THE MECHANISM OF ACCUMULATION OF PROLINE AND QUATERNARY AMMONIUM COMPOUNDS AND THEIR ADAPTIVE SIGNIFICANCE IN TOMATO (*Lycopersicon esculentum*, Mill.) AND WHEAT (*Triticum aestivum*) EXPOSED TO SALT STRESS

By

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Dedicated to my parents

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LIST OF ABBREVIATIONS

3-OMG	3-orthomethylglucose
A	Assimilation
ABA	Abscisic acid
ATP	Adenosine triphosphate
BA	Benzyladenine
C-3	Carbon cycle 3 of photosynthesis
C-4	Carbon cycle 4 of photosynthesis
Ca^{++}	Calcium
$CaSO_4$	Calcium sulphate
Cl^-	Chloride
CO_2	Cardon dioxide
CO_3^{2-}	Carbonate
CRI	Chloride regulation index
D_2O/HDO	Deutriated water
DNP	Dinitrophenol
dw	Dry weight
E	Transpiration rate
f w	Fresh weight
G	Conductance of water vapour
g	Grams
G2	Generation 2 of the cell cycle
GB	Glycinebetaine
h	hour
HCO_3^-	Bicarbonate
HH	High humidity
Jv	Water flow
K_2S0_4	Potassium sulphate
KCl	Potassium chloride
LH	Low humidity
Log (e)	Natural log
Lp	Hydraulic conductivity
LSD	Least significant difference
m.Osm.	Milli osmol
mbar	Milli bar
meq/l	Milliequivalent per litre
mg	Milligram
$MgCl_2$	Magnesium chloride

MHz	Megahertz
ml	Millilitre
mM	Millimolar
mmole	Millimole
MPa	Megapascal
N	Nitrogen
N	Normality
NA	Nicotiamide
Na^+	Sodium
Na^+(ext.)	Sodium in the rooting medium
Na_2SO_4	Sodium sulphate
NaCl	Sodium chloride
NAD (H)	Nicotiamide adenine dinucleotide (reduced)
NAD	Nicotinamide adenine dinucleotide
NADP(H)	Nicotiamide adenine dinucleotide phosphate (reduced)
ng	Nanogram
NMN	Nicotiamide mononucleotide
NMR	Nuclear magnetic resonance
NO_3	Nitrate
NS	Not significant
OH^-	hydroxide
P-5-C	Pyrroline-5-carboxylate
Pa	Ambiant CO_2 partial pressure
PAI	Proline accumulation index
PEG	Polyethylene glycol
PEP	Phosphoenolpyruate
pH	Negative logarithm of hydrogen ion activity
Pi	Intercellular CO_2 partial pressue
ppm	parts per million
PVC	Polyvinylchloride
Q_{10}	Diffusion coeffient
QAC	Quaternary ammonium compound
R	Regression coefficient
R	Resistance
RO	Reverse osmosis
Ra	Boundary layer resistance
Rb	Rubidium
RUBISCO	Ribulose biphosphate carboxylase oxygenase
RuBP	Ribulose biphosphate
SO_4^{2-}	Sulphate

Sq.M	Meter square
SRI	Sodium regulation index
t-butanol	Tertiary butanol
TAI	Trigonelline accumulaiton index
TAMO	Trimethyl amino N-oxide
TLC	Thin layer chromatography
VR	Variance ratio
W	mole fraction of water vapour

SYMBOLS

*	Probability 0.50
* *	Probability 0.10
* * *	Probability 0.01
o	Degree
°C	Degree centigrade
ß	Beta
µ	Micro
µM	Micromolar
∂	Gamma
Δ	Delta
Δe	Saturation deficit
Δp	Pressure differential
µ Ein	Micro Einstein
Ψ	

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STATEMENT

I hereby declare that the thesis here presented contains no material which has been accepted for the award of any other degree or diploma in any University and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due references are made in the text of this thesis.

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SUMMARY

The present study is aimed at investigating the effects of salt stress in triggering or modulating accumulation of proline and quaternary ammonium compounds (QAC) in tomato (*Lycopersicon esculltentum*, Mill.) cv. Duke and wheat (*Triticum aestivum*) cv.Sun-9E. The physiological significance of accumulation of these compounds in relation to (1) salt tolerance and (2) maintenance of photosynthesis during salt stress are also investigated in *Lycopersicon* spp. and wheat respectively.

Proline accumulated in both tomato and wheat challenged with salt. QACs, trigonelline in tomato and glycinebetaine in wheat also accumulated in response to salt stress. The accumulation of these compounds correlated with decreases in $\psi_{ext.}$, ψ_{leaf}, ψ_s, ψ_p and increases in tissue Na^+ and Cl^- concentrations. The results summarised hereunder are aimed at investigating the potential causal factor triggering accumulation of these compounds

The hypothesis that ψ_p was the causal factor in triggering proline and QAC accumulation was evaluated by subjecting these species to progressive salinization and prolonged exposure to salinity at a steady state to ensure turgor maintenance. Proline, trigonelline and glycinebetaine accumulated despite a lack of change in ψ_p, eliminating ψ_p as the causal factor.

The possibility that the correlation between ψ_s and accumulation of these compounds is due to the increase in concentration of these solutes *per se* was considered, but the computation of the contribution of these compounds to the osmotic pool demonstrated a negligible effect, suggesting that the relationship between ψ_s and accumulation was not due to the increase in the solute concentration *per se*.

A distinction as to whether the accumulation of these compounds was due to bulk changes in ψ_s or to the presence of specific ions was attempted by subjecting these

species to stress with a non-ionic, non-permeating osmoticum, PEG_{4000}, and different ionic osmotica. The accumulation response was not specific to salt stress alone however, the concentration of proline, in particular, was influenced by specific ions. At an equal $\psi_{ext.}$ (-1.065 MPa) more proline accumulated with $MgCl_2$ salinity than with NaCl. The differences in proline accumulation correlated with differences in ψ_s in each case. However, the accumulation of equal concentration of proline in plants under NaCl, $MgCl_2$ or KCl salinities at iso-ionic strengths despite differences in ψ_s suggest that ψ_s may not control accumulation of this compound. The observation of constant tissue glycinebetaine concentration, despite continued decrease in ψ_s and of substantial increase in glycinebetaine concentration in unstressed control plants with no change in ψ_s suggest that ψ_s also does not control glycinebetaine accumulation. Trigonelline accumulation was also not proportionate to the decrease in ψ_s of $MgCl_2$ salinised plants. Conclusive evidence that ψ_s was not the controlling factor in accumulation of these compounds was obtained from experiments where these plants were subjected to stress at high humidity, and proline and QACs accumulated without any decrease in ψ_s

The accumulation of equal concentrations of proline in plants stressed with NaCl,$MgCl_2$ or KCl despite differences in tissue anion or cation concentrations suggest that neither leaf cation nor anion concentrations control accumulation of proline or trigonelline. Conclusive evidence that leaf ion concentration is not involved in proline or trigonelline accumulation was obtained from experiments in which ion uptake was inhibited by Ca^{++} and high humidity and yet proline and trigonelline accumulation occured. The maintenance of constant tissue glycinebetaine concentration despite a continued increase in leaf ion concentration and a substantial increase in glycinebetaine accumulation without an increase in leaf ion concentration, together with a lack of correlation between glycinebetaine accumulation and leaf ion concentration in plants subjected to high humidity suggests that neither leaf cation nor anion concentration is the cause of glycinebetaine accumulation. The variation in proline concentration in plants stressed with NaCl and $MgCl_2$ at iso-osmotic potential however, was related to

variation in Cl^- strength of the external medium. Stressing plants with NaCl or $MgCl_2$ at iso-anionic strength resulted in equal proline concentration. This was further confirmed by comparing KCl and NaCl (which enabled achievement of an equivalence of $\psi_{ext.}$, cation and anion strength in the rooting medium) where proline accumulation was equal.

Similarly, ψ_{leaf} is not the controlling factor in accumulation of these compounds, there was a substantial increase in proline in tomato with a steady ψ_{leaf} and equal accumulation of proline in NaCl and $MgCl_2$ salinised plants at iso-anionic strengths despite differences in ψ_{leaf}, glycinebetaine accumulated in unstressed plants with no decrease in ψ_{leaf} and proline and QACs accumulated at high humidity without a change in ψ_{leaf}.

Substantial accumulation of proline and QACs occurred without any reduction in dry matter production, eliminating the contention that growth reduction is a prerequsite for accumulation of these compounds.

These results show that the observed correlations between changes in leaf water status or ion concentration and accumulation of proline and QACs are only apparent, not absolute. This suggests that the roots play a role in the accumulation of these compounds in the leaves other than that of modulating the ion supply to the shoot. Salinity in the rooting medium influences the root derived hormones especially ABA and cytokinins. However, accumulation of proline or QACs could not be ascribed to ABA, as there was no increase in ABA concentration in salt stressed tomato or wheat. Preliminary studies show that proline and QACs do not accumulate in NaCl stressed wheat and tomato when BA (benzyladenine) was applied exogenously suggesting that reduction in cytokinin supply during salt stress (Itai and Vaadia, 1965, 1971;Itai *et al.*, 1968; Walker and Dumbroff, 1981) may be the trigger influencing accumulation of proline or QAC. The extent of proline or QAC accumulation was influenced by humidity, nature of ion in the rooting medium and wind velocity. These variations in

concentrations of proline and QACs at different humidities and wind velocities may be attributed to variation in synthesis or transport of a root-derived active factor.

When the physiological significance of accumulation of proline and trigonelline in relation to salt tolerance, measured as growth, was examined in selected *Lycopersicon* species of varying ecological habitats, *L. cheesmanii*, belonging to a saline-coastal habitat, was found to be the most tolerant and *L. pinnellii* the most sensitive. The commercial cultivar, *L. esculentum* cv. Duke, ranked 7th in the order of relative tolerance to salt. All species accumulated proline in all organs in response to salinity; but there was no general relationship between the ability of these species to accumulate proline and their relative salt tolerance. Relative trigonelline accumulation in the meristematic tissues of NaCl stressed plants correlated with the salt tolerance of these species, however, as did their ability to (1) maintain turgor in the expanding leaves, (2) exclude Na^+ from the expanded leaves and (3) exclude Cl^- from the root tissues.

Experiments on the physiological significance of the accumulation of proline and glycinebetaine in sustaining photosynthesis during salt stress in wheat *in vivo* showed that pre-treatement with glycinebetaine, but not proline, alleviated NaCl-induced stomatal and non-stomatal inhibition of photosynthesis completely. A permeating and non-dissociating osmoticum, 3-orthomethyl-glucopyranose, also alleviated NaCl-induced perturbations of photosynthesis suggesting that glycinebetaine may work by maintaining chloroplast volume and not by specific effects on photosynthetic processes.

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CHAPTER 1

General Introduction and objectives

CHAPTER 1 GENERAL INTRODUCTION AND OBJECTIVES

The growth of glycophytes is reduced in a saline environment by either osmotic or specific ionic effects on cell expansion and metabolism. The growth and survival in such an environment depend, in part, on the ability of the plant to osmoregulate and maintain turgor by accumulating ions and organic compounds (Greenway and Munns, 1980). A variety of low molecular weight organic compounds, such as proline (Stewart and Lee,1974; Chu *et al.*, 1976; Rajasekaran,1979), glycinebetaine (Storey and Wyn Jones,1978), stachydrine (Parameshwara,1984), trigonelline (Parameshwara,1984) and sucrose (Nightingale and Farenham,1935-1936; Gauch and Evans, 1942; Bernstein and Ayers,1953) have been found to accumulate as a consequence of salt stress. It has been well established that proline and Quaternary Ammonium Compounds (QAC) accumulate in response to salt stress in halophytes (Stewart and Lee, 1974; Coughlan and Wyn Jones, 1980) and physiological roles of these compounds have been postulated in osmoregulation (Stewart and Lee,1974; Wyn Jones *et al.*, 1977; Grumet and Hanson,1986), protection of proteins against biologically unfavorable consequences of dehydration-induced thermodynamic perturbation (Paleg *et al.*, 1984), and in the protection of enzyme activity (Pollard and Wyn Jones,1979). In glycophytes also, proline and QACs accumulate with salt stress (Chu *et al.*, 1976; Wyn Jones and Storey, 1978) but, the rate and extent of accumulation may be modified by factors other than the water status of the plants and the external medium, and their role in such plants is less well established. It may also lie in areas other than the mere osmotic role. Further, the significance of inter-genotypic variations in accumulation is also disputed.

The evidence presently available indicates that accumulation of proline and glycinebetaine is correlated with many of the concomitant events such as the decrease in leaf water, osmotic and turgor potentials (Chu,1974; Greenway and Leahy,1972) and the increase in ion concentrations (Storey and Wyn Jones,1978) when plants are

subjected to salt stress. However, there is no discrimination between the importance of these various physical effects of ionic stress in triggering or modulating accumulation of proline and QACs. To date, no attempt has been made to determine whether the observed relationships are absolute or only apparent. It is generally assumed that there cannot be a multiplicity of initiating factors for a particular response and it is useful to ascertain the active factor. A knowledge of this will then assist in elucidating the roles of these compounds in plants during salt stress.

The question of separating the various consequences of a saline environment on solute accumulation can be approached by extending and exploiting the ion uptake mechanisms using different ionic species differing in respect of uptake, to alter internal ion concentrations and water relations. We can also control atmospheric humidity to manipulate the environment in which plants are challenged with salt.

The present investigation is directed to investigate the physical effects of different species of salt, in triggering or modulating proline or QAC accumulation. To relate growth to these changes and to elucidate the reality of relationships between accumulation of proline and QACs and associated factors in the salt stress environment. Tomato (*Lycopersicon esculentum*, Mill.) and wheat (*Triticum aestivum*), both classified as medium salt tolerant species (USDA,1954), were used as test plants. Finally, a special focus is drawn on the diversity of solutes accumulated during salt stress by *Lycopersicon spp.* in relation to eco-typic differences.

CHAPTER-2

Review of literature

CHAPTER 2: REVIEW OF LITERATURE

This review is a highly personal analysis of what we currently know (or do not know) of plant responses to salt stress, with specific reference to growth, metabolic consequences of stress, accumulation of solutes, and their role(s) in plants during stress and on relief; of some pitfalls in data interpretation and of needs for the present investigation. It is hoped that strongly advocated views, many of which will likely be proven wrong, and the little-tested working hypothesis or speculations, will serve as an impetus to progress. No pretense is made here as to completeness in covering the literature. Extensive reviews are available on the various responses of unicellular micro-algae and vascular plants from halophytic and non-halophytic habitats concerning, physiological, biochemical and ion regulatory mechanisms which contribute to sustained growth or survival in salt stress. The publications by Strogonov,(1964) and Weisal(1972) presented some understanding of the physiological basis of salt tolerance in halophytes and these were extended by the reviews of Flowers *et al.,* (1977) and Munns *et al.,* (1983) to cover the responses of unicellular organisms. Further reviews by Mass and Neiman(1977); Pitman(1977); Wyn Jones *et al.,* (1977) and Stewart *et al.,(* 1979) on tolerance mechanisms, ion uptake, osmoregulation and nitrogen metabolism of salt stressed plants have further increased our understanding of the respective fields. To keep the subject matter within reasonable bounds, attention here is focussed on the primary effects of salt through alterations in water, ion and hormonal status and the secondary responses to these changes in the metabolism of nitrogenous compounds.

2.1 WATER RELATIONS

2.1.1 Water in the saline environment

If we regard the chemical potential of pure water at a certain reference state as zero, then the water potential (ψ) in the soil, atmosphere or plant has a negative value (Kozlowski,

1964; Hillel,1965,1971; Slatyer,1967). Water potential is expressed in MPa. Differences in water potential between points drive water movement within the system. Plants obtain most of their water from the rooting medium. In a saline soil, the total water potential will be determined by the osmotic potential of the soil solution ($\psi\pi$soil) and matric forces exerted by the soil colloids and surfaces (ψm). It is difficult, if not impossible, to distinguish between these component potentials. According to many investigators (Wadleigh and Ayers,1945; Slatyer,1967) both components have a cumulative effect on the plants and during short term exposures their effects are inseparable. The relationship is described by the following equation:

$$\psi w = \psi s + \psi p + \psi m$$

where ψw is the sum of solute(ψs) pressure (ψp) and matric (ψm) potentials. The matric potential component is considered negligible and the equation reduces to :

$$\psi w = \psi s + \psi p$$

Plants exposed to saline conditions experience a shortage of water and this concept persuaded Schimper (1903) to formulate his 'physiological drought' theory which postulated that both halophytes and glycophytes suffer from dehydration in saline conditions. A consequent low hydration of protoplasm was deemed to be responsible for the changes in metabolic activity evident in plants subjected to low tissue water potential (Iljin, 1953; Kramer, 1959; Levitt,1956,1962; Kozlowski,1964).

2.1.2 Salt stress and water flow

Flow of water through plants has been described as a catenary process analogous to the flow of electricity through a series of resistances (Gradmann,1928; Vanden Honert,1948). Thus, the flow of water across any section of the flow path is proportional to the gradient in water potential between the shoot and root and inversely proportional to the resistances to flow imposed by soil, root, vascular system and leaves (Cowan and Milthorpe, 1968). Thus the equation:

$$J_v = L_p(\Delta\psi_w) = \frac{R_{root} - R_{leaf}}{R_{root} + R_{stem} + R_{leaf}}$$

where, J_v is the water flow ($cm^3\ cm^{-2}\ sec^{-1}$) and L_p is the hydraulic conductivity ($cm\ sec^{-1}\ MPa^{-1}$) of the pathway, R is resistance ($sec\ cm^{-1}\ MPa^{-1}$) (Slatyer,1969; Cowan and Milthorpe, 1968). Water transfer across barriers in the roots and leaves, as well as the movement of water vapour transpired from the leaf, are diffusive processes. They are proportional to vapour pressure or water concentration differences, which are in turn exponentially related to the water potential. For small water potential differences, always found in living plants, it is permissible to set the rate of diffusive water movement approximately proportional to the water potential difference. In this sense, $J_v = L_p - \Delta p$ where, Δp represents the pressure differential between the external medium and the xylem. The total force driving the water equals the osmotic potential (ψ_s)and transpirationally-induced hydrostatic forces (Weatherly, 1982). The equation describing flow is properly described by

$$J_v = L_p(\Delta p - \infty\Delta\psi_s)$$

where, $\Delta\psi_s$ is the rise in the osmotic pressure across the membrane and ∞ is the reflection co-efficient that accounts for membrane permeability to a given solute (Passioura, 1984). In the diffusive water pathway major resistances are due to cuticle, boundary layer (Ra) and stomata. The degree of stomatal opening governs the plant's transpiration rate (Raschke, 1979) when Ra is small, i.e, in a well circulated atmosphere.

Stomata are the major pathway for the CO_2 and water transfers. Stomatal conductance, therefore, determines the rate of both water loss and CO_2 uptake, so that, conservation precludes high rates of CO_2 assimilation (Cowan and Farquhar, 1977). Stomata tend to open or close with changes in the external and internal environment, influencing both carbon fixation and water loss. Thus, their function has the effect of reducing the average loss of water relative to the average rate of carbon fixation (Cowan, 1977, Farquhar *et*

al., 1980, Hall and Schulze, 1980). Reduction in stomatal conductance is frequently observed in plants in saline environments (Seeman and Crichely, 1985, West *et al.*, 1986), influencing transpiration and CO_2 exchange. The dependence of transpiration rate (E) on conductance of water vapour (G) is simply expressed as :

$$E = G (W_1 - W_0)$$

where, E and G are in moles m^{-2} sec^{-1}, and W_1 and W_0 are mole fractions of water vapour inside and outside the leaf. Diffusivity of water vapour through stomata is influenced by the moisture saturation levels of the atmosphere where the plants are grown. Thus, air-humidity influences the rate of diffusion of water vapour from the plant and is high in low humidity (Oertli,1966). In situations of high humidity, the total potential drop of water across the plant is small. Because of a small total water potential drop, the rate of water movement should also be small (though not proportionately smaller). The actual behaviour of water in plants cannot be predicted from consideration of the total water potential drop and the resistance functions alone. The mechanism of salt uptake has also to be considered. Under a steady state of salt uptake against a concentration gradient, a small drop in total water potential will result in positive turgor causing bleeding and guttation, as observed in barley (Oertli, 1966). However, this depends on the concentration of the salt solution. A concentration- dependent steady-state guttation has been found in barley (Oertli, 1966). Similarly, under conditions of high humidity and low transpiration the tissue water content of salt stressed plants has been found to be similar to that of control plants (Neiman and Poulsen, 1967). Less negative water potential in salt stressed beet, onion and radish has also been found under high humidity (Hoffman and Rawlins, 1971). In addition, factors such as soil texture and hydraulic conductivity have been reported to affect water flow into the root because of their effects on resistance to water movement through the medium to the root surface. Aeration, temperature, degree of suberization of roots and ionic species in the root medium also affect the rate of uptake chiefly by modifying the resistance of the roots. The contribution of individual factors to water absoption under stress have been detailed by

Kramer (1980). Root systems exposed to salt show reduced permeability to water due to inhibition of root extension and increased suberization of existing roots (Klepper, 1967). Even a small decrease in osmotic potential causes a measurable decrease in root permeability. The hydraulic conductivity of roots is also influenced by ABA, a hormone which increases many fold during salt stress (Walton *et al.*, 1977). Dramatic increase in water uptake in excised roots and root systems on application of ABA have been demonstrated in tomato (Tal and Imber, 1971), sunflower (Glinka, 1973), maize (Collins and Kerrigan, 1973,1974). This increase in water uptake has been attributed to an increase in the hydraulic conductivity of roots. However, such an influence is not to be expected in salt stressed plants as salt itself increases hydraulic resistance (O'Leary, 1969).

2.1.3 Osmotic adjustment

When the osmotic potential of the solution in the root medium decreases the osmotic potential of the plant also decreases, usually proportionally. Such a decrease in osmotic potential could be obtained by:(1) a reduction in cell volume either by loss of turgor or cell wall elasticity (2) uptake of ions from the root medium (3) synthesis of organic solutes or (4) a combination of these responses. The decrease in osmotic pressure caused by 'net' changes in the content or concentration of osmotic solutes is termed "osmotic adjustment" (cf, Munns *et al.*, 1982). Such osmotic adjustment ensures turgor maintenance. Evidence for osmotic adjustment has been found for many stressed plants (Eaton, 1927, 1948; Black, 1960; Slatyer, 1967; Lagerwerff and Eagle, 1969). The initial imbalance in water relation observed when the plants are first exposed to salt stress is not permanent and is at least partly negated in some species and fully in others by accumulation of organic solutes (Meiri and Poljakoff Mayber,1969). Such osmoregulatory response is observed not only in higher plants but also in algae, fungi and bacteria (Kauss, 1974; Cram, 1976; Helleburst,1976). In some species of plants, high salinity leads to a higher water content expressed as succulence(Burggin, 1947; Boyer, 1965) due to uptake of anions (Strogonov, 1964). This indicates that plants in saline

environments are not necessarily suffering from dehydration.

2.2 ION RELATIONS

The presence of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, CO_3^{2-} and HCO_3^- ions at high concentrations in the rooting environment causes salt stress. A multiplicity of effects on ion uptake, nutrition, water relations and metabolism flow from their presence.

2.2.1 Ions and water flow

Ions in the rooting medium influence the root in a variety of ways including a lowering of water potential which affects: (1). water flow and (2). root metabolism. A high root conductance reduces the extent of water deficit in the shoot, but measurements on plants kept in saline conditions for long periods suggest that conductance decreases. Exposure of bean plants to 84 mM NaCl for 3 weeks reduced the hydraulic conductance six fold. After boiling the roots, the conductance was still three fold lower than that of control roots (O'Leary, 1969), indicating that the decrease was at least partly due to an increase in suberization and the low conductance persisted for at least 2 days after the transfer of the plants from 84 to 0 mM NaCl. However, these data were obtained by the measurement of exudation from excised roots in de-ionised water under an applied pressure of 0.2 MPa and artefacts cannot be excluded. More recent experiments indicate that the changes in root conductance are slow and are linked with growth reduction. In 'split root' experiments with tomatoes and sunflowers lasting only 3 days there was no decrease in root conductance nor in growth of the shoot (Shalhevet *et al.*, 1976). A monocotyledonous halophyte, *Puccinella peisonis* , has a multiple endodermis and heavy suberization (Stelzer and Lauchli, 1977) and the root conductance of these plants is low. These plants have both a relatively low shoot Cl^- and Na^+ concentration and reduced water flow. If suberization seals the extracellular pathway for water flow, there would be a bigger reduction in ion transport than in water flow.

2.2.2 Ion uptake in relation to salt stress

In some cases, ions are readily absorbed by diffusion or mass flow and in other cases ions are exchanged for hydrogen ions (Jenny and Ayers 1939; Epstein and Stout,1952). Such an exchange and the consequent decrease in pH around the absorbing root was demonstrated by Sachs as early as 1875. Transport of ions into the symplasm occurs at the plasmalemma of epidermal, and to some degree, cortical cells. Plasmadesmata between cells in the root connect the cytoplasm of adjascent cells to form the symplast which is highly selective for ion transport across the root. Transport across the cell membrane controls entry of ions into the symplasm and possibly release into the xylem also. The cell walls constituting the apoplast, form another pathway through which water flows carrying ions from the external solution (Lauchli, 1972; Luttge, 1974; Clarkson and Hanson, 1980; Pitman, 1976,1982). Barriers to water flow at the endodermis restrict the non-selective flow of ions to the xylem.

2.2.3 Mechanisms of ion uptake

Various models have been proposed to describe the ion uptake mechanism (Epstein,1966; Laties, 1969). The concept of two distinct mechanisms was derived from studies on the kinetic response of ion absorption to substrate concentration(Epstein and Hegen 1952; Rains and Epstein, 1967 a,b; Rains, 1969). The rate of absorption is characterised by two distinct kinetic responses over a wide concentration range, one mechanism operating below approximately 1 mM and the second operating within the concentration range of 1-50 mM (Epstein, 1966). The properties of these kinetically separable mechanisms are important in regulating the intracellular ionic milieu of cells exposed to a high salt environment. There is a higher affinity for K^+ at the low concentration range (mechanism 1) than at high concentrations (mechanism 2). The high affinity for K^+ is demonstrated by the transport of these ions across cell membranes from a substrate medium of low K^+ concentration in preference to transport of the chemically similar Na^+ ion. Such a high affinity mechanism has been found in barley (Epstein, 1966), corn

roots and leaves (Rains, 1969), and halophytes (Rains and Epstein,1967 a,b). This characteristic depends largely on the presence of Ca^{++} (Lauchli and Epstein, 1970). In a recent study, Wrona and Epstein,(1985) described differences in K^+ and Na^+ absorption by excised roots of *Lycopersicon esculetum and L. cheesmanii* using $^{86}Rb^+$ (as an analogue for K^+) and $^{22}Na^+$. At a (low) concentration range of 0.01-0.2 mM both species demonstrated saturation kinetics for K^+ (^{86}Rb) but not for Na^+. *L. esculentum* showed a higher affinity for K^+ than did *L. cheesmanii.* At a higher concentration range (5-50 mM), however, similar uptake patterns were observed for both ions, although relatively more Na^+ was taken up by *L. esculentum* than by *L. cheesmanii.*

The site of uptake for both system has been suggested to be either at the plasmalemma (Rains and Epstein, 1967a,b; Epstein, 1966) or at the tonoplast (Torrie and Laties, 1966; Luttge and Laties, 1966,1967; Osmond and Laties, 1968) and statistical evaluation by Nissen (Weisal,1972) suggests that both sites may be active.

2.2.4 Energetics of ion uptake

When salt is administered to the rooting medium, respiration of the root cells increases (Lundegardh, 1955; Robertson, 1968). Lundegardh (1955) linked the enhancement of respiration to an increase in the anion concentration in the substrate, a relationship characterised by the term 'anion respiration'. Mitchell (1966) and Robertson (1967,1968) followed Ludegardh with the claim that the transfer of electrons along the electron chain supplies the potential for the accumulation of anions against a concentration gradient and that cations flow in a passive manner to maintain electroneutrality. A second view holds that ATP or high energy intermediates generated by respiratory or photosynthetic processes are utilised by plants to accumulate ions (Van Steveninck, 1965; Luttge and Laties, 1966; Rains, 1968). Although the existence of a dual mechanism of ion uptake has been demonstrated, nothing is known about the energetics of these mechanisms. Enhanced respiration has been observed at concentrations of salt well above the concentration range of mechanism 1 (Robertson,1968) which reveals that the respiratory response is related to ion

transport by mechanism 2. Polya and Atkinson, (1969) studied the absorption of K^+, Na^+ and Cl^- by beet discs as a function of time and anti-metabolites. The external concentration was maintained at 0.5 mM which effectively limited uptake of ions through mechanism 1 (Mengel,1967). By relating ATP levels to fluxes and studying the effects of various anti-metabolites they concluded that transport by mechanism 1 is dependent upon electron transport, not on the utilization of ATP or high energy intermediates. Rains and Epstein (1967) compared DNP, an uncoupler of oxidative phosphorylation, with anaerobic conditions over both ranges of concentrations and concluded that the energy utilised (ATP) in transporting ions is not significantly different either in source, or the amount required for absorption of ions by both mechanisms. In contrast, Luttge and Laties (1967) compared relative sensitivity of both mechanisms to inhibitors and concluded that absorption by mechanism 1 is more sensitive to inhibitors than is absorption by mechanism 2. The two sets of conflicting data can partly be explained by the differences in plant species and tissues used in the studies viz., barley root and corn seedlings and the use of different ions in their investigations. However, it is attractive to consider that less energy is required to accumulate ions from high concentrations since the concentration gradient is lower at the higher concentration range of mechanism 2. This might then be manifest in the lower sensitivity to anti-metabolites observed by Luttge and Laties (1967).

Implication of ATP in the transport of ions along with demonstrations of ATPase in plant tissues (Atkinson and Polya, 1967; Fisher and Hodges, 1969; Kylin and Gee,1970) has prompted research on the possible link between these enzymes and ion transport. A monovalent ion- stimulated ion transport has been characterised in oat roots (Fisher and Hodges, 1969) and ATPase activity was positively correlated with K^+ transport. Leonard (1983,1985) concluded that ATPase activity is a measure of the ATPase-driven ion pump which has been extensively characterised.

2.2.5 Selectivity of ions

Whether ion uptake occurs by an active or passive mechanism, ion uptake is selective and

not proportional to the external concentration. Most halophytes take up large quantities of both Na^+ and Cl^- into the shoot (Flowers *et al.*, 1977) whereas glycophytes tend to exclude Na^+ or Cl^- from the shoot (Hodson *et al.*, 1985). Such ion exclusion mechanisms have been reported from wheat (Torres and Bingham,1973), *Festuca rubra* (Rozema *et al.*,1978), *Agropyron elongatum* (Shanon, 1978), soybean (Lauchli and Weieneke, 1979) and tomato (Wrona and Epstein, 1985). Differences in ion uptake selectivity have been found within a single species eg. barley cv. California Mariout and Arimar (Storey and Wyn Jones, 1978) or within a single genus eg. tomato (Wrona and Epstein, 1985). In tomato, some wild species and domestic cultivars have been reported to be 'salt excluders' (Tal, 1971; Fong, 1983) while some are described as 'salt accumulators' (Rush and Epstein, 1976; Phills *et al.*, 1979). A comparative study (Rush and Epstein, 1981) with *L. esculentum* and *L. cheesmanii* , a wild salt tolerant species of coastal habitat, has shown that at high salt concentrations, the wild salt-tolerant species accumulated Na^+ and Cl^- freely in the leaves while the salt sensitive cultivar excluded Na^+ and Cl^- from the leaves and accumulated more salt in the roots. It was also evident that the salt tolerant species partially substitutes Na^+ for K^+ in metabolism whereas the domesticated cultivar did not. Such a difference between these two species was not observed for Cl^- accumulation. The accumulated Na^+ in *L. cheesmanii* has been suggested to function as a 'cheap' osmoticum (Wrona and Epstein,1985) as for halophytes (Yeo *et al.*, 1980).

In glycophytes two efficient systems for K^+/Na^+ selectivity have been proposed (a). K^+/Na^+ exchange at the plasmalemma (Pitman and Sandler,1967) leading to selective K^+ uptake (Rains and Epstein, 1967 a,b) coupled with extrusion of Na^+ from the root symplasm (Jeschke, 1979) and (b) Ion transport at the tonoplast favouring vacuolar accumulation of sodium. Little is known about the extent to which root systems contribute to the performance of glycophytes under salt stress. However, the experiments of Wolf and Jeschke (1986) with *Hordeum vulgare* cv.California Mariout, a salt tolerant cultivar and *H. distichon*, cv. Villa, a salt sensitive cultivar (Greenway, 1962) on sodium fluxes of 0.1-50 mM Na^+ showed a higher sodium flux and content in the cytoplasm and

vacuole in Villa than in California Mariout. K^+ dependent Na^+ extrusion across the plamalemma has also been found to be higher with Villa than California Mariout which indicates that a high degree of K^+/Na^+ selectivity at the plamalemma is not *per se* decisive for salt tolerance in barley. However, at high external Na^+ concentrations the xylem transport of Na^+ was not higher in the salt tolerant California Mariout than in Villa. In both cultivars Na^+ transport was independent of $Na^+_{(ext)}$ over the range 10-50 mM NaCl.

In addition to modifications in the uptake selectivity for Na^+ brought about by changes in external concentration, variables such as rate and magnitude of salt increment, duration of stress, rate of growth and ion translocation also modify the selectivity processes. In barley, variation in plant Na^+ levels due to the nature of administration of stress has been found, being high at low salinities in a pseudo-steady state treatment compared to those in an incremental increase treatment (Storey and Wyn Jones, 1978). Similar variations were not observed in plant Cl^- concentration suggesting influences on the mobility of Na^+. Other experiments have led to the conclusion that Cl^- is more mobile than Na^+ (Pitman, 1977) and that Cl^- uptake attains equilibrium rapidly compared to Na^+ (Greenway,1973).

2.2.6 Internal and environmental factors influencing salt absorption,transportation and accumulation

Salt accumulation in root cells and transport to the shoots are both affected by a variety of internal (species, condition of tissue, respiration, sugar, internal ion concentration and hormones) and environmental (aeration, temperature, composition and concentration of ions at the rooting medium, pH, humidity) factors. Such influences on ion uptake and accumulation result in altered water relations in plants under stress and associated metabolic processes.

2.2.6.1 *Species*

There is a wide diversity in the ability of the plants to take up or exclude ions. Most

halophytes tolerate high external salt concentrations by taking in high amounts of Na^+ and Cl^- ions and efficiently compartmenting them (Hajibagheri, 1984). The upper limit for survival among the halophytes varies from 300 mM for *Atriplex hastata* (Black,1966) to 1020 mM NaCl for *Salicornia europea* (Montfort and Brandup, 1927). Variation in relative quantities of ion uptake also exist in glycophytes within genera (gramineae and legumes), within species (*L. esculentum and L.cheesmanii),* between varieties (barley, Arimar and California Mariout), and amongst cell lines. Their tolerance depends on their ability either to exclude (Lauchli,1984) or to take up and efficiently compartment excess ions. Such mechanisms have been reported for tomato species (Wrona and Epstein, 1985) where significant quantities of Na^+ are taken up by *L.cheesmanii*, a salt tolerant species in comparison to *L . esculentum*, which excludes Na^+ from the leaf.

2.2.6.2 *Condition of tissue*

The accumulation of salt in tissues is closely related to growth and metabolism. Enlarging cells have a high accumulation rate but mature cells which have lost their ability to enlarge also lose their ability to accumulate ions (Kramer,1980). The synthesis of new binding sites and carrier molecules is also involved and the cessation of growth brings an end to these activities (Steward and Sutcliffe, 1959; Kramer, 1980). As a result of this, there are differences in the distribution of ions between expanded, expanding and meristematic tissues (Barlow,1986; Hodson *et al*, 1985; Flowers and Yeo,1986). Organ by organ analysis of *Agrostis stolenifera* during NaCl stress revealed high accumulation of Na^+ and Cl^- in older leaves with a decrease in amounts in leaves towards the tip (Hodson *et al.*, 1985). Similar observations have been reported for *Suaeda maritima* (Hajibagheri *et al.*, 1985) Such a difference has been interpreted in terms of growth rapidity, suggesting rapidly growing leaves have higher Na^+ fluxes than slowly growing leaves (Flowers and Yeo, 1986).

2.2.6.3 *Internal ion concentration*

As the concentration of salt in the cell increases, its capacity to absorb additional salt

decreases (Wrona and Epstein,1985). Hence, the curve for absorption against time tends to flatten, suggesting that the time required for absorption to saturate is only a few hours in a concentrated solution but several days in a dilute solution. Such a response has been explained as resulting from the difference between ion influx and effux with efflux increasing with internal ion concentration until it balances influx. This seems unlikely as efflux of labelled ions from the vacuole is slow (Kramer, 1980). An alternative explanation is that an increasing proportion of the carrier molecules are combined with ions and block the transport system. In support of this view, Hodges and Vaadia (1964) found that roots high in chloride both accumulated less labelled chloride and transported less to the xylem.

II. Exogenous factors

2.2.6.4 *Nature of accompanying ion and effects of counter ions*

Despite suggestions that cation uptake follows anion uptake (Lundegardh and Burstrom, 1935; Robertson,1968) it seems that the uptake mechanisms for cations and anions differ and are, to a great extent, independent. In barley roots chloride and sodium behave differently, sodium being retained by root cells while a steady-state equilibrium between uptake and leakage has been established for chloride (Ganmore-Neumann,1970). Uptake of Na^+, K^+, Rb^+, or Ca^{++} also is not correlated with uptake of the anions (Ganmore-Neumann, 1970; Hiatt, 1970). The production of negatively charged macro-molecules unable to diffuse from their positions in the cell, results in the establishment of a Donnan equilibrium favouring the uptake of cations rather than anions. Anions enter the cell either by exchange for OH^-or via a metabolic transport system. The uptake of cations is normally independent of the uptake of the counter anion in plants with a capacity for rapid organic acid production, but in plants lacking that capacity the uptake of cations is dependent on the counter anion. The uptake of cations, especially K^+ (Sutcliffe, 1952), and Na^+ (Rains and Epstein,1965), is influenced by the nature of the accompanying anion. If this is Cl^-, uptake is higher than when this is sulphate, sulphate seeming to inhibit the uptake of both Na^+ and K^+.

2.2.6.5 *Calcium*

The role of Ca^{++} in ion transport has not been clearly defined but it is involved in a variety of cellular functions, particularly membrane function (Leopold and Willing,1984), cell wall extension (Taiz,1984) and recovery from stress(Hanson,1984). Salt stress inhibits the supply of Ca^{++} to the shoot in several species (Gauch and Waldleigh,1945; Bajwa and Bhumbla,1971; LeSais,1976) and a high Na^{+}/Ca^{++} ratio in the plant tissues may be one cause of salt injury (Kent and Lauchli,1985). An increase in membrane permeability is the probable primary result of low Ca^{++} and, in the absence of NaCl, does not affect growth (La haye and Epstein,1971). Alternatively, it has been argued that low Ca^{++} *per se* increases membrane permeability, leading to an increase in passive Na^{+} and Cl^{-} transport becoming conspicuous only at high external ion concentrations (Greenway and Munns, 1980). The increase in tissue Na^{+} concentration can be inhibited by increasing external Ca^{++} concentration (La haye and Epstein,1971) and Ca^{++} increases the tolerance of plants to high salt (La haye and Epstein,1969).

2.2.6.6 *Humidity*

The level of humidity in the environment is a major determinant of transpiration rate which is one of the most obvious factors with potential to influence ion transport. This could occur through changes in the bulk flow of water and ions to the root surface and distribution of ions within the plant. High humidity lowers ion uptake (Neiman and Poulsen, 1967; O'Leary and Knecht, 1971) and it is generally accepted that an increase in transpiration can cause an increase in the rate of salt uptake (Russell and Barber, 1960). However, the effect of transpiration on ion uptake is by no means clear. Cheesman and Wickens (1986) for instance, claimed that transpiration did not influence the uptake of Na^{+} and K^{+} significantly. Salt movement into the root system of rapidly transpiring plants involves mass flow as well as active transport (Kramer, 1959; Kylin and Hylmo,1957). An interaction between ion transport and water flow can either be direct (the mass flow of water sweeping the ions through an apoplastic or symplastic

route to the xylem) or indirect (the water flow increasing the net trans-root flow of ions by reducing xylem concentration and favouring unloading from the symplasm). Pitman (1982) discussed these relationships, indicating that ion movement from the xylem back to the root symplast is not negligible but is dependent on the concentration of ions in the xylem. Increasing water flow increases total solute flow by decreasing xylem ion concentration. Clearly, systems more complex than simple diffusion from the symplast down a concentration gradient are probable though not yet well understood (Clarkson *et al.*, 1984). Ion distribution in the plant may change with humidity; for instance, bean and cotton grown in high humidity accumulated ions in the stem and petiole rather than in the leaf blade (Neiman and Poulsen, 1967).

Transpiration, and, presumably, ion uptake, will also be modified by air velocity and turbulence through their effects on the boundary layer resistance. If transpiration influences ion uptake then an effect of wind and turbulence will surely follow. There have been no studies on this factor but it cannot be ignored.

2.2.7 Ion distribution within the plant

On average, more than 90% of the Na^+ accumulated in halophytes is in the shoot, of which at least 80% is in the leaves (Flowers,1975). This contrasts with the distribution in glycophytes which tend to exclude excessive ions from the leaves eg. tomato (Rush and Epstein, 1981). The wild species *Lycopersicon cheesmanii* and *Solanum pinnellii* exhibited a typical halophytic response whereas cultivated tomato responds as a glycophyte (Dehan and Tal,1978). This suggests that the greater tolerance of wild tomatoes to high internal Na^+ and Cl^- may be due to an ability to compartment the ions. The association between a high ion concentration in the leaves of a glycophyte and salt sensitivity is by no means general, however. In rice, one tolerant variety had a low leaf Na^+ content but another, equally tolerant, had high Na^+(Greenway and Munns,1980). Similar observations have been made by Aswathappa and Bachelard(1986) with *Casuarina spp.*; maize varieties, differing in tolerance, had similar leaf ion concentrations (Lessani and Marschner,1978). Such varietal differences in salt

tolerance, despite similar leaf ion concentrations, may be due to tolerance to low external water potential, to differences in ion compartmentation, to differences in ion concentration in the roots or to differences in ion distribution between leaves of different age.

Once ions arrive at the leaf in the transpiration stream they either accumulate in compartments within the cells, remain in the apoplast or are re-exported. Ions accumulated in the leaf are distributed between various compartments, the storage capacity of which is the product of their volume and ion concentration. The major contributor to total leaf volume (excluding air space) is the protoplast, particularly the vacuole. The cytoplasm constitutes relatively a small fraction of the total protoplast volume (5-10%) and the ion concentration within it must be maintained within rather narrow limits to maintain metabolic function. The cytoplasm is, therefore, insignificant as an ion storage site. Compartmental analysis of accumulated ions shows noticeable differences between halophytes (Flowers *et al.*, 1977; Yeo,1981; Harvey *et al.*, 1981; Gorham and Wyn Jones,1983) and a glycophyte (Harvey and Thrope,1986). *Suaeda maritima,* a halophyte contained more Na^+ in the vacuole than in the cytoplasm (Hajibagheri, 1984) whereas a glycophyte, wheat, had more Na^+ in the cytoplasm(Harvey and Trope, 1986). There is a separate constraint on ion accumulation in the apoplast, which constitutes about 3% of total leaf mesophyll volume (1.9 % in *Hellopsis helianthoides* : 2.9% in *Coriopsis triptens* : Parkhurst,1982; 3.7% in *Gossypium hirsutum:* Berline *et al.*, 1982; 2.8% in *Phaseolus vulgaris* : Briarty,1980; 3.3% in *Suaeda maritima,*: Hajibagheri *et al.*, 1984; 3.0% in *Triticum aestivum* :Flowers and Yeo, 1986), as ion accumulation in the apoplast will lower the water potential and have adverse effects on protoplast water relations (Oertli, 1966; Greenway and Munns,1980).

2.3 ACCUMULATION OF SOLUTES IN SALT STRESS

When plants are subjected to salt stress, a variety of low molecular weight organic compounds such as proline (Stewart and Lee, 1974; Chu *et al.*, 1976), many quaternary

ammonium compounds such as glycinebetaine (Wyn Jones and Storey, 1981), prolinebetaine (stachydrine), trigonelline (Parameshwara,1984) and ß-alaninebetaine (Lahrer and Hameline, 1975), and sucrose (Nightingale and Farenham, 1936) accumulate. In this review, most attention is paid to the accumulation of proline and quaternary ammonium compounds especially glycinebetaine and trigonelline, which are pertinent to the crops studied.

2.3.1 The response

2.3.1.1 Proline

Proline accumulation was first reported in wilted tissues of excised Perennial Ryegrass by Kemble and Mac Pherson (1954). The amount accumulated was greater than that which could be accounted for by proteolysis alone and, indeed, concentrations as high as 10% of the dry weight have been reported in stressed plants (Stewart and Lee,1974). Proline accumulation has been observed in mesophytes and halophytes (Stewart and Lee 1974; Storey and Wyn Jones,1978), resurrection plants (Tymms and Gaff,1979), lower organisms (Schobert,1977; Brown and Hellebust,1978; Greenway and Setter,1979) and the diatom *Phaedactylum tricornutum* (Schobert,1977). An increase in free proline in the leaves in response to a saline root medium was first observed by Palfi and Juhasz (1970). A similar response has been reported for barley (Singh *et al.,* 1973; Storey and Wyn Jones,1978a,b; Chu *et al.,* 1976; Buhl and Stewart,1983), tomato (Rajasekaran,1979; Tal *et al.,* 1979), wheat (Dreier,1983), *Abelmoschus esculentus* (Sampathkumar,1980), *Agropyron elongatum,* (Weimberg,1986) and *Thinopyrum bessarabicum* (Gorham *et al.,* 1985). A substantial increase in proline concentration has also been found in several halophytes (Storey and Wyn Jones, 1978 a,b) and in *Chlorella emersonii* (Setter and Greenway,1979). A more complete list of responsive plants is tabulated in Aspinall and Paleg,(1981), and this demonstrates that proline accumulation in response to salt stress is a common phenomenon. Proline accumulation has also been found to occur in excised leaf segments of barley (Chu *et al.,* 1976; Bhul and Stewart, 1983; Voetberg and Stewart,1984; Pesci and Beffagna,1986),

cultured barley embryos (Lone *et al.*,1987) and even in cell suspension cultures (Treichel,1979,1986) challenged with salt.

2.3.1.2 Quaternary Ammonium Compounds (QAC)

The quaternary ammonium compounds are a group of 'onium' compounds, formed by an addition reaction(Wheeland,1953). Among this array of QACs, glycinebetaine was the first discovered by Husemann and Marme in 1863, as a constituent of *Lycium barbarum,* an arid zone shrub, and was given the trivial name lycine (see Karrer,1958). From that time, many QACs have been characterized viz., ß-alaninebetaine, 2-trimethylamino-6-ketoheptanoate, prolinebetaine (stachydrine), (-)4 hydroxyprolinebetaine (betonicine), (+) 4-hydroxyprolinebetaine (turicine), 3-hydroxy prolinebetaine (hypaphorne), 2-mercaptohistidinebetaine (ergothonine), pipecolatebetaine (homostachydrin), trigonelline, and choline as choline-o-phosphate, choline-o-sulphate and chlorocholinechloride (Table 1). In 1936, using a method developed by Blood and Cransfield, Schulze and Stannk established that these compounds occur in high concentrations in *Atriplex spp.* and beet (cf Guggenheim,1958). In relevance to this study, the review will be focussed on glycinebetaine and trigonelline.

2.3.1.2.1 Glycinebetaine

Betaines and related compounds have been found in invertebrates (Beer,1967; Schoffeniels and Gills,1972), fungi (Ardeeva,1971) marine algae (Takemota and Sai,1964) and halophytic bacteria (Rafaeli-Eshkol and Avi-dor,1968). Glycinebetaine, N,N,N,trimethylglycine with a simple, free dipolar structure, is widely distributed and occurs in large amounts. Wyn Jones and Storey (1981) surveyed the taxonomic distribution of accumulation of this compound. Their data suggest that although the distribution is related to halophytic or saline and arid zone conditions, accumulation is not universal in either halophytes or glycophytes. Accumulation as a response to exposure to salt has been demonstrated in *Chloris gayana* (Storey and Wyn Jones,1975), *Hordeum vulgare* (Wyn Jones and Storey, 1978a), *Diplachrie fusca* (Sandhu *et al.*,1981),

TABLE R 1
Names and structures of major betaines.

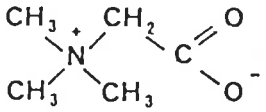
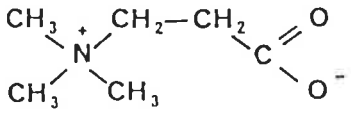
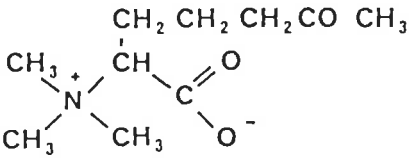
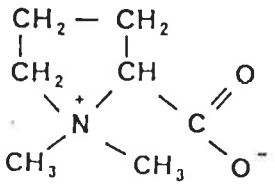
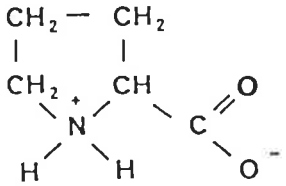
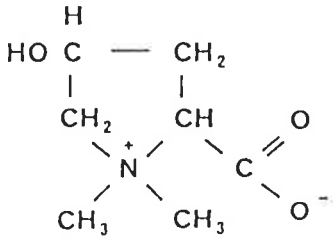
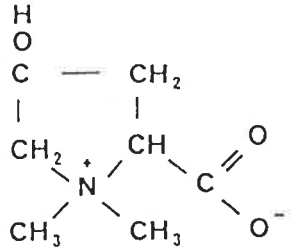
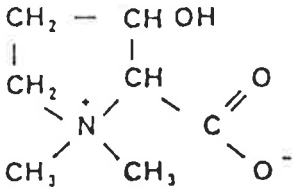
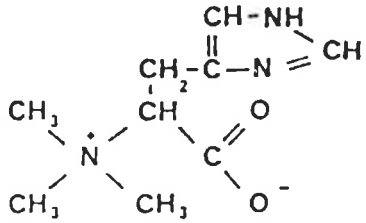
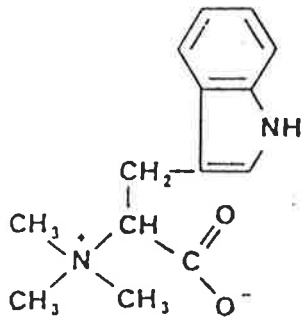
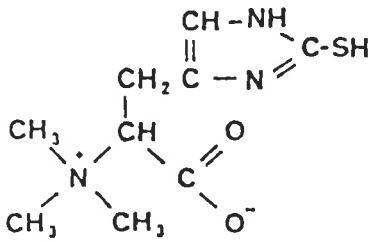
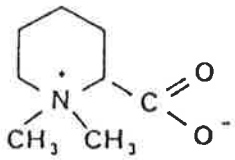
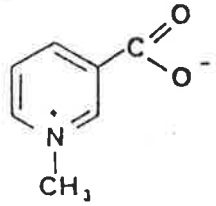
Structures	Preferred trivial names	Other trivial names
	Glycinebetaine	Oxyneurin, Betaine
	β-alaninebetaine	Homobetaine
	2-trimethylamino-6-ketoheptanoate	
	Prolinebetaine	Stachydrine
	Proline	
	(−)4-hydroxyprolinebetaine	Betonicine
	(+)4-hydroxyprolinebetaine	Turicine

Table RI continued

Names and Structures of major betaines.

Structures	Preferred trivial names	Other trivial names
	3-hydroxyprolinebetaine	3-oxystachydrine
	Histidinebetaine	Herzynine Ercinine
	Tryptophanbetaine	Hypaphorine
	2-mercaptohistidine-betaine	Ergothioneine
	Pipecolatebetaine	Homostachydrine
	Trigonelline	

Spartina x townsendii (Storey and Wyn Jones,1978b), *Atriplex spongiosa* , *Suaeda monoica* (Storey and Wyn Jones, 1977), *Suaeda maritima* (Flowers and Hall, 1978), sorghum (Grieve and Mass, 1984), *Salicornia europaea* (Guy *et al.*, 1984), *Lymus sabulosus* (Gorham *et al.*, 1984), spinach (Hanson *et al.*,1985; Robinson and Jones,1986), wheat (Grattan and Grieve, 1985) and sugar beet (Hanson *et al.*, 1985). The concentration accumulated varies from 0.5 to 2 µmole g^{-1} f.wt. in *Zea mays* to about 100-533 µmole g^{-1} f.wt. in halophytes such as *Aster trifolium, Minuria leptophylla, Atriplex halitmus* and many Chenopods (Wyn Jones and Storey,1981).

2.3.1.2.2 Trigonelline

Trigonelline, a nicotinic acid derived quaternary ammonium compound, usually the most abundant molecule in the pyridine nucleotide metabolic pathway for the production of NAD (Preiss and Handier,1957), has been found in a wide range of species belonging to the Leguminoseae, Solanaceae, Cruciferae, Compositae and Graminae (Klein *et al.*, 1931). It has also been reported to occur in the seeds of 27 different species (Blaim,1962). It exists in cotyledons of the dry seeds of various legumes (Evans *et al.* 1979; Evans and Tramontano, 1984; Tramontano *et al.* 1982, 1983) and has been postulated to have a hormonal function (Evans and Tramontano, 1984). Such information led Tramontano *et al.*,(1986) to survey the entire Dicotyledoneae for its presence. It was hoped that applying this phytochemical data to phylogeny would help elucidate the overall function of this compound within the plant kingdom. The review of Wyn Jones and Storey (1981) pointed out that although many species of arid, saline and arid-saline habitats contained this compound, it did not accumulate to high levels in vegetative tissues. The only exceptions have been reports of Blaim (1962), Parameshwara(1984) and Gorham *et al.*(1984), who found accumulation in response to salt stress.

2.3.2 Tissue distribution

2.3.2.1 Proline

Although, most of the studies of proline accumulation in response to salt stress have been

confined to leaf tissues, proline concentration also increased in excised roots of *Tamarix* and *Pisum* (Bar-nun and Poljakoff-Mayber,1977), the leaf sheath and roots of intact barley plants (Chu,1974), the growing regions of barley (Riazi *et al.* 1985), the shoots and roots of *Agropyron elongatum* (Weimberg,1986) and isolated chloroplasts of *Mesembryanthemum crystallinum*,L. (Demming and Winter,1986). The accumulation of proline in excised organs seemed to differ as to whether it is water or salt stressed. Although proline accumulated to higher concentration in different organs of intact plants when water stressed, it did not in excised organs (Palfi,1971). Provision of precursors of proline to excised water stressed barley roots did not induce proline accumulation, although similar provision to etiolated or chlorotic leaves initiated accumulation (Singh *et al.*, 1973). This led to their speculations that 1. proline is synthesised in the leaf and transported to the root and 2. chlorophyll is essential for synthesis. However, excised root tips of *Pisum* and *Tamarix* accumulated proline when exposed to salinity (Bar-nun and Poljakoff-Mayber,1977). This may show that the responses to water and salt stress are different or that species differ in the ability to accumulate proline in different organs.

2.3.2.2 Quaternary Ammonium Compounds

2.3.2.2.1. Glycinebetaine

Cromwell and Rannie (1953) examined the tissue distribution of glycinebetaine in *Beta vulgaris* and found it to occur generally in highest concentration in the leaves in older plants although in young seedlings, the concentration in the root exceeded that in the shoot.The young actively growing leaves contained a higher concentration than the mature, fully expanded leaves (Simenaurer,1975), and Storey and Wyn Jones(1977) also found a higher concentration of this compound in the shoot than in the root. In wheat, also less mature leaves have higher concentrations which decreased with age (Ahmad, 1978) and it was suggested that glycinebetaine was transferred from the flag leaves to the developing grains which also accumulated glycinebetaine as they matured. Similarly, Grieve and Mass(1984) recorded the highest concentration of glycinebetaine in the youngest mature leaf blades rather than in the shoot as a whole or the leaf sheath of sorghum.

Glycinebetaine has been found in seeds of cotton (Pollock and Stevens, 1965) and beet (Storey,1976) and in mature wheat aleurone and embryo but not in the starchy endosperm (Chittenden *et al.*,1978). Analysis of wheat ear tissues (Pearce *et al.*, 1976) revealed a 4-5 times higher (96 μmoles g^{-1}dw.) glycinebetaine concentration in the anthers than in the leaves.

2.3.2.2.2 Trigonelline

There are many reports of the occurrence of trigonelline in seeds. It has also been found in leaves, stem, roots and nodules of alfalfa (Parameshwara,1984) and in the root meristematic cells of peas (Evans and Tramontano, 1981).

2.3.3 Localization

Many hypotheses which concern the effect of the presence of accumulated proline and quaternary ammomium compounds hinge upon the assumption that all, or at least most, of the accumulated proline and QACs is present in cytoplasm. The significance of site of localization is important for two reasons: 1. in relation to a postulated role as an osmoticum to balancing the gradient in osmotic potential within the cell (Wyn Jones *et al.*, 1977) and 2. in respect of function in protecting organelles from perturbations due to ion-induced dehydration (Stewart and Lee, 1974). Goring *et al.*,(1977) suggested from indirect evidence that proline was preferentially located in the cytoplasm of roots of salinity-stressed corn. Leigh and Brandon (1976) however found proline in both the vacuole and the cytoplasm although the concentration was higher in cytoplasm. They suggested that there was an active and reversible movement of proline across the tonoplast in response to osmotic stress experienced by the cell. Neither of these studies excluded the possibility of proline accumulation in the vacuole but, if vacuolar accumulation does occur, transport across the tonoplast must be rapid and bi-directional as accumulated proline is rapidly metabolised when stress is relieved (Boggess *et al.*, 1976). Proline did not appear to accumulate exclusively in the chloroplast or other organelles in the cell as they did not occupy a sufficient volume to be considered as a feasible, exclusive location

for the amount of proline accumulated. Δ'pyrroline-5-carboxylase reductase,an enzyme in the synthetic pathway between proline and glutamate, has been found in the cytoplasm and chloroplasts but not in the mitochondria (Noguchi *et al.*, 1966,1968; Stewart and Lee,1974), lending further support to the suggestion that proline accumulates in the cytoplasm and essentially in the chloroplast. However, it is possible that the site of synthesis can be remote from the site of storage. The evidence by Wyn Jones *et al.*, (1977) strongly favoured the hypothesis that glycinebetaine is concentrated in the cytoplasm rather than the vacuole, contributing a concentration of 210-420mM depending on cell size, which would contribute 600 m.Osm. Glycinebetaine accumulates in isolated protoplasts and chloroplasts of spinach (Hanson *et al.*, 1985). It has also been shown to accumulate in the chloroplasts of intact spinach plants during salt stress (Robinson and Jones,1986). The precise localization of trigonelline has not been examined.

2.3.4 Water relations and accumulation of proline and QACs

It has been suggested that the accumulation of many organic compounds contributes to osmotic adjustment in plants (Wyn Jones *et al.*, 1977). But, the mechanism by which accumulation is initiated is still unclear. Accumulation of proline (Chu, 1974), glycinebetaine (Wyn Jones and Storey, 1978) and trigonelline (Parameshwara, 1984) is correlated with decreased leaf water potential, the concentration in the leaves being also a function of the time of exposure to stress and translocation to other organs. This correlation between accumulation and decrease in water potential is most likely due to changes in one or more components of the water potential. It is not an experimentally easy task to separate unequivocally the effects of a change in any single component. The use of solutes of varying penetrance suggests that changes in tissue turgor potential or cell volume control the accumulation of proline in *Chlorella* (Greenway and Leahy, 1972). In higher plants, however, it is difficult to find evidence for such control. Chu *et al.*, (1976) with proline accumulation and Wyn Jones and Storey (1978) with glycinebetaine found that accumulation was correlated with changes in plant osmotic

potential but not with turgor. Such a correlation may well not be causal as both proline and glycinebetaine would contribute to osmotic potential and hence a correlation between them is to be expected. Trigonelline accumulation in lucerne is also correlated with water potential (Parameshwara, 1984) but the response has not been analysed further.

2.3.5 Ion relations and accumulation of proline and QACs

Compartmentation of ions in the vacuoles of halophytic plants has been reported (Hajibhageri,1984). Such a situation creates an imbalance in the osmotic equilibrium between the vacuole and the cytoplasm. Proline and glycinebetaine accumulated during salt stress is exclusively localised in the cytoplasm and they are suggested to maintain osmotic equilibrium within the cells of halophytes (Wyn Jones *et al.*, 1977; Hall *et al.*, 1976). In glycophytes proline and glycinebetaine also accumulate during salt stress and it is possible that inefficient compartmentation of ions and Na^+ in the cytoplasm (Harvey and Thorpe, 1986) influences the synthesis and metabolism of proline and QACs.

2.3.5.1 Proline

If the suggestion that the proline accumulated in the cytoplasm balances the solute potential of the vacuole consequent upon vacuolar ion accumulation (Stewart and Lee, 1974; Wyn Jones *et al.*, 1977) is correct, proline accumulation would be directly proportional to salt accumulation in the tissue. In mature leaves of two halophytes, proline concentration indeed reached a steady proportionality with tissue Na^+ and Cl^- concentration (Treichel, 1975). Similarly, leaf proline concentration was directly proportional to leaf osmotic potential due primarily to accumulated Na^+, Cl^- and K^+ ions (Storey and Wyn Jones, 1978; Neals and Sharkey, 1981) and in a glycophyte (sorghum) proline concentration was linearly related to total monovalent cation concentration (Weimbergh *et al.*, 1982). Voetberg and Stewart (1984) also showed a linear relationship between proline accumulation and tissue Na^+ concentration. However, in shoots of salt stressed spinach, proline concentration increased exponentially with decreasing cell sap osmolality (Coughlan and WynJones, 1980). If the assumption is

valid that the accumulation of proline maintains osmotic equilibrium in the cell, it is to be expected that it should continue to accumulate at a steady state as long as ions accumulate in the cell, ultimately reaching a steady state when further ion accumulation ceases. Both a rapid decrease in proline content (Chu *et al.*, 1976a; Ahmed and Wyn Jones, 1979) and continued proline accumulation (Voetberg and Stewart,1984) with no change in internal ion concentration have been reported, however(Greenway ,1962; Voetberg and Stewart, 1984). This evidence for glycophytes suggests that internal Na^+ and Cl^- concentration may not exert control over proline synthesis, and the reported relationships may be coincidental. Further, the role of proline may be other than maintaining osmotic equilibrium in the cell.

Although proline accumulates in intact barley seedlings in response to salt (Chu *et al.*, 1976a), there is no proline accumulation in leaf sections exposed to NaCl (Chu *et al.*, 1976b). This led to an investigation of the influence of a variety of ionic species on proline accumulation which revealed a specific inhibition of proline accumulation by monovalent cations (Na^+ and K^+). Such inhibition was not observed when divalent cations were employed. Anions were also found to influence proline accumulation, higher accumulation occurring in Cl^- salinity than in SO_4^{2+}or NO_3^-salinity. These effects were independent of changes in water relations. This response was assumed to be due to an inhibition of proline synthesis by Na^+ (Chu *et al.*, 1976b) but Buhl and Stewart (1983) and Voetberg and Stewart (1984) found no such inhibition at 410 mM NaCl. Studies of Treichel (1986) also support this view as even at 400 mM or higher NaCl concentrations *in vitro* inhibition of specific activity of Δ' P-5-C dehydrogenase or complete inhibition of proline accumulation was not observed. High concentrations of Na^+ and NH_4^+ have been reported (Noguchi et al., 1966) to reduce the incorporation of ^{14}C glutamic acid into proline in tobacco leaves however, and Δ' pyrroline-5-carboxylate (P-5-C) dehydrogenase, a key enzyme in proline synthesis, is strongly inhibited by 0.2 M NaCl or KCl, but not by K_2SO_4 or Na_2SO_4 (Boggess and Stewart, 1974; Boggess *et al.*,1975).

2.3.5.2 Quaternary Ammonium Compounds

2.3.5.2.1 Glycinebetaine

Although many halophytic and non-halophytic species accumulate glycinebetaine, this response is not universal (Wyn Jones and Storey, 1981). The accumulation of glycinebetaine is related to the decrease in cell osmotic pressure due primarily to accumulation of Na^+ and Cl^- (Wyn Jones and Storey,1977; Storey and Wyn Jones, 1978a,1978b). A similar role as that suggested for proline in maintaining osmotic equilibrium in the cells has been postulated for glycinebetaine (Wyn Jones *et al.,* 1977). Variations in the water potential threshold for initiating accumulation have been observed between different plant species being 0.65 MPa for *S X townsendii* (Wyn Jones *et al.*, 1977); 1.08 MPa for *Atriplex spongiosa*, 3.24MPa for *Suaeda monoica* (Storey and Wyn Jones, 1979) and 1.08 MPa for barley cv. California Mariout (Storey and Wyn Jones, 1978). Although there is no detailed evidence on the influence of ion species on accumulation, glycinebetaine accumulates in *Atriplex spongiosa* in response to KCl, K_2SO_4 and $MgCl_2$ as well as NaCl stress (Wyn Jones and Storey, 1981).

2.3.5.2.2 Trigonelline

Trigonelline(Parameshwara,1984) accumulates in lucerne in significant concentrations within 24 hours of exposure of plants to 500 mM NaCl. A similar accumulation in response to salt stress has also been reported in *Leymus sabulosus* (Gorham *et al.*, 1984). Stress relief did not influence the trigonelline concentration at any NaCl concentration range (Parameshwara,1984). There is no evidence on the relationship between ion concentration and accumulation of trigonelline.

2.3.6 Hormonal relations and accumulation of proline and QACs

Salt stress in addition to influencing plant water and ion relations affects endogenous hormones such as auxin (Itai *et al.*, 1968), ABA (Hoad, 1975; Walton *et al.*, 1977; Itai 1978; Walker and Dumbroff, 1981), cytokinin (Itai and Vaadia, 1965; Itai, 1978;

Walker and Dumbroff, 1981) and ethylene (Garcia and Einset, 1985). The resulting altered hormonal balance will have an impact on growth through effects on physiological and biochemical processes.

Salt or water stress produces a rapid increase in ABA (Hoad, 1975; Walton *et al.*,1977; Itai, 1978) and a decrease in cytokinin activity (Itai and Vaadia, 1965; Itai *et al.*, 1968; Itai 1978; Walker and Dumbroff, 1981) with the hormonal balance returning to normal levels on stress relief (Itai *et al.*, 1968; Boussiba and Richmond, 1976; Sivakumaran and Hall, 1979; Walker and Dumbroff, 1981). There is a linear negative relationship between increasing salinity and decreased tissue cytokinin concentration (Itai *et al.*, 1968). Kinetin exerts considerable influence on several functions such as cell expansion and differentiation (Skoog and Tsui, 1948), apical dominance (Thimann and Wickson, 1957), polarity of growth (Sommer, 1961) and net protein synthesis (Itai *et al.*, 1968) may be affected. A reduction in endogenous cytokinin concentration during salt stress has also been postulated to alter salt and water balance (Livne and Vaadia, 1965; Collins and Kerigan, 1974), and decrease hydraulic conductance (Tal and Imbar, 1971).

An increase in ABA concentration during salt stress may control stomatal aperture (Little and Kidt, 1968), inhibit protein synthesis (Chrispeels and Varner, 1967), increase water and ion uptake and decrease root resistance (Glinka, 1975, 1977). Reduced electron transport (Bauer *et al.*, 1976), CO_2 fixation (Sunkhla and Heber, 1974), chlorophyll biosynthesis (Bengston *et al.*, 1977), RuBP carboxylase and PEP carboxylase activity (Popova *et al* 1982), and photosynthesis (Popova *et al* ., 1987) also result from increased ABA concentration .

The kinetics of accumulation of proline and ABA during water stress, and their disappearance on relief of stress, permit the hypothesis that proline accumulation is controlled by ABA. Studies of Stewart and Voetberg (1985) and Stewart *et al.*, (1986) support this hypothesis as ABA accumulation precedes proline accumulation and no proline accumulation occurred in the absence of ABA (Stewart and Voetberg, 1985). Preventing transcription and translation in ABA synthesis also inhibited proline

accumulation (Guerrero and Mullet, 1985; Stewart *et al.*, 1986) suggesting that gene activation is required for these two compounds to accumulate (Stewart *et al.*,1986). The accumulation of proline in response to exogenous application of ABA is not universal, however, as both sunflower (Wample and Bewley, 1975) and tobacco (Aspinall *et al* .,1973) do not accumulate proline in response to applied ABA although they do so in response to stress. These suggests that the two processes are not necessarily linked, which is supported by the observation that *flacca*, a wilty, ABA deficient, tomato mutant, showed significant proline accumulation without an increase in ABA (Stewart and Voetberg, 1987).

There is no detailed evidence on the influence of endogenous cytokinins on proline accumulation but application of benzyl adenine, a member of the purine group prevents proline accumulation induced by wilting, ABA or salt stress (Stewart *et al.*, 1986).

The ability of ABA to induce glycinebetaine accumulation appears to be contentious. Huber and Sankhla (1980) reported that ABA, at 10 and 100µM concentration, induced glycinebetaine accumulation in 4 and 14 day *Pennisetum* seedlings within 4h. Mc Donnell *et al* .,(1982), using the same species, found that ABA treatment did not initially induce glycinebetaine accumulation even when the incubation period was extended to 7h. Nevertheless, glycinebetaine accumulated in significant amounts in these seedlings when grown in ABA solution for 4 days. The only evidence that ABA may influence the accumulation of other QACs is the demonstration by Parameshwara (1984) that ABA, at a concentration of 5µg/ml, caused accumulation of stachydrine and trigonelline in alfalfa.

2.4 BIOSYNTHESIS AND METABOLISM OF PROLINE AND QACs

2.4.1 Proline

Proline synthesis in lower organisms and plants occurs by two pathways, originating from either glutamate or ornithine. They differ in the reactions leading to conversion of the precursor amino acid to the related intermediate products Δ' pyrroline-5-carboxylate and Δ' pyrroline-2-carboxylic acid (Vogal and Bonner, 1954; Mizusaki *et*

al., 1964; Morris *et al.*, 1969; Boggess *et al.*, 1976; Liu and Hellebust, 1976). Little is known about the relative contributions of the ornithine and glutamate pathways to the intracellular pool of proline, but in bacteria the relative activity of these systems depends upon the species, and all cells are not capable of performing all conversions that lead to proline formation (Shevyakova,1982).

2.4.1.1 Glutamate pathway

Evidence for the presence of this pathway (Plate 1) in higher plants was obtained by Mizusaki *et al.*, (1964), Noguchi *et al.*, (1968), Goas *et al.*, (1970) and Boggess *et al.*,(1976). Most of the proline accumulated is believed to be derived from glutamate via. four steps involving 3 enzymic reductions and one non-enzymic cyclization. The first step, the conversion of the ∂-carbamyl group of glutamate to produce an intermediate acetyl phosphate is catalysed by ∂-glutamyl kinase (EC 2.7.2.11) (Krishna and Liesinger, 1979). ∂-glutamyl P reductase, the second enzyme in the pathway, catalyses the NADPH dependant reduction of ∂-glutamyl phosphate to yield glutamyl-∂-semialdehyde (Krishna *et al.*, 1979). ∂-glutamyl-semi aldehyde exists in rapid non-enzymatic equilibrium with Δ' pyrroline-5-carboxylate, formed by cyclization and liberation of one water molecule. P-5-C reductase (EC 1.5.1.2) catalyses the final step in the biosynthesis of proline, reduction of the double bond in P-5-C to yield proline. The co-factor for this reaction may be NAD(P)H. The preference for NADH or NAD(P)H varies in different tissues: for example, in rat liver (Piesach and Strecker, 1962), blow fly (Balboni, 1978) and pseudomonas (Krishna *et al.*, 1979), NADH is the preferred reductant while NAD(P)H is preferred in tobacco(Noguchi *et al.*, 1966) and *E. Coli* (Baich, 1969). The enzymology of the successive transformations of glutamate into proline has been studied most fully in bacteria, but reactions are not fully characterized due to instability of activity in crude extracts (Shevyakova, 1982).

The enzyme pyrroline-5-carboxylate reductase, has been reported to be localised in the chloroplast in tobacco leaves (Noguchi *et al.*, 1968) and the reduction to be closely associated with non-cyclic phosphorylation. The reports of Noguchi et al., (1966),

PLATE. 1 Pathways of biosynthesis and metabolism of proline*

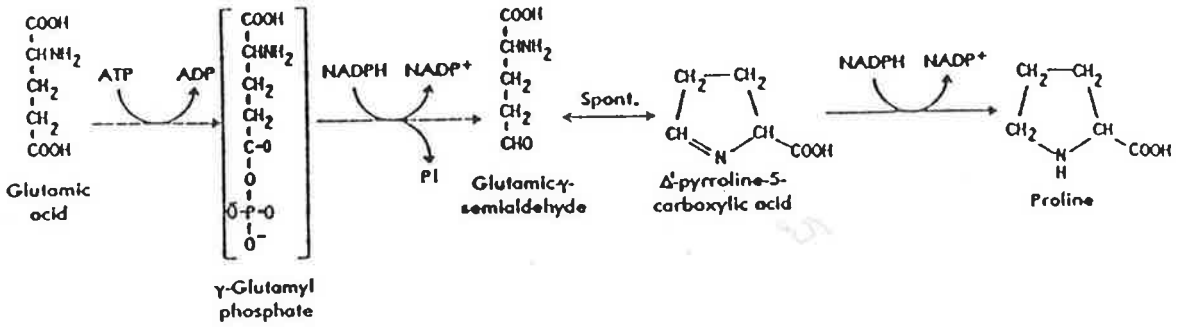
A. Biosynthetic pathway

B. Pathways of conversion of arginine and ornithine to proline

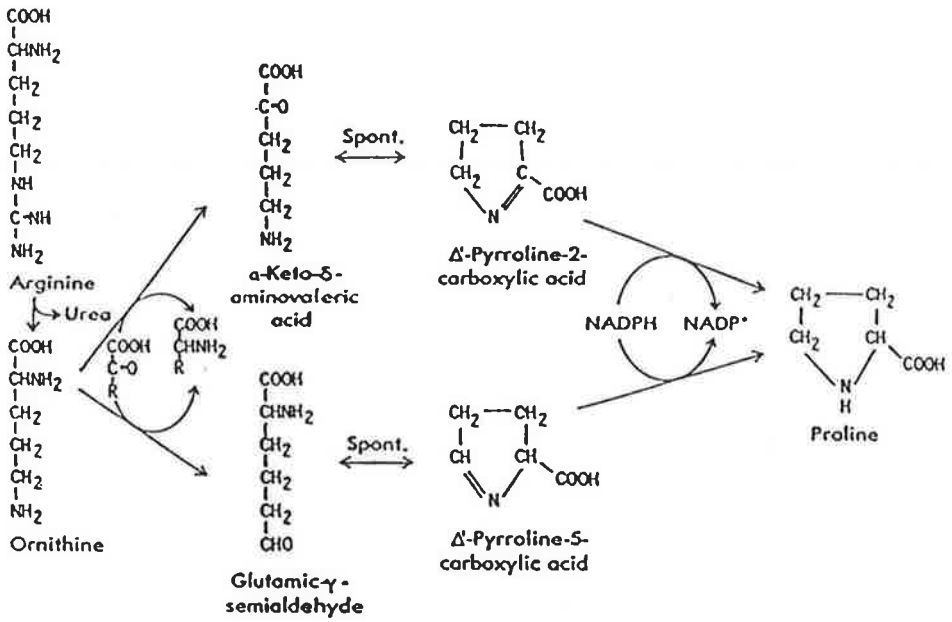
C. Pathway of proline oxidation

(From Stewart, 1981)

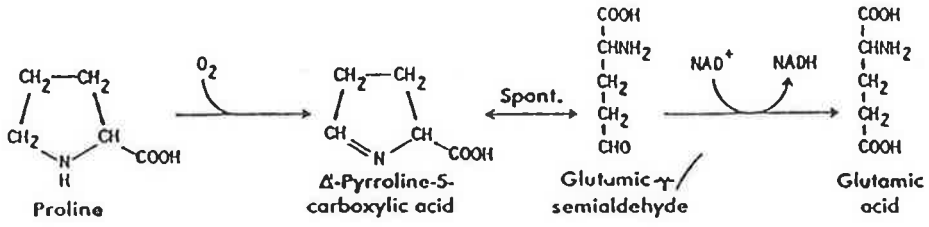
* see text for the enzymes involved



A —— The proline biosynthetic pathway. Solid arrows represent documented reactions. Dotted arrows refer to postulated reactions.



