1983). Total number of leaves of wheat decreased with an increasing concentration of NaCl salinity (Khan, 1996, Maas and Grieve, 1990). Jafri and Ahmad (1995) reported that leaf area in cotton is significantly reduced with an increasing concentration of salinity (EC 16, 22 and 24 dS m^{-1}). Reduction in leaf area of plants under salinity has also been reported in cotton (Hoffman *et al.*, 1971).

Leaf thickness in the young leaves increased when 150 mol m^{-3} NaCl (alone) and 150 mol m^{-3} NaCl + 150 mol m^{-3} KCl were applied compared with other treatments. Also, leaf thickness in the old leaves was significantly greater in highly saline than in low saline treatments probably due to a large amount of Na^+ and Cl^- in the leaves (Table 5.4). Jafri and Ahmad (1995) found that increase in

leaf thickness with corresponding increase in the ratio of spongy mesophyll in cotton also observed under different salinity regimes. Such increase was reported earlier in bean and cotton (Longstreth and Nobel, 1979).

In Experiment 5.3, growth parameters of cotton were lower in NaCl (alone) treatment. External K^+ with its various anions increased vegetative growth significantly, but the effect on plant height was more pronounced with K_2SO_4 and KCl. Rao and Yadav (1997) found that foliar application of 20 or 40 ppm K^+ as K_2SO_4 at the vegetative and/or onset of flowering stages of Indian mustard on saline soils increased seed yield compared with the controls given no K^+. Ismail (1996) reported that 200 mol m^{-3} NaCl significantly reduced the growth of *Phaseolus vulgaris*, while addition of 200 g KNO_3/m^3 to the salinized medium considerably reduced the adverse effect of NaCl salinity. Generally, various K^+ sources did not show significant differences among each other on the growth of cotton. However, the growth of plants treated with K-phosphate was lower compared with other K^+ sources.

The adverse effects of salinity on net photosynthesis and the rate of transpiration were found in Experiment 5.1. Net photosynthesis decreased much more in foliar-applied salinity rather than in soil salinity treatments. On the other hand, additional KCl brought about an increase in photosynthesis and transpiration, but the effect was more via foliar rather than soil application (Table 5.1). Mosojidek *et al.* (1991) reported that generally the rate of photosynthesis declines under high salt stress. Increase in salinity of the medium resulted in decrease in transpiration in mustard, wheat and pearl millet (Qadar, 1994). In Experiment 5.2, photosynthetic rate was also decreased at 150 mol m^{-3} NaCl. Additional 10 mol m^{-3} K^+ significantly increased net photosynthesis, rate of transpiration and stomatal conductance under highly saline conditions. Bohra and Dörffling (1993) found that K^+ application of 50 and 75 mg K^+ kg^{-1} soil significantly increased potential photosynthetic activity in two varieties of rice under salinity. Leidi *et al.* (1992) reported that photosynthesis was affected by salinity (200 mol m^{-3} NaCl) in cotton, the decrease was due to salinity-induced K^+-deficiency.

Chlorophyll measurements decreased significantly with increasing salinity. Additional 10 mol m^{-3} KCl significantly increased chlorophyll measurements in highly saline treatment (Table 5.5). Reduction in green area due to salinity has been reported by Rawson (1986), Wyn Jones and Gorham (1989). Soil salinization to EC value of 10 mmhos/cm reduced chlorophyll content, as also photosynthetic rate in all varieties of rice (Pandey and Saxena, 1987).

In Experiment 5.1, Na^+ concentrations in the leaves were significantly higher via foliar rather than soil application of salinity in accordance with the results described in Chapter 4, this was probably due to a greater Na^+ import from direct absorption through the wetted leaves, i.e. an increased Na^+ uptake in the salt spray treatment. It could be possible that, the rate of absorption increased rapidly as the solution evaporated from the leaf and the salt became concentrated. Although the mechanism for the entry of foliar-applied salts into a leaf is not fully understood, there is evidence that direct stomatal penetration does not account for significant uptake, whereas the cuticles of guard cells are important for absorption (Humphreys *et al.*, 1986). Grattan (1994) reported that foliar uptake bypasses the exclusion mechanism by which roots growing in saline soil restrict the transport of Na^+ to the shoot. Root absorption is a selective process and most crop plants are relatively effective at excluding much of the salt in the soil solution (larger extent of Na^+) from entering the leaves (Grattan *et al.*, 1994). It therefore appeared from Experiment 5.1 that when Na^+ ions were absorbed by the leaves there were fewer opportunities for Na^+ exclusion than when these ions were absorbed by the roots and transported to the leaves. The entry of Na^+ through root is via root cortical, epidermal cells, plasmalemma of the xylem-parenchyma cells and the casparian strip that provide barriers that minimizes this ion influx to apoplast of the root xylem and transpiration stream. Benes *et al.* (1996) reported that the path of apoplastic and symplastic flow to the leaves is much shorter when Na^+ and Cl^- are absorbed primarily through leaves rather than through roots endodermis. It is suggested that a physiological mechanism might be involved in cotton roots that more efficiently control the uptake of Na^+ when salt was applied to the soil.

The data presented in Table 5.2 show that when 200 mol m^{-3} NaCl was applied to the soil and leaves, K^+ concentrations in both young and old leaves were lower in leaf treatments compared with soil treatments. Similar results were also obtained in experiments of Chapter 4 (see Tables 4.4 and 4.9). There are some explanations of K^+ concentrations variations, 1) Na^+ build up in the leaf tissue may trigger some (unknown) hormonal signal down to the roots that inhibits transport of K^+ ions to the shoot. 2) The increase of Na^+ ions could create an increased osmotic pressure in the plant and causes the suppression of uptake of essential nutrients such as K^+. 3) K^+ from old leaves may be translocated to the petiole, stem, young leaves and roots via the phloem when leaves were treated with salinity. This recirculation may be due to a build up of Na^+ ions that promote the exchange of K^+ for Na^+. 4) A combination of the above possibilities may occur. Flowers and Yeo (1986) reported that for many dicots, Na^+ is the predominant cation, and K^+, although sufficient for normal plant metabolism, is depressed as external salinity rises, i.e. Na^+ replaces K^+. Reductions in leaf K^+ concentrations in plants sprayed with saline water were also reported by Maas *et al.* (1982). Further experiments would be needed to verify that K^+ uptake is inhibited under soil and foliar-applied salinity.

As predicted (Chapter 4), Na^+ concentrations were higher in the old than in young leaves, while the opposite was true for K^+ concentrations (see Tables 5.2, 5.6 and 5.10). High salt loads are usually found in older leaves, because once growth is finished, ion concentrations are not any more compensated by volume increases, and continued transpiration will cause those concentrations to rise. It was observed that several of the old leaves were shed during foliar application of salt. It could be possible that the capacity for compartmentation within the cell became saturated, and the build-up of salts in the cytoplasm and/or the cell wall killed the old leaves. Munns and Termaat (1986) noted that if the rate of leaf death is greater than the rate of leaf expansion the supply of carbohydrates will decrease in proportion to the reduction in photosynthetically active leaf area. With time, the young leaves will be unable to sustain the growth of the whole plant. Munns *et al.* (1983) reported that lower rates of Na^+ accumulation in

expanding leaves might be the result of higher leaf growth and/or a mechanism that controls the transport or uptake of Na^+ by roots.

A striking feature of the ion composition of salinized plants was that Na^+ partitioned away from the young and old leaves (see Tables 5.2 and 5.6) and also from the young and old petioles (Table 5.10) with KCl application at even low concentrations (10 mol m^{-3}). This indicates a strong interaction between external KCl and salinity effects on Na^+ concentrations. However, with increasing concentrations of KCl, there was no further reduction of Na^+ concentrations. Bar-Tal *et al.* (1991) found that external K^+ at 30 mol m^{-3} caused significant reduction in Na^+ concentrations of corn plant at high salinity.

In experiment 5.1, Mg^{2+} concentration decreased in cotton leaves in response to the NaCl either applied to the soil or leaves, but the decrease was high via foliar application. Mg^{2+} concentrations also decreased in salinized plants with additional KCl (Table 5.2 and 5.6). Bohra and Dörffling (1993) reported that external K^+ decreased Mg^{2+} concentration and consequently increased the K/Mg ratios in rice under salinity. Ca^{2+} concentrations in both leaves were inversely related to the NaCl concentrations and were much higher than the Mg^{2+} concentrations. Marschner (1995) reported that Ca^{2+} is the main competitor of Mg^{2+} and the binding sites on the root plasma membrane appear to have less affinity for the high Mg^{2+} than for Ca^{2+}. Calcium induced Mg^{2+} deficiency is reported in sesame (Nassery *et al.*, 1979) and in barley (Carter *et al.*, 1979). Ca^{2+} concentrations decreased in both leaves when NaCl and KCl were applied to the leaves compared with soil application. Benes *et al.* (1996) found that Ca^{2+} concentrations were decreased in maize when 30 mol m^{-3} NaCl was applied to leaves as well as in soil, but the decrease was more pronounced via foliar than soil application. Similar results were obtained by Gorham *et al.* (1994) in barley, with 150 mol m^{-3} NaCl applied to soil and leaves, Ca^{2+} concentrations were lower in foliar than in soil treatments. In Experiment 5.2, Mg^{2+} concentrations significantly decreased in highly saline treatments, but the effect was more pronounced in older leaves than in younger ones. Gorham and Bridges (1995) found that Mg^{2+} concentrations in *Gossypium hirsutum* cv. Acala SJ2 only decreased significantly

in the older leaves at 150 mol m^{-3} NaCl, while young leaves were not greatly affected.

Leaf Cl^- concentrations data (Table 5.3) indicates that both soil and foliar absorption processes are responsible for increasing leaf Cl^- concentrations with associated NaCl salinity. Foliar salinity increased Cl^- concentrations in the old leaves much more than soil salinity as for Na^+ concentrations. The results are in agreement with the statement of Benes *et al.* (1996) who reported that in barley, the plants generally had higher leaf Cl^- concentrations when salts were applied to leaves rather than when salt was applied to the soil. Hence, it is suggested that cotton may rank differently for foliar uptake of Cl^- than for uptake through the roots. Gorham *et al.* (1994) found that Cl^- concentrations in two barley varieties were significantly higher in soil salinity rather than in foliar salinity (150 mol m^{-3} NaCl) treatments. Generally, Cl^- concentrations in the young leaves of salinized plants decreased due to the application of external KCl, while in the control, Cl^- concentrations increased with increasing concentration of external K^+ as KCl.

In experiment 5.2, Cl^- concentrations were increased in both leaf tissues consistently with increasing concentrations of KCl in low salinity treatments. Additional KCl reduced Cl^- concentrations in both leaves under highly saline conditions with an exception at 150 mol m^{-3} KCl treatment in old leaves. Cl^- concentrations were substantially higher in the old rather than in young leaves. Jeschke and Pate (1991) found that Cl^- concentrations in *Ricinus* increased with age of leaves. The pattern of Cl^- concentrations in the presence of high salt and external KCl was similar to that observed in experiment 5.1. In experiment 5.3, Cl^- concentrations in leaves and petioles were also decreased with the application of various accompanying anions of K^+. Cl^- concentrations did not increase with an application of KCl in leaf and petiole tissues. Cl^- concentrations in young leaves and old petioles were low in K-acetate treatment (Table 5.10).

The pattern of malate concentration in the presence of salt was similar to that observed in the previous experiment (see section 4.3.6), that increased Cl^- concentrations depressed malate concentrations. In the control and soil salinity treatments, malate concentrations were higher in the old than in young leaves,

while the opposite was true for foliar salinity treatments (Table 5.3). Klagges *et al.* (1993) found that malate concentrations in *Leptochloa fusca* rose with leaf age. A severe reduction in malate concentrations in the old leaves with foliar application of salt was found in all experiments of this Chapter. It could be possible that these leaves were overloaded with Na^+ and Cl^- which might be a consequence of the reduction in malate concentrations. Negative correlations in young (-0.44**) and in old leaves (-0.87**) were found between malate and Cl^- concentrations in experiment 5.2. Generally, negligible NO_3^- was found in both leaf tissues in all experiments of this Chapter.

In Experiment 5.1, cotton leaves exhibited symptoms of leaf injury i.e. leaf tips and margin were necrotic when 200 mol m^{-3} NaCl alone was applied on the leaves, but old leaves were much more affected than young leaves. Older leaves have a longer opportunity to accumulate salt by foliar and/or root absorption processes than do younger leaves. However, leaf injury was not found in soil salinity treatments. Grattan *et al.* (1994) found that barley which is classified as salt-tolerant exhibited substantially more foliar injury (i.e., chlorotic and necrotic) particularly in older leaves in uncovered plants than those covered during saline irrigation. In Experiment 5.2, leaf injury was observed in 150 mol m^{-3} NaCl and 150 mol m^{-3} NaCl + 150 mol m^{-3} KCl treatments. In 10 mol m^{-3} NaCl + 150 mol m^{-3} KCl treatment, some leaves showed leaf injury that leaf tips and margin were necrotic. In Experiment 5.3, visual symptoms of foliar burn on leaves were lower when NaCl was applied with K_2SO_4, KCl, KNO_3 and CH_3COOK than in NaCl (alone) followed by KH_2PO_4 treatment. Leaf burn can disrupt cell membrane integrity and photosynthesis, resulting in decreased carbon fixation (Chang and Oosterhuis, 1994).

5.5 Conclusions

- Cotton roots under soil salinity restricted the accumulation of Na^+, both in young and old leaves, and maintained adequate concentrations of K^+ under soil salinity with 200 mol m^{-3} NaCl. However, when the same concentrations of salt was applied to the leaves, Na^+ and Cl^- built-up steadily, while those of K^+ concentrations fell dramatically particularly in the old leaf tissues.
- Soil and foliar salinities caused substantial reduction in vegetative growth, but the effect was more pronounced in foliar rather than in soil salinity treatments.
- Foliar application of 150 and 200 mol m^{-3} NaCl caused severe leaf injury in cotton, and the effect was more serious in old leaves.
- External KCl at 10 and 25 mol m^{-3} was more beneficial than higher concentrations of KCl to increase vegetative growth, net photosynthesis, rate of transpiration, leaf chlorophyll, K^+ concentrations and reduce leaf Na^+ and Cl^- concentrations.
- Under non-saline conditions, external KCl did not significantly increase vegetative growth of cotton or K^+ concentrations.
- Shoot fresh weight, photosynthesis and K^+ concentrations were higher and Na^+ and Cl^- concentrations were lower in K-acetate rather than other accompanying anions of K^+.
- Method of soil and foliar-applied NaCl and KCl produced different results i.e. a decrease in growth and K^+ concentrations in foliar-applied NaCl compared with soil-applied NaCl, whereas the opposite was true for KCl application.

CHAPTER SIX

EFFECTS OF SALINITY ON K^+ UPTAKE

CHAPTER 6

EFFECTS OF SALINITY ON K^+ UPTAKE

6.1 Introduction

Excess of Na^+ and Cl^- creates ionic imbalance that may impair the selectivity of root membranes (Bohra and Dörffling, 1993). Excess of salt leads to the loss of K^+ due to membrane leakage and loss of Ca^{2+} from plasmalemma in roots due to displacement by Na^+ ions (Cramer *et al.*, 1985 and 1986). High Na^+ concentrations may disrupt K^+ and Ca^{2+} transport and interfere with growth of many plant species including cotton (Cramer *et al.*, 1987). K^+ uptake by plants can be affected by high salinity and particularly by high concentration of Na^+ in the solution (Bar-Tal *et al.*, 1991, Chow *et al.*, 1990, Helal and Mengel, 1979 and Kawasaki *et al.*, 1983). Potassium deficiency can be induced under saline conditions because high concentration of Na^+ can interfere with K^+ uptake, even though the uptake of K^+ and Na^+ occur through independent pathways (Lazof and Cheeseman, 1988).

Cl^- and SO_4^{2-} accumulate in plants treated with NaCl and Na_2SO_4 respectively. Thus, these anion accumulations may contribute to osmotic adjustment. The mechanisms by which various salts reduce plant growth are not entirely clear and may be different for plants of different salt tolerance (Abd El-Samad and Shaddad, 1996).

The root is the plant organ in direct contact with the saline environment. Its structure and function therefore regulate ion uptake and transport. The root is the primary barrier to solute movement into the plant and as a result the ion concentrations delivered to the shoot are different from those in the external medium (Shannon *et al.*, 1994). In plants growing under saline conditions roots are directly exposed to high external salinity. Tolerance of the plant, therefore, would appear to depend on salt tolerance of the root. This tolerance would include the capability of continued uptake of essential elements like K^+ and NO_3^- amongst other ions (Jeschke and Wolf, 1988). In many cases, root growth is less affected by salt than shoot growth (Lüttge, 1983).

Salinity reduces K^+ concentrations in the shoots and roots of cotton (Kent and Läuchli, 1985), maize (Zidan *et al.*, 1992), faba bean (Cordovilla *et al.*, 1995), sweet pepper (Gómez *et al.*, 1996) and citrus (Walker and Douglas, 1983). Läuchli and Stelter (1982) reported that in a salt tolerant cultivar of cotton, however, K^+ concentration was not affected by 144 mol m^{-3} NaCl. Uptake from low external K^+ levels is highly specific for K^+, while at higher K^+ concentrations (>0.5 mol m^{-3}) Na^+ can competitively inhibit K^+ influx (Epstein *et al.*, 1963). The application of high K^+ might enhance the capacity for osmotic adjustment of plants growing in saline habitats (Cerdá *et al.*, 1995), whereas in tropical grasses, high K^+ supply was not found to enhance osmotic adjustment of water-stressed leaves (Wilson and Ludlow, 1983).

The main objective of the experiments described in this Chapter was to determine the effect of root and foliar-applied salinity on K^+ uptake by cotton. It was observed from previous experiments of Chapter 4 and 5 that foliar application of NaCl significantly decreased K^+ concentrations in the leaves. This could be due among other mechanisms to inhibit K^+ uptake rate by the roots and its translocation to the shoot. To test this hypothesis, some experiments were conducted in hydroponic culture. An experiment was also carried out to compare the effect of NaCl and Na_2SO_4 salinities when applied to the roots and shoots on uptake and translocation of K^+. I also investigated whether K^+ deficiency caused by salinity could be decreased by foliar application of KCl.

6.2 Materials and Methods

6.2.1 Experiment 6.1 (Root & shoot-applied 10 and 50 mol m^{-3} NaCl)

This experiment was designed to examine the effect of 10 and 50 mol m^{-3} NaCl applied by different methods i.e. root and shoot application, on the net uptake rate of potassium.

Seeds of *Gossypium hirsutum* L., Acala SJ-2 were used in this experiment. Seeds were delinted (see section 2.2) and germinated as described in section 2.3. After germination, the seeds were transferred to hydroponic culture as mentioned in Chapter 2.4. The experiment was conducted in a growth cabinet

(Fisons 600THTL, 28 °C day/20 °C night, 16 h photoperiod, with 350 µmol photons m^{-2} s^{-1} photosynthetically active radiation (400-700 nm) supplied by warm white fluorescent tubes plus tungsten light bulbs).

Plant nutrition was applied as; 1 mol m^{-3} $NH_4H_2PO_4$, 4 mol m^{-3} $Ca(NO_3)_2$, and 2 mol m^{-3} $MgSO_4$ (Hoaglands No. 1) with 0.5 cm^3 micronutrients (Hoagland and Arnon, 1950). These nutrients were applied from transferring the seedlings into hydroponic culture until harvesting the experiment. The nutrient solution was initially changed every other day, but when the treatments were applied to plants 21 days after sowing, the nutrient solution was changed daily.

The experiment comprised 5 treatments with 6 replicates. There were three levels of NaCl (0, 10 and 50 mol m^{-3}) and two modes of salt application i.e. via root and shoot. KCl at 0.1 mol m^{-3} concentration was applied for 2 weeks from the sowing then applied at 0.5 mol m^{-3} to all the treatments with nutrient solution i.e. via root application. The plants were treated for 15 days and then harvested. Before harvesting, the shoots and roots of the plants were washed thoroughly with distilled water and dried shoots and roots were measured for fresh weight. The shoots and roots were then kept in a oven at 70 °C for 48 hours for dry weight.

Net uptake rate of K^+ was measured according to its depletion, i.e. disappearance of K^+ from the nutrient solution with time. The first sample from the nutrient solution was taken one hour after the solution was changed and the second sample was taken after 5 hours. Both samples were measured for K^+ using a flame photometer. Calculations of net uptake rate of K^+ were calculated as; NUR-K^+ = K^+ in the 1^{st} sample - K^+ in the 2^{nd} sample. NUR-K^+ was measured daily for 15 days from the date of starting the treatments. After taking the final reading, the plants were harvested and NUR-K^+ was calculated on dry root basis. Dry shoots and roots were extracted (see section 2.11) and the extract was analysed for Na^+ and K^+ ions on flame photometer.

Statistical Analysis

Data was analysed using Describe and one way Anova by using 5 treatments with 5 replicates. The data are presented with standard error of the

means, and were tested for significant differences between means at P>0.05 using Tukey's test.

6.2.2 Experiment 6.2 (Root and shoot-applied 50 mol m^{-3} NaCl)

This experiment was conducted to investigate the effect of 50 mol m^{-3} NaCl when applied to shoots and roots on inhibition of K^+ uptake. The same materials and methods were used in this experiment as for experiment 6.1. This experiment was carried out in a Vindon growth room in the basement of the Thoday Building, University of Wales, Bangor with the same temperature and photoperiod as in experiment 6.1. There were 3 treatments with 6 replicates i.e. 0 mol m^{-3} NaCl, 50 mol m^{-3} NaCl (shoot treatment) and 50 mol m^{-3} NaCl (root treatment). 0.5 mol m^{-3} KCl was applied for the first 2 weeks then applied at 1 mol m^{-3} KCl to all the treatments via root application. The plants were treated after 19 days from the sowing and continued treatment for 7 days. The first reading was taken 10 minutes after the solution was changed and the second after 3 hours.

Statistical Analysis

Same as in experiment 6.1, but using 3 treatments with 6 replicates.

6.2.3 Experiment 6.3 (Cl^- versus SO_4^{2-})

This experiment was designed to examine the effect of 50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4 salinities when applied to shoots and roots on the net uptake rate of K^+. The same materials and methods and growth conditions were used as in experiment 6.2. The experiment was treated for 7 days.

Statistical Analysis

Same as in experiment 6.2, but using 5 treatments with 6 replicates.

6.2.4 Experiment 6.4 (Foliar-applied 10 mol m^{-3} KCl)

This experiment was designed to investigate the effect of 10 and 150 mol m^{-3} NaCl applied to the roots in 3 different concentrations of KCl (0.1, 1 and 10 mol m^{-3}) on inhibition of K^+ uptake. I further investigated whether foliar application of KCl (10 mol m^{-3}) could reduce the effect of NaCl on K^+

concentrations. The same materials and methods and growth conditions were used as for experiment 6.3. KCl at 0.1, 1 and 10 mol m^{-3} was applied to roots from the sowing of seeds. The plants were treated for 13 days.

Statistical Analysis

Three way Anova i.e. 2 levels of NaCl x 3 levels of KCl (root) x 2 levels of KCl (shoot) was done by using 12 treatments with 5 replicates.

6.3 Results

6.3.1 Experiment 6.1 (Root & shoot-applied 10 and 50 mol m^{-3} NaCl)

6.3.1.1 Shoot, root dry weight and net uptake rate of K^+

Analysis of variance revealed that there were no significant differences in dry weight of shoot and root between the treatments. However, dry shoot and root weight was lower when 10 mol m^{-3} NaCl was applied to the shoots compared with the other treatments (Fig. 6.1a and 6.1b).

50 mol m^{-3} NaCl applied to the roots or shoots caused significant ($p \leq 0.039$) inhibition in NUR-K^+ compared with the control, but NUR-K^+ was more inhibited in root rather than in shoot treatments (Fig. 6.2). 10 mol m^{-3} NaCl applied to the roots or shoots also caused inhibition in NUR-K^+ by 45% and 24% respectively compared with the control.

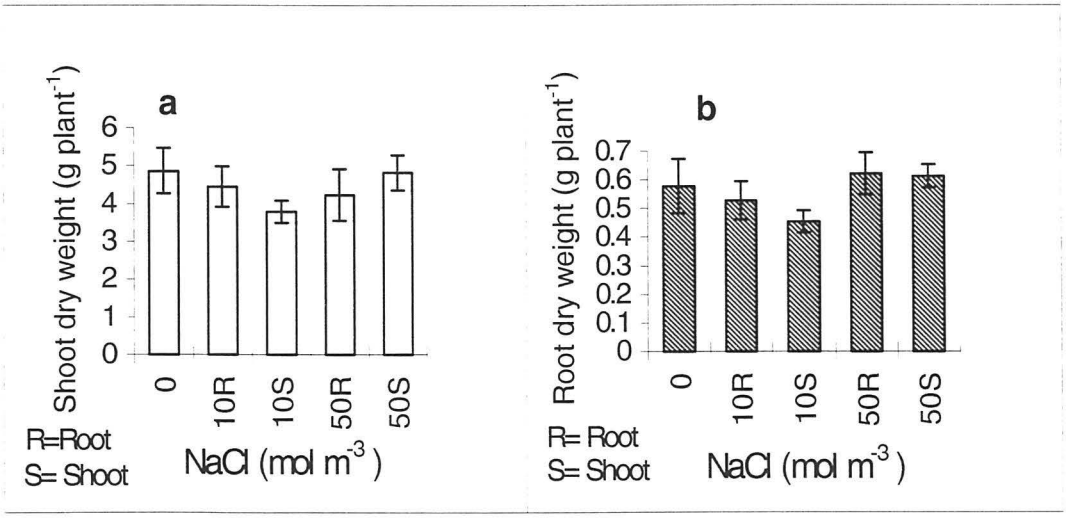

Fig. 6.1 Effect of 10 and 50 mol m^{-3} NaCl applied to the roots or shoots on **(a)** shoot and **(b)** root dry weight of 36 day old cotton plants (15 days treatment). Each column is the mean of 5 replicates and vertical bars indicate standard errors of the means (Experiment 6.1).

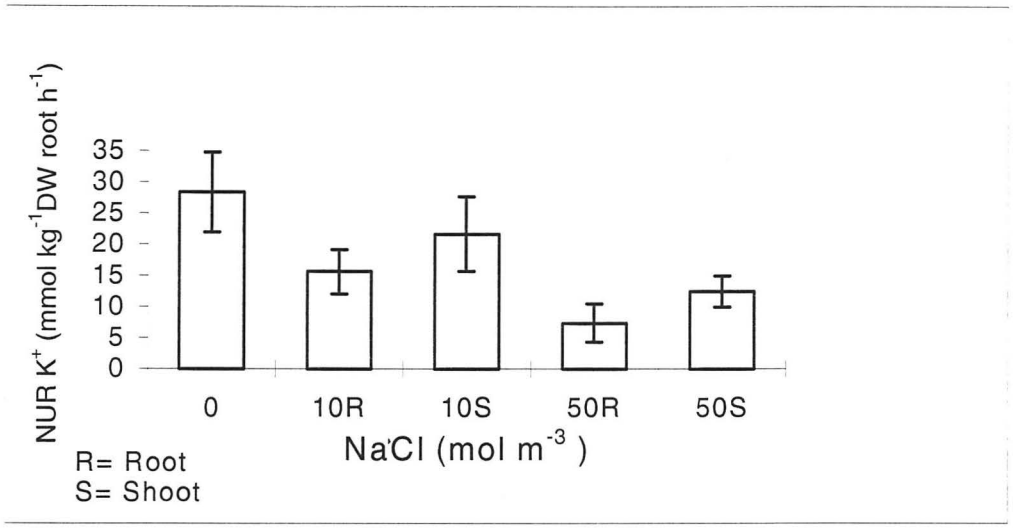

Fig. 6.2 Effect of 10 and 50 mol m^{-3} NaCl applied to the roots or shoots on K^+ Net Uptake Rate (NUR, mmol kg^{-1} DW root h^{-1}) determined by disappearance from solutions of 36 day old cotton plants (15 days treatment). Each column is the mean of 5 replicates and vertical bars indicate standard errors of the means (Experiment 6.1).

6.3.1.2 Shoot Na^+ and K^+ concentrations (mmol kg^{-1} DW shoots) (Fig. 6.3a)

Na^+ concentrations in the shoots were significantly ($p \leq 0.000$) higher when 10 and 50 mol m^{-3} NaCl were applied to the roots compared with the shoot application and the control. There was no great difference in Na^+ concentrations between high salinity shoot and low salinity root treatment.

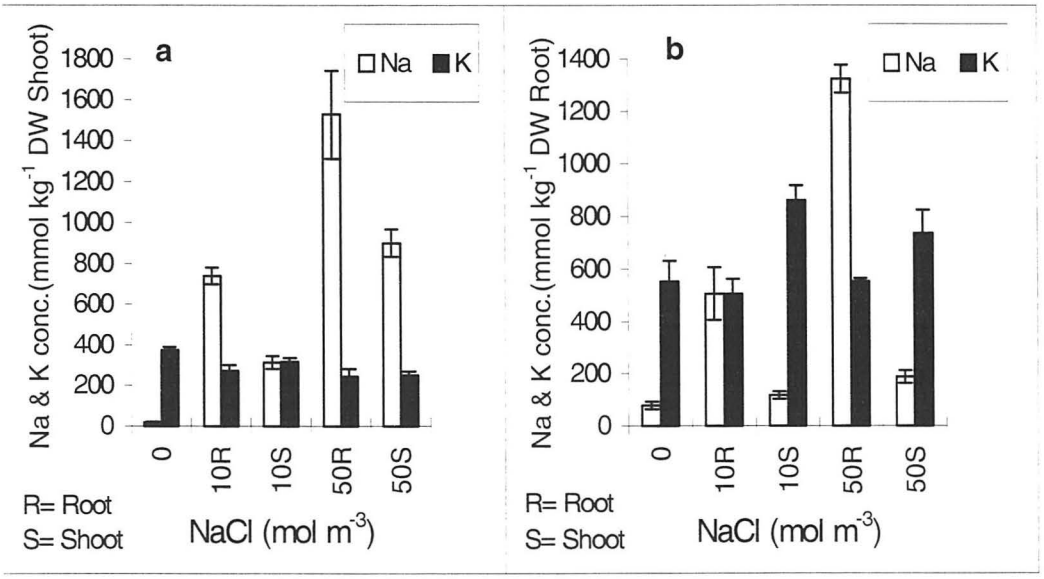

Fig. 6.3 Effect of 10 and 50 mol m^{-3} NaCl applied to the roots or shoots on Na^+ and K^+ concentrations (mmol kg^{-1} DW) in **(a)** dry shoot and **(b)** dry root of 36 day old cotton plants (15 days treatment). Each column is the mean of 5 replicates and vertical bars indicate standard errors of the means (Experiment 6.1).

10 mol m^{-3} root-applied NaCl and 50 mol m^{-3} NaCl either applied to the roots or shoots significantly ($p \leq 0.009$) decreased K^+ concentrations compared with the control. K^+ concentrations were higher in low salinity than in high salinity treatments, but the difference was not significant.

6.3.1.3 Root Na^+ and K^+ concentrations (mmol kg^{-1} DW roots) (Fig. 6.3b)

Na^+ concentrations in the roots were significantly ($p \leq 0.000$) higher when 10 and 50 mol m^{-3} NaCl was applied to the root medium compared with low and high NaCl applied to the shoots and the control. Na^+ concentrations in the roots were significantly higher when 10 mol m^{-3} NaCl was applied to the roots rather than when 50 mol m^{-3} NaCl was applied to the shoots. This shows that very little Na^+ was transported from the shoots to the roots.