B —— Pathways of conversion of arginine and ornithine to proline.



C —— The pathway of proline oxidation.

showed that ^{14}C incorporation from glutamic acid into proline was inhibited by salt and suggested that this might be due to the effect of Na^+ on Δ'- P-5-C reductase. However, Huber (1974) found promotion of *in vitro* activity of the enzyme by both NaCl and ABA and Treichel(1986) also showed that even a concentration of 400mM NaCl did not completly inhibit the activity of this enzyme in cell suspension cultures.

2.4.1.2 Ornithine pathway

In higher plants, the ornithine pathway (Plate 1) of proline biosynthesis is characteristic of germinating seeds and cotyledons where hydrolytic processes enrich the pool of amino acids with ornithine liberated from storage proteins. Under these conditions, ornithine appears to be a product of the hydrolysis of the amidine group of arginine by arginase (EC 3.5.3.1). The synthesis of proline from ornithine is catalysed by ornithine transaminase to yield glutamyl-∂-aldehyde which cyclizes to P-5-C which is then converted to proline by P-5-C reductase. By examining the transfer of ^{14}C from arginine to proline in tubers of Jerusalem artichoke, Wrench *et al.*, (1977) concluded that the conversion occurred via ornithine and glutamyl- ∂ -semialdehyde without passing through a glutamate pool. That ornithine can serve as a precursor for proline synthesis, has also been shown in peanut cotyledons (Mazelis and Fowden, 1969) and bean leaves (Stewart and Boggess, 1978). Using ^{15}N ornithine, Duranton and Wartz (1965) obtained a similar conversion of ornithine to proline in Jerusalem artichoke tubers.

2.4.1.3 Metabolism of proline

Conversion of proline to glutamate proceeds through pyrroline-5-carboxylate in the majority of biological systems with further oxidation to glutamic acid or transamination to ornithine (Plate 1) (Streeker, 1960). More rarely, pyrroline-2-carboxylate or glutamate semi-aldehyde act as intermediates. A specific requirement for the ring closed condensation product Δ' P-5-C makes direct conversion from glutamyl semi-aldehyde to glutamate impossible (Bender, 1975). The proposed pathway of proline oxidation via Δ'P-5-C involving proline oxidase (EC 1.5.1.2) is closely associated with the electron

transport chain that interacts directly with cytochromes. Unlike Δ' P-5-C reductase, which is the NAD(P) linked enzyme associated with the formation of proline from glutamate, proline oxidase is an oxygenase. This enzyme has been reported in plants (Boggess *et al.*, 1978; Huang and Cavalieri, 1979). Earlier, the enzyme proline dehydrogenase (EC 1.5.1.2) had been reported to be responsible for the oxidation of proline requiring NAD^+ as the electron acceptor, in the cotyledons of pumpkin (Rena and Splittstoesser, 1974), leaves of *Triticum vulgare* (Mazelis and Creveling, 1974) and barley (Boggess *et al.*, 1975). Doubt as to the role of proline dehydrogenase in the *in vivo* oxidation of proline arose from the necessity to assay it at high pH (>10) and from the observation that this enzyme co-purifies with P-5-C reductase. In addition this enzyme has not been proven to catalyse P-5-C formation *in vivo* . Further, mitochondria isolated from 3 day old wheat shoots with no proline dehydrogenase activity are able to oxidise proline, suggesting the presence of a proline oxidising system similar to that observed in mitochondria isolated from animals and other organisms (Boggess *et al* 1978). Proline oxidation by mitochondria isolated from etiolated shoots of corn, wheat, barley, soybean and mungbean has also been demonstrated by Boggess *et al.*, (1978). This oxidation is dependant on oxygen, but not on NAD, and mitochondria lacked the enzyme proline dehydrogenase. Huang and Cavalieri (1979) also found indirect evidence for an oxygen-dependant proline oxidase in mitochondrial fractions of spinach leaves and castor bean endosperm which was suggested to be linked to the electron transport system.

The second step in the oxidation of proline to glutamate is the conversion of P-5-C to glutamate, catalysed by the enzyme Δ'P-5-C dehydrogenase (EC 1.5.1.12). The possibility of formation of P-2-C is remote as (a) the enzyme P-5-C dehydrogenase cannot utilize P-2-C and (b) feeding ^3H proline resulted in non-labelled glutamic acid (Stewart and Boggess, 1978). The enzyme Δ' P-5-C dehydrogenase has been reported to exist in mitochondria from root and shoot tissues of pea and corn, castor bean endosperm, pumpkin cotyledons (Stewart and Lai, 1974) and barley roots (Boggess *et al.*, 1975). The reaction has been found to be clearly distinct and unrelated to the reduction of glutamate to glutamate semi-aldehyde (Frank and Ranhand, 1964). The reaction has a pH

optimum of 7.6 and NAD^+ is the preferred electron acceptor (Stewart and Lai, 1974; Boggess *et al.*, 1975).

2.4.1.4 Regulation of proline synthesis

Feed-back control of proline biosynthesis plays a considerable role in regulation of the proline levels in the absence of stress (Noguchi *et al.*, 1966; Oaks *et al.*, 1971)but this is not universal (Baich and Pierson, 1965). Krishna and Leisinger(1979) found 40% inhibition of the conversion of glutamate to glutamate semi-aldehyde at 5 mM proline and complete inhibition at 30 mM proline. Similar results have been obtained with the diatom *Cyclotella cryptica* (Liu and Hellebust, 1976), maize roots (Oaks *et al.*, 1970) and barley leaves (Boggess *et al.*, 1976). Further, the reports of Noguchi *et al.*,(1966) and Krishna *et al.*(1979) showed that the reduction of glutamyl-∂-semialdehyde to proline is unrestrained confirming that the glutamate to glutamyl -∂- carboxylate step is the rate limiting step.

2.4.1.5 Regulation of proline metabolism

Stimulation of oxidation of proline to glutamic acid by an increase in proline concentration has been reported in bean (Stewart, 1972), and tobacco and barley (Boggess *et al.*, 1976). This response appears to be due to regulation of the proline pool rather than to proline stimulating enzyme activity (Stewart *et al.*,1977). There is no evidence for an influence of proline, or any other amino acid, on plant P-5-C dehydrogenase.

2.4.1.6 Mechanism of proline accumulation

Accumulation of proline in stress conditions conceivably could happen through 1. an increase in *de novo* synthesis from glutamate or ornithine; 2. degradation of protein; 3. a reduction in oxidation of proline; or 4. release from bound to free form (Kudrev, 1967); or 5. a combination of one or more of these. A decline in the protein content of tissue under salt stress may be due to a decrease in protein synthesis or increased

proteolysis or both (Strogonov, 1964; Benzioni *et al.*, 1967; Rajasekaran, 1979). Reduction in the incorporation of amino acids during salt stress (Kahane and Poljakoff-Mayber,1968) suggests an inhibited protein synthesis. Such a reduced incorporation of amino acids into protein might be expected to increase the free amino acid pool. An increased free amino acid pool during salt stress has been reported for halophytes (Stewart and Lee, 1974), subtropical sea grasses (Pulrich, 1980), *Thinopyrum bassabaricum* (Gorham *et al.*, 1985) and barley (Chu, 1974) and attributed to an increased level of proline. Impaired protein synthesis or protein breakdown during water stress was concluded to be unlikely to be the sole cause of proline accumulation , since 1. inhibiting synthesis was not sufficient to cause proline accumulation (Boggess and Stewart, 1980); 2. the amount of free proline accumulated greatly exceeded that which could be accounted for by protein breakdown (Kemble and Mac Pherson, 1954; Thompson *et al.*, 1966); and 3. stressed cells of *Cyclotella cryptica* showed no proteolysis and pre-labelled proteins showed no loss of activity, yet significant amounts of proline accumulated .

The total free amino acid pool may also increase following reduced transport from the organ to other parts of the plant. Dove (1968) has shown significant translocation of nitrogen compounds in water stressed tomato even at a fairly low relative water content while Tully *et al* (1979) maintain that translocation is not reduced during the first two days of stress but is significantly reduced after this period. This reduction in translocation coincided with the commencement of proline accumulation and when transport in wilted plants was interrupted by a cold jacket or steam, proline accumulation was accelerated. The total nitrogenous compounds exported out of the leaf fell from 203 µg/day in turgid to 16 µg/day in stressed plants at -3.0MPa external water potential and leaf proline content increased from 0.3 µg to 67 µg. The accumulated proline is probably synthesised *de novo* (Hanson and Tully, 1979). Proline contributed only 9 µg/day (3 % of the total amino acid exported) to the exported N while glutamic acid and glutamine together provided 76 µg/day (30% of the total amino acid exported). At the same time about 64.5 µg N as proline accumulated within 3 days when nitrogen export

from the tissue was reduced to 10 % of that from turgid tissue. Although proline accumulated in response to an inhibition of translocation from the leaf, a simultaneous stress condition was required to enhance proline accumulation. Massive proline accumulation does not occur in turgid tissues even when the transport system is severed (Chu *et al.*, 1976; Buhl and Stewart, 1983).

2.4.1.7 Precursors for proline synthesis

Arginine (Wrench *et al.*, 1977) and glutamic and aspartic acids (Hubac *et al.*, 1969) have been suggested as precursors for proline synthesis. Synthesis of proline from arginine via. ornithine, without passage through a glutamate pool, has been demonstrated in osmotically stressed Jerusalem artichoke (Wrench *et al.*, 1977). In this case it was calculated that arginine was quantitatively the most important precursor for proline synthesis. A decrease in arginine content prior to a stress-induced increase in proline has also been reported in Spruce (Duranton, 1973), and the subterranean parts of *Carex pachystylis* (Hubec *et al.*,1969), but in this latter species the precursor was not arginine but glutamic and aspartic acids in the leaves. Glutamic acid was also the major precursor for proline synthesis in barley leaves (Boggess and Stewart, 1976) where the synthesis of proline from ornithine was not stimulated by wilting unless the leaves were pre-wilted to allow proline accumulation to commence. In this system, in which conversion of arginine and ornithine to proline was delayed until 9 hours after imposition of stress, glutamic acid was converted to proline within 3 to 6 hours at which time proline accumulation was already dectected. When turgid leaves of turnip were infiltrated with various amino acids no proline accumulated (Stewart *et al.*, 1966), suggesting that the presence of increased precursors alone is not sufficient for proline to accumulate and a stress stimulus is essential.

2.4.2 Quaternary Ammonium Compounds

2.4.2.1 Glycinebetaine

Cromwell and Rannie(1953) and Delwiche and Bregoff (1957) proposed two pathways

for the biosynthesis of glycinebetaine in plants; (1) by the sequential methylation of the parent amino acid, glycine; and (2) by the oxidation of choline formed by sequential methylation of ethanolamine (Plate 2). Later investigations by Bowman and Rohiinger(1970) in wheat, Hanson and Nelson(1986) in barley and Hanson *et al.* (1985) and Weigel *et al.* (1986) in spinach, suggested the second pathway was operative and that glycinebetaine is syntheised from choline in stressed plants. A similar synthesis of glycinebetaine from choline has been found in mammalian liver (Hanson and Grumet, 1985; Le Rudulier *et al.*, 1984). Tracer studies by Hanson and Hitz, (1982) in both unstressed and stressed plants showed that glycinebetaine is synthesised by two step oxidation from choline. The increase in glycinebetaine accumulation during stress has been reported to be due to an increase in the activity of choline oxidase, this step being rate limiting (Hanson *et al.*, 1985).

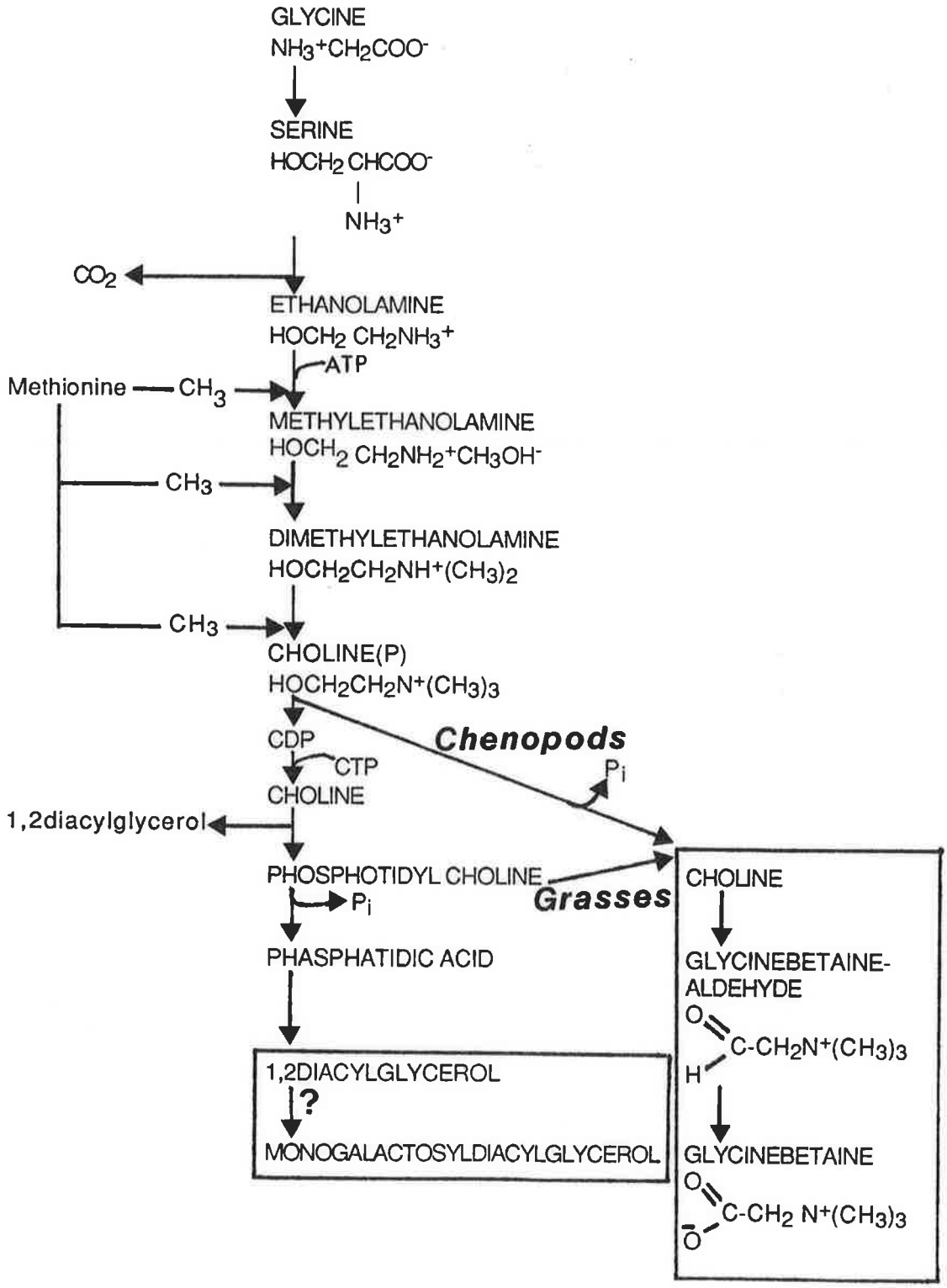
2.4.2.1.1 Metabolism of glycinebetaine

There is conflicting evidence on the degradation of glycinebetaine accumulated as a consequence of salt stress. Ladyman *et al.* (1980) monitored the glycinebetaine concentration in barley shoots for 12 days during two cycles of transient drought stress and found no reduction in total glycinebetaine content. Similarly, there was only a slight decrease in the concentration of glycinebetaine in *Spartina alterifolia* following the relief of salt stress and this could be attributed to dilution by plant growth (Cavalieri, 1983). However, Hanson and Nelson(1978) claimed that there was a 70% decrease in leaf glycinebetaine concentration after removal of water stress, but this experiment was not designed to differentiate between actual glycinebetaine degradation and glycinebetaine translocation from the leaf blade. A degradative pathway postulated to occur by demethylation of glycinebetaine to glycine (Kortstee,1970; Wyn Jones *et al.*,1973) has yet to be demonstrated and it is generally concluded that glycinebetaine is a stable end-product of metabolism, any apparent reduction in level observed on stress relief being due to dilution from plant growth or to translocation (Grattan and Grieve, 1985).

PLATE 2 Pathways of biosynthesis of glycinebetaine

[P-phosphoryl derivatives of the bases] The boxes indicate that the enclosed reaction sequences are compartmented, possibly in the same sub-cellular compartment. In this scheme the choline produced from hydrolysis of phosphoryl choline (chenopods) and from that of phosphotidyl choline (graminae) is kept apart from the bulk free choline of the cell and is directed towards glycinebetaine synthesis.

[compiled from Stewart and Larhar, 1981; Mc Donnell and Wyn Jones, 1988]


GLYCINE
NH3+CH2COO-
SERINE
HOCH2 CHCOO-
NH3+
CO2
ETHANOLAMINE
HOCH2 CH2NH3+
ATP
Methionine
CH3
METHYLETHANOLAMINE
HOCH2 CH2NH2+CH3OH-
CH3
DIMETHYLETHANOLAMINE
HOCH2CH2NH+(CH3)2
CH3
CHOLINE(P)
HOCH2CH2N+(CH3)3
CDP
CTP
CHOLINE
Chenopods
Pi
1,2diacylglycerol
PHOSPHOTIDYL CHOLINE
Pi
Grasses
PHASPHATIDIC ACID
1,2DIACYLGLYCEROL
?
MONOGALACTOSYLDIACYLGLYCEROL
CHOLINE
GLYCINEBETAINE-
ALDEHYDE
O
C-CH2N+(CH3)3
H
GLYCINEBETAINE
O
C-CH2 N+(CH3)3
O-

2.4.2.2 Trigonelline

Trigonelline is a methylated nicotinic acid; feeding plants with nicotinic acid can increase the trigonelline content (Blake,1954) and conversion of nicotinic acid to trigonelline has been demonstrated (Newmann *et al.*,1979). Cell cultures of several legumes (*Phaseolus vulgaris, Glycine max, and Cicer arietinum*) convert nicotinic acid to trigonelline (Leienbach *et al.*, 1975). Joshi and Handler(1960) showed that trigonelline synthesis by pea preparations is due to methyl transfer from S-adenosyl methionine to nicotinic acid. Trigonelline has been proposed by Tramontano *et al.* (1983) to function as a storage form of nicotinic acid since high concentrations of nicotinic acid may be toxic. When plants are supplied with labelled trigonelline, NAD, NADP and NMN become labelled (Tramontano *et al.*, 1982) as trigonelline is converted to other members of the pyridine nucleotide pathway. Despite the demonstration of such potential inter-conversions in plants, the response of stressed tissue is unknown. However, the increase in trigonelline concentration in Fenugreek caused by supplying proline (Klein and Linser, 1932) suggests an inter-relationship between proline and trigonelline metabolism.

2.5 ADAPTIVE SIGNIFICANCE OF PROLINE AND QACs

As proline and glycinebetaine accumulate to high concentrations in plants as a consequence of stress it is natural that an adaptive significance for these compounds has been sought. Since the response has survived through evolution in a range of plants and other organisms it would suggest that accumulation benefits plants during encounter with adverse environmental conditions. Demonstration of a high capacity to accumulate proline in a halophyte, *Aster tripolium* , grown in saline water (Goas *et al.*, 1970), stimulated Stewart and Lee (1974) to suggest that the capacity to accumulate proline is correlated with the salt tolerance of 11 halophytic species. This correlation was not found with domestic and wild salt tolerant species of tomato (Tal *et al* ., 1979) nor with a wide range of crop plants(Chu ,1974). With glycinebetaine, the evidence for a correlation between salinity tolerance and relative accumulation is possibly stronger (Storey and Wyn Jones,

1977) although many salt tolerant plants accumulate no glycinebetaine (Wyn Jones and Storey, 1981).

Basically any compound that accumulates in the cell to a high concentration must fulfil certain requirements to be 'compatible' with the functioning of the cell or disruption of normal cellular activity will occur. Both proline and glycinebetaine fulfil these expectations in that they are highly soluble, carry a neutral charge at neutral pH, are retained within the cell by the plasma membrane against a large concentration gradient and do not interfere with essential plant metabolism (Borowitzka, 1981). In addition to these negative or neutral properties, several positive adaptive roles for proline and glycinebetaine have been postulated.

2.5.1 Osmoregulation

When plants are subjected to salt stress, there is an initial decrease of turgor which is regained in time. This recovery has been attributed to an increase in osmotic concentration within the cells following the synthesis of organic solutes or the intake of ions or both. In *Chlorella* , proline accumulation increased with an increase in the concentration of an external non-permeating medium (Setter and Greenway, 1979). Proline also contributed substantially (66% to 80%) to osmoregulation in *Chlorella* adapted to 335 mM NaCl for 2 to 3 days (Setter and Greenway, 1979). A similar function for proline in some halophytes, where proline accumulation is marked, has also been suggested (Stewart and Lee, 1974). However, the contribution of proline to this function in glycophytes such as barley appears to be limited (Chu *et al.*, 1976). Glycinebetaine is also reported to contribute substantially to osmoregulation (Wyn Jones *et al.,* 1977). In a study with isolated spinach chloroplasts (Robinson and Jones, 1986) the glycinebetaine accumulated during salt stress has been calculated to contribute 36 % of chloroplast osmotic potential.

The large quantities of ions taken into the plant during salt stress particularly by halophytes (Yeo, 1981), are expected to be compartmented into the vacuole rather than

the cytoplasm, where adverse metabolic consequences would flow (Hajibagheri, 1984). If ions are so sequestered, then a potential imbalance in osmotic equilibrium within the cell would occur, creating a demand for the accumulation of compatible solutes in the cytoplasm. Both glycinebetaine and proline accumulate exclusively in the cytoplasm of stressed beet cells (Wyn Jones *et al.*, 1977) and histochemical studies with *Suaeda maritima* showed a cytoplasmic location of glycinebetaine (Hall *et al.*, 1978). In glycophytes, however, the lack of such efficient compartmentation of ions leads to accumulation of ions in the cytoplasm perturbing the function of cytoplasmic organelles (Harvery and Trope, 1986). Little is known of the adaptive significance of these compounds in glycophytes under such situations. High concentrations of glycinebetaine and proline were claimed (Wyn Jones and Storey, 1978) to occur only when growth was severely reduced. Further, higher concentrations of proline (Tal *et al.*, 1979) and glycinebetaine (Wyn Jones and Storey, 1978) accumulated in salt sensitive than in salt tolerant species. Such results led Greenway and Munns (1980) to suggest that the accumulation of these two compounds in glycophytes may serve a role in survival rather than in the maintenance of growth during stress.

2.5.2 Protection

Glycinebetaine and proline have been postulated to be involved in protecting many cellular functions. Heber *et al.*, (1971) found that the phosphorylating ability of thylakoids inactivated at -25°C was regained in the presence of proline and proline has also been found to protect against the effects of heat, pH, salt and dilution (Ahmad *et al.*, 1982; Nash *et al.*, 1982; Paleg *et al.*, 1981). Glutamine synthetase, a key enzyme in nitrogen metabolism, was protected against PEG-induced precipitation by proline (Paleg *et al.*, 1985) and both proline and glycinebetaine alleviated the effects of dehydration-induced thermodynamic perturbation on this enzyme (Paleg *et al.,* 1985).

There are only two clear examples of a protective role for glycinebetaine in salt-induced perturbations. One is the demonstration by Pollard and Wyn Jones, (1979) that glycinebetaine (500 mM) alleviated the inhibitory effects of 200mM NaCl on barley

malic enzyme and the other is the protection of RuBP carboxylase oxygenase against dissociation during KCl stress (Incharoensakdi *et al.*, 1986). Other QACs such as sarcosine and TAMO(tri-methyl amino N-oxide) protected this enzyme in a similar way.

2.5.3 Other effects of proline and QACs

In addition to these defined roles, many others have also been suggested. At the whole organism level, pre-treatment with proline prevented the inhibition of wheat germination by salt (Palfi *et al.*, 1974). Exogenous application of proline has also been found to stimulate the growth of *Salmonella* subjected to osmotic stress (Christian, 1955) and enhance shoot elongation in cultured barley embryos in the presence of salt (Lone *et al.*, 1987). Proline has been suggested to serve as storage compound for C and N which is rapidly reutilised when water or salt stress is relieved (Barnett and Naylor, 1966; Stewart *et al.*, 1966). Such a role cannot be claimed for glycinebetaine or trigonelline accumulation as degradation after relief of stress has not been observed (Parameshwara, 1984; Grattan and Grieve, 1985). Trigonelline, has been postulated to function as a hormone (Evans and Tramontano, 1981).

2.6 SALT STRESS AND GROWTH

A reduction in leaf expansion is generally the major growth response of plants to both short and long term salinity stress. In the short term, the reduction in leaf area is not due to limited water uptake, reduced carbohydrate supply, inhibited photosynthesis or ATP production, but to a reduced NO_3^- supply (Cram, 1973; Deane-Drumond and Glass,1982; Munns and Termaat, 1986) acting on protein synthesis (Aspinall, 1986). In addition, Munns and Termaat, (1986) speculated that some additional factor, originating in the root, exerts control over shoot growth during salt stress. During a long term stress other factors also become important including a reduction in leaf area expansion consequent upon specific effects of NaCl (Munns and Termatt, 1986) and salt accumulation to toxic levels causing the death and shedding of expanded leaves. Sustained carbon fixation is important to sustain turgor maintenance during salt stress through the

synthesis of sugars (Jones *et al.*, 1980), and other compounds (Hitz *et al.*, 1982). There is also a respiratory cost in compartmenting the incoming ions. Schwarz and Gale (1981,1983) and Shone and Gale (1983) determined the maintenance respiratory costs during salt stress for several species with differing salt tolerance. This indicated that salt sensitive species such as *Zea mays* incurred a higher maintenance respiratory cost than salt tolerant species such as *Atriplex hemimus*. Maintenance respiration necessitates an adequate supply of respiratory energy originating from photosynthesis (Kriedemann, 1986). Photosynthesis is affected by salt in many ways, however. If ions accumulate in the apoplast, a considerable stress is exerted on adjacent mesophyll cells and photosynthesis (Munns and Passioura, 1984). Reduced photosynthesis could also be due to a reduced stomatal conductance (Seeman and Critchely 1985; West *et al.*, 1986;Gale *et al.*, 1967 and Yeo *et al.*, 1985). In *Phaseolus vulgaris,* a 30% reduction in internal CO_2 was reported during salt stress (Seeman and Critchley, 1985), this reduction being directly related to the internal Cl^- concentration. In halophytes, however, salinity has a lesser effect upon the extent to which stomates limit photosynthesis (Osmond, 1980).

Salt also influences non-stomatal factors in photosynthesis including electron transport from photosystem II to photosystem I (Smillie and Nott., 1982; Downton and Milhouse, 1983) , the ability to fix CO_2, the regeneration capacity of RuBP carboxylase, and the capacity or efficiency of the RUBISCO enzyme. (Von Caemmerer and Farquhar, 1981; Badger *et al.*, 1984; Seeman and Critchely, 1985) a reduction in photosynthesis due to such non-stomatal effects of salt has been reported for a number of salt sensitive species (Gale *et al.*, 1967; Downton, 1977; Walker *et al.*, 1983; Seeman and Critchley, 1985). However, the biochemical basis for such altered photosynthetic capacity during salt stress is still unclear. A decrease in RUBP carboxylase activity with salt has been claimed (Osmond and Greenway, 1972 ; Kaiser and Heber, 1981), but in *Phaseolus* (Seeman and Critchley, 1985) no such inhibition was observed. However, in this latter study, a deflection in the initial slope of the A/P_i (assimilation versus internal CO_2 concentration) curve at low external CO_2 partial pressure, indicated that the capacity and

/or efficiency of RUBISCO activity was reduced (Seeman and Critchley, 1985). It is also possible that end product inhibition of certain metabolic processes reduces assimilation in which case growth limitations may not be directly related to photosynthesis. Rather, the inhibition of photosynthesis may alter source-sink relationships which in turn affect growth (Greenway and Munns, 1980; Munns *et al.*, 1982; Rawson and Munns, 1984).

CHAPTER-3

Materials and Methods

CHAPTER 3 MATERIALS AND METHODS

3.1 MATERIALS

Seeds of tomato, (*Lycopersicon esculentum,* Mill.), CV.Duke, an F1 hybrid, obtained from Yates Seed Co., Australia; *Lycopersicon cheesmanii,* obtained from Dr. B.Patterson, CSIRO, Division of Food Research, N.S.W, Australia and multiplied at Waite Agricultural Research Institute (WARI), *L. chilense, L. chmielewskii, L. esculentum v cerasiforme, L. hirsutum, L. parviflorum, L. pinnellii, L. peruvianum, L. pimpinellifolium* supplied by Prof. C.M.Rick, University of California, Davis; and wheat (*Triticum aestivum*) CV. Sun-9-E obtained from Dr. C.F. Jenner, Dept. of Plant Physiology WARI were used.

3.2. METHODS

3.2.1 Plant environmental control

Plants were grown in either growth cabinets (Zankel, Australia) or a plant growth room. The photon flux density was 350-450 μEin. sec^{-1} provided by sodium vapour lamps and the temperature was 20±1°C during the day and 17±1°C at night with a photoperiod of 16 hours. In specific experiments the relative humidity was also regulated with humidifiers (Defensor -505). These humidity regulators were standardised, using Electronic humidity sensors (Solomat) and continuously monitored with thermo-hygrographs. The position of the pots was changed daily before irrigation, to minimise effects due to gradients of light, temperature, and air velocity within the cabinet.

Air velocity over the plants was controlled in specific experiments by regulating shutters fixed in the horizontal air flow across the cabinet. Air turbulence was minimised in one experiment by fixing a glass sheet in the center of the cabinet at right angles to the air flow leaving the top open, thus reducing turbulence on one side of the

cabinet. The air velocity in the two compartments thus created was monitored with a miniature electronic anemometer probe attached to a solomat. Humidity, temperature and light were monitored and CO_2 concentrations in the split chambers measured with a calibrated Infra-red gas analyser (IRGA) ADC Type 225-2B-SS. For leaf-feeding experiments with wheat, plants were grown in a glasshouse compartment at a similar temperature to that in the growth cabinet.

3.2.2 Plant culture

Seeds of tomato (*L. esculentum*) CV. Duke and wheat, (*T. aestivum*), CV.Sun-9-E, were germinated in petri-dishes in the dark at 24 ±1°C. After 24 hours, excess water was decanted from the petri dishes and incubation continued until the radicle emerged, this took 3-4 days with tomato and 2 days with wheat. Seeds of wild *Lycopersicon spp.* with hard seed coats were treated with 4% sodium hypochlorite for 2-3 hours, washed to neutral pH, soaked with 5 mM $CaSO_4$ $2H_2O$, (to absorb the sodium hypochlorite adhering to the seed coat) for 5-10 minutes and washed to neutral pH again before sowing. The various species differed in the rate of emergence, ranging from 7 to 21 days, even after seed treatment. Care was taken to avoid variation between individual plants by selecting uniform sized seeds, with recently-emerged radicles.

In all experiments a Christies Beach sand washed to reduce silt to less than 5 per cent was used. The grain size ranged from 0.1 to 3.0 mm with 15 per cent 2-3 mm sized particles. Water holding capacity of the sand was 12-15 per cent and the pH of the saturation extract around 7. Black polythene pots of 15.5cm (tomato) and 12.5cm (wheat) diameter were used. A layer of well washed granite gravel was placed in the bottom of the pot to facilitate drainage. Pots were filled with 1.0 kg (12.5 cm diameter pot) or 2.25 kg (15.5cm diameter pots), and washed with R.O water to remove ions before settling for a day prior to planting. Seeds were planted at 5 plants/pot for tomato at a spacing of 6.5 cm and 25 plants/pot for wheat at a spacing of 1.5cm. Two uniform seeds were placed in each hole and thinned to one plant 2 (wheat) and 5 (tomato) days after emergence. Pots were irrigated with R.O water until 5 days after

emergence and thereafter with 0.25 strength Hoagland nutrient solution (Hoagland and Arnon, 1938) gradually increased to full strength on the day of stress imposition. 250 ml. per pot was supplied twice daily. This was more than sufficient to maintain field capacity. *L. cheesmanii* plants were grown singly in pots of 12.5cm as they are spreading in nature.

3.2.3 Imposition of stress

Twenty five (tomato) or ten (wheat) days after seedling emergence the stress treatments were imposed, either abruptly or gradually. In abrupt stress treatments, the nutrient solution was replaced by the required salt solution (in full strength Hoagland solution) immediately. In progressive salinization treatments, the osmotic potential of the root medium was decreased by 0.1 MPa day^{-1} (or as described later). Once the described level was achieved, the plants continued to receive the appropriate solution for the remainder of the experiment. The various osmotic solutions were prepared with AR grade reagents one day prior to administration and stored in the growth cabinet to equilibrate temperature. The concentration of individual salts required to achieve the desired osmotic potential, were computed from data on freezing point depression and osmotic co-efficients tabulated by Robinson and Stokes, (1955) and Hodgman (1955). The osmotic potentials of the solutions used were measured with a Spanner psychrometer, the full strength Hoagland nutrient solution contributing -0.065 MPa. 250 ml of solution was applied twice a day to each pot, which was more than sufficient to maintain field capacity. The excess drained away, removing accumulated salt and facilitating adjustment to the desired osmotic potential.

3.2.4 Plant growth measurements

A. Fresh and Dry weight

Shoots were separated, sealed in air-blown polythene bags and transported in a "Themocol" box, (to minimize weight loss from transpiration and respiration) and weighed using a Mettler P1200 balance. After leaf area measurement (see below) they

were placed in paper bags and dried in an forced air oven at 80°C for 48 hours. Roots were washed twice to free them of sand, blotted dry and weighed (freshweight) then dried as for the shoots.

B. Leaf area

The leaf area of the whole plant was determined using a Patons digital electronic planimeter. The machine was calibrated with a 10 cm^2 metal plate during every use.

3.2.5 Photosynthesis

3.2.5.1 Porometry

Stomatal resistance of the upper and lower surfaces of the primary leaflet of the fully expanded leaf of tomato, was measured with a pre-calibrated Li-Cor LI 60, diffusive resistance porometer and represented as sec cm^{-1}. Measurement was commenced 3 hours after the start of the photoperiod.

3.2.5.2 Gas exchange

The rate of CO_2 assimilation of an attached flag leaf of wheat was measured with an open-ended gas exchange system constructed as described by Von Caemmerer and Farquhar (1981). Leaves to be measured were enclosed with an aluminium and glass cuvette maintained at a constant temperature of 20.5 ±0.2°C. Flow rate through the cuvette was 1.0 litre min^{-1}. Air passing over the leaf was conditioned by removing CO_2 with soda lime and then humidified with a dew point set when the air was passed through the glass condensor at 18°C. Water vapour content was determined by a Vaisala HM 606 (Vaisala, OY, Holsinki, Finland) capacitive sensor. The CO_2 concentration was established by injecting 10 per cent carbon dioxide in air into the air stream through a mass flow controller. The CO_2 depletion caused by the leaf was measured with an ADC Mark III Infra red gas analyser. Air was circulated inside the cuvette by a small fan. The light source was a tungsten halogen lamp. Assimilation and evaporation rates, leaf conductance and intercellular CO_2 concentrations were calculated on an APC III

computer attached to the system. Calculation of results followed those set out by Von Caemmerer and Farquhar (1981). On the day of measurement the plants were taken from the growth room or cabinet to the gas exchange unit. The penultimate leaf was laid across the cuvette. The top of the cuvette was then laid over the leaf and tightened with clamp screws until the inside cuvette was completely sealed from outside air. After 15-30 min. equilibration the partial pressure of CO_2 (pCO_2) within the cuvette was increased from 350 to 900 µbars and triplicate gas exchange measurements were made at an ambient CO_2 concentration of 350 µbars. The partial pressure of CO_2 was then reduced down to 50 µbars and measurements were made at low pCO_2 concentrations of 50-350 µbars to obtain a curvilinear relationship.

3.2.5.3 Leaf tip feeding

The intact wheat flag leaf was used for examination of the responses of gas exchange parameters to organic solutes as the leaf was long enough to extend beyond the gas exchange chamber. Gas exchange at various external CO_2 partial pressures was measured, the leaf tip then was cut under water (Plate-3) and solutions fed through the cut tip. Transpiration was expected to be high in the leaf chamber due to the high light intensity, so solutions fed through the cut end would have reached the site of gas exchange rapidly.

3.2.6 Measurement of plant water status

3.2.6.1 Leaf water potential

Leaf water potential was measured with a Spanner thermocouple psychrometer (Barrs, 1968). One entire leaf (tomato) or 3 leaf blades (wheat) were cut, rolled gently and placed immediately in the chamber which was sealed with 2 layers of parafilm. The chambers were then attached to the thermocouples and equilibrated in a water bath maintained at 25° C for 2 hours. The thermocouple output was read once every 30 min. on 5-6 occasions. The water potential was calculated from 3-4 readings once the output had stabilised.

PLATE 3 Feeding solutes to the flag leaf of wheat through the cut end for gas exchange measurements

P -Plant
FL Flag leaf
TT Test tube
S Solutes
TS Test tube stand
GC Gas exchange chamber


GC
FL
P
TT
S

3.2.6.2 Osmotic potential

The water potential of the tissue can be analysed into solute and turgor potentials and written as $\psi = \psi_s + \psi_p$ where, ψ_s is solute potential and ψ_p is turgor or pressure potential. In dead cells, ψ_p= zero (Barrs, 1968) so the water potential is equal to the osmotic potential. After, measurement of leaf water potential, the chambers containing leaf samples were separated from the thermocouple assemblies and wiped clean with paper tissues. At the same time the leaf samples were blotted to absorb water droplets that had fallen when detaching the thermocouple assembly. The chambers containing the leaf samples were stoppered with rubber bungs wrapped with parafilm and aluminium foil (to prevent moist air entering) and immersed in liquid nitrogen (~ -150°C) for 5 minutes before the chambers were allowed to equilibrate to 25°C.The rubber bungs were removed rapidly and the chambers fixed to the thermocouple assembly, sealed and placed in the water bath. The same procedure as described for the measurement of leaf water potential was followed thereafter.

3.2.6.3 Turgor potential

Turgor potential was calculated from the values derived for total water potential and osmotic potential as $\psi_p = \psi_s - \psi$. The values of total water potential, osmotic potential and turgor potential were expressed as MPa.

3.2.7 Transpiration

Transpiration measurements on intact plants were made by enclosing the pot with two layers of polythene bags which were tied tightly around the base of the stem 3 hours after watering. An initial weight was taken (W_0) at this time and pots were re-weighed (W_1) next day at the time the plants were dissected and leaf area measured. Transpiration rate was expressed as mg water loss cm^2 leaf $area^{-1}h^{-1}$.

3.2.8 Preservation of plant materials

3.2.8.1 For biochemical analysis

Samples were harvested using separate scissors for control and stress treatments, gathered in a glass vial and immediately frozen with liquid nitrogen. They were stored at -20°C until freeze drying for 2-3 days after which they were maintained at 2-3°C until analysis.

3.2.8.2 For ionic analysis

Samples were harvested separately, dried in a forced air oven at 80°C for 48 hours and stored in a desiccator with silica gel until analysis.

3.2.9 Chemical analysis

3.2.9.1 Extraction

Weighed, freeze-dried samples were ground in a 50ml glass centrifuge tube using an ultraturrax and extracted with 15 ml methanol:chloroform:water (12:5:3 v/v). The tubes were immersed in ice to keep the temperature near zero to prevent heat associated degradation. 5-8 ml of water were added to break the stable emulsion which otherwise formed and to wash the grinding head. The extract was shaken well on a rotary shaker before centrifugation at 3500-4500 rpm for 30 min. The supernatent (methanol:water fraction) was collected and measured. Extraction was repeated once and the supernatent solutions were pooled. The pooled sample was stored at 2-3°C until analysis for proline and QACs. Normally, proline analysis was carried out on either the same or subsequent day as extraction and analysis for the QACs began on the following day.

3.2.9.2 Proline estimation

Chinard (1952), developed an acid-ninhydrin method for proline estimation, which was subsequently studied for the effects of interferences due to hydroxyproline,

lysine, hydroxy-lysine, ornithine, pipecolic acid, citrulline, tryptophan, and histidine by Scheweet, (1954); and Troll and Lindsley (1959). This method modified by Singh *et al.*, (1973) was used in the present study.

A. Resin preparation:

Zerolite-225 (Diamond Shamrock, Polymers Ltd.) described in Singh *et al* (1973) could not be used as it is not available and a similar resin, Amberlite (IR-20 (Na), 0.30-1.18mm, 14-52 mesh, standard grade (BDH, England) was substituted. The resin was treated with 1N NaOH (1:2 V/V) in a glass column at a flow rate of 2 ml $min^{-1}cm^{-2}$. The column was then washed with water, until the pH fell to 8.

B. Analysis:

Pooled extracts of known volume were added to the resin (~300-400mg) in a glass tube, shaken well with 3 ml of water and decanted into boiling tubes. The resin was shaken with a further 2 aliquots of 3 - 4 ml of water which were pooled with the first aliquot. Standard solutions of L-proline containing amounts from 0 µg to 60 µg, were treated similarly. The final volume of the pooled extract was 10 ml. 5 ml of glacial acetic acid was added to adjust the pH to 1. Then, 5ml of ninhydrin reagent (prepared by dissolving 125 mg of ninhydrin in 3 ml of glacial acetic acid and 2 ml of 6M orthophosphoric acid) was added to each sample. Two or three glass beads were added to each tube which was shaken thoroughly and boiled in a water bath at 90-95°C for 1 hour.The tubes were then transferred to an ice bath to stop the reaction, 5 ml of toluene was added and the tubes were shaken vigorously and allowed to settle overnight. The colour developed was read at 520 nm using a Beckman PC/600 colorimeter. The amount of proline was calculated from a standard curve.

3.2.9.3 Quaternary ammonium compounds

The remaining solution left after proline analysis, was used to determine quaternary ammonium compounds. Extracts were adjusted to pH 5-7, before ion exchange chromatography.

A. Column preparation:

300 X 12 mm glass columns were packed with glass wool at the base and filled with 5 g of Dowex -50W (Sigma,USA), a sulphonated cation exchange resin in H^+ form with 2% cross linkage (50-100 mesh). The resin was made into a slurry with water and flushed into the column, allowed to settle and then eluted with water until the eluate pH was 6-6.5. The column was then allowed to settle overnight. The resin was converted to the H^+ form by addition of 25 ml of 8N HCl and was then washed with deionised water until the eluate pH was 5.5.

B. Ion exchange chromatography:

The column was then loaded with the extract, without disturbing the settled column and washed with 100 -150 ml of deionised water at a minimal flow rate. Quaternary ammonium compounds, together with some amino acids, were eluted with 100 ml 4N HCl. The columns were charged with 8N HCl again and the whole procedure repeated to continue with subsequent samples.

The acid eluate was dried *in vacuo* at 50-60°C. The residue was dissolved in 5-10 ml of ethanol, dried and dissolved in 5 ml of distilled water. The extract was then dried *in vacuo* followed by a forced air oven maintained at 80-90°C for a few minutes and placed in a stream of N_2 to eliminate traces of water. The dried extract was dissolved in 0.8ml of D_2O, 0.6ml of the solution was then transferred to a 5 mm NMR tube, to which 2 μ moles of t-butanol was added as an internal standard. The sample was at a pH of 2-3. For TLC (Thin Layer Chromatography) the samples were dissolved and taken up in a known volume of 9:1 methanol:water to facilitate application to the silica gel.

3.2.9.4 Thin-layer chromatography

Thin layer chromatography was employed to identify QACs in the crops studied and to evaluate the ion exchange chromatography methods used as even a small quantity of the sample could be traced. The TLC procedure is tedious for large number of samples however, and this method was not adopted as the routine procedure for estimation.

A. Preparation of plates

Glass plates of 20 x 20 cm were thoroughly washed with detergent, rinsed with de-ionised water and dried in an oven at 100°C. The cooled plates were cleaned with methanol before a slurry of Kieselgel 60 G Art 7731 (Merk, USA) (38g in 65 ml of water) was poured into the reservoir of a Desaga spreader with 0.20mm clearance. The plates were stored at room temperature until required, and activated at 110 °C for 30 mins, 2 h. before applying the samples.

B. Application of sample

Samples dissolved in 9:1 v/v methanol:water were preferred. Samples were applied either as spots or as bands 2cm above the lower edge of the layer. The band application was carried out with a Cameg Linomat III, mechanical spotter/streaker.

C. Development of plates

Plates were developed for 1-2 hours in chloroform:methanol:ammonia (60:30:10 v/v) in an air-tight glass tank, equilibrated for 12 h. The plates were then air dried, and, in some cases, kept in an oven maintained at 80°C for few minutes to remove ammonia, which interfered with the Dragondoff reagent reaction.

D. Detection of QACs

QACs were visualised with Dragondoff's reagent (Radeka *et al.*,1971). The stock solution was prepared with bismuth carbonate (2.5g) and sodium iodide (7g) in glacial acetic acid (25 ml), boiled until dissolved to give a clear red solution and cooled to room temperature, before 10 ml of ethyl acetate was added. The spray reagent consisted of a mixture of 10 ml of stock solution, 3 ml of conc.HCl, 11 ml of deionised water and 10 ml of ethanol. The dried plates were taken to the fume hood and sprayed, using an atomiser. A second spray was applied 10-15 min. later, if required.

3.2.9.5 NMR quantitation

This method of quantitating solutes in plant samples was described by Jones *et al*., (1986). Similar methods have been followed to quantify the QACs in feeds (Chastellain and Hirsbrunner, 1976).

The QACs were measured by obtaining 'H NMR spectra on a JEOL FX 90Q Fourier transform NMR Sprectrometer operating at a frequency of 90 MHz at an ambient probe temperature of 24°C. Routinely, 32 acquisitions were accumulated in the 8K memory address using a specified width of 1000MHz and 15 micro seconds 45° pulse width with a recycle time of approximately 4.2 sec. The pulse width and delay between successive acquisitions, ensured complete relaxation of the NMR signals of interest (Spin-lattice relaxation times ranged between 0.6 and 1.8 sec) which enabled peak areas to be used to quantitate the compounds of interest. The method of exponential line broadening was not applied to the free induction delay prior to Fourier transformation. In the case of tomato samples, where the trigonelline peak fell near the water peak, the water peak was suppressed by homo-nuclear gated decoupling. Peaks of QACs were identified and confirmed using authentic standards (Appendix-1) and were integrated to the internal standard (t-butanol) over various concentration ranges. The standards were subjected to linear regression analysis and the quantities in unknown samples were computed by reference to this regression.

3.2.10 Abscisic acid

A. Extraction

100 mg of freeze dried leaf material was placed in 50 ml glass centrifuge tubes and 10-15 ml of boiling water was added to the samples. These were then placed in a water bath maintained at 90-100°C for 10 min. Boiling water was used in order to avoid enzymic hydrolysis of conjugated forms of ABA (Loveys and Van Dick, personal communication). Extracts were stored at 2°C overnight. 1µg of (±)-[C-6 $^{2}H_{3}$] ABA was added to each sample, mixed thoroughly and centrifuged at 3000 rpm for 15 min.

The supernatents were collected in a 125 ml conical flask. Extraction was repeated once more using 15-20 ml of R.O water, centrifuged and the aliquots pooled.

B. Filtration and Elution

25 ml plastic syringes (Piston removed) were used as column manifolds. A layer of glass wool was placed at the bottom of the column and polyvinyl polypyrralidone (PVP) was filled to occupy 4-5 ml volume. Water was run through the column to settle the PVP. The pooled aliquots were adjusted to p^H 2.5 with 1N HCl and passed through the PVP to separate pigments and the filtered solution was collected. ABA was eluted with 3 volumes of water, the p^H adjusted to remain about 2.5. These filtrates were poured into a separating funnel with 15-20 ml of ethylacetate shaken for a minute and allowed to settle. The ethylacetate layer was collected and the water fraction extracted twice more with 20 ml of ethylacetate. The pooled ethyl acetate fractions were reduced to dryness, taken up in 1.0 ml of ethylacetate and transferred to 1.5 ml epindoff tubes. The extract volume was reduced to 50 µl using a stream of dry N_2 before TLC.

C. Thin layer chromatography

Preparation of plates:

Glass plates of 20 X20cm were thoroughly washed with detergent, rinsed with de-ionised water and dried at 100°C. The cooled plates were cleaned with methanol before a slurry of kieselgel 60G Art.7731 (Merk, USA) (38g in 75ml R.O water) was poured into the reservoir of a Desaga spreader with 0.25mm clearance. The plates were stored at room temperature until required and were activated at 110°C for 30 min. 2 h before applying samples.

Application of samples:

The samples from the epindoff tubes were applied as bands 2 cm above the lower edge. The band application was carried out with a Cameg Linomatte mechanical spotter/streaker. Authentic *cis* ABA was co-chromatographed in each TLC plates.

Development of plates:

Plates were developed for 1-11/2 h in ethyl acetate:toluene: acetic acid (25:15:2 V/V) in an air-tight glass tank, equilibrated for 12 h. The plates were dried in an oven maintained at 80°C for a few minutes to remove toluene.

Elution:

Standard *cis* ABA was identified by a short exposure to UV (Short wave length) and the regions corresponding to standard *cis* ABA was marked as sample ABA. Gel was carefully scraped from the plates avoiding the standard. The gel containing ABA was transferred to a centrifuge tube and extracted 4 times with 10 ml of ethylacetate each time centrifuging at 3000 rpm for 20 min. These extracts were dried *in vacuo* and extracted with 1 ml of ethylacetate and transferred to 2 ml glass vials. These were then dried down under a stream of N_2.

D. Gas chromatography/ mass spectrometry (GC/MS)

ABA was estimated by combined gas chromatography/mass spectrometry. For GC/MS the samples were dissolved in 100µl of hexane and injected onto bonded phase capillary column (BP 1SGE, Melbourne). Oven temperature was programmed to raise from 70° to 240° at 15° min^{-1}. ABA methyl ester was eluted at 14 min. The column was directly coupled to a Hewlett Packard 5970B mass selective detector. Ions at M/2 134 and 137 were monitored and ABA in the extracts was estimated by interpolation from a standard curve relating 134/137 peak ratio to ABA/D_2-ABA mass ratio (Loveys *et al.*, 1987) [Plates 4,5].

3.2.11 Ionic analysis

3.2.11.1 Extraction for Cl^-, Na^+ and K^+

Dried samples (about 100mg) were ground with a pestle and mortar and partitioned into aliquots for the separate estimation of Na^+,K^+, and Cl^-. The ground samples were weighed, and transferred to polythene tubes. 5 ml of 1M HNO_3 was added and glass

PLATE 4 Ion chromatogram of *cis* ABA

137 amu pertains to internal ABA standard
134 amu pertains to sample ABA

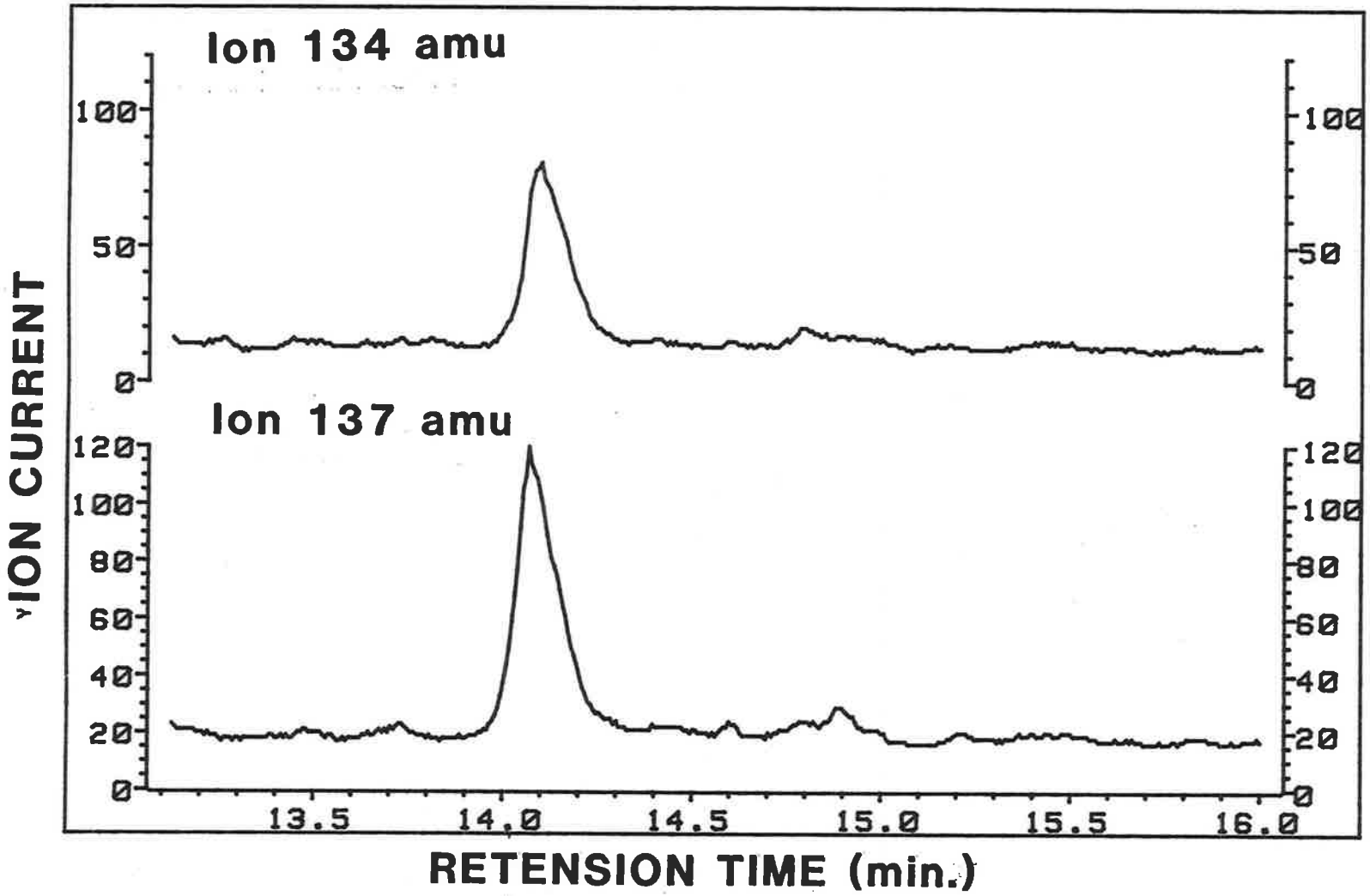
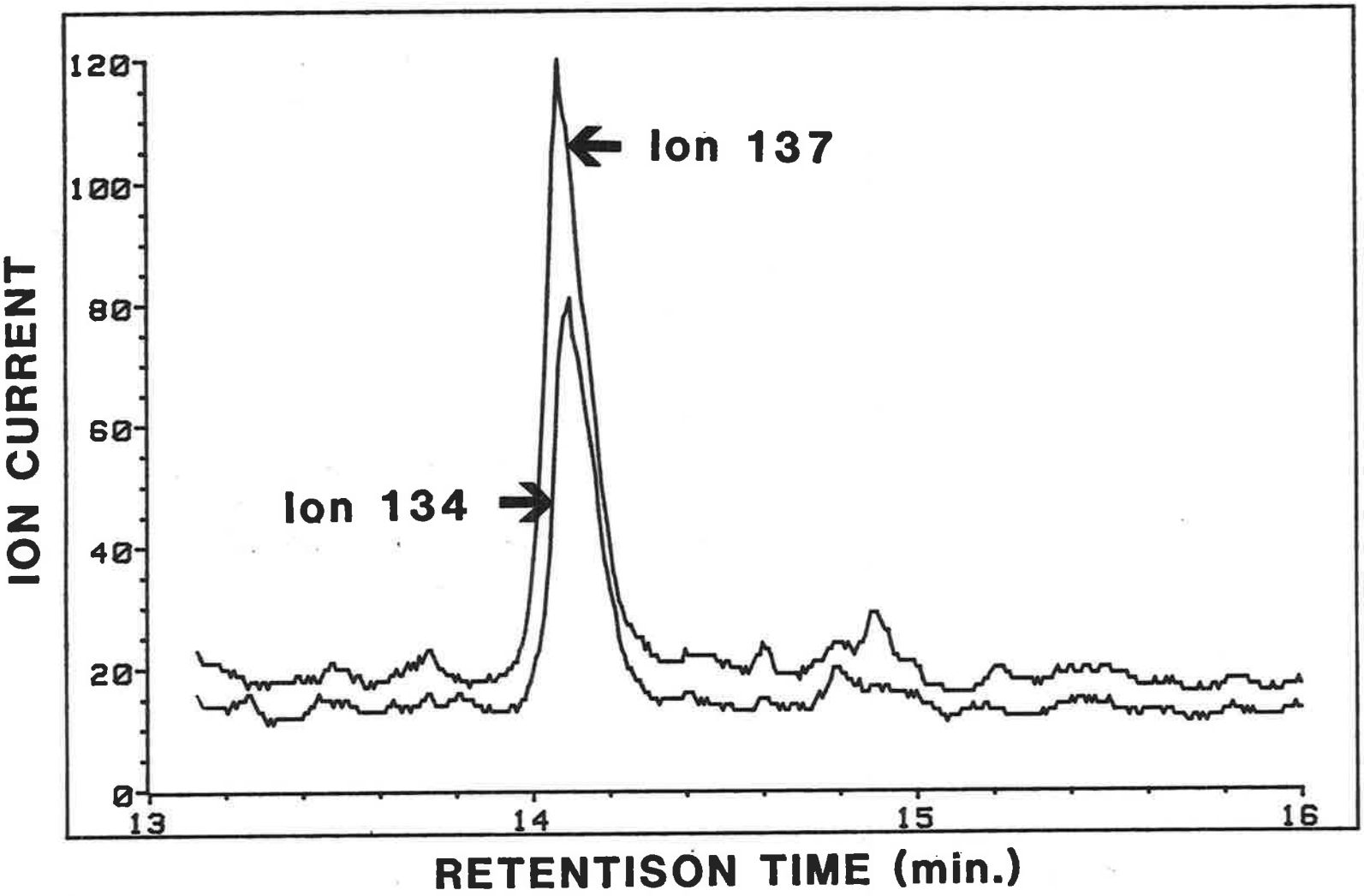

Ion 134 amu
100
50
0
Ion 137 amu
120
100
80
60
40
20
0
13.5
14.0
14.5
15.0
15.5
16.0
ION CURRENT
RETENSION TIME (min.)

PLATE 5 Coupled ion chromatogram of 134 and 137 amu peaks of *cis* ABA

137 amu pertains to internal ABA standard
134 amu pertains to sample ABA


ION CURRENT
120
100
80
60
40
20
0
13
14
15
16
Ion 137
Ion 134
RETENTISON TIME (min.)

marbles were placed on the tubes before they were incubated in a hot water bath at 90-95°C for 30 min. The samples were then spun at 3000 rpm for 20 min. at room temperature. The supernatant was decanted into a graduated tube and the pellet was re-extracted with 5 ml deionised water for 30 min., centrifuged, the supernatants were pooled and made up to a volume of 10 ml and stored at 2°C.

3.2.11.2 Chloride analysis

Chloride analysis was carried out using a Butchler-Cotlov Chloridometer 4-2008.

A. Reagents:

1. Nitric acid reagent (0.1N HNO_3 and 10% glacial acetic acid): 6.4 ml of conc. HNO_3 and 100 ml of glacial acetic acid were added to 900 ml water, mixed thoroughly and stored in a glass bottle.

2. Gelatin reagent: This was purchased from Kemtech, Australia and contained 60:1:1 of gelatin (Knox unflavoured gelatin #1), thymol blue (water soluble,National Aniline) and thymol.

3. Sodium chloride (0.5 meq/l) in 0.1 N nitric acid with 10% acetic acid reagent.

B. Method

Nitric acid reagent was added to an aliquot of the sample (0.1 to1 ml) to obtain a total volume of 2 ml. 2 drops of the gelatin reagent was added and the mixture was titrated using the silver ion electrodes of the instrument in either the low or medium ranges of the titration mode. Before the samples were titrated, the instrument was equilibrated and adjusted to room temperature. Triplicate blank and 0.5 meq/lit NaCl solutions were titrated and the time required (in sec) was recorded. Amount of chloride in the sample was calculated and expressed as mmole g dw^{-1}.

3.2.11.3 Extraction for Mg^{++}:

An aliquot of oven dried material (100 mg) was weighed and placed in a 100 ml Corning digestion tube together with 1.0 ml of conc. sulphuric acid, 5 ml of conc. nitric acid and 1ml of perchloric acid. This mixture was heated gently until the initial reaction subsided, the temperature was then increased to 350-400 °C for 60 min. The digest was cooled to room temperature and made up to 10 ml and then diluted 40 fold with 0.5 N nitric acid. The final solution contained 5 ml of 0.4% lanthanum oxide solution. The extracts were stored in 4°C until analysis.

3.2.11.4 Measurement

The extracts were diluted as necessary and K^+,Na^+ and Mg^{++} estimated on a PYE UNICAM SP9 Atomic absorption spectrophotometer, along with appropriate standards and the concentrations expressed as mmole g dw^{-1}.

3.2.12 Statistical analysis

Designs adopted are recorded under the respective experiments. Data were analysed on the VAX-FORTRAN computer net-work facility of the University of Adelaide using Genstat statistical analysis programmes.

CHAPTER-4

Results and Discussion

CHAPTER 4 RESULTS AND DISCUSSION

SECTION 4.1 THE EFFECT OF THE MODE OF CAUSING SALT STRESS ON THE ACCUMULATION OF PROLINE AND QUATERNARY AMMONIUM COMPOUNDS (QACs)

4.1.1 Introduction

Plants accumulating organic compounds such as proline and glycinebetaine in response to salinity stress do so in the presence of modification in tissue water status (turgor, osmotic and water potential), cellular ion content(both cations and anions) and hormone levels. Any of these changes are candidates for causing accumulation. Some separation of the roles of these various consequences of exposure to salt stress in the accumulation mechanism can be attempted by manipulating the manner in which the plant is exposed to salinity stress. Sudden salinization with NaCl can lead to massive, non-selective uptake of $Na^+$and Cl^-, compared with gradual salinization where uptake is less (Pitman, 1984; Storey and Wyn Jones, 1978a). Variation in the water status of the leaves due to modification of the nature of the salinization treatment has also been accomplished with barley (Chu, 1974), and modification of the manner of administration of stress may provide information on the response of accumulated compounds to the factors associated with salt stress.

In the following experiments, the primary objective was to investigate accumulation of proline and QACs in two glycophytes, tomato and wheat belonging to the group showing medium salt tolerance (U.S.D.A. 1954) in order to establish relationships between accumulation and the tissue consequences of salt stress under different salinization systems.

4.1.2 Salt shock

4.1.2.1 Methods

Tomato, cv.Duke and wheat cv. Sun-9-E were grown at 20±1°C day and 17±1°C night temperature, with a 16 hour photoperiod and a photon flux density of 350 to 450 μ Ein sec^{-1} (Materials and methods 3.2.1 and 3.2.2). NaCl was supplied 25 and 10 days after the emergence of tomato and wheat seedlings respectively by replacing the nutrient solution with a range of NaCl solutions of -0.065, -0.265, -0.565, -1.065 and -2.065 MPa external water potential ($\psi_{ext.}$) containing Full strength Hoagland solution. 250 ml of these several solutions being applied twice daily. First true leaves were sampled 12, 24, 48 and 72 hours after commencement of stress treatments for the measurement of leaf water, osmotic and turgor potential (3.2.6), proline (3.2.9.2) , QACs (3.2.9.3) and of leaf ion content (3.2.11). The experimental design was a Factorial Randomised Block with three replications of each treatment.

4.1.2.2 Results

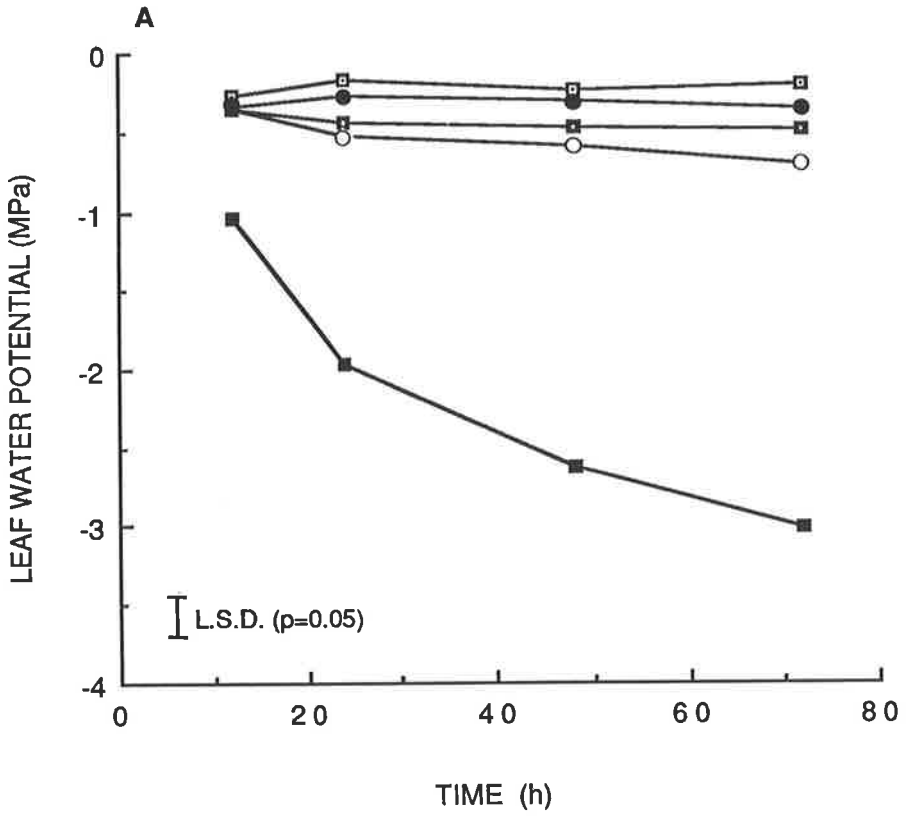
4.1.2.2.1 Leaf water potential

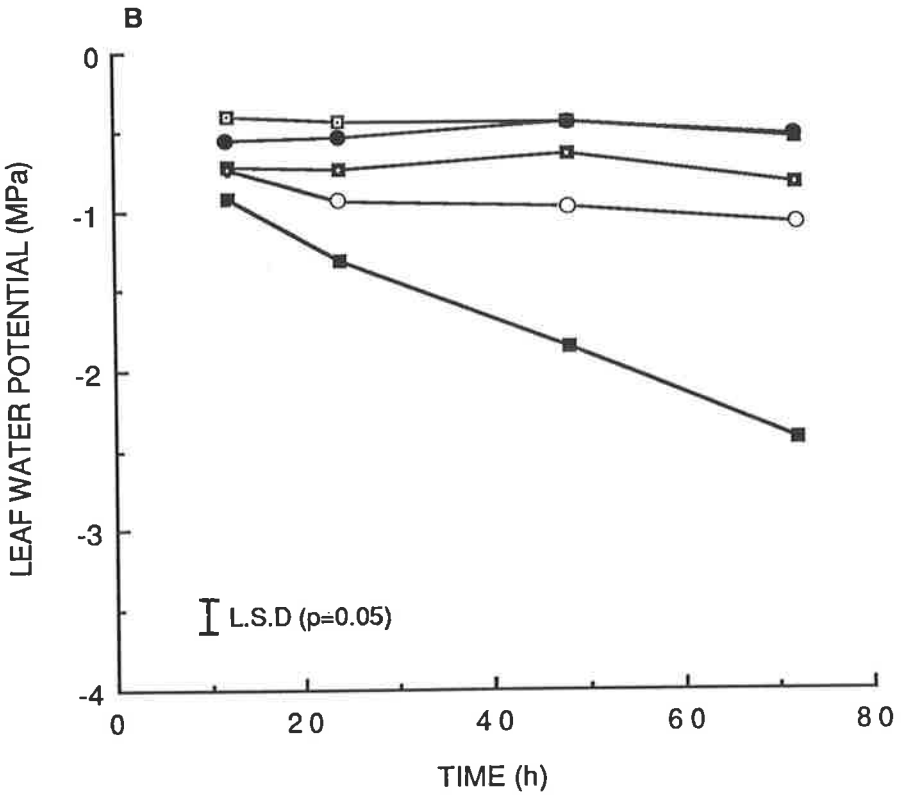
The leaf water potential of the control tomato plants (Fig.1A) ranged from -0.20 to -0.30 MPa and of wheat (Fig 1B) from -0.40 to -0.53 MPa and did not change significantly during the period of the experiment. In the stress treatments, leaf water potential fell in parallel with the external water potential. At the lower salt concentrations, with ψ_{ext} of -0.565 MPa and above in tomato and -0.265 MPa in wheat, the leaf water potential was not significantly different from that of control plants throughout the stress period. The leaf water potential of tomato at -1.065 MPa was at no time below the external water potential in the solution bathing the roots; evidently the shock salinity treatment caused rapid stomatal closure which equalised ψ within the plant.

Fig. 1 Changes in leaf water potential (-MPa) of tomato and wheat subjected to salt shock

A. tomato

B. wheat


A
0
-1
-2
-3
-4
LEAF WATER POTENTIAL (MPa)
L.S.D. (p=0.05)
0
20
40
60
80
TIME (h)


B
0
-1
-2
-3
-4
LEAF WATER POTENTIAL (MPa)
L.S.D (p=0.05)
0
20
40
60
80
TIME (h)

4.1.2.2.2 Leaf osmotic potential

Leaf osmotic potential of unstressed tomato (Fig 2A) was slightly higher (-0.6 to -0.7 MPa) than that of wheat (-0.8 to -0.9 MPa, Fig 2B) and did not change during the experiment. A reduction in leaf osmotic potential in both species followed the decrease in external water potential. Appreciable reduction in leaf osmotic potential was observed within 12 hours of subjecting plants to stress at the lowest external water potential but not until 24 in tomato or 48 hours in wheat at higher $\psi_{ext.}$ There was little further reduction in leaf osmotic potential at the higher levels of $\psi_{ext.}$ At the lowest $\psi_{ext.}$ in wheat, leaf ψ_s fell consistently for 72 hours reaching -2.75 MPa and in tomato it fell for 48 hours reaching -2.55 MPa but thereafter remained constant.

4.1.2.2.3 Turgor potential

Turgor measurements derived from the difference between ψ_s and ψ ranged from +0.360 to +0.425 MPa in unstressed tomato (Fig 3A) and from +0.400 to +0.575 MPa in unstressed wheat (Fig 3B). External salinity to -1.065 MPa did not affect tomato turgor but it fell below zero with $\psi_{ext.}$-2.065 MPa and did not recover within the 72 hours of the experiment. In wheat, estimated turgor in all treatments varied unpredictably and no reasonable conclusions can be drawn for the data.