K^+ concentrations were significantly ($p \leq 0.003$) higher when low NaCl was applied to the shoots compared with low and high NaCl applied to the root medium and the control. Fig. 6.1b shows that shoot and root dry weights were lower at 10 mol m^{-3} NaCl applied to the shoots compared with other treatments. The highest K^+ concentrations in this treatment could be due to low growth of shoot and root thereby, roots showed high K^+ concentrations. Root K^+ concentrations were more or less the same as at the control and in low and high root salinity treatments. K^+ concentrations were higher in the roots than in the shoots with both modes of low and high salt application, while the opposite was true for Na^+ concentrations. In the control, K^+ concentrations were also higher in the roots than in the shoots.

6.3.2 Experiment 6.2 (Root and shoot-applied 50 mol m^{-3} NaCl)

6.3.2.1 Shoot, root dry weight and net uptake rate of K^+

50 mol m^{-3} NaCl applied to the roots significantly ($p \leq 0.00$) reduced shoot dry weight compared with the control and saline shoot treatment (Fig. 6.4). There was no difference in shoot dry weight between the control and saline shoot treatment. No differences in root dry weight were found between the treatments.

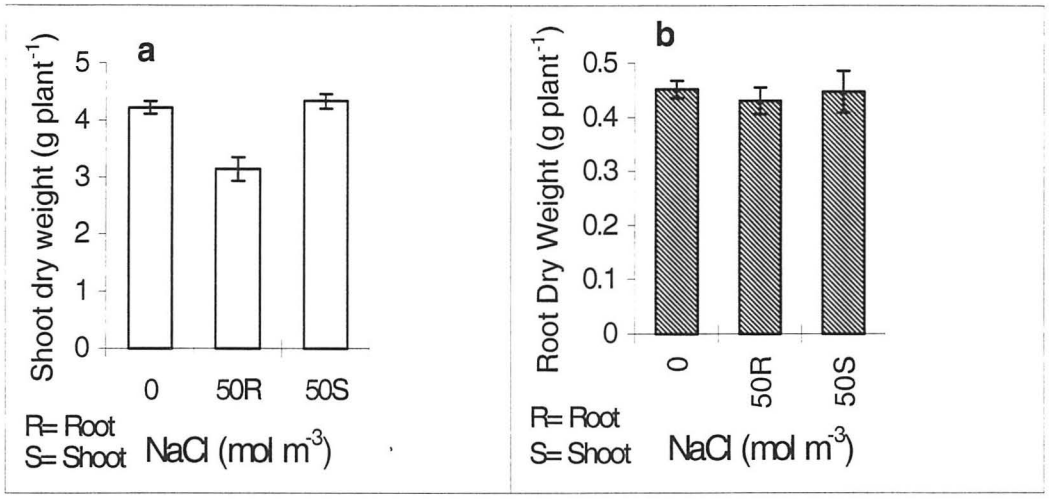

Fig. 6.4 Effect of 50 mol m^{-3} NaCl applied to the roots or shoots on **(a)** shoot and **(b)** root dry weight of 26 day old cotton plants (7 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.2).

50 mol m^{-3} NaCl either applied to the roots or shoots significantly ($p \leq 0.001$) inhibited net uptake rate of K^+ compared with the control (Fig. 6.5).

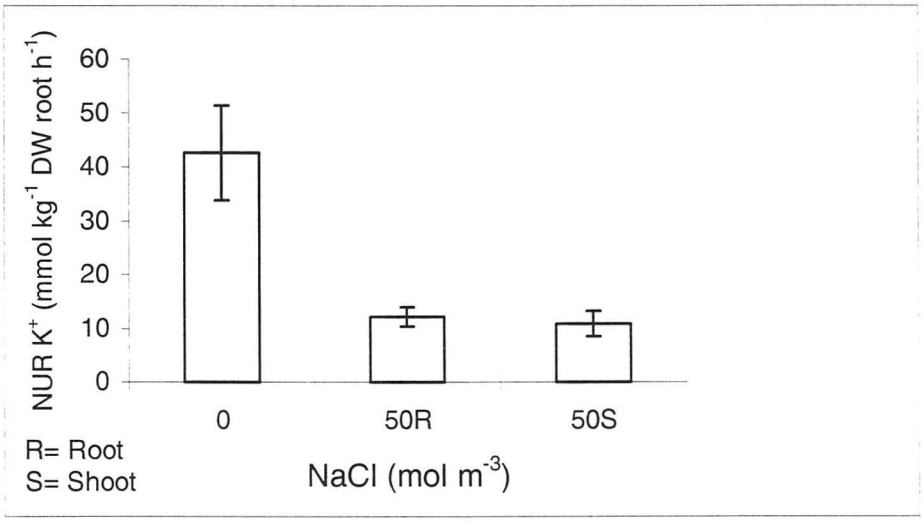

Fig. 6.5 Effect of 50 mol m^{-3} NaCl applied to the roots or shoots on K^+ Net Uptake Rate (NUR, mmol kg^{-1} DW root h^{-1}) determined by disappearance from solutions of 26 day old cotton plants (7 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.2).

6.3.2.2 Shoot Na^+ and K^+ concentrations (mmol kg^{-1} DW shoots) (Fig. 6.6a)

No significant differences in K^+ concentrations of shoots between the treatments were found. However, K^+ concentration in shoots decreased by 16% in

saline root and 29% in saline shoot treatments compared with the control. Na^+ and K^+ concentrations were higher in saline root than in saline shoot treatments.

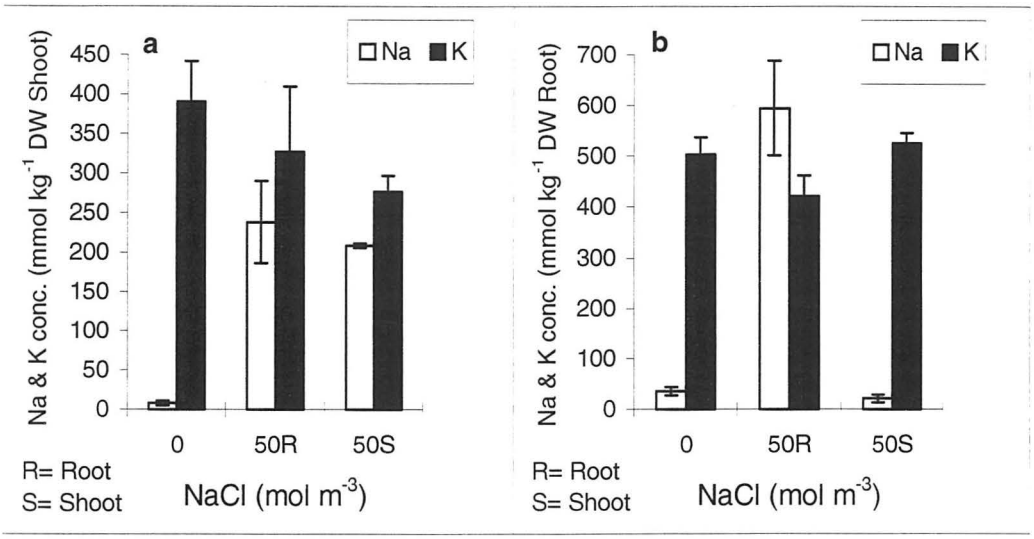

Fig. 6.6 Effect of 50 mol m^{-3} NaCl applied to the roots or shoots on Na^+ and K^+ concentrations (mmol kg^{-1} DW) in **(a)** dry shoot and **(b)** dry root of 26 day old cotton plants (7 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.2).

6.3.2.3 Root Na^+ and K^+ concentrations (mmol kg^{-1} DW roots) (Fig. 6.6b)

50 mol m^{-3} NaCl applied to the root medium significantly ($p \leq 0.000$) increased Na^+ concentration in the roots compared with the control and saline shoot treatment. Na^+ concentrations in the roots did not increase with an application of 50 mol m^{-3} NaCl when applied to shoots. This shows that Na^+ was not transported from the shoots to the roots.

No significant difference in K^+ concentrations in the roots was found among the treatments. However, K^+ concentrations were lower in saline root treatment than in the control and saline shoot treatment.

6.3.3 Experiment 6.3 (Cl^- versus SO_4^{2-})

6.3.3.1 Shoot, root dry weight and net uptake rate of K^+

No significant difference in dry weight of shoots or roots was found between the treatments. Dry weight of shoots and roots was slightly higher in both Na_2SO_4 treatments compared with NaCl treatments (Fig. 6.7).

Net uptake rate of K^+ was significantly ($p \leq 0.000$) inhibited with the application of 50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4 either applied to the roots or shoots compared with the control (Fig 6.8). Inhibition of K^+ uptake was greater with NaCl rather than with Na_2SO_4. Both salts inhibited NUR-K^+ more when applied to the roots than when applied to the shoots.

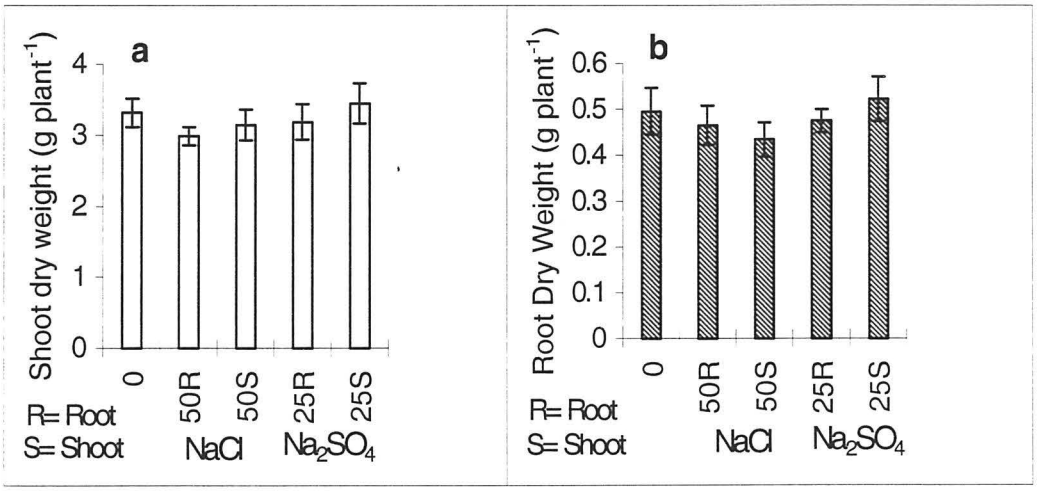

Fig. 6.7 Effect of 50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4 applied to roots or shoots on **(a)** shoot and **(b)** root dry weight of 32 day old cotton plants (7 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.3).

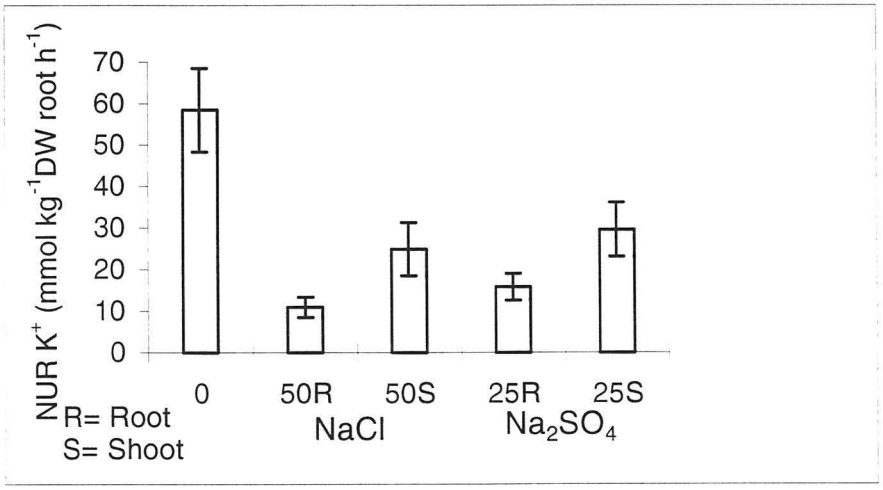

Fig. 6.8 Effect of 50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4 applied to the roots or shoots on K^+ Net Uptake Rate (NUR, mmol kg^{-1} DW root h^{-1}) determined by disappearance from solutions of 32 day old cotton plants (7 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.3).

6.3.3.2 Shoot Na^+ and K^+ concentrations (mmol kg^{-1} DW shoots) (Fig. 6.9a)

Na^+ concentrations in the shoots were significantly ($p \leq 0.000$) lower when Na_2SO_4 was applied to the shoots compared with root-applied Na_2SO_4 as well as compared with NaCl when applied to the shoots and roots.

Both salts either applied to the roots or shoots significantly ($p \leq 0.000$) lowered K^+ concentrations in the shoots compared with the control. K^+ concentrations were the same in the root and shoot treatment with NaCl. However, K^+ concentrations were significantly lower when Na_2SO_4 was applied to the shoots rather than when applied to the roots.

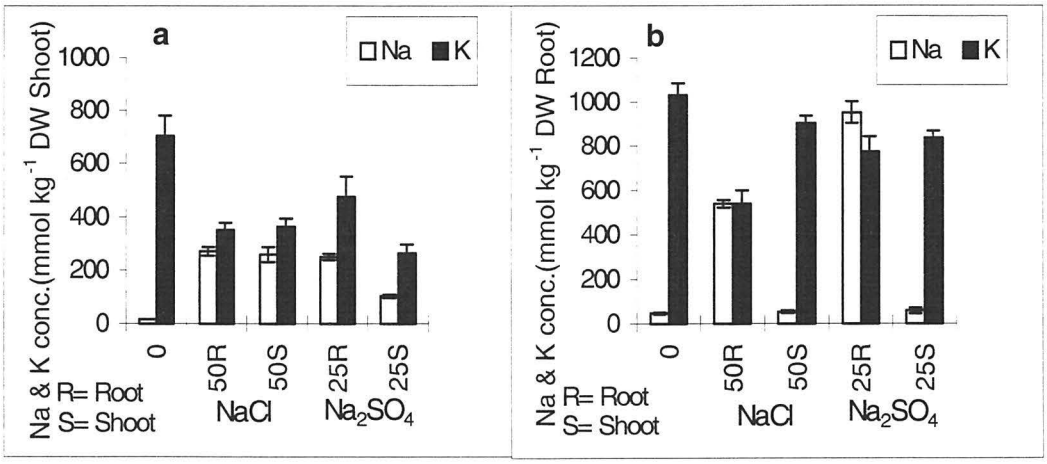

Fig. 6.9 Effect of 50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4 applied to the roots or shoots on Na^+ and K^+ concentrations (mmol kg^{-1} DW) in **(a)** dry shoot and **(b)** dry root of 32 day old cotton plants (7 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.3).

6.3.3.3 Root Na^+ and K^+ concentrations (mmol kg^{-1} DW roots) (Fig. 6.9b)

Na^+ concentrations in the roots were significantly ($p \leq 0.000$) higher when 25 mol m^{-3} Na_2SO_4 was applied to the roots compared with other treatments. 50 mol m^{-3} NaCl applied to the roots significantly increased Na^+ concentrations in the roots compared with the shoot treatment of NaCl, Na_2SO_4 and the control. When either forms of salt were applied to the shoot, Na^+ concentrations in the roots did not increase due to negligible transport of Na^+ from the shoots to roots as observed in experiment 6.1 and 6.2.

Both treatments of Na_2SO_4 and root treatment of NaCl significantly ($p \leq 0.000$) decreased K^+ concentrations in the roots compared with the control. 50

mol m^{-3} NaCl applied to the roots significantly reduced K^+ concentrations compared with shoot treatment of NaCl and also shoot and root treatment of Na_2SO_4.

6.3.3.4 Shoot Cl^- and SO_4^{2-} concentrations (mmol kg^{-1} DW shoot) (Fig. 6.10a)

Shoot and root application of NaCl significantly ($p \leq 0.000$) increased Cl^- concentrations in the shoots compared with the control and both treatments of Na_2SO_4. Cl^- concentrations were lower in both treatments of Na_2SO_4 than in the control. No large differences in Cl^- concentrations were observed when NaCl was applied to the roots or shoots.