4.1.2.2.4 Leaf sodium concentration

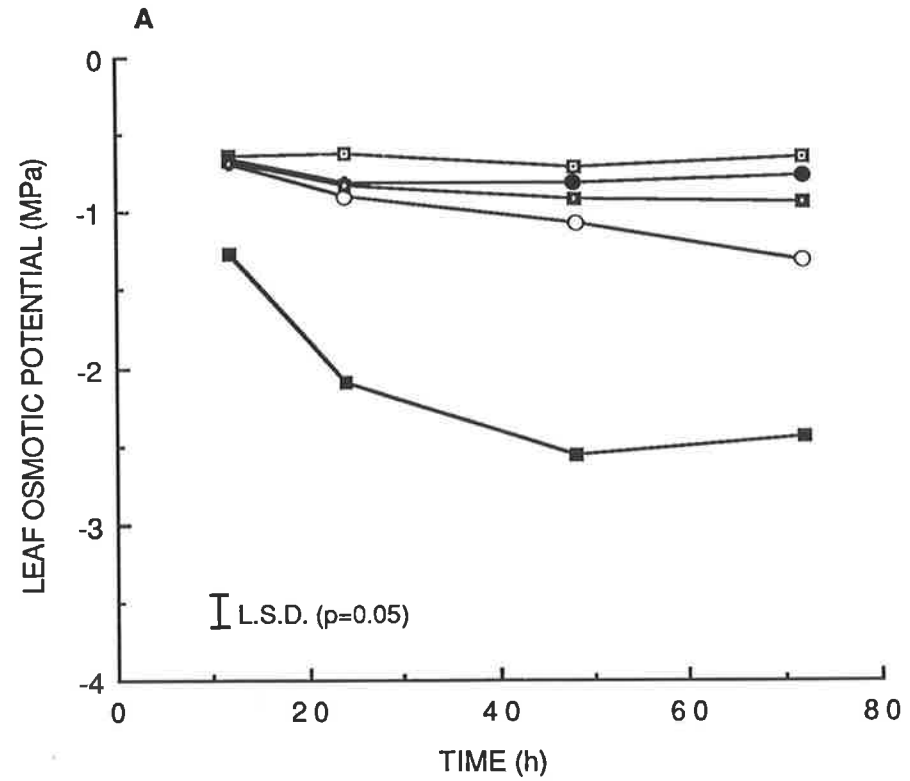
The sodium concentration in control plants was 0.09 to 0.14 mmole g^{-1} dw. in tomato (Fig 4A) and 0.06 to 0.16 mmole g^{-1}dw. in wheat (Fig. 4B) and did not change during the 72 hours of the experiment. Tomato plants only accumulated significant amounts of Na^+ at an external ψ_s of -1.065 MPa and below and wheat showed a similar response, although here Na^+ accumulation at -0.565 $\psi_{ext.}$ reached significance due to the lesser variability of the data. Substantial Na^+ uptake occurred within 12 hours in both wheat and tomato at -2.065 MPa external water potential. This initial increase was greater in tomato than wheat but the concentrations after 72 hours of exposure were similar (2.49 tomato and 2.22 wheat). There was no evidence that accumulation had ceased at

Fig. 2 Changes in leaf osmotic potential (-MPa) of tomato and wheat subjected to salt shock

A. tomato

B. wheat

-0.065MPa
-0.265MPa
-0.565MPa
-1.065MPa
-2.065MPa


A
0
-1
-2
-3
-4
LEAF OSMOTIC POTENTIAL (MPa)
L.S.D. (p=0.05)
0
20
40
60
80
TIME (h)

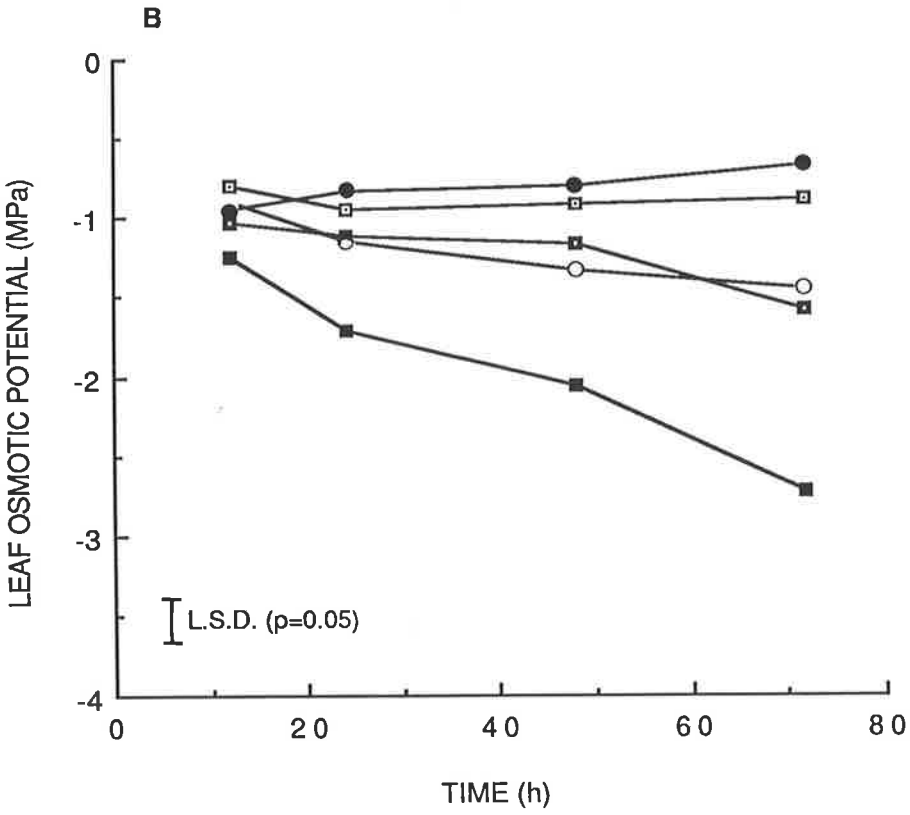
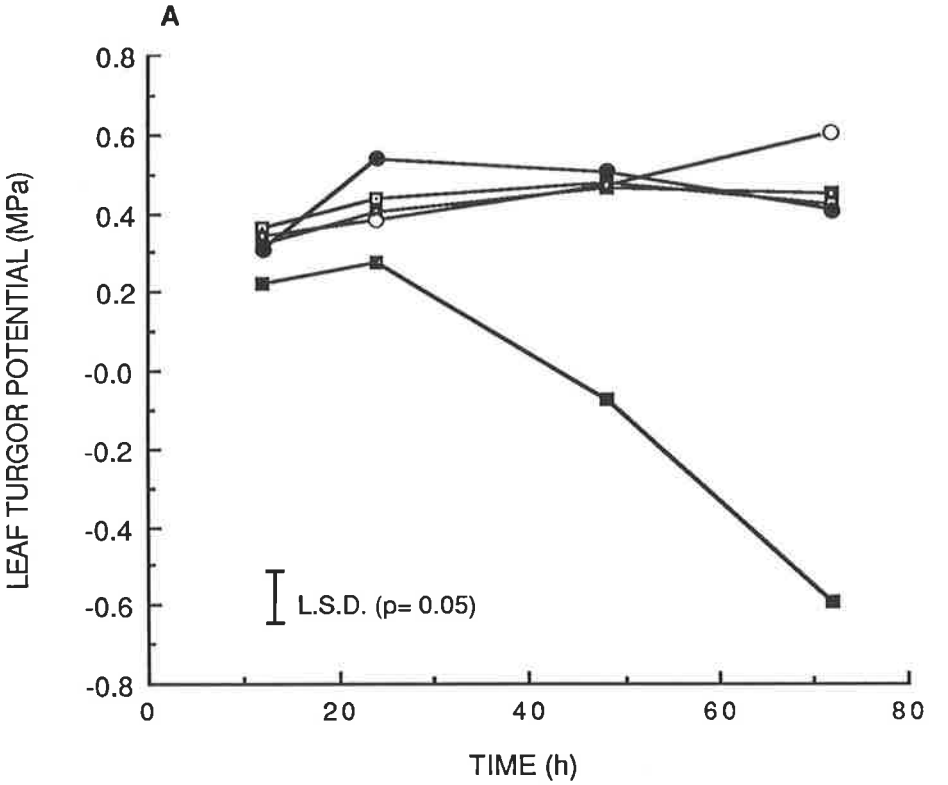

B
0
-1
-2
-3
-4
LEAF OSMOTIC POTENTIAL (MPa)
L.S.D. (p=0.05)
0
20
40
60
80
TIME (h)

Fig. 3 Changes in leaf turgor potentials (MPa) of tomato and wheat subjected to salt shock

A. tomato

B. wheat

-0.065MPa
-0.265MPa
-0.565MPa
-1.065MPa
-2.065MPa


A
LEAF TURGOR POTENTIAL (MPa)
0.8
0.6
0.4
0.2
-0.0
-0.2
-0.4
-0.6
-0.8
L.S.D. (p= 0.05)
0
20
40
60
80
TIME (h)

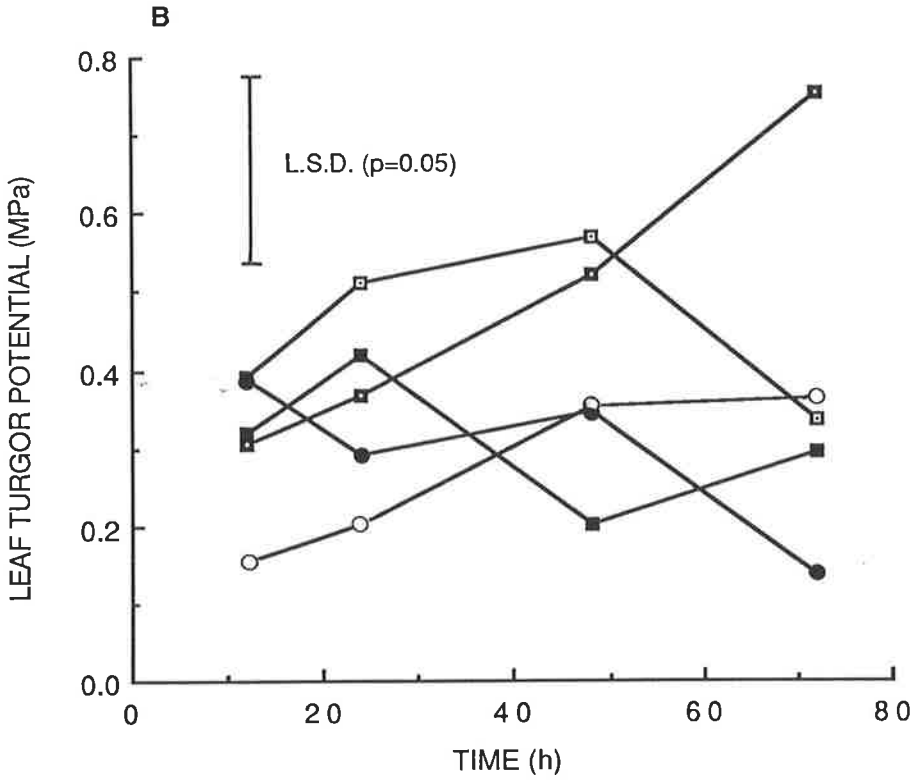
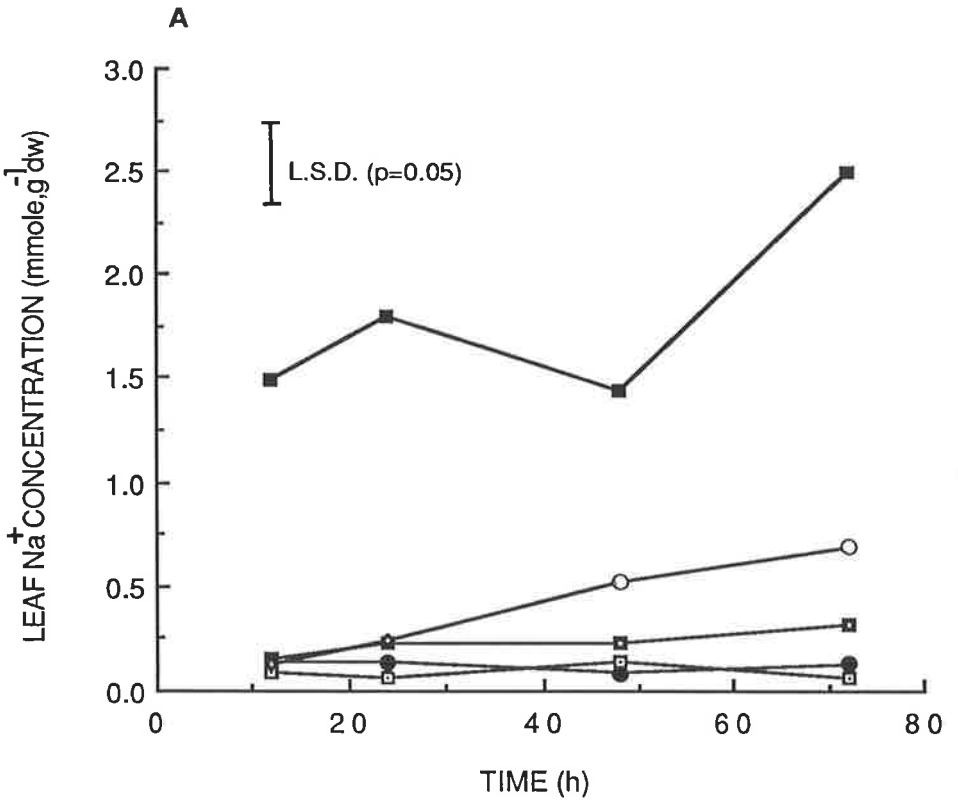

B
LEAF TURGOR POTENTIAL (MPa)
0.8
0.6
0.4
0.2
0.0
L.S.D. (p=0.05)
0
20
40
60
80
TIME (h)

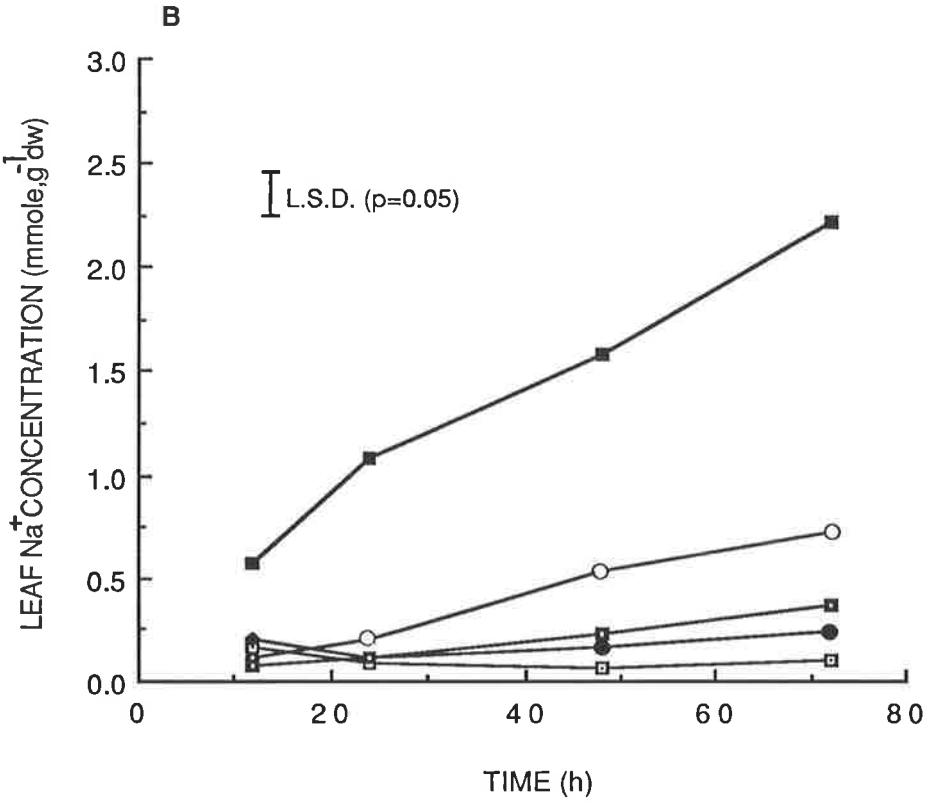
Fig. 4 Leaf Na^+ concentrations (mmole,g^{-1}dw) of tomato and wheat subjected to salt shock

A. tomato

B. wheat

-0.065MPa
-0.265MPa
-0.565MPa
-1.065MPa
-2.065MPa


A
3.0
2.5
2.0
1.5
1.0
0.5
0.0
L.S.D. (p=0.05)
LEAF Na+ CONCENTRATION (mmole,g-1dw)
0
20
40
60
80
TIME (h)


B
3.0
2.5
2.0
1.5
1.0
0.5
0.0
L.S.D. (p=0.05)
LEAF Na+ CONCENTRATION (mmole,g-1dw)
0
20
40
60
80
TIME (h)

that time.

4.1.2.2.5 Leaf chloride concentration

The leaf chloride concentration in unstressed tomato was low (0.05 to 0.06 mmole $g^{-1}dw$) whereas, that of wheat was considerably greater at 0.29 to 0.385 mmole $g^{-1}dw$(Fig 5A and 5B). Chloride uptake was promoted by external sodium chloride, although tomato excluded Cl^- at lower concentrations more efficiently than did wheat. External chloride up to 226 mM did not significantly increase uptake in tomato whereas in wheat there was increased uptake even at 113 mM $Cl^-_{ext.}$ However, rapid uptake took place within 12 hours of exposure to salt at the highest $NaCl_{ext.}$ (454 mM). Wheat accumulated more chloride (2.44 mmole $g^{-1}dw$) in 72 hours than tomato (1.00 mmole $g^{-1}dw$.). This data suggests that wheat takes up more chloride than does tomato under conditions of salt shock.

4.1.2.2.6 Proline

The proline concentration in control plants was about 0.20 mg g^{-1} dw. in tomato (Fig 6A) and wheat (Fig. 6B) and did not vary in the time of the experiment. A significantly higher concentration of proline was observed within 24 hours at the lowest external water potential of -2.065 MPa. But, by 72 hours the tomato plants at -1.065 MPa $\psi_{ext.}$ had accumulated more proline than those at -2.065 MPa $\psi_{ext.}$ The threshold for accumulation of proline was between -0.565 and -1.065 MPa $\psi_{ext.}$ for both wheat and tomato. The quantity of proline accumulated by these two crops differed substantially; at -2.065 MPa $\psi_{ext.}$ wheat accumulated 6.6 fold more proline than tomato. There was no evidence that accumulation had ceased at 72 hours.

4.1.2.2.7 Quaternary Ammonium Compounds (QAC)

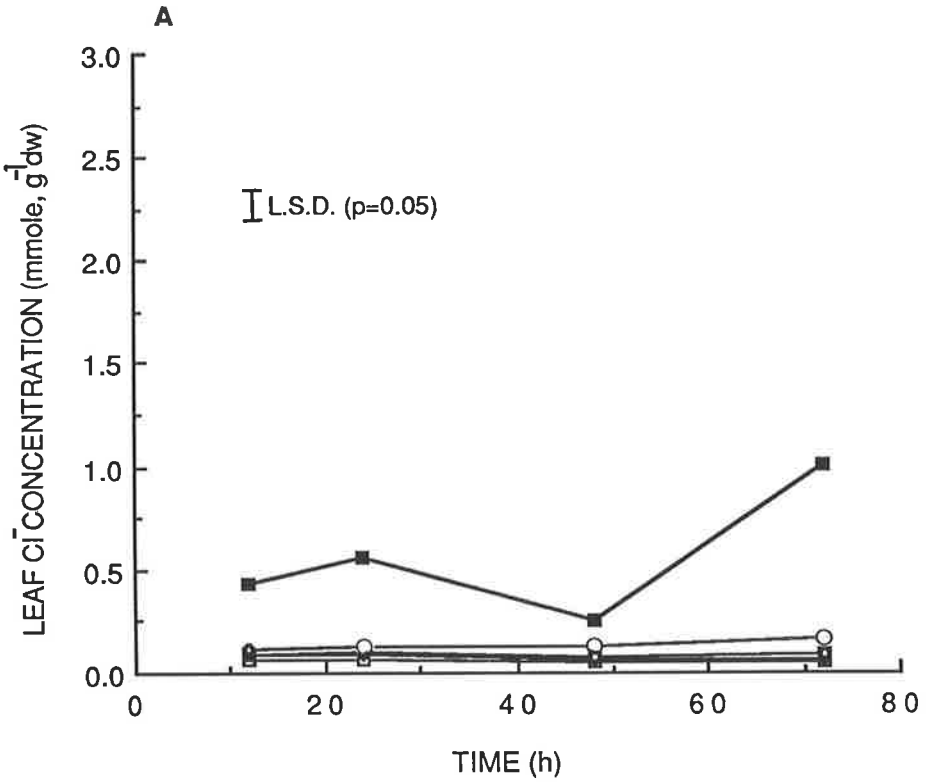
Trigonelline in tomato and glycinebetaine in wheat were found by TLC and confirmed by NMR Spectroscopy in comparison with authentic standards (Appendix 1).

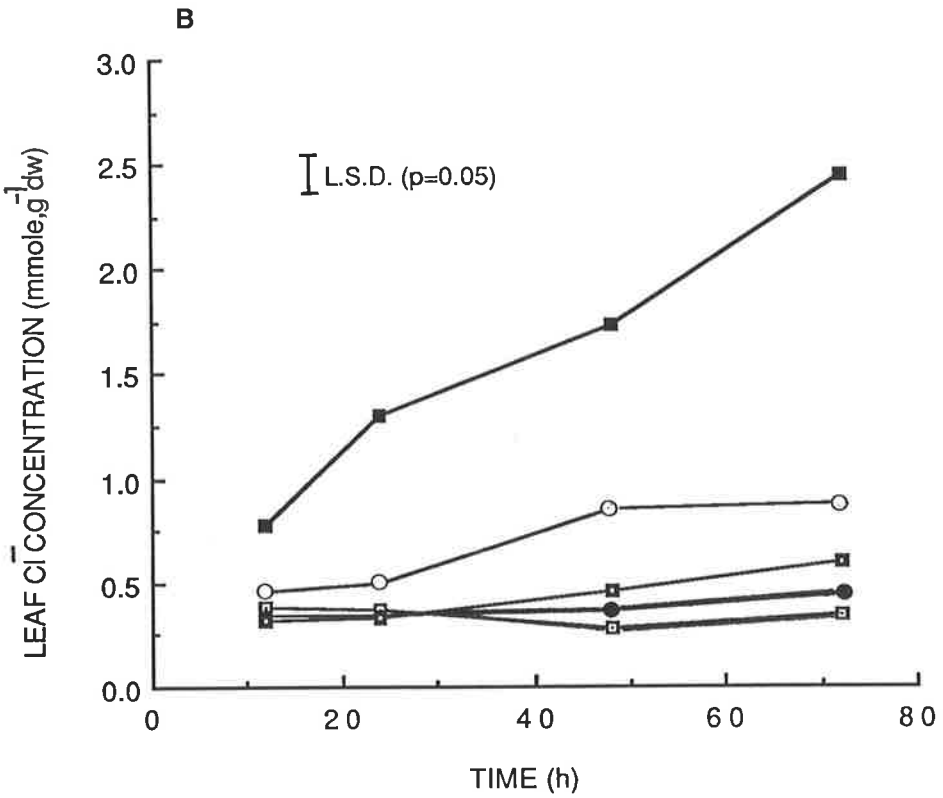
Fig. 5 Leaf Cl^- concentrations (mmole,g^{-1}dw) of tomato and wheat subjected to salt shock

A. tomato

B. wheat

-0.065MPa
-0.265MPa
-0.565MPa
-1.065MPa
-2.065MPa


A
3.0
2.5
2.0
1.5
1.0
0.5
0.0
LEAF Cl⁻ CONCENTRATION (mmole, g⁻¹dw)
L.S.D. (p=0.05)
0
20
40
60
80
TIME (h)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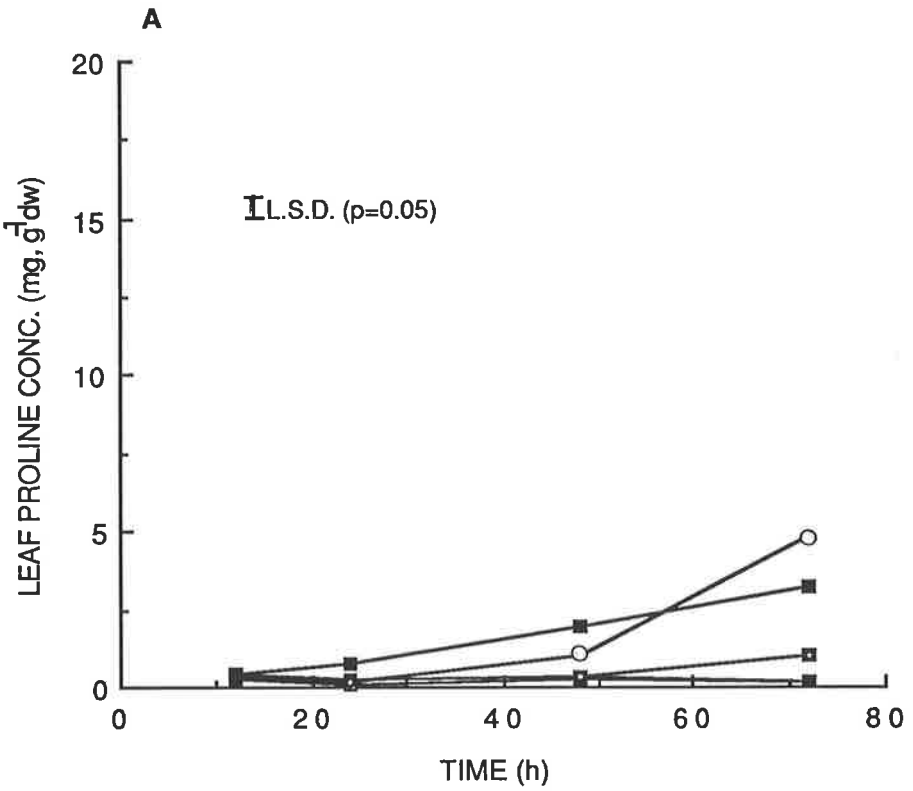
2.5
2.0
1.5
1.0
0.5
0.0
LEAF Cl⁻ CONCENTRATION (mmole,g⁻¹dw)
L.S.D. (p=0.05)
0
20
40
60
80
TIME (h)

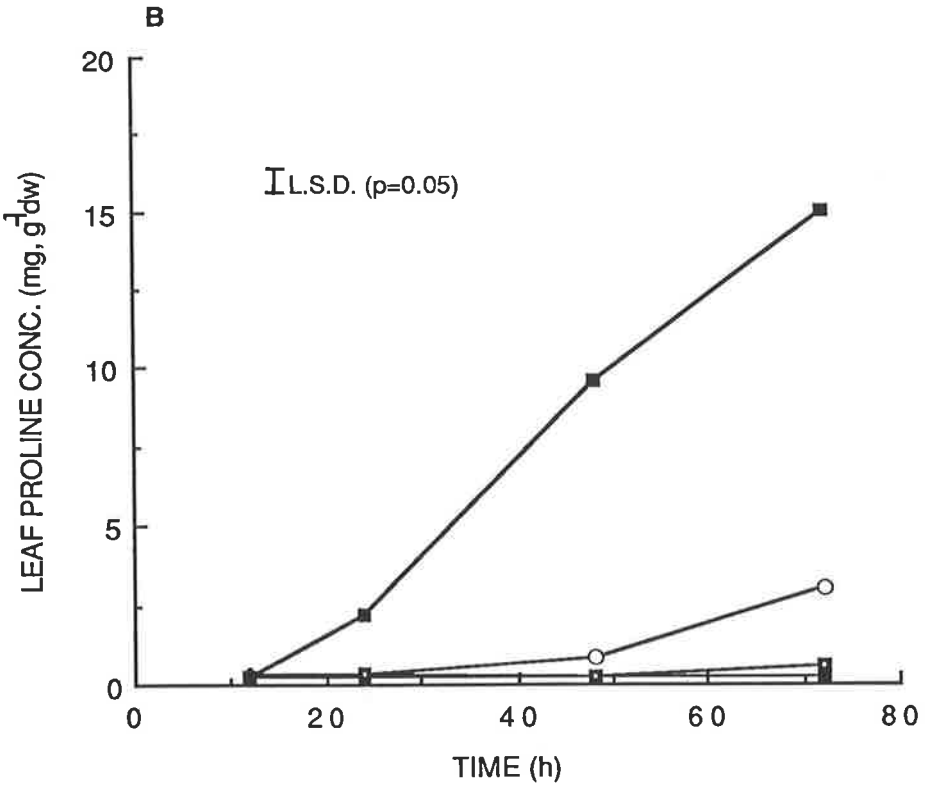
Fig. 6 Leaf proline concentrations (mg,g^{-1}dw) of tomato and wheat subjected to salt shock

A. tomato

B. wheat

-0.065 MPa
-0.265 MPa
-0.565 MPa
-1.065 MPa
-2.065 MPa


A
20
15
10
5
0
LEAF PROLINE CONC. (mg, g⁻¹dw)
L.S.D. (p=0.05)
0
20
40
60
80
TIME (h)


B
20
15
10
5
0
LEAF PROLINE CONC. (mg, g⁻¹dw)
L.S.D. (p=0.05)
0
20
40
60
80
TIME (h)

A. Trigonelline

Trigonelline concentrations in control tomato plants ranged from 0.75 to 1.00 mg g^{-1} dw and there was no accumulation with time (Table 1). Salt in the external medium induced trigonelline accumulation in the leaves in the first 12 hours of exposure, the response being significant at -2.065 MPa ψ_{ext}, but there was no further accumulation in the following 60 hours.

B. Glycinebetaine

A higher concentration of glycinebetaine than of proline was present in control plants of wheat (Fig 7) and this did not change in the period of the experiment. Glycinebetaine accumulated in response to salt stress, significantly even at -0.265 MPa ψ_{ext}. Most accumulated at -1.065 MPa ψ_{ext}, the concentration at -2.065 MPa ψ_{ext}. being initially depressed in comparison with the control plants, rising sharply to 24 hours then remaining constant. In all other treatments, the glycinebetaine content increased with time and showed no evidence of having reached a maximum by the end of the experiment.

4.1.3 Discussion

The present investigation has confirmed previous reports on the accumulation of proline (Strogonov, 1964; Palfi and Juhaz, 1970; Stewart and Lee, 1974; Chu *et al.*, 1976), glycinebetaine (Storey and Wyn Jones, 1978b; Wyn Jones and Storey, 1981) and trigonelline (Parameshwara, 1984; Gorham *et al.*, 1984) in response to salt stress. When the water potential of the external medium was decreased abruptly by NaCl, the leaf water potential of tomato and wheat fell with the ψ_{ext}. (Fig. 1A,B). In tomato it did not fall below ψ_{ext}. which suggests a suspension of water flow and conservation of tissue water by stomatal closure. Water flow within the plant would be expected to follow the gradient in ψ from the leaves to the rooting medium but resistances to reverse water flow (from leaves to root) may have been sufficient to reduce water flow along this gradient to a negligible value.

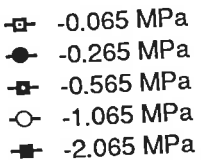
Table 1 Effect of NaCl on trigonelline concentration during abrupt salinization in tomato (mg/g d.wt.)

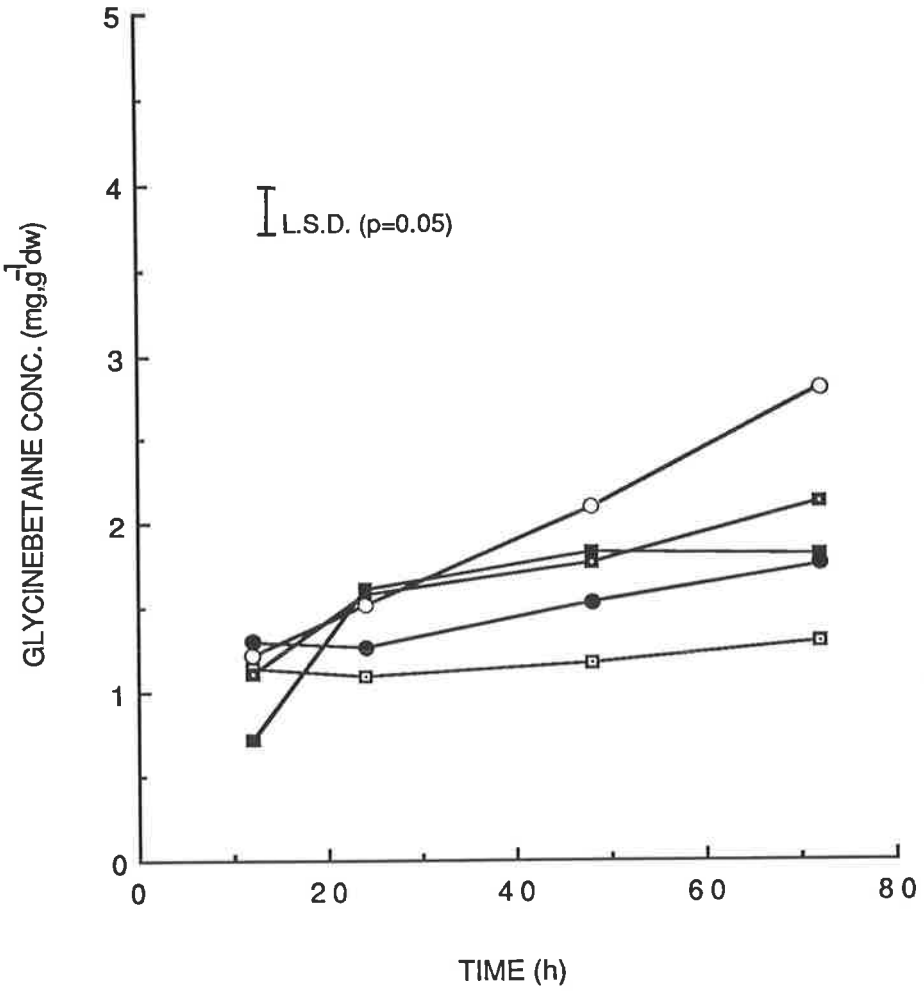
Time (Hours)	External water potential (MPa)				
	-0.065	-0.265	-0.565	-1.065	-2.065
12	0.828	0.872	1.103	1.267	1.583
24	0.887	0.866	1.085	1.337	1.663
48	0.759	0.808	1.135	1.374	1.697
72	0.810	0.817	1.167	1.392	1.773
MEAN	0.821	0.841	1.123***	1.342***	1.679***

Source of variation	VR	L.S.D(p=0.05)
Time	0.842 NS	
Ext. water potential	134.736***	0.089
Time X Ext.Water potential	0.724 NS	

*** (p=0.01)
NS (Not significant)

Fig. 7 Leaf glycinebetaine concentration (mg,g^{-1}dw) of wheat subjected to salt shock




GLYCINEBETAINE CONC. (mg.g-1dw)
L.S.D. (p=0.05)
0
1
2
3
4
5
0
20
40
60
80
TIME (h)

Fall in leaf water potential may be accompanied by a loss of turgor or confined to a change in osmotic potential depending on the concentration of salt, rapidity of salt stress imposition, and the ability of the plant species to take up ions, and synthesise organic solutes. In salt-stress conditions, plant turgor is regulated by the uptake of Na^+ and Cl^- ions (Greenway and Munns, 1980). Both tomato and wheat took up almost equal quantities of Na^+, the uptake being proportional to the $Na^+_{ext.}$ concentration. Saturation of Na^+ accumulation in sampled leaves was observed between 24 and 48 hours at the lower $Na^+_{ext.}$ concentration but not at 454 mM Na^+ where a linear increase in internal Na^+ was evident. A similar response was observed for Cl^- concentration in wheat at the highest $Cl^-_{ext.}$ concentration; however, there was no substantial increase in Cl^- concentration up to a concentration of 225 mM $Cl^-_{ext.}$ in tomato. Although a significant amount of Cl^- was translocated to the leaf at 454 mM Cl^-_{ext}, this was 2.5 fold lower than in wheat. An increase in $Cl^-_{ext.}$concentration had no significant influence on the internal Cl^- concentration, demonstrating a Cl^- exclusion mechanism in tomato. Such a Cl^- exclusion mechanism has been reported previously in tomato (Rush and Epstein, 1981). Any exclusion mechanism appeared to breakdown in both wheat and tomato at 454 mM $NaCl_{ext.}$ due, possibly, to plasmolysis of the root cells and breakdown of selective control at the symplastic pathway (Pitman, 1984; Storey and Wyn Jones, 1978 a) leading to massive non-selective uptake of ions and loss of membrane properties.

Even though rapid uptake of ions occurred at 454mM $NaCl_{ext.}$ the ions accumulated appeared to be insufficient to maintain turgor in tissues experiencing a rapid decline in ψ. There are numerous reports of negative turgor pressure in plant cells (Kreeb, 1963; Hellmuth, 1969; Greive, 1961; Slatyer, 1960; Noymeir and Ginzburg, 1969) but the existence of negative turgor has been disputed (Tyree, 1976; Noymeir and Ginzburg, 1967). Claims for negative turgor as a fact or a fallacy seem to depend primarily on differences in the method of determination of ψ_s. In reports of negative turgor, ψ_s was estimated on frozen and re-thawed tissues using a vapour equilibrium technique. Tyree (1976) claims that estimation of ψ_s by such means could

erroneously underestimate ψ_s as solutes in the protoplast are diluted by pure water in the apoplast. The possible error has been estimated to range up to a maximum of 82% of ψ , depending on the volume and water content of the apoplast. Negative turgor potential has never been reported from pressure bomb estimations. However, use of the pressure bomb technique has been questioned by Acock (1975) who claims that the many untested assumptions involved in estimating ψ_s with a pressure bomb lead to a lack of evidence for negative turgor. Iljin's (1930, 1931) theory of the mechanism of drought-induced damage to protoplasm supposes that death occurs in water-stressed cells when small protoplasmic tensions (negative turgor pressures) arise as these would propagate through protein molecules causing deformation of tertiary structures. Negative turgor in salt stressed plants has never been reported but it is possible that abrupt salinization at -2.065 MPa $\psi_{ext.}$ would have altered membrane structure and properties causing deformation resulting in a negative turgor, as suggested for drought stressed cells. The question is difficult to resolve as apoplastic water dilution does not seem a feasible explanation for negative turgor in a salt-stressed plant where salt accumulation would be expected in the apoplast. On the other hand, it seems unlikely that appreciable negative turgor could develop in cells of a plant such as the tomato.

Proline accumulated in both species, accompanied by trigonelline in tomato and glycinebetaine in wheat showed a greater ability to accumulate proline at a comparable $\psi_{ext.}$ of -2.065 MPa. In tomato, although a significant accumulation occurred at -2.065 MPa initially, the rate of accumulation was inhibited by 72 hours and the plants at -1.065 MPa $\psi_{ext.}$ eventually accumulated most proline. This inhibition in proline accumulation at -2.065 MPa $\psi_{ext.}$ would seem to be most likely due to massive uptake of Na^+ following the breakdown in ion exclusion and/or loss of turgor. Both factors would lead to rapid inhibition of protein synthesis, enhanced protein breakdown and cellular disruption leading to inhibition of synthetic function. Similar inhibition of proline accumulation in barley at high $NaCl_{ext.}$ concentration has been suggested to be due to high tissue Na^+ concentration (Chu *et al.*, 1976a).

The sensitivity to inorganic salts of two of the enzymes which are involved in proline accumulation has been examined. *In vitro* activity of Δ^1 -P-5-C dehydrogenase, which catalyses the final step in the biosynthetic pathway from proline to glutamic acid is inhibited by a variety of inorganic ions, but such inhibition would favour proline accumulation rather than oxidation, so cannot be involved in the observed inhibition of proline synthesis. Huber (1974) has reported promotion of the *in vitro* activity of the enzyme Δ^1-P-5-C reductase by NaCl but this response also is not consistent with the inhibition of proline accumulation observed in the present experiment. In contrast, Noguchi *et al.*, (1966) found a high concentration of Na^+ to inhibit incorporation of glutamic acid into proline in tobacco which would lead to reduced proline accumulation. If this is general, then sustained proline accumulation under conditions of high internal Na^+ concentration would depend on Na^+ being sequestered in areas other than the cytoplasm. Hence, the lack of inhibition of proline accumulation in wheat which contained a similar concentration of Na^+(2.49 mmole g^{-1} dw.) as did tomato (2.22 mmole g^{-1}dw) suggests that relatively greater amounts of Na^+ are sequestered in wheat. Lack of compartmental analysis of ion distribution in these two crops limits further discussion on this point.

Accumulation of glycinebetaine was also inhibited at the highest $NaCl_{ext.}$ concentration. The reason for this inhibition is unknown and it has not been reported previously. It is possible that certain enzyme steps in the pathway of synthesis would have been inhibited by high internal Na^+ and /or Cl^- concentration.

The response of trigonelline accumulation in stressed tomato contrasts with the reports of others (Storey and Wyn Jones, 1978; Wyn Jones and Storey, 1981) who found no significant increase in the concentration of trigonelline in tomato subjected to salt stress. This apparent discrepancy in the response observed may be explained by differences in the concentrations of salt solution administered. In the previous experiments the plants were subjected to 100 mM NaCl (-0.48 MPa), whereas, from this study, the apparent threshold for accumulation of trigonelline is -0.565 MPa

$\psi_{ext.}$ However, an increase in trigonelline concentration in response to NaCl stress has been reported for alfalfa (Parameshwara, 1984) and *Leymus sabulosus* (Gorham *et al.*, 1984).

Correlations were obtained in the present experiment (Table 2,3) and by others between accumulation of proline and leaf osmotic potential (Chu *et al.*, 1976a), turgor (Greenway and Leahy, 1972), Na^+ concentration (Voetberg and Stewart, 1984) and between glycinebetaine and osmotic potential (Storey and Wyn Jones, 1978 b). Such correlations may be causal, in either direction or due to relationships through other factors. If accumulation is due to a primary factor, then it is unlikely to be water status as cold stress (Chu *et al.*, 1976) causes proline accumulation. The present experiment does not elucidate this point but the following experiments were planned to explore this issue further.

Table 2 Correlation coefficients of the regression analysis between proline, trigonelline and other factors of salinity in tomato

48 HOURS

	PROLINE	TRIGONELLINE
LEAF WATER POTENTIAL	-0.81*** (L)	-0.87***(CL)
LEAF OSMOTIC POTENTIAL	-0.82***(L)	-0.91***(CL)
LEAF TURGOR POTENTIAL	-0.73** (L)	-0.67** (L)
LEAF Na^+CONCENTRATION	0.90*** (L)	0.93*** (CL)
LEAF Cl^- CONCENTRAION	0.88*** (L)	0.91*** (CL)

72 HOURS

LEAF WATER POTENTIAL	-0.69*** (CL)	-0.92*** (CL)
LEAF OSMOTIC POTENTIAL	-0.78*** (CL)	-0.94*** (CL)
LEAF TURGOR POTENTIAL	-0.71*** (CL)	-0.85*** (CL)
LEAF Na^+CONCENTRATION	0.82*** (CL)	0.94 *** (CL)
LEAF Cl^- CONCENTRATION	0.60 ** (CL)	0.86 *** (CL)

*** (p=0.01) (L) Linear relationship

** (p=0.10) (CL) Curvilinear relationship

Table 3 Correlation coefficients of the regression analysis between proline,glycinebetaine and other factors of salt stress in wheat

48 HOURS

	PROLINE	GLYCINEBETAINE
LEAF WATER POTENTIAL	-0.93*** (L)	-0.81***(CL)
LEAF OSMOTIC POTENTIAL	-0.89***(L)	-0.65***(CL)
LEAF TURGOR POTENTIAL	-0.51NS(L)	-0.22 NS (CL)
LEAF Na^+CONCENTRATION	0.97*** (L)	0.82*** (CL)
LEAF Cl^- CONCENTRATION	0.94*** (L)	0.83*** (CL)

72 HOURS

LEAF WATER POTENTIAL	-0.99***(L)	-0.70***(CL)
LEAF OSMOTIC POTENTIAL	-0.89*** (L)	-0.59*** (CL)
LEAF TURGOR POTENTIAL	-0.16 NS (L)	-0.12NS (CL)
LEAF Na^+CONCENTRATION	0.96*** (L)	0.82 *** (CL)
LEAF Cl^- CONCENTRATION	0.98 *** (L)	0.88 *** (CL)

*** (p=0.01) (L) Linear relationship

NS NOT SIGNIFICANT (CL) Curvilinear relationship

4.1.3 Progressive salinization

The close relationships between solute concentration and a variety of factors in the previous experiment did not allow isolation of the controlling factor in accumulation. Gradual salinization would be expected to allow time for osmotic and metabolic adjustment facilitating turgor maintenance (Chu *et al.*, 1976a) so isolating any influence of turgor on accumulation.

Growth is reduced during salt stress particularly in glycophytes (Greenway and Munns, 1980; Munns and Termaat, 1986) and it has been claimed that proline and glycinebetaine accumulate only when growth is severely reduced (Storey and Wyn Jones, 1978, 1978a; Hanson and Nelson, 1978). It has been surmised that the growth reduction and accumulation of these compounds are inter-related.

The present experiment was planned to examine the growth responses, proline, glycinebetaine and trigonelline accumulation and water and ion status of tomato and wheat during progressive salinization. The concentration of salt applied was limited to 221 mM (-1.065 MPa) NaCl to avoid the the consequences of the higher concentration of salt applied in the previous experiment.

4.1.3.1 Methods

Seedlings of tomato cv. Duke and wheat cv. Sun-9-E were grown at 20 ±1°C day and 17 ± 1°C night temperature, with a 16 hour photoperiod and a photon flux density of 350 to 450 μEin sec^{-1} (Materials and methods 3.2.1 and 3.2.2). NaCl containing full strength Hoaglands solution nutrients was supplied 25 and 10 days after the emergence of the seedlings of tomato and wheat respectively. The water potential of the rooting medium was decreased gradually by -0.1MPa/day for 10 days. On the final day of stress the plants were experiencing -1.065 MPa ψ_{ext}. Youngest fully expanded leaves were sampled on 0, 2, 5 and 11 (tomato) and 0, 2, 5, 9 and 11 days (wheat) from

commencement of stress for the measurement of leaf water, osmotic and turgor potential (3.2.6) proline(3.2.9.2) QACs (3.2.9.3) and leaf ion content (3.2.11). Leaf area and shoot fresh and dry weight were measured (3.2.4). Each treatment was replicated thrice.

4.1.3.2 Results

4.1.3.2.1 Leaf water potential

The leaf water potential of unstressed tomato (Fig 8A) stayed at about -0.25 MPa and of wheat (Fig 8B) ranged from -0.37 to - 0.28 MPa. In stressed tomato plants the leaf water potential dropped in parallel with the external water potential, the reduction being significant only after day 5, whereas in wheat ψ_{leaf} decreased significantly on day 2 but then remained unchanged for 3 days before falling rapidly by day 9-10 increasing thereafter.

4.1.3.2.2 Leaf osmotic potential

Leaf osmotic potential remained unchanged in unstressed tomato (Fig 9A) and wheat (Fig 9B) during the 11 day period of the experiment. The leaf osmotic potential of the control wheat plants was lower than that of tomato. There was a linear decrease in leaf osmotic potential in tomato which matched the decrease in external water potential. In wheat as with the leaf water potential, there was an initial decrease, observed on day 2, which remained unchanged until day 5 followed by a rapid decrease by day 9 and 10 increasing thereafter.

4.1.3.2.3 Leaf turgor potential

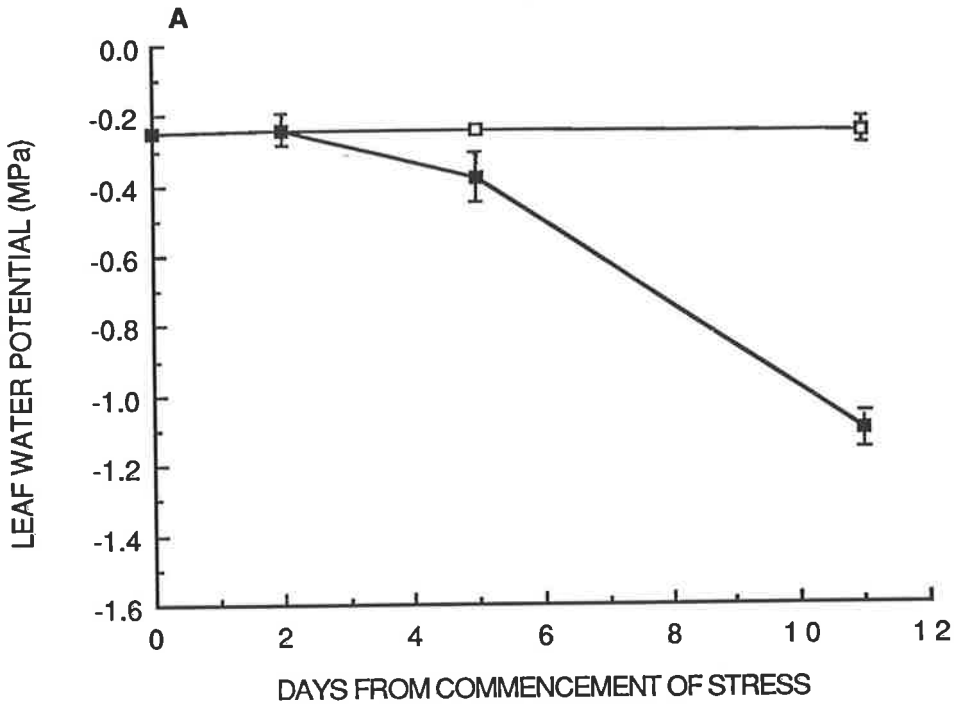
The calculated turgor potential of control wheat plants (Fig 10B) was higher by 0.2 MPa than that of tomato (Fig 10A) and did not change appreciably over the 11 day period of the experiment. The turgor pressure of the stressed tomato plants rose above that of control plants for the first five days and then fell to the control level on day 11. Wheat maintained turgor for the first five days of stress but then dropped significantly

Fig. 8 Changes in leaf water potential (-MPa) of tomato and wheat exposed to progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
LEAF WATER POTENTIAL (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

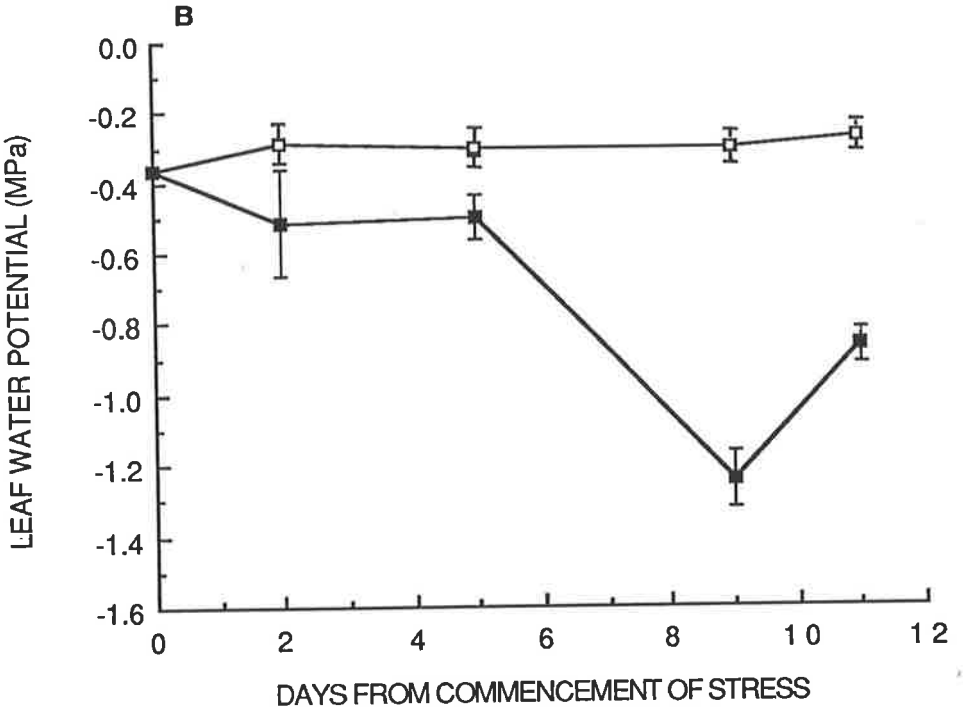
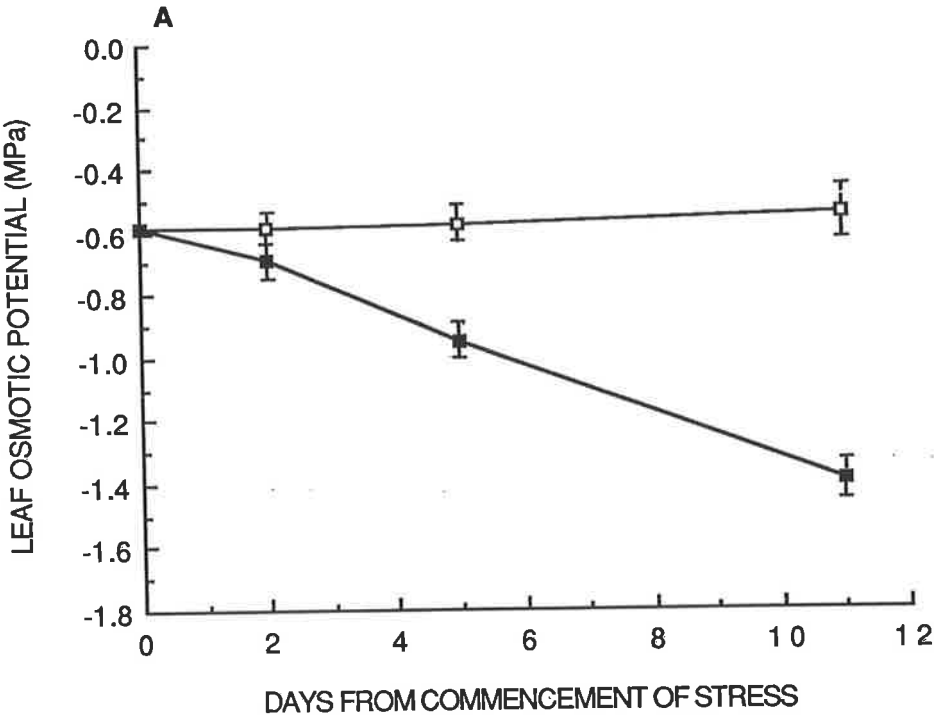

B
LEAF WATER POTENTIAL (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Fig. 9 Changes in leaf osmotic potential (-MPa) of tomato and wheat exposed to progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
LEAF OSMOTIC POTENTIAL (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

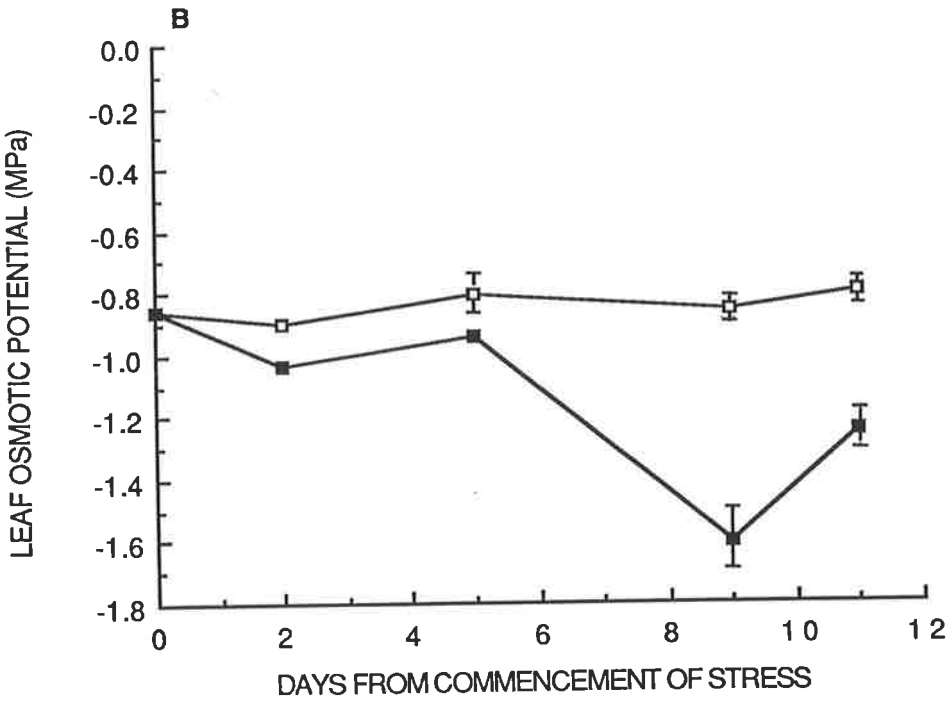
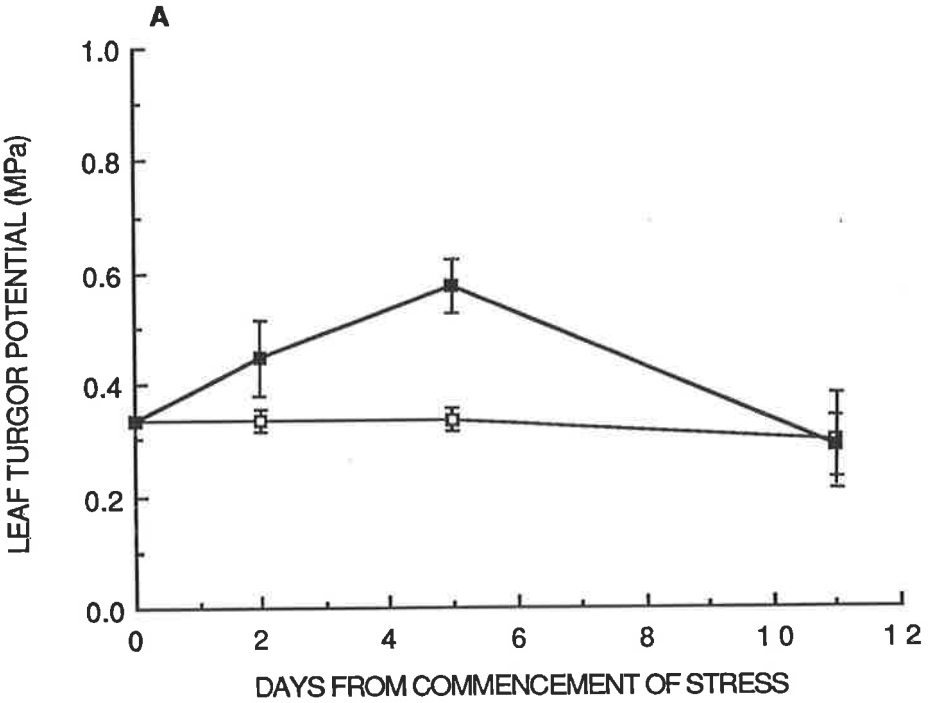

B
LEAF OSMOTIC POTENTIAL (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

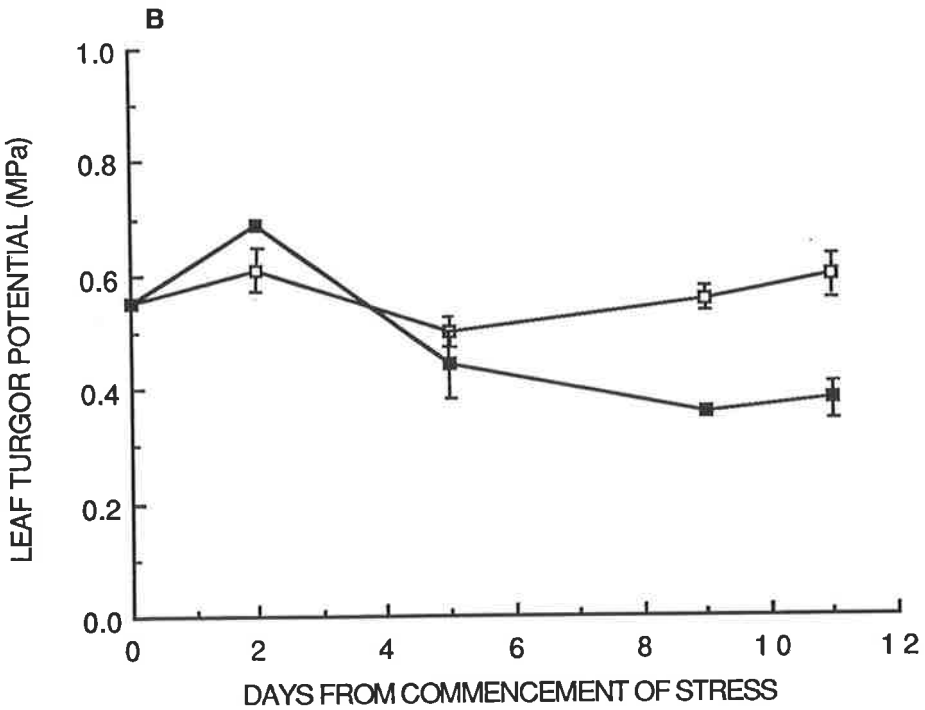
Fig. 10 Changes in leaf turgor potential (MPa) of tomato and wheat exposed to progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
LEAF TURGOR POTENTIAL (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


B
LEAF TURGOR POTENTIAL (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

below the control value. Nevertheless, the NaCl stressed wheat plants maintained positive turgor during the entire period of stress.

4.1.3.2.4 Leaf sodium concentration

The leaf sodium concentration in unstressed plants of wheat (Fig 11B) and tomato (Fig 11A) was low and remained unchanged throughout the experimental period. The leaf Na^+ concentration increased significantly on day 2 in wheat whereas an increase was observed only after day 5 in tomato. Substantial Na^+ uptake occurred only after day 5 in both species. On day 11, tomato contained a higher (0.91 mmole g^{-1}dw or 235.0 mM) concentration of Na^+ compared to wheat (0.39 mmole/g.d.wt. or 94.7 mM). The increase in Na^+concentration was proportional to the $Na^+_{ext.}$

4.1.3.2.5 Leaf chloride concentration

The leaf chloride concentration of control tomato plants (Fig 12A) was lower than that of wheat (Fig 12B) and did not change over the 11 day period of the experiment. In wheat, a significant increase in leaf Cl^- concentration was recorded on day 2 reaching 0.64 mmole g^{-1}dw(251.8 mM) by day 11. In tomato, however, leaf Cl^- did not increase until day 5 with a concentration of 0.48 mmole g^{-1}dw (196.4 mM) being reached by day 11. Cl^- uptake was in proportion to $Cl^-_{ext.}$

4.1.3.2.6 Proline

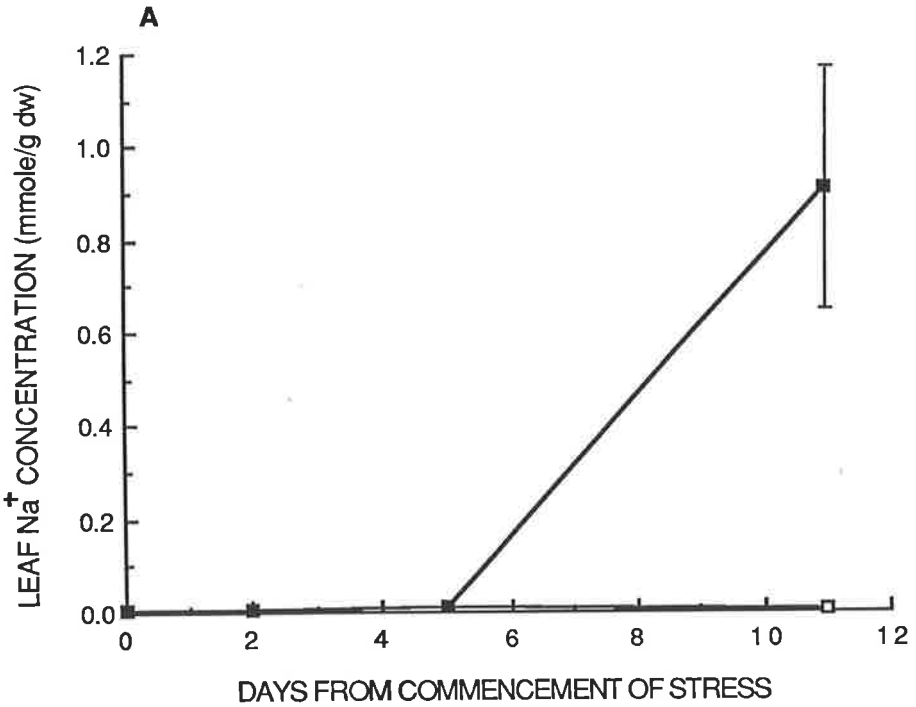
The leaf proline concentration of control plants of tomato (Fig 13A) and wheat (Fig 13B) was low and remained unchanged during the 11 day period of the experiment. Accumulation commenced at or just after day 5 in both species, but the rate of accumulation was greater in tomato. Tomato had accumulated 6.6 fold more proline (8.20 mg g^{-1} dw) than wheat (1.25 mg g^{-1} dw) by day 11. There was no evidence that proline accumulation had ceased by day 11.

Fig. 11 Leaf Na^+ concentration (mmole,g^{-1}dw) of tomato and wheat exposed to progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LEAF Na+ CONCENTRATION (mmole/g dw)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

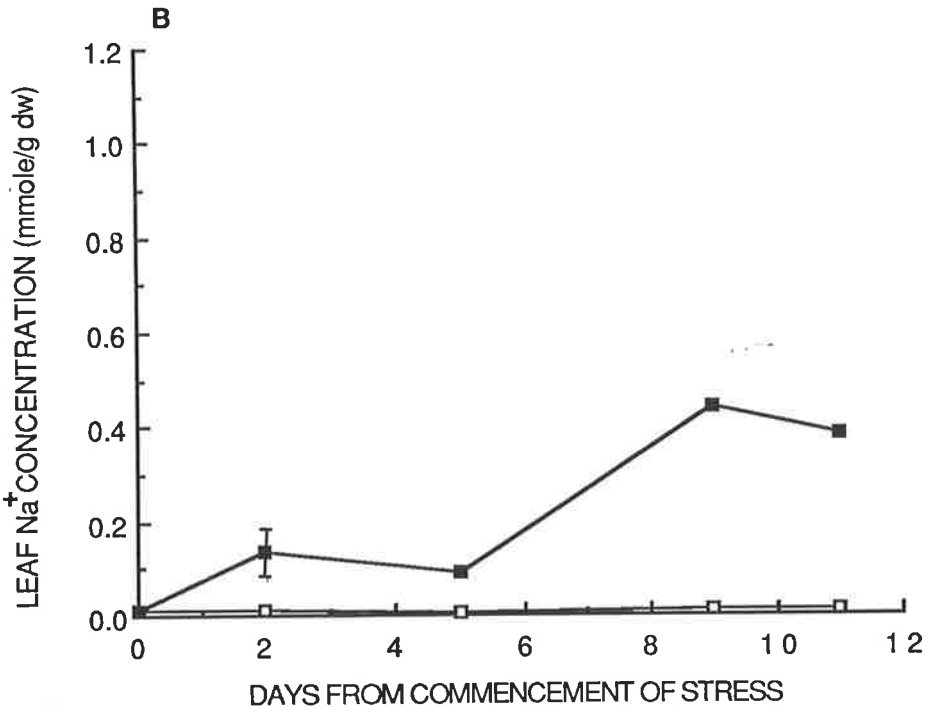
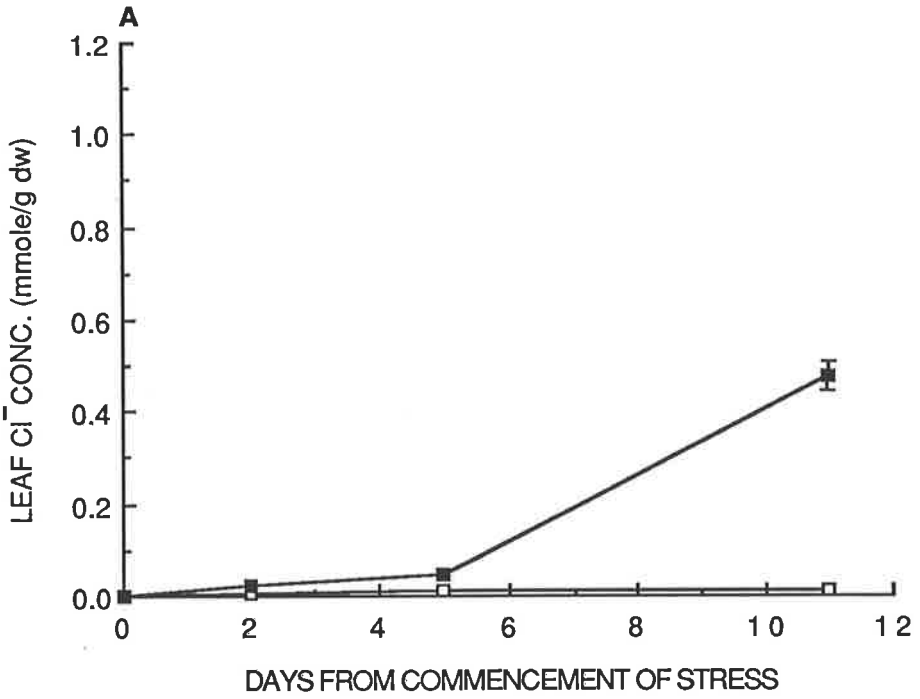

B
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LEAF Na+ CONCENTRATION (mmole/g dw)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Fig. 12 Leaf Cl^- concentration (mmole,g^{-1}dw) of tomato and wheat exposed to progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LEAF Cl⁻ CONC. (mmole/g dw)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

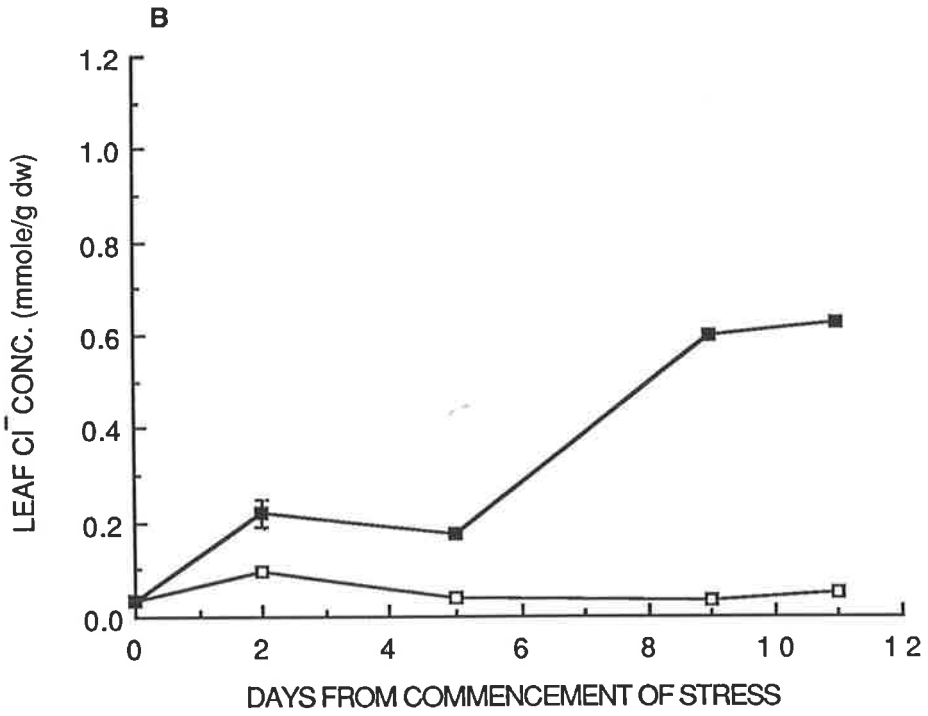
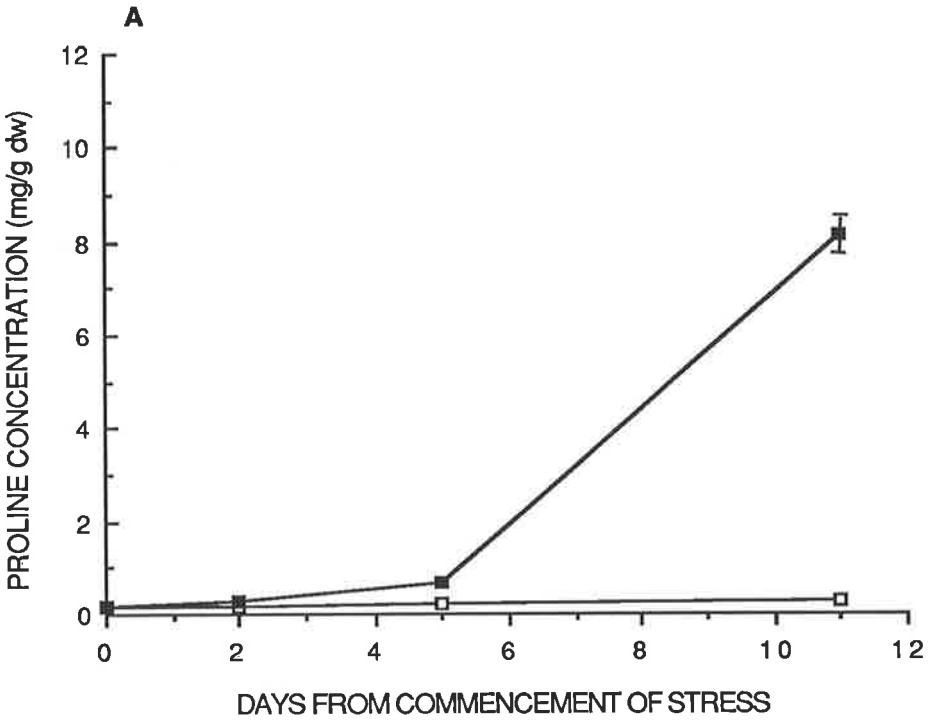

B
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LEAF Cl⁻ CONC. (mmole/g dw)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

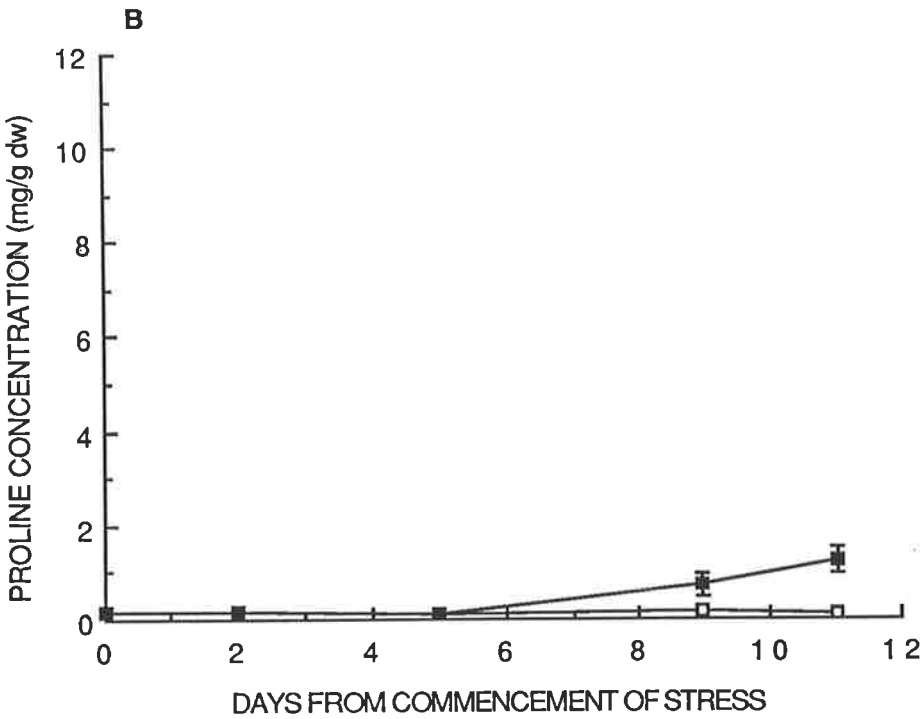
Fig. 13 Leaf proline concentration (mg,g^{-1}dw) of tomato and wheat exposed to progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
PROLINE CONCENTRATION (mg/g dw)
12
10
8
6
4
2
0
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


B
PROLINE CONCENTRATION (mg/g dw)
12
10
8
6
4
2
0
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

4.1.3.2.7 Quaternary Ammonium Compounds

A. Trigonelline

Unstressed tomato plants contained about 0.80 mg g^{-1} dw trigonelline (Fig 14A). There was a small but significant increase in the concentration of trigonelline with stress by day 2 but this did not continue and stressed plants contained only 1.1 mg g^{-1} dw on day 11.

B. Glycinebetaine

The leaf glycinebetaine concentration in control wheat plants (Fig 14B) was about 1.0 mg g^{-1} dw and did not change significantly with time. In NaCl stressed plants however, glycinebetaine accumulated from day 5, to reach a concentration of 5.6 mg g^{-1}dw. on day 11. As with proline, there was no evidence that glycinebetaine accumulation had then ceased.

4.1.3.2.8 Growth

A. Shoot fresh weight

Although fresh weight of both tomato (Fig 15A) and wheat (Fig 15B) appeared to be reduced by the salinity treatment, this reduction was at no time statistically significant.

B. Leaf area

A reduction in leaf area was observed in salt stressed plants of tomato (Fig 16A) and wheat (Fig 16B) with a relative reduction of 40 and 37 per cent respectively compared to the control plants on day 11. It is noteworthy that stressed plant leaf area continued to expand even as the stress intensified.

C Shoot dry weight

The dry matter production of both tomato and wheat when stressed was similar to that

Fig. 14A Leaf trigonelline concentration (mg,g^{-1}dw) of tomato exposed to progressive salinization

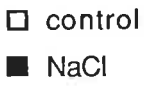
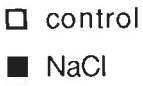
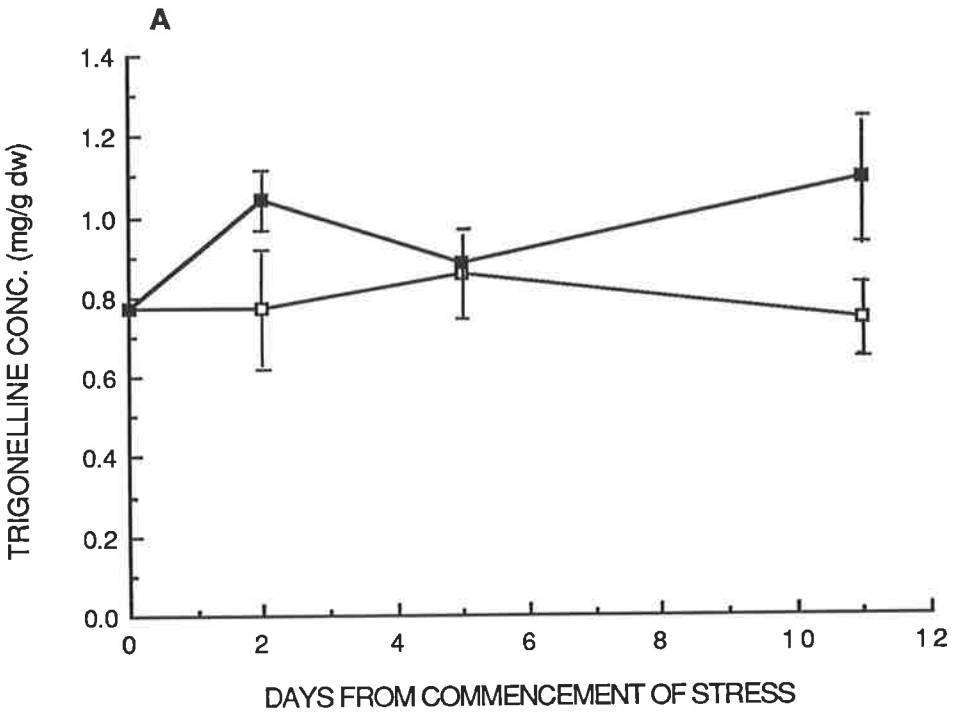


Fig. 14B Leaf glycinebetaine concentration (mg,g^{-1}dw) of wheat exposed to progressive salinization




A
TRIGONELLINE CONC. (mg/g dw)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

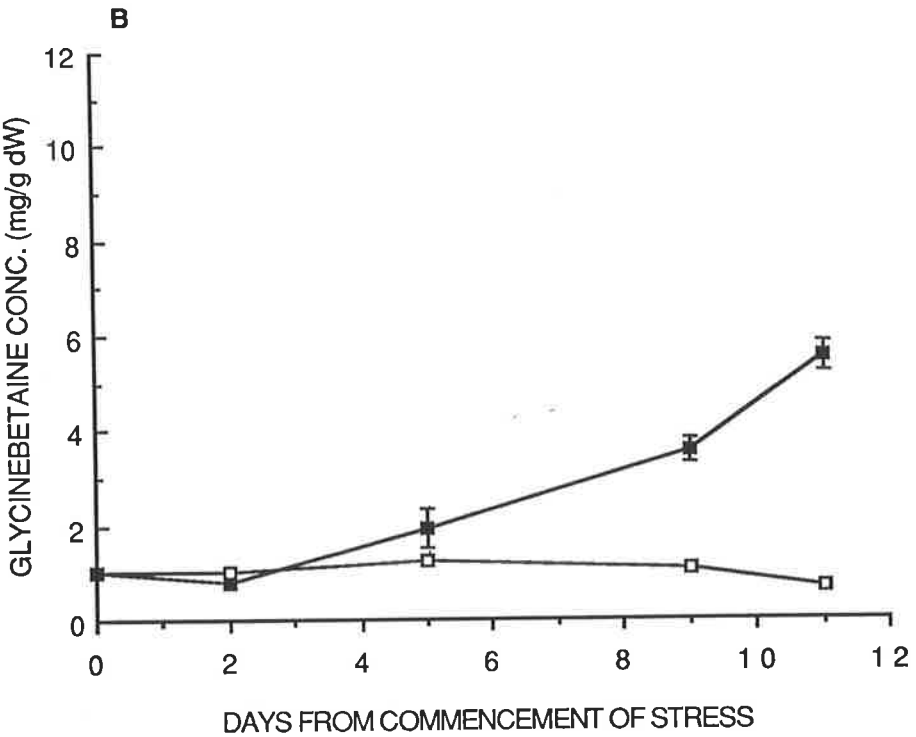
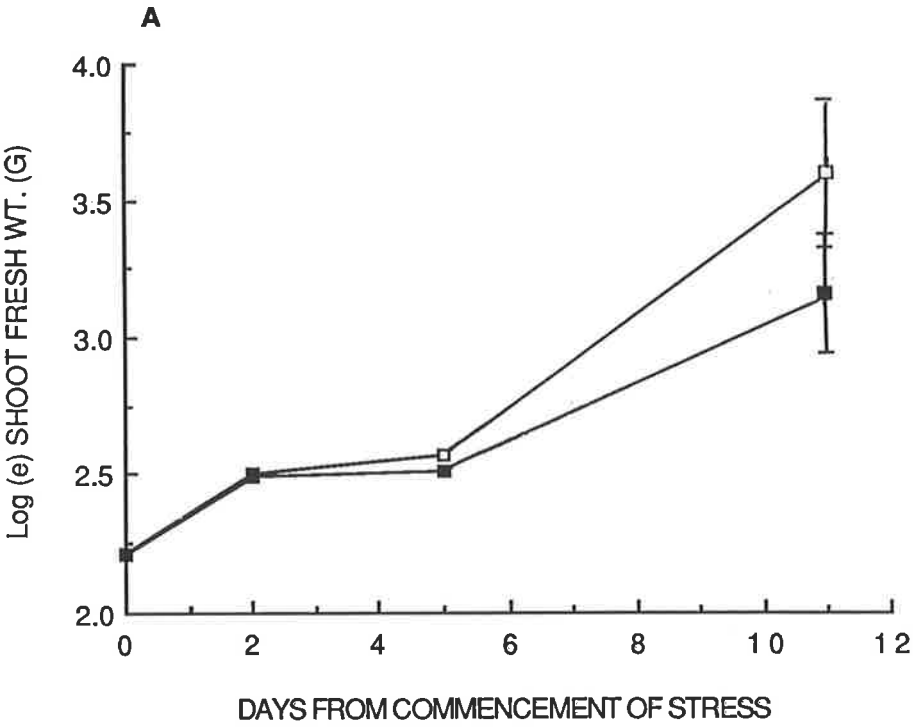

B
GLYCINEBETAINE CONC. (mg/g dW)
12
10
8
6
4
2
0
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Fig. 15 Shoot fresh weight of tomato and wheat as influenced by progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
4.0
3.5
3.0
2.5
2.0
Log (e) SHOOT FRESH WT. (G)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

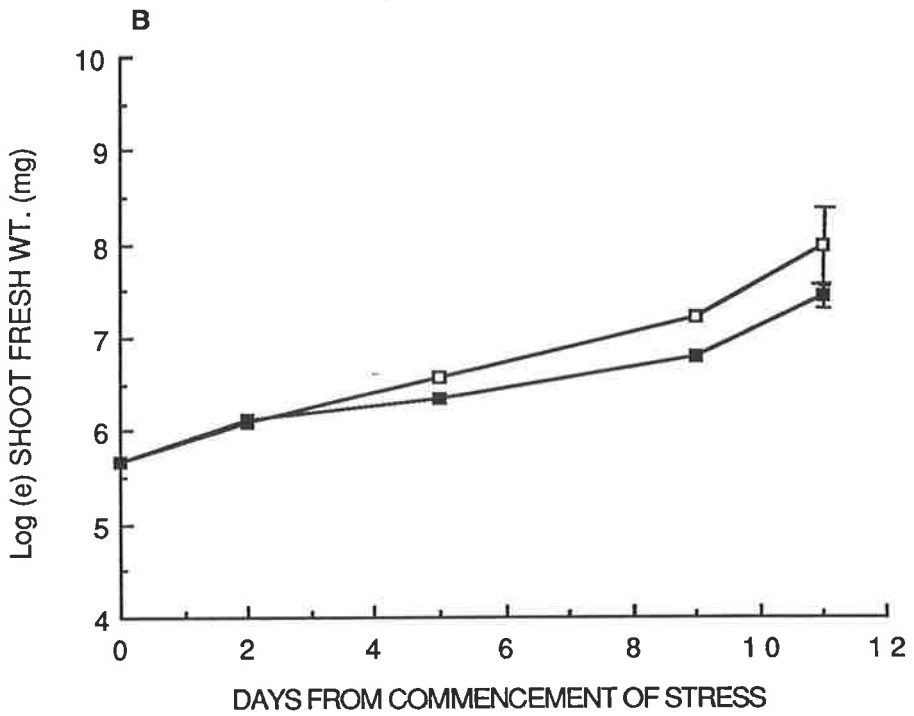
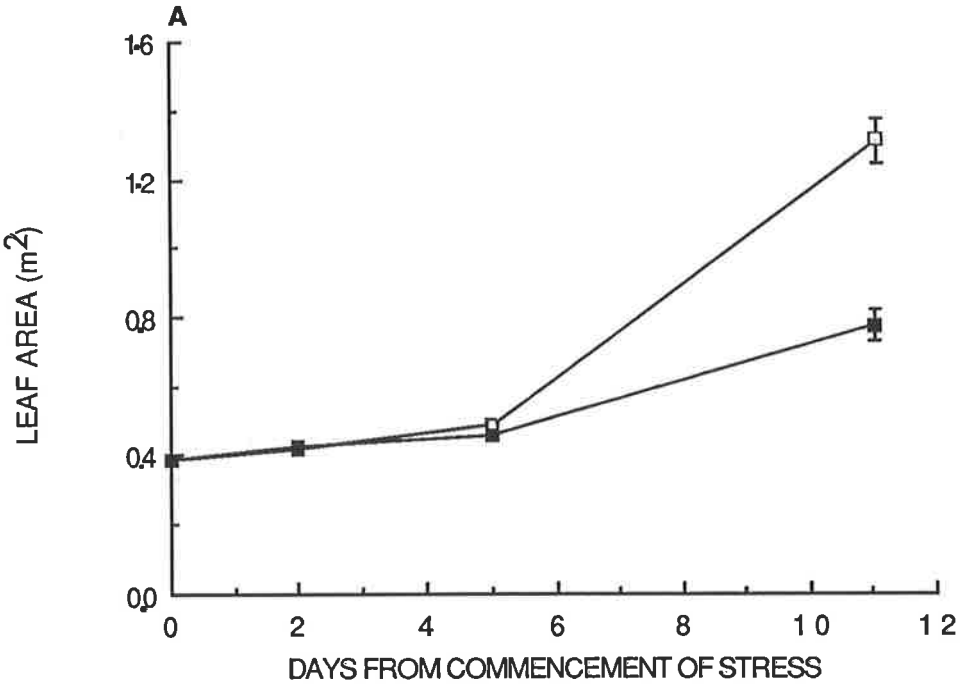

B
10
9
8
7
6
5
4
Log (e) SHOOT FRESH WT. (mg)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

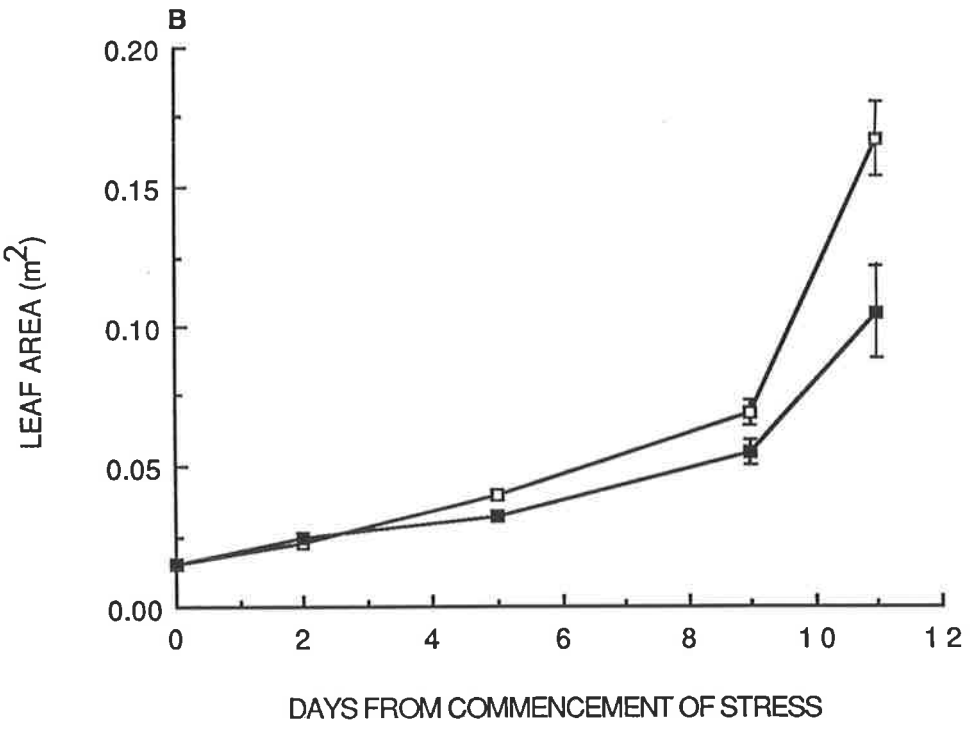
Fig. 16 Leaf area expansion of tomato and wheat as influenced by progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
1·6
1·2
0·8
0·4
0·0
LEAF AREA (m2)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


B
0.20
0.15
0.10
0.05
0.00
LEAF AREA (m2)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

of control plants throughout (Fig 17A,B). The apparent initial reduction in tomato was due to fluctuation in the control, not the stress plants.

4.1.3.3 Discussion

Accumulation of proline and glycinebetaine was correlated with the decreases in leaf water, osmotic potential and Na^+ and Cl^- concentrations but not with turgor. Both proline and glycinebetaine accumulated despite the plants maintaining turgor, which would appear to rule out turgor as the controlling factor. As the salt stress was imposed gradually there was time for osmotic adjustment in the plants to cope with decreasing $\psi_{ext.}$ by the uptake of ions and synthesis of organic solutes. Acceptance of the conclusion that turgor does not control accumulation rests on the accuracy of the determination of turgor potential. Turgor potential was derived from differences between water and osmotic potential measured psychrometrically. The procedure for osmotic potential with frozen, re-thawed tissue (Ehling, 1961) has been questioned (Oertli, 1966) as, in salt-stressed plants salt may accumulate in cell walls external to the protoplast and contribute to the measured osmotic potential. As a consequence, the measured osmotic potential would be erroneously low and the turgor potential erroneously high. No direct estimate of this possible error can be made for the present data but visual observations support the measured turgor and the negative turgor observed in the previous experiment suggests that apoplastic salt levels were low or they would have lowered the measured osmotic potential and negative turgor would not have been realised. The increase in turgor above control levels may have been due to a significant increase in internal Na^+ and Cl^- lowering osmotic potential relatively more than the decrease in $\psi_{leaf.}$ It is also possible that reduced transpiration due to stomatal closure together with the decreasing ψ_s led to higher turgor (Oertli,1966).

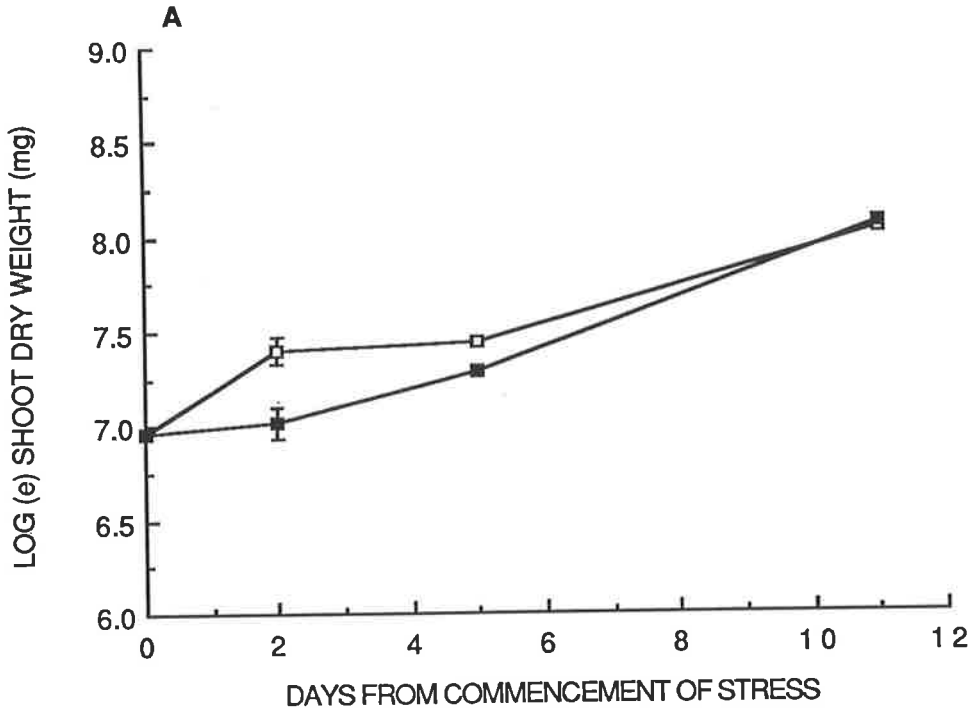
The conclusion that turgor here did not control proline accumulation is in contrast with the reports of several others (Ordin, 1970; Greenway and Leahy,1970,1972; Jones,1973; Setter and Greenway,1979). Manipulating turgor by administering 3-orthomethylglucose to *Chlorella emersonii,* Greenway *et al.*(1982) concluded that

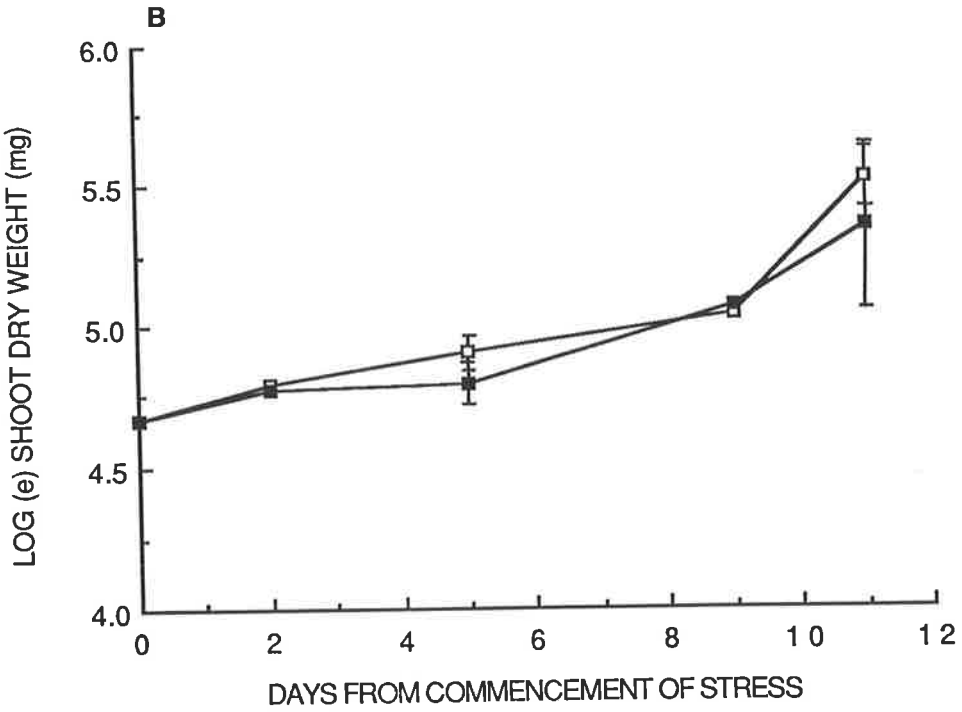
Fig. 17 Shoot dry matter production of tomato and wheat as influenced by progressive salinization

A. tomato

B. wheat

□ control
■ NaCl


A
9.0
8.5
8.0
7.5
7.0
6.5
6.0
LOG (e) SHOOT DRY WEIGHT (mg)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


B
6.0
5.5
5.0
4.5
4.0
LOG (e) SHOOT DRY WEIGHT (mg)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

turgor or cell volume is the primary signal for proline accumulation. This apparent difference in response may reflect differences in the location or activity of the enzymes concerned in the various metabolic responses or be an artifact caused by difference in the experimental system. In particular, the reports of Greenway and Leahy(1972), wherein ethylene glycol was used to stress excised tissue, differ from the present experiment. Ethylene glycol is a rapidly permeating osmoticum with a reflection coefficient of 0.5 and the influence of ethylene glycol on the metabolism of proline itself is not known. In the other study (Setter and Greenway, 1979), a fresh water alga, *Chlorella emersonii,* was used as the test system and cells were transferred suddenly from a low to a high concentration. It is not known how realistic it is to extrapolate the mechanism existing in a fresh-water alga to a higher plant. In addition, in both studies neither the water nor the ion status of the test system was reported. The reports of Chu *et al.*,(1976) also showed proline accumulation despite turgor maintenance, in barley. However, it could be argued that turgor falls temporarily with each incremental decrease in $\psi_{ext.}$ but readjusts through ion uptake and solute synthesis within 24 h; this could only be checked by continuous recording of turgor or by demonstrating continuous proline accumulation at a steady $NaCl_{ext.}$ over a period of days.

Trigonelline, although accumulated initially, did not continue to accumulate and the concentration was low compared to that induced by abrupt salinization. Suspended accumulation despite changes in $\psi_{ext.}$ and internal water and ion status, suggests that none of these factors exert control over trigonelline accumulation. Trigonelline has been suggested as a storage form of nicotinic acid (NA) and a member of a pyridine nucleotide pathway, of which the function is to generate NAD (Godavari and Waywood, 1970). From the available evidence it would seem that large amounts of radio-labelled NA and NAD are converted to trigonelline and trigonelline is converted back to its intermediates in the pyridine nucleotide pathway (Tramontano *et al.*, 1982). It is possible that reduced availability of NA and NAD would result in reduced trigonelline accumulation. NAD is a preferred electron acceptor for proline synthesis (Stewart and

Lai, 1974; Boggess *et al.*, 1975) and if large amounts of NAD are trapped in the synthesis of proline, trigonelline accumulation may be suspended. Thus, the accumulation of proline may exert some control over trigonelline accumulation. An increased trigonelline concentration was observed as a consequence of exogenous proline application in fenugreek (Klein and Linser, 1932) which also suggests an inter-relationship between proline and trigonelline accumulation.

If the possibility that turgor controls accumulation is eliminated, ψ_s remains. A decrease in ψ_s can be due either to loss of water or to an increase in dissolved solutes, but water loss is unimportant where turgor is maintained. Both Na^+ and Cl^- accumulated and would have undoubtedly contributed to the osmotic pool, together with organic solutes. It is essential to estimate the contribution of the accumulated organic solutes to the osmotic pool in order to establish whether the observed correlation with ψ_s is due to the increase in organic solutes itself. If we postulate that all these compounds are evenly distributed throughout the cell, the maximum concentration observed would have exerted an osmotic potential of -0.086 (proline), -0.035 (trigonelline) and -0.052 MPa (glycinebetaine). These contributions are relatively insignificant compared to the total decrease in osmotic potential which suggests that it is unlikely that the apparent relationship with ψ_s is due to their contribution to the osmotic potential.

Rate of salinization altered the pattern of proline accumulation in both species. The concentration of proline accumulated was low in wheat exposed to gradual salinization compared to salt shock but the reverse was true with tomato. It is generally assumed that gradual salinization allows the plant to undergo metabolic adjustment which reduces the metabolic impact of stress. This accounts for lower proline accumulation in wheat (this study) and barley, (Chu *et al.*, 1976 a; Storey and Wyn Jones, 1978b). However, such an explanation cannot be generalised as tomato accumulated more proline during progressive salinization than during salt shock. Glycinebetaine accumulation was also influenced by the nature of salinity imposition, a relatively higher concentration being observed following progressive rather than abrupt

salinization. A similar response has been reported for barley (Storey and Wyn Jones, 1978b). Choline oxidase, an important enzyme in glycinebetaine synthesis, has been reported to be less active during short term stress and to account for the low concentration of glycinebetaine in salt shock (Hanson *et al.*, 1985). Such a shift in response of proline and glycinebetaine in these two salinization systems occurred without marked changes in leaf water or ion status.

The rate of salinization also altered the uptake pattern of Na^+ and Cl^- in both species. Tomato excluded Cl^- at 221 mM $NaCl_{ext.}$ during salt shock but accumulated a high concentration of Cl^- during gradual salinization to that external concentration. Wheat on the other hand, imported more Cl^- during abrupt than in progressive salinization. In tomato, a similar alteration in the pattern of Na^+ uptake was observed. Barley also has been found to take up less Na^+ and Cl^- during abrupt than during a pseudo- steady state of salinization (Storey and Wyn Jones, 1978a). A low rate of uptake of Na^+ and Cl^- during progressive salt stress has been attributed to preservation of membrane properties and selectivity of the root cells (Pitman, 1984). The concentration of ions in the shoot will also depend upon the duration of the treatment and the growth rate, with dilution of ions by growth in salinity stress extending for a period of time (Gates *et al.*, 1970). In this case, however, the response would be unlikely to be due to growth-induced dilution in concentration because Na^+ and Cl^- were estimated for the youngest fully expanded leaf and expressed on a dry weight basis.

It has been suggested that accumulation of both proline and glycinebetaine occurs only when growth is severely reduced (Storey and Wyn Jones, 1978) suggesting that the role of proline and glycinebetaine accumulation is related to survival rather than maintenance of growth. The results obtained in the present investigation are contrary to these claims as significant amounts of proline and glycinebetaine accumulated with no reduction in dry matter gain. Although shoot fresh and dry weights were not influenced by salt stress, leaf expansion was inhibited signifying that dry matter production would be eventually reduced.

4.1.4 Prolonged salinization

Prolonged exposure of plants to a particular salt concentration could be expected to lead to a constant internal ion concentration, as found in *Suaeda maritima* (Yeo, 1974) and rice (Ladenburger and Albert,1981). A constant $\psi_{ext.}$ could also be expected to lead to a steady mean $\psi_{leaf,}$ apart from diurnal variations. Plants in a steady state of internal ion and water status would be useful material to facilitate further understanding of the accumulation of compatible solutes and their relationships with these variables of salt stress. The present investigation has this aim.

4.1.4.1 Methods

Seedlings of tomato cv. Duke and wheat cv. Sun-9-E were raised at 20±1°C day and 17±1°C night temperature with a 16 hour photoperiod and a photon flux density of 350 to 450 μEin sec^{-1} (Materials and Methods 3.2.1). Seedlings grown in Hoagland nutrient solution until 25 (tomato) or 10 (wheat) days after emergence were provided with either full strength Hoagland nutrient solution alone or full strength Hoagland solution containing NaCl. The water potential of the external rooting medium was reduced gradually by -0.10 MPa day^{-1} until 10 days reaching -1.065 MPa (221 mM) $NaCl_{ext.}$ concentration and was then maintained at this level for a further period of 40 days after reaching the final salt concentration. The youngest fully expanded leaves of tomato and wheat were harvested 1, 10, 20 and 40 days for measurement of leaf water, osmotic and turgor potential (3.2.6), proline (3.2.9.2), QACs(3.2.9.3) and leaf ion content(3.2.11). Leaf area and shoot fresh and dry weights were also measured (3.2.4). Relative growth rate was calculated (Hunt,1982) using the formula $_{1\text{-}2}R = (\log_e W_2 - \log_e W_1)/ (T_2-T_1)$ where, R is relative growth rate, $\log_e W_1, W_2$ are $\log_e$ dry weights at times T_1 and T_2. The experimental design was a Factorial Randomised Block with three replications of each treatment.

4.1.4.2 Results

4.1.4.2.1 Leaf water potential

There was a significant decrease in ψ_{leaf} of salt stressed tomato plants, reducing $\psi_{ext.}$ by 1.0 MPa, led to a fall in ψ_{leaf} of nearly the same extent. This fall was almost complete within one day, and the interaction between stress and time was not significant (Table 4). In wheat (Fig 18), however, this interaction was significant as ψ_{leaf} decreased with time despite $\psi_{ext.}$ remaining constant. Here a reduction in $\psi_{ext.}$ by 1.0 MPa produced a fall of more than 2.0 MPa in ψ_{leaf}. ψ_{leaf} of control plants also fell slightly during this period.

4.1.4.2.2 Leaf osmotic potential

ψ_s in salt stressed tomato plants decreased compared to the control by about the same amount as $\psi_{ext.}$ decreased. This change was essentially complete within one day and the interaction between treatments and duration of stress was not significant (Table 5). In wheat, ψ_s decreased gradually but significantly until the 40th day, reaching -3.10 MPa (Fig 19). Again, as with ψ_{leaf}, ψ_s in control plants tended to fall over the 40 days but the decrease was not statistically significant.

4.1.4.2.3 Leaf turgor potential

The calculated turgor potential of stressed tomato plants remained above the control values until day 20 but decreased significantly below the control level on day 40 (Table 6). There were no significant differences in turgor between control and salt-stressed wheat plants at any time, although the values fluctuated over the 40 day period (Table 7).

4.1.4.2.6 Leaf sodium concentration

The leaf Na^+ concentration of both control tomato (Fig 20A) and wheat plants (Fig 20B) was low and remained unchanged until the end of the experiment whereas in

Table 4 Effect of steady state of salinization on leaf water potential of tomato (MPa)

Treatments	Days at -1.065 MPa				
	1	10	20	40	MEAN
CONTROL	-0.25	-0.34	-0.31	-0.27	-0.29
NaCl	-1.11	-1.15	-1.21	-1.32	-1.20***
DIFFERENCE	0.87	0.81	0.91	1.04	0.91

SOURCE OF VARIATION		L.S.D (p=0.05)
TREAT	493.207 ***	0.085
PERIOD	1.451 NS	
TREAT X PERIOD	1.474 NS	

*** (p=0.01)
NS -Not significant

Fig. 18 Changes in leaf water potential (-MPa) of wheat exposed to steady state of salinization

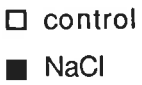
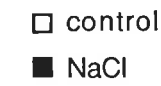
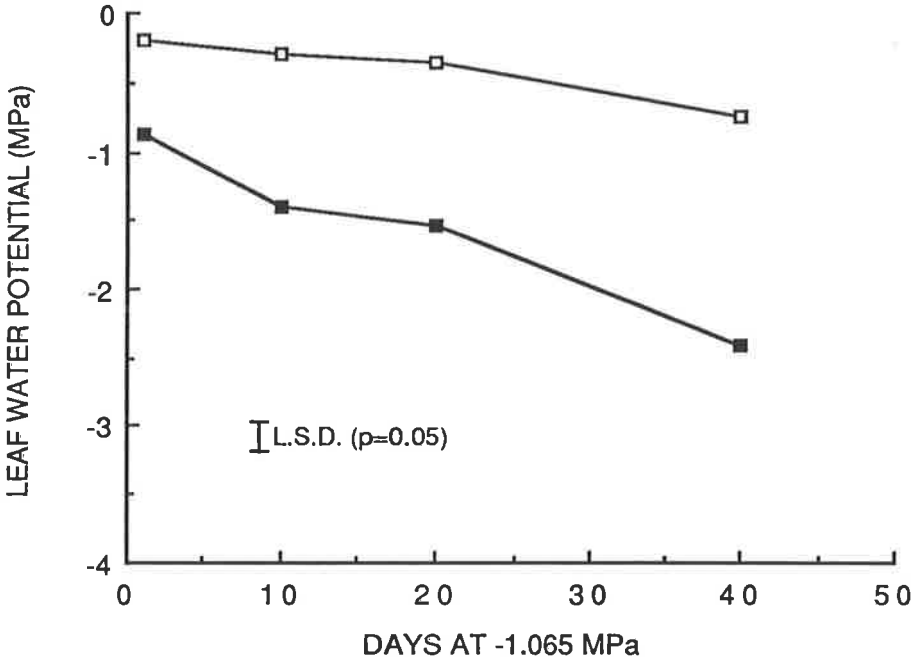


Fig. 19 Changes in leaf osmotic potential (-MPa) of wheat exposed to steady state of salinization




LEAF WATER POTENTIAL (MPa)
0
-1
-2
-3
-4
L.S.D. (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

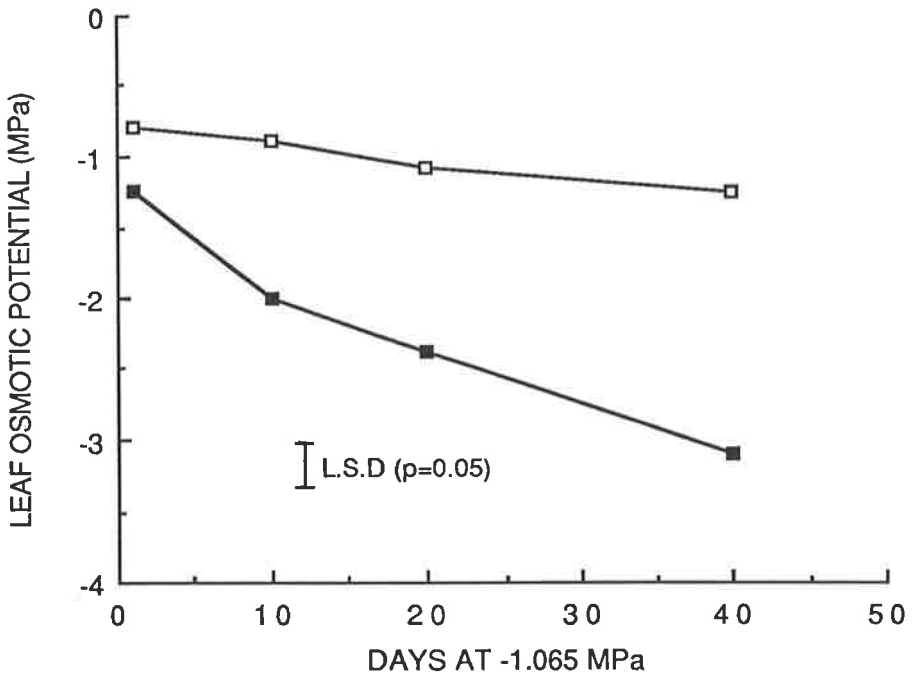

LEAF OSMOTIC POTENTIAL (MPa)
0
-1
-2
-3
-4
L.S.D (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

Table 5 Effect of steady state of salinization on leaf osmotic potential of tomato (MPa)

Treatments	Days at -1.065 MPa				
	1	10	20	40	MEAN
CONTROL	-0.55	-0.62	-0.68	-0.68	-0.64
NaCl	-1.36	-1.62	-1.68	-1.45	-1.53***
DIFFERENCE	0.81	1.00	0.99	0.77	0.89

SOURCE OF VARIATION		L.S.D (p=0.05)
TREAT	316.50 ***	0.104
PERIOD	3.701 ***	0.148
TREAT X PERIOD	1.466 NS	

*** (p=0.01)
NS -Not significant

Table 6 Effect of steady state of salinization on leaf turgor potential of tomato (MPa)

Treatments	Days at -1.065 MPa				
	1	10	20	40	MEAN
CONTROL	0.31	0.28	0.38	0.36	0.33
NaCl	0.25	0.47	0.46	0.13	0.33
DIFFERENCE	-0.06	+0.19	+0.08	-0.23	

SOURCE OF VARIATION		L.S.D (p=0.05)
TREAT	0.056 NS	
PERIOD	2.124 NS	
TREAT X PERIOD	5.561***	0.22

*** (p=0.01)
NS -Not significant

Table 7 Effect of steady state of salinization on leaf turgor potential of wheat (MPa)

Treatments	Days at -1.065 MPa				
	1	10	20	40	MEAN
CONTROL	0.60	0.59	0.74	0.52	0.61 NS
NaCl	0.38	0.62	0.83	0.69	0.63 NS
DIFFERENCE	-0.26	+0.03	+0.09	+0.17	+0.02

SOURCE OF VARIATION	
TREAT	0.054 NS
PERIOD	2.169 NS
TREAT X PERIOD	1.042 NS

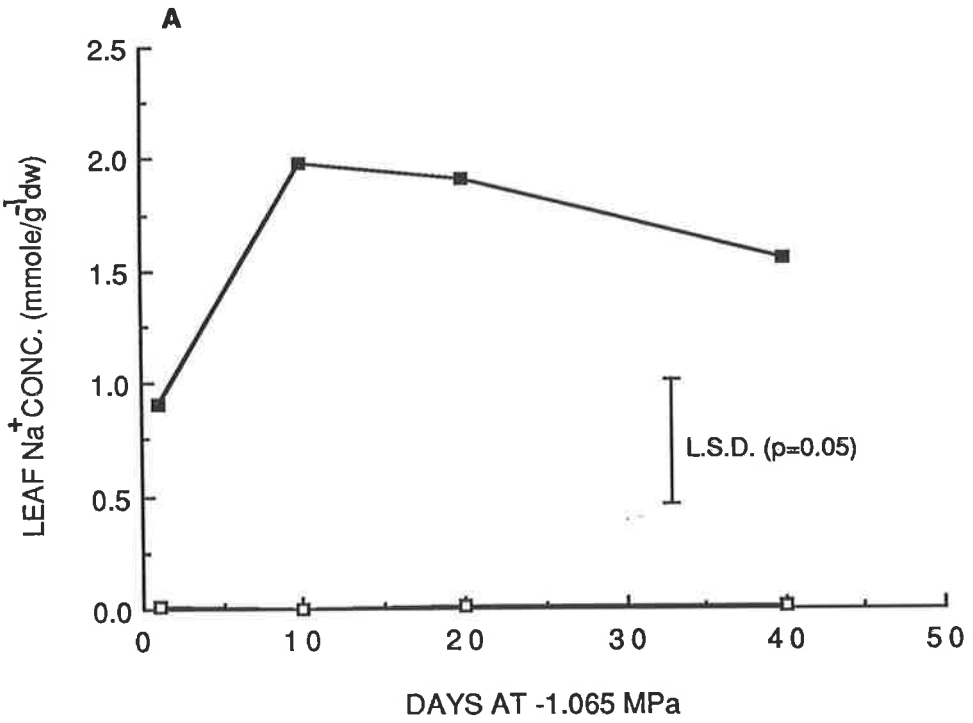
NS -Not significant

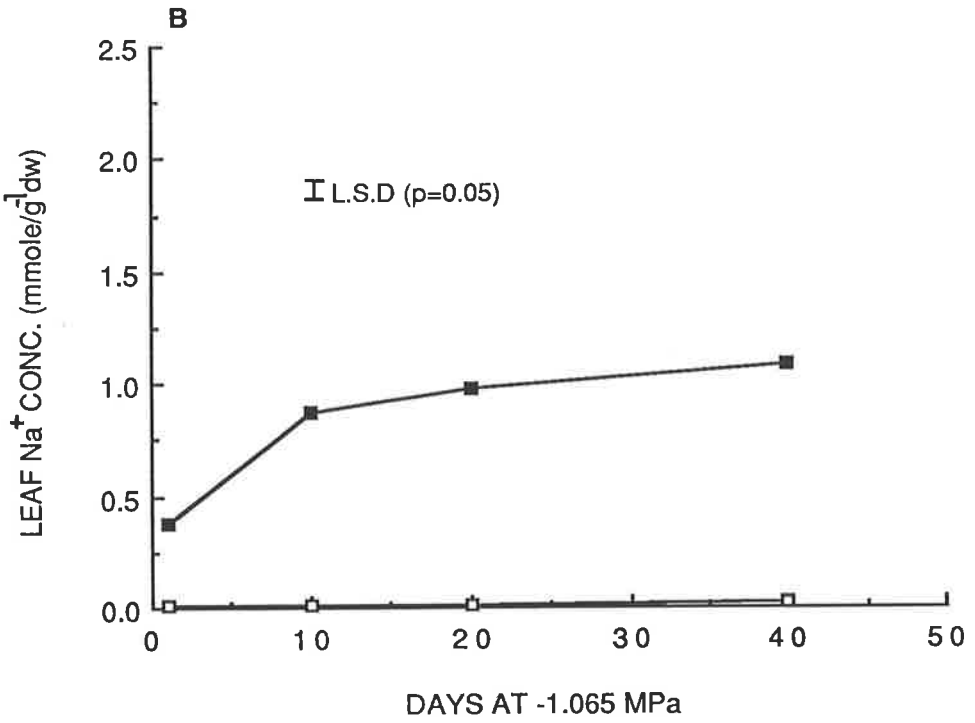
Fig. 20 Leaf Na^+ concentrations (mmole, $g^{-1}dw$) of tomato and wheat as influenced by steady state of salinization

A. tomato

B. wheat

□ control
■ NaCl


A
2.5
2.0
1.5
1.0
0.5
0.0
LEAF Na+CONC. (mmole/g-1dw)
L.S.D. (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa


B
2.5
2.0
1.5
1.0
0.5
0.0
LEAF Na+CONC. (mmole/g-1dw)
L.S.D (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

stressed tomato and wheat there was a significant uptake of Na^+ until day 10 with leaf concentration remaining relatively constant thereafter until the end of the experiment. Tomato accumulated almost twice as much Na^+ as did wheat.

4.1.4.2.7 Leaf chloride concentration

The leaf chloride concentration of unstressed tomato(Fig. 21A) and wheat (Fig 21B) was also low and unaltered over the 40 day period of the experiment. A similar trend to that for Na^+ was observed for Cl^- uptake by stressed plants of both species. Leaf Cl^- concentrations increased significantly until day 10 and thereafter remained relatively unchanged. In contrast to the results for Na^+, however, tomato and wheat contained similar amounts of chloride on day 40.

4.1.4.2.8 Proline

The leaf proline concentration was low and remained unchanged in unstressed wheat (Fig 22B) and tomato (Fig 22A). The leaf proline concentration in stressed tomato increased until day 10, but then fell significantly by day 40. In wheat, in contrast, accumulation began more slowly but it continued to the end of the experiment. There was no evidence that accumulation had ceased by day 40 in wheat.

4.1.4.2.9 Quaternary Ammonium Compounds

A. Trigonelline

The trigonelline concentration in both control and stressed tomato (Fig.23A) leaves fluctuated considerably, but there was a trend for trigonelline content always to be higher in stressed plants (0.98 -control ; 1.65 mg g^{-1} dw -stress)

B. Glycinebetaine

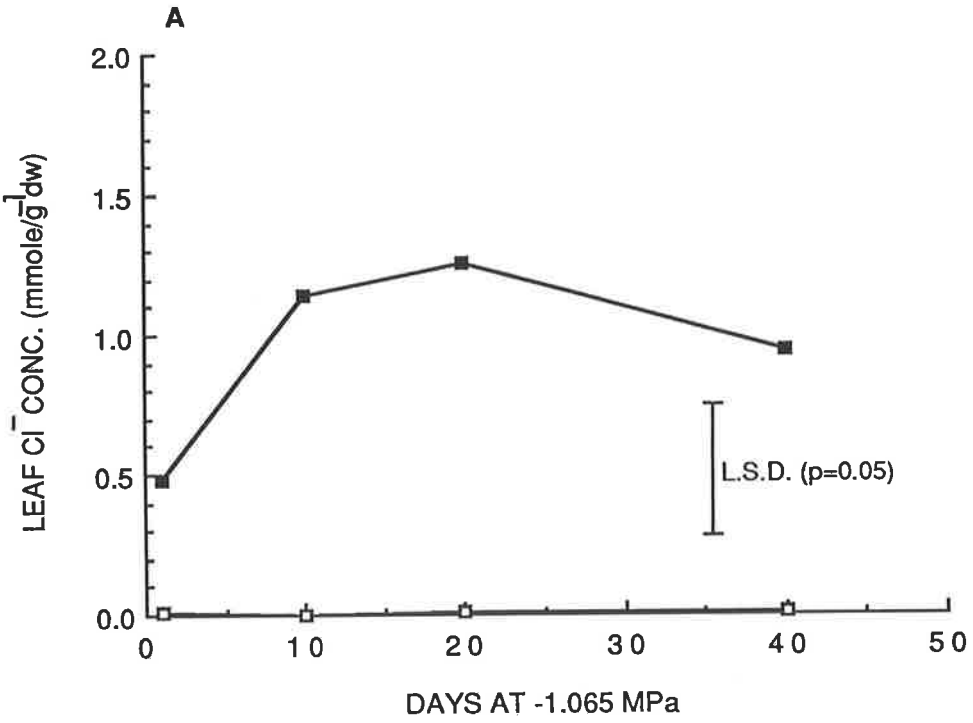
Evaluation of stress effects on glycinebetaine accumulation (Fig. 23B) was complicated by a rapid accumulation in control plants between days 2 and 20. This cannot be attributed to random fluctuations. Although levels in stressed leaves were initially

Fig. 21 Leaf Cl^- concentrations (mmole, $g^{-1}dw$) of tomato and wheat as influenced by steady state of salinization

A. tomato

B. wheat

□ control
■ NaCl


A
2.0
1.5
1.0
0.5
0.0
LEAF Cl⁻ CONC. (mmole/g⁻¹dw)
L.S.D. (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

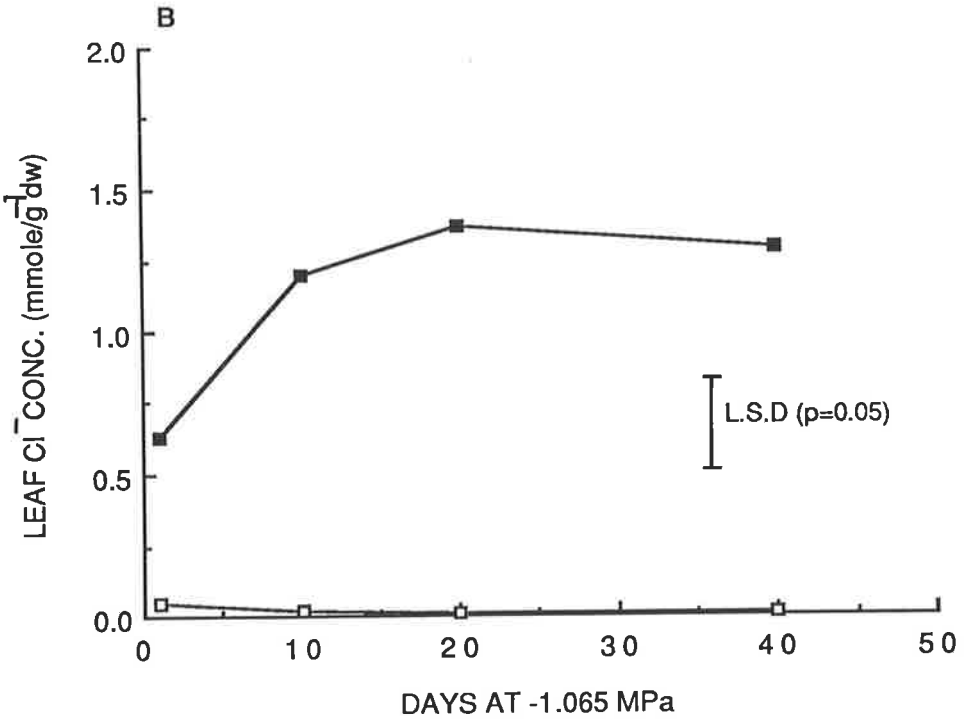
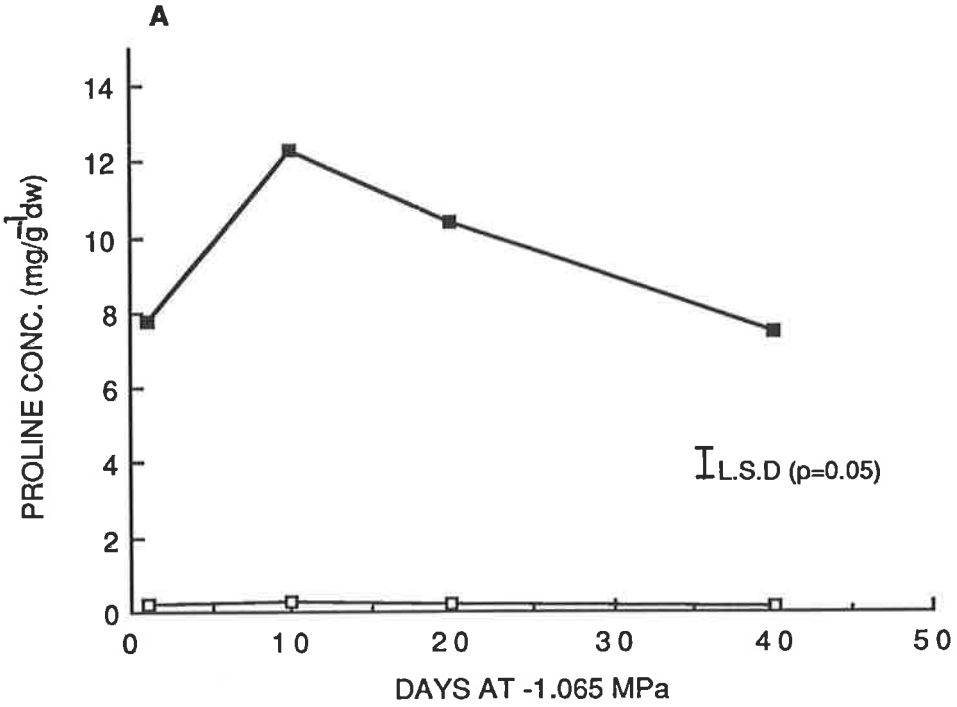

B
2.0
1.5
1.0
0.5
0.0
LEAF Cl⁻ CONC. (mmole/g⁻¹dw)
L.S.D (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

Fig. 22 Leaf proline concentration (mg, g^{-1}dw) of tomato and wheat as influenced by steady state of salinization

A. tomato

B. wheat

□ control
■ NaCl


A
PROLINE CONC. (mg/g⁻¹dw)
14
12
10
8
6
4
2
0
0
10
20
30
40
50
DAYS AT -1.065 MPa
L.S.D (p=0.05)

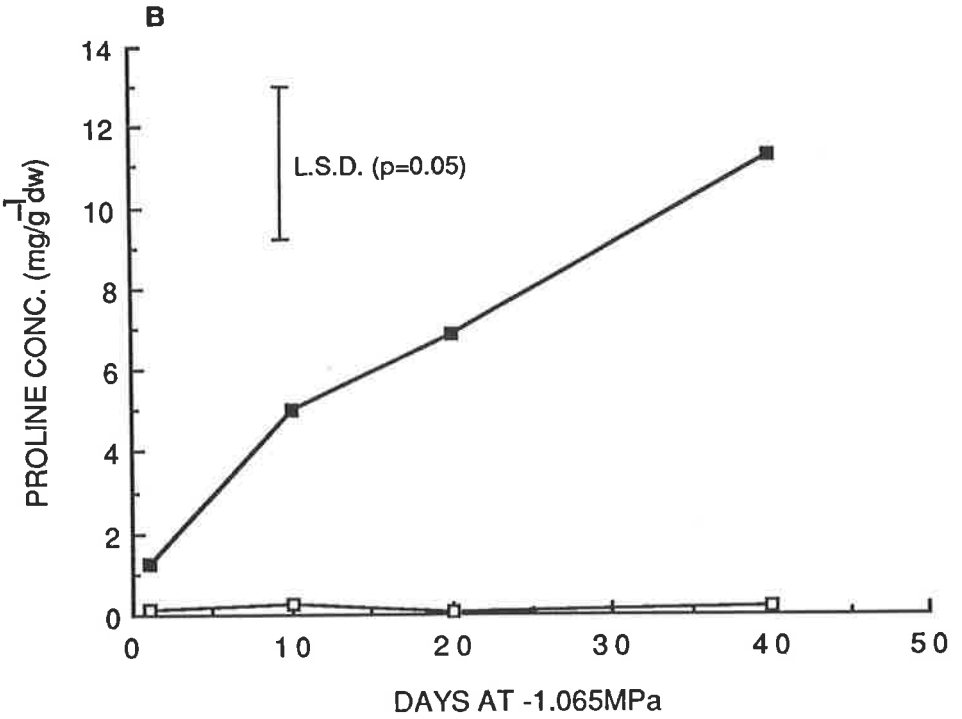

B
PROLINE CONC. (mg/g⁻¹dw)
14
12
10
8
6
4
2
0
0
10
20
30
40
50
DAYS AT -1.065MPa
L.S.D. (p=0.05)

Fig. 23A Leaf trigonelline concentration ($mg,g^{-1}dw$) of tomato exposed to steady state of salinization

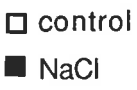
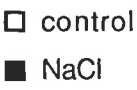
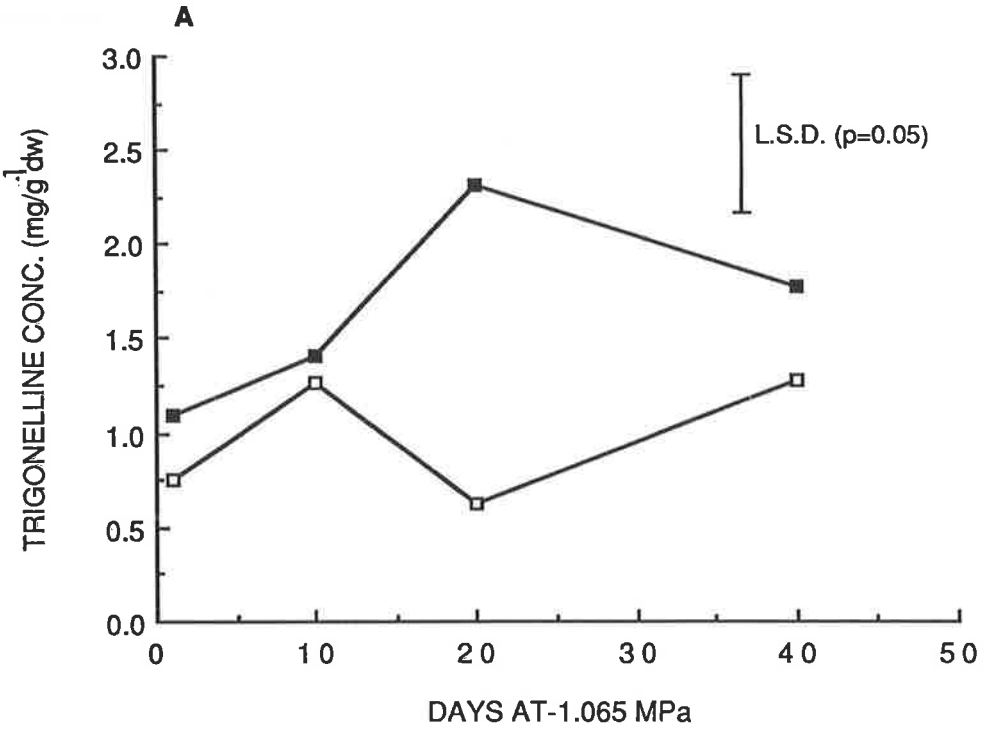
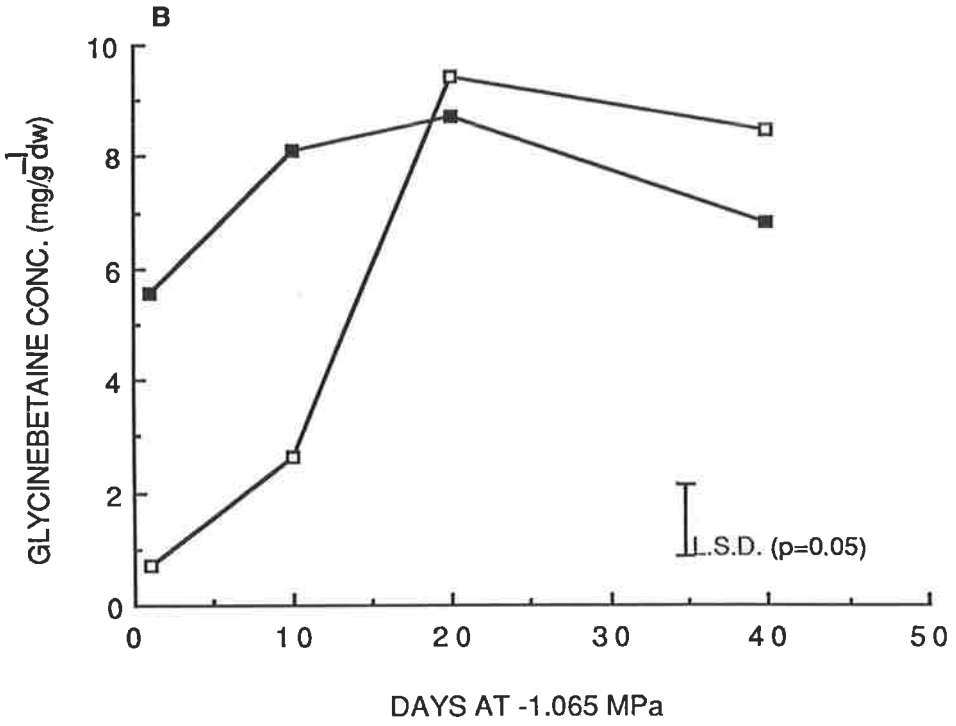


Fig. 23B Leaf glycinebetaine concentration ($mg,g^{-1}dw$) of wheat exposed to steady state of salinization




A
TRIGONELLINE CONC. (mg/g^{-1}dw)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
L.S.D. (p=0.05)
DAYS AT-1.065 MPa


B
GLYCINEBETAINE CONC. (mg/g^{-1}dw)
10
8
6
4
2
0
0
10
20
30
40
50
L.S.D. (p=0.05)
DAYS AT -1.065 MPa

higher than those in the control, there was little difference towards the end of the experiment. There was less change in glycinebetaine content of stressed leaves with time than in the control leaves.

4.1.4.2.10 Growth

A. Shoot fresh weight

NaCl significantly reduced shoot fresh weight increase in both tomato (Fig. 24A) and wheat (Fig 24B). However, the shoot fresh weight of both salt stressed tomato and wheat continued to increase throughout the experiment. The reduction in the increase in fresh weight of the shoot in the stressed plants of both species was similar (84 per cent) on day 40.

B. Leaf area

Leaf area expansion continued at a significant rate in unstressed tomato (Fig. 25A) throughout the 40 days, whereas in wheat it had ceased by day 20 (Fig 25B). In stressed tomato, leaf expansion was severely inhibited but continued at a slow rate throughout whereas in wheat, expansion ceased by day 10 and older leaves senesced thereafter leading to a reduction in viable leaf area.

C Shoot dry weight

The rate of dry matter production was significantly reduced in stressed plants (Figs 26 A,B), but they continued to grow at a slow rate (Table 8). The reduction in dry matter production on day 40 was 78 and 83 per cent compared to the respective controls of tomato and wheat .

4.1.4.3 Discussion

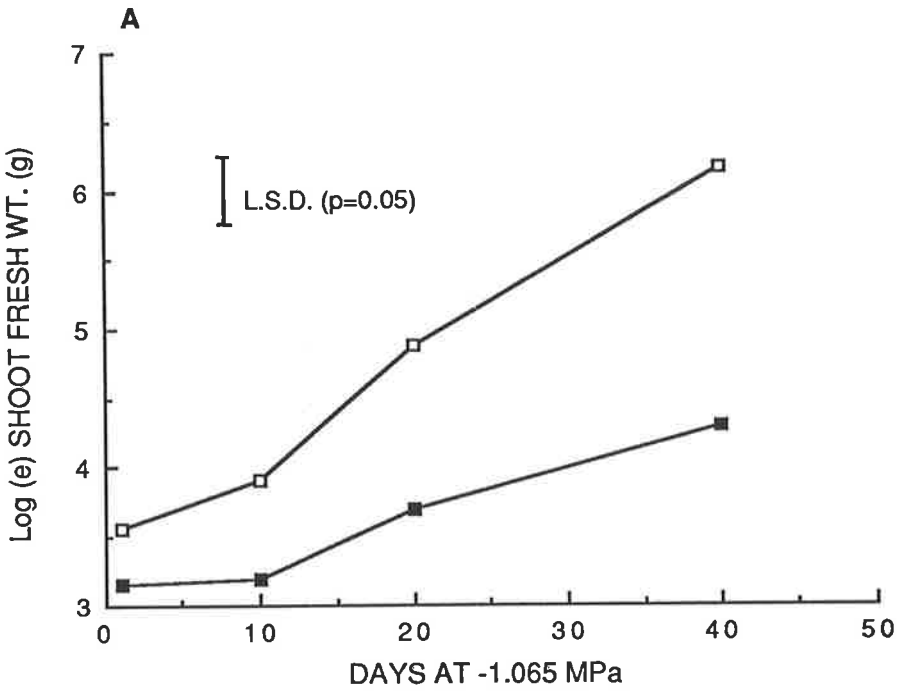
In a constant environment ψ_{leaf} is proportional to $\psi_{ext.}$ (Slavik, 1974). Plants subjected to salt stress at a constant $\psi_{ext.}$ will maintain a constant ψ_{leaf} unless the resistance in the water flow pathway alters or salt accumulates in the apoplast (Oertli,

Fig. 24 Shoot growth of tomato and wheat as influenced by steady state of salinization

A. tomato

B. wheat

□ control
■ NaCl


A
Log (e) SHOOT FRESH WT. (g)
7
6
5
4
3
L.S.D. (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

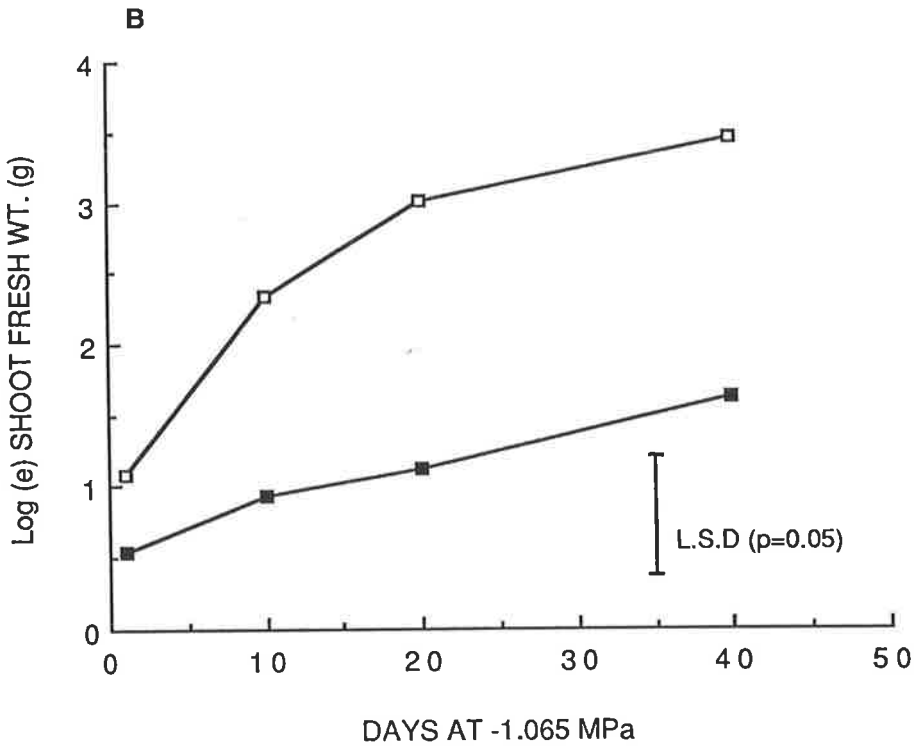
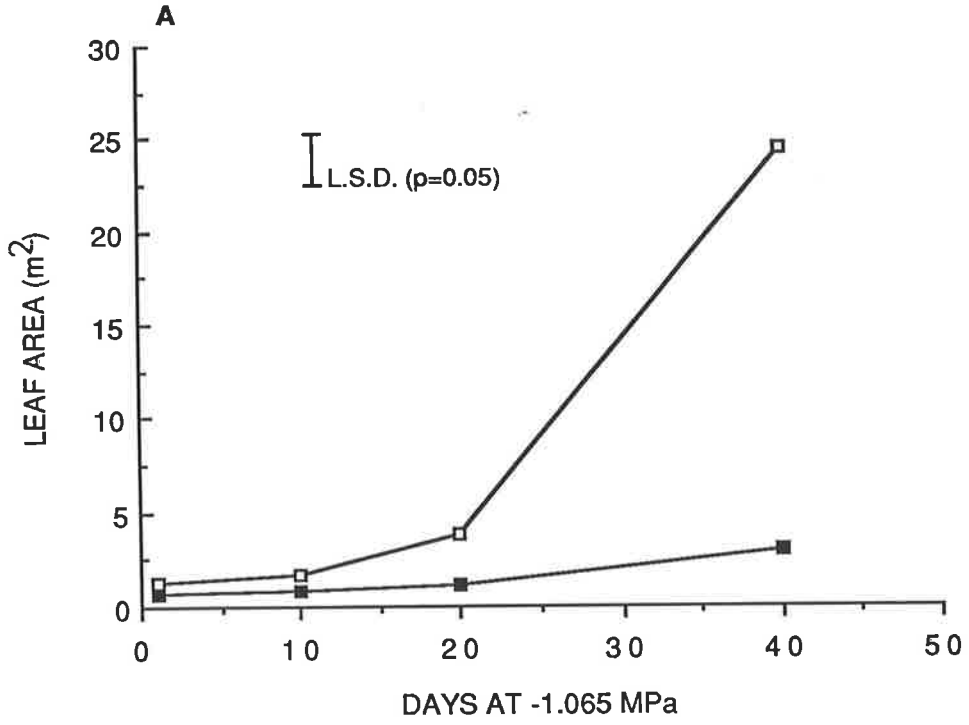

B
Log (e) SHOOT FRESH WT. (g)
4
3
2
1
0
L.S.D (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

Fig. 25 Leaf area expansion of tomato and wheat as influenced by.steady state of salinization

A. tomato

B. wheat

□ control
■ NaCl


A
30
25
20
15
10
5
0
LEAF AREA (m^2)
L.S.D. (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

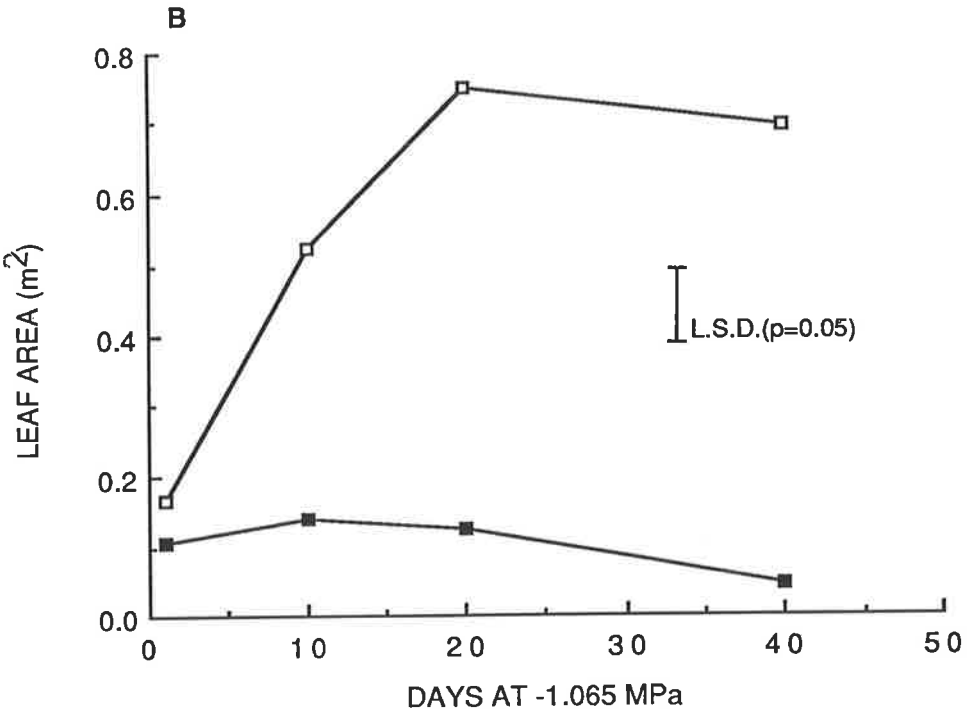
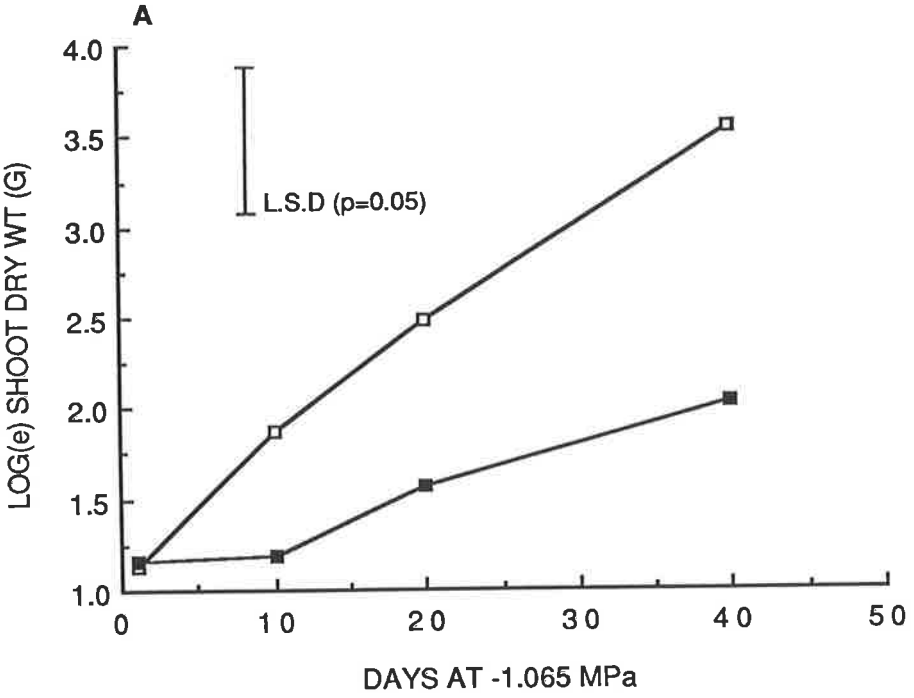

B
0.8
0.6
0.4
0.2
0.0
LEAF AREA (m^2)
L.S.D.(p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

Fig. 26 Shoot dry matter production as influenced by steady state of salinization in tomato and wheat

A. tomato

B. wheat

□ control
■ NaCl


A
4.0
3.5
3.0
2.5
2.0
1.5
1.0
LOG(e) SHOOT DRY WT (G)
L.S.D (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

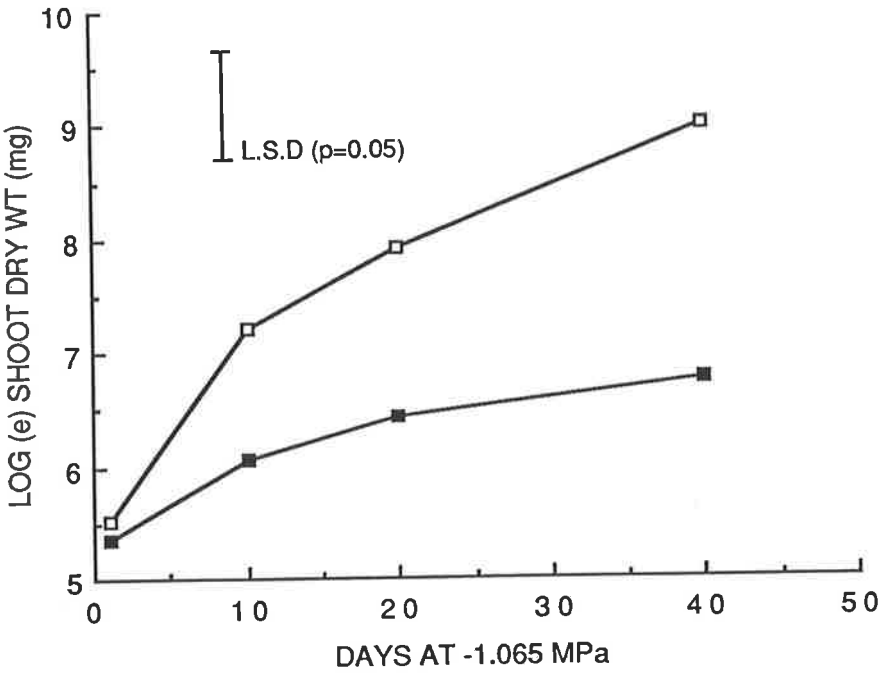

10
9
8
7
6
5
LOG (e) SHOOT DRY WT (mg)
L.S.D (p=0.05)
0
10
20
30
40
50
DAYS AT -1.065 MPa

Table 8 Effect of steady state of salinization on relative growth rates of tomato and wheat (g,g^{-1},day^{-1})

	Treatment	Time interval (days from commencement of stress)			
		0-11	11-21	21-31	31-51
TOMATO	CONTROL	0.098	0.075	0.061	0.053
	NaCl	0.102	0.002	0.038	0.023
WHEAT	CONTROL	0.060	0.170	0.069	0.036
	NaCl	0.052	0.070	0.039	0.017

1966). In tomato, prolonged exposure to a constant $\psi_{ext.}$ resulted in a steady ψ_{leaf}. But, in wheat, a continued decline in ψ_{leaf} occurred despite a constant $\psi_{ext.}$ suggesting a change in resistance in the water flow pathway or salt saturation of the accumulation sites in the root and shoot. Increased hydraulic resistance in the roots has been suggested to follow from increased suberization (Kramer, 1980; O'Leary,1975) and such a response to prolonged exposure has been suggested to be the cause of increased resistance to water flow (O'Leary,1975). This may have been the reason for the consistent decrease in ψ_{leaf} of wheat. The constant $NaCl_{ext.}$ also apparently induced a stabilised internal $Na^+$and Cl^- concentration as reported for *Suaeda maritima* (Yeo,1974) and rice (Ladenburger and Albert, 1980). These steady states of salt stress also facilitated turgor maintenance in both species. However, the interpretation of a steady internal water and ion status must be viewed with some caution as these parameters were measured on different leaves at each sampling occasion.

Accumulation of proline under such a steady state of ion and water status in the external rooting medium mirrored the changes in leaf ψ_s, Na^+, and Cl^- concentration, but not turgor which is consistent with the relationships observed in the previous experiment. A significant increase in proline accumulation up to day 10, and a decrease thereafter, occurred without any change in leaf water potential for tomato which suggests that ψ_{leaf} does not directly regulate accumulation. In wheat, however, due to the continued decrease in ψ_{leaf}, such a separation could not be realised. The activity of the enzyme Δ^1-P-5-C dehydrogenase is a potential source of control for endogenous free proline concentration and inhibition of Δ^1-P-5-C dehydrogenase could account, at least partially for proline accumulation. An *in vitro* study with barley (Boggess *et al.*, 1975) showed a dramatic inhibition of this enzyme by Cl^-. Promotion of Δ^1-P-5-C reductase, which converts glutamic acid to proline, would also result in proline accumulation. An *in vitro* study on this enzyme obtained from *Pennisetum typhoides* (Huber, 1974) demonstrated promotion of this enzyme by NaCl. These experiments suggest that tissue Cl^- may directly control proline accumulation in NaCl stress. Although $Na^+$and Cl^- are major contributors to the ψ_s pool, the correlation

between ψ_s and proline accumulation cannot be directly attributed to either Na^+ or Cl^- until the effects of these factors are separated.

Glycinebetaine accumulation also followed the changes in leaf ψ_s , and Na^+ and Cl^- concentration, conforming with the previous experiment. Wyn Jones *et al.*,(1977) suggested that accumulation of glycinebetaine compensates osmotic disequilibrium in the cytoplasm due to Na^+ and Cl^- accumulation in the vacuole. This describes a potential role rather than elucidating the mechanism of accumulation. Further, such a logical role cannot be attributed to accumulation in wheat which amounts to only 4 per cent at most of total Na^+ and Cl^- taken up into the vacuole (Harvey and Thorpe, 1986). This suggests that the role of glycinebetaine could lie in areas other than mere osmoregulation. In this experiment, the accumulation of glycinebetaine does not appear to be specific to NaCl as significant accumulation was observed in unstressed plants. Continued growth of wheat at a 20°C day temperature for a prolonged period may have induced accumulation. The critical temperature for glycinebetaine accumulation in wheat has been found to be around 18°C (Naidu, Personal communication) and this may have led to continued accumulation as it is not usually catabolised further (Ladyman *et al.*, 1980; Hanson and Wyse, 1982). Moreover, the plants in the growth cabinet approached the light source as they grew, receiving a high photon irradiance which may have increased leaf temperature. Accumulation may alternatively be linked directly to light stimulated oxidation of choline and betainal due to high electron transport via. oxidant regeneration (Rabinowitch and Fridowitch, 1983) or indirectly via. enzyme activation (Buchenon, 1980). Increased accumulation of glycinebetaine at high irradiance has been reported for the spinach chloroplast (Hanson *et al.*, 1985). The possibility that increase in accumulation of glycinebetaine in the control plants could also be due to ontogenetic changes cannot be ruled out. A similar increase in glycinebetaine in wheat grown in nutrient solutions for a prolonged period has been identified in the reports of Grattan and Grieve, (1985) however, lack of information on the level of irradiance under which the plants were grown restricts consideration as to whether this was an ontogenetic or an environmental effect.