At the control, SO_4^{2-} concentrations in the shoots were significantly ($p \leq 0.001$) higher than in the root and shoot treatments of NaCl. SO_4^{2-} concentrations in the shoots did not increase with foliar application of Na_2SO_4 as it was significantly lower than at the control. Significantly higher SO_4^{2-} concentrations were found when 25 mol m^{-3} Na_2SO_4 was applied to the roots compared with the shoot treatment of Na_2SO_4 and root and shoot treatments of NaCl.

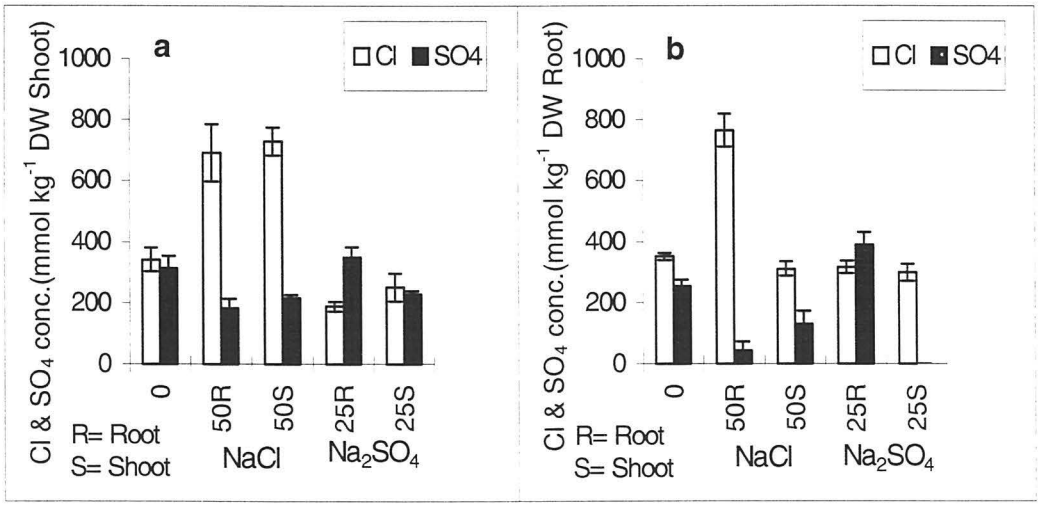

Fig. 6.10 Effect of 50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4 applied to the roots or shoots on Cl^- and SO_4^{2-} concentrations (mmol kg^{-1} DW) in **(a)** dry shoot and **(b)** dry root of 32 day old cotton plants (7 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.3).

6.3.3.5 Root Cl^- and SO_4^{2-} concentrations (mmol kg^{-1} DW roots) (Fig. 6.10b)

Cl^- concentrations in the roots were significantly ($p \leq 0.000$) higher in the root treatment of NaCl than other treatments. Foliar application of NaCl did not increase Cl^- concentrations in the roots as they were lower than the control.

At the control, SO_4^{2-} concentrations in the roots were significantly higher than both treatments of NaCl and shoot-applied Na_2SO_4. SO_4^{2-} concentrations significantly increased with root-applied Na_2SO_4 compared with other treatments. It was surprising that with foliar application of Na_2SO_4, roots showed a negligible SO_4^{2-} concentration.

6.3.4 Experiment 6.4 (Foliar-applied 10 mol m^{-3} KCl)

6.3.4.1 Shoot and root dry weight (Fig. 6.11)

Increasing salinity significantly ($p \leq 0.000$) decreased shoot dry weight, whereas increasing concentrations of KCl (root medium) significantly ($p \leq 0.000$) increased shoot dry weight. Interaction between NaCl x KCl had significant ($p \leq 0.049$) effects on shoot dry weight. Foliar application of KCl did not produce significant effects on shoot dry weight. Shoot dry weight was higher when 1 and 10 mol m^{-3} KCl was applied to the roots compared with 0.1 mol m^{-3} KCl in both saline treatments. No clear differences in shoot dry weight between 1 and 10 mol m^{-3} KCl treatments were found.

Salinity, KCl and their interaction produced significant ($p \leq 0.008$, $p \leq 0.000$, $p \leq 0.031$ respectively) effects on the root dry weight. Root dry weight was significantly greater at 1 and 10 mol m^{-3} KCl compared with 0.1 mol m^{-3} KCl treatments under both saline conditions. 150 mol m^{-3} NaCl did not decrease root dry weight in 0.1 and 1 mol m^{-3} KCl treatments, but decreased it in root-applied 10 mol m^{-3} KCl. Foliar application of KCl slightly increased root dry weight at 1 mol m^{-3} KCl in both saline treatments. Generally, root dry weight was slightly higher when 10 mol m^{-3} KCl was applied rather than when 1 mol m^{-3} KCl was applied to the roots.

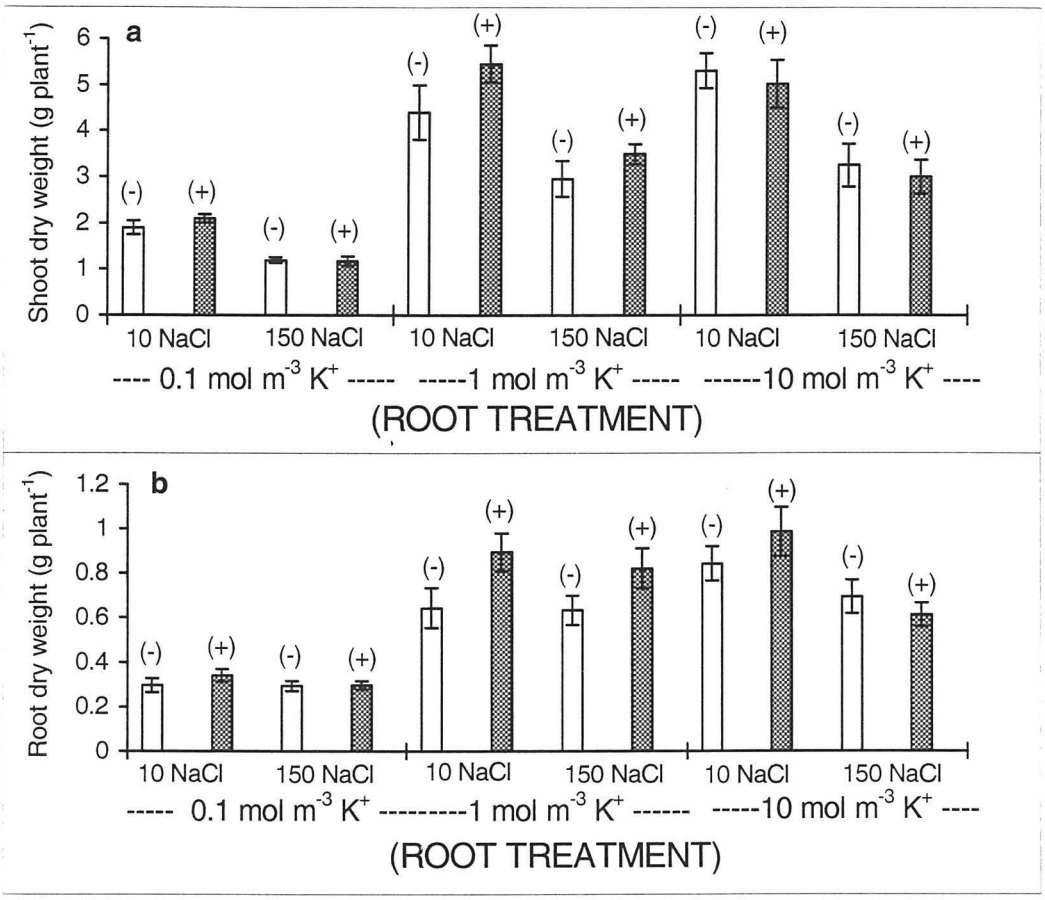

Fig. 6.11 Effect of 10 and 150 mol m^{-3} NaCl with 0.1, 1 and 10 mol m^{-3} KCl applied to the roots and (+) and (-) 10 mol m^{-3} KCl applied to the shoots on **(a)** shoot and **(b)** root dry weight of 44 day old cotton plants (13 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.4).

6.3.4.2 Net uptake rate (NUR) of K^+ (Fig 6.12)

150 mol m^{-3} NaCl significantly ($p \leq 0.000$) inhibited K^+ uptake. None of the other factors produced significant effects on NUR-K^+. Net uptake rate of K^+ in low salinity treatments was slightly lower with foliar application of KCl when K^+ concentration was 0.1 mol m^{-3}. This suggests that at low KCl, foliar application of KCl could meet the plant requirement of K^+, thus the shoot did not require more K^+ from roots. However, NUR-K^+ was much higher with foliar application of KCl at 1 mol m^{-3} KCl in both salinity treatments. At 10 mol m^{-3} NaCl, K^+ uptake was significantly higher at 0.1 mol m^{-3} KCl compared with 1 mol m^{-3} KCl (no foliar K^+) treatments. This shows that the requirement of the shoot for K^+ was higher when roots were given low K^+ than when roots were given 1 mol m^{-3} KCl.

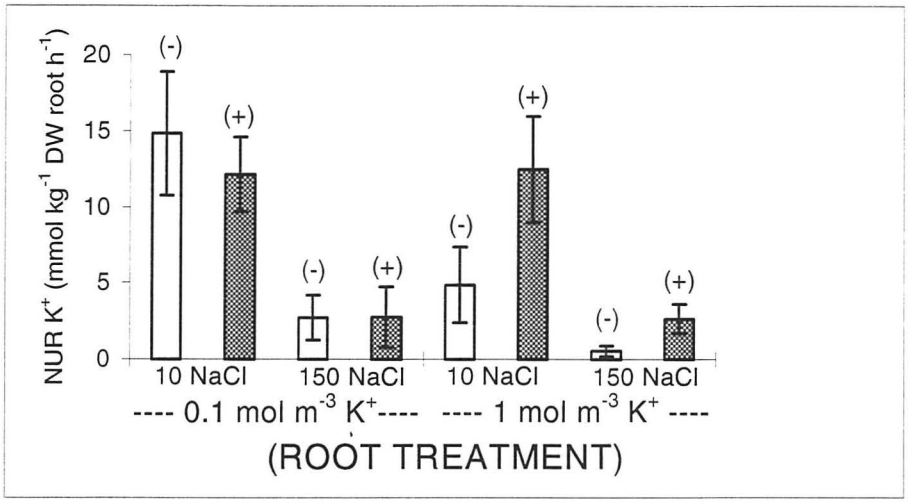

Fig. 6.12 Effect of 10 and 150 mol m^{-3} NaCl with 0.1, 1 and 10 mol m^{-3} KCl applied to roots and (+) and (-) 10 mol m^{-3} KCl applied to shoots on K^+ Net Uptake Rate (NUR, mmol kg^{-1} DW root h^{-1}) determined by disappearance from solutions of 44 day old cotton plants (13 days treatment). Each column is the mean of 6 replicates and vertical bars indicate standard errors of the means (Experiment 6.4).

When 10 mol m^{-3} KCl was applied to the root medium, K^+ was higher in the nutrient solution at second observation than at first observation. It could be possible that the solution became more concentrated for K^+ due to evaporation of water from the nutrient solution. Hence, the data are not presented in the Fig. 6.12.

6.3.4.3 Shoot Na^+ and K^+ concentrations (mmol kg^{-1} DW shoots) (Fig. 6.13).

Salinity, KCl and foliar application of KCl produced significant ($p \leq 0.000$, $p \leq 0.008$ and $p \leq 0.022$ respectively) effects on Na^+ concentrations in the shoots. A consistent decrease in Na^+ concentrations of shoots was observed in both saline treatments with increasing concentrations of KCl in the root medium. Foliar application of KCl did not affect Na^+ concentrations in both saline treatments at all concentrations of KCl applied to the roots.

150 mol m^{-3} NaCl significantly ($p \leq 0.000$) decreased K^+ concentrations, while increasing concentrations of KCl in the root medium significantly ($p \leq 0.000$) increased K^+ concentrations in the shoots. Foliar-KCl significantly increased K^+ concentrations in only low saline at 10 mol m^{-3} KCl root treatment. NaCl x KCl interaction had significant ($p \leq 0.000$) effects on K^+ concentrations of the shoots.

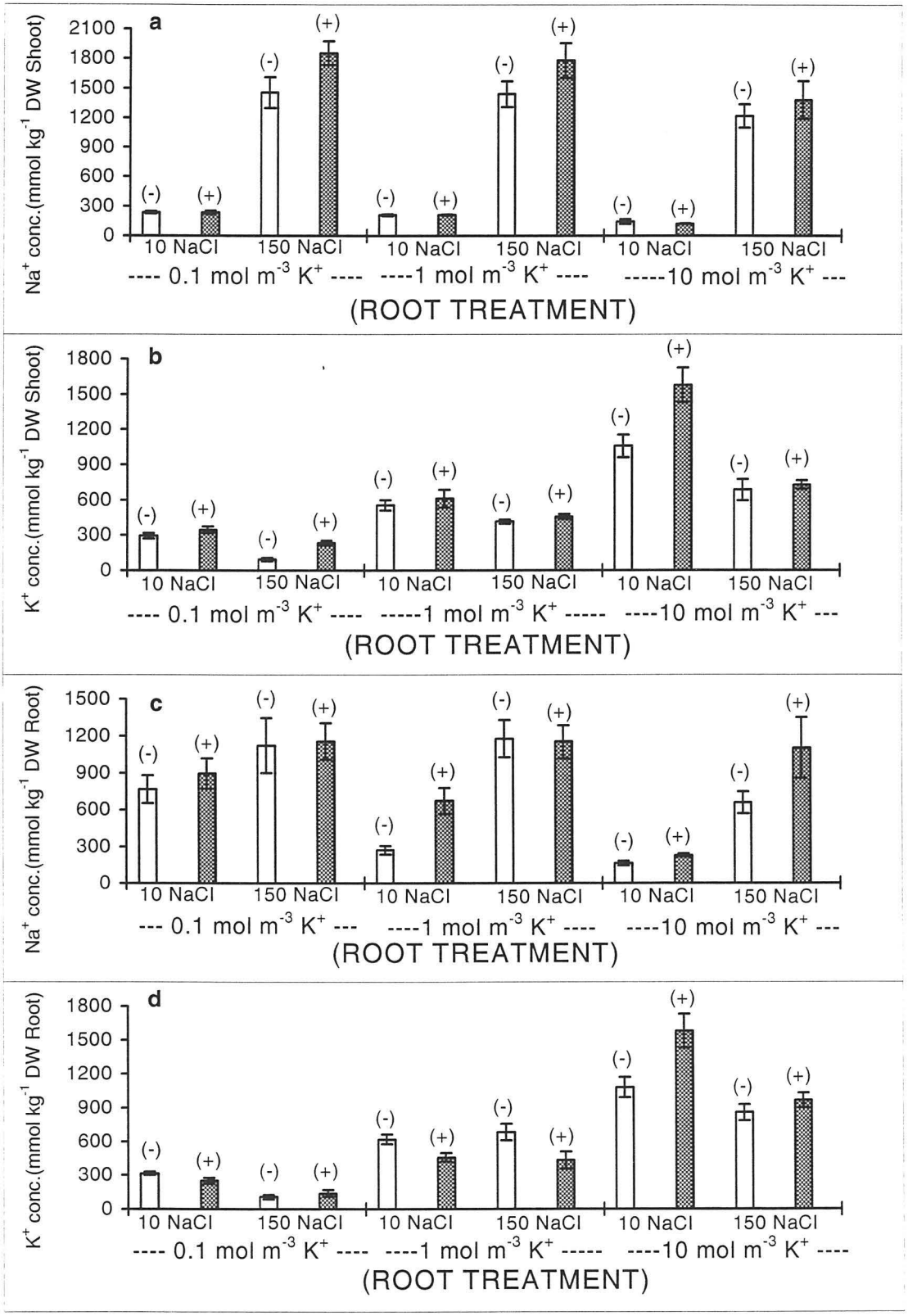

Fig. 6.13 Effect of 10 and 150 mol m^{-3} NaCl with 0.1, 1 and 10 mol m^{-3} KCl applied to the roots and (+) and (-) 10 mol m^{-3} KCl applied to the shoots on **(a)** Na^+ and **(b)** K^+ concentrations (mmol kg^{-1} DW) in dry shoot and **(c)** Na^+ and **(d)** K^+ concentrations (mmol kg^{-1} DW) in dry roots of 44 day old cotton plants (13 days treatment). Each column is the mean of 6 replicates and vertical bars indicate SE. of the means (Exp. 6.4).

6.3.4.4 Root Na^+ and K^+ concentrations (mmol kg^{-1} DW roots) (Fig. 6.13)

Increasing concentrations of NaCl significantly ($p \leq 0.000$) increased Na^+ concentrations in the roots, while Na^+ concentrations were significantly ($p \leq 0.000$) decreased in low salinity treatments with increasing concentrations of KCl in the root medium. Na^+ concentrations in the roots were lower in high saline treatments when 10 mol m^{-3} KCl was applied to the roots compared with 1 and 0.1 mol m^{-3} KCl treatments. Foliar application of KCl produced significant ($p \leq 0.030$) effects on Na^+ concentrations in the roots. Generally, foliar application of KCl increased Na^+ concentrations in both saline treatments.

Salinity, KCl and their interaction produced significant ($p \leq 0.000$) effects on K^+ concentrations of roots. K^+ concentrations were lower in 0.1 and 10 mol m^{-3} KCl treatments at 150 mol m^{-3} NaCl compared with 10 mol m^{-3} NaCl. K^+ concentrations were significantly greater with increasing concentrations of KCl in the root medium. K^+ concentrations in the roots were significantly higher with foliar application of KCl in low saline treatment when 10 mol m^{-3} KCl was applied to the root medium compared to other treatments.

6.4 Discussion

The effect of salinity on the growth of shoot and root of cotton was assessed by measurements of dry weight. 10 and 50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4 did not significantly affect shoot or root dry weight with an exception in experiment 6.2 for shoot dry weight which was significantly reduced when 50 mol m^{-3} NaCl was applied to the root medium (Fig. 6.4). Cordovilla *et al.* (1995a) reported that 50 mol m^{-3} NaCl significantly affected shoot and root dry weight of pea plant (salt sensitive), while soybean (salt tolerant) was not affected with the same concentration. Botella *et al.* (1997) found that shoot and root dry weight were not significantly affected by 100 mol m^{-3} NaCl when maize plants were grown hydroponically in 1 mol m^{-3} K^+ solution. Faba bean was given salinity of 7.8 and 13.7 dS/m, salinity decreased shoot growth more than root growth (Sharma, 1991). Khan *et al.* (1994b) reported that a plant (alfalfa) could allocate more photosynthate to roots for the synthesis of organic acids to maintain osmotic

balance under adverse conditions; therefore the effect of salt was relatively less on the roots than the shoots. This is in accordance with Munns and Termaat (1986), who postulated that the roots are often less affected by salinity than shoots. Although the roots are directly exposed to saline environments, they are less affected by salinity than the shoots (Maas *et al.*, 1972).

In experiment 6.3, shoot and root dry weight were not significantly decreased when 50 mol m^{3-} NaCl or 25 mol m^{-3} Na_2SO_4 were applied to shoots and roots. Shoot and root dry weight were slightly higher in both treatment of Na_2SO_4 than in both treatment of NaCl (Fig. 6.7). Abd El-Samad and Shahdad (1996) found that plants treated with the SO_4^{2-} (Na_2SO_4) produced greater shoot and root dry matter than those plants treated with Cl^- (NaCl) salts. When callus culture of chinese cabbage was given an equimolar concentration of NaCl and Na_2SO_4 salts, the reduction in growth by Na_2SO_4 was much greater than that by NaCl (Paek *et al.*, 1988). Ghanem and Salama (1995) found that fresh and dry weight of callus from seeds of *Hyoscyamus muticus* decreased with increasing salinity of the medium and were greater on media containing NaCl than on Na_2SO_4 media.

150 mol m^{-3} NaCl induced inhibition of shoot growth in experiment 6.4 at all three concentrations of KCl in the root medium. Shoot and root growth were significantly lower at 0.1 mol m^{-3} KCl compared with 1 and 10 mol m^{-3} KCl in low and high salinity treatments (Fig 6.11a and b). It could be possible that K^+ in the nutrient solution was not enough to maintain a growth rate similar to that of the plants grown at 1 mol m^{-3} KCl. Similar responses have been found in spinach plants, which responded to an increasing K^+ concentration, reducing the differences in shoot growth between plants grown in low and high salinity (Chow *et al.*, 1990). Botella *et al.* (1997) found that shoot dry weight was significantly affected by 100 mol m^{-3} NaCl when maize plants were grown hydroponically in 0.1 mol m^{-3} K^+ solution. These results suggest that K^+ deficiency in the shoots was the main limiting factor for plant growth. Generally, shoot and root dry weight was more or less the same between 1 and 10 mol m^{-3} KCl root treatments, indicating that plants do not need high concentration of K^+ for their growth.

Inhibitory effects on K^+ uptake with 10, 50 and 150 mol m^{-3} NaCl or 25 mol m^{-3} Na_2SO_4 were found, but the effect was more pronounced when salts were applied to the roots rather than when applied to the shoots (except 150 mol m^{-3} NaCl which was not applied to the shoots) (Fig. 6.2, 6.5, 6.8 and 6.12). It may be possible this occurs as the roots are in direct contact with the saline water for a longer period, whereas the leaves are sprayed saline water for a short time. Uptake may occur when the laves are wetted.

K^+ uptake inhibition by Na_2SO_4 as well as NaCl indicates that the response is not Cl^- specific. However, NaCl produced a more inhibitory effect on K^+ uptake than Na_2SO_4. High concentration of Na^+ is well known to affect K^+ uptake (Hsiao and Läuchli, 1986). Botella *et al.* (1997) found that 100 mol m^{-3} NaCl significantly inhibited K^+ uptake in maize plants. High Na^+ can replace Ca^{2+} from root membranes, thus affecting the selectivity for K^+ uptake (Cramer *et al.*, 1987). Leidi *et al.* (1992) reported that K^+ uptake in cotton plants when grown in nutrient solution was affected by increase in salt level (0-300 mol m^{-3} NaCl) and caused a decrease of K^+ concentration in the plant as well as an increase of Na^+ and Cl^- levels.

The effect of salinity on K^+ uptake, growth and translocation depends on K^+ concentration in the root medium. Fig. 6.3b shows that, K^+ concentrations in the roots were higher when low NaCl (10 mol m^{-3}) was sprayed on the shoot rather than when high NaCl (50 mol m^{-3}) was applied to the shoot. Taking into accounts that shoot and root growth was lower at 10 mol m^{-3} NaCl shoot treatment than 50 mol m^{-3} NaCl shoot treatment. It is possible to conclude that the highest concentrations of K^+ in the roots might be due to low growth of shoot and root. In experiment 6.1, K^+ concentrations were higher in the roots than in the shoots, while the opposite was true for Na^+ concentrations. The findings are in agreement with the results of Botella *et al.* (1997), who found that K^+ concentrations were higher in roots than in shoots, while for Na^+ the opposite occurred when maize plants were grown hydroponically in 100 mol m^{-3} NaCl with 1 mol m^{-3} K^+ solution.

50 mol m^{-3} NaCl and 25 mol m^3 Na_2SO_4 either applied to the roots or shoots reduced K^+ concentrations in the shoots compared with the control. Generally, the effect of salts on K^+ concentrations was more pronounced with shoot application rather than root application (Fig 6.3a, 6.6a and 6.9a). Fig. 6.3b shows that root K^+ concentrations in 10 and 50 mol m^{-3} NaCl shoot treatment were significantly higher than the control. This indicates that the roots limited the transport of K^+ to the shoots when salt was applied to the shoots, thus the root showed high concentration of K^+. 150 mol m^{-3} NaCl also reduced K^+ concentrations in the shoots and roots in low, medium and high KCl treatments (Fig.6.13b and d). Cordvilla *et al.* (1995) reported that under salinity stress, K^+ concentration in shoots and roots was reduced with increasing salinity level. Zhong and Läuchli (1994) found that 150 mol m^{-3} NaCl in growth solution caused a significant decline in K^+ content in cotton roots. When 10 mol m^{-3} KCl was applied to the roots, Na^+ concentrations decreased in the shoots and roots in both saline treatments compared with 0.1 mol m^{-3} KCl treatments (see Fig. 6.13 a and c). This shows that high K^+ could compete with Na^+ and reduced its concentrations in the shoots and roots.

Cotton roots showed negligible Na^+ concentrations when shoots were sprayed with saline water. This indicates that phloem could not translocate Na^+ from shoot to root, and thereby excessive build up of Na^+ was found in the shoots. Benes *et al.*, (1996) suggest that in maize, Na^+ exclusion appears to be very poor when ions are absorbed through the leaves. A model proposed by Wolf and Jeschke (1987) of ion flows in barley shows that translocation of Na^+ in the phloem was remarkably small and for all organs it was below that of K^+.

To summarise, high Na^+ concentrations in the shoot in response to external salinity via foliar application could be an indication of a signal to the root produced by the shoot to slow down K^+ uptake which resulted in low accumulation of K^+ in the shoot. This may be explained that the roots release ions as a response to a signal from the shoot, as the shoot was overloaded with Na^+ and the capacity for compartmentation within the cell became saturated with Na^+, then the shoot did not require K^+ to compensate in the compartments. Na^+

transportation from the shoot to the root was negligible. If there was high transport of Na^+ from shoot to root, compartments could be unsaturated. There are some explanations made by other workers on inhibition of K^+ uptake under salinity. Lynch and Läuchli (1984) reported that salinization of the medium (45 mol m^{-3}) inhibits both K^+ uptake by excised barley roots and K^+ release from their steles. They further found that xylem exudation of K^+ was inhibited from excised roots of barley seedlings. Läuchli (1976) suggested that stelar K^+ release is an active process, therefore, a direct inhibition of stelar K^+ release by salinization may reflect the inhibition of a K^+ channel. Shuman (1994) reported that the first phase in the absorption of K^+ by the root is transport through the plasma membrane of the cortical cells. Mills *et al.* (1985) reported that Na^+ does not interfere with K^+ fluxes into the xylem as strongly as it does at the root plasma membrane. Most of the K^+ supply to the root comes from K^+ translocated from older tissues, including the shoot. In older tissues the endodermis is more strongly suberized, and movement into and out of the stele may be restricted to plasmodesmata (Gorham, 1992a). The transport in old tissues may be exchange of K^+ in the cortex for Na^+ in the xylem, as in *Zea mays* (Johanson and Cheeseman, 1983). Munns and Passioura (1984) reported that the toxic effects of salt in leaves may result from high accumulation within the cells or from the accumulation of solutes in the apoplast, resulting in loss of turgor.

Low concentration of K^+ in the salinized shoot suggests that a first and decisive step of regulating high K^+ in the shoots is K^+/Na^+ discrimination occurring during K^+ uptake and xylem loading in the root. Läuchli (1984) reported that in Fabaceae it has been shown that any Na^+ that has crossed the root to the xylem may be removed from the xylem stream in exchange for K^+, particularly in the proximal region of the root and base of the stem. Jeschke and Wolf (1985) reported that in salt-treated leaves of barley K^+ export in the phloem exceeded import via xylem, thereby reducing the K^+ content of the leaf. Any K^+ imported into leaves in excess of demand during expansion apparently was returned to the root by retranslocation via the phloem (Jeschke and Wolf, 1985). They further reported that the magnitude of net K^+ export from leaves by Na^+ is a process of

ion recycling under conditions of K^+ deficiency. By this process K^+ ions imported via xylem into a leaf are returned via phloem transport. According to the model designed by Wolf and Jeschke (1987), K^+ imported via xylem in leaf 1 (mature) of barley and then recirculated via phloem (89%) to the root and young leaves when 1 mol m^{-3} NaCl and K^+ were in the external medium. However, when 100 mol m^{-3} NaCl and 10 mol m^{-3} K^+ were in the external medium, uptake and net flows of K^+ were substantially decreased in the presence of external NaCl despite a higher external K^+ concentration. They suggest that the strong decrease in phloem export of K^+ from leaf 1 appears to contradict the small inhibition of phloem exudation in presence of NaCl. They calculated phloem recirculation of K^+ from shoot to root under salinity which was 18%, whereas, it was 89% in the control. Jeschke *et al.* (1992) found that 40 mol m^{-3} NaCl strongly reduced K^+ uptake, root to shoot transport in the xylem and even more severely phloem export from mature leaves and return flow via phloem from shoot to root in white lupin. Radiotracer experiments will be needed to detect K^+ movement when salt is applied to the leaves.

6.5 Conclusions

- 50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4 either applied to the roots or shoots significantly inhibited net K^+ uptake rate by roots of cotton, but the effect was more pronounced via root rather than shoot application. 150 mol m^{-3} NaCl applied to the roots significantly decreased K^+ uptake rate.
- K^+ concentrations were reduced in the shoots with root and shoot-applied salts (50 mol m^{-3} NaCl and 25 mol m^{-3} Na_2SO_4) compared with the control.
- Cotton shoot restricted the transport of Na^+ to the root as root Na^+ concentrations were negligible when shoots were sprayed with NaCl and/or Na_2SO_4.
- Generally, the shoot and root dry weight were not significantly affected at 50 mol m^{-3} NaCl or 25 mol m^{-3} Na_2SO_4. However, at 150 mol m^{-3} NaCl, shoot growth was significantly decreased, while the root growth was less affected.

- 10 mol m^{-3} KCl in the root medium significantly reduced Na^+ concentration in the shoots and roots compared with 0.1 mol m^{-3} KCl treatments.
- Foliar application of KCl had no advantage to increase growth and K^+ concentrations and reduce Na^+ concentrations when 150 mol m^{-3} NaCl was applied in the root medium.
- Na^+ and K^+ concentrations were higher in the roots than in the shoots.
- Inhibition of K^+ uptake and K^+ concentrations with 50 mol m^{-3} NaCl or 25 mol m^{-3} Na_2SO_4 did not decrease growth of cotton.

To improve the experimental design

- Ca^{2+} concentrations in the nutrient medium should be increased when NaCl concentrations were 150 mol m^{-3}. Ca^{2+} counteracts the unfavourable effect of saline conditions that effect both the K^+ uptake of roots and the dry matter.
- To avoid osmotic shock to the plants due to the application of 150 mol m^{-3} NaCl suddenly, it should be given in increments. It was observed that when 150 mol m^{-3} NaCl was applied, plants grown in 0.1 and even 1 mol m^{-3} KCl solutions became wilted. It was also observed that plant growth stopped in those plants that were grown in 0.1 mol m^{-3} KCl solution.
- K^+ uptake rate may be measured by the loss of K^+ in the nutrient solution and calculate on the basis of water losses in the solution. The weight of each vessel used in the uptake measurements should be recorded to enable accurate correction of evapotranspiration losses of water during uptake period.

CHAPTER SEVEN

GENERAL DISCUSSION

CHAPTER 7

GENERAL DISCUSSION

The ultimate objective of this work was to determine if the additional nutrients applied under saline conditions could reduce the effect of salinity and improve the vegetative growth and ion contents of cotton. In Chapter 3, the effect of additional nitrogen with various levels of NaCl salinity was studied. I also investigated which form of nitrogen was beneficial for cotton plants. In Chapter 4, the effect of exogenous glycinebetaine when applied to the leaves and soil was studied. In Chapter 5, I studied the effect of external potassium applied to the soil and leaves under soil and foliar-salinity. The optimum level of potassium supply for cotton was examined. Further the best accompanying anion of potassium was determined. In Chapter 6, the effect of NaCl and Na_2SO_4 salinities on K^+ uptake by cotton and translocation of potassium was investigated.