SECTION 4.2 VARIATION IN THE ACCUMULATION OF PROLINE AND QACs WITH DIFFERENT SALTS IN THE ROOTING MEDIUM

4.2.1 Introduction

The experiments described in section 4.1 showed consistent relationships between proline and glycinebetaine accumulation and leaf ψ_s, Na^+, and Cl^- concentrations. A decision on whether accumulation is responsive to bulk changes in ψ_s, or is specific to variation in cation (Na^+) or anion (Cl^-) could not be made. Exposing plants to stress with permeating and non-permeating osmotic species could throw light on this issue. Further, specific ionic effects on accumulation may also occur. Evidence for an influence of ion concentration on promotion of Δ^1-P-5-C reductase (Huber, 1974) and inhibition of Δ^1-P-5-C dehydrogenase activity (Boggess *et al.*, 1975) is available. Any influence on these enzymes could be assumed to be reflected on the accumulation of proline *in vivo*. However, nothing is known concerning the influence of ions on accumulation of QACs.

4.2.2 The effect of osmotic species on the accumulation of proline and QACs

This first experiment was designed to compare the effect of exposing tomato and wheat to a variety of inorganic ions and a non-permeating solute (PEG_{4000}) in the rooting medium.

4.2.2.1 Methods

Seedlings of tomato CV. Duke and wheat CV. Sun-9-E were raised as described in Materials and Methods (3.2.1) until 25 (tomato) or 10 (wheat) days after emergence when the seedlings were supplied with full strength nutrient solution alone or containing PEG_{4000}, NaCl (221.4), KCl (225.4), K_2SO_4(188.1), KNO_3 (255.5), $CaCl_2$ (169.9), or $MgCl_2$ (152.9 mM). The osmotic potential of Hoagland solution alone was -0.065MPa and of all other solutions -1.065 MPa. In each case, seedlings were supplied with the appropriate solution immediately. Iso-osmotic solutions were prepared as described in section 3.2.3 and

ψ_s was confirmed with a Spanner psychrometer. The youngest fully expanded leaf was sampled 48 h after exposure to the respective osmotic solutions for measurement of leaf water, osmotic and turgor potential (3.2.6), proline (3.2.9.2) and QACs(3.2.9.3). The experimental design was a randomised block with four replications of each treatment.

4.2.2.2 Results

4.2.2.2.1 Leaf water potential

At 48 hours, leaf ψ had fallen in all plants exposed to stress . In tomato(27A), ψ_{leaf} fell in the plants subjected to the non-permeating osmoticum by rather less than the fall in external ψ whilst in those exposed to the various ions it fell even less and to the same extent in each case. In wheat (Fig.27B), the fall in ψ_{leaf} in both PEG and KNO_3 was approximately equivalent to the fall in $\psi_{ext,}$ with a lesser fall in all other solutions.

4.2.2.2.2 Leaf osmotic potential

The leaf osmotic potential of wheat (Fig. 28B) dropped significantly below the control level but differences in ψ_s between the various stress treatments were not great. In tomato (Fig. 28A) ψ_s decreased significantly in all except KNO_3 and $CaCl_2$ where ψ_s remained at the control level. ψ_s in $MgCl_2$ decreased more than in KNO_3 and $CaCl_2$ and ψ_s decreased more in response to PEG than to most ionic species. In both species the responses to the two divalent cations differed considerably, the fall in ψ_s being greater in $MgCl_2$ than $CaCl_2$ stress.

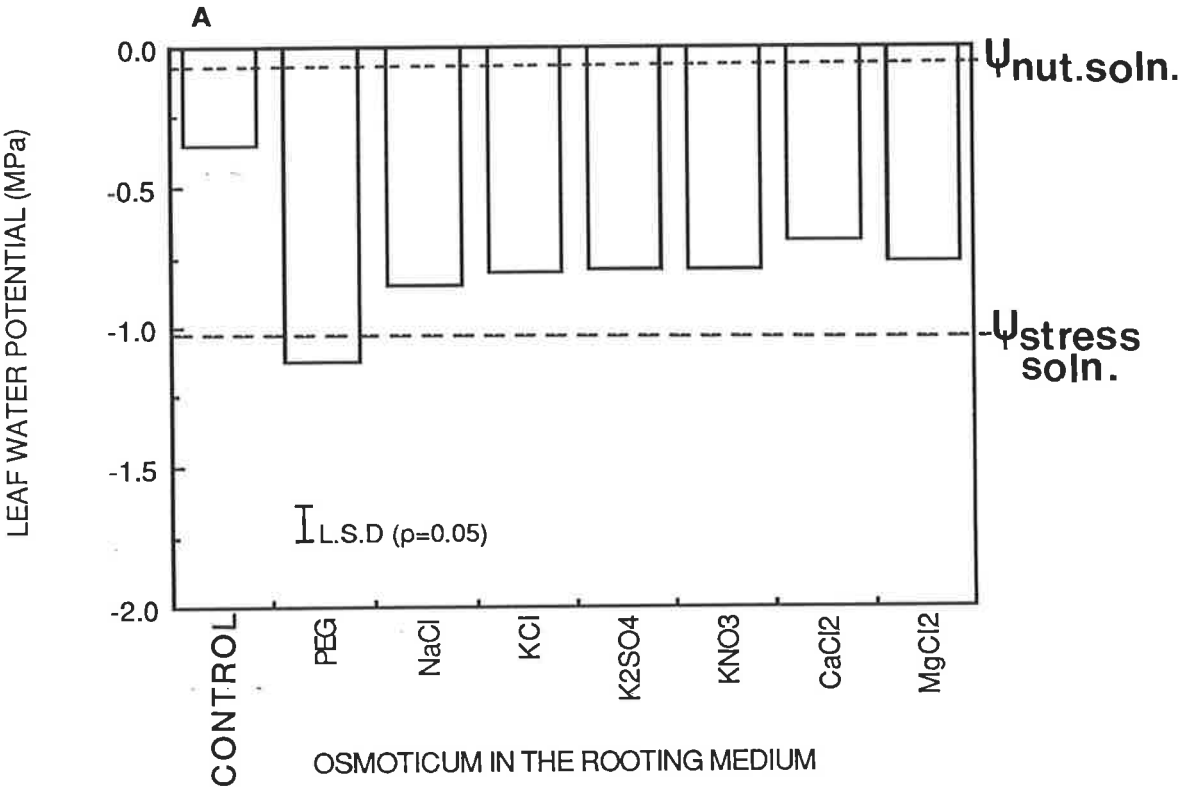
4.2.2.2.3 Leaf turgor potential

Leaf turgor was lost in both species when plants were exposed to PEG in the rooting medium(Figs. 29A,B). In contrast, turgor remained the same as in the control in both species when exposed to $MgCl_2$ stress. In tomato (Fig. 29A), leaf turgor was also maintained by plants subjected to K_2SO_4 stress with turgor being lost to a greater or lesser extent in all other treatments. In wheat (Fig. 29B), turgor was maintained in KCl.

Fig. 27 Leaf water potential (-MPa) of tomato and wheat as effected by various osmotica at the rooting medium

A. tomato

B. wheat


A
0.0
-0.5
-1.0
-1.5
-2.0
LEAF WATER POTENTIAL (MPa)
Ψnut.soln.
Ψstress soln.
L.S.D (p=0.05)
CONTROL
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
OSMOTICUM IN THE ROOTING MEDIUM

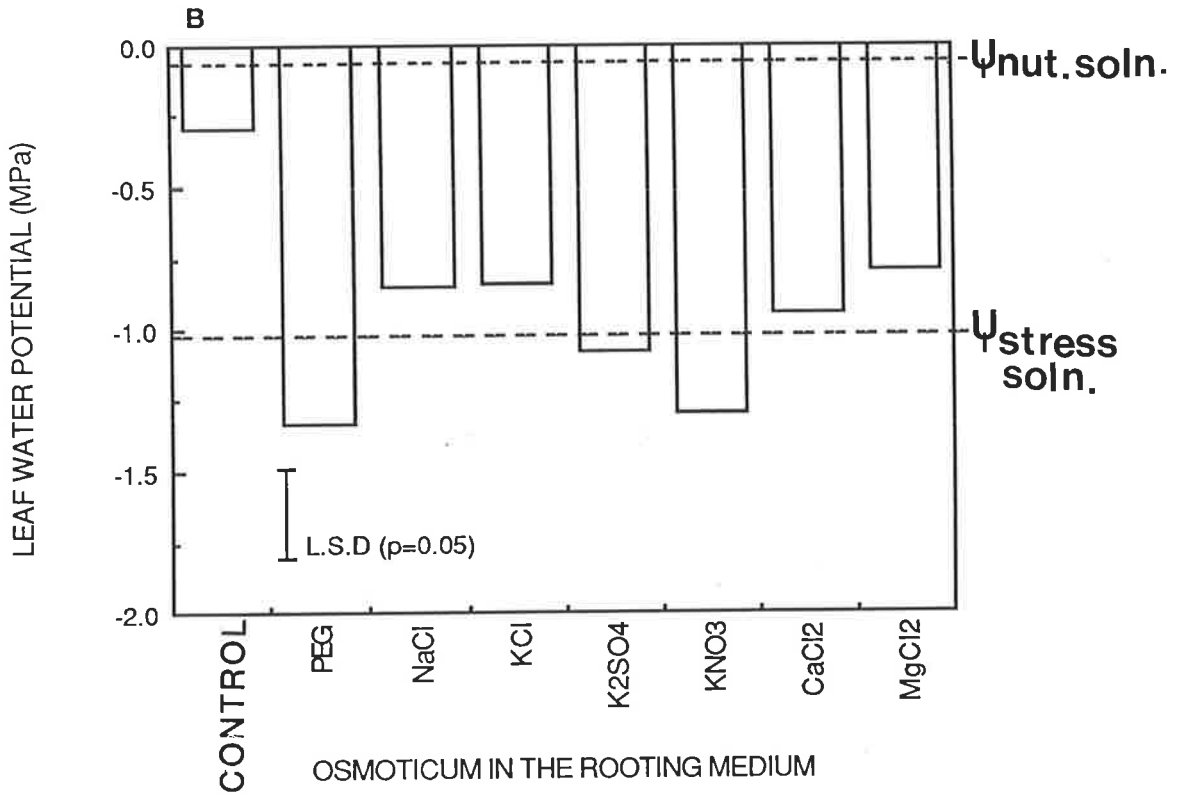
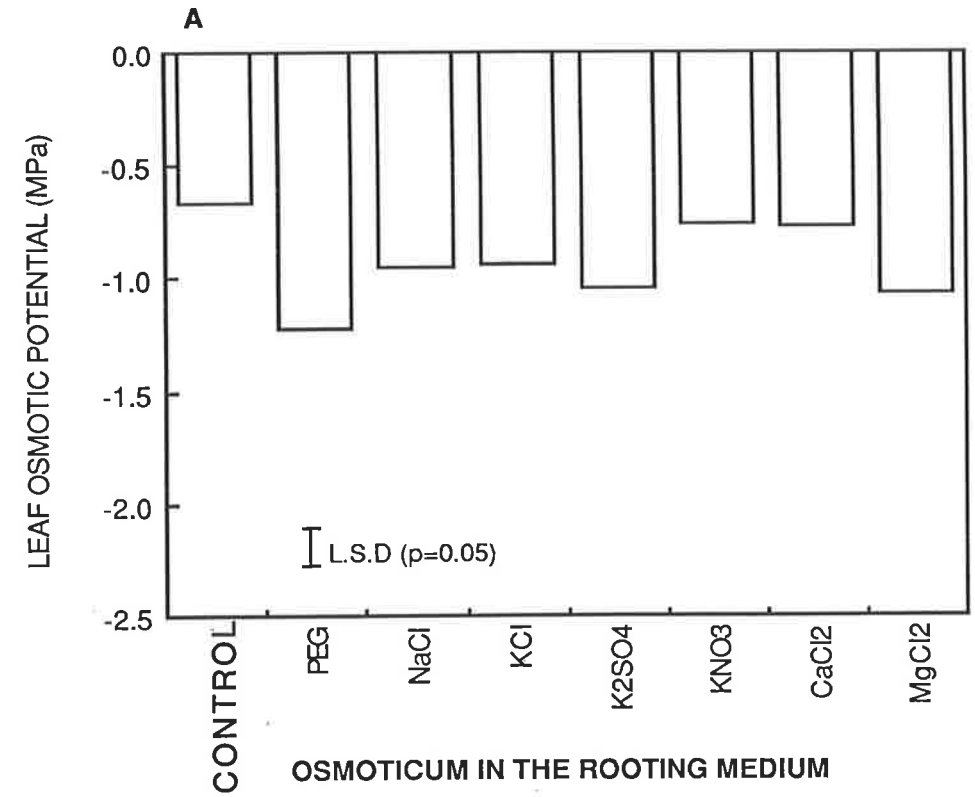

B
0.0
-0.5
-1.0
-1.5
-2.0
LEAF WATER POTENTIAL (MPa)
Ψnut.soln.
Ψstress soln.
L.S.D (p=0.05)
CONTROL
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
OSMOTICUM IN THE ROOTING MEDIUM

Fig. 28 Leaf osmotic potential (-MPa) of tomato and wheat as effected by various osmotica at the rooting medium

A. tomato

B. wheat


A
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
LEAF OSMOTIC POTENTIAL (MPa)
L.S.D (p=0.05)
CONTROL
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
OSMOTICUM IN THE ROOTING MEDIUM

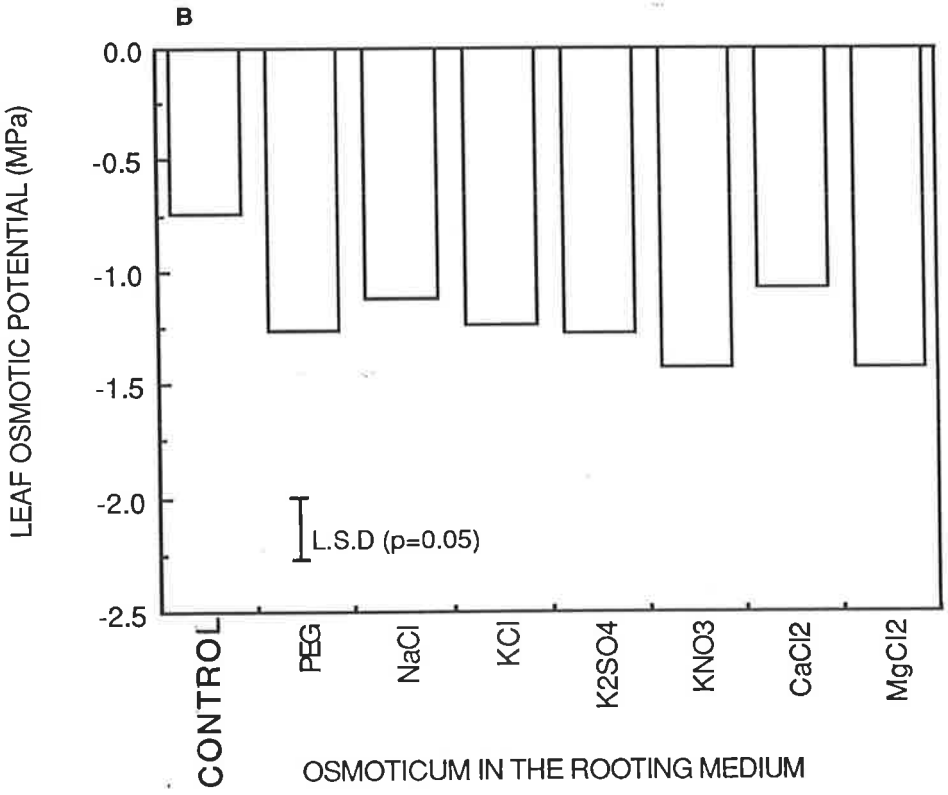
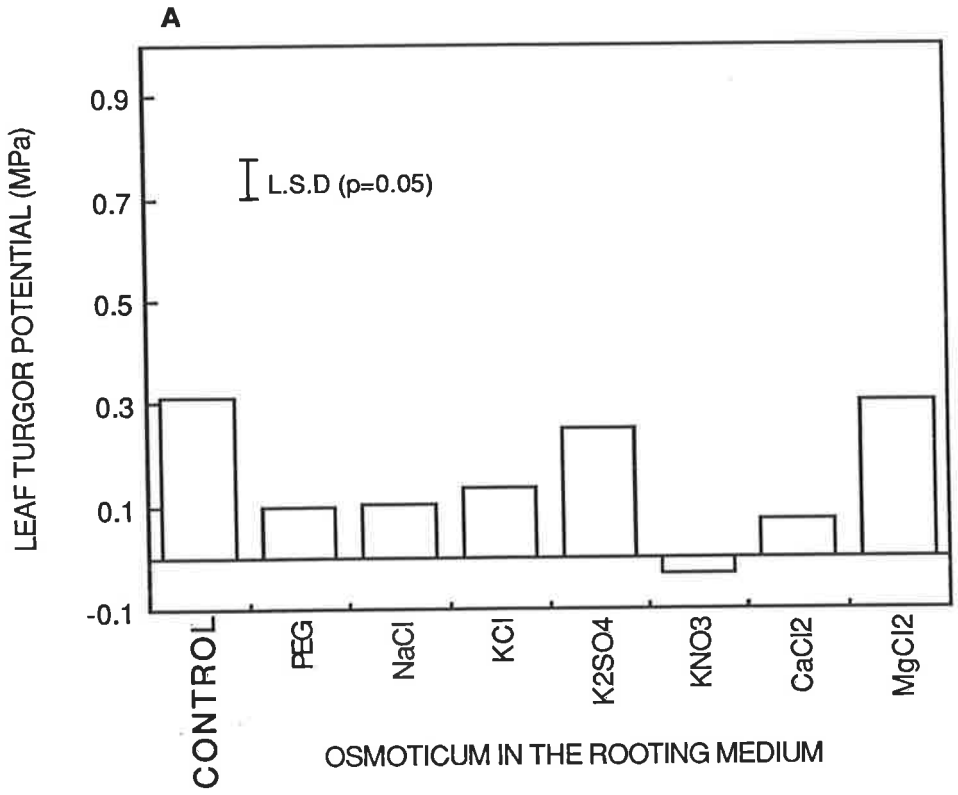
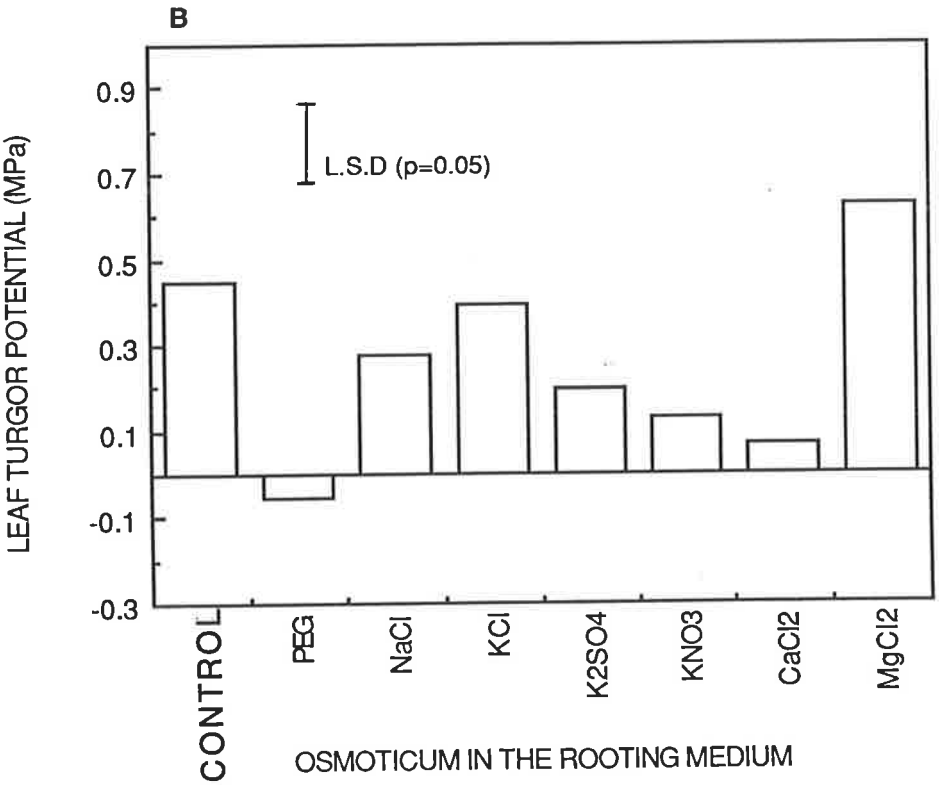

B
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
LEAF OSMOTIC POTENTIAL (MPa)
L.S.D (p=0.05)
CONTROL
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
OSMOTICUM IN THE ROOTING MEDIUM

Fig. 29 Leaf turgor potential (MPa) of tomato and wheat as effected by various osmotica at the rooting medium

A. tomato

B. wheat


A
LEAF TURGOR POTENTIAL (MPa)
0.9
0.7
0.5
0.3
0.1
-0.1
L.S.D (p=0.05)
CONTROL
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
OSMOTICUM IN THE ROOTING MEDIUM


B
LEAF TURGOR POTENTIAL (MPa)
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
L.S.D (p=0.05)
CONTROL
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
OSMOTICUM IN THE ROOTING MEDIUM

4.2.2.2.4 Proline

Proline accumulated most rapidly in plants of both species exposed to the non-permeating osmoticum. In tomato (Fig. 30A), the only other treatments to induce significant proline accumulation were KNO_3 and $CaCl_2$. In contrast, wheat (Fig. 30B) accumulated proline with all inorganic salts except NaCl. Wheat stressed with salts of the divalent cations, Ca^{++} and Mg^{++} accumulated more proline than wheat stressed with salts of monovalent cations, $Na^{+}$and K^{+}.

4.2.2.2.5 Quaternary Ammonium Compounds

A. Trigonelline

The trigonelline concentration in the tomato plants did not vary significantly between treatments in this experiment (Table 9)

B. Glycinebetetaine

The glycinebetaine concentration in wheat (Fig. 31) increased significantly when plants were stressed. The amount accumulated was the same in plants stressed with PEG and divalent cationic salts but rather less in plants exposed to monovalent cationic salts particularly KNO_3.

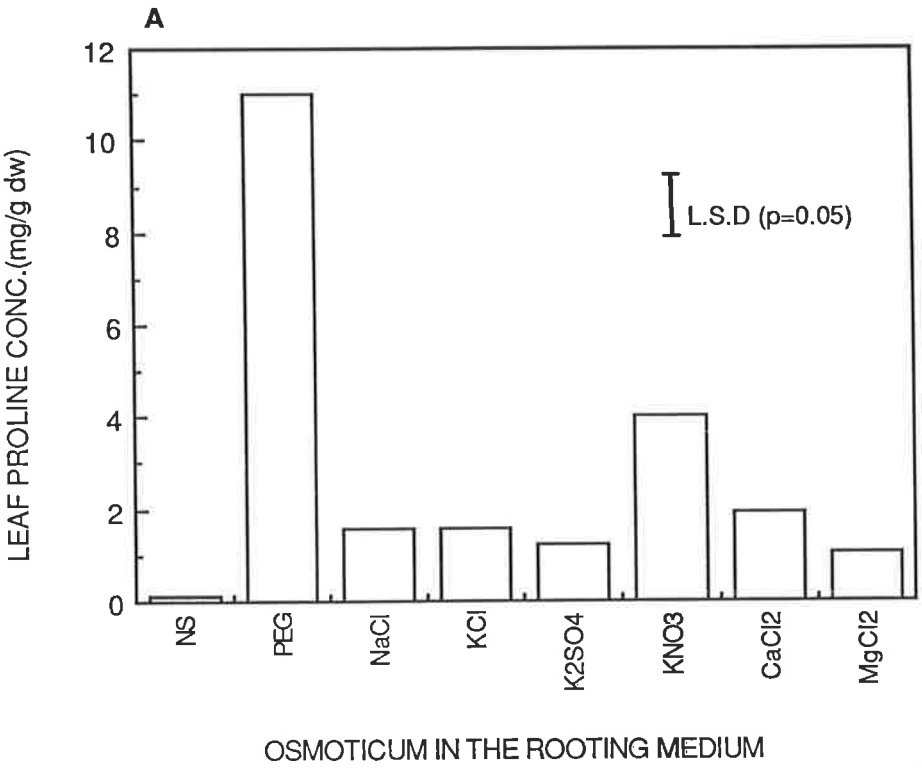
4.2.2.3 Discussion

In the plants subjected to the non-permeating osmoticum, PEG, the decrease in ψ_s was due to both water loss and solute accumulation, as turgor was lost (Fig. 29B). The decrease in ψ_s varied in plants experiencing ionic stress, depending on the mobility of the ionic species into the plant. Although no leaf ion concentration was measured in this experiment, it is possible that the less negative ψ_s recorded in plants under $CaCl_2$ stress may have been due to less Ca^{++} uptake (Mengel and Kirkby, 1979); further, Cl^{-} uptake would have also been inhibited by the accompanying cation, Ca^{++}(Yeo and Flowers, 1985) resulting in a low turgor potential. Similarly, turgor was lost (only in tomato) even in KNO_3 stress. K^{+} is a preferred osmoticum in plants and is highly mobile against an electro-chemical

Fig. 30 Leaf proline concentrations ($mg,g^{-1}dw$) of tomato and wheat under various osmotic stress

A. tomato

B. wheat


A
LEAF PROLINE CONC.(mg/g dw)
12
10
8
6
4
2
0
L.S.D (p=0.05)
NS
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
OSMOTICUM IN THE ROOTING MEDIUM

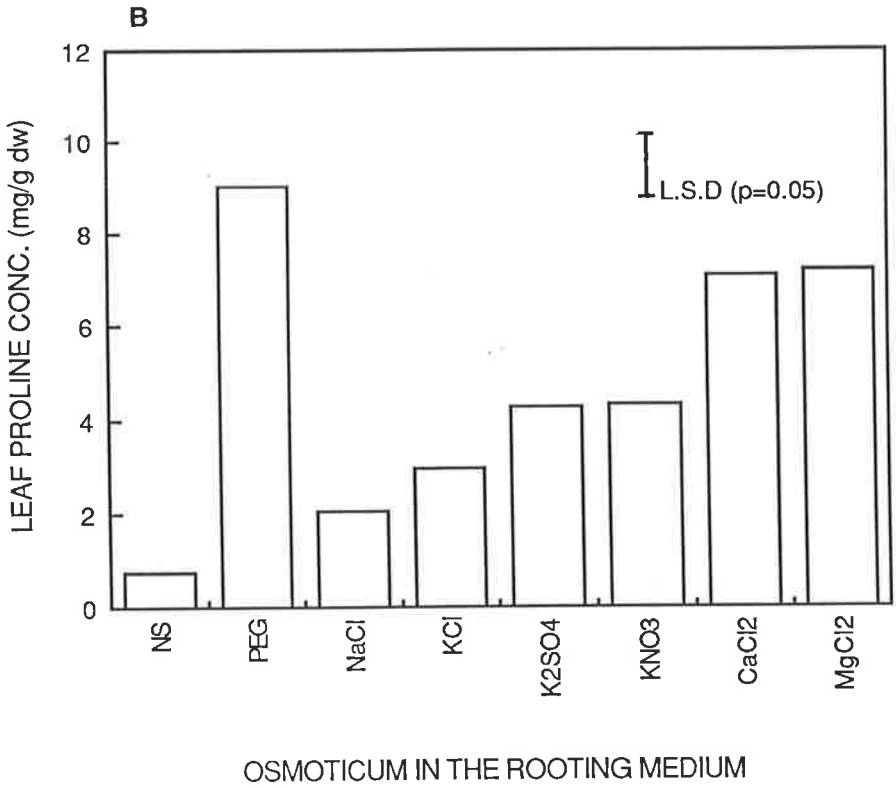

B
LEAF PROLINE CONC. (mg/g dw)
12
10
8
6
4
2
0
L.S.D (p=0.05)
NS
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
OSMOTICUM IN THE ROOTING MEDIUM

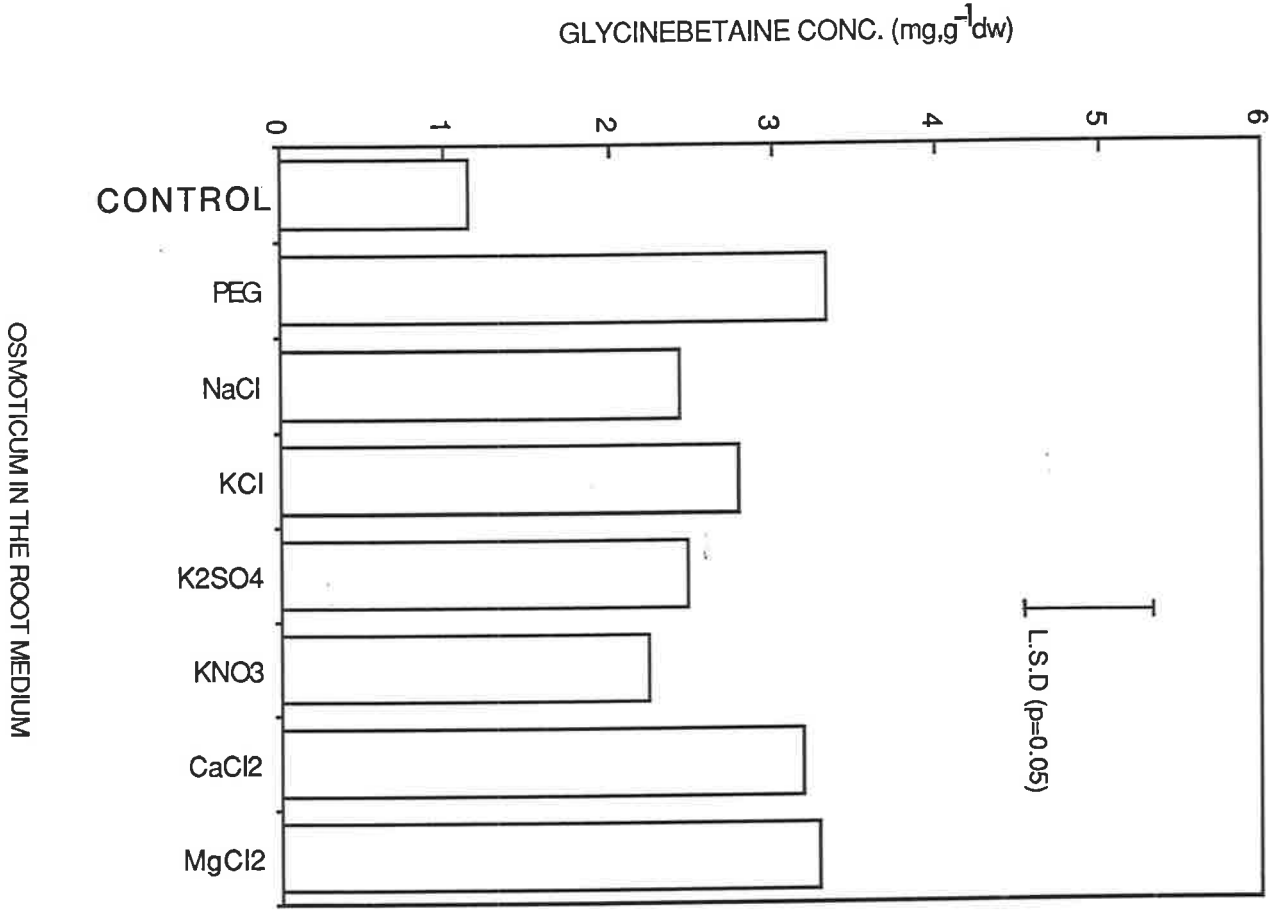
Table 9 The effect of different osmotic species in the rooting medium on trigonelline concentration of tomato ($mg.g^{-1}dw.$)

OSMOTIC SPECIES	TRIGONELLINE
CONTROL	0.996
$PEG_{(4000)}$	1.997
NaCl	1.026
KCl	0.777
K_2SO_4	0.949
KNO_3	1.425
$CaCl_2$	1.771
$MgCl_2$	1.524
MEAN	1.306

SOURCE OF VARIATION	
TREATMENT	NS

NS = NOT SIGNIFICANT

Fig. 31 Leaf glycinebetaine concentration ($mg,g^{-1}dw$) in wheat under various osmotic stresses


GLYCINEBETAINE CONC. (mg,g-1dw)
0
1
2
3
4
5
6
CONTROL
PEG
NaCl
KCl
K2SO4
KNO3
CaCl2
MgCl2
L.S.D (p=0.05)
OSMOTICUM IN THE ROOT MEDIUM

gradient(Mengel and Kirkby, 1979) in which case a greater decrease in ψ_s would have been expected. However, the loss of turgor in tomato under KNO_3 stress suggests a barrier to ion uptake. It is not known whether NO_3^- and K^+ compete for the same transport carrier ATPase, whether NO_3^- inhibits K^+uptake or whether the NO_3^- taken up is metabolised. All these possibilities would result in less osmoregulation, resulting in turgor loss. In contrast, the osmotic potential of both species exposed to $MgCl_2$ decreased significantly, and this was probably due to rapid ion uptake and solute synthesis, rather than to water loss, as turgor remained at the control level or above.

Proline accumulation was also influenced by osmotic species in the rooting medium. The most striking response was the accumulation of proline under PEG stress in both species. Proline accumulation was relatively low in plants under ionic stress, but greater accumulation was recorded with divalent (Ca^{++}, Mg^{++}) than monovalent cationic salts in wheat as was found by Chu *et al.*, (1976b) in barley. However, tomato only accumulated proline and then to small extent in the presence of KNO_3 and $CaCl_2$. The low proline concentration found in tomato exposed to NaCl stress confirms the results in section 4.1.2. This response could be attributed neither to insufficient time of exposure to stress, as proline accumulated in significant quantities under PEG stress, nor to a stress level below threshold $\psi_{ext.}$, as the $\psi_{ext.}$ was constant in all the stress treatments. Tomato appears to respond more to a gradual, rather than to an abrupt, imposition of salt stress.

Glycinebetaine accumulation was also influenced by the osmotic species present in the rooting medium. Plants under PEG and divalent cation salt stress accumulated more than those under monovalent cation salt stress. Storey and Wyn Jones (1978b) also observed a greater glycinebetaine accumulation under PEG than NaCl stress. This indicates that the response is not specific to salt stress alone. Further, variation in accumulation response in different ionic osmotica despite a constant $\psi_{ext.}$ indicates that glycinebetaine synthesis is influenced by specific ions. Although the pathway of glycinebetaine synthesis is well proven (Hanson *et al.*, 1985; Weigal *et al.*, 1986), specific ionic effects on the enzymes involved in the pathway of synthesis have not been reported.

Although this experiment demonstrates specific ion effects on proline and glycinebetaine accumulation, it is not informative on the mechanism of the response as measurements were taken at 48 h only. The amount of proline or glycinebetaine accumulated by 48 h would not be determined by ψ_s or ion status at that time but most likely it would be correlated with some integrated function of ψ_s or ion content over time. Further, such correlations could work both ways, and it is at least as likely that proline or glycinebetaine concentration determine ψ_s (at least partly) as the reverse. Differences in ψ_s among different osmotica could then be partly due to differences in accumulated compounds. Furthermore, as it is argued that accumulation is largely determined by events occurring before the measurement of concentration, and not by contemporary events, it follows that a poor correlation with a simultaneous measurement of turgor would be unlikely to eliminate that factor as a possible control mechanism.

4.2.3 The influence of a divalent and a monovalent cationic salt on the accumulation of proline and QACs

In the previous experiment although proline accumulated in response to ionic stress, a similar increase in accumulation was not observed with tomato. It is evident from the data in section 4.1 that tomato responds to a gradual rather than to an abrupt salinization. Subjecting tomato to gradual salinization with a divalent and a monovalent cationic salt would explore whether the divalent cation induced more proline to accumulate as with wheat. Mg^{++} and Na^{+} were chosen as candidate cations, with Cl^{-} as the accompanying anion. Cl^{-} was preferred to SO_4^{2-}, or NO_3^{-} as it would not be subject to metabolism (Mengel and Kirkby, 1979).

4.2.3.1 Methods

Seedlings of tomato CV. Duke and wheat CV. Sun-9-E were grown at a 20±1°C day and 17±1°C night temperature, with a 16 hour photoperiod and a photon flux density of 350-420 μEin sec^{-1} (Materials and Methods 3.2.1,3.2.2). NaCl and $MgCl_2$ were added to the full strength Hoagland solution supplied 25 and 10 days after emergence of the seedlings of tomato and wheat respectively. The water potential of the rooting medium was gradually decreased by -0.10 MPa day^{-1} for 10 days. On the final day of stress the plants were experiencing -1.065MPa (221.4 mM NaCl; 152.9 mM $MgCl_2$) $\psi_{ext.}$ The youngest fully expanded leaves were sampled on 0, 2, 5, 11 (tomato) and 0, 2, 5, 9, 11 (wheat) days from commencement of stress for measurement of leaf water, osmotic and turgor potential (3.2.6), proline (3.2.9.2) QACs (3.2.9.3) and leaf ion content (3.2.11). Leaf area and shoot fresh and dry weights were measured (3.2.4). The design was a factorial randomised block with three replications of each treatment.

4.2.3.2 Results

4.2.3.2.1 Leaf water potential

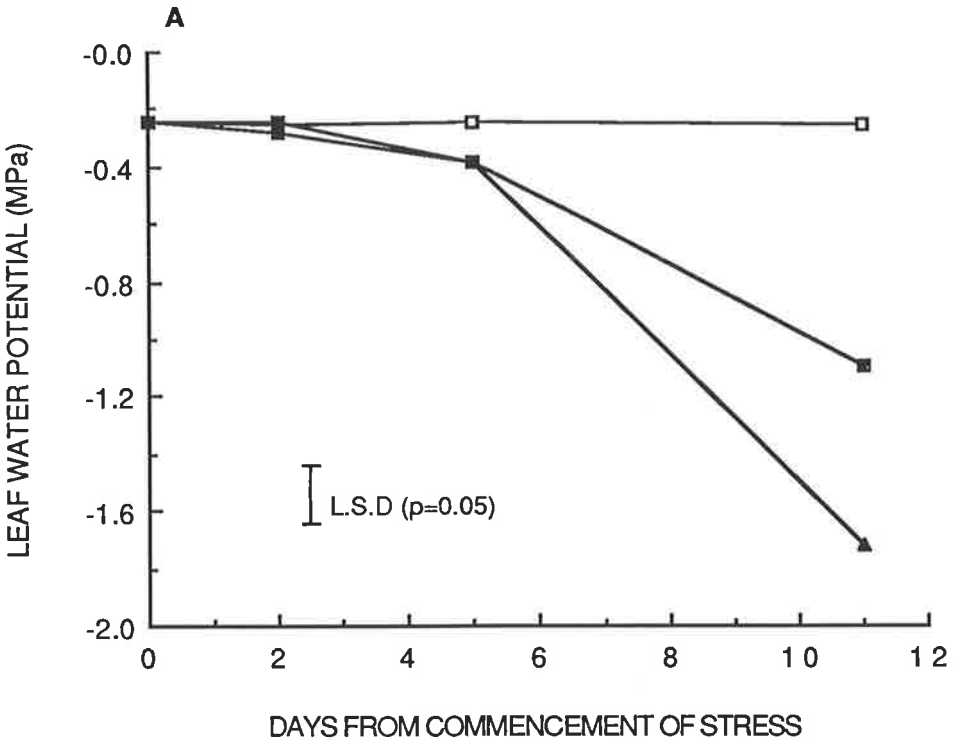
The leaf water potential of unstressed tomato (Fig 32A) stayed at about -0.25 MPa and of wheat (Fig 32B) between -0.37 and -0.28 MPa. There was no significant difference in

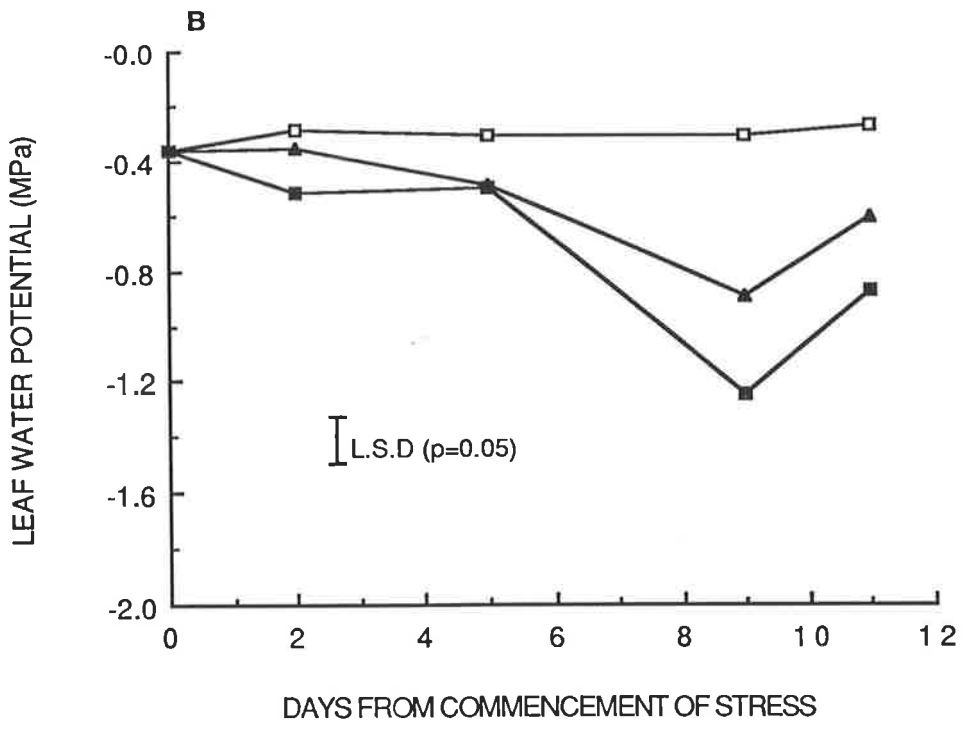
Fig. 32 Changes in leaf water potential (-MPa) of tomato and wheat as influenced by NaCl and $MgCl_2$

A. tomato

B. wheat

□ control
■ NaCl
▲ $MgCl_2$


A
-0.0
-0.4
-0.8
-1.2
-1.6
-2.0
LEAF WATER POTENTIAL (MPa)
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


B
-0.0
-0.4
-0.8
-1.2
-1.6
-2.0
LEAF WATER POTENTIAL (MPa)
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

ψ_{leaf} of tomato between the salinity treatments up to day 5. Subsequently, the fall in ψ_{leaf} on day 11 was significantly greater with $MgCl_2$ than with NaCl stress although $\psi_{ext.}$ was the same. In wheat, also, a similar response was observed until day 5, but here the subsequent fall in ψ_{leaf} was greater with NaCl than $MgCl_2$ salinity by day 9 and there was evidence for some recovery in the last 2 days.

4.2.3.2.2 Leaf osmotic potential

The leaf osmotic potential of control tomato (Fig 33A) and wheat (Fig 33B) remained constant during the experiment.ψ_s of control wheat was lower (more negative) than that of tomato by -0.38 MPa.ψ_s of tomato under both salinities decreased significantly by day 5 and fell with $\psi_{ext,}$ it fell more rapidly in the presence of $MgCl_2$ than with NaCl stress. By day 11, ψ_s was 0.83 MPa lower in the $MgCl_2$ treatment than in NaCl. In wheat ψ_s commenced at a lower level than in tomato but fell more slowly in both salinities and there was evidence for an increase in ψ_s at the end of the period. Wheat ψ_s did not differ significantly between the two salt treatments on any day of the experiment.

4.2.3.2.3 Leaf turgor potential

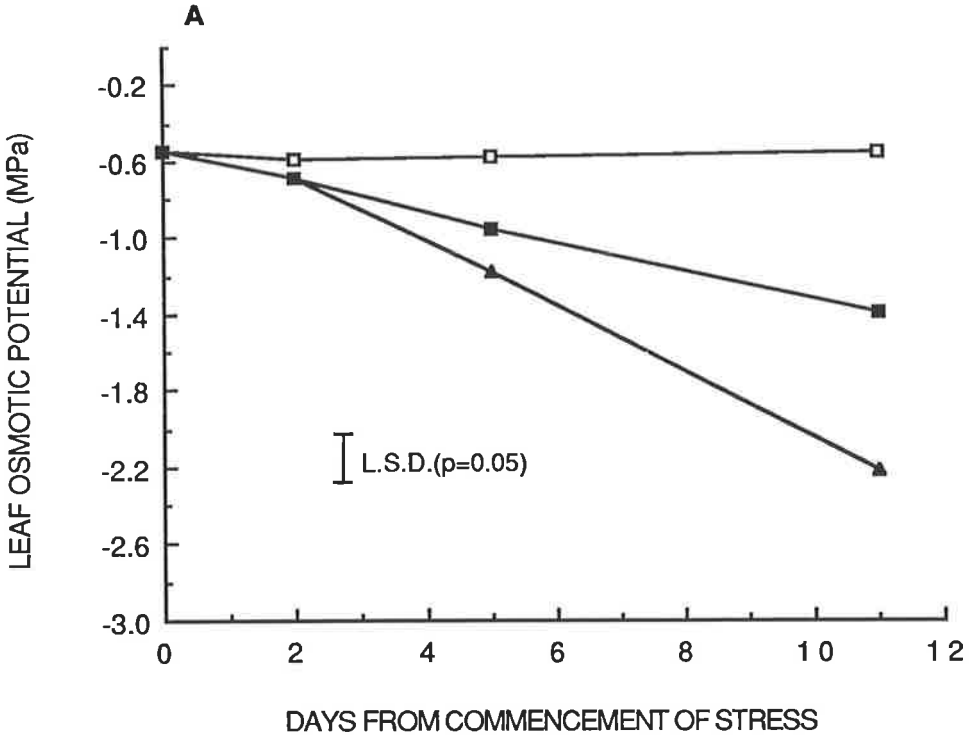
The turgor potential of control wheat (Table 10B) was higher than that of tomato (Table 10A) and both remained unchanged throughout the experimental period. The turgor potential of NaCl stressed tomato increased initially above the control value but returned to the control value by day 11; at no time did it decrease below the control turgor. The leaf turgor potential of $MgCl_2$ stressed plants was significantly higher than that of those under NaCl stress and remained above control levels throughout. In wheat, the interaction between treatment and time in the data was not significant. Considering the mean turgor potential over the period of stress there was less turgor in plants under NaCl stress than in control plants, although turgor was not completely lost. $MgCl_2$ stress had no significant effects on turgor.

Fig. 33 Changes in leaf osmotic potential (-MPa) of tomato and wheat as influenced by NaCl and $MgCl_2$

A. tomato

B. wheat

□ control
■ NaCl
▲ $MgCl_2$


A
LEAF OSMOTIC POTENTIAL (MPa)
-0.2
-0.6
-1.0
-1.4
-1.8
-2.2
-2.6
-3.0
L.S.D.(p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

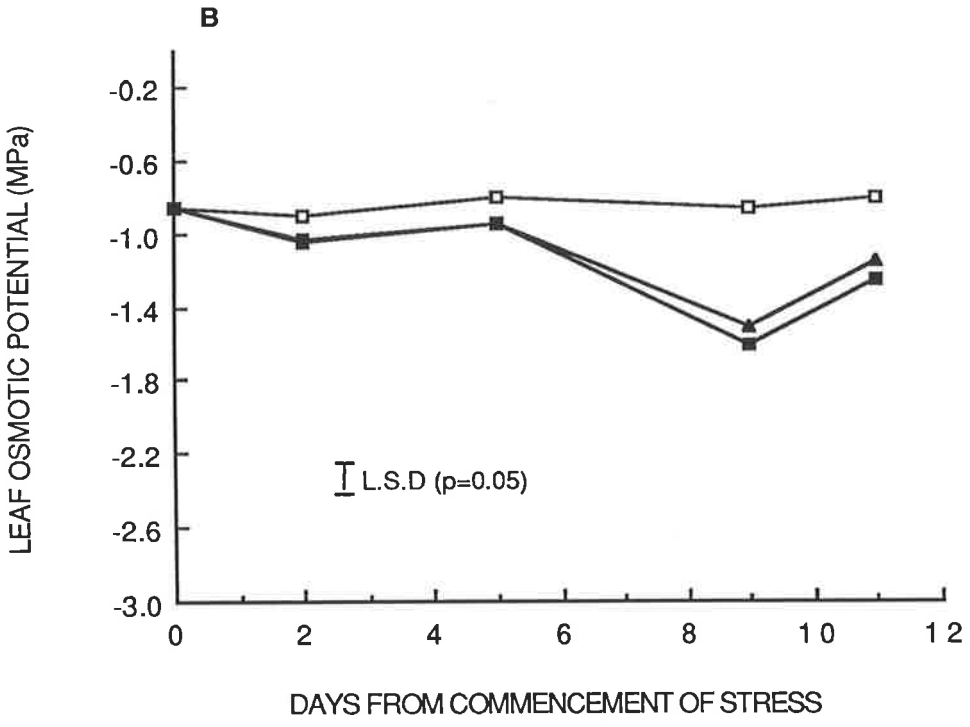

B
LEAF OSMOTIC POTENTIAL (MPa)
-0.2
-0.6
-1.0
-1.4
-1.8
-2.2
-2.6
-3.0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Table 10A Effect of a divalent and a monovalent cationic salt on leaf turgor potential of tomato (MPa)

TREATMENT	DAYS FROM COMMENCEMENT OF STRESS				
	0	2	5	11	MEAN
CONTROL	0.32	0.34	0.34	0.30	0.32
NaCl	-	0.45	0.58	0.29	0.44
$MgCl_2$	-	0.42	0.81	0.50	0.58***
MEAN	0.32	0.40	0.57***	0.36	0.46

SOURCE OF VARIATION	VR	L.S.D(p=0.05)
TREATMENT	16.99***	
PERIOD	13.44***	
TREAT X PERIOD	4.59***	0.15

*** (P=0.01)

Table 10B Effect of a divalent and a monovalent cationic salt on leaf turgor potential of wheat (MPa)

TREATMENT	DAYS FROM COMMENCEMENT OF STRESS					
	0	2	5	9	11	MEAN
CONTROL	0.55	0.61	0.50	0.56	0.60	0.56
NaCl	-	0.69	0.36	0.38	0.38	0.47***
$MgCl_2$	-	0.69	0.46	0.61	0.54	0.58
MEAN		0.66	0.47	0.51	0.51	0.54

SOURCE OF VARIATION	VR	L.S.D (P=0.05)
TREATMENT	3.33***	0.096
PERIOD	5.17***	0.111
TREAT X PERIOD	1.49 NS	

NS =NOT SIGNIFICANT
*** (p=0.01)

4.2.3.2.4 Leaf sodium concentration

The leaf sodium concentration in the control and $MgCl_2$ stressed plants of both tomato (Fig 34A) and wheat (Fig 34B) remained low throughout. In NaCl stressed tomato plants, leaf Na^+ concentration increased by day 5 and reached a concentration of 0.91 mmole g^{-1} dw by day 11. This was more rapid than in wheat where the leaf Na^+ concentration on day 11 was only 0.38 mmole g^{-1} dw.

4.2.3.2.4 Leaf magnesium concentration

Leaf Mg^{++} concentrations in control and NaCl stressed tomato(Fig. 35A) and wheat (Fig. 35B) plants were similar, the concentration decreasing over the 11 days. In $MgCl_2$ stressed wheat, the concentration increased until day 9 but then showed a tendency to fall with further stress. In tomato, Mg^{++} accumulated throughout the period to reach a similar concentration to that found in wheat leaves.

4.2.3.2.5 Leaf chloride concentration

The leaf Cl^- concentration of control plants of both tomato (Fig 36A) and wheat (Fig 36B) remained low until the end of the experiment. In tomato under NaCl and $MgCl_2$ stress leaf Cl^- increased until day 11, the rate of uptake being significantly higher in plants under $MgCl_2$ than under NaCl stress. As with tomato, leaf chloride concentration in wheat was significantly higher in plants in $MgCl_2$ than in NaCl salinity. Although, wheat under NaCl salinity took up a higher concentration of Cl^- than did tomato, tomato accumulated more Cl^- than wheat in $MgCl_2$ stress.

4.2.3.2.6 Proline

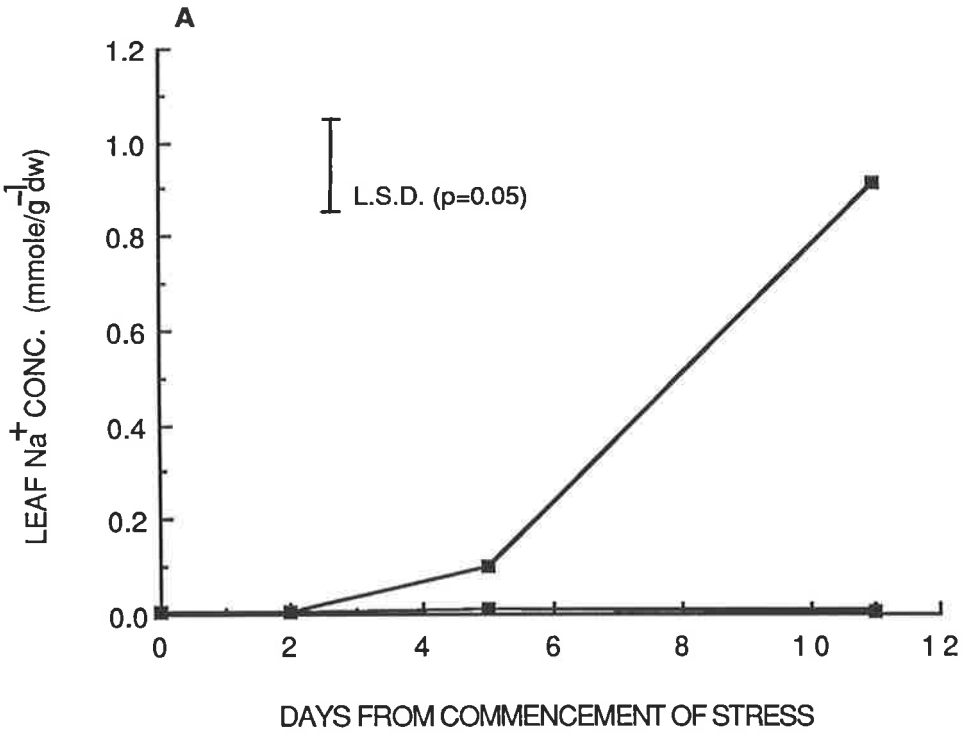
The proline concentration in the control plants of tomato (Fig 37A) and wheat (Fig 37B) remained low and unchanged throughout the experimental period. Accumulation had commenced before day 5 in tomato under both salinities but plants subjected to $MgCl_2$ salinity accumulated more than those in NaCl stress. In wheat, the accumulation commenced later than day 5 in both salinity treatments but by day 9, the proline concentration in plants

Fig. 34 Leaf Na^+ concentrations (mmole,g^{-1} dw) of tomato and wheat under NaCl and $MgCl_2$ stress

A. tomato

B. wheat

□ control
■ NaCl
▲ $MgCl_2$


A
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LEAF Na^+ CONC. (mmole/g^{-1}dw)
L.S.D. (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

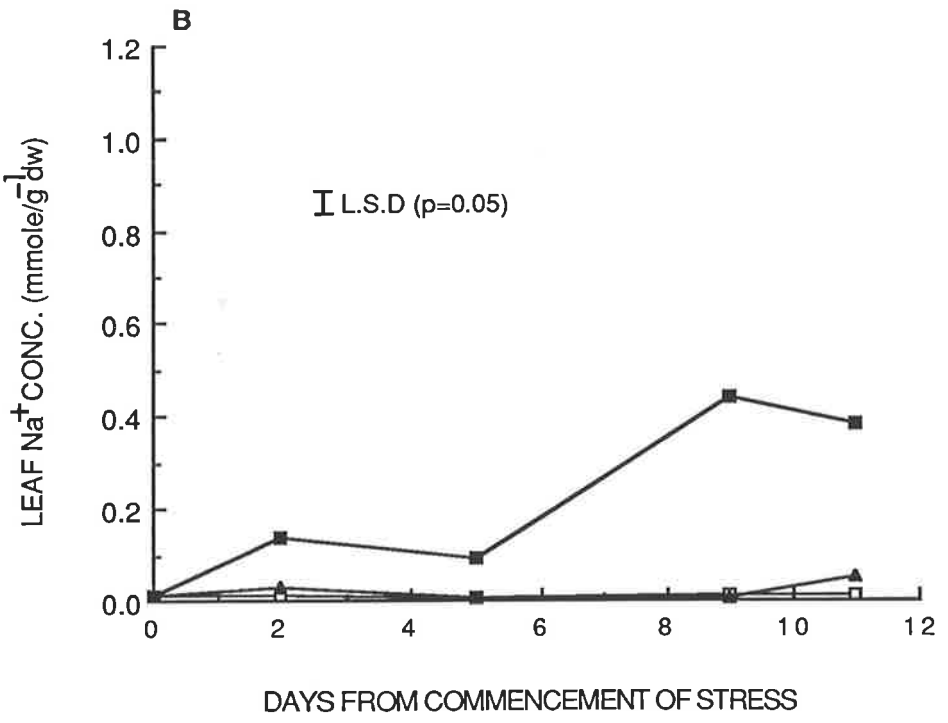
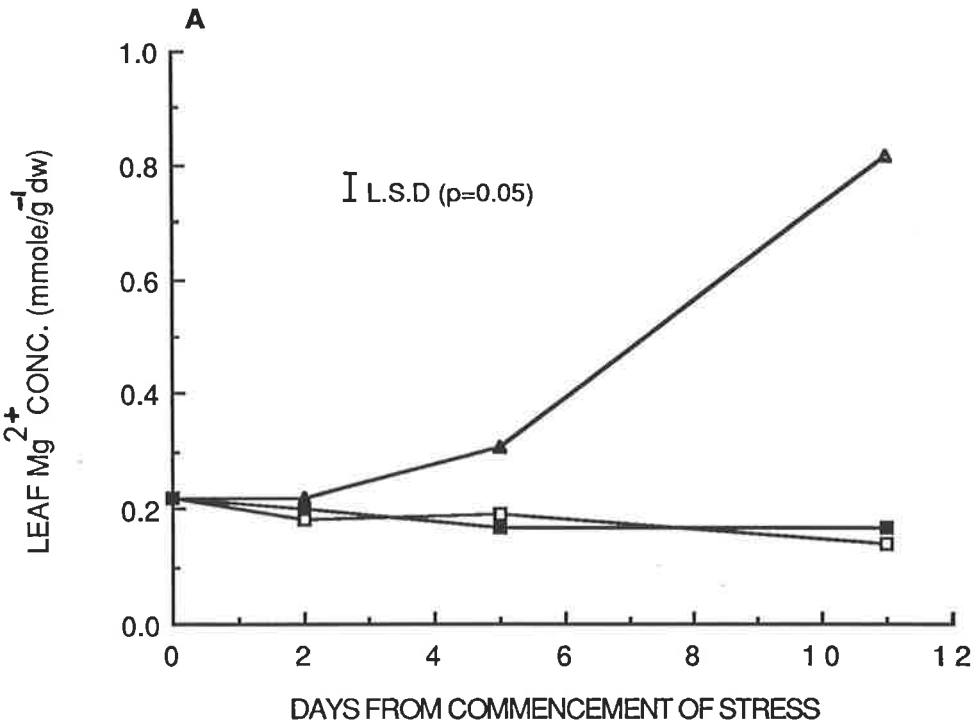

B
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LEAF Na^+ CONC. (mmole/g^{-1}dw)
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Fig. 35 Leaf Mg^{++} concentrations (mmole,g^{-1} dw) of tomato and wheat under NaCl and $MgCl_2$ stress

A. tomato

B. wheat

□ control
■ NaCl
▲ $MgCl_2$


A
1.0
0.8
0.6
0.4
0.2
0.0
I L.S.D (p=0.05)
LEAF Mg^{2+} CONC. (mmole/g dw)$^{-1}$
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

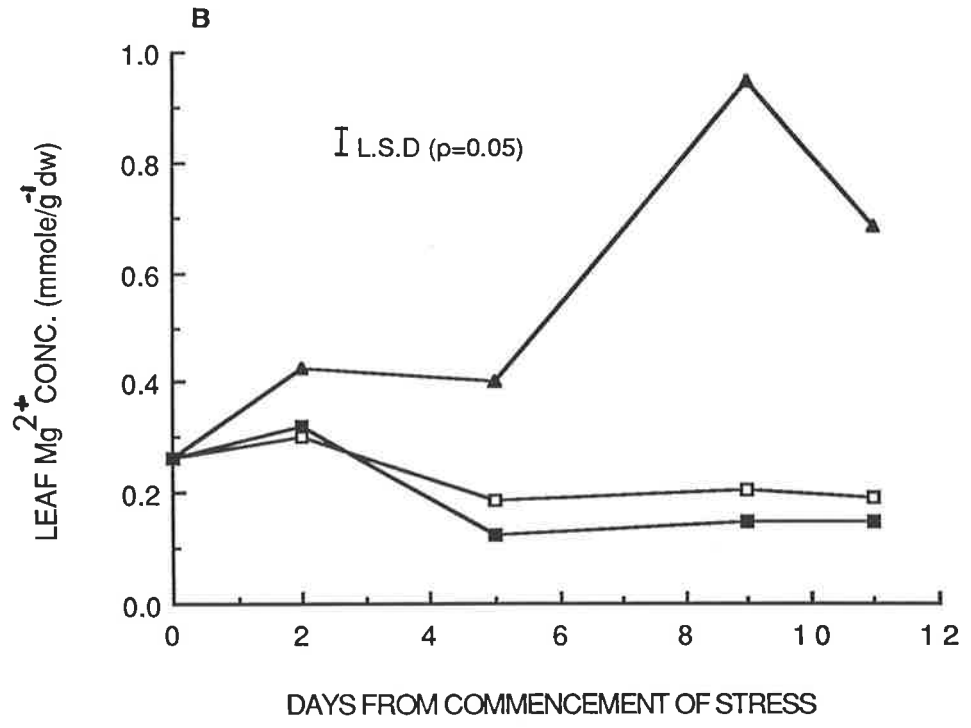
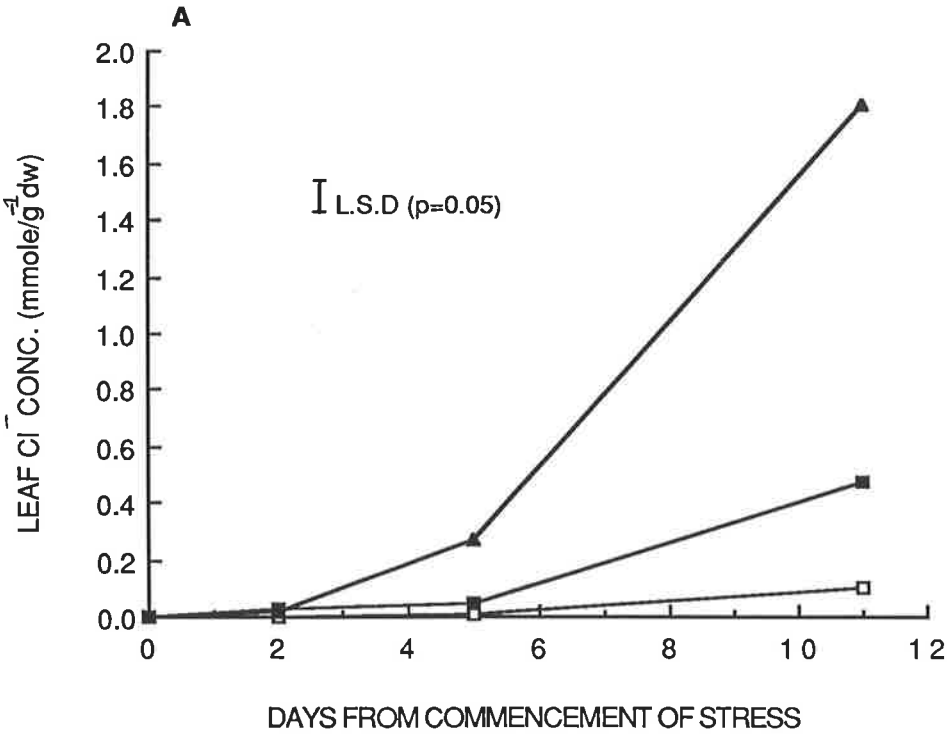

B
1.0
0.8
0.6
0.4
0.2
0.0
I L.S.D (p=0.05)
LEAF Mg^{2+} CONC. (mmole/g dw)$^{-1}$
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Fig. 36 Leaf Cl^- concentrations (mmole,g^{-1} dw) of tomato and wheat under NaCl and $MgCl_2$ stress

A. tomato

B. wheat

□ control
■ NaCl
▲ $MgCl_2$


A
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LEAF Cl⁻ CONC. (mmole/g⁻¹dw)
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

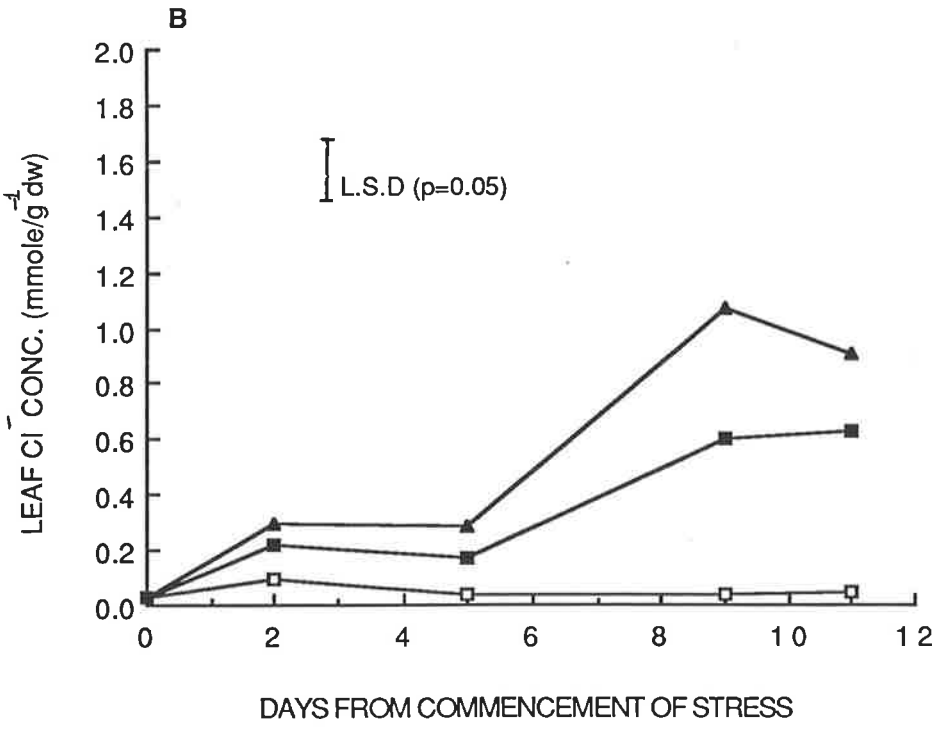
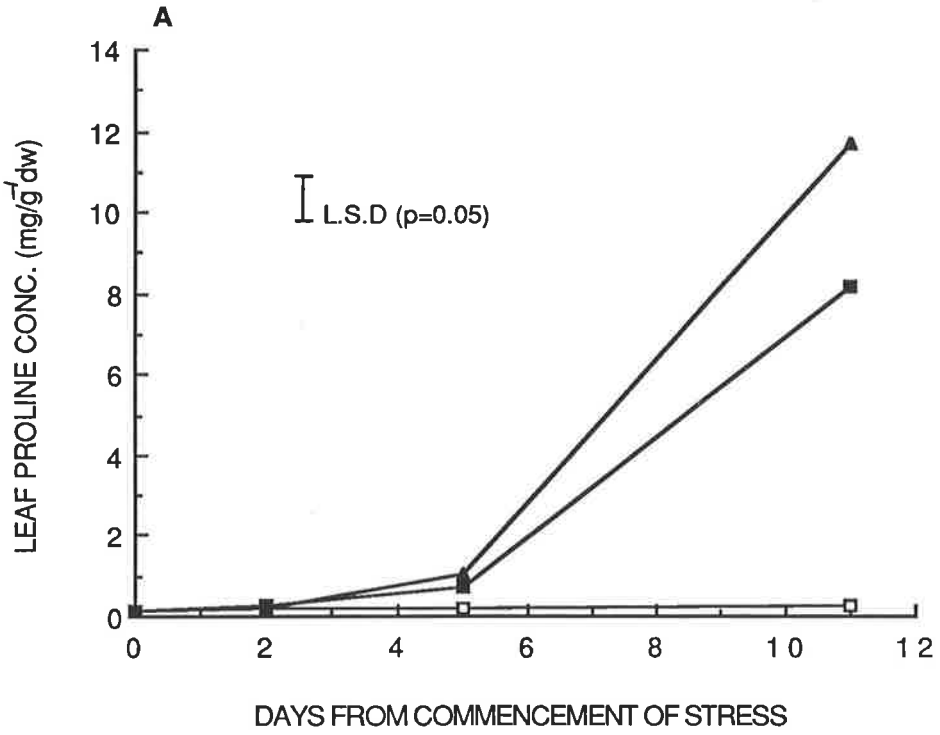

B
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LEAF Cl⁻ CONC. (mmole/g⁻¹dw)
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

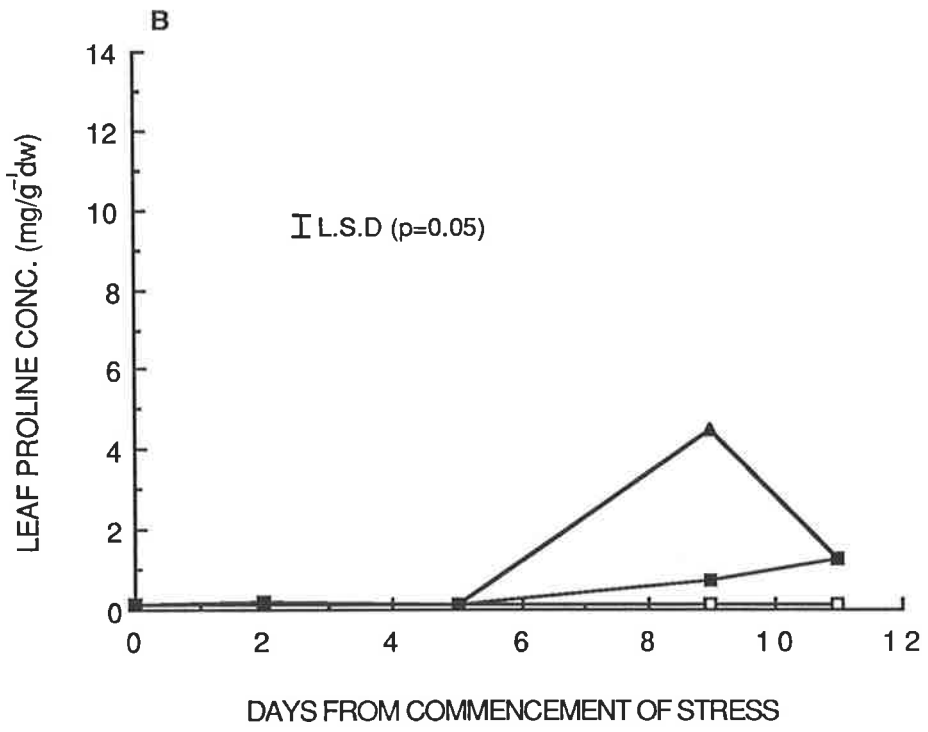
Fig. 37 Leaf proline concentration (mg,g^{-1} dw) of tomato and wheat under NaCl and $MgCl_2$ stress

A. tomato

B. wheat

□ control
■ NaCl
▲ $MgCl_2$


A
LEAF PROLINE CONC. (mg/g⁻¹dw)
14
12
10
8
6
4
2
0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


B
LEAF PROLINE CONC. (mg/g⁻¹dw)
14
12
10
8
6
4
2
0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

under $MgCl_2$ salinity had increased to 4.5 mg g^{-1} dw compared to 0.73 mg g^{-1} dw in NaCl stress. Leaf proline concentration apparently fell in $MgCl_2$ treated plants late in the experiment but both NaCl and $MgCl_2$ stressed plants then had a significantly higher concentration than the control. In general, the accumulation in tomato under both salinities was greater than in wheat.

4.2.3.2.7 Quaternary Ammonium compounds

A. Trigonelline

The trigonelline concentration in control tomato plants remained the same throughout (Table 11). Both NaCl and $MgCl_2$ caused some small increase in leaf trigonelline concentration, although it is difficult to reach firm conclusions on time trends in the data.

B. Glycinebetaine

The glycinebetaine concentration of control wheat leaves varied only little during the experimental period (Fig 38). Both NaCl and $MgCl_2$ induced a considerable accumulation of glycinebetaine, the rates of accumulation being very similar in both cases. There was a significant difference in glycinebetaine concentration between the two treatments on day 9 but this was not sustained and may have been due to sampling effects.

4.2.3.2.8 Growth

A. Fresh weight of shoot

The shoot fresh weight of both tomato and wheat was reduced to a similar extent by both NaCl and $MgCl_2$ stress. However, growth in fresh weight continued throughout the experiment (Fig 39A,B).

B. Leaf area

Salt stress, irrespective of ionic species, reduced leaf area expansion to a similar extent in both species (Fig 40 A,B). The relative reduction being 40 (NaCl), 44 ($MgCl_2$) and 37 (NaCl), 43 ($MgCl_2$) per cent in tomato and wheat respectively. Leaf expansion continued at

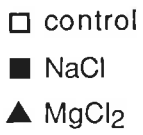
Table 11 Effect of a divalent and a monovalent cationic salt on the trigonelline concentration of tomato (mg g^{-1}d.wt.)