7.1 General dissussion

7.1.1 Additional nitrogen under salinity

Seed emergence of cotton was decreased and delayed with increasing concentration of salt (50-200 mol m^{-3} NaCl). The adverse effect was both a result of impaired water absorption and ion toxicity leading to mortality of embryo/young seedlings (Qadar, 1994). No potential improvement in seed emergence under saline conditions due to supplemental nitrogen was apparent in this study (Table 3.1).

Plant growth is usually impaired under saline conditions resulting in decreased crop growth. The changes in vegetative growth of cotton are shown in the Table 3.2 and 3.6, which showed a significant reduction in growth of cotton under increasing level of NaCl. Additional N did not interact with negative effects of salinity on growth. The low growth in a low N treatment, and the same reduction pattern in growth (% of controls) by salinity at two N concentrations, showed that N and salinity effects were independent. Khan *et al.*, (1994a) reported that increased salinity (0-100 mol m^{-3} NaCl) substantially reduced the dry weight

of roots and shoots, and relative growth rate of alfalfa, but additional nitrogen supplied either as ammonium-N or Nitrate-N at 6 mol m^{-3} considerably moderated the salinity effects on these parameters. Wheat grown in saline soils with a mixture of nitrate and ammonium nitrogen produced an acceptable yield (Shaviv *et al.*, 1990).

Additional NH_4NO_3 significantly increased chlorophyll measurements and was positively correlated with leaf nitrogen (0.56**). Wood *et al.* (1992) found that chlorophyll-meter reading (SPAD) was highly correlated to N concentration of cotton leaves.

Every increase in salt concentration was accompanied by an increase of Na^+ concentration in the leaf, petiole and stem tissues. The most significant findings of the experimentation above were that supplemental 7 mol m^{-3} NH_4NO_3 reduced Na^+ concentrations and increased K^+ concentrations in the leaf, petiole and stem tissues (Table 3.4). Applied N depressed accumulation of the Na^+, but stimulated accumulation of K^+ by barley plants (Shen *et al.*, 1994). The decrease in Na^+ content of leaf could promote growth of plant and increase dry matter weight (Liu *et al.*, 1987).

7.1.1.1 NH_4^+ versus NO_3^-

The contrast of the salinity response of the plants fed by two nitrogen forms is amplified by the fact that under non-saline condition nitrate-fed plants were perceptibly larger in vegetative growth than the ammonium-fed plants. However, in saline conditions the growth was more or less the same with both forms of nitrogen (see Table 3.6). Lewis *et al.* (1989) found that total plant mass of maize was greater with ammonium-fed than nitrate-fed plants at the control, while, the opposite was true for saline treatments. Bar-Y *et al.* (1987) suggested that by raising the NO_3^- concentration in the irrigation water, the tolerance of avocado plants to salinity was increased. Leidi *et al.* (1992) found that ammonium grown cotton plants produced less biomass than did plants grown on NO_3^-, whereas peanut plants followed similar growth patterns in both nitrogen forms.

In Experiment 3.2, plants grown with NO_3^- solution accumulated less Na^+ than the plants grown with NH_4^+ solution while for Cl^- concentrations, the opposite was true under both saline and non-saline conditions. The results are in accordance with Leidi *et al.* (1992) who reported that peanut and cotton accumulated more Na^+ and less Cl^- with NH_4^+ than NO_3^--fed solutions. Ammonium is a metabolically more expensive N source for roots than NO_3^- (Blacquire, 1987) because it is exclusively assimilated in the roots (Lewis *et al.*, 1989). Martinez and Cerdá (1989) have shown that NO_3^- solution increased Cl^- accumulation, but did not affect Na^+ contents. Silberbush and Lips (1988) reported that increasing concentration of nitrate in the nutrient solution resulted in about 50% reduction of Na^+ in the leaves of plants grown with 50 mol m^{-3} NaCl, and a similar but more moderate reduction of Cl^-. They suggested that the main effect of NO_3^- was on the Na^+ concentration in the leaves, while Cl^- followed because of the cation-anion balance.

7.1.1.2 Nitrogen content

In experiments described in Chapter 3, nitrogen concentrations in the leaves did not decrease with increasing concentration of NaCl salinity. When NaCl salinity (EC= 2.4 mmho/cm) was applied to wheat, N-uptake was equal or greater in the salinized than in the non-saline soil (Shaviv and Hagin, 1993). Niazi *et al.* (1995) found that N content decreased with increasing salinity (3, 6, 9 or 12 dS/m) in chickpea. Chloride induced N deficiency in wheat plants salinized with NaCl (Torres and Bingham, 1973). Pessarakli and Zhou (1990) reported that total N uptake was significantly higher in *Phaseolus vulgaris* given NaCl compared with the controls suggesting plants under NaCl obtain most of their required N through uptake rather than N fixation. Bernstein *et al.* (1974) found that despite decrease in total-N uptake, leaf N concentration of some grain and vegetables crops increased with increasing salinity at all N fertilisation levels. N uptake by cotton plants increased at -0.4 and -0.8 Mpa osmotic potential of NaCl salinity (Pessarakli and Tucker, 1985). However, total N uptake by salt-sensitive green bean plants significantly decreased with increasing salinity (-0.5 Mpa osmotic

potential of NaCl) of the nutrient solution (Pessarakli, 1991). It is suggested that differences in N concentration of these crops (cotton as compared with green bean) under salt stress are probably due to differences in their salt tolerance. To explain these results, a dilution or concentration effect was reported by Pessarakli and Tucker (1985) as a cause of the fluctuations in N content in plant.

7.1.2 Exogenous glycinebetaine

Additional betaine of different levels when applied to the soil and leaves neither increased growth of cotton nor reduced Na^+ concentrations from the various parts of the plant tissues. This may be explained by the fact that exogenous betaine was lower when applied to the leaves therefore, probably could not show any response. The data show that at highest level of exogenous betaine (10 mol m^{-3}), generally, there were no significant increases in betaine concentration in various tissues compared with not adding betaine (see Appendix 2). Makela *et al.* (1996) found that the optimum betaine concentration producing significant effects of growth in turnip rape and wheat was 100 and 300 mol m^{-3} respectively. No response in growth or Na^+ concentrations in the soil-applied betaine was found. This may be due to betaine being degraded by micro-organisms in the soil. This lack of betaine in the soil may also explain why the growth and Na^+ concentrations were not affected, despite the beneficial role of glycinebetaine in osmotic adjustment. Foliar application of betaine (4 and 6 kg/ha) significantly increased grain yield of maize and sorghum (Agboma *et al.*, 1997).

In general, betaine was higher in the young than in old tissues of leaf, petiole and stem under both soil and foliar-saline conditions. Nakamura *et al.* (1996) found that the level of glycinebetaine in young leaf blades of *Hordeum vulgare* was approximately three times that in old leaf blades. Betaine concentrations were much higher in young than old leaves is also reported by Gorham (1996) in cotton and Colmer *et al.* (1995) in wheat. In young and old leaves, petiole and stems, betaine concentrations were generally higher when 200 mol m^{-3} NaCl was applied to the soil compared with the control.

7.1.3 Additional $CaCl_2$

Results presented in Table 4.4 show that K^+ concentrations in most of the young and old parts of the plant tissues did not decrease when 200 mol m^{-3} NaCl was applied to the soil. However, when the same concentrations of NaCl were applied in experiment 5.1, K^+ concentrations decreased in young and old leaves (see Table 5.2). This might be interpreted as an indication that in experiment 5.1, additional Ca^{2+} was not given as applied in experiment 4.1 (10 mol m^{-3} $CaCl_2$). The role of Ca^{2+} in maintaining membrane selectivity is already discussed. With low Ca^{2+}, membranes become more permeable and there is a leakage of K^+ (efflux) out of the cell. Elzam (1971) reported that K^+ uptake by barley roots growing in 100 mol m^{-3} NaCl was reduced by 95% relative to the non-saline control when external Ca^{2+} concentrations were low (0.5 mol m^{-3}), but this reduction was smaller (72%) with 4 mol m^{-3} external Ca^{2+}. Similar results were reported for cotton (Kent and Luchli, 1985) when comparing K^+ concentrations in roots of plants growing without NaCl or with 200 mol m^{-3} NaCl. In experiments conducted by Gorham *et al.* (1990 and 1991) under saline conditions, $CaCl_2$ was added to maintain a nominal Na:Ca ratio of 20:1 (ignoring the small amount of Ca^{2+} present in the nutrient solution). Gorham and Bridges (1995) found that additional $CaCl_2$ (5 mol m^{-3}) significantly increased K^+ concentrations and decreased Na^+ concentrations compared with no added Ca^{2+} treatments in cotton growing at 100 mol m^{-3} NaCl. They suggest that optimum Ca^{2+} concentration for growth of cotton is in the range of 1 to 15 mol m^{-3} for plants growing in hydroponic culture with 100-150 mol m^{-3} NaCl.

7.1.4 Shoot & root growth at various concentration of NaCl and KCl

The results from the experiments described in Chapter 6 revealed that growth of shoot and root dry weight of cotton plants was not generally inhibited by 50 mol m^{-3} NaCl applied to the roots and shoots. However, 150 mol m^{-3} NaCl significantly decreased shoot dry weight at various KCl concentrations in the root medium while generally, root dry weight was less affected with the same concentrations (see Fig. 6.11). These results can be interpreted in two different

ways: either the reduction of K^+ uptake produced the weight decrease or the decrease in K^+ concentration in shoots was the consequence of the lower weight. Pessarakli (1995) reported that shoot dry weight of cotton was reduced more than root dry weight by increasing salinity. He suggested that, with the common knowledge of plant physiology that plant roots under stress conditions grow more and penetrate deeper in the soil in search of water and nutrients. The low effect of salinity on root growth is further confirmed by Khan *et al.* (1994b), Munns and Termaat (1986) and Maas *et al.* (1972). They reported that roots are often less affected by salinity than shoots.

Shoot and root dry weight of the plants grown at 0.1 mol m^{-3} KCl in low and high salinity treatments were significantly lower than the plants grown at 1 and 10 mol m^{-3} KCl (see Fig. 6.11). This indicates that K^+ deficiency in the root medium is the main limiting factor for shoot and root growth under low and highly saline conditions. No significant difference in shoot and root dry weight was found between the plants grown under 1 and 10 mol m^{-3} KCl solution. It means that plants do not require high levels of K^+ when grown under low and highly saline environment.

7.1.5 Leaf thickness

Leaf thickness was significantly increased when the leaves were sprayed with highly saline water, but the effect was more in the old rather than in the young leaves (Appendix 5 and Table 5.4). The inclusion of high amounts of Na^+ and Cl^- in the old leaves probably increased the leaf thickness. Gausman and Cardenas, (1968) reported that increase in thickness of cotton leaves in plants growing at high salinity was due to development of palisade and spongy mesophyll cells.

7.1.6 Leaf injury

Cotton leaves exhibited symptoms of leaf injury (i.e., chlorotic and necrotic) when leaves were sprayed with 150 and 200 mol m^{-3} NaCl, but the old leaves were much more affected than the young leaves. However, these symptoms

were not found in soil salinity treatments. Maas *et al.* (1982) reported that when plants are irrigated with saline waters through soil, they initially experience salinity problems when the roots encounter excess salts in the soil water. This is not the case with sprinkler irrigation when foliage is wetted by saline irrigation waters, and salt absorption occurs directly through the leaves. Therefore, the tolerance of crops to foliar application of saline waters may not be the same as their tolerance to soil salinity.

7.1.7 Additional KCl under saline and non-saline conditions

The drastic inhibitory effects of 150 mol m^{-3} NaCl on growth, photosynthesis, transpiration, stomatal conductance, leaf chlorophyll, K^+, Mg^{2+}, Ca^{2+}, malate and SO_4^{2-} concentrations were significantly counteracted with additional foliar-KCl at 10 and 25 mol m^{-3}. The growth increased substantially and Na^+ concentrations decreased from 134 to 20 mol m^{-3} in the young and from 283 to 187 in the old leaves with additional 10 mol m^{-3} KCl (see Tables 5.4 and 5.6). In the presence of K^+, exclusion of Na^+ via phloem could occur, and K^+ also prevented the entry of Na^+, thus there was a strong selection for K^+ over Na^+. However, with increasing concentration of KCl, no significant effects were observed on the above parameters. It means that K^+ had beneficial effects on growth and ion content of cotton to certain levels (10 mol m^{-3}). K^+ application can reduce the deleterious effects of salinity on plant development as has been reported by Ben-Hayyim *et al.* (1987), Benlloch *et al.* (1994) and Kafkafi (1984). The maintenance of higher K^+/Na^+ ratio in tissues subjected to saline conditions is considered one of the important physiological mechanisms contributing to salt tolerance of many plant species (Ashraf, 1994, Gorham, 1993 and Gorham *et al.*, 1997, Greenway and Munns, 1980 and Wyn Jones *et al.*, 1984).

Foliar application of KCl (10 mol m^{-3}) in experiment 6.4 did not increase the shoot and root growth and K^+ concentrations in the shoots and roots when low (10 mol m^{-3}) and high (150 mol m^{-3}) NaCl was applied in the root medium. It was observed in experiment 5.1 and 5.2 that foliar-KCl increased the growth and K^+ concentrations under K^+ deficiency that was caused by foliar

application of NaCl and a strong interaction was found between Na^+ and K^+ when NaCl and KCl were applied on the leaves. If there would be a treatment of shoot applied NaCl, then it could be possible that foliar-applied KCl would have an effect on the growth and K^+ concentrations.

In the controls and at 10 mol m^{-3} NaCl, additional KCl did not significantly increase vegetative growth, photosynthesis, transpiration and K^+ concentrations in the leaves. (see data of Experiment 5.1 and 5.2). This shows that enough K^+ was available in the soil to meet the K^+ demand of the plant. Under non-saline conditions localised supply of K^+ can support root growth in other parts of the root system (Drew, 1975) and provide K^+ for transport towards the meristematic root tip (Marschner and Richter, 1973). Jeschke and Wolf (1988) were able to demonstrate in *Ricinus* that external K^+ supply is not required even in the presence of 100 mol m^{-3} NaCl to maintain K^+ concentration at the high level that is needed for growth. They suggested that K^+ is imported via the phloem, thus, roots are capable of growing as part of the whole plant under the adverse conditions of salinity and local K^+ deficiency.

7.1.7.1 Accompanying anions of potassium

To test the best accompanying anions of potassium, K-chloride, K-sulphate, K-phosphate, K-nitrate and K-acetate were used with 150 mol m^{-3} NaCl. All anions produce significant increase in growth and reduced Na^+ concentrations of young and old leaves and petioles compared with NaCl (alone) (Table 5.8 and 5.10). Among all different accompanying anions of K^+, K-acetate slightly increased plant biomass, photosynthesis and K^+ and Ca^{2+} concentrations and decreased Na^+ and Cl^- concentrations compared with other accompanying anions of K^+. K-acetate is an organic anion, and may play an important role in maintaining the charge balance with potassium. Vegetative growth, photosynthesis and ion content were more or less the same with K-chloride, K-sulphate and K-nitrate sources. However, the plants grown with K-phosphate solution were generally lower in growth, photosynthesis, transpiration, stomatal conductance,

K^+, Ca^{2+}, malate and higher in Na^+ and Cl^- concentrations compared with other anions (see data of Experiment 5.3).