TREATMENT	DAYS FROM COMMENCEMENT OF STRESS				
	0	2	5	11	MEAN
CONTROL	0.90	0.77	0.86	0.75	0.79
NaCl	-	1.04	0.89	1.09***	1.08
$MgCl_2$	-	0.83	1.35***	0.57	0.92
MEAN		0.88	1.03	0.80	0.91

SOURCE OF VARIATION	VR	L.S.D (p=0.05)
TREATMENT	3.378	
PERIOD	3.880	
TREAT X PERIOD	6.348***	0.299

*** (p=0.01)

Fig. 38 Leaf glycinebetaine concentration (mg,g^{-1} dw) of wheat as influenced by NaCl and $MgCl_2$ at the rooting medium



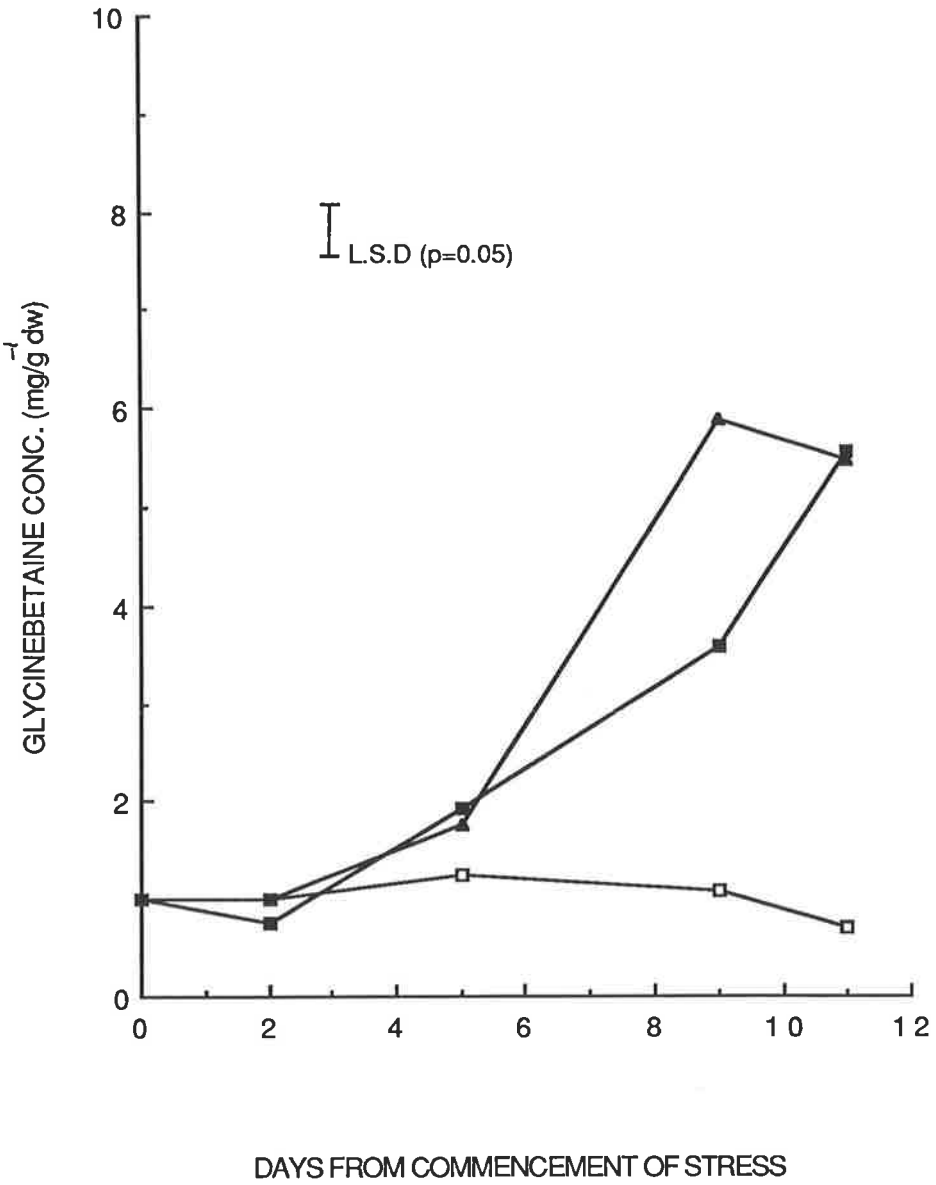
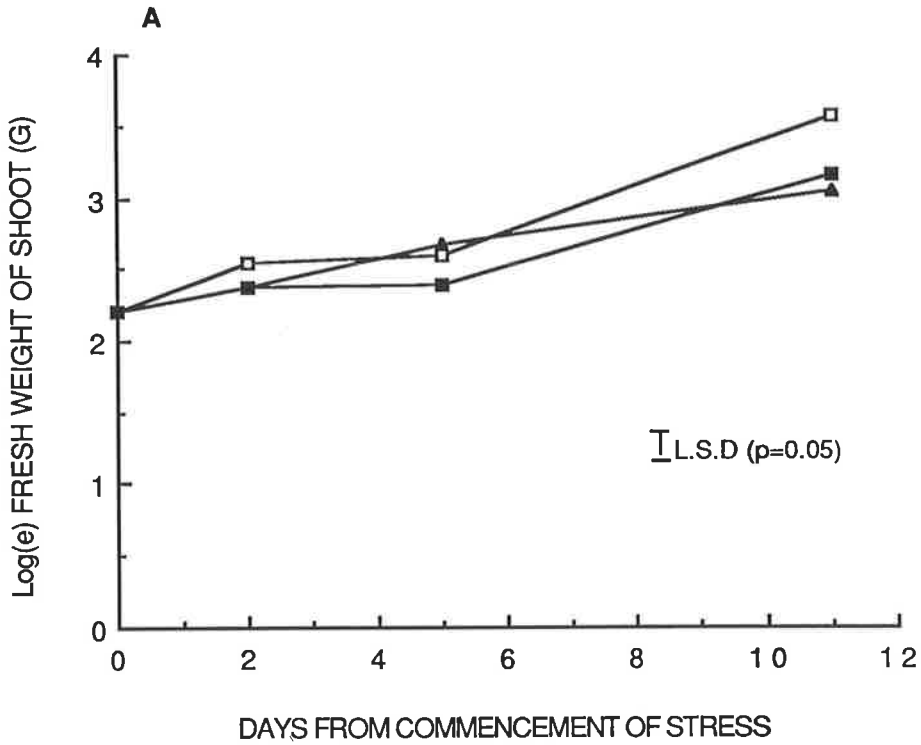

10
8
6
4
2
0
0
2
4
6
8
10
12
L.S.D (p=0.05)
GLYCINEBETAINE CONC. (mg/g dw)$^{-1}$
DAYS FROM COMMENCEMENT OF STRESS

Fig. 39 Shoot fresh weight of tomato and wheat as influenced by NaCl and $MgCl_2$ at the rooting medium

A. tomato

B. wheat

□ control
■ NaCl
▲ $MgCl_2$


A
Log(e) FRESH WEIGHT OF SHOOT (G)
4
3
2
1
0
0
2
4
6
8
10
12
L.S.D (p=0.05)
DAYS FROM COMMENCEMENT OF STRESS

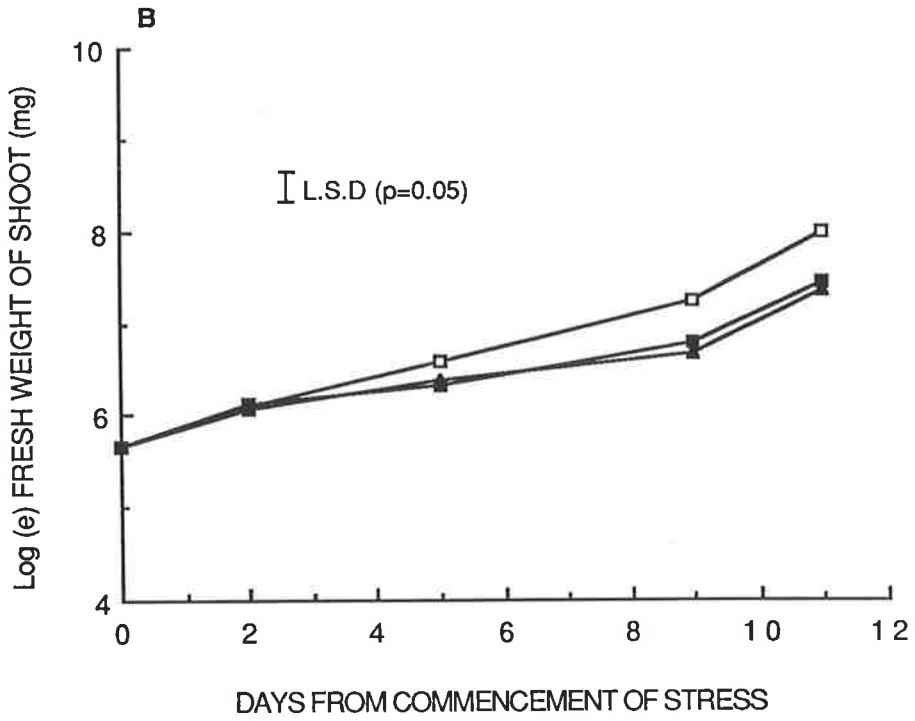
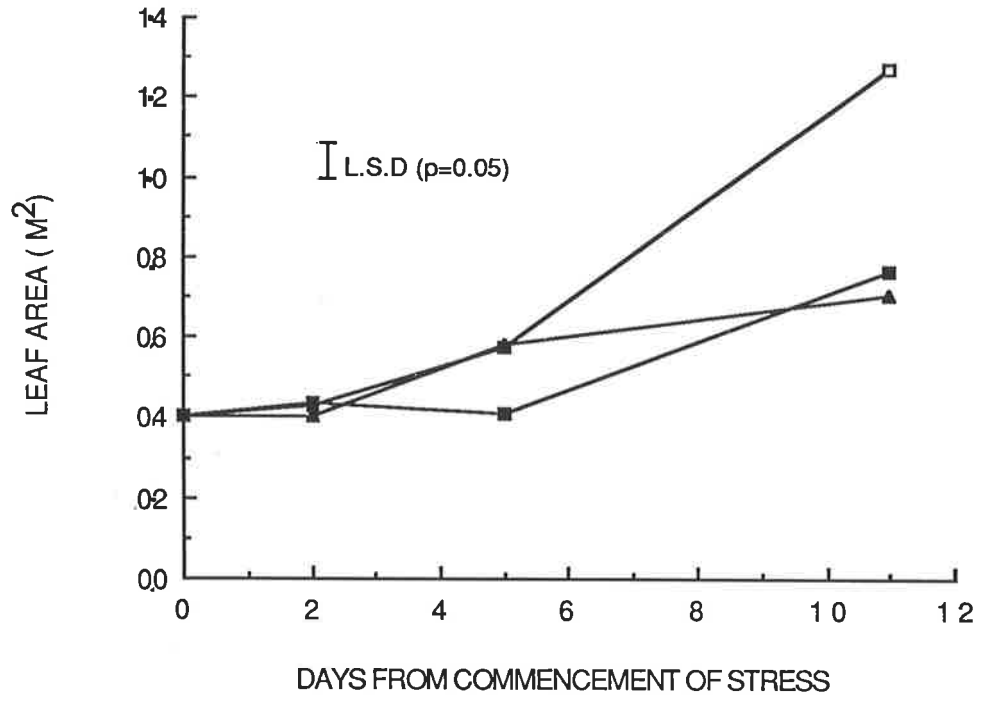

B
Log (e) FRESH WEIGHT OF SHOOT (mg)
10
8
6
4
0
2
4
6
8
10
12
L.S.D (p=0.05)
DAYS FROM COMMENCEMENT OF STRESS

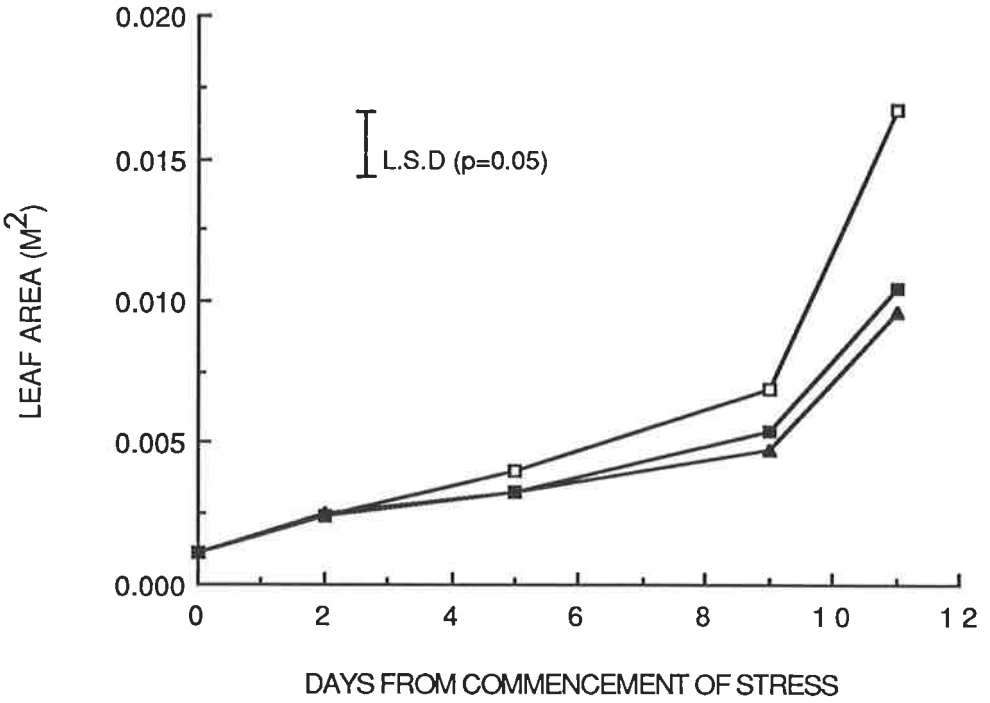
Fig. 40 Leaf area expansion in tomato and wheat as influenced by NaCl and $MgCl_2$ at the rooting medium

A. tomato

B. wheat

□ control
■ NaCl
▲ $MgCl_2$


LEAF AREA (M2)
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0·0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


LEAF AREA (M2)
0.020
0.015
0.010
0.005
0.000
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

a significant rate under both salinities in both species even as the stress intensified.

C. Dry weight of shoot

In NaCl and $MgCl_2$ stressed wheat and tomato shoot dry matter production continued as in control plants (Table 12 A,B). The apparent reduction in dry weight on day 2 in tomato in NaCl stress may be due to sampling error.

D. Fresh weight /dry weight ratio

The fresh weight : dry weight ratio (a measure of succulence) increased with time in control tomato and wheat plants until the end of the experiment (Table 13 A,B). Although the ratio was small in control plants of wheat compared to tomato before the stress began, by day 11 it increased by 3.35 times and was similar to that in tomato. In NaCl and $MgCl_2$ stressed wheat succulence also increased and it did not differ significantly from control until day 5; however, the increase thereafter to day 11 was less in these two stress treatments, resulting in significantly lower values compared to control. Plants experiencing NaCl stress were found to be more succulent than those in $MgCl_2$ stress. With regard to tomato, although there was a slight increase by day 2 in NaCl stressed plants only, the ratio decreased thereafter, remaining constant until day 11, and the ratio did not vary greatly between the two salinity treatments.

4.2.3.3 Discussion

Proline accumulated in significant amounts in both species, being greater in plants supplied with a divalent cationic salt, $MgCl_2$ than those given a monovalent cationic salt, NaCl, which supports the previous data on wheat (section 4.2.2). The difference in proline concentration could either be due to differences in rapidity of initiation of accumulation or to differences in the rate of accumulation. In both species under both salinities, proline accumulation commenced apparently on or after day 5, and as there were no significant differences in proline concentration between divalent and monovalent cationic salt stress on that day it seems unlikely that the difference in proline accumulation was due to a difference in the rapidity of initiating accumulation. The difference in proline accumulation between NaCl

Table 12A Effect of a divalent and a monovalent cationic salt on Log(e) shoot dry weight of tomato (g)

TREATMENT	DAYS FROM COMMENCEMENT OF STRESS				
	0	2	5	11	MEAN
CONTROL	0.77	0.97	0.99	1.40	1.03
NaCl	0.77	0.73	0.89	1.44	0.96
$MgCl_2$	0.76	0.88	1.13	1.32	1.03
MEAN	0.77	0.86	1.01	1.39	1.00

SOURCE OF VARIATION	VR		L.S.D(p=0.05)
TREATMENT	2.93		
PERIOD	90.52		
TREAT X PERIOD	3.55	***	0.142

*** (p=0.01)

Table 12B Effect of a divalent and a monovalent cationic salt on Log(e) shoot dry weight of wheat (mg)

TREATMENT	DAYS FROM COMMENCEMENT OF STRESS					
	0	2	5	9	11	MEAN
CONTROL	4.72	4.80	4.90	5.03	5.51	4.99
NaCl	4.72	4.78	4.79	5.07	5.21	4.91
$MgCl_2$	4.72	4.93	4.93	4.96	5.32	4.95
MEAN	4.72	4.81	4.87	5.02***	5.35***	4.95

SOURCE OF VARIATION	VR	L.S.D(p=0.05)
TREATMENT	0.589 NS	
PERIOD	14.286***	0.186
TREAT X PERIOD	0.491 NS	

NS - NOT SIGNIFICANT
*** (p=0.01)

Table 13A Effect of a divalent and a monovalent cationic salt on fresh weight : dry weight ratio of tomato

TREATEMENT	DAYS FROM COMMENCEMENT OF STRESS				
	0	2	5	11	MEAN
CONTROL	7.58	7.72	10.05	11.60	9.24
NaCl	7.58	10.22	7.46	7.42	8.17
$MgCl_2$	7.58	7.58	7.02	7.67	7.46
MEAN	7.58	8.51	8.18	8.90	8.29

SOURCE OF VARIATION	VR	L.S.D (p=0.05)
TREATMENT	9.834***	1.10
PERIOD	0.913NS	
TREATMENT X PERIOD	7.084***	1.92

*** (p=0.01)
NS Not significant

Table 13B Effect of a divalent and a monovalent cationic salt on fresh: dry weight ratio of wheat

TREATMENTS	DAYS FROM COMMENCEMENT OF STRESS					
	0	2	5	9	11	MEAN
CONTROL	2.50	3.65	5.37	9.21	11.61	6.47
NaCl	2.50	3.87	4.62	5.64	9.05	5.14
$MgCl_2$	2.50	3.37	4.33	5.37	7.28	4.57
MEAN	2.50	3.63	4.77	6.74	9.32	5.39

SOURCE OF VARIATION	VR	L.S.D (p=0.05)
TREATMENT	18.663***	
PERIOD	58.669***	
TREAT X PERIOD	3.933***	1.61

*** (p=0.01)

and $MgCl_2$ stress increased during the course of stress intensification suggesting that the rate of accumulation would have been influenced differently by the salt species. Table 14 shows that the rate of accumulation was higher in $MgCl_2$ than in NaCl salinity. These differences in proline accumulation, although they reflect changes in leaf water and osmotic potential in both NaCl and $MgCl_2$ stress in tomato, may not be due directly to these factors because the greater proline accumulation in wheat under $MgCl_2$ stress was accompanied by changes in neither ψ_{leaf} nor ψ_s . A similar observation was recorded for barley (Chu *et al.*, 1976 b) in a short term stress experiment. There also, proline accumulation differed between divalent and monovalent cationic salts but leaf ψ and ψ_s did not. This led to the suggestion that the variation was due to a specific ion effect on proline metabolism.

Proline accumulation in salt stressed tissues may be explained by either increased activity of Δ^1-P-5-C reductase (Huber, 1974) or decreased oxidation of proline to glutamic acid with Δ^1-P-5-C dehydrogenase (Boggess *et al.*, 1976). The higher proline concentration in tomato than in wheat with NaCl stress may have been due to the high leaf Na^+ concentration in tomato stimulating the activity of Δ^1-P-5-C reductase. NaCl has been demonstrated to increase the activity of Δ^1-P-5-C reductase and although this may explain the increase in proline accumulation *in vivo* with NaCl stress, lack of information on the effect of Mg^{++} on this enzyme restricts an explanation of the greater proline accumulation in plants under $MgCl_2$ salinity.

It is evident from the studies of Boggess *et al.*, (1975) that osmotic potential *per se* was without any effect on the enzyme Δ^1-P-5-C dehydrogenase and inhibition was attributed to the presence of ions, particularly Cl^-. Further, inhibition of this enzyme was greater when $MgCl_2$ rather than NaCl was used as the osmoticum. Inhibition of this enzyme would reduce the oxidation of proline resulting in increased proline concentration if proline synthesis continued unaltered. This would contribute to the greater accumulation of proline in $MgCl_2$ stress *in vivo* as leaf Cl^- concentration in both species was significantly higher in $MgCl_2$ than in NaCl stress. This could also explain the reduced accumulation under ionic stress in tomato as compared to wheat in the previous experiment. Tomato excludes Cl^- under conditions of salt shock (Section 4.1.2) and so this enzyme would not have been inhibited

Table 14 Effect of a divalent and a monovalent cationic salt on rate of proline accumulation in tomato and wheat ($\mu g\ h^{-1}$)

DAYS	TOMATO			DAYS	WHEAT		
	NaCl	$MgCl_2$	Difference		NaCl	$MgCl_2$	Difference
0-2	1.80	-	-	0-2	0.52	1.56	+1.04
2-5	5.80	11.89	+6.09	2-5	-	-	-
5-11	51.80	74.06	+22.26	5-9	6.51	45.31	+38.8
				9-11	11.45	-68.75	-80.2

resulting in continued oxidation of the proline synthesised. SO_4^{2-} is also a poor inhibitor of this enzyme which would explain the lower proline accumulation in SO_4^{2-} salinity in the previous experiment.

Although inhibition of proline oxidation could account for proline accumulation in salt stressed plants, the case under PEG stressed tomato and wheat in the previous experiment is more tenuous because significant accumulation of proline occurred with only 30 per cent loss in water content (calculated from fresh weight and dry weight) , a loss that was unlikely to concentrate the cell sap to a level inhibitory to this enzyme. To account for a level of Cl^- equivalent to that in NaCl stressed plants, PEG stressed plants must concentrate the cell sap by 300 per cent. Therefore, significant accumulation following inhibition of this enzyme by Cl^- seems an unlikely explanation for the PEG-stimulated accumulation in the previous experiment.

Although $\psi_{ext.}$ in NaCl and $MgCl_2$ stress were the same (-1.065 MPa) the leaf chloride concentration increased more rapidly in $MgCl_2$ stress than in NaCl stress. Cl^- uptake is proportional to external concentration and this ion has been demonstrated to move against an electro-chemical gradient (Mengel and Kirkby, 1979). In the present case, $MgCl_2$ and NaCl were supplied at the same osmotic potential so the higher leaf Cl^- concentration in $MgCl_2$ stressed plants could be due to the higher Cl^- concentration in the external medium 316 mM compared to 221.4 mM. Alternatively, it has been suggested (Leonard, 1985) that Na^+ and Cl^- compete for the same site for transport into the cell, reducing Cl^- uptake in NaCl salinity. The high leaf Cl^- concentration in the presence of $MgCl_2$ might be expected to result in a lower leaf ψ_s than with NaCl. This was true for tomato but not so in wheat.

In contrast to proline, glycinebetaine accumulation was not influenced by ionic species. The greater leaf Cl^- concentration in $MgCl_2$ salinity did not influence glycinebetaine accumulation, suggesting that accumulation is independent of changes in tissue Cl^- concentration. Further, despite a 485 per cent higher leaf cation concentration (Mg^{++}) in plants under $MgCl_2$ compared to NaCl salinity stress (Na^+), glycinebetaine accumulation did not increase proportionately which suggests that accumulation is unlikely to be related to changes in bulk leaf cation concentration. However, it cannot be dismissed as a controlling

factor until glycinebetaine accumulation is demonstrated without any changes in internal ion concentration or vice-versa.

Accumulation of both proline and glycinebetaine occurred without any reduction in the rate of dry matter production confirming the data in section 4.1.3 and contradicting the views of others(Storey and Wyn Jones, 1978; Hanson and Nelson, 1978).

4.2.4 Effects of divalent and monovalent cations and an anion at iso-ionic strength in the rooting medium on proline accumulation

In the previous experiment, plants were salinized with NaCl and $MgCl_2$ at iso-osmotic potential. In that case, the cation and anion strength of the $MgCl_2$ solution was higher than that of the NaCl solution. Internal ion concentration is proportional to the ion concentration in the external medium (Section 4.1.2) which would account for the greater leaf ion concentration in $MgCl_2$ than NaCl. This could enhance proline accumulation by directly influencing the enzymatic reactions leading to proline accumulation. This experiment tested the hypothesis that the greater accumulation of proline in plants under $MgCl_2$ than NaCl stress found previously was due to higher ionic strength in the rooting medium.

4.2.4.1 Methods

Seedlings of tomato CV. Duke were grown at a 20 ± 1°C day and 17±1°C night temperature with a 16 h photoperiod and a photon flux density of 350 to 420 µEin sec^{-1} (Materials and Methods 3.2.1, 3.2.2). NaCl and $MgCl_2$ containing full strength Hoagland's nutrient solution was supplied 25 days after emergence of the seedlings. Cation (Na^+ or Mg^{2+}) or anion (Cl^-) strength of the rooting medium was increased gradually by 0.02 cal/mole day^{-1}. Molarity of the respective salt solutions equivalent to the appropriate ionic strength was computed using the formula $\mu_i = 1/2 \{ C_i Z_i^2 \}$ (Harned and Owen, 1958) where μ_i is ionic strength (cal/mole), C_i= concentration of ion (M), Z_i= valency of the ion. Details of the various physical characteristics of NaCl and $MgCl_2$ solutions at iso-ionic strengths of Cl^-, Na^+ and Mg^{2+} are given in Table 15. The youngest fully expanded leaves of the stressed plants were sampled on 0, 2 (0.02), 4 (0.06), 6 (0.10), 8 (0.14), and 10 (0.18 cal/mole) days after imposition of stress, and of plants in nutrient solution on day 0 and 10 for measurement of leaf water, osmotic and turgor potential (3.2.6), proline (3.2.9.2) and leaf ion content (3.2.11). Stomatal resistance of both upper and lower surfaces of the expanded leaves was measured using a Li-Cor LI 60 diffusive resistance porometer (3.2.5.1). The design was a factorial randomised block with three replications of each treatment.

Table 15 Physical characteristics of NaCl and $MgCl_2$ solutions

CATION/ANION	IONIC STRENGTH (cal/mole) cation	anion	total	CONC. (mM)	$\Psi_{ext.}$ (MPa)
Cl(Na)/Na(Cl)	0.02	0.02	0.04	40	0.23
	0.06	0.06	0.12	120	0.59
	0.10	0.10	0.20	200	0.95
	0.14	0.14	0.28	280	1.32
	0.18	0.18	0.36	360	1.67
Cl(Mg)	0.04	0.02	0.06	20	0.09
	0.12	0.06	0.18	60	0.38
	0.20	0.10	0.30	100	0.67
	0.28	0.14	0.42	140	0.96
	0.36	0.18	0.54	180	1.25
Mg(Cl)	0.02	0.01	0.03	10	0.02
	0.06	0.03	0.09	30	0.16
	0.10	0.05	0.15	50	0.31
	0.14	0.07	0.21	70	0.45
	0.18	0.09	0.27	90	0.59

4.2.4.2 Results

4.2.4.2.1 Leaf water potential

In stressed plants (Fig 41) ψ_{leaf} decreased after day 2 in plants under Cl(Na) and Cl(Mg), while in Mg(Cl) stress the decrease was significant only on day 10. The decrease in ψ_{leaf} in Na(Cl) and Cl(Mg) was similar to day 6, but thereafter there was a more rapid decrease in plants under Cl(Mg) despite the fact that $\psi_{ext.}$was less negative than with Na(Cl). However, in all stress treatments, ψ_{leaf} decreased below $\psi_{ext.}$ on the final day of the experiment.

4.2.4.2.2 Leaf osmotic potential

A significant decrease in ψ_s was observed in plants subjected to Na(Cl) and Cl(Mg) stress by day 4 but in the Mg(Cl) treatment the decrease was significant only on day 6 and remained constant thereafter (Fig 42). The decrease in ψ_s of plants under Na(Cl) and Mg(Cl) stress was similar and did not differ until day 8, thereafter decreasing rapidly in plants under Na(Cl) stress. The decrease in ψ_s was greater in plants under Cl (Mg) than in NaCl stress.

4.2.4.2.3 Leaf turgor potential

The interaction between treatments and period of stress in the data was non-significant (Table 16). However, main effects showed a higher turgor in plants under Cl(Mg) or Mg(Cl) than those under NaCl stress. The mean turgor potential was higher in all stress treatments than the control(+0.29 MPa).

4.2.4.2.4 Stomatal resistance

A. Upper surface

Stomatal resistance of the upper surface (Table 17A) of the control tomato plants was about 3 sec cm^{-1} both before the stress began and on day 10. In plants subjected to NaCl stress, stomatal resistance increased progressively as stress intensified. Stomatal resistance also increased, although not to the same extent, in the Cl(Mg) treatment but not in the Mg(Cl) treatment where stomatal resistance was similar to the control throughout.

Fig. 41 Changes in leaf water potential (-MPa) of tomato stressed with iso-ionic strengths of NaCl and $MgCl_2$

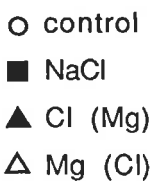
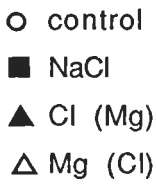
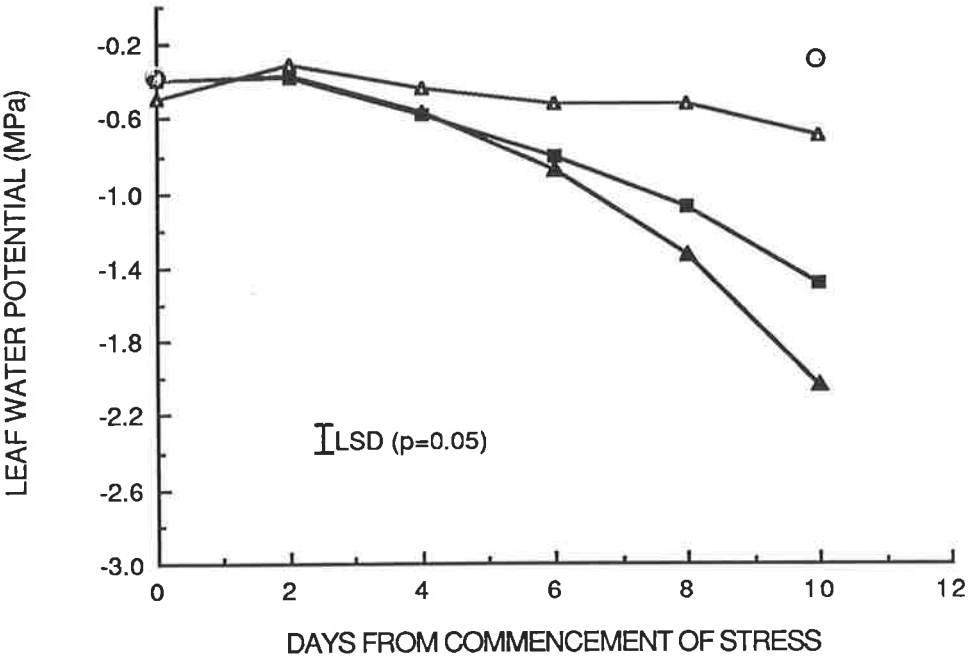


Fig. 42 Changes in leaf osmotic potential (-MPa) of tomato stressed with iso-ionic strengths of NaCl and $MgCl_2$




LEAF WATER POTENTIAL (MPa)
-0.2
-0.6
-1.0
-1.4
-1.8
-2.2
-2.6
-3.0
0
2
4
6
8
10
12
LSD (p=0.05)
DAYS FROM COMMENCEMENT OF STRESS

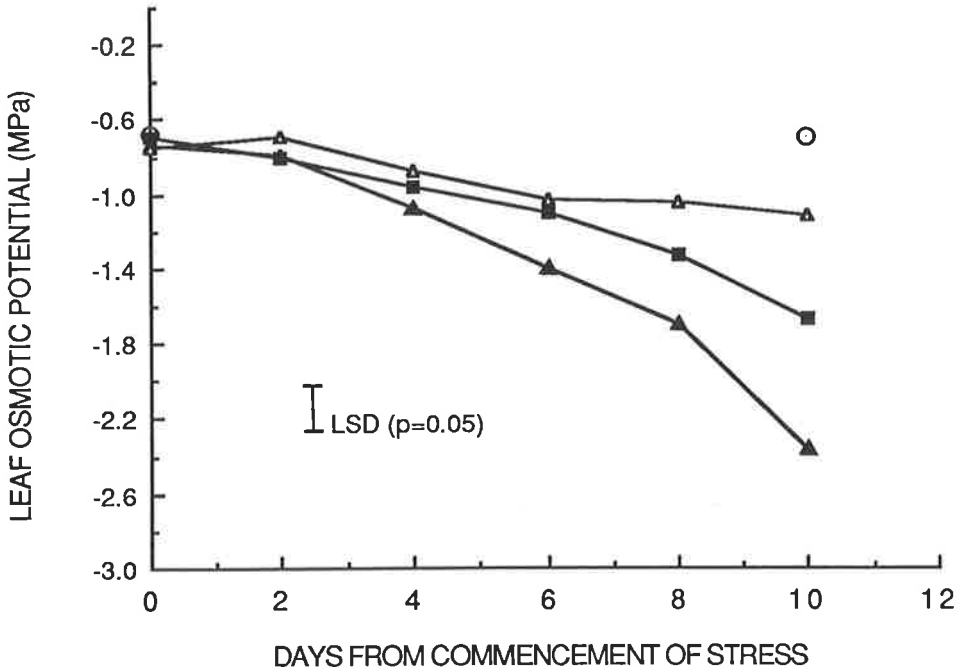

LEAF OSMOTIC POTENTIAL (MPa)
-0.2
-0.6
-1.0
-1.4
-1.8
-2.2
-2.6
-3.0
0
2
4
6
8
10
12
LSD (p=0.05)
DAYS FROM COMMENCEMENT OF STRESS

Table 16 Effect of cations and an anion at iso-ionic strength in the rooting medium on the leaf turgor potential of tomato (MPa)

CATION/ANION	DAYS FROM COMMENCEMENT OF STRESS						MEAN
	0	2	4	6	8	10	
CL(Na)	0.29	0.43	0.39	0.34	0.25	0.20	0.32
Cl(Mg)	0.33	0.41	0.52	0.52	0.37	0.33	0.41
Mg(Cl)	0.26	0.39	0.43	0.51	0.51	0.43	0.42
[Nut.soln.	0.28	-	-	-	-	0.29	0.29]
MEAN	0.29	0.42	0.45	0.46	0.37	0.32	

SOURCE OF VARIATION	VR		LSD (p=0.05)
IONS	6.82	***	0.063
PERIOD	4.56	***	0.089
IONS X PERIOD	1.81	NS	

*** (p=0.01)
NS - Not significant

Table 17A Effect of cations and an anion at iso-ionic strength in the rooting medium on stomatal resistance (upper surface) of tomato (sec cm^{-1})

CATION/ANION	DAYS FROM COMMENCEMENT OF STRESS						MEAN
	0	2	4	6	8	10	
Cl(Na)/Na(Cl)	3.1	5.8	11.9	12.6	13.3	17.5	10.7
Cl(Mg)	3.2	4.9	6.2	6.2	6.4	7.3	5.7
Mg(Cl)	2.6	4.0	3.3	3.6	4.5	3.1	3.5
[NUT. SOLUTION	3.0	-	-	-	-	2.9	2.9]
MEAN	3.0	4.9	7.1	7.5	8.1	9.3	

SOURCE OF VARIATION	VR	LSD (p=0.05)
IONS	58.98	
PERIOD	11.60	
IONS X PERIOD	5.41***	3.38

*** (P=0.01)

B. Lower surface

The stomatal resistance of the lower surface was less than that of the upper surface (Table 17B). The stomatal resistance of the lower surface in both Cl(Mg) and Mg(Cl) increased gradually until day 10. This contrasts with the response of stomates on the upper surface of the leaf where the ions had much less effect. As with the upper surface, the stomatal resistance of the plants under NaCl stress increased throughout as the external NaCl strength increased.

4.2.4.2.5 Leaf sodium concentration

The leaf Na^+ concentration in control and Mg(Cl) or Cl(Mg) stressed tomato (Fig 43) remained low but increased significantly with time in plants under NaCl stress. The increase in leaf Na^+ concentration was proportional to the increase in $Na^+_{ext.}$

4.2.4.2.6 Leaf magnesium concentration

Leaf Mg^{++} concentration in control and NaCl stressed plants (Fig 44) was low and remained unchanged until day 10 whereas it increased significantly in plants under Mg(Cl) and Cl(Mg) stress. The leaf Mg^{++} concentration increased until day 6 [Cl(Mg)] or day 4 [Mg(Cl)] and maintained a high level until day 8, declining thereafter.

4.2.4.2.7 Leaf chloride concentration

Leaf Cl^-concentration (Fig 45) increased by day 4 in all the stress treatments, being greater in plants under Cl(Mg) than NaCl or Mg(Cl) stress, accumulation continued until day 10. Leaf Cl^- concentration in NaCl and Mg(Cl) was similar until day 6 but thereafter there was a slightly greater accumulation in NaCl. The leaf chloride concentration in plants under Cl(Mg) increased most rapidly, giving twice the concentration of leaves subjected to NaCl by day 10. This continued increase occurred despite the fact that the $Cl^-_{ext.}$ concentration was maintained constant.

4.2.4.2.8 Leaf proline concentration

Leaf proline concentration increased progressively in plants under stress, the rate of

Table 17B Effect of cations and an anion at iso-ionic strength in the wrooting medium on the stomatal resistance (lower surface) of tomato (sec cm^1)

CATION/ANION	DAYS FROM COMMENCEMENT OF STRESS						MEAN
	0	2	4	6	8	10	
Cl(Na)/Na(Cl)	1.1	2.6	4.7	5.9	12.9	15.3	7.1
Cl(Mg)	0.9	1.6	2.9	3.2	9.9	13.2	5.3
Mg(Cl)	1.0	1.4	1.2	2.7	5.0	10.6	3.7
[NUT. SOLUTION	1.0	-	-	-	-	1.6	1.3]
MEAN	1.0	1.8	3.0	4.0	9.3	13.1	

SOURCE OF VARIATION	VR	LSD (p=0.05)
IONS	24.15	
PERIOD	94.99	
IONS X PERIOD	2.85 *	2.45

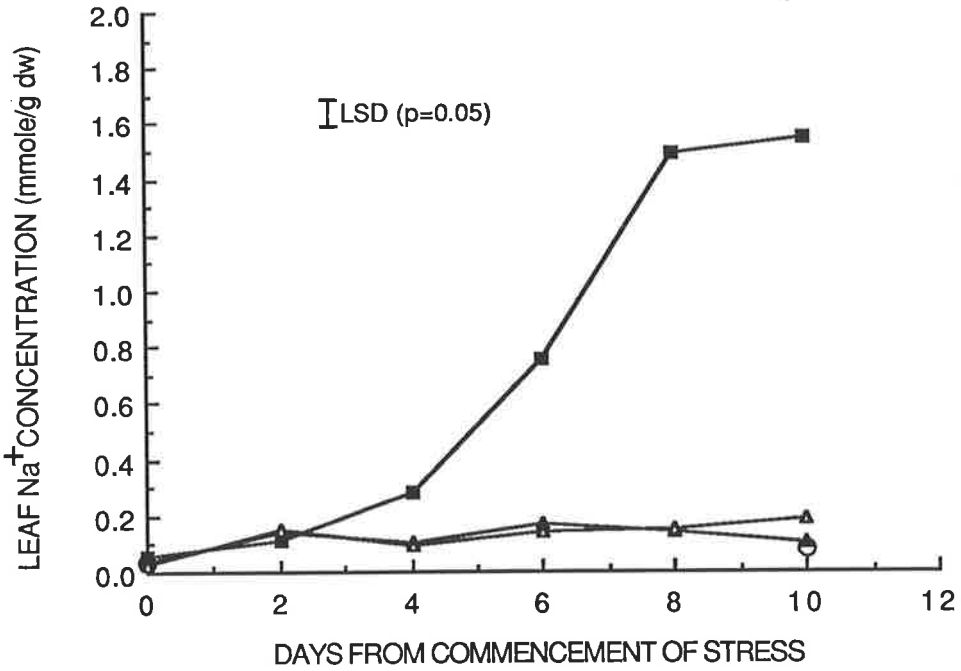
* (P=0.5)

Fig. 43 Leaf Na^+ concentration (mmole,g^{-1}dw) of tomato stressed with iso-ionic strengths of NaCl and $MgCl_2$

- ○ control
- ■ NaCl
- ▲ Cl (Mg)
- △ Mg (Cl)

Fig. 44 Leaf Mg^{++} concentration (mmole,g^{-1}dw) of tomato stressed with iso-ionic strengths of NaCl and $MgCl_2$

- ○ control
- ■ NaCl
- ▲ Cl (Mg)
- △ Mg (Cl)


LEAF Na^+ CONCENTRATION (mmole/g dw)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LSD (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

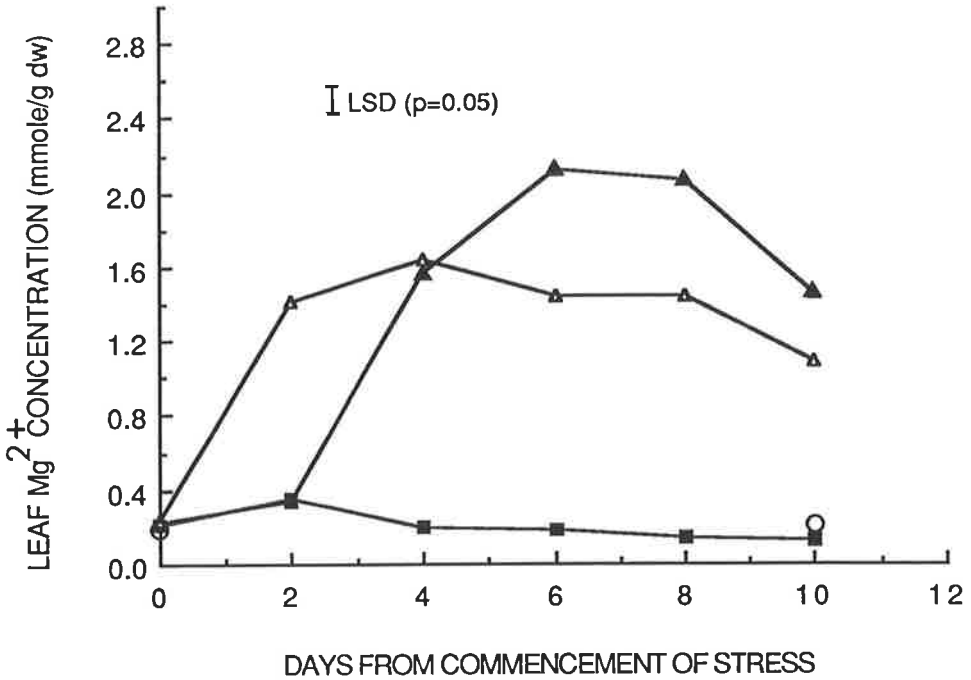

LEAF Mg^{2+} CONCENTRATION (mmole/g dw)
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
LSD (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Fig. 45 Leaf Cl^- concentration (mmole,g^{-1}dw) of tomato stressed with iso-ionic strengths of NaCl and $MgCl_2$

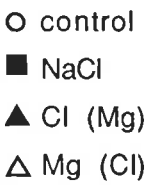
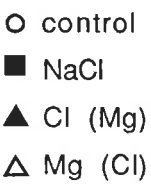
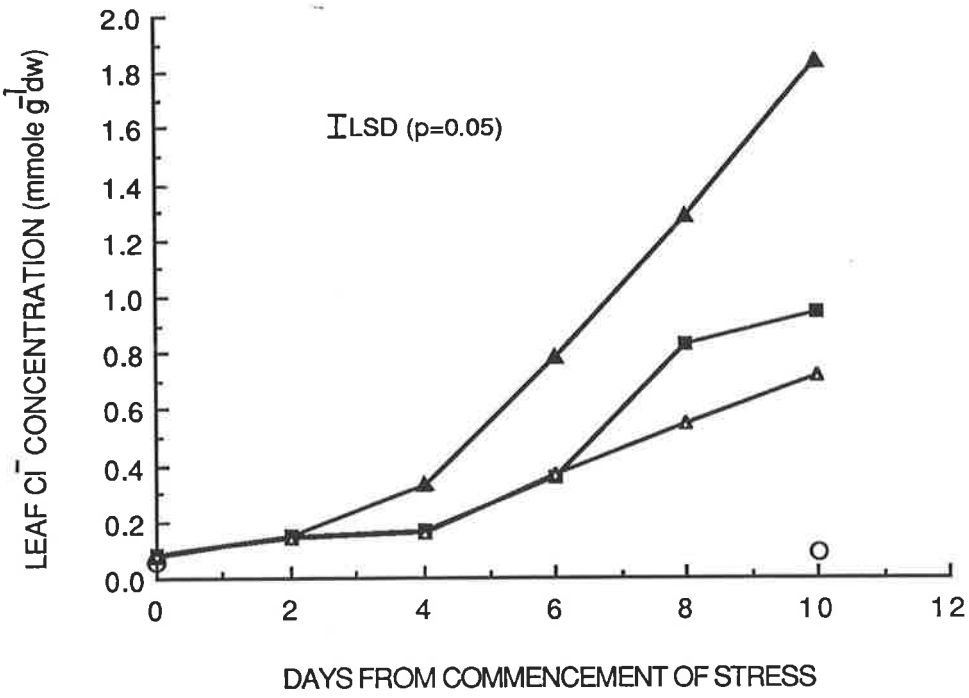
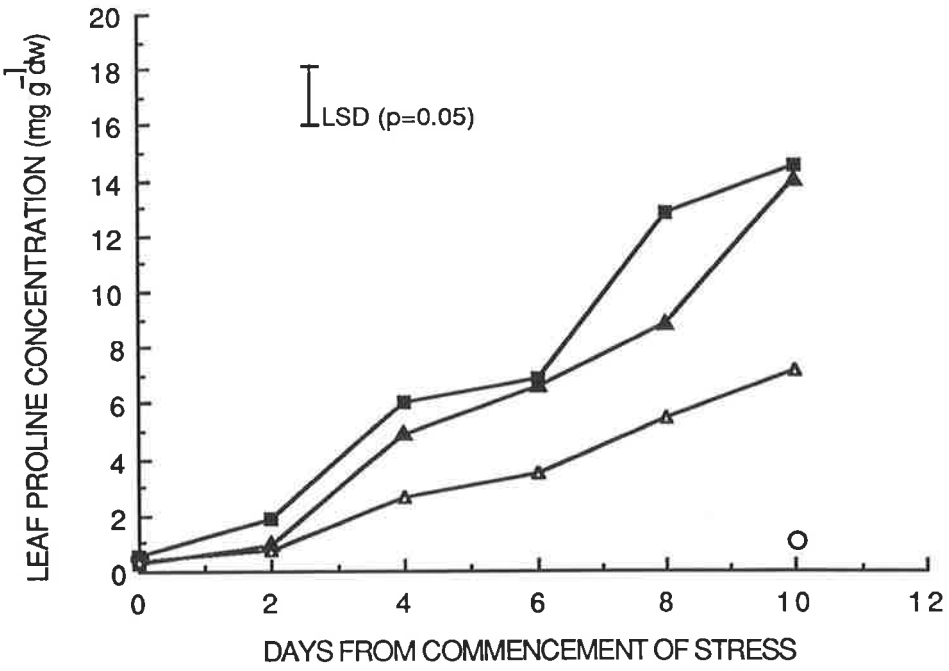


Fig. 46 Leaf proline concentration (mg,g^{-1} dw) of tomato stressed with iso-ionic strengths of NaCl and $MgCl_2$




LEAF Cl⁻ CONCENTRATION (mmole g⁻¹dw)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
LSD (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


LEAF PROLINE CONCENTRATION (mg g⁻¹dw)
20
18
16
14
12
10
8
6
4
2
0
LSD (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

accumulation being greater with NaCl and Cl(Mg) than Mg(Cl) (Fig. 46). The proline concentrations in plants under iso-anionic stress did not differ significantly from each other. In general, plants under divalent cationic salt stress accumulated less proline than those under a monovalent cationic stress at iso-ionic strength of the external medium.

4.2.4.3 Discussion

A decrease in ψ_{leaf} is determined chiefly by the osmotic potential of the rooting medium and the resistances in the water flow pathway (Slavik, 1974; Cowan and Milthorpe, 1968). ψ_{leaf} in Cl (Mg) decreased greatly compared to that in NaCl despite the fact that $\psi_{ext.}$ was low (less negative) [Fig. 47, Table 18]. The possible reasons are: 1. an effect on water flow to the leaf, for instance through a change in resistance in the liquid pathway, although, there is no evidence to support Mg^{++} increasing resistance in the literature. 2. the apparent greater decrease in ψ_{leaf} in Cl(Mg) could be an artifact due to ion accumulation in the apoplast decreasing ψ_{cell} (Oertli, 1966). The difference in ψ_{leaf} cannot be due to this phenomenon as ψ_{leaf} was determined by psychrometry. 3. a lower stomatal resistance in plants under Cl(Mg) than in those under NaCl. Stomatal resistance increases in the presence of divalent cations more than with monovalent cations (Wilson and Mansfield, 1970) but the evidence obtained in the present study contradicts this response. Stomatal resistance has also been found to be proportional to the decrease in $\psi_{ext.}$ (Mansfield and Davis, 1981). The lower $\psi_{ext.}$ in Cl(Mg) than in NaCl may be a reason for the lower stomatal resistance in Cl(Mg), and this could account for the greater transpiration in Cl(Mg) leading to a greater decrease in ψ_{leaf} than in NaCl.

It is impossible to achieve an equivalence of anion and cation strength of $\psi_{soln.}$ for a divalent and a monovalent salt, so any discussion of the effects of these factors on plant metabolism is tempered by this fact. Discussion in this section is hence primarily oriented towards comparing the responses in NaCl with Cl(Mg)[similar anion strength, different cation strength and $\psi_{ext.}$] and NaCl with Mg(Cl) [similar cation strength, different anion strength and $\psi_{ext.}$]. Before discussing the response of proline accumulation to different ionic strength of salt, it is important to establish whether accumulation is influenced by ψ_{ext}, which differed under all ionic treatments. A comparison between the slopes of the regression

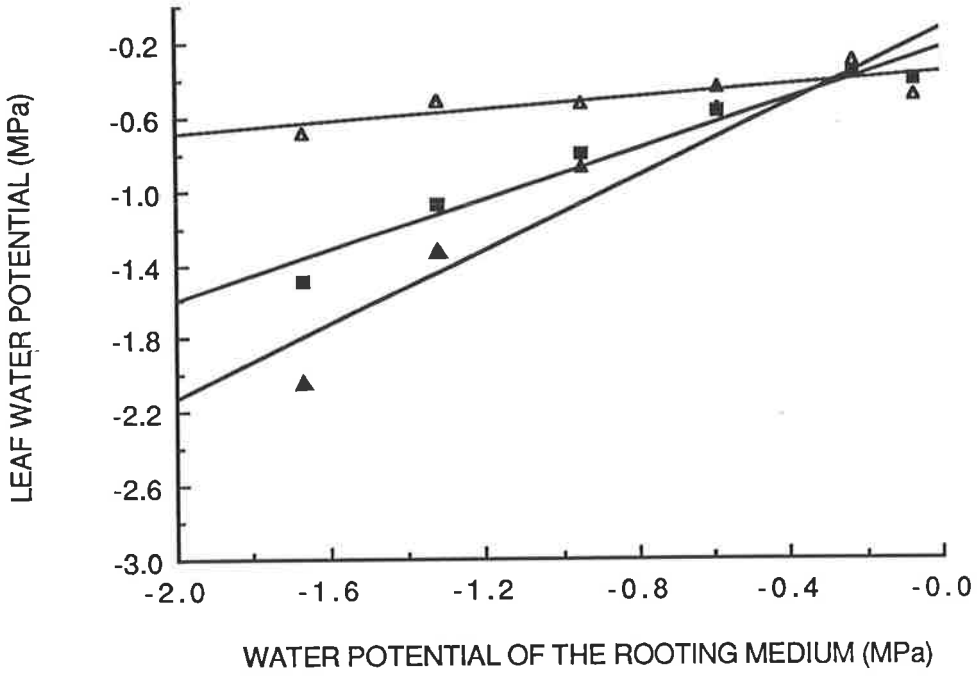
Fig. 47 Relationships between water potential of the rooting medium and of leaf in tomato stressed with iso-ionic concentrations of NaCl and $MgCl_2$ (Data obtained from Fig. 41)

Y= -0.2224 + 0.6581x	R=0.97***	■ NaCl
Y= -0.1363 + 1.2705x	R=0.96***	▲ Cl (Mg)
Y= -0.3587 + 0.4343x	R=0.82***	△ Mg (Cl)

Fig. 48 Relationships between water potential of the rooting medium and leaf proline concentraion in tomato stressed with iso-ionic concentrations of NaCl and $MgCl_2$ (Data obtained from Fig. 46)

Y= -0.0453 - 8.9127x	R= 0.99***	■ NaCl
Y= -0.1329 - 10.6426x	R= 0.99***	▲ Cl (Mg)
Y= -0.2073 - 11.6162x	R= 0.99***	△ Mg (Cl)

*** R values significant at 0.1%


LEAF WATER POTENTIAL (MPa)
-0.2
-0.6
-1.0
-1.4
-1.8
-2.2
-2.6
-3.0
-2.0
-1.6
-1.2
-0.8
-0.4
-0.0
WATER POTENTIAL OF THE ROOTING MEDIUM (MPa)

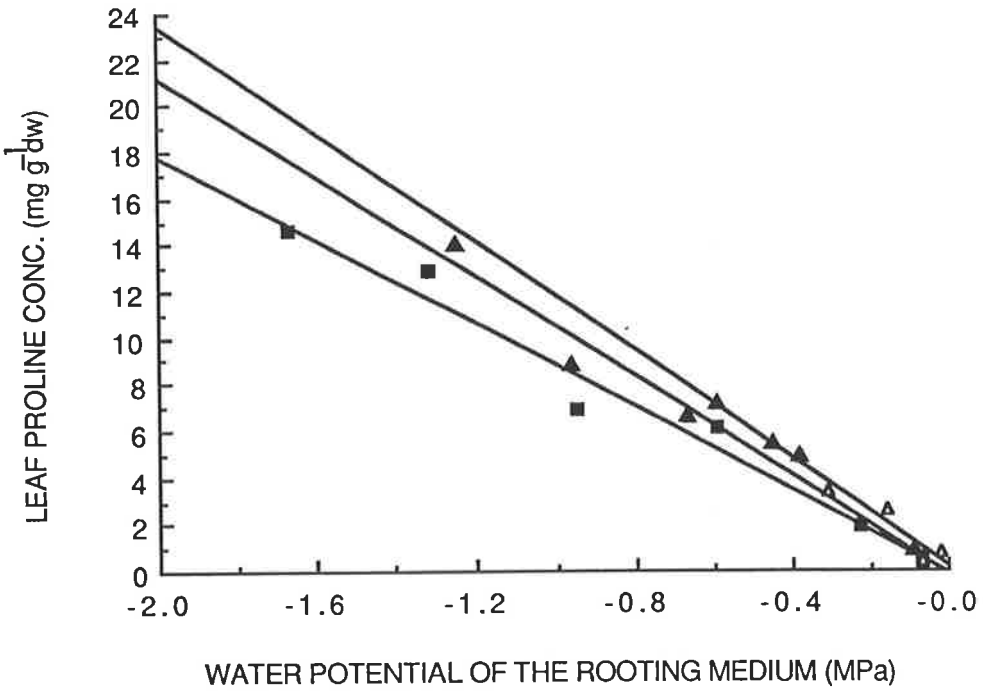

LEAF PROLINE CONC. (mg g⁻¹dw)
24
22
20
18
16
14
12
10
8
6
4
2
0
-2.0
-1.6
-1.2
-0.8
-0.4
-0.0
WATER POTENTIAL OF THE ROOTING MEDIUM (MPa)

Table 18 Test of significance of slopes in the regression analysis between leaf water potential of the external medium and leaf water potential

TREATMENT	REGRESSION EQUATION	R
NaCl	y=-0.2224 + 0.6581X	0.97***
Cl(Mg)	y=-0.1363 + 1.2705X	0.96***
Mg(Cl)	y= -0.3587 +0.4343X	0.82***

SE of the mean difference of slopes	0.4329
Error df (n-4)	12
$t_{(n-4),0,05}$	2.179
LSD (SE_d x $t_{(n-4),0.05}$)	0.5446
	NaCl Cl(Mg) Mg(Cl)

*** (p=0.01)

Table 19 Test of significance of slopes in the regression analysis between leaf proline concentration and water potential of the rooting medium

TREATMENT	REGRESSION EQUATION	R
NaCl	y=-0.0453 -8.9217X	0.99***
Cl(Mg)	y=-0.1329 -10.6426X	0.99***
Mg(Cl)	y= 0.2073 - 11.6162X	0.99***

SE of the mean difference of slopes	0.7877
Error df (n-4)	12
$t_{(n-4),0,05}$	2.179
LSD (SE_d x $t_{(n-4),0.05}$)	1.7164
	NaCl Cl(Mg) Mg(Cl)

between $\psi_{ext.}$ and leaf proline concentration (Fig. 48; Table 19) shows a significant difference between the slopes for NaCl and Cl(Mg) which suggest that proline accumulation may be influenced by specific ions. Figure 49, however, shows that NaCl has a slope intermediate to the Mg(Cl) and Cl(Mg) slopes (Table 20). This argues that the effect, if real, is not a specific ion effect. An interpretation is that $\psi_{ext.}$ controls proline accumulation and that the ion species modifies the relationship between $\psi_{ext.}$ and ψ_{leaf}. However, the results reported in section 4.2.2 where a significant difference in proline concentration between a divalent and monovalent salt occurred despite iso-osmotic potential in the rooting medium supports the conclusion that bulk $\psi_{ext.}$ does not control proline accumulation. A higher proline concentration with a divalent compared to a monovalent salt at iso-osmotic potential in barley (Chu, 1974) also suggests a specific ion effect rather than a effect due to bulk $\psi_{ext.}$

Given that proline accumulation is influenced by specific ions, it is necessary to establish whether this is due to cations or anions in the rooting medium or in the leaf. There was no correlation between leaf Mg^{++} concentration and proline accumulation but leaf Na^{+} concentration and proline accumulation was correlated (Fig. 50). This evidence suggests that the greater concentration of proline accumulated in $MgCl_2$ stress (Section 4.2.3) would be unlikely to be due to a greater Mg^{++} concentration in the leaf. It is also unlikely that the higher proline concentration recorded in section 4.2.3 in $MgCl_2$ salinity was due to the higher $Mg^{++}_{ext.}$ strength because greater proline accumulation occurred with Na^{+} than with Mg^{++} despite a two fold high Mg^{++} concentration in the rooting medium (Fig. 51), suggesting that proline accumulation may be more sensitive to Na^{+} than Mg^{++} in the rooting medium.

It was presumed that the higher Cl^{-} concentration in $MgCl_2$ salinised plants (Section 4.2.3) was due to high $Cl^{-}_{ext.}$ strength at iso-osmotic potential in the rooting medium. However, despite an equal anion strength, leaf Cl^{-} concentration was still significantly higher in $MgCl_2$ than in NaCl (Fig. 52) which suggests an effect of the accompanying cation. Although there is evidence suggesting an anion influence on cation uptake (Rains and Epstein, 1965; Sutcliffe, 1952) there is no evidence for cation influencing anion uptake. It has been presumed that proline accumulation is promoted by Cl^{-} in the leaves due to an inhibition of Δ^{1}-P-5-C

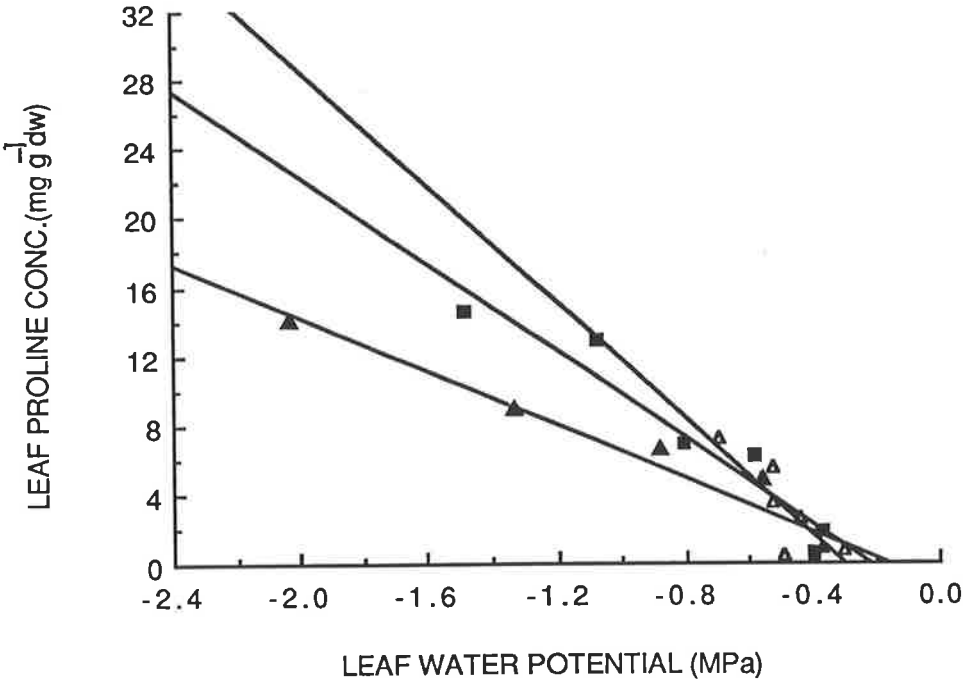
Fig. 49 Relationships between leaf water potential and proline concentration in tomato stressed with iso-ionic concentrations of NaCl and $MgCl_2$ (Data obtained from Figs. 41 and 46)

Y= -2.7434 - 12.5488x R=0.96*** ■ NaCl
Y= -1.2353 - 7.7055x R=0.97*** △ Cl (Mg)
Y= -5.0961 - 16.8809x R=0.81*** ▲ Mg (Cl)

Fig. 50 Relationships between leaf cation concentration and leaf proline concentration in tomato stressed with iso-ionic concentrations of NaCl and $MgCl_2$ (Data obtained from Figs 43, 44 and 46)

Y= 1.3898 + 8.129x R=0.97*** ■ Na
Y= -0.3935 + 3.8008x $R=0.53^{ns}$ ▲ Mg

*** R values significant at 0.1%
ns Not significant


LEAF PROLINE CONC.(mg g^{-1}dw)
32
28
24
20
16
12
8
4
0
-2.4
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
LEAF WATER POTENTIAL (MPa)

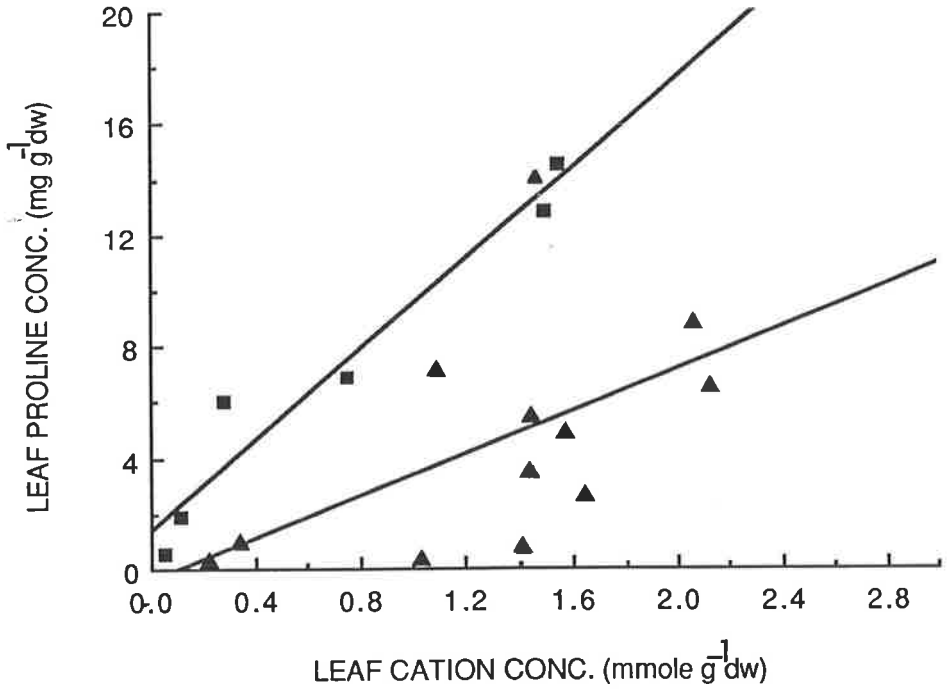

LEAF PROLINE CONC. (mg g^{-1}dw)
20
16
12
8
4
0
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
LEAF CATION CONC. (mmole g^{-1}dw)

Table 20 Test of significance of slopes in the regression analysis between leaf proline concentration and leaf water potential

TREATMENT	REGRESSION EQUATION	R
NaCl	y=-2.7434 - 12.5488X	0.96***
Cl(Mg)	y=-1.2353 - 7.7055X	0.97***
Mg(Cl)	y= -5.0961 - 16.8809X	0.81***

SE of the mean difference of slopes	2.6501
Error df (n-4)	12
$t_{(n-4),0,05}$	2.179
LSD (SE_d x $t_{(n-4),0.05}$)	5.7746

NaCl	Cl(Mg)	Mg(Cl)

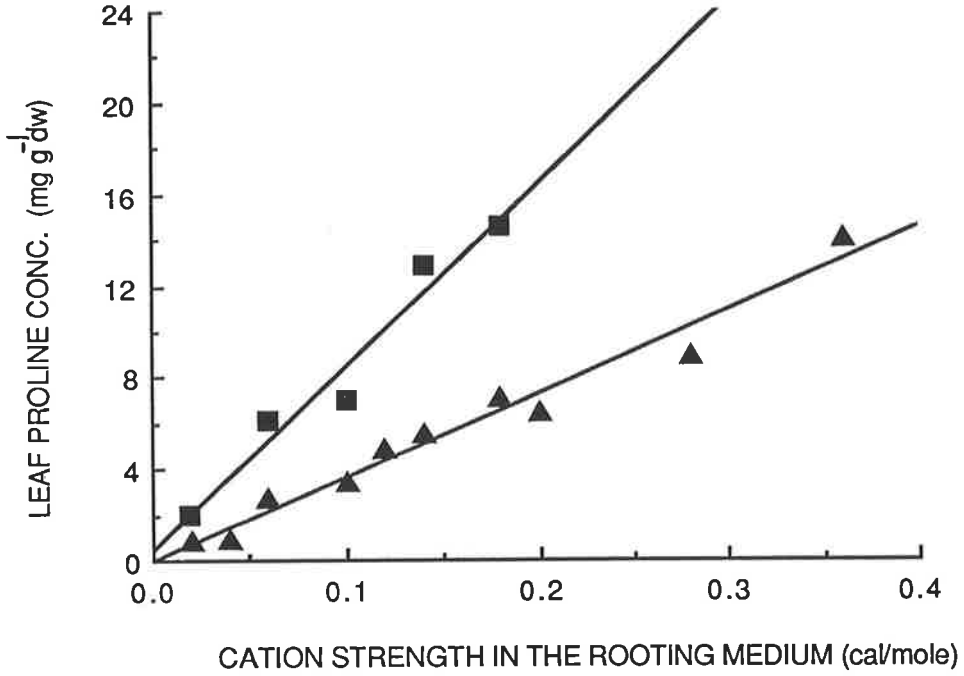
Fig. 51 Relationships between cation strength in the rooting medium and proline concentration in tomato stressed with iso-ionic concentrations of NaCl and $MgCl_2$ (Data obtained from Table 15 and Fig 46)

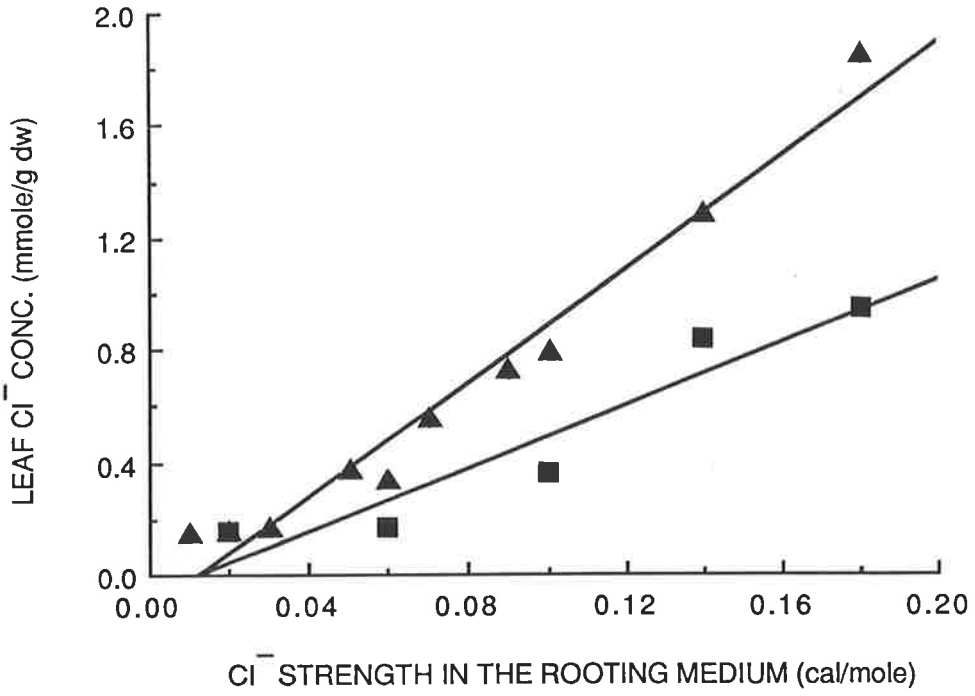
Y= 0.4335 + 80.205x	R=0.98***	■ Na
Y= -0.019 + 36.4887x	R=0.98***	▲ Mg

Fig. 52 Relationships between Cl^- strength of the rooting medium and leaf Cl^- concentration in tomato stressed with iso-ionic concentrations of NaCl and $MgCl_2$ (Data obtained from Table 15 and Fig 46)

Y= -0.0671 + 5.5837x	R=0.95***	■ NaCl
Y= -0.1246 + 10.097x	R=0.98***	▲ $MgCl_2$

*** R values significant at 0.1%


LEAF PROLINE CONC. (mg g⁻¹dw)
24
20
16
12
8
4
0
0.0
0.1
0.2
0.3
0.4
CATION STRENGTH IN THE ROOTING MEDIUM (cal/mole)


LEAF Cl⁻ CONC. (mmole/g dw)
2.0
1.6
1.2
0.8
0.4
0.0
0.00
0.04
0.08
0.12
0.16
0.20
Cl⁻ STRENGTH IN THE ROOTING MEDIUM (cal/mole)

dehydrogenase (Boggess *et al.*, 1975). However, despite a 106 per cent higher leaf Cl^- concentration in plants in Cl(Mg) than NaCl, proline accumulation was similar or even more in NaCl. At a similar internal Cl^- concentration in NaCl and Mg(Cl) on day 6, a greater proline accumulation resulted in NaCl than in Mg(Cl). Proline accumulation in plants under Cl(Mg) was at no time greater than that in NaCl when $Cl^-_{ext.}$ strength was equivalent (Fig. 53). This suggests that maintaining an equivalent anion strength in the medium may lead to equivalent proline accumulation and that greater proline accumulation in $MgCl_2$ salinity (section 4.2.3) is not due to differences in internal Cl^- concentration, but may be due to greater $Cl^-_{ext.}$ strength. If proline accumulation is related to $Cl^-_{ext.}$, then this is not mediated through $Cl^-_{int.}$ and the mechanism by which leaf proline accumulation is initiated is not known. It may presumably be triggered by some factor at the root level, sensitive to either a decrease in $\psi_{soln.}$ or anion concentration or both. Proline accumulates in response to a low $\psi_{ext.}$ for instance caused by PEG (Section 4.2.2, Singh, 1970, Chu, 1974). This demonstrates that ions in the rooting medium are not an exclusive mechanism triggering proline accumulation. It is also possible that $\psi_{ext.}$ controls proline accumulation and the ions in the rooting medium modulate the factor triggering proline accumulation.

There is a parallel response between stomatal resistance and proline accumulation in different salinity treatments in the present study and there is a highly significant correlation between these two responses (Fig. 54). A similar correlation between stomatal resistance and endogenous proline level has been reported for NaCl treated *Commellina communis* (Itai *et al.*, 1988). This suggests at least two possibilities: firstly, the underlying mechanism between these two processes may be the same, resulting in a parallel response. Secondly, the endogenous proline accumulated in response to salt stress regulates stomatal movements. ABA has been shown to increase stomatal resistance (Jones and Mansfield, 1972; Walton, 1980), and to enhance proline accumulation. However, increasing stomatal resistance in epidermal strips of *Commellina communis* floated on varying proline concentrations (Itai *et al.*, 1988) was independent of ABA.

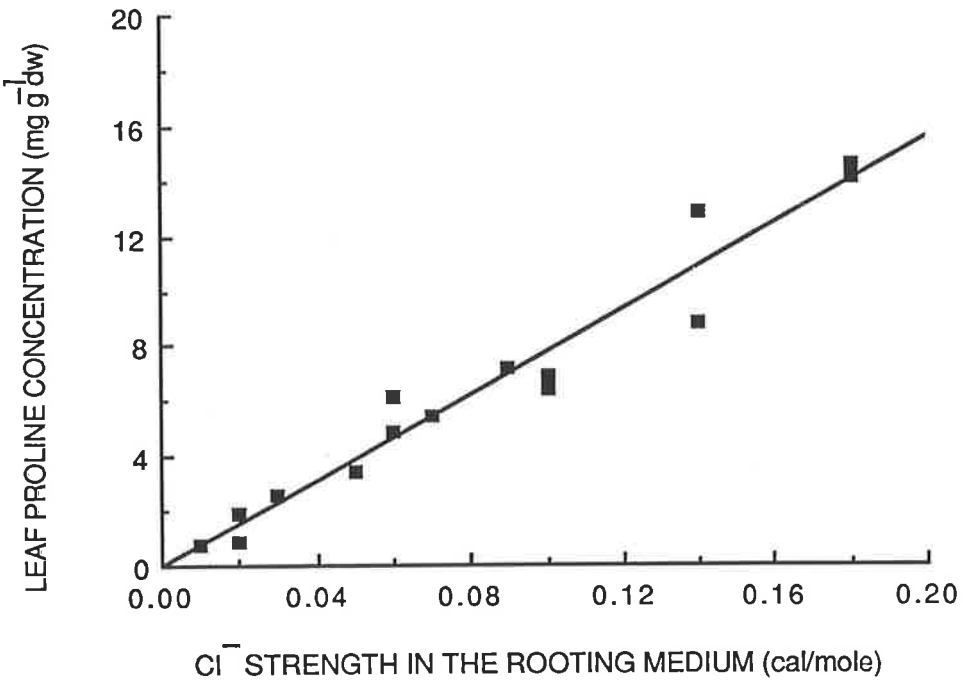
Fig. 53 Relationships between Cl^- strength in the rooting medium and proline concentration in tomato stressed with iso-ionic concentrations of NaCl and $MgCl_2$ (Data obtained from Table 15 and Fig. 46)

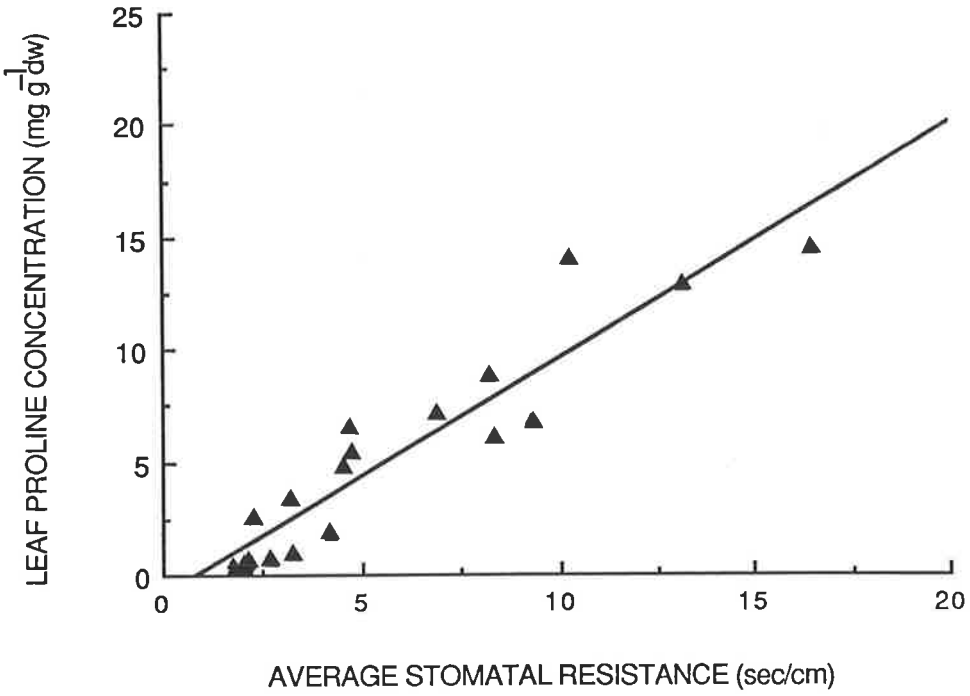
$Y = -0.0284 + 77.7987x$ $R=0.98^{***}$

Fig. 54 Relationships between stomatal resistance and leaf proline concentration in tomato stressed with iso-ionic concentrations of NaCl and $MgCl_2$ (Data obtained from Table 15 and Fig. 46)

$Y = -0.8095 + 1.0467x$ $R=0.93^{***}$

*** R values singificant at 0.1%


LEAF PROLINE CONCENTRATION (mg g⁻¹dw)
20
16
12
8
4
0
0.00
0.04
0.08
0.12
0.16
0.20
Cl⁻ STRENGTH IN THE ROOTING MEDIUM (cal/mole)


LEAF PROLINE CONCENTRATION (mg g⁻¹dw)
25
20
15
10
5
0
0
5
10
15
20
AVERAGE STOMATAL RESISTANCE (sec/cm)

4.2.5 The effect of length of period of exposure to a low concentration of NaCl or KCl on proline and trigonelline accumulation

Previous experiments with tomato suggest that gradual salinization or prolonged exposure to stress results in greater proline accumulation than abrupt exposure to stress. This response could be a time related phenomenon, with proline accumulation requiring time for full display. Further, the absence of proline accumulation at or below $\psi_{ext.}$ -0.565 MPa may also be due to an insufficient period being allowed for accumulation to develop rather than to a true threshold effect.

Variation in proline accumulation under NaCl and $MgCl_2$ salinity (Section 4.2.3) may be due to differences in anion strength of the rooting medium (Section 4.2.4). Maintaining the iso-anionic strength of NaCl and $MgCl_2$ solutions in the rooting medium resulted in the accumulation of a similar proline concentration (Section 4.2.4). However, at iso-anionic strength, $\psi_{ext.}$ of NaCl and $MgCl_2$ solutions differed, as it is impossible to achieve an equivalence of all physical characteristics for divalent and monovalent cationic salts. In an investigation of potential ion effects on proline accumulation a comparison of Na^+ with another monovalent cation, K^+, is more realistic as equivalent cation and anion strengths, and osmotic potentials in the rooting medium, can be achieved.

This experiment was planned to establish, firstly, whether proline and trigonelline accumulation are enhanced in plants exposed to a low concentration of salt for a prolonged period and, secondly, whether proline accumulation differs between KCl and NaCl.

4.2.5.1 Methods

Seedlings of tomato CV. Duke were grown at 20±1 °C day and 17±1°C night temperature with a 16 h photoperiod and a photon flux density of 350 -420 µEin sec^{-1} (Materials and Methods 3.2.1, 3.2.2). NaCl and KCl were added to full strength Hoagland nutrient solution (-0.065 MPa) supplied 25 days after emergence of the seedlings. The concentration of the solution in

the rooting medium was increased by 25 mM day^{-1} for 4 days, to reach 100 mM (-0.45 MPa). The total cationic and anionic strengths of NaCl and KCl were identical (0.025 cal $mole^{-1}$). The plants were maintained at this level for a further period of 10 days. The youngest fully expanded leaves were harvested 2, 4, 6, 8 and 10 days after reaching the final concentration, for measurement of leaf water, osmotic and turgor potential (3.2.6), proline (3.2.9.2), QAC (3.2.9.3) and leaf ion content (3.2.11). Shoot and root dry weights were also measured (3.2.4). Relative growth rate (RGR) and net assimilation rate (NAR) were calculated (Hunt, 1982).

4.2.5.2 Results

4.2.5.2.1 Leaf water potential

The leaf water potential of control plants remained close to -0.2 MPa throughout the experiment (Table 21). The leaf water potential of both NaCl and KCl stressed plants decreased significantly and equally by day 2. It fell further in NaCl stress during the next 2 days but then increased to the level found on day 2 and remained close to that level thereafter. In KCl stressed plants ψ_{leaf} did not change between days 2 and 8, but increased at the end of the experiment.

4.2.5.2.2 Leaf osmotic potential

Both NaCl and KCl caused a decrease in ψ_s, although KCl caused a greater decline(Table 22). Although there was some changes in ψ_s with time, there was no difference in the response in the two salt stress treatments.

4.2.5.2.3 Leaf turgor potential

The leaf turgor potential of control plants varied between +0.32 and +0.50 MPa during the10 day period of the experiment (Table 23). Positive turgor was also maintained throughout in the two stress treatments and only fell below that in control plants on day 4 in NaCl stress. The turgor potential of KCl stressed plants was higher than that of control plants throughout, the greatest difference being 0.35 MPa on day 4.

Table 21 Effect of length of period of exposure to NaCl or KCl on leaf water potential (MPa)

TREATMENT	DAYS AT 100 mM CONCENTRATION					
	2	4	6	8	10	MEAN
CONTROL	-0.18	-0.23	-0.21	-0.14	-0.19	-0.19
NaCl	-0.52	-0.84	-0.66	-0.56	-0.46	-0.61
KCl	-0.45	-0.45	-0.46	-0.51	-0.29	-0.43
MEAN	-0.39	-0.50	-0.44	-0.40	-0.31	

SOURCE OF VARIATION	VR	LSD(p=0.05)
PERIOD	16.44	
TREATMENT	239.15	
PERIOD X TREATMENT	8.20***	0.09

*** (p=0.01)

Table 22 Effect of length of period of exposure to NaCl or KCl on leaf osmotic potential (MPa)

TREATMENT	DAYS AT 100 mM CONCENTRATION					
	2	4	6	8	10	MEAN
CONTROL	-0.68	-0.70	-0.58	-0.60	-0.50	-0.61
NaCl	-0.95	-1.11	-1.15	-0.87	-0.78	-0.97
KCl	-1.14	-1.27	-1.17	-0.97	-1.05	-1.12
MEAN	-0.92	-1.03	-0.97	-0.81	-0.78	

SOURCE OF VARIATION	VR	LSD (p=0.05)
PERIOD	8.63***	0.10
TREATMENT	89.82***	0.08
PERIOD X TREATMENT	1.45NS	

*** (p=0.01)
NS Not significant

Table 23 Effect of length of period of exposure to NaCl on leaf turgor potential (MPa)

TREATMENT	DAYS AT 100 mM CONCENTRATION					
	2	4	6	8	10	MEAN
CONTROL	0.50	0.48	0.38	0.46	0.32	0.43
NaCl	0.42	0.27	0.49	0.32	0.33	0.37
KCl	0.68	0.83	0.71	0.46	0.76	0.69
MEAN	0.53	0.52	0.53	0.41	0.47	

SOURCE OF VARIATION	VR		LSD (p=0.05)
PERIOD	2.39	NS	
TREATMENT	42.23	***	0.08
PERIOD X TREATMENT	3.59	***	0.17

NS = NOT SIGNIFICANT

*** (p=0.01)

4.2.5.2.4 Leaf sodium concentration

The leaf Na^+ concentrations in control and KCl stressed plants were low and remained unchanged throughout (Fig 55). The leaf Na^+ concentration in NaCl stressed plants increased prior to day 2 and continued to increase until day 4 remaining constant thereafter.

4.2.5.2.5 Leaf potassium concentration

The leaf K^+ concentration of control plants ranged between 0.88 and 1.41 mmole g^{-1} dw (Fig. 56). The leaf K^+ concentration of both control and NaCl stressed plants followed a linear decrease over the10 day period, being lower in NaCl-treated plants throughout. The leaf K^+ concentration in KCl stressed plants was significantly higher than that of control plants on day 2 and continued to increase for a further 2 days and was maintained thereafter. Leaf K^+ concentration was higher than leaf Na^+ concentration on nearly all occasions.

4.2.5.2.6 Leaf chloride concentration

There was no significant interaction between the effects of treatment and stress period on leaf Cl^- concentration;however, NaCl and KCl stress caused a rapid initial increase in leaf Cl^- which remained high thereafter. The level in KCl stressed plants was higher than that in NaCl stressed plants (Table 24).

4.2.5.2.6 Proline

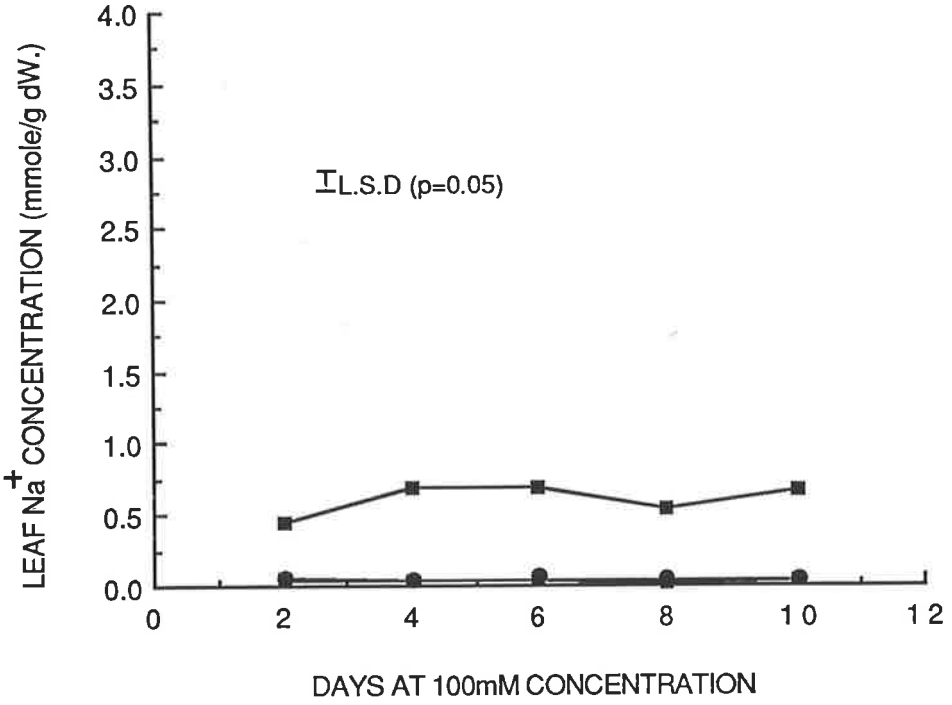
Proline accumulated prior to day 2 in both stress treatments and the apparent increase in concentration with further exposure to stress was not significant (Table 25). There was no significant difference in proline concentration between NaCl or KCl, but it is noteworthy that an appreciable proline concentration accumulated in response to this minimal stress.

4.2.5.2.9 Trigonelline

Trigonelline accumulated in plants exposed to KCl alone, accumulation occurring before day 2 (Table 26).

Fig. 55 Length of period of exposure to NaCl and KCl on leaf Na^+ concentration (mmole, g^{-1} dw)

Fig. 56 Length of period of exposure to NaCl and KCl on leaf K^+ concentration (mmole, g^{-1}dw)


LEAF Na^+ CONCENTRATION (mmole/g dW.)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS AT 100mM CONCENTRATION

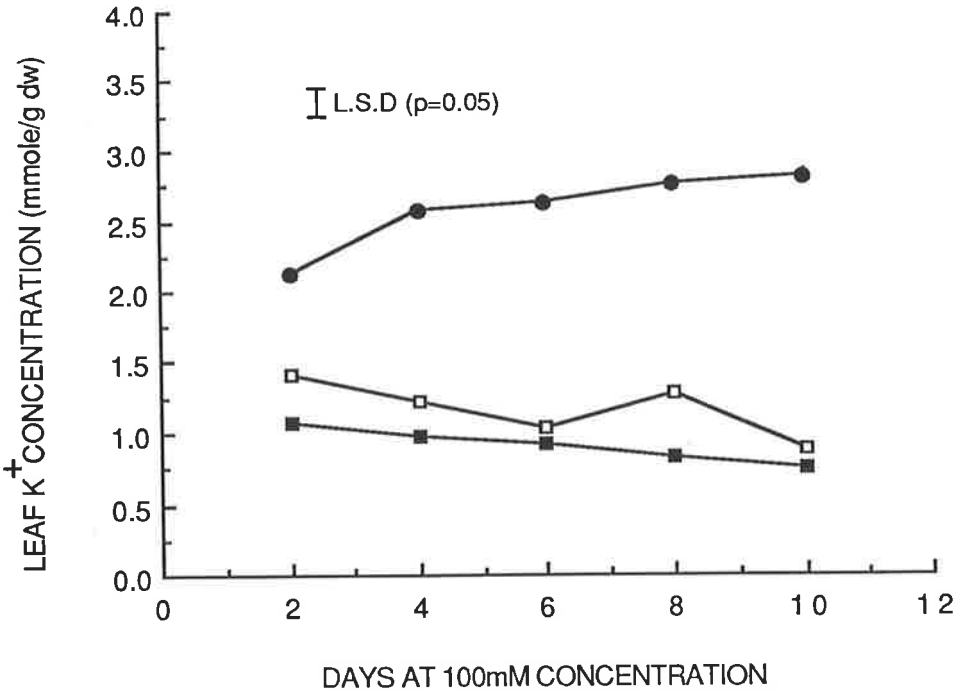

LEAF K^+ CONCENTRATION (mmole/g dw)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS AT 100mM CONCENTRATION

Table 24 Effect of length of period of exposure to NaCl or KCl on leaf chloride concentration (mmole g^{-1}d.wt.)

TREATMENT	DAYS AT 100 mM CONCENTRATION					
	2	4	6	8	10	MEAN
CONTROL	0.0015	0.0038	0.0035	0.0056	0.0018	0.0032
NaCl	0.0971	0.1602	0.1415	0.1013	0.1313	0.1263
KCl	0.1447	0.2237	0.2622	0.0973	0.1637	0.1783
MEAN	0.0811	0.1293	0.1358	0.0681	0.0989	

SOURCE OF VARIATION	VR	LSD (p=0.05)
PERIOD	2.280	
TREATMENT	35.331 ***	0.0437
PERIOD X TREATMENT	1.035 NS	

NS= NOT SIGNIFICANT

*** (p=0.01)

Table 25 Effect of length of period of exposure to NaCl or KCl on leaf proline concentration (mg, g^{-1}d.wt.)

TREATMENT	DAYS AT 100 mM CONCENTRATION					
	2	4	6	8	10	MEAN
CONTROL	0.24	0.75	0.56	0.66	0.42	0.53
NaCl	5.18	5.01	6.84	6.57	6.24	5.97
KCl	4.80	5.88	6.27	6.90	7.70	6.31
MEAN	3.41	3.88	4.56	4.71	4.79	

SOURCE OF VARIATION	VR		LSD (p=0.05)
PERIOD	2.09	NS	
TREATMENT	101.85	***	0.93
PERIOD X TREATMENT	0.81	NS	

NS= NOT SIGNIFICANT

*** (p=0.01)

Table 26 Effect of length of period of exposure to NaCl or KCl on trigonelline concentration (mg g^{-1}d.wt.)

TREATMENT	DAYS AT 100 mM CONCENTRATION					
	2	4	6	8	10	MEAN
CONTROL	0.79	1.42	1.17	1.23	1.17	1.16
NaCl	0.96	2.21	2.38	1.42	2.11	1.81
KCl	3.17	3.08	2.05	1.46	2.15	2.38
MEAN	1.64	2.24	1.87	1.37	1.81	

SOURCE OF VARIATION	VR	LSD (p=0.05)
PERIOD	0.87NS	
TREATMENT	5.37***	0.767
PERIOD X TREATMENT	0.91NS	

NS = NOT SIGNIFICANT

*** (p=0.01)

4.2.5.2.10 Dry weight of plants

There was a significant and similar reduction in shoot dry matter production in both NaCl and KCl stressed plants (Table 27). Root growth continued at a similar rate throughout in the control plants, whereas in both NaCl and KCl stressed plants it slowed down, but not cease, from day 2 onwards (Table 28). The relative growth rates of the plants in KCl and NaCl stress, computed from these dry matter data, did not differ significantly from those of the control plants, nor did the net assimilation rates.

4.2.5.3 Discussion

This experiment demonstrates considerable proline accumulation even at a $\psi_{ext.}$ of -0.515 MPa. The results in section 4.1.2 suggested that proline did not accumulate in plants kept for 3 days at or above -0.565 MPa $\psi_{ext.}$ Although many reports concerning proline accumulation in response to salt stress are available (Stewart and Lee, 1974; Storey and Wyn Jones, 1978; Palfi and Johasz, 1970; Buhl and Stewart, 1983; Tal *et al.*, 1979), a threshold $\psi_{ext.}$ for initiation of proline accumulation has not been agreed, although Chu *et al.*, (1976) postulated a threshold $\psi_{ext.}$ of -0.57 MPa. In section 4.1.2, a threshold ψ_s above -0.94 MPa was suggested and for barley, thresholds ψ_s of -1.1 MPa for cv. Prior (Chu *et al.*, 1976) and -0.75 MPa (300 osmol m^3) for cv California.Mariout (Storey and Wyn Jones, 1978) have been reported. In the present experiment, proline accumulated at $\psi_{ext.}$ -0.515MPa and ψ_s -0.95 suggesting that previous estimates of the threshold were overestimates or that no fixed threshold exists.

An alternative hypothesis is that proline accumulation is controlled by leaf cation concentration. A threshold leaf cation concentration of 200 µmole g^{-1}fw has also been suggested for the initiation of proline accumulation in sorghum (Weimberg *et al.*, 1982). However, a significant amount of proline accumulated by day 2 in NaCl stressed plants whilst the leaf ion concentration was at 150 µmole g^{-1} fw on that day (calculated from Na^+ and K^+ data -Fig.55,56). Moreover, if this were indicative of a threshold internal ion concentration for proline accumulation, proline should have accumulated in unstressed control plants as the leaf K^+ concentration was 150µmole g^{-1}fw. Taken together, these

Table 27 The effect of length of period of exposure to NaCl or KCl on Log (e) dry weight of shoot (mg) [As the interaction was not significant,the overall means of the treatments are furnished]

A.TREATMENT

CONTROL	NaCl	KCl	L.S.D. (p=0.05)
6.95	6.65	6.76	0.16

B. PERIOD

DAYS AT 100 mM CONCENTRATION					L.S.D (P=0.05)
2	4	6	8	10	
6.28	6.32	6.81	7.08	7.45	0.209

Table 28 Effect of length of period of exposure to NaCl or KCl on Log(e)dry weight of root (mg)

TREATMENT	DAYS AT 100mM CONCENTATION					
	2	4	6	8	10	MEAN
CONTROL	4.887	5.025	5.718	5.835	6.710	5.633
NaCl	4.699	4.580	4.994	5.268	5.589	5.026
KCl	4.767	4.942	5.017	5.249	5.211	5.037
MEAN	4.781	4.849	5.243	5.450	5.836	5.232

SOURCE OF VARIATION	VR	L.S.D (p=0.05)
TREATMENT	58.39***	
PERIOD	61.58***	
TREEATMENT X PERIOD	8.90***	0.2867

*** (P=0.01)

responses suggest that the notion of leaf ion concentration, or ψ_s , controlling proline accumulation may be erroneous and that a signal other than leaf ion concentration may be required to initiate proline accumulation.

Despite similar leaf Na^+ (0.367; 0.441) and Cl^- (0.088; 0.0971 mmole g^{-1} dw) concentrations a four fold higher proline concentration accumulated in the present experiment at an equivalent $\psi_{ext.}$ than in that described in section 4.1.2. Further, proline had accumulated 2 days after the plants reached the final concentration, but no more accumulated. These indicate that gradual imposition rather than a long exposure seems to be an essential determinant of proline accumulation. This response however, contrasts with those of barley (Chu *et al.*, 1976) and wheat (Section 4.1.2;4.1.3) where more proline accumulated in response to a sudden salt shock rather than to a gradual imposition of stress. This suggests that plant species differ in proline accumulation depending on the mode of causing salinity. The reason for such differential responses between plant species is not known. Proline accumulated on day 2 at 100 mM $NaCl_{ext.}$ in the present study, contrasting with results of Tal *et al.*,(1979) with tomato, where proline did not accumulate in NaCl (100 mM) stressed plants until 8 days despite a gradual imposition of stress. It is possible that this discrepancy in response between Tal *et al.*, (1979) and the present study may be due to the differences in cultivar tested, age of the seedlings stressed, and the method of plant culture.

Leaf trigonelline concentration increased 2 fold in response to KCl salinity alone, despite a similar $\psi_{ext.}$, cation and anion concentration in the rooting medium. Trigonelline has been shown to accumulate in *Leymus sabulosus* (Gorham *et al.*, 1984) and alfalfa (Parameshwara, 1984) in response to NaCl. The present study differs from that of Gorham and others in respect of the concentration of NaCl administered (200 mM) and the length of period of exposure to NaCl (12 days at 200 mM). The significant increase in trigonelline concentration within 72 h of exposing alfalfa to 50 mM NaCl (Parameshwara, 1984) suggests that trigonelline accumulation could be a crop specific response. Trigonelline is synthesised by methylation of nicotinic acid involving the nicotinic acid methylpherase enzyme (Jindra, 1967; Antony *et al.*, 1975). It is possible that high trigonelline

accumulation in KCl stress may be due to a higher leaf K^+ concentration promoting the activity of this enzyme but there is no evidence of an ionic influence on this enzyme.

The leaf cation(K^+) concentration in KCl stressed plants was 4 fold higher than the cation concentration in NaCl stressed plants(Na^+). The K: Cl ratio was also 3.56 fold higher than Na: Cl (Table 29). Tomato takes up K^+ preferentially; as Wrona and Epstein (1986) also found a 2 fold increase in K^+ over Na^+ at iso-ionic concentration. The internal K^+ concentration increased from 250 mM on day 2 to 330 mM on day 4 despite the fact that the external K^+ concentration was only 100 mM which suggests that K^+ is transported against an electrochemical gradient (Spanswick and Williums, 1964; Dunlop and Bowling, 1971; Ansari and Bowling, 1972). A high leaf K^+ concentration would partly account for the greater decrease in ψ_s and higher ψ_p in KCl stressed plants. A leaf K^+ concentration of 330 mM contributed to about 66 per cent of the leaf osmotic potential (-0.74 MPa) whereas leaf Na^+ (92 mM) contributed to only 21 per cent (-0.21 MPa) of leaf osmotic potential in NaCl stressed plants. The contribution of Cl^- to the osmotic pool in NaCl (-0.028 MPa; 2.93 per cent) and in KCl (-0.048 MPa; 3.58 per cent) stressed plants was low. Accumulated proline and trigonelline also contribute to osmoregulation. Although proline accumulation was greater (2.5 times- 43.44 µmole g^{-1} dw with NaCl : 2.58 times- 69.50 µmole g^{-1}dw with KCl) than that of trigonelline (12.46 µmole g^{-1}dw-NaCl : 17.87 µmole g^{-1}dw -KCl) in both salinities, an equal contribution to osmoregulation was made by proline and trigonelline [proline: -0.088 (9.07); -0.089 (7.85): trigonelline :- -0.086 (8.86); -0.087 MPa (7.76 per cent ψ_s)] in NaCl and KCl stressed plants, respectively. These calculations of osmotic contribution were based on the assumption that the solutes are distributed throughout the cell, and the relative water content of the tissue could be used to calculate leaf water content. A parallel increase in Na^+ and proline concentration led Voetberg and Stewart (1984) to suggest that proline acts as a compatible solute balancing salt accumulation in the vacuole. If proline accumulation fulfils this role alone then it would not be expected to accumulate in plants under KCl stress to the same extent as in those under NaCl stress. K^+ itself acts as an osmoticum (Baker and Weatherly, 1969; Mengel and Pfluger, 1969) and is transported across the tonoplast and equalises any differences in ψ_s between the cytoplasm and the vacuole(Mengel, 1985). If this is so, the accumulation of proline in KCl stressed

Table 29 Na^+: Cl^- and K^+: Cl^- ratios in NaCl and KCl salinised plants of tomato

	DAYS AT 100 mM CONCENTRATION					MEAN
	2	4	6	8	10	
Na^+: Cl^-	4.54	4.32	4.83	5.43	5.13	4.85±0.20
K^+ : Cl^-	14.74	11.58	10.05	28.58	17.31	16.45±3.28

± S.E of the mean of the data

plants suggests that proline has other functions than solely osmoregulation although its accumulation would inevitably contribute to the osmotic pool.

4.3 MANIPULATION OF HUMIDITY OF THE STRESS ENVIRONMENT AND ITS EFFECTS ON ACCUMULATION OF PROLINE AND QUATERNARY AMMONIUM COMPOUNDS

4.3.1 Introduction

Atmospheric humidity can affect transpiration by (1) changing the potential gradient of water diffusion from the leaf, or (2) directly affecting r_s independently of ψ_{leaf}. Under conditions of high humidity and low transpiration, the tissue water content of salt stressed plants has been found to be similar to that of control plants (Neiman and Poulsen, 1967) and a less negative water potential has also been recorded in the leaves of plants salt stressed at high humidity (Hoffmann and Rawlins, 1971).

The transpiration rate influences ion transport through the bulk flow of water and ions to the root surface and distribution of ions within the plant. A lowered ion uptake in high atmospheric humidity (O'Leary and Knect, 1971; Neiman and Poulsen, 1967), and increased salt uptake due to increased transpiration (Russell and Barber, 1960), have both been reported.

The evidence reported in sections 4.2.4 and 4.2.5 suggests that there is only a weak link between proline accumulation and leaf water and ion status, indicating that these may not be the primary factors initiating proline accumulation. However, they cannot be discounted unless proline accumulation can be demonstrated to be independent of change in leaf water or ion status. Accordingly, the experiments in this section were designed to measure proline and quaternary ammonium compound accumulation under conditions in which leaf water and ion status during salt stress was manipulated through the humidity of the stress environment.

4.3.2 The effect of varying atmospheric humidity on accumulation of proline and trigonelline

This experiment aimed to examine the influence of humidity on leaf water and ion relations and their associated influence on proline and trigonelline accumulation in tomato.

4.3.2.1 Methods

Seedlings of tomato cv. Duke were grown at 20±1°C day and night temperature with a 16 hour photoperiod and a photon flux density of 350-420 μEin sec^{-1}. They were supplied with Hoagland nutrient solution until 25 days after emergence of the seedlings (Materials and Methods 3.2.1;3.2.2). On the 25th day, these seedlings were supplied with either full strength Hoagland nutrient solution or full strength Hoagland nutrient solution containing NaCl or $MgCl_2$. A day before stress was imposed the plants were placed in an environment of either 70 or 94 per cent relative humidity (Section 3.2.1). These humidity ranges will be referred to as low (70) or high (94 per cent). The equivalence in saturation deficit (Δe) was 6.6 (70) and 0.9 mbar (94 per cent). Relative humidity in the plant growth cabinets was achieved by placing two humidifiers inside the cabinets near the air-inlet so that incoming air was humidified constantly. A humidity sensor placed in the air-exit controlled cabinet humidity. The shutters at the lower levels of the cabinets were completely closed and the ones at the top were partly opened. This facilitated conservation of humidity in the cabinet. A humidity lower than 70 per cent could not be achieved as the ambient humidity was around 60-65 per cent and the cabinets lacked any dehumidification facility. Salinity stress was imposed on the plants in both relative humidities at a rate of 0.1 MPa day^{-1} until 10 days to reach -1.065 MPa. The youngest fully expanded leaves were harvested 0, 5, 9, and 11 days from commencement of stress for measurement of leaf water, osmotic and turgor potential (3.2.6), proline (3.2.9.2), trigonelline (3.2.9.3) and leaf ion content (3.2.11). The design was a factorial randomised block with three replications of each treatment.

4.3.2.2 Results

4.3.2.2.1 Leaf water potential

The interaction between humidity and salinity and time of sampling was not significant (Table 30) although the overall interaction between humidity and salinity treatment was significant. In plants stressed in high atmospheric humidity the leaf water potential decreased to a significantly lesser extent than those stressed in low humidity. The overall interaction between stress period and humidity showed an increase in leaf water potential in plants in high humidity until day 9 tending to decrease thereafter, whereas in plants growing at a low humidity the leaf water potential decreased from day 5 onwards. Although high humidity did not completely abolish the salinity-induced fall in ψ_{leaf}, it did reduce the extent of the fall.

4.3.2.2.2 Leaf osmotic potential

As with leaf water potential, the level of humidity in the stress environment influenced leaf osmotic potential (Table 31)in NaCl stressed plants, being low in low humidity and high in high humidity. This influence of humidity on ψ_s was not observed in control or $MgCl_2$ stressed plants.

4.3.2.2.3 Leaf turgor potential

The leaf turgor potential of plants before stress imposition was +0.33 MPa and this remained constant in control plants until the end of the experiment (Table 32). Stressing at a high humidity favoured maintenance of high turgor potential in plants under $MgCl_2$ salinity but not in NaCl . In plants stressed with NaCl, leaf turgor fell in both humidity environements from day 0 onwards, there being little evidence of greater turgor retention at high humidity. In contrast, in plants stressed with $MgCl_2$, turgor was only lost at low humidity, the plants growing at high humidity being similar to control plants throughout.

Table 30 The effects of low and high humidity on leaf water potential of tomato under NaCl and $MgCl_2$ salinity (-MPa)

TREATMENT	HUMIDITY	DAYS FROM COMMENCEMENT OF STRESS				MEAN
		0	5	9	11	
CONTROL	LOW	-0.37	-0.35	-0.34	-0.29	-0.34
	HIGH	-0.37	-0.16	-0.13	-0.13	-0.20
	MEAN	-0.37	-0.25	-0.24	-0.21	-0.27
NaCl	LOW	-0.37	-0.46	-0.84	-1.12	-0.67
	HIGH	-0.37	-0.32	-0.53	-0.83	-0.51
	MEAN	-0.37	-0.39	-0.68	-0.97	-0.61
$MgCl_2$	LOW	-0.37	-0.38	-0.61	-1.01	-0.59
	HIGH	-0.37	-0.35	-0.49	-0.79	-0.50
	MEAN	-0.37	-0.37	-0.56	-0.90	-0.55
MEAN		-0.37	-0.34	-0.49	-0.69	-0.47

SOURCE OF VARIATION	VR	LSD (p=0.05)
HUMIDITY	72.37***	0.03
TREATMENT	164.01***	0.04
PERIOD	97.57***	0.05
HUMIDITY X TREATMENT	2.71*	0.06
HUMIDITY X PERIOD	10.17***	0.06
TREATMENT X PERIOD	44.34***	0.08
HUMIDITY X TREATMENT X PERIOD	1.14NS	- - - -

*** (p=0.01)
* (p=0.05)
NS Not significant

Table 31 The effects of low and high humidity on leaf osmotic potential of tomato under NaCl and $MgCl_2$ salinity (-MPa)

TREATMENT	HUMIDITY	DAYS FROM COMMENCEMENT OF STRESS				MEAN
		0	5	9	11	
CONTROL	LOW	-0.69	-0.67	-0.52	-0.57	-0.61
	HIGH	-0.69	-0.53	-0.40	-0.51	-0.53
	MEAN	-0.69	-0.60	-0.46	-0.54	-0.57
NaCl	LOW	-0.69	-0.73	-0.97	-1.29	-0.92
	HIGH	-0.69	-0.58	-0.77	-0.92	-0.74
	MEAN	-0.69	-0.65	-0.87	-1.10	-0.83
$MgCl_2$	LOW	-0.69	-0.76	-0.89	-1.14	-0.87
	HIGH	-0.69	-0.61	-0.88	-1.24	-0.85
	MEAN	-0.69	-0.69	-0.89	-1.19	-0.86
MEAN		-0.69	-0.65	-0.74	-0.94	-0.75

SOURCE OF VARIATION	VR	LSD (p=0.05)
HUMIDITY	13.25***	0.05
TREATMENT	52.29***	0.06
PERIOD	26.84***	0.07
HUMIDITY X TREATMENT	3.36*	0.09
HUMIDITY X PERIOD	1.57NS	---
TREATMENT X PERIOD	14.42***	0.13
HUMIDITY X TREATMENT X PERIOD	1.68NS	---

*** (p=0.01)

* (p=0.05)

NS Not significant

Table 32 The effects of low and high humidity on leaf turgor potential of tomato under NaCl and $MgCl_2$ salinity (MPa)

TREATMENT	HUMIDITY	DAYS FROM COMMENCEMENT OF STRESS				MEAN
		0	5	9	11	
CONTROL	LOW	0.33	0.32	0.18	0.28	0.27
	HIGH	0.33	0.37	0.27	0.38	0.33
	MEAN	0.33	0.34	0.22	0.33	0.30
NaCl	LOW	0.33	0.26	0.13	0.17	0.22
	HIGH	0.33	0.26	0.18	0.09	0.21
	MEAN	0.33	0.26	0.16	0.13	0.22
$MgCl_2$	LOW	0.33	0.38	0.26	0.13	0.27
	HIGH	0.33	0.26	0.38	0.51	0.37
	MEAN	0.33	0.32	0.32	0.32	0.32
MEAN		0.33	0.31	0.23	0.26	0.28

SOURCE OF VARIATION	VR	LSD (p=0.05)
HUMIDITY	4.66**	0.45
TREATMENT	8.06***	0.56
PERIOD	2.98*	0.64
HUMIDITY X TREATMENT	1.67NS	- - -
HUMIDITY X PERIOD	2.63*	0.09
TREATMENT X PERIOD	1.99NS	- - -
HUMIDITY X TREATMENT X PERIOD	2.88*	0.16

*** (p=0.01)

* (p=0.05)

NS Not significant

4.3.2.2.4 Leaf sodium concentration

The leaf Na^+ concentrations of control and $MgCl_2$ stressed plants were low and remained constant throughout the entire stress period. The leaf Na^+ concentration of plants under NaCl stress was influenced by the humidity at which the plants were stressed (Table 33), being significantly lower in plants stressed at a high humidity compared with those at a low humidity. Most of the Na^+ was taken up between days 0 and 9 and further increase, though significant statistically, was not substantial. At high humidity there was a fall in leaf Na^+ concentration between days 9 and 11 that was substantial.

4.3.2.2.5 Leaf magnesium concentration

In contrast to Na^+ uptake into the leaf, Mg^{++} accumulation into the leaves of plants stressed with $MgCl_2$ was entirely unaffected by atmospheric humidity (Table 34).

4.3.2.2.6 Leaf chloride concentration

The leaf chloride concentration of control plants was low, remained constant and was not influenced by the humidity of the stress environment over the entire stress period (Table 35). Chloride uptake was inhibited in NaCl stressed plants at the high humidity but there was no difference in the chloride content of the leaves of plants grown at different humidities and stressed with $MgCl_2$. The mean leaf chloride concentration was significantly lower in plants in NaCl than in $MgCl_2$ at both humidities.

4.3.2.2.7 Leaf proline concentration

The data were transformed (Log_e) with the objective of distributing the error homogeneously. Leaf proline concentration in control plants was low and was not influenced by humidity over the entire period of the experiment (Fig. 57). In plants stressed with either NaCl or $MgCl_2$, leaf proline concentration was significantly higher in low humidity than in high humidity. Proline accumulation continued in plants under both salinities at either humidity until day 11. The leaf proline concentration

Table 33 The effects of low and high humidity on leaf sodium concentration of tomato under NaCl and $MgCl_2$ salinity (mmole g^{-1} dw)

TREATMENT	HUMIDITY	DAYS FROM COMMENCEMENT OF STRESS				MEAN
		0	5	9	11	
CONTROL	LOW	0.0070	0.0089	0.0099	0.0089	0.0087
	HIGH	0.0070	0.0073	0.0089	0.0061	0.0073
	MEAN	0.0070	0.0081	0.0094	0.0075	0.0080
NaCl	LOW	0.0070	0.1951	0.4393	0.4736	0.2788
	HIGH	0.0070	0.1517	0.3500	0.1741	0.1707
	MEAN	0.0070	0.1734	0.3947	0.3238	0.2247
$MgCl_2$	LOW	0.0070	0.0082	0.0061	0.0093	0.0076
	HIGH	0.0070	0.0065	0.0064	0.0065	0.0066
	MEAN	0.0070	0.0073	0.0063	0.0063	0.0071

SOURCE OF VARIATION	VR	LSD (p=0.05)
HUMIDITY	13.47***	0.020
TREATMENT	208.23***	0.025
PERIOD	32.90***	0.029
HUMIDITY X TREATMENT	12.61***	0.035
HUMIDITY X PERIOD	5.02***	0.040
TREATMENT X PERIOD	32.53***	0.040
HUMIDITY X TREATMENT X PERIOD	4.78***	0.070

*** (p=0.01)

Table 34 The effects of low and high humidity on leaf magnesium concentration of tomato under NaCl and $MgCl_2$ salinity (mmole g^{-1} dw)

A. TREATMENT X PERIOD

TREATMENT	DAYS FROM COMMENCEMENT OF STRESS				MEAN	L.S.D (P=0.05)
	0	5	9	11		
CONTROL	0.2310	0.2339	0.2899	0.2820	0.2592	
NaCl	0.2310	0.2342	0.2567	0.2364	0.2426	
$MgCl_2$	0.2310	0.6256	0.9435	1.045	0.7112	
MEAN	0.2310	0.3640	0.4967	0.5211	0.4043	**0.080**

B. HUMDITY

LOW	HIGH	L.S.D (0.05)
0.3879	0.4208	**0.032**

Table 35 The effects of low and high humidity on leaf chloride concentration of tomato under NaCl and $MgCl_2$ salinity (mmole g^{-1} dw)

TREATMENT	HUMIDITY	DAYS FROM COMMENCEMENT OF STRESS				MEAN
		0	5	9	11	
CONTROL	LOW	0.0085	0.0064	0.0041	0.0034	0.0056
	HIGH	0.0085	0.0043	0.0039	0.0059	0.0056
	MEAN	0.0085	0.0054	0.0040	0.0046	0.0056
NaCl	LOW	0.0085	0.0455	0.2041	0.2260	0.1210
	HIGH	0.0085	0.0776	0.0710	0.0730	0.0575
	MEAN	0.0085	0.0616	0.1375	0.1495	0.0893
$MgCl_2$	LOW	0.0085	0.0869	0.2571	0.3933	0.1865
	HIGH	0.0085	0.0953	0.2730	0.3258	0.1757
	MEAN	0.0085	0.0911	0.2651	0.3596	0.1811

SOURCE OF VARIATION	VR	LSD (p=0.05)
HUMIDITY	3.14**	0.025
TREATMENT	64.78***	0.030
PERIOD	38.12***	0.034
HUMIDITY X TREATMENT	3.11*	0.043
HUMIDITY X PERIOD	2.372NS	----
TREATMENT X PERIOD	14.203***	0.060
HUMIDITY X TREATMENT X PERIOD	1.708NS	----

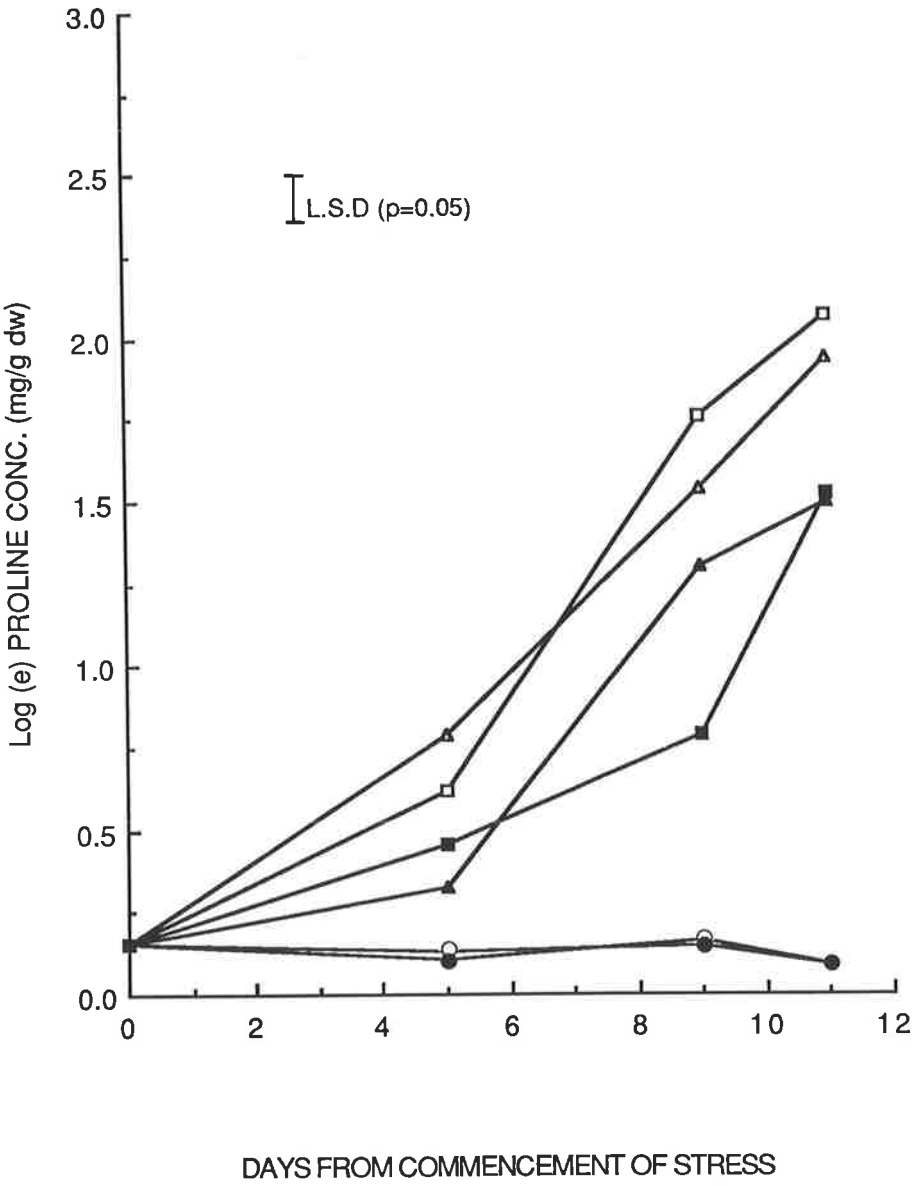
*** (p=0.01)

* (p=0.05)

NS Not significant

Fig. 57 Log (e) proline concentration (mg, g^{-1} dw) of NaCl and $MgCl_2$ stressed tomato at low (LH) and high (HH) humidities

○ LH control
□ LH NaCl
△ LH $MgCl_2$
● HH control
■ HH NaCl
▲ HH $MgCl_2$


3.0
2.5
2.0
1.5
1.0
0.5
0.0
L.S.D (p=0.05)
Log (e) PROLINE CONC. (mg/g dw)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

increased by day 5 in plants under both salinities at either humidity and continued to increase thereafter. There were some variations in proline accumulation with NaCl and $MgCl_2$ stress but these were not consistent and by day 11 the plants had accumulated similar amounts of proline in both salinity treatments.

4.3.2.2.7 Leaf trigonelline concentration

As with proline, the humidity in the stress environment influenced trigonelline accumulation in plants under both NaCl and $MgCl_2$ salinity treatments, being greater in low than high humidity (Table 36) in plants stressed with NaCl, trigonelline accumulated to a significant level at the low humidity only.

4.3.3 Discussion

Subjecting tomato to stress at a high relative humidity had two major effects. Firstly, it diminished the decrease in leaf water potential (Table 30;Fig. 58) and secondly it reduced the increase in leaf ion concentration, especially Na^+ and Cl^- (Tables 33, 35; Fig. 59; 61). As a result, ψ_{leaf} was independent of $\psi_{ext.}$ in NaCl and $MgCl_2$ stressed plants at high humidity. A high relative water content in bean and cotton (Neiman and Poulsen, 1967) and a high (less negative) leaf water potential and osmotic potential in beet, cotton, onion and radish (Hoffmann and Rawlins, 1971) salinised at high humidity, support the results obtained in this experiment. Proline accumulated in significant amounts (240 per cent compared to control) in NaCl and $MgCl_2$ stressed plants at high humidity by day 5 despite the lack of change in leaf water potential (Table 30; Fig.57) or osmotic potential (Table 31; Fig.57). Trigonelline also accumulated in significant amounts in $MgCl_2$ stressed plants at high humidity by day 5 (Table 36). A close relationship between ψ_{leaf} or ψ_s and proline or trigonelline accumulation was found previously (section 4.1.2) and proline accumulation has been related to ψ_s in salt stressed barley (Chu *et al.*, 1976). A parallelism between ψ_{leaf} and trigonelline accumulation was also observed in alfalfa (Parameshwara, 1984) and in the plants subjected to low humidity in the present experiment. However, a significant increase in proline and trigonelline content in the absence of changes in

Table 36 The effects of low and high humidity on leaf trigonelline concentration of tomato under NaCl and $MgCl_2$ salinity (mg g^{-1} dw)

TREATMENT	HUMIDITY	DAYS FROM COMMENCEMENT OF STRESS				MEAN
		0	5	9	11	
CONTROL	LOW	0.79	1.42	1.32	1.13	1.17
	HIGH	0.79	1.35	1.17	1.00	1.08
	MEAN	0.79	1.38	1.25	1.07	1.12
NaCl	LOW	0.79	2.20	1.89	2.58	1.87
	HIGH	0.79	1.26	1.34	1.27	1.17
	MEAN	0.79	1.73	1.61	1.93	1.52
$MgCl_2$	LOW	0.79	1.89	3.73	2.40	2.21
	HIGH	0.79	2.09	2.70	1.95	1.88
	MEAN	0.79	1.99	3.22	2.17	2.04

SOURCE OF VARIATION	VR	LSD (p=0.05)
HUMIDITY	16.25***	0.19
TREATMENT	33.64***	0.23
PERIOD	33.46***	0.26
HUMIDITY X TREATMENT	3.82*	0.32
HUMIDITY X PERIOD	2.54NS	--
TREATMENT X PERIOD	8.87***	0.45
HUMIDITY X TREATMENT X PERIOD	1.76NS	--

*** (p=0.01)

* (p=0.05)

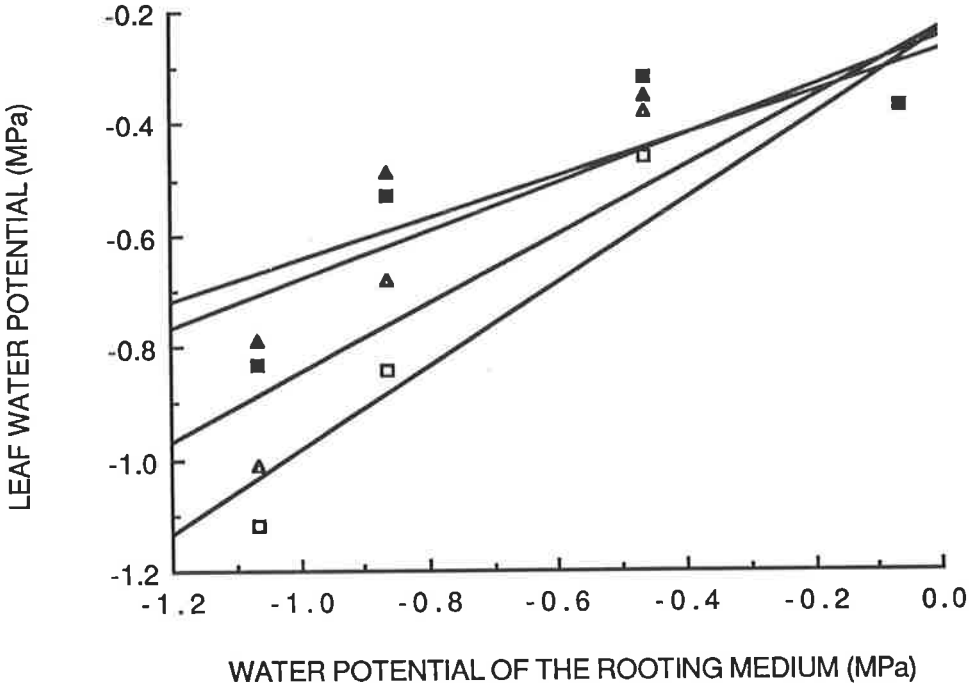
NS Not significant

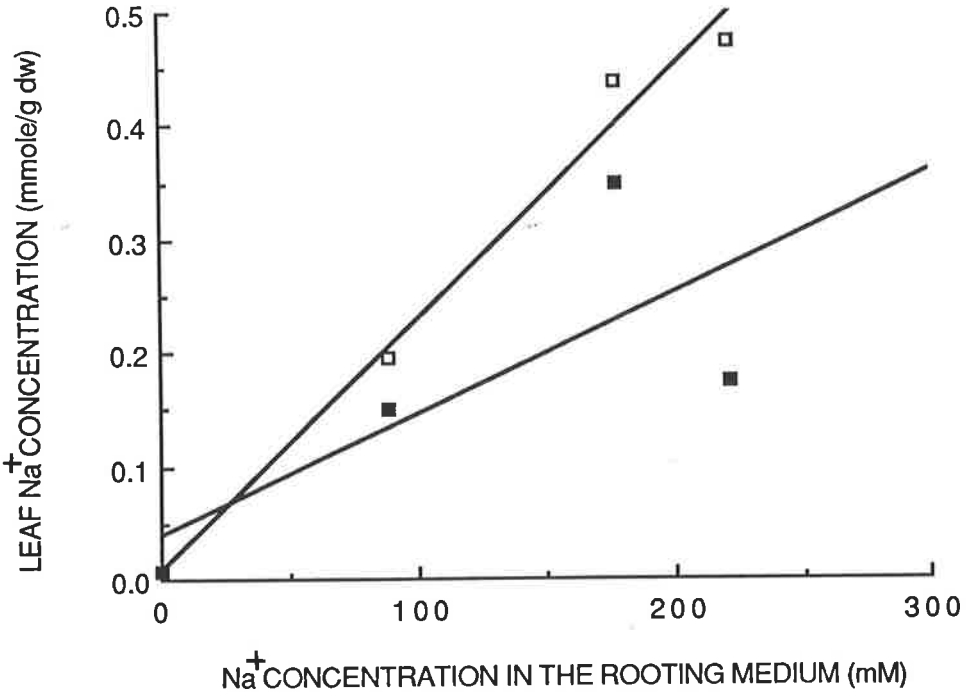
Fig. 58 Relationships between water potential of the rooting medium and leaf water potential of NaCl and $MgCl_2$ stressed tomato at low (LH) and high(HH) humidities (Data obtained from Table 30)

$Y= -0.2373 + 0.7482x$	$R=0.95^{**}$	□ NaCl (LH)
$Y= -0.2472 + 0.4314x$	$R=0.83^{ns}$	■ NaCl (HH)
$Y= -0.2306 + 0.6169x$	$R=0.90^{*}$	△ $MgCl_2$(LH)
$Y= -0.2686 + 0.3763x$	$R=0.82^{ns}$	▲ $MgCl_2$(HH)

Fig. 59 Relationships between Na^+ concentration of the rooting medium and leaf Na^+concentration of NaCl stressed tomato at low (LH) and high (HH) humidities
(Data obtained from Table 33)

$Y= 0.0088 + 0.0022x$	$R=0.99^{***}$	□ LH
$Y= 0.0411 + 0.0011x$	$R=0.74^{ns}$	■ HH


LEAF WATER POTENTIAL (MPa)
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
WATER POTENTIAL OF THE ROOTING MEDIUM (MPa)


LEAF Na+ CONCENTRATION (mmole/g dw)
0.5
0.4
0.3
0.2
0.1
0.0
0
100
200
300
Na+ CONCENTRATION IN THE ROOTING MEDIUM (mM)

ψ_{leaf} or ψ_s indicates that neither factor controls accumulation. The observed relationships recorded earlier in the present study and elsewhere (Chu *et al.*, 1976; Parameshwara, 1984) are presumably due to parallel changes occurring in plants subjected to salt stress.

High humidity in the stress environment also influenced the leaf ion concentrations especially $Na^+$and Cl^- (Fig 59; 61) in plants exposed to NaCl salinity. A complete inhibition of Cl^- uptake and about 63 per cent inhibition of Na^+ uptake was observed in NaCl stressed plants at high humidity. Such a reduction in ion uptake presumably is effected through a reduction in transpiration rate in plants at high humidity. Transpiration, in addition to influencing bulk water flow, also influences ion uptake. An increased leaf ion concentration in a low humidity (Russel and Barber, 1960) and a reduced ion concentration in a high humidity (O'Leary and Knecht, 1971; Neiman and Poulsen, 1967) has been demonstrated previously. Salt movement into the root system involves both mass flow (passive) as well as active transport involving a specific ATPase (Leonard, 1983,1985). Complete inhibition of Cl^- uptake in NaCl stressed plants at high humidity suggests that Cl^- is transported by passive means alone. Leaf Na^+ concentration was also substantially reduced in NaCl stressed plants at high humidity; however, complete inhibition of accumulation did not result. This suggests that Na^+ uptake, at least to some extent, is through active means. Rains and Epstein (1967) also found evidence for active transport of Na^+ in NaCl salinised cotton. The leaf Na^+ concentration increased in proportion to the $Na^+_{ext.}$ concentration in NaCl stressed plants at low humidity (Fig. 59), but at high humidity, the relationship between $Na^+_{ext.}$ and Na^+_{leaf} concentration was not significant, due to a reduction in leaf Na^+ concentration on day 11. This reduction was presumably due to re-export of Na^+ to other organs.

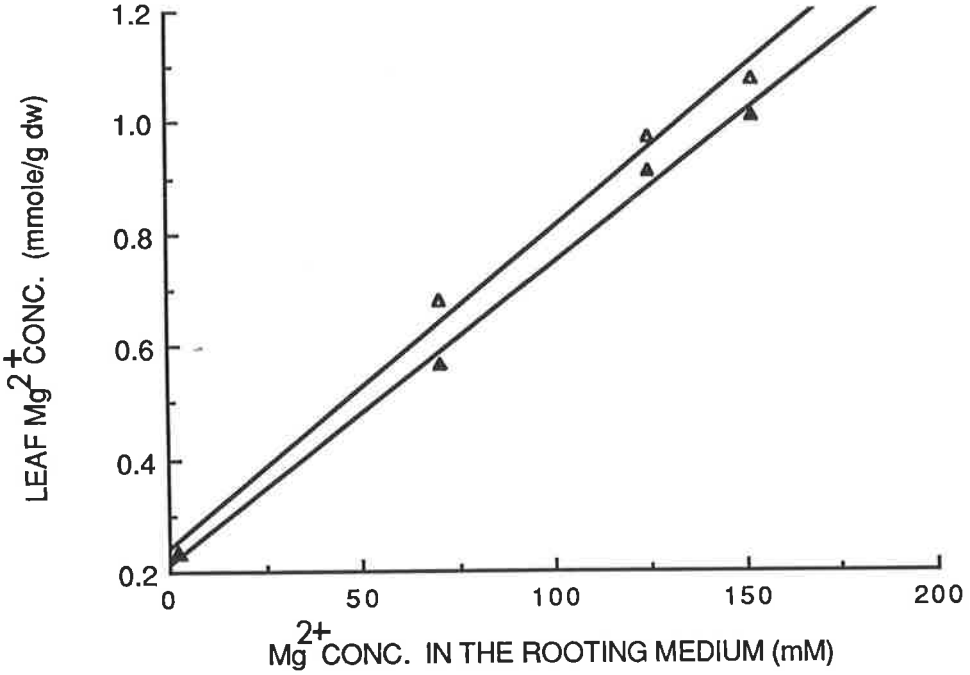
Manipulation of humidity in the stress environment did not influence leaf Mg^{++} concentration (Fig. 60), indicating that this ion is principally transported through active means. Leonard (1983, 1985) suggested an involvement of a specific ATPase for Mg^{++} transport. Differences between cation and anion accumulation were observed

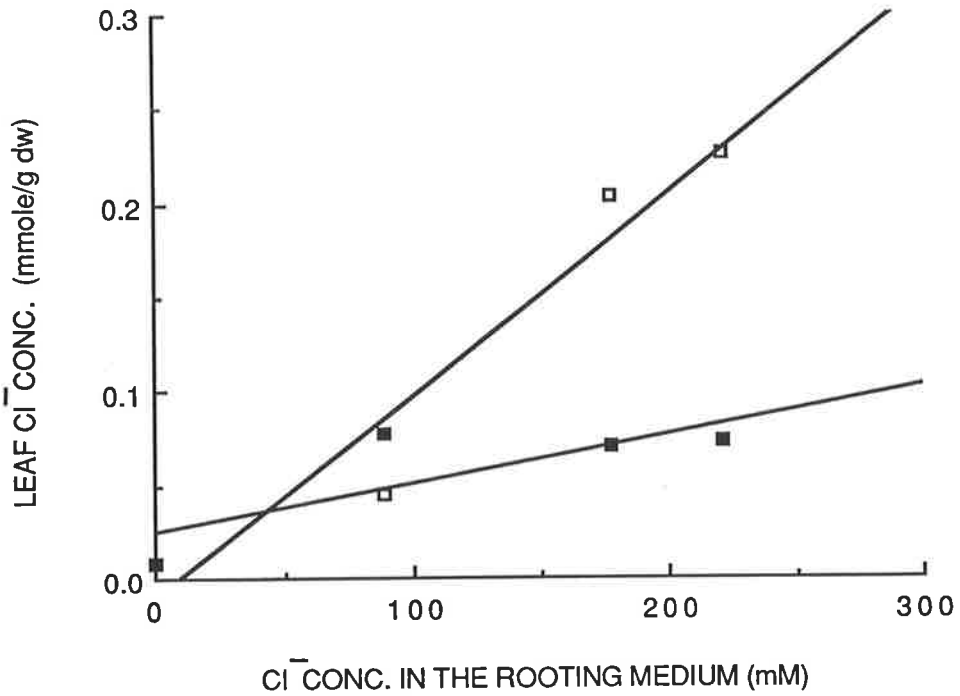
Fig. 60 Relationships between Mg^{++} concentration of the rooting medium and leaf Mg^{++} concentration of $MgCl_2$ stressed tomato at low (LH) and high(HH) humidities
(Data obtained from interactions of the Table 34)

Y= 0.2445 + 0.0057x	R=1.00***	△ LH
Y= 0.2149 + 0.0053x	R=1.00***	▲ HH

Fig. 61 Relationships between Cl^- concentration of the rooting medium and leaf Cl^-concentration of NaCl stressed tomato at low (LH) and high (HH) humidities
(Data obtained from Table 35)

Y= -0.0105 + 0.0011x	R=0.97***	□ LH
Y= 0.0256 + 2.622e-4x	$R=0.78^{ns}$	■ HH


1.2
1.0
0.8
0.6
0.4
0.2
0
50
100
150
200
LEAF Mg^{2+} CONC. (mmole/g dw)
Mg^{2+} CONC. IN THE ROOTING MEDIUM (mM)


0.3
0.2
0.1
0.0
0
100
200
300
LEAF Cl^- CONC. (mmole/g dw)
Cl^- CONC. IN THE ROOTING MEDIUM (mM)

in NaCl and $MgCl_2$ stressed plants at different humidities. This could lead to a charge imbalance in the cells. The data on Na^+: Cl^- (Table 37), however, indicates that there was no significant difference in Na:Cl ratios between control and NaCl stressed plants at either humidity. The Mg^{++}: Cl^- ratio in $MgCl_2$ stressed plants was found to be 33 (control) and 7-10 (stressed) fold higher than Na^+: Cl^-. This indicates that tomato takes up Mg^{++} preferentially over Na^+. A significantly lower Mg^{++}: Cl^- ratio in $MgCl_2$ salinised plants compared to controls may suggest an increased Cl^- uptake. The high Cl^- uptake in $MgCl_2$ salinised plants contrasts with that in NaCl salinised plants, especially in plants at high humidity. It has been suggested that the uptake mechanisms for cations and anions differ and are, to a great extent, independent (Gannonmore-Newmann, 1970). Anions have been suggested to enter the cell by exchange for OH^- or via a metabolic transport system. The higher Cl^- uptake in plants exposed to $MgCl_2$ may be due to Mg^{++} inducing production of OH^- ions or to greater Cl^- uptake in $MgCl_2$ salinised plants neutralising the charge imbalance created by Mg^{++} uptake.

As the possibility that ψ_s controls proline accumulation has been eliminated, the suggestion that leaf ion concentration acts through solute potential is also eliminated. However, accumulated ions may influence the activity of the enzymes leading to proline accumulation. However, proline accumulation occurred to a significant concentration despite a lack of increase in leaf Cl^- concentration in NaCl stressed plants grown at high humidity. Accordingly, relationships observed between leaf Cl^- concentration and proline accumulation in this and the previous experiments may not signify a direct causal relationship.

Leaf Na^+ concentration is also correlated with leaf proline accumulation (Section 4.1). Na^+ has been claimed to affect proline accumulation by influencing the activity of Δ-P-5-C reductase which converts Δ-P-5-C carboxylate to L-proline (Huber, 1974). Although, a significant correlation between leaf Na^+ and proline concentration was found in NaCl stressed plants at low humidity, there was no relationship between them at high humidity. Such a control by Na^+ cannot be discounted completely, however,

Table 37 Na^+: Cl^- and Mg^{++}: Cl^- ratios of NaCl and $MgCl_2$ stressed plants at various humidities

HUMIDITY	Na^+: Cl^-		Mg^{++}: Cl^-	
	CONTROL	NaCl	CONTROL	$MgCl_2$
LOW	1.73± 0.53	1.47± 0.54	55.62± 18.52	10.39± 8.78
HIGH	1.46± 0.47	2.52± 1.23	49.22± 13.63	9.90± 8.20

± S.E of the mean difference calculated over the entire period of the experiment

unless proline accumulation is demonstrated with no change in leaf Na^+ concentration. Proline accumulation in $MgCl_2$ salinity was significantly correlated with leaf Mg^{++} concentration across both humidities (Fig.60). Previously, however, leaf Mg^{++} and proline concentration were found not be correlated (Section 4.2.4) so this relationship may only be circumstantial.

Proline concentration in NaCl or $MgCl_2$ stressed plants at the high humidity was lower than that at low humidity. A similar variation in proline concentration due to humidity in the stress environment was found in water stressed barley (Naidu, 1988) and the differences were ascribed to variation in ψ_{leaf}. However, the variation in proline concentration evident on day 5 in $MgCl_2$ stressed plants occurred without any difference in ψ_{leaf}. Moreover, the evidence that ψ_{leaf} does not control proline accumulation in salt-stressed plants suggests that it is unlikely that variation in proline concentration between humidities is affected through differences in ψ_{leaf}. Proline accumulation in $MgCl_2$ salinity at low humidity was differed from the response described in section 4.2.3 where more proline accumulated in $MgCl_2$ than in NaCl salinity at iso-osmotic potential. No such difference was observed in the present experiment. This could be due to the difference in humidity prevailing during these two experiments. The previous experiment was conducted in December when the ambient humidity was around 50 per cent and in the present experiment the humidity in the low range was maintained at 70 per cent. It is also possible that the variation in proline accumulation between the two experiments could be due to differences in air velocity. This was different in these two experiments, due to the regulation of the air-inlet shutters to maintain humidity.

Trigonelline accumulated to a significant concentration in plants stressed in low but not high humidity, being greater in $MgCl_2$ salinity than NaCl salinity. Trigonelline accumulation was related neither to ion accumulation in the leaf nor to leaf water status.

4.3.3 The effect of differences in humidity in the stress environment on proline accumulation in NaCl and $MgCl_2$ salinity at iso-osmotic potential of the rooting medium

The results of the previous experiment were different to those described in section 4.2.3 in that a higher concentration of proline accumulated in plants under $MgCl_2$ than NaCl salinity (4.2.3), while previously, accumulation was similar. This occurred despite external salinity being maintained at the same osmotic potential in both experiments. The two experiments differed in (1) time of conduct and (2) incoming air-velocity. The first experiment (Section 4.2.3) was conducted in December and humidity (not controlled) was about 40-50 per cent, whereas the humidity in the second experiment (Section 4.3.2) was controlled at 70 per cent. Proline accumulation is influenced by the level of humidity in the stress environment, being greater at low humidity and lower at high humidity(Section 4.3.2).

The present experiment was designed to test the hypothesis that the variation in proline accumulation was due to the differences in humidity between the experiments.

4.3.3.1 Methods

Tomato cv. Duke seedlings were grown at 20 ±1°C day and night temperature with a 16h photoperiod and a photon flux density of 350 - 420 µEin sec^{-1} (Material and Methods 3.2.1 ; 3.2.2). Twenty five days after emergence of the seedlings, stress was imposed with full strength Hoagland's solution containing NaCl or $MgCl_2$. A day before stress was imposed the plants were equilibrated at either 51 (Δe 10.9) or 94 per cent (0.5 mbar)relative humidity as in the previous experiment. This experiment was carried out during January which allowed maintenance of the low humidity. The air-inlet shutters were maintained as in experiment 4.3.2 in both cabinets. Stress was imposed at -0.1 MPa day^{-1} until 10 days to reach -1.065 MPa. On day 11(after the commencement of stress), the youngest fully expanded leaves were sampled for

measurement of leaf water, osmotic and turgor potential (Section 3.2.6), proline (Section 3.2.9.2) and leaf ion concentrations (Section 3.2.11). Prior to the harvest stomatal resistance of the upper and lower surface of the expanded leaves was measured using a Li-cor LI 60 diffusive resistance porometer (Section 3.2.5.1). Transpiration measurements were made on the plants in both humidities on the final sampling day (Section 3.2.7). The design was a factorial randomised block with three replications of each treatment.

4.3.3.2 Results

4.3.3.2.1 Leaf water potential

Humidity of the stress environment significantly influenced the leaf water potential of plants in both salinities, the potential being lower in plants at 51 than at 94 per cent humidity. Variation in the humidity of the atmosphere had no effect on the control plants(Fig. 62).

4.3.3.2.2 Leaf osmotic potential

As with leaf water potential, leaf osmotic potential was influenced by the atmospheric humidity in control and NaCl stressed plants, being more negative in 51 than in 94 per cent humidity (Fig. 63). In $MgCl_2$ salinised plants, however, ψ_s was not affected by humidity.

4.3.3.2.3 Leaf turgor potential

There was no significant influence of humidity on leaf turgor potential of NaCl stressed plants, which remained similar to the control (Fig. 64). In $MgCl_2$ stressed plants at high humidity there was a significant increase in ψ_p (+0.24 MPa) over both those at low humidity and the control plants.

Fig. 62 Effect of different humidities in the environment on leaf water potential (-MPa) of NaCl and $MgCl_2$ stressed tomato

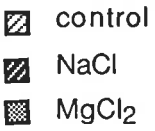


Fig. 63 Effect of different humidities in the environment on leaf osmotic potential (-MPa) of NaCl and $MgCl_2$ stressed tomato

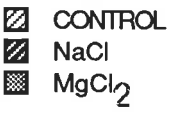
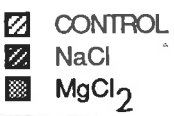
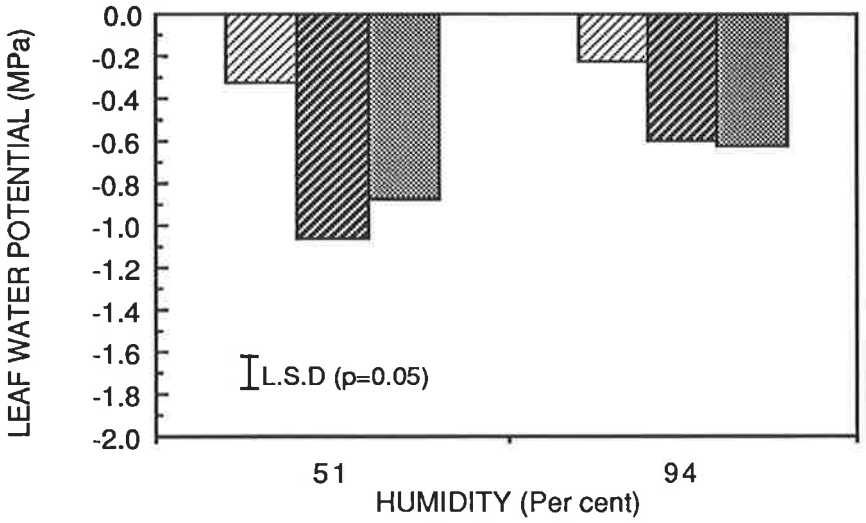
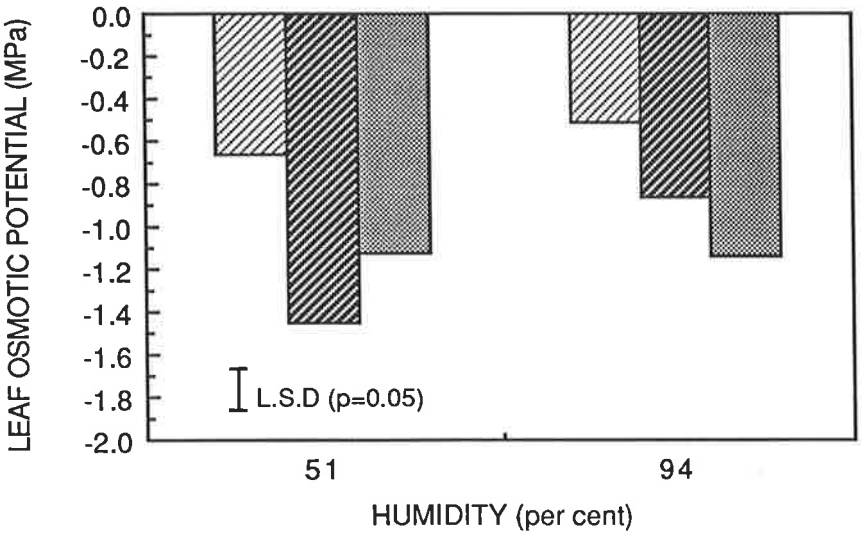
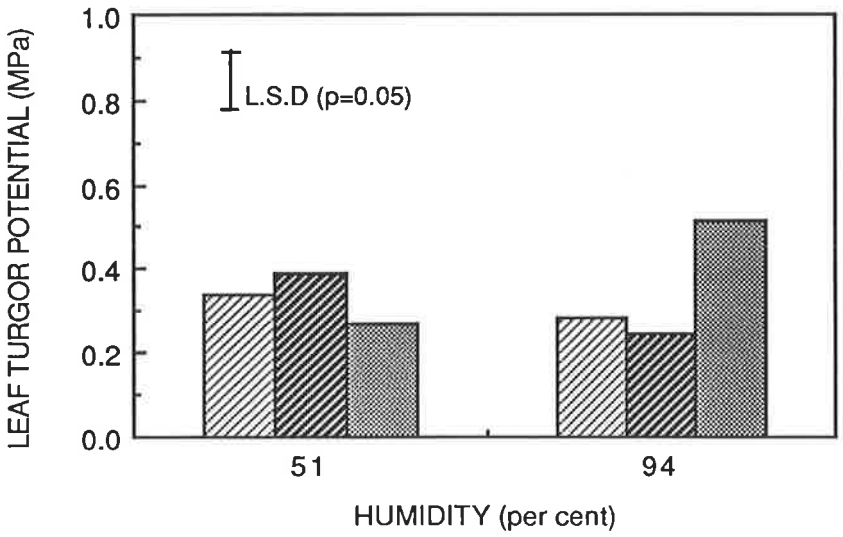


Fig. 64 Effect of different humidities in the environment on leaf turgor (MPa) potential of NaCl and $MgCl_2$ stressed tomato




LEAF WATER POTENTIAL (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
L.S.D (p=0.05)
51
94
HUMIDITY (Per cent)


LEAF OSMOTIC POTENTIAL (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
L.S.D (p=0.05)
51
94
HUMIDITY (per cent)


LEAF TURGOR POTENTIAL (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
L.S.D (p=0.05)
51
94
HUMIDITY (per cent)

4.3.3.2.4 Stomatal resistance

A. Upper surface

The level of humidity in the stress environment influenced the stomatal resistance of the upper surface of the leaf in both control and stressed plants, being lower at 94 than 51 per cent humidity (Fig 65A). Stomatal resistance was higher in $MgCl_2$ than NaCl stressed plants at 51 per cent humidity whereas at 94 per cent humidity, NaCl stressed plants recorded a higher resistance than $MgCl_2$ stressed plants.

B. Lower surface

At 94 per cent humidity in the atmosphere, the response of stomata on the lower surface was similar to that of those on the upper surface at both salinities (Fig 65B). At 51 per cent humidity, however, stomatal resistance was higher in NaCl than $MgCl_2$ salinised plants, which contrasts with the response of stomata on the upper surface.

4.3.3.2.5 Transpiration rate

Transpiration rate was differently influenced by the level of humidity in the atmosphere in both control and stressed plants (Fig 66). High humidity in the atmosphere enhanced the transpiration rate in control plants whereas in plants under salinity stress the transpiration rate remained low and similar. At 51 per cent humidity, however, the transpiration rate was lower in $MgCl_2$ than NaCl salinity.

4.3.3.2.6 Leaf sodium concentration

Leaf Na^+ concentration in NaCl stressed plants was significantly influenced by humidity of the stress environment being lower in 94 than 51 per cent humidity (Fig. 67). The reduction in leaf Na^+ concentration in plants at high humidity being 72 per cent.