7.1.8 Distribution of K^+ and Na^+

An inverse relationship between the distribution of Na^+ and K^+, i.e. low K^+ and high Na^+ in the old leaves while high K^+ and low Na^+ in the young leaves was found in all the experiments described in this manuscript. Gorham and Wyn Jones (1983) found that Na^+ was higher in old than in young leaves of *Suaeda maritima* whereas the opposite was true for K^+ concentration. This supported the suggestion that for turgor, the old leaves depended on Na^+, an energetically less expensive mineral ion than K^+, which was retranslocated to growing tissues and the youngest leaves for nutrition and turgor (Bhatti *et al.*, 1993). They further reported that younger leaves being close to the shoot apex would derive their mineral requirement initially from the phloem. Their composition would, therefore, point to the composition of phloem sap, which may be rich in K^+ and low in Na^+ (MacRobbie, 1971). Similar trends in Na^+ concentrations have been reported by Greenway and Munns (1980) and Rashid (1986). They suggested that these patterns are probably due to rapid volume increase in expanding leaves and prolonged intake of ions by expanded leaves via the transpiration stream. A sudden and rapid rise in Na^+ concentrations in the old leaves may be due to the fact that at the time of sampling, older leaves had been exposed to saline irrigation for a long time, and it has to be remembered that the ions present in the solution (Na^+, Cl^- and Ca^{2+}) are very immobile in the phloem. Jeschke and Wolf (1985) reported that in barley these differences arise from a high K^+/Na^+ selectivity of phloem loading, which allows for a significant retranslocation of K^+ from old to young leaves while limiting the export of Na^+ from mature leaves.

In Experiment 5.3, K^+ and NO_3^- concentrations were very low in both young and old leaves while petioles were rich in these ions (Table 5.10). This suggests that when plants were sprayed with saline water, replacement of K^+ and NO_3^- occurred only in the leaves while the petioles were not affected. It could be

possible that these ions were translocated from leaves and accumulated in their corresponding petioles. As K^+ and NO_3^- were sufficient in the petioles, therefore Na^+ could not replace them. Foliar application of NaCl reduced leaf K^+ as has been reported by Benes *et al.* (1996) and Gorham *et al.* (1994).

7.1.9 Na^+ translocation

Foliar application of salts on the leaves did not produce an accumulation of Na^+ in the roots (see Figs. 6.6 and 6.9). This indicates that phloem could not translocate Na^+ from shoots to roots. Jeschke and Pate (1991) found that little Na^+ was translocated out of *Ricinus* leaves.

Foliar uptake

The inorganic nutrients supplied in the form of spray have to be first absorbed by the leaf before translocation to other parts is possible. The uptake occurs through both upper and lower surfaces of the leaf. It was suggested by Haynes and Goh (1977) that salts enter the foliage through the cuticle rather than the stomata. Differences in cuticular composition or thickness may exist between young and old leaves, thus resulting in a greater or lesser degree of salt absorption through the foliage.

The first barrier to the entry of solutes through the leaf is the cuticular membrane that envelops the leaf. The inner surface of the cuticle adjoins the walls of the epidermal cells, and therefore the ions have to enter the cell wall before absorption by the epidermal cells. However, the cell wall itself is not a barrier to absorption because it constitutes the apoplastic region for transport (Kannan, 1986). Marschner (1995) reported that in plants the uptake of solutes by the surface of leaves and other aerial parts is severely restricted by the outer wall of the epidermal cells. This outer wall is covered by the cuticle and a layer of epicuticular waxes. The solutes, after permeating the cuticle and the cell wall, reach the intercellular space from where they are absorbed by the leaf cells. There are two pathways for the movement of solutes within the leaf, i.e. apoplastic and symplastic. The apoplastic pathway is essentially passive. The transport through

the symplast is faster than through the apoplast (van Steveninck and Chenoweth, 1972).

Franke (1967) suggested that the process of foliar absorption takes place in three stages. In the first stage, substances supplied to the surface of leaves penetrate the cuticle and the cellulose wall via limited or free diffusion. In the second stage, these substances, having penetrated the free space, are adsorbed to the surface of the plasma membrane by some form of binding, while in the third stage, the adsorbed substances are taken up into the cytoplasm in a process requiring metabolically derived energy.

7.1.10 Salinity reduced K^+ concentrations and K^+ uptake rate

The most interesting data for cation concentrations in the leaves of cotton treated with 200 mol m^{-3} NaCl are shown in Tables 4.4 and 4.9. Leaf sap concentrations of K^+ reduced from 148 to 68 mol m^{-3} in the young and from 130 to 24 mol m^{-3} in the old leaves with foliar application of salt (mean values of 5 replicates). In Experiment 5.1 and 5.2, K^+ concentration was also significantly decreased when NaCl (200 and 150 mol m^{-3}) was applied to the leaves. What is surprising about these results is the large accumulation of Na^+ in the leaves of cotton plants when exposed to a short period of salt spray. The effect of Na^+ storage via foliage on K^+ concentrations is particularly interesting since it implies, and provides a tool for investigating, the regulation of leaf K^+ concentrations by the leaf rather than by the roots. This pattern indicates translocation of K^+ from the leaves and enhancement of this process by Na^+/K^+ replacement within the leaf tissue (Marschner, 1995). K^+ concentrations were substantially reduced in the old leaves as the translocation rate increases with leaf age (Wetselaar and Farquhar, 1980). Taleisnik and Grunberg (1994) reported that the decrease in K^+ concentration in salinized plants due to high Na^+ concentration suggests that root membranes may have become more leaky under salinity.

A number of factors might be involved in decreasing K^+ concentration due to continuous build-up of Na^+ in the leaves via foliar application of salt. One factor is inhibition of K^+ uptake by roots and translocation to the shoot. It is well

known that high external concentration of Na^+ decreases K^+ uptake and K^+ translocation from root to shoot (Botella *et al.*, 1997). Some experiments were carried out in hydroponic culture to examine the uptake rate of K^+ under soil and foliar-applied salinity. Both methods of salt application (hydroponic culture) were responsible for inhibition of K^+ uptake. High Na^+ in the leaves might have given a signal to the root to slow down uptake of K^+ that resulted in low transport of K^+ to the shoot. As suggested by Gorham (1995a), the main fluxes involved in controlling ion transport in root are: a) selective uptake of K^+ at the plasmalemma and exchange of K^+ for Na^+ in the cytoplasm, b) selective export of Na^+ across the tonoplast to the vacuoles and selective exchange of Na^+ for K^+ in the vacuoles, c) selectivity during transport into and out of the xylem, d) supply of solute from the phloem.

The low concentration of K^+ in the leaves and/or shoot in the presence of high Na^+ could be due to low selectivity during influx and low efficiency of K^+/Na^+ exchange at the plasmalemma in cotton roots. Stelter and Jeschke (1983) found that low discrimination between K^+ and Na^+ in xylem transport in *Atriplex hortensis* roots could be due to limited selectivity during release of ions from the symplasm to the xylem sap i.e. at the plasmalemma of the xylem parenchyma cells. They further reported that addition of Na^+ increased the K^+ efflux from the cortical cells of *Atriplex hortensis* roots, and this was followed by a decrease in the K^+ efflux from the xylem vessels. This decrease corresponds to the inhibition of K^+ transport by Na^+. K^+ concentrations in the shoots declined in saline treatments, indicating low K^+ uptake rates from the xylem. Such a function has been proposed for petioles of *Trifolium alexandrinum* (Winter, 1982). Wolf and Jeschke (1987) reported that external NaCl strongly inhibited uptake and xylem flow of K^+. Lynch and Läuchli (1984) suggest that inhibition of K^+ uptake under salinity is a direct inhibition of stelar K^+ release which may reflect the inhibition of a K^+ channel or K^+-ATPase situated in the plasmalemma of stelar cells. Thiel and Blatt (1991) also reported that Na^+ is known to block several types of inward and outward K^+ channels in plants. Remobilization of K^+ from old to young leaves may have a role in raising the K^+ concentrations in expanding leaves. A high

accumulation of Na^+ may cause poor K^+ recirculation via the phloem from old to young leaves. This could be an other possible mechanism for decreasing K^+ content. Two mechanisms for K^+ uptake have been proposed by Kochian and Lucas (1982): a high affinity saturable transport system subjected to feedback regulation from K^+ in the roots, and a low affinity nonsaturating transport system. Further experiments would be needed by using a radio labelling technique to detect K^+ circulation in different parts of cotton plant.

Soil-applied 200 mol m^{-3} NaCl did not decrease K^+ concentrations in young and old tissues of leaf, petiole and stem (see Table 4.2). Benes *et al.* (1996) reported that salt-tolerant crops possess the ability to maintain sufficient K^+ concentration in leaves in the presence of high Na^+ concentrations in the soil medium. According to Maas and Hoffman (1977), cotton is classified as a salt-tolerant crop. However, the opposite results were observed in foliar-applied salinity treatments as leaf K^+ concentrations were severely inhibited by NaCl. Reductions in leaf K^+ concentrations in plants sprayed with saline water were also reported by Maas *et al.* (1982). Gorham *et al.* (1994) found that K^+ concentrations were significantly decreased in barley leaves with 250 mol m^{-3} NaCl sprayed on the leaves. Benes e*t al.* (1996) reported that saline spraying increased leaf Na^+ and decreased K^+ concentrations in maize much more than soil salinity treatments, even though the saline spray (30 mol m^{-3} NaCl) was given only two or three times per week for 30 minutes. The results of these experiments (4.1 and 4.2) suggest that cotton leaves lack the ability to maintain K^+ in the presence of high Na^+ as K^+ was replaced by Na^+ when leaves were sprayed with saline water. However, this is not the case when saline water was applied to the soil. Grattan *et al.* (1994) suggest that root uptake is a selective process and most crops are relatively effective at excluding much of the salts in the soil solution from entering the leaves, while foliar uptake bypasses the exclusion mechanism of salts.

In summarizing, excessive accumulation of Na^+ in the leaf via foliar application of NaCl resulted in decreasing K^+ concentrations in the leaf tissues. It is suggested that high concentrations of Na^+ in the leaves might have given a signal to the roots, via a messenger that regulates metabolic processes in the roots

that reduces transport of ions to the shoot. That messenger would probably be a hormone or growth regulators (abscisic acid). Rate of transpiration flow could be reduced due to a great accumulation of Na^+ in the leaves, this process might reduce K^+ uptake rate in the nutrient solution, as plants take up nutrients through the transpiration stream. However, in this study, the rate of transpiration was not significantly decreased when 150 and/or 200 mol m^{-3} NaCl was applied on the leaves compared with the control as well as with 10 mol m^{-3} NaCl treatments with an exception in the old leaves in experiment 4.2 (see Tables 4.6, 5.1 and 5.5). Na^+ replaces K^+, which could be an alternative explanation for low K^+ concentrations. This indicates export of K^+ from the leaves and enhancement of this process by Na^+/K^+ replacement within the leaf tissue. It could be possible that the K^+ was exported from the leaves and accumulated in the petioles, stems and roots. A high amount of Na^+ in the leaves and/or shoots via foliage saturated the compartmentation within the cell, then probably K^+ was not required by the shoot to compensate any more in the compartments. A net decrease in the K^+ contents of the leaves by foliar-applied salinity may also contribute to poor recirculation of K^+. Young leaves are supplied with K^+ via phloem, while Na^+ accumulates in old leaves, often replacing K^+ already accumulate there. The K^+ in old leaves is thus available for recirculation via the phloem to sink tissue. This recirculation process could be impaired by high amount of Na^+ directly absorbed by the leaves through foliar-applied salinity. It might be possible to determine K^+ movements in various plant parts by using radio labelling techniques.

To develop cotton cultivars with tolerance to saline spraying, the characters affecting foliar absorption, such as leaf wettability and waxes would need to be evaluated and modified to reduce foliar absorption of NaCl.

7.2 General Conclusions

* Additional N did not interact with negative effects of salinity on growth of cotton. Additional NH_4NO_3 (7 mol m^{-3}) significantly increased K^+ concentrations in the leaf, petiole and stem tissues and decreased Na^+ concentrations in the same tissues compared with no applied nitrogen. Increasing concentrations of NaCl (50-200 mol m^{-3}) did not decrease nitrogen concentrations in the leaves of cotton.
* Nitrate-N significantly increased the growth of cotton compared with ammonium-N at the control. Na^+ concentrations were lower in the leaf, petiole and stem tissues with NO_3^- than NH_4^+-fed plants while the opposite was true for Cl^- concentrations.
* Exogenous betaine (0.1-10 mol m^{-3}) either applied to the soil or sprayed on the leaves neither increased growth, photosynthesis nor betaine concentrations nor decreased Na^+ concentrations in the leaf, petiole and stem tissues.
* The method of soil and foliar application of NaCl and KCl produced different results. Growth, photosynthesis and K^+ concentrations were decreased and Na^+ concentrations substantially increased during foliar-applied NaCl compared with soil-applied NaCl, whereas, the opposite was true for KCl application.
* Na^+ concentrations were significantly higher in the old rather than in the young leaves, whereas the opposite was true for K^+ concentrations.
* Under non-saline conditions and even at low NaCl (10 mol m^{-3}), external KCl did not significantly increase growth of cotton and leaf K^+ concentrations.
* In substrate salinity (200 mol m^{-3} NaCl), cotton roots restricted an accumulation of Na^+ in the leaves and maintained adequate K^+ concentrations in the young and old leaves. However, when the same concentration of salt was sprayed on the leaves, Na^+ and Cl^- concentrations rose continually, while K^+ concentrations were severely reduced in the leaves.

* Foliar application of 150 and 200 mol m^{-3} NaCl caused severe leaf injury in cotton, and the effect was more serious in the old leaves. However, these symptoms were not found under soil applied salinity.
* External KCl at 10 and 25 mol m^{-3} combined with 150 mol m^{-3} NaCl when sprayed on the leaves accelerated the growth, photosynthesis, transpiration, leaf chlorophyll and leaf K^+ concentrations, and reduced leaf Na^+ and Cl^- concentrations compared with application of NaCl alone or with higher concentrations of KCl.
* Among all different accompanying anions of K^+, K-acetate slightly increased plant biomass, photosynthesis and K^+ and Ca^{2+} concentrations and decreased Na^+ and Cl^- concentrations compared with other accompanying anions of K^+.
* Na^+ concentrations were significantly higher in the leaves whereas, the opposite was true for petiole K^+ concentrations.
* 50 mol m^{-3} NaCl and/or 25 mol m^{-3} Na_2SO_4 either applied to the roots or shoots and 150 mol m^{-3} NaCl applied in the root medium significantly inhibited K^+ uptake by roots, and decreased K^+ concentrations in the shoots compared with the control. K^+ uptake rate was more inhibited with NaCl than Na_2SO_4 salinity.
* Cotton shoots, when sprayed with salt, did not transport Na^+ to the roots.
* 10 mol m^{-3} KCl in the root medium significantly decreased Na^+ concentrations in the shoots and roots compared with 0.1 mol m^{-3} KCl treatments.

7.3 Possible future experiments

K^+ concentration decreases when saline water is sprayed on the leaves of cotton. Recirculation of K^+ might be poor due to a great accumulation of Na^+ in the leaves. Foliar-applied NaCl and its effects on K^+ mobility to other plant parts including the roots could be measured by using a radio labelling technique. Research has been greatly facilitated since 1951 by the use of radioisotopes that permit accurate measurements of uptake and transport, and allow a distinction between nutrients absorbed by the foliage and those taken up by the roots. Franke (1967) suggested that the best method to measure passage of ions is radioactive

labelling of the solutions since exact determinations are possible in association with paper chromatography and autoradiography. K^+ and Rb^+ are two such ions which in experiments on absorption behave virtually as isotopes of the same element. Consequently, ^{86}Rb has often been used as a radioactive tracer for K^+ in investigations concerning ion absorption by excised tissues of higher plants. ^{86}Rb is more convenient to use than ^{42}K as a label for K^+ because the half-life of ^{86}Rb is 18.6 days, compared with 12.27 hours for ^{42}K (Läuchli and Epstein, 1970).

a) Apply radiolabelled Rb^+ to an old leaf.