Fig. 65 Effect of different humidities in the enviornment on the stomatal resistance (sec cm^{-1})of the upper and lower surface of NaCl and $MgCl_2$ stressed tomato

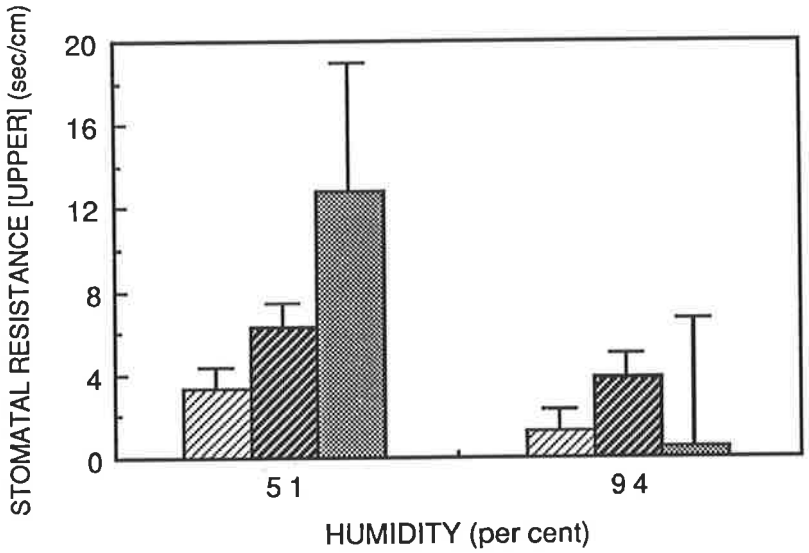
A.Upper

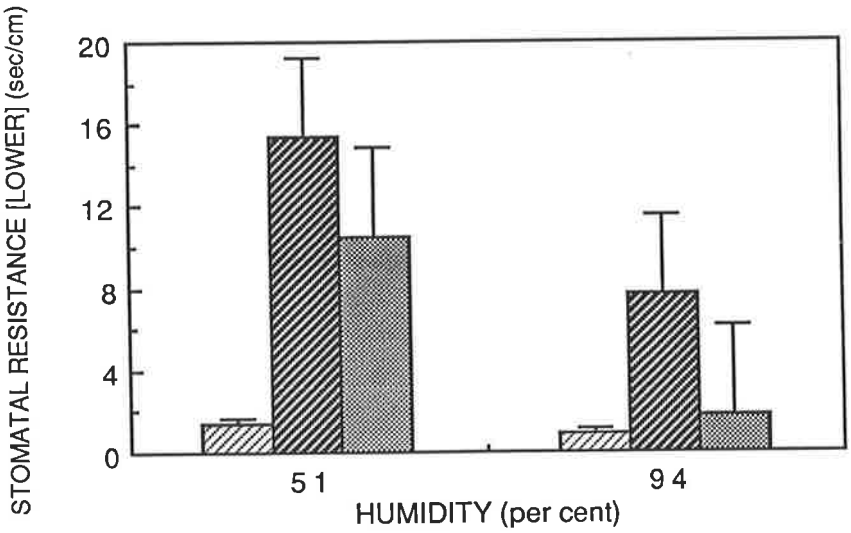
B.Lower

CONTROL
NaCl
$MgCl_2$

Fig. 66 Effect of different humidities in the environment on transpiration rate ($mg,m^{-2}\ h^{-1}$) of NaCl and $MgCl_2$ stressed tomato

CONTROL
NaCl
$MgCl_2$


STOMATAL RESISTANCE [UPPER] (sec/cm)
20
16
12
8
4
0
5 1
9 4
HUMIDITY (per cent)


STOMATAL RESISTANCE [LOWER] (sec/cm)
20
16
12
8
4
0
5 1
9 4
HUMIDITY (per cent)

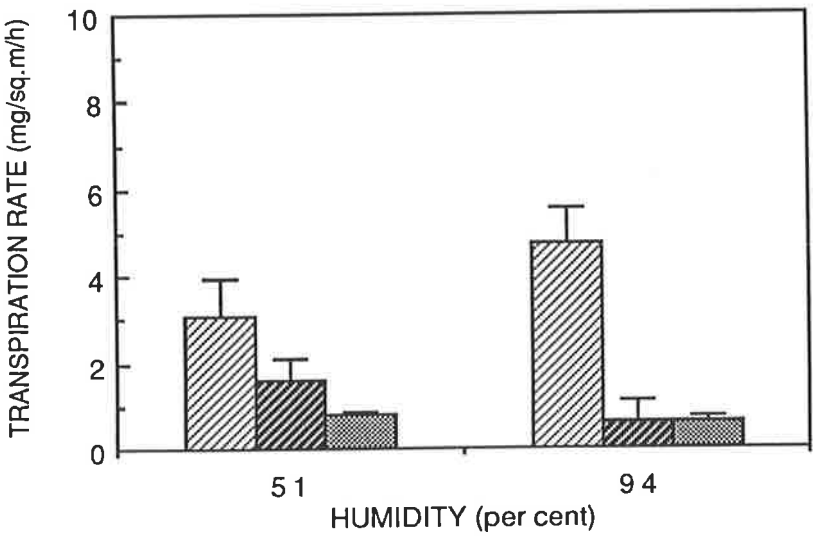

TRANSPIRATION RATE (mg/sq.m/h)
10
8
6
4
2
0
5 1
9 4
HUMIDITY (per cent)

Fig. 67 Effect of different humidities in the enviornment on leaf Na^+ (mmole, g^{-1} dw) concentration of NaCl and $MgCl_2$ stressed tomato

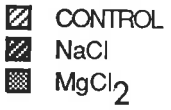
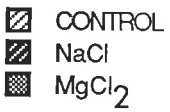
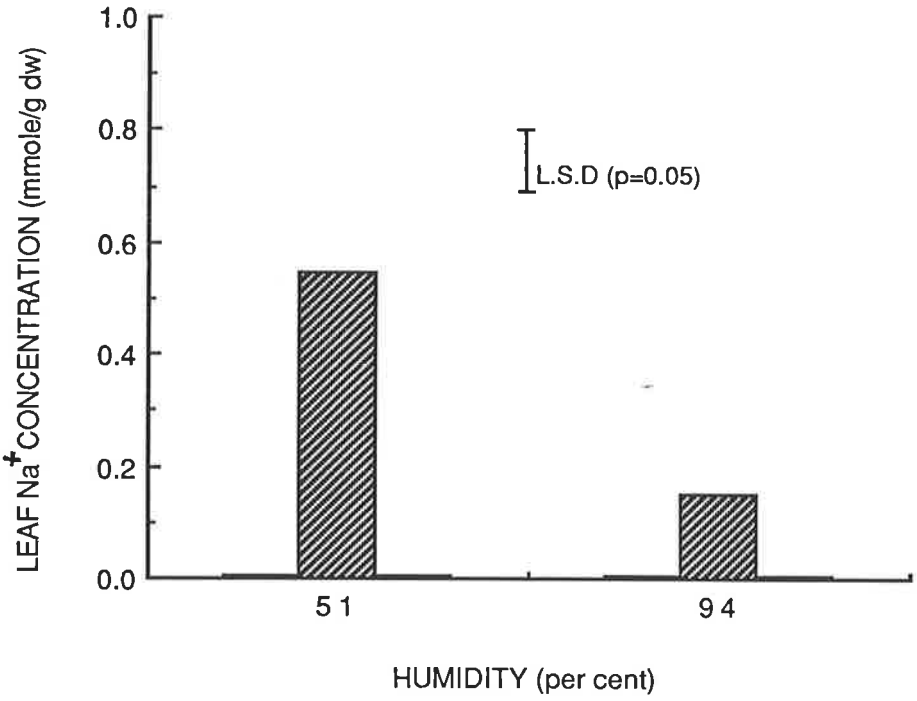
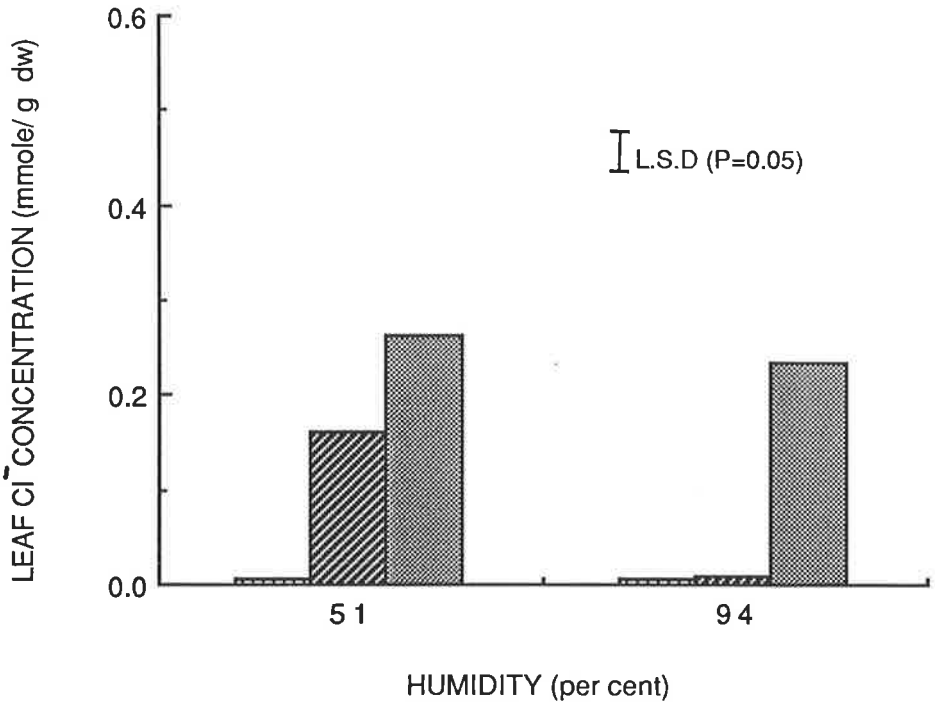


Fig. 68 Effect of different humidities in the environment on leaf Cl^- (mmole, g^{-1} dw) concentration of NaCl and $MgCl_2$ stressed tomato




1.0
0.8
0.6
0.4
0.2
0.0
LEAF Na^+ CONCENTRATION (mmole/g dw)
L.S.D (p=0.05)
5 1
9 4
HUMIDITY (per cent)


0.6
0.4
0.2
0.0
LEAF Cl^- CONCENTRATION (mmole/ g dw)
L.S.D (P=0.05)
5 1
9 4
HUMIDITY (per cent)

4.3.3.2.7 Leaf magnesium concentration

The interaction between the effects of the treatment and humidity on leaf Mg^{++} concentration was not significant (data not presented).

4.3.3.2.8 Leaf chloride concentration

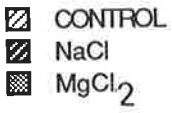
Leaf chloride concentration was significantly influenced by humidity. A complete inhibition of Cl^- accumulation was found in NaCl stressed plants at high humidity (Fig. 68) whereas in $MgCl_2$ salinity the leaf chloride concentration was similar at both humidities. In general, $MgCl_2$ salinised plants accumulated more Cl^- than NaCl stressed plants.

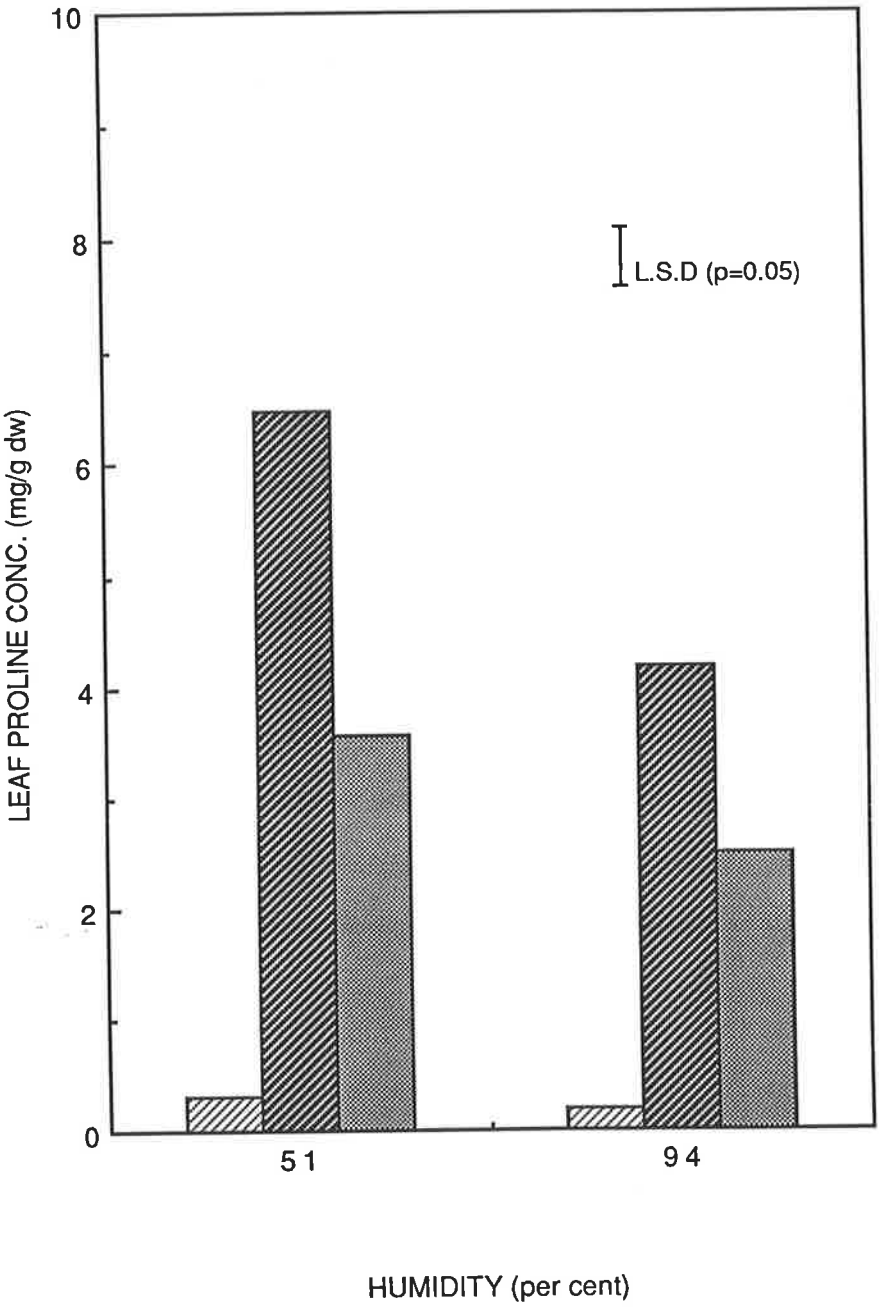
4.3.3.2.9 Leaf proline concentration

Leaf proline concentration in control plants was unaffected by the level of humidity whereas in salinised plants leaf proline concentration was greater in low than in high humidity (Fig. 69). NaCl stressed plants accumulated more proline than those in $MgCl_2$ stress at either humidity.

The results of the present experiment show that leaf proline concentration in $MgCl_2$ salinised plants was lower than that in NaCl even at the low humidity, suggesting that the differences in response between the experiments described in sections 4.2.3 and 4.3.2 was not due to the low humidity during experiment 4.2.3 but may have been due to the variation in wind velocity. The next experiment was designed to test this possibility.

Fig. 69 Effect of different humidities on leaf proline concentration (mg, g^{-1}dw) of NaCl and $MgCl_2$ stressed tomato




10
8
6
4
2
0
LEAF PROLINE CONC. (mg/g dw)
L.S.D (p=0.05)
51
94
HUMIDITY (per cent)

4.3.4 The effect of wind velocity in modifying proline accumulation in NaCl and $MgCl_2$ stress at low humidity

In experiment 4.3.2, the shutters for air circulation in the cabinet were closed except for those above the canopy. This gave better control of humidity but led to a 9 fold difference in incoming air velocity which was 1.04 m sec^{-1} in experiment 4.2.3 and 0.12 m sec^{-1} in experiment 4.3.2. The wind speed affects the boundary layer resistance (Nobel, 1982) and contributes to leaf resistance which, in turn, controls transpiration and leaf temperature (Nobel, 1982; Hall *et al.*, 1976).

Proline accumulation has been postulated to be initiated by some active factor moving from the root(Section 4.3.2). Variation in the transpiration rate would influence the transport of any factor moving in the xylem, and could lead to differences in proline accumulation. It is also possible that the differences in proline accumulation could be due to differences in CO_2 concentration between the two experiments. At the low wind velocity replenishment of CO_2 in the plant growth compartment could be expected to be slower. Reduced CO_2 concentration would influence the production of photosynthates (Farquhar and Sharkey, 1982) and a variation in CO_2 concentration could influence proline accumulation through differences in photosynthate supply (Thompson *et al.*, 1966; Aiyer, 1981).

4.3.4.1 Methods

Tomato cv. Duke seedlings were grown at 20±1°C day and night temperature with a 16 h photoperiod and photon flux density of 350 -420 µEin sec^{-1}(Materials and Methods 3.2.1; 3.2.2). Twenty five day old seedlings were placed in the split-compartment described below. The conditions described for experiments 4.2.3 and 4.3.2 were simulated in one cabinet. Air-turbulence was minimised in one compartment of the cabinet by fixing a glass sheet in the center of the cabinet at right angles to the air-flow leaving a gap of 15 cm at the top. In this compartment the air-velocity was 0.12

m sec^{-1} (low). The shutters were completely opened in the other compartment which experienced a wind velocity of 1.04 m sec^{-1} (high). The air-velocities in those two compartments were monitored using a miniature electronic anemometer probe attached to a solomat placed at the center of the compartments. Temperature and light intensity in both compartments were similar and humidity was maintained at 51 per cent. The CO_2 concentrations in both compartments was constantly sampled through PVC tubes placed at the center of the cabinet and measured with a pre-calibrated infra-red gas analyser (IRGA) ADC type 225-2B-SS. On the 25 th day the plants were supplied with either full strength Hoagland solution or stressed with full strength Hoagland's solution containing either NaCl or $MgCl_2$ at 0.1 MPa day^{-1} until 10 days to reach -1.065MPa. The youngest fully expanded leaves were harvested from both compartments 11 days after commencement of stress for the measurement of leaf water, osmotic and turgor potential (Section 3.2.6), proline (Section 3.2.9.2) and leaf ion content (Section 3.2.11). In plants exposed to either high or low wind velocity, transpiration rates were measured as described in section 4.3.3.1. Stomatal resistance was measured on both upper and lower surfaces (Section 3.2.5.1). The design was a factorial randomised block with three replications of each treatment.

4.3.4.2 Results

4.3.4.2.1 CO2 concentration and leaf water relations

There was no difference in CO_2 concentration between the two compartments in the course of the experiment (Table 38). There were also no differences between the compartments in leaf water, osmotic or turgor potentials in any of the salinity treatments (data not presented).

4.3.4.2.2 Stomatal resistance

A. Upper surface

The Fig.70A shows that the higher wind velocity increased the stomatal resistance of the upper leaf surface in both NaCl and $MgCl_2$ stressed plants although control plants

Table 38 The effect of wind velocity on CO_2 concentration of the split-compartments (ppm)

DAYS	CO_2 CONCENTRATION#	
	CHAMBER-1(0.12 m sec^{-1})	CHAMBER-2(1.04 m sec^{-1})
1	386.3	388.9
2	376.1	378.7
3	376.2	376.2
4	374.6	376.5
5	402.1	402.1
6	350.5	351.1
7	368.5	368.5
8	410.5	409.1
9	425.5	425.5
10	390.5	390.5
11	366.9	368.8

CO_2 was sampled every 4 min. from each compartment 24 h a day

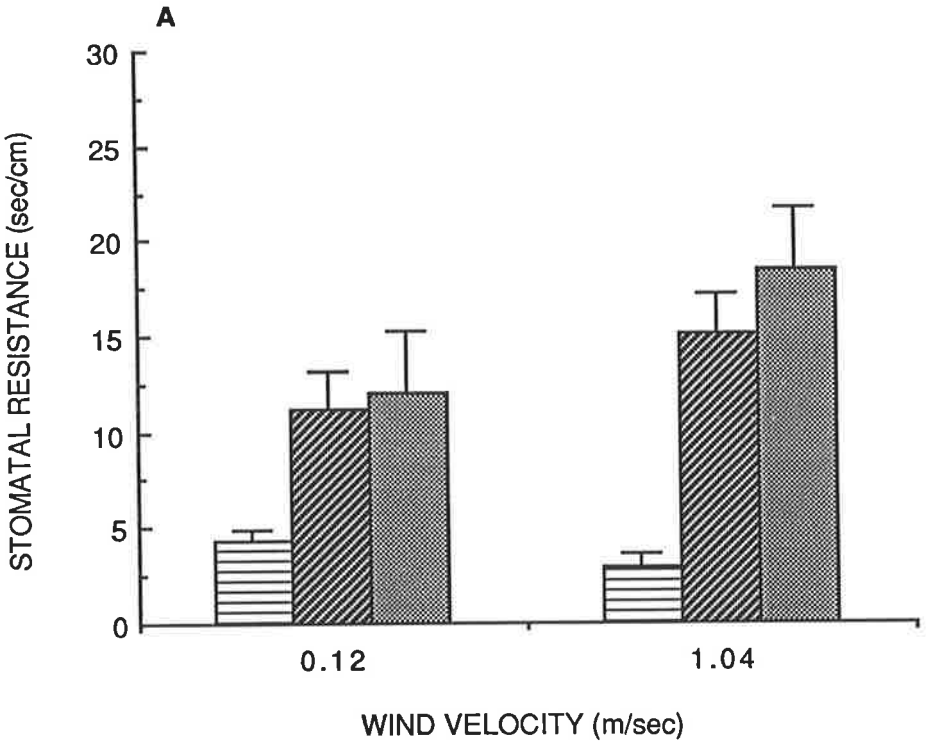
Values are averages of 100 random readings

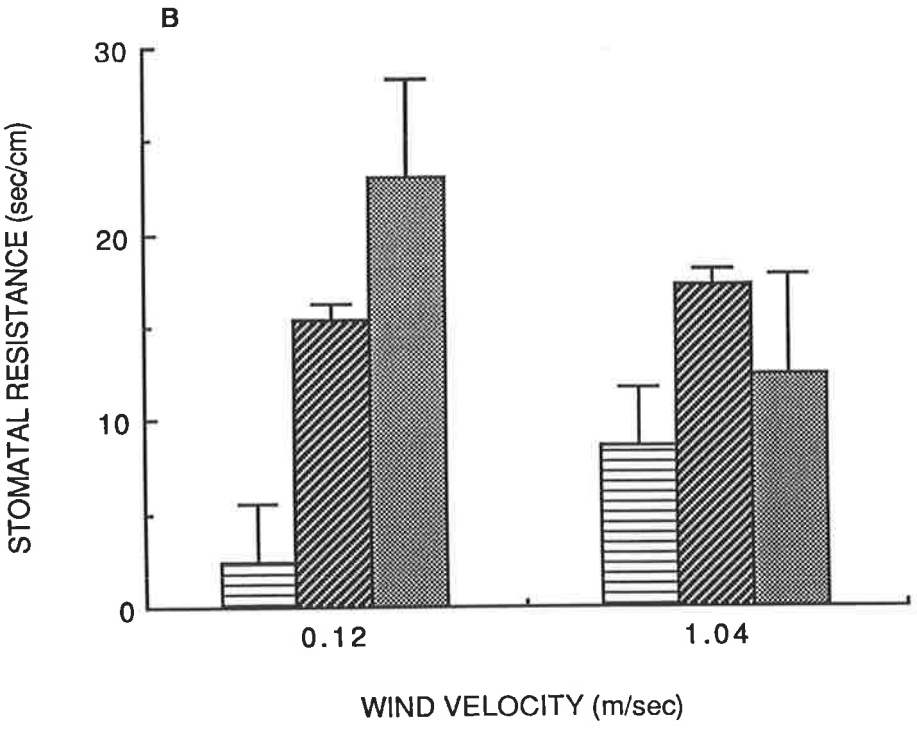
Fig. 70 Effect of different wind velocities on stomatal resistance (sec,cm^{-1}) of upper and lower surface of the leaves of NaCl and $MgCl_2$ stressed tomato

A.Upper

B.Lower

CONTROL
NaCl
$MgCl_2$


A
30
25
20
15
10
5
0
STOMATAL RESISTANCE (sec/cm)
0.12
1.04
WIND VELOCITY (m/sec)


B
30
20
10
0
STOMATAL RESISTANCE (sec/cm)
0.12
1.04
WIND VELOCITY (m/sec)

were unaffected. The plants exposed to $MgCl_2$ salinity had a higher resistance than those in NaCl salinity.

B. Lower surface

The stomates of the lower surface of the leaf responded in a similar way to the upper surface in control and NaCl stress, giving a higher resistance at high than at low wind velocity (Fig 70B). However, the stomates of the $MgCl_2$ stressed plants gave a higher resistance at the lower wind velocity.

4.3.4.2.6 Transpiration rate

Salinity treatments reduced the transpiration rate irrespective of the difference in wind velocity, the reduction being greater in plants under $MgCl_2$ than NaCl salinity (Fig.71). The transpiration rate was higher in plants under $MgCl_2$ stress at high wind velocity than those at low wind velocity which, however, was similar to that of NaCl stressed plants. In contrast, the transpiration rate of NaCl stressed plants was lower at high wind velocity than at low wind velocity.

4.3.4.2.7 Leaf sodium and magnesium concentration

Neither Na^+ nor Mg^{++} concentration in the leaf was affected by wind velocity (data not presented).

4.3.4.2.9 Leaf chloride concentration

There was a significant reduction in leaf chloride concentration in NaCl stressed plants at high wind velocity whereas in $MgCl_2$ salinity the leaf chloride concentration increased significantly (Fig. 72) in parallel with transpiration.

4.3.4.2.10 Leaf proline concentration

Leaf proline concentration was increased by the increase in wind velocity in $MgCl_2$ salinity (Fig. 73). The leaf proline concentration was the same in NaCl and $MgCl_2$ salinity at the low wind velocity. Although it increased in NaCl stressed plants

Fig. 71 Effect of different wind velocities on transpiration rates of NaCl and $MgCl_2$ stressed tomato

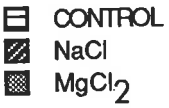
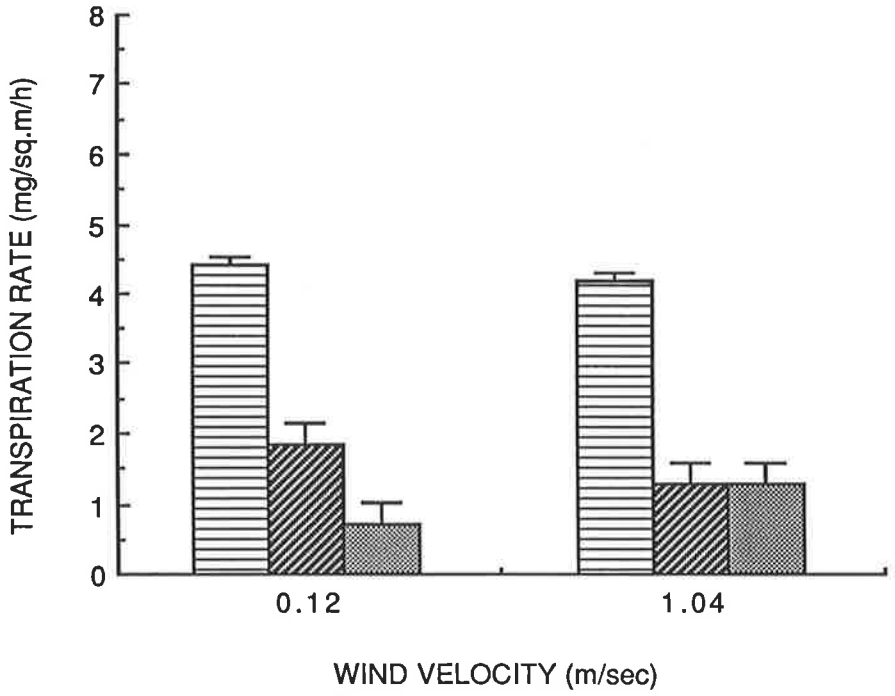


Fig. 72 Effect of different wind velocities on leaf chloride concentration (mmole g^{-1} dw) of NaCl and $MgCl_2$ stressed tomato


TRANSPIRATION RATE (mg/sq.m/h)
8
7
6
5
4
3
2
1
0
0.12
1.04
WIND VELOCITY (m/sec)

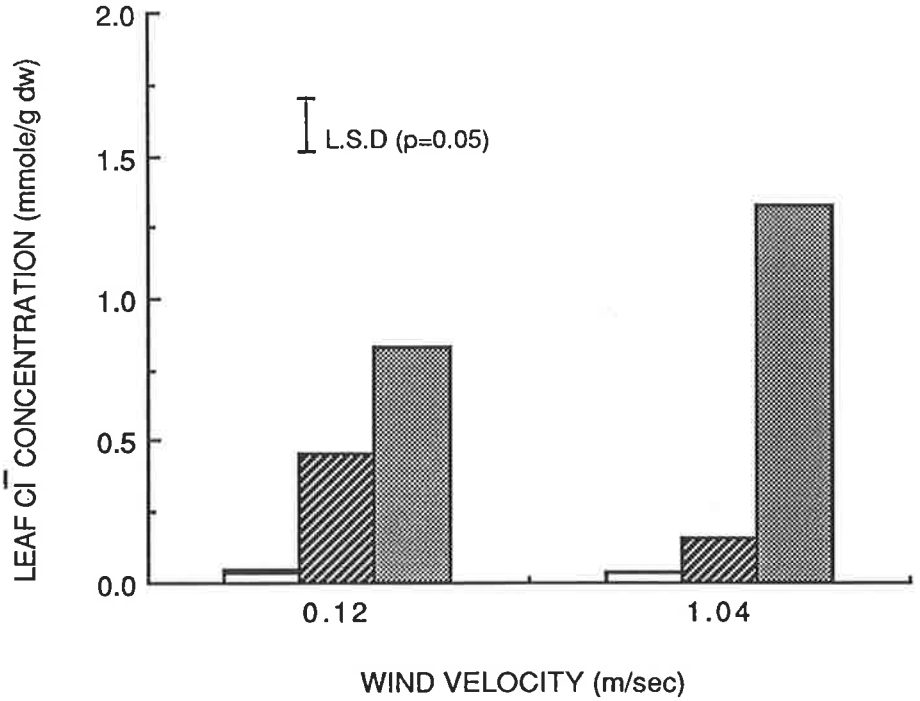
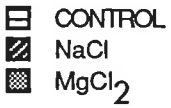
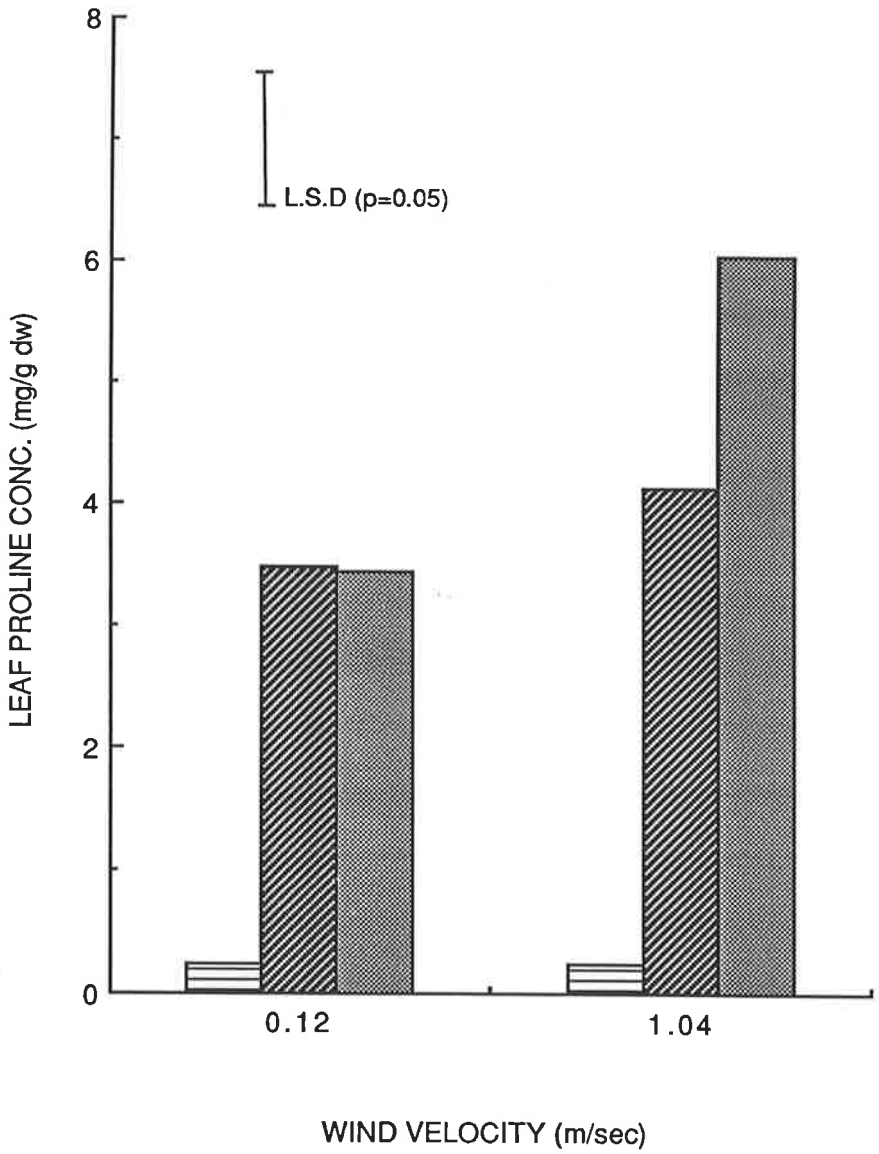

LEAF Cl⁻ CONCENTRATION (mmole/g dw)
2.0
1.5
1.0
0.5
0.0
L.S.D (p=0.05)
0.12
1.04
WIND VELOCITY (m/sec)

Fig. 73 Effect of different wind velocities on leaf proline concentration of NaCl and $MgCl_2$ stressed tomato




8
6
4
2
0
L.S.D (p=0.05)
LEAF PROLINE CONC. (mg/g dw)
0.12
1.04
WIND VELOCITY (m/sec)

(slightly but not significantly) in response to high wind velocity, it increased considerably in $MgCl_2$ treated plants so that there was a 75.6 per cent difference between NaCl and $MgCl_2$ at the higher wind velocity.

4.3.4.3 Discussion

The variation in proline accumulation between $MgCl_2$ and NaCl stressed plants despite a similar $\psi_{ext.}$ in experiments 4.3.2 and 4.2.3, was suggested to be due to the low humidity prevailing during experiment 4.2.3. as variations in proline concentration with differences in humidity were found (Section 4.3.2). The significantly higher amount of proline accumulated in both salinity treatments at low rather than at high humidity in section 4.3.3 is similar to the response observed in section 4.3.2. However, the lower proline concentration in $MgCl_2$ stressed plants than in NaCl stressed plants at the low humidity shows that the difference in proline concentration between NaCl and $MgCl_2$ in experiments 4.2.3 and 4.2.3 may not be due to low humidity alone.

The results in section 4.3.4 show a higher proline concentration in $MgCl_2$ stressed plants exposed to a high wind velocity than in NaCl stressed plants, which suggests that the previous difference in response (Sections 4.2.3, 4.3.2) may have been due to differences in wind velocity interacting with low humidity rather than to low humidity alone. A similar CO_2 concentration in the two compartments despite variation in wind velocity rules out the possibility of this being responsible for the difference in proline concentration between experiments. The effect on proline accumulation in $MgCl_2$ salinity cannot be attributed to a direct physical effect of high wind velocity as there was no change in proline concentration in control plants, which suggests that variation in wind velocity modifies the response in stressed plants alone. The variation in proline concentration was not due to differences in leaf water or cation status as these were not affected by high wind velocity. Leaf Cl^- concentration was influenced by differences in wind velocity, however, and this response depended on the accompanying cation. Although leaf Cl^- concentration varied with wind velocity, it cannot be the sole

cause for variation in proline concentration because (1) a reduction in leaf Cl^- concentration in NaCl stressed plants did not result in a low proline concentration (Fig. 72; 73), (2) variations in leaf Cl^- concentrations in NaCl and $MgCl_2$ salinity were not accompanied by variations in leaf proline concentration (Section 4.3.4), and (3) leaf Cl^- concentration was not related to proline accumulation in previous experiments (Section 4.3.2). The variation in the leaf Cl^- concentration in different wind velocities is most likely due to variations in transpiration rates (Fig. 71; 72) and the nature of the accompanying cation (Sections 4.2.3; 4.2.4;4.3.2). Variation in leaf ion concentration due to variation in the transpiration rate has been demonstrated previously (Russell and Barber, 1960).

Wind velocity influenced the response of upper and lower surface stomates of NaCl and $MgCl_2$ salinised plants. There is evidence that high wind velocity increases the stomatal resistance through modification of the boundary layer and leaf temperature (Grace *et al.*, 1975; Hall *et al.*, 1976), but an interaction between salinity and wind velocity has not been previously reported. Any influence on stomata would have an effect on the transpiration rate (Raschke, 1970). Fig. 71 shows that the high wind velocity unexpectedly decreased the transpiration rate of control and NaCl stressed plants. However, the response differed in $MgCl_2$ stressed plants where there was a higher transpiration rate at high than at low wind velocity (Fig. 70A,B).

It has been postulated (Section 4.2.4; 4.3.2) that proline accumulation is controlled by a factor produced in the roots. It is possible that the greater proline accumulation in $MgCl_2$ salinity compared to NaCl salinity at high wind velocity is due to the transpiration rate of $MgCl_2$ stressed plants being higher at high wind velocity than at low wind velocity. The variation in proline concentration between high and low humidities in experiment 4.3.3 may also have been due to differences in transpiration rate. However, the higher proline concentration in $MgCl_2$ than in NaCl stressed plants at high wind velocity occurred without any difference in transpiration rate. This suggests two possibilities. Firstly, that the higher proline concentration in plants stressed with $MgCl_2$ is due to the synthesis of more root -derived active factor. Even if

the active factor is transported through the transpiration stream, the more rapid synthesis of the active factor would lead to greater proline accumulation. Alternatively, the transpiration measurements on day 10 -11 may not be related to proline accumulation on that day. Events occurring prior to day 11 might be more relevant than those happening coincidentally.

It can be concluded that the discrepancy in proline concentration between experiments 4.3.2 and 4.2.3 in NaCl and $MgCl_2$ stressed plants at iso-osmotic potential of the rooting medium was due to variation in wind velocity at low humidity during the experiments.

4.3.5 The effect of atmospheric vapour pressure deficit on glycinebetaine accumulation in wheat

Glycinebetaine accumulation has been found to be correlated with a decrease in leaf water and osmotic potential and an increase in leaf Na^+ and Cl^- concentration (section 4.1.2). Similar relationships between ψ_s and glycinebetaine accumulation have been reported previously(Storey and Wyn Jones, 1978; Wyn Jones *et al.*, 1977; Grumet and Hanson, 1986). However, glycinebetaine has been found to accumulate without a change in leaf water status or ion content (Section 4.1.4), high internal Mg^{++} concentration did not cause accumulation (Section 4.2.3), and glycinebetaine content varied greatly with a constant leaf water status and ion content (Sections 4.1.2; 4.1.3; 4.1.4). These various relationships between glycinebetaine accumulation, leaf water status and internal ion concentration have been established by a variety of techniques and no systematic attempt has been made to investigate the mechanism triggering the accumulation of glycinebetaine. Subjecting plants to high humidity can serve as a method of preventing a decrease in leaf water and osmotic potential (Section 4.3.2). This system is ideal to examine the relationships between the various factors which may be involved in glycinebetaine accumulation.

4.3.5.1 Methods

Seedlings of wheat cv. Sun-9-E were grown at 20 ± 1°C day and night temperature with a 16 h photoperiod and a photon flux density of 350-420 µEin sec^{-1} (Materials and Methods, 3.2.1 and 3.2.2). NaCl and $MgCl_2$ containing full strength Hoagland solution nutrients was supplied 10 days after emergence of the seedlings. A day before stress imposition the plants were placed in separate cabinets maintained at 51 or 90 per cent humidity. Both cabinets experienced a wind velocity of 1.04 m sec^{-1}. Humidity and wind velocity in the cabinets were constantly monitored. Stress was imposed on plants at both humidities at 0.1 MPa day^{-1} until 10 days to reach -1.065

MPa. The youngest fully expanded leaves were harvested 0,5,9, and 11 days from commencement of stress for measurement of leaf water, osmotic and turgor potentials (Section 3.2.6), proline (Section 3.2.9.2), glycinebetaine (Section 3.2.9.3) and leaf ion contents (Section 3.2.11). The first leaf was sampled on days 0,5,and 9 and the second leaf on day 11, as these were the youngest fully expanded leaves at the time. However, sampling a different leaf on day 11 led to a systematic variation in all measurements made and the responses measured on day 11 will not be discussed. The design was a factorial randomised block with three replications of each treatment.

4.3.5.2 Results

4.3.5.2.1 Leaf water potential

Stressing wheat at the high humidity inhibited the decrease in leaf water potential in both NaCl and $MgCl_2$ stress until day 9 (Fig. 74). In plants exposed to NaCl and $MgCl_2$ stress at low humidity, leaf water potential decreased significantly by day 4 and decreased further to day 9.

4.3.5.2.2 Leaf osmotic potential

High humidity significantly increased leaf osmotic potential in control plants by day 5 , but ψ_s decreased thereafter to a value similar to that in control plants at low humidity (Fig. 75). In NaCl stressed plants at high humidity, the leaf osmotic potential was lower than that in control plants at high humidity(but similar to that in controls at low humidity). At low humidity, the leaf osmotic potential of NaCl stressed plants fell between days 5 and 9. The leaf osmotic potential of $MgCl_2$ stressed plants had decreased by day 5 at low humidity and day 9 at high humidity. The decrease was similar in both humidities by day 9.

4.3.5.2.3 Leaf turgor potential

The leaf turgor potential of control plants at high humidity remained significantly lower than that of those in low humidity (Fig. 76) throughout the experimental period.

Fig. 74 Leaf water potential (-MPa) of NaCl and $MgCl_2$ stressed wheat at low (LH) and high (HH) humidities

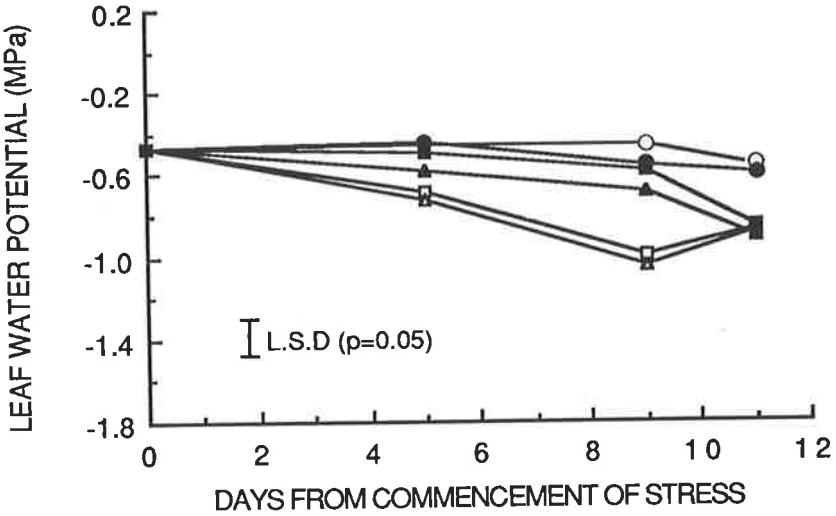
- ○ LH control
- □ LH NaCl
- △ LH $MgCl_2$
- ● HH control
- ■ HH NaCl
- ▲ HH $MgCl_2$

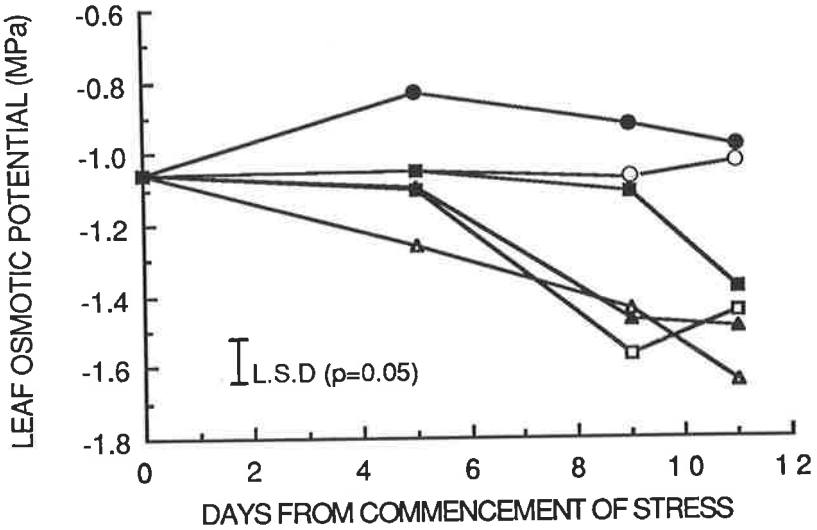
Fig. 75 Leaf osmotic potential (-MPa) of NaCl and $MgCl_2$ stressed wheat at low (LH) and high (HH) humidities

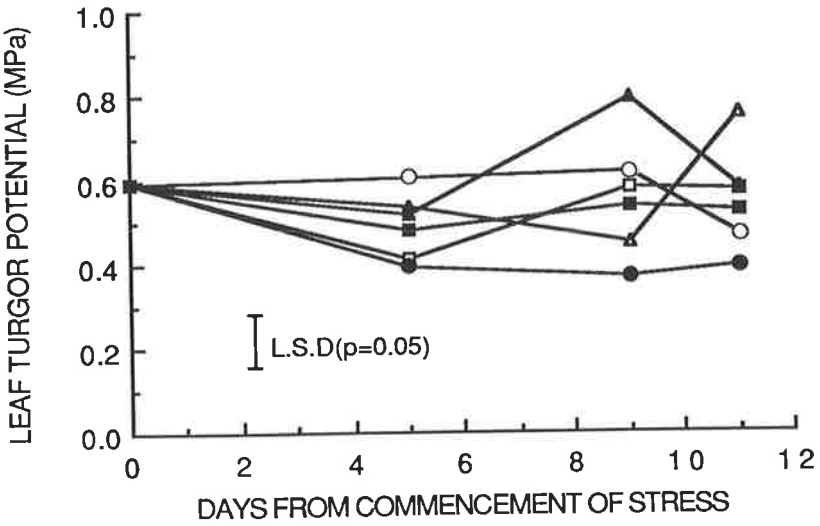
- ○ LH control
- □ LH NaCl
- △ LH $MgCl_2$
- ● HH control
- ■ HH NaCl
- ▲ HH $MgCl_2$

Fig. 76 Leaf turgor potential (MPa) of NaCl and$MgCl_2$ stressed wheat at low (LH) and high (HH) humidities

- ○ LH control
- □ LH NaCl
- △ LH $MgCl_2$
- ● HH control
- ■ HH NaCl
- ▲ HH $MgCl_2$


LEAF WATER POTENTIAL (MPa)
0.2
-0.2
-0.6
-1.0
-1.4
-1.8
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


LEAF OSMOTIC POTENTIAL (MPa)
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


LEAF TURGOR POTENTIAL (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
L.S.D(p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

There were some variations in leaf turgor potential on different days, but the turgor of stressed plants remained similar to or above the control throughout.

4.3.5.2.4 Leaf sodium concentration

Leaf Na^+ concentration was significantly influenced by the humidity of the stress environment (Fig. 77). High humidity in the stress environment resulted in lower Na^+ accumulation with a 48 per cent reduction on day 9.

4.3.5.2.5 Leaf magnesium concentration

High humidity also reduced leaf Mg^{++} concentration, the leaf Mg^{++} concentration in plants at high humidity increased significantly by day 5, increasing further to day 9 (Fig. 78). However, high humidity led to a 19.6 % lower leaf Mg^{++} concentration on day 9.

4.3.5.2.6 Leaf chloride concentration

The leaf chloride concentration of control plants at both humidities remained low (Fig 79). In both NaCl and $MgCl_2$ stressed plants, the leaf chloride concentration increased by day 5, however, the subsequent increase in NaCl stressed plants was substantially reduced by high humidity. In $MgCl_2$ stressed plants at high humidity, leaf chloride concentration increased more rapidly than that in plants at low humidity between days 5 and 9.

4.3.5.2.7 Leaf proline concentration

Subjecting plants to stress at different humidities had a significant influence on leaf proline concentration (Fig.80), the concentration being low at high humidity and high at low humidity irrespective of the salinity treatment. Proline accumulation commenced at or prior to day 5 and continued to increase in plants under both salinities, being greater in $MgCl_2$ than NaCl stress at either humidity.

Fig. 77 Leaf Na^+ concentrations (mmole,g^{-1}dw) of NaCl and $MgCl_2$ stressed wheat at low (LH) and high (HH) humidities

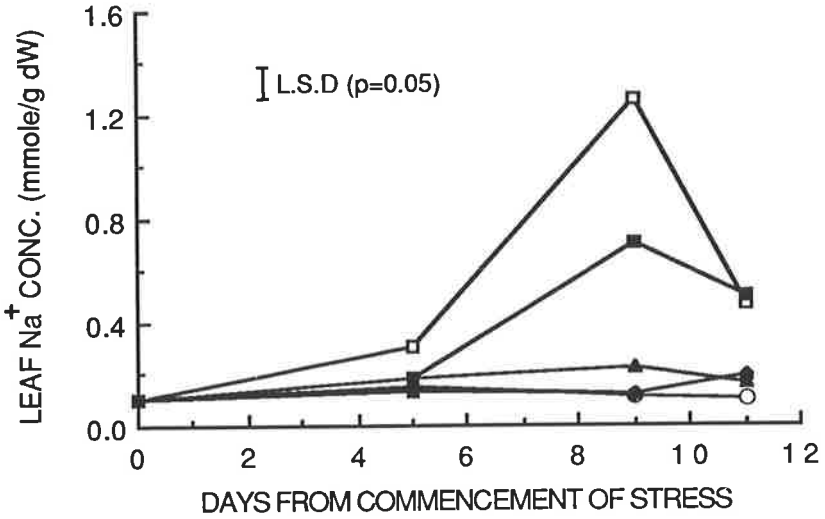
○ LH control
□ LH NaCl
△ LH $MgCl_2$
● HH control
■ HH NaCl
▲ HH $MgCl_2$

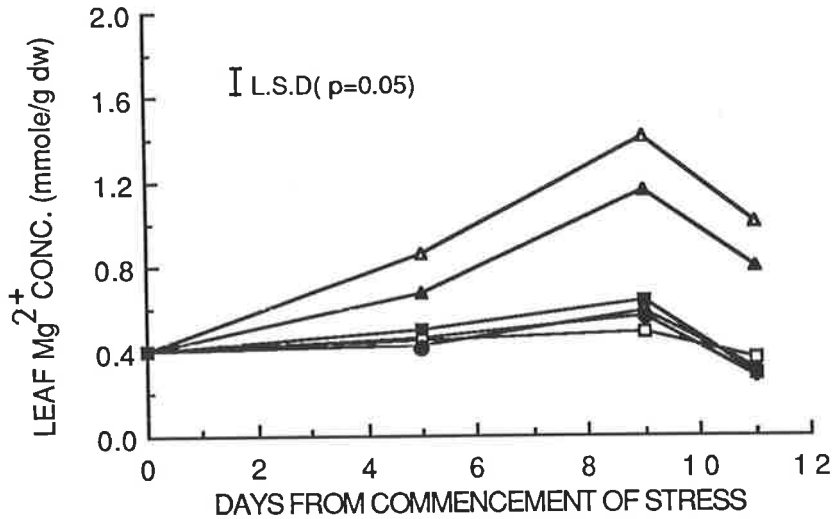
Fig. 78 Leaf Mg^{++} concentrations (mmole,g^{-1}dw) of NaCl and $MgCl_2$ stressed wheat at low (LH) and high (HH) humidities

○ LH control
□ LH NaCl
△ LH $MgCl_2$
● HH control
■ HH NaCl
▲ HH $MgCl_2$

Fig. 79 Leaf Cl^- concentrations (mmole,g^{-1}dw) of NaCl and$MgCl_2$ stressed wheat at low (LH) and high (HH) humidities

○ LH control
□ LH NaCl
△ LH $MgCl_2$
● HH control
■ HH NaCl
▲ HH $MgCl_2$


LEAF Na^+ CONC. (mmole/g dW)
1.6
1.2
0.8
0.4
0.0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


LEAF Mg^{2+} CONC. (mmole/g dw)
2.0
1.6
1.2
0.8
0.4
0.0
L.S.D(p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

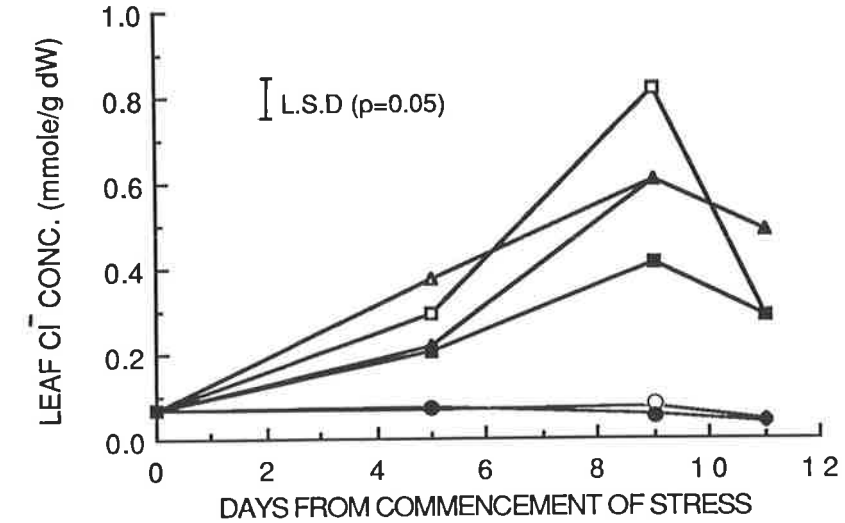
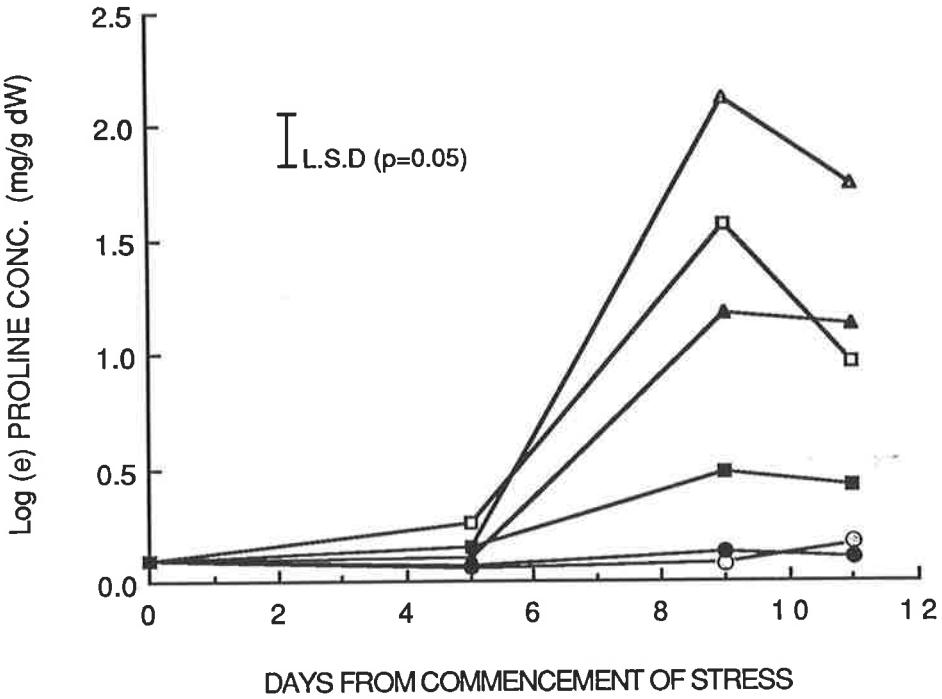

LEAF Cl^- CONC. (mmole/g dW)
1.0
0.8
0.6
0.4
0.2
0.0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

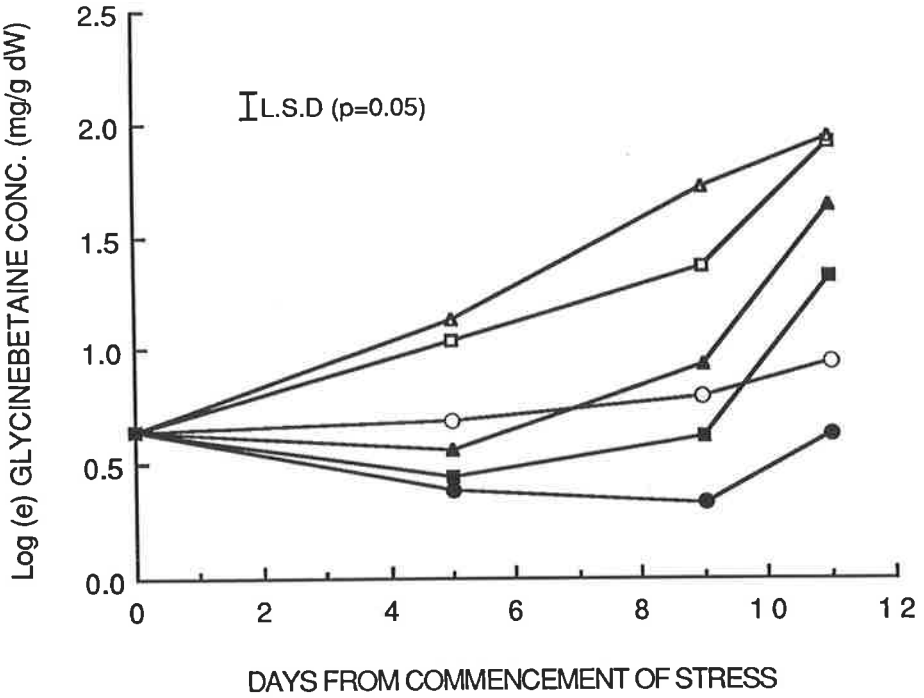
Fig. 80 Log (e) leaf proline concentration ($mg,g^{-1}dw$) of NaCl and $MgCl_2$ stressed wheat at low (LH) and high (HH) humidities

- ○ LH Control
- □ LH NaCl
- △ LH $MgCl_2$
- ● HH Control
- ■ HH NaCl
- ▲ HH $MgCl_2$

Fig. 81 Log (e) leaf glycinebetaine concentration ($mg,g^{-1}dw$) of NaCl and $MgCl_2$ stressed wheat at low (LH) and high (HH) humidities

- ○ LH Control
- □ LH NaCl
- △ LH $MgCl_2$
- ● HH Control
- ■ HH NaCl
- ▲ HH $MgCl_2$


Log (e) PROLINE CONC. (mg/g dW)
2.5
2.0
1.5
1.0
0.5
0.0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS


Log (e) GLYCINEBETAINE CONC. (mg/g dW)
2.5
2.0
1.5
1.0
0.5
0.0
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

4.3.5.2.8 Leaf glycinebetaine concentration

Humidity in the stress environment influenced glycinebetaine accumulation in both control and stressed plants(Fig 81). The leaf glycinebetaine concentration, which was 0.91 mg g^{-1}dw before the commencement of stress, fell significantly in control plants at high humidity, whereas in salt stressed plants glycinebetaine concentration increased significantly by day 5 ($MgCl_2$) or day 9 (NaCl). However, the leaf glycinebetaine concentration in stressed plants at high humidity remained similar to or below the levels in control plants at low humidity until day 9. In plants stressed at low humidity, however, leaf glycinebetaine concentration increased significantly prior to day 5 and continued to increase until the end of the experiment. The plants under $MgCl_2$ stress accumulated more glycinebetaine than those under NaCl salinity in either humidity.

4.3.5.2.9 Growth

A. Dry weight of shoot

Humidity had no influence on shoot growth (Table 39). Shoot growth was not influenced by salinity until day 9 ; thereafter shoot growth continued to increase in plants under both salinities, but at a slower rate than in control plants.

B. Dry weight of root

In contrast to the lack of effect on shoot growth, humidity significantly influenced root growth of both control and stressed plants (Table 40), being significantly lower at high humidity. At low humidity root growth continued at a faster rate in control and stressed plants until day 5, reducing thereafter in both salinities. However, growth continued at a slower rate compared to the controls. By day 11, a significant addition to root dry matter was evident in salinised plants at low humidity.

Table 39 The effects of low and high humidity on Log(e) shoot dry weight (mg) of wheat under NaCl and $MgCl_2$ stress [Humidity X treatment X period interaction was not significant]

A. TREATMENT X PERIOD

TREATMENT	DAYS FROM COMMENCEMENT OF STRESS				MEAN	**L.S.D**
	0	5	9	11		(p=0.05)
CONTROL	0.0398	0.0890	0.1230	0.2136	0.1164	
NaCl	0.0398	0.0896	0.1203	0.1623	0.1021	
$MgCl_2$	0.0398	0.0798	0.1240	0.1588	0.1006	
MEAN	0.0398	0.0861	0.1224	0.1783		**0.03**

B.HUMIDITY

	LOW	HIGH	**L.S.D** (p=0.05)
	0.1133	0.1001	0.01

Table 40 The effects of low and high humidity on Log(e) root dry weight of wheat under NaCl and $MgCl_2$ stress (mg)

TREATMENT	HUMIDITY	DAYS FROM COMMENCEMENT OF STRESS				MEAN
		0	5	9	11	
CONTROL	LOW	0.0529	0.0908	0.1667	0.3157	0.1565
	HIGH	0.0529	0.1151	0.1027	0.1085	0.0712
	MEAN	0.0529	0.0748	0.1214	0.2065	0.1139
NaCl	LOW	0.0529	0.1151	0.1027	0.1085	0.0948
	HIGH	0.0529	0.0521	0.0612	0.0774	0.0609
	MEAN	0.0529	0.0836	0.0819	0.0930	0.0778
$MgCl_2$	LOW	0.0529	0.0692	0.0837	0.1173	0.0808
	HIGH	0.0529	0.0512	0.0574	0.0794	0.0602
	MEAN	0.0529	0.0602	0.0706	0.0984	0.0705
	MEAN	0.0529	0.0729	0.0913	0.1326	0.0874

SOURCE OF VARIATION	VR	LSD (p=0.05)
HUMIDITY	164.39***	
TREATMENT	54.37***	
PERIOD	87.29***	
HUMIDITY X TREATMENT	29.49***	
HUMIDITY X PERIOD	29.74***	
TREATMENT X PERIOD	23.50***	
HUMIDITY X TREATMENT X PERIOD	17.37***	0.025

*** (p=0.01)

4.3.5.3 Discussion

The results of this experiment suggest that the trigger for glycinebetaine accumulation may not be a change in leaf water status. Significant amounts of glycinebetaine accumulated although there was no change in leaf water relations in plants at high humidity. Further, it does not seem that the internal ion concentration would be the possible trigger as glycinebetaine concentration was not proportional to the internal ion concentration (Wyn Jones *et al.*, 1977). Although there was a significant increase subjected leaf Na^+ concentration in plants in NaCl stress at high humidity (day 9), the leaf glycinebetaine concentration remained below the level of the control plants kept at low humidity. This was not due to these plants being below a threshold Na^+ concentration as the leaf Na^+ concentration in plants at high humidity on day 9 was higher than the concentration in plants on day 5 at low humidity which had accumulated glycinebetaine. The results with $MgCl_2$ stress also do not favour the possibility that leaf cation concentration is a trigger controlling glycinebetaine accumulation. For a similar leaf Mg^{++} concentration (difference of 19 per cent) between plants at high and low humidity, the difference in glycinebetaine concentration was 3.5 fold . The low glycinebetaine concentration in $MgCl_2$ stressed plants grown at high humidity cannot be attributed to failure to reach a threshold ion concentration as this was significantly higher than in those in low humidity on day 5 which had accumulated glycinebetaine. The possibility that leaf chloride controls glycinebetaine accumulation may also be ruled out. In $MgCl_2$ salinised plants, leaf chloride increased to a similar extent in both humidities yet the glycinebetaine concentration differed considerably. This evidence suggests that relationships between glycinebetaine accumulation and leaf water, osmotic potential and Na^+ or Cl^- concentration observed here (section 4.1.2) and else where (Storey and Wyn Jones, 1978; Wyn Jones *et al.*, 1977; Grumet and Hanson, 1986) do not indicate controlling mechanisms.

There are a few possible explanations for the low concentration of glycinebetaine accumulated in control and stressed plants at high humidity. Firstly, a reduction in

transport of a root-derived active factor may be involved. High humidity significantly reduces transpiration, and although no transpiration measurements were made in this experiment, the evidence of Hoffman and Jabes(1978), for wheat grown at high humidity, indicates a reduction in transpiration rate in both control and salt stressed plants reducing the transport of root-derived factors. Secondly, the high humidity suspended root growth in both control and stressed plants. Similar reductions in root growth in Cacao (Sale, 1970) and cotton (Hoffman *et al.*, 1971) subjected to high humidity have been reported. Any reduction in root growth could reduce the amount of root-derived factor synthesised and this may be another reason for the low concentration of glycinebetaine in plants at high humidity.

SECTION 4.4 INHIBITION OF Na^+ ABSORPTION AND ITS EFFECT ON PROLINE ACCUMULATION IN TOMATO

4.4.1 Introduction

An increase in leaf Na^+ concentration has been suggested to control proline accumulation, with a threshold of 200 µmole cations g^{-1} fw (Voetberg and Stewart, 1984). Results in section 4.1.2 and 4.2.4 also show a linear relationship between leaf proline and Na^+ concentration. An increase in the activity of Δ^1 P-5-C reductase (Huber, 1974) and inhibition of Δ^1 P-5-C dehydrogenase (Boggess *et al.*, 1975) with increasing NaCl concentration have been demonstrated and both would increase the proline pool. However, an inhibition of proline accumulation due to an increase in leaf Na^+ concentration has been shown (Section 4.1.2; Chu *et al.*, 1981). The various responses of proline accumulation in the present study (Sections 4.2.5; 4.3.2; 4.3.5) do not support the hypothesis that leaf cation concentration is a triggering mechanism for proline accumulation. This experiment explores this relationship further by examining proline accumulation under conditions of inhibited Na^+ absorption.

Anaerobic conditions, low temperature and 2,4 dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, have been demonstrated to inhibit Na^+ absorption (Rains and Epstein, 1967). An increase in Ca^{++} concentration in the rooting medium at high NaCl concentration also virtually abolishes Na^+ uptake (Rains and Epstein, 1967; Lahaye and Epstein, 1969). Ion uptake and transport can also be varied through manipulating transpiration rates (Pitman, 1977, 1982). Limiting transpiration by subjecting plants to high humidity reduces leaf Na^+ concentration in tomato (Section 4.3.2)and other crop species (Pitman, 1984). In the present study Ca^{++} was preferred to DNP or azide as these compounds inhibit Na^+ absorption by inhibiting phosphorylation activity. Any influence on phophorylation activity would be expected to influence proline synthesis itself (Aiyer, 1981).

4.4.2 Methods

Seedlings of tomato CV Duke were grown at 20±1°C day and night temperature with 16 h photoperiod and a photon flux density of 350-420 μEin sec^{-1} (Materials and Methods, 3.2.1; 3.2.2). A day prior to stress imposition, the plants were equilibrated at 94 per cent (Δ_e 0.4 mbar) relative humidity. Humidity in the cabinet was constantly monitored and maintained. On the 25th day after the emergence of the seedlings, plants were supplied with full strength Hoagland's nutrient solution or NaCl containing 0, 4, 20 mM Ca^{++} as $Ca(NO_3)_2$ in Hoagland nutrient solution. NaCl concentration in the rooting medium was increased 22.4 mM day^{-1} (-0.1MPa). On the final day of stressing the plants experienced 179 mM NaCl(-0.8 MPa). Appropriate controls were run with the same concentrations of $Ca(NO_3)_2$ as in the NaCl solutions. Youngest fully expanded leaves were harvested on days 5 and 9 for the measurement of leaf water, osmotic and turgor potential (Section 3.2.6), proline (Section 3.2.9.2) and leaf ion content (Section 3.2.11). The design was a factorial randomised block with three replications of each treatment.

4.4.3 Results

4.4.3.1 *Leaf water potential*

Varying the level of Ca^{++} in the rooting medium did not influence the leaf water potential of either control or stressed plants (Fig.82).

4.4.3.2 *Leaf osmotic and turgor potentials*

The responses of leaf osmotic potential (Fig. 83) paralleled the changes in leaf water potential and as a result there was no difference in turgor potential between treatments.

4.4.3.4 *Leaf sodium concentration*

An increase in Ca^{++} concentration in NaCl solutions of the rooting medium significantly reduced the Na^{+} concentration in leaves of stressed plants on both days 5 and 9 (Fig.

Fig. 82 Leaf water potential (-MPa) of NaCl stressed tomato as influenced by various levels of Ca^{++} in the rooting medium

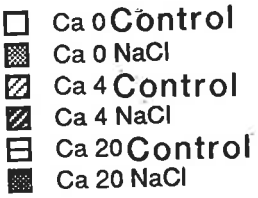
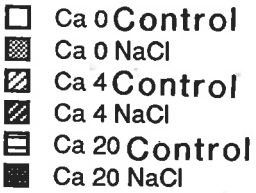
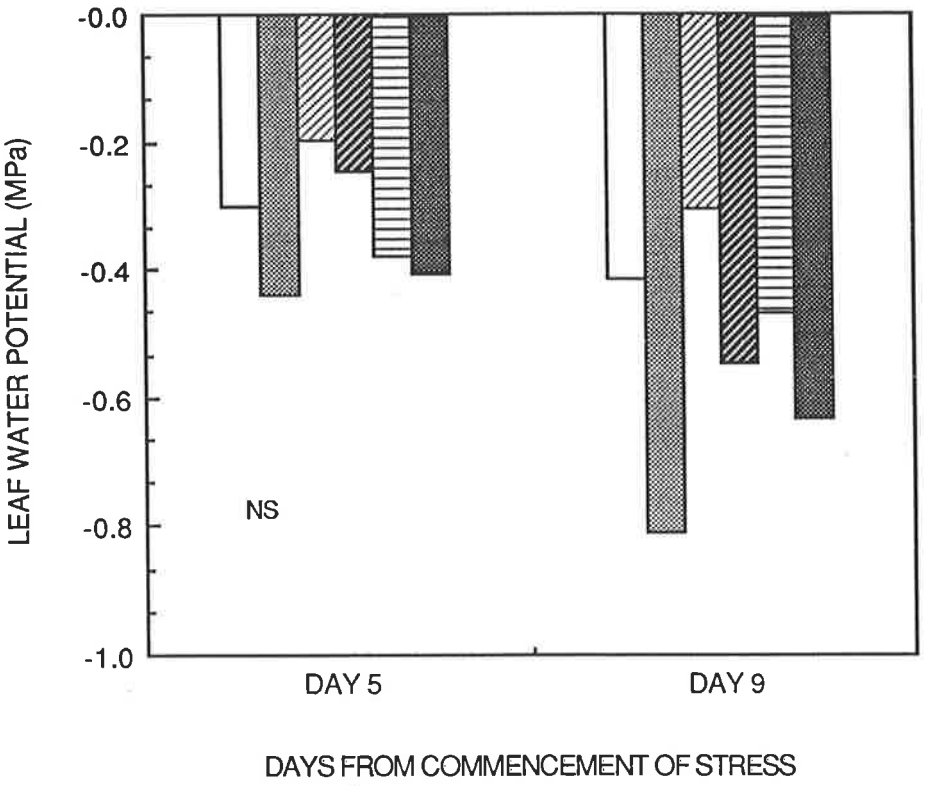
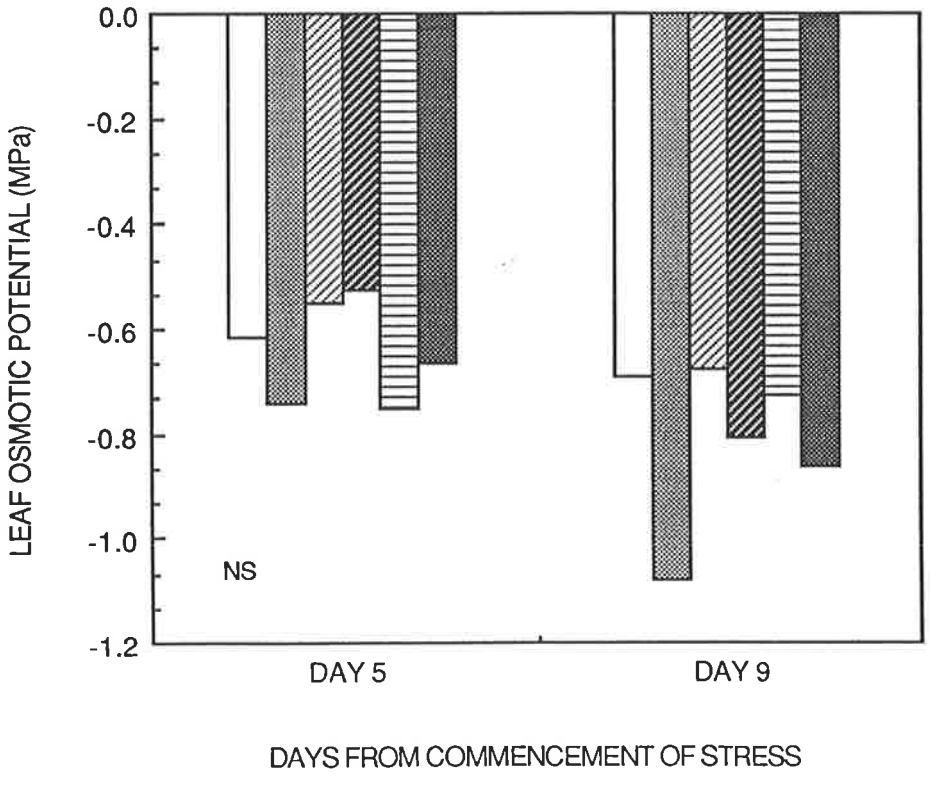


Fig. 83 Leaf osmotic potential (-MPa) of NaCl stressed plants as influenced by various levels of Ca^{++}




LEAF WATER POTENTIAL (MPa)
-0.0
-0.2
-0.4
-0.6
-0.8
-1.0
NS
DAY 5
DAY 9
DAYS FROM COMMENCEMENT OF STRESS


LEAF OSMOTIC POTENTIAL (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
NS
DAY 5
DAY 9
DAYS FROM COMMENCEMENT OF STRESS

84). Leaf Na^+ concentration continued to increase with time but the increase between day 5 and 9 in the presence of 20 mM Ca^{++} was not significant.

4.4.3.5 *Leaf chloride concentration*

The increase in Ca^{++} (or NO_3^-) concentration in the saline rooting medium had no appreciable influence on leaf Cl^- concentration (Fig. 85).

4.4.3.6 *Leaf proline concentration*

The increase in Ca^{++} (or NO_3^-) concentration in the saline rooting medium significantly enhanced proline accumulation in stressed plants (Fig. 86). An increase in Ca^{++} concentration in the rooting medium did not influence proline concentration in control plants.

4.4.4 Discussion

Subjecting plants to NaCl in a high atmospheric humidity substantially reduced the effects of salinity on leaf water relations and allowed observation of the effects of varying $Ca(NO_3)_2$ concentration in the absence of changes in leaf water relations. Increasing the Ca^{++} concentration in the rooting medium had a large influence on leaf Na^+ concentration. Leaf Na^+ accumulation between days 5 and 9 was reduced by Ca^{++}, little if any accumulation occurring at 20 mM Ca^{++}. Ca^{++} has frequently been shown to inhibit Na^+ absorption (Rains,1972). Ca^{++} affects transport processes at the cell surface (Epstein, 1961; Rains *et al.*, 1964), membrane structure (Wildes and Neales, 1971; Hepler and Wayne, 1985) and translocation and re-distribution of ions in plants exposed to salinity (Lahaye and Epstein, 1969).

Proline accumulated in the first 5 days despite a lack of change in leaf water relations or leaf Na^+ and Cl^- concentration. The observed relationships between each of these factors and proline accumulation recorded earlier in the present study, and with leaf turgor potential (Setter and Greenway, 1979; Greenway and Leahy, 1972), leaf osmotic potential (Chu *et al.*, 1976), and leaf Na^+ concentration (Voetberg and

Fig. 84 Effect of varying levels of Ca^{++} (mM) at the rooting medium on leaf Na^{+} concentration (mmole, g^{-1}dw) of NaCl stressed tomato

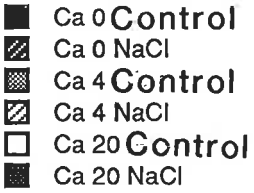


Fig. 85 Effect of varying levels of Ca^{++} (mM) on leaf Cl^{-} concentration (mmole,g^{1}dw) of NaCl stressed tomato

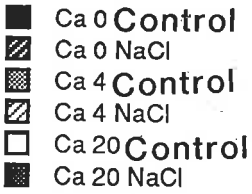