The isotopically labelled solutions may be applied at the time of foliar application of NaCl. A small drop of the radioactive solution is placed in the centre of the upper surface of the old leaf. At various intervals after treatment the plant may be harvested and partitioned. A circular disc should be removed which includes the spot to which the radioactive solution was applied. Then the plant may be separated into the remainder of the treated leaf, non-treated leaf, stem, petiole, and the roots. The plant parts may be cut into small segments and placed in a paper bag and dried at 70 °C and dried samples assayed directly for radioactivity. Mobility of Rb^+ and its distribution may be measured by determining the percent of applied isotope recovered in non-treated plant parts. This may be calculated from the total radioactivity applied to each plant and the radioactivity of the individual parts. For radioactive counting, five replicates of a single plant each, may be assayed for each time interval after treating with isotope. In this way, relative mobility of isotopically labelled element could be measured. If Rb^+ is not present in young leaf, stem, petiole or root tissue, it means K^+ is not recirculated.

b) Add radiolabelled Rb^+ to root medium

Again as previous, after applying saline water on the leaves, the plant may be harvested, the different organs may be cut and the radioactive Rb^+ activity may be measured, both under control and foliar salinity treatments. If Rb^+ is not present in the plant or present at a lower concentration than control, then we know that foliar application of salt has inhibited the uptake of Rb^+ from the root medium.

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APPENDICES

Appendix 1: Mean values showing the effect of various concentrations of betaine with and without NaCl (200 mol m^{-3}) both applied to the soil on growth parameters, photosynthesis, (µmol m^{-2} s^{-1}), transpiration (mmol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), SPAD and betaine concentrations (mol m^{-3} expressed sap) of *Gossypium hirsutum* L. (Experiment 4.1).

Parameters	Control				Salinity			
	Betaine Concentration				Betaine Concentration			
	0	0.1	1	10	0	0.1	1	10
Total shoot height (mm)	1128	1140	1122	1138	890	852	836	830
Total number of nodes	12	12	11	12	10	10	10	11
FW/DW ratio	4.3	4.1	4.1	4	4.9	4.9	4.6	5.1
Thickness of young leaf (mm)	14	14	13	14	17	17	18	19
Thickness of old leaf (mm)	15	15	16	16	18	20	19	21
Photosynthesis (young leaf)	19	17	19	22	18	19	17	15
Photosynthesis (old leaf)	15	15	16	17	17	15	17	16
Transpiration (young leaf)	13	12	13	13	12	11	10	11
Transpiration (old leaf)	12	11	12	12	11	10	10	11
Stomatal cond. (y. leaf)	539	498	533	636	455	397	355	365
Stomatal cond. (o. leaf)	475	413	439	484	405	336	375	397
SPAD	46	45	45	53	51	50	50	54
Betaine conc. (young leaf)	94	91	78	127	117	119	130	126
Betaine conc. (old leaf)	77	72	86	98	116	92	106	122
Betaine conc. (young petiole)	55	47	63	63	82	59	70	58
Betaine conc. (old petiole)	46	36	57	64	65	64	67	76
Betaine conc. (young stem)	52	59	55	67	73	77	75	67
Betaine conc. (old stem)	45	48	60	75	59	78	64	61

Appendix 2: Mean values showing the effect of various concentrations of betaine with and without NaCl (200 mol m^{-3}) both applied on the leaves of *Gossypium hirsutum* L. on growth parameters, photosynthesis, (µmol m^{-2} s^{-1}), transpiration (mmol m^{-2} s^{-1}), stomatal conductance (mmol m^{-2} s^{-1}), SPAD and betaine concentrations (mol m^{-3} expressed sap). (Experiment 4.2).

Parameters	Control				Salinity			
	Betaine Concentration				Betaine Concentration			
	0	0.1	1	10	0	0.1	1	10
Total shoot height (mm)	904	1106	990	1030	1046	1216	1072	1056
Total number of nodes	10	12	11	11	11	13	11	12
FW/DW ratio	4.2	3.7	3.8	3.6	5.4	5.2	5.0	6.6
Thickness of young leaf (mm)	13	16	15	16	15	15	17	18
Thickness of old leaf (mm)	16	16	16	16	25	24	26	23
Photosynthesis (young leaf)	19	17	17	18	20	18	20	19
Photosynthesis (old leaf)	14	19	20	18	11	12	9	11
Transpiration (young leaf)	13	12	11	12	12	10	11	10
Transpiration (old leaf)	12	13	13	12	9	9	6	7
Stomatal cond. (y. leaf)	530	461	391	485	589	439	542	478
Stomatal cond. (o. leaf)	498	533	558	480	292	308	186	268
SPAD	51	50	47	49	39	42	43	44
Betaine conc. (young leaf)	114	110	103	79	103	107	98	97
Betaine conc. (old leaf)	98	85	88	102	70	73	72	66
Betaine conc. (young petiole)	75	63	75	79	58	70	60	57
Betaine conc. (old petiole)	73	61	77	83	49	63	51	60
Betaine conc. (young stem)	56	72	64	72	59	63	52	43
Betaine conc. (old stem)	55	69	78	62	58	61	51	47

Appendix 3: Mean values showing the effect of various concentrations of betaine with and without NaCl (200 mol m^{-3}) both applied to the soil on ion concentrations (mol m^{-3} expressed sap) of *Gossypium hirsutum* L. (Experiment 4.1).

Parameters	Control				Salinity			
	Betaine Concentration				Betaine Concentration			
	0	0.1	1	10	0	0.1	1	10
Na^+ conc. (young leaf)	11	3	4	6	55	97	71	104
Na^+ conc. (old leaf)	11	8	4	6	112	112	113	138
Na^+ conc. (young petiole)	5	2	2	1	46	49	48	56
Na^+ conc. (old petiole)	5	5	3	3	67	76	75	84
Na^+ conc. (young stem)	4	3	1	3	48	45	55	74
Na^+ conc. (old stem)	3	4	3	3	69	76	68	72
K^+ conc. (young leaf)	129	123	127	137	116	148	134	114
K^+ conc. (old leaf)	89	99	83	109	94	82	84	80
K^+ conc. (young petiole)	217	218	213	223	229	233	240	232
K^+ conc. (old petiole)	270	263	278	284	276	276	313	283
K^+ conc. (young stem)	195	181	190	195	194	200	211	190
K^+ conc. (old stem)	162	163	169	162	123	125	110	123
Mg^{2+} conc. (young leaf)	40	35	42	43	26	32	24	29
Mg^{2+} conc. (old leaf)	56	46	57	68	56	53	42	45
Mg^{2+} conc. (young petiole)	29	26	30	31	24	24	25	27
Mg^{2+} conc. (old petiole)	34	33	32	52	33	39	37	34
Mg^{2+} conc. (young stem)	33	29	31	31	26	27	28	29
Mg^{2+} conc. (old stem)	20	21	19	26	17	21	17	17
Ca^{2+} conc. (young leaf)	81	73	86	95	53	66	45	55
Ca^{2+} conc. (old leaf)	168	169	155	201	134	117	106	94
Ca^{2+} conc. (young petiole)	50	46	49	46	30	32	26	30
Ca^{2+} conc. (old petiole)	58	61	55	73	53	56	52	47
Ca^{2+} conc. (young stem)	35	32	31	28	22	21	20	22
Ca^{2+} conc. (old stem)	20	21	20	18	16	15	15	15
Cl^- conc. (young leaf)	207	205	201	194	264	256	257	275
Cl^- conc. (old leaf)	200	204	241	250	384	360	375	386
Cl^- conc. (young petiole)	153	159	170	152	230	226	219	218
Cl^- conc. (old petiole)	83	88	85	95	139	151	136	167
Cl^- conc. (young stem)	216	207	270	211	276	324	323	312
Cl^- conc. (old stem)	108	117	113	116	132	109	162	118
Malate conc. (young leaf)	37	48	41	42	26	20	27	13
Malate conc. (old leaf)	71	63	61	75	47	44	39	20
Malate conc. (young petiole)	32	35	33	30	29	32	24	23
Malate conc. (old petiole)	35	37	42	34	28	19	21	25
Malate conc. (young stem)	83	97	74	109	53	38	43	41
Malate conc. (old stem)	35	38	51	18	25	10	11	54
NO_3^- conc. (young leaf)	23	11	9	32	30	31	22	29
NO_3^- conc. (old leaf)	44	48	35	81	46	37	36	40
NO_3^- conc. (young petiole)	15	9	4	30	27	20	18	25
NO_3^- conc. (old petiole)	5	5	2	21	14	13	11	15
NO_3^- conc. (young stem)	0	1	0	3	0	0	0	0
NO_3^- conc. (old stem)	0	0	0	1	0	0	0	1

Appendix 4: Mean values showing the effect of various concentrations of betaine with and without NaCl (200 mol m^{-3}) both applied on the leaves on ion concentrations (mol m^{-3} expressed sap) of *Gossypium hirsutum* L. (Experiment 4.2).

Parameters	Control				Salinity			
	Betaine Concentration				Betaine Concentration			
	0	0.1	1	10	0	0.1	1	10
Na^+ conc. (young leaf)	6	3	4	14	153	124	167	159
Na^+ conc. (old leaf)	13	24	19	22	321	340	406	317
Na^+ conc. (young petiole)	2	2	1	2	23	18	21	22
Na^+ conc. (old petiole)	4	4	3	4	70	69	69	61
Na^+ conc. (young stem)	2	2	2	1	23	12	18	17
Na^+ conc. (old stem)	5	4	3	4	42	34	36	41
K^+ conc. (young leaf)	152	188	118	133	70	59	72	71
K^+ conc. (old leaf)	118	122	143	137	24	23	21	26
K^+ conc. (young petiole)	242	223	199	203	199	167	194	191
K^+ conc. (old petiole)	265	255	271	280	266	245	284	258
K^+ conc. (young stem)	190	173	168	160	148	132	148	157
K^+ conc. (old stem)	159	156	146	153	138	122	137	138
Mg^{2+} conc. (young leaf)	61	57	37	44	26	29	38	30
Mg^{2+} conc. (old leaf)	84	68	71	83	29	30	31	32
Mg^{2+} conc. (young petiole)	35	34	27	33	28	29	30	31
Mg^{2+} conc. (old petiole)	43	42	39	46	42	41	50	47
Mg^{2+} conc. (young stem)	34	33	31	32	21	27	28	30
Mg^{2+} conc. (old stem)	18	33	22	26	21	23	24	24
Ca^{2+} conc. (young leaf)	99	87	93	66	47	48	64	48
Ca^{2+} conc. (old leaf)	183	168	166	184	54	67	59	56
Ca^{2+} conc. (young petiole)	37	35	29	31	36	36	32	31
Ca^{2+} conc. (old petiole)	49	46	43	44	51	51	58	51
Ca^{2+} conc. (young stem)	22	21	21	19	19	19	20	24
Ca^{2+} conc. (old stem)	16	17	14	15	16	13	15	16
Cl^- conc. (young leaf)	67	72	67	68	168	134	146	125
Cl^- conc. (old leaf)	72	82	67	89	233	252	246	212
Cl^- conc. (young petiole)	75	63	61	70	125	105	124	112
Cl^- conc. (old petiole)	27	28	20	27	72	72	80	59
Cl^- conc. (young stem)	79	118	106	64	332	369	445	284
Cl^- conc. (old stem)	90	67	67	113	105	136	154	147
Malate conc. (young leaf)	65	64	60	60	35	43	47	38
Malate conc. (old leaf)	93	94	90	100	47	69	73	76
Malate conc. (young petiole)	51	54	31	43	28	30	31	39
Malate conc. (old petiole)	45	46	38	43	36	35	33	41
Malate conc. (young stem)	129	96	134	120	7	17	17	23
Malate conc. (old stem)	31	67	53	38	35	20	31	19
NO_3^- conc. (young leaf)	39	37	41	30	31	31	20	37
NO_3^- conc. (old leaf)	89	81	65	37	80	59	54	87
NO_3^- conc. (young petiole)	34	21	26	20	32	24	16	23
NO_3^- conc. (old petiole)	16	14	16	7	17	13	12	16
NO_3^- conc. (young stem)	2	2	5	2	0	0	0	0
NO_3^- conc. (old stem)	2	2	0	1	0	2	0	0

Appendix 5. Growth parameters of *Gossypium hirsutum* L. treated with various concentrations of NaCl and KCl either sprayed on the leaves (L) or applied to the soil (S). The values are means of 5 replicates ± standard errors. (Experiment 5.1).

Data set	Treatments NaCl (mol m^{-3})	Treatments KCl (mol m^{-3})	Total shoot height (mm)	Height above marked leaf (mm)	Total shoot fresh weight (g)	Weight above marked leaf (g)	Total No. of nodes	No. of nodes ab. marked leaf	FW/DW ratio	Thickness young leaf	Thickness old leaf
1	0 S	0 S	990 ± 23	683 ± 22	251 ± 6	178 ± 4	12 ± 1	8 ± 0	4.6 ± 0.0	15 ± 0	19 ± 1
	0 S	1 S	1018 ± 7	700 ± 6	259 ± 6	180 ± 5	12 ± 0	8 ± 0	4.4 ± 0.1	15 ± 1	21 ± 1
	0 S	10 S	1072 ± 20	742 ± 11	262 ± 9	188 ± 8	13 ± 0	8 ± 0	4.4 ± 0.1	16 ± 1	21 ± 1
2	0 L	0 L	962 ± 40	643 ± 25	268 ± 13	189 ± 4	11 ± 0	9 ± 0	4.6 ± 0.1	15 ± 0	20 ± 1
	0 L	1 L	998 ± 22	680 ± 15	270 ± 9	196 ± 9	12 ± 0	8 ± 0	4.4 ± 0.2	15 ± 1	21 ± 1
	0 L	10 L	1082 ± 13	742 ± 28	288 ± 7	201 ± 12	14 ± 0	9 ± 0	4.5 ± 0.1	16 ± 1	19 ± 1
3	200 S	0 S	700 ± 29	513 ± 17	145 ± 4	97 ± 4	9 ± 1	7 ± 1	5.1 ± 0.0	17 ± 0	22 ± 1
	200 S	1 S	748 ± 22	480 ± 19	158 ± 8	114 ± 4	10 ± 0	7 ± 0	5.0 ± 0.1	16 ± 1	18 ± 1
	200 S	10 S	886 ± 28	628 ± 39	165 ± 7	126 ± 6	11 ± 0	8 ± 0	4.5 ± 0.2	16 ± 0	21 ± 0
4	200 S	0 L	665 ± 52	460 ± 55	117 ± 29	87 ± 23	8 ± 0	6 ± 1	5.3 ± 0.4	16 ± 1	22 ± 1
	200 S	1 L	796 ± 13	530 ± 19	176 ± 8	125 ± 7	11 ± 0	7 ± 0	5.3 ± 0.1	16 ± 1	20 ± 1
	200 S	10 L	908 ± 14	604 ± 20	203 ± 8	146 ± 10	12 ± 0	8 ± 0	4.8 ± 0.1	16 ± 0	21 ± 1
5	200 L	0 S	570 ± 50	308 ± 52	76 ± 19	64 ± 17	9 ± 1	6 ± 1	9.0 ± 1.3	21 ± 1	28 ± 2
	200 L	1 S	650 ± 95	386 ± 91	116 ± 35	91 ± 28	9 ± 1	7 ± 1	7.2 ± 0.7	19 ± 1	25 ± 1
	200 L	10 S	864 ± 79	588 ± 77	178 ± 10	152 ± 7	12 ± 0	8 ± 1	7.2 ± 0.3	19 ± 1	26 ± 1
6	200 L	0 L	615 ± 117	377 ± 107	90 ± 38	76 ± 33	9 ± 2	7 ± 2	8.0 ± 0.9	20 ± 1	25 ± 1
	200 L	1 L	918 ± 26	610 ± 14	262 ± 31	209 ± 26	13 ± 0	8 ± 0	6.2 ± 0.2	17 ± 0	23 ± 1
	200 L	10 L	966 ± 34	648 ± 40	287 ± 21	210 ± 22	12 ± 0	8 ± 0	6.3 ± 0.4	17 ± 0	21 ± 1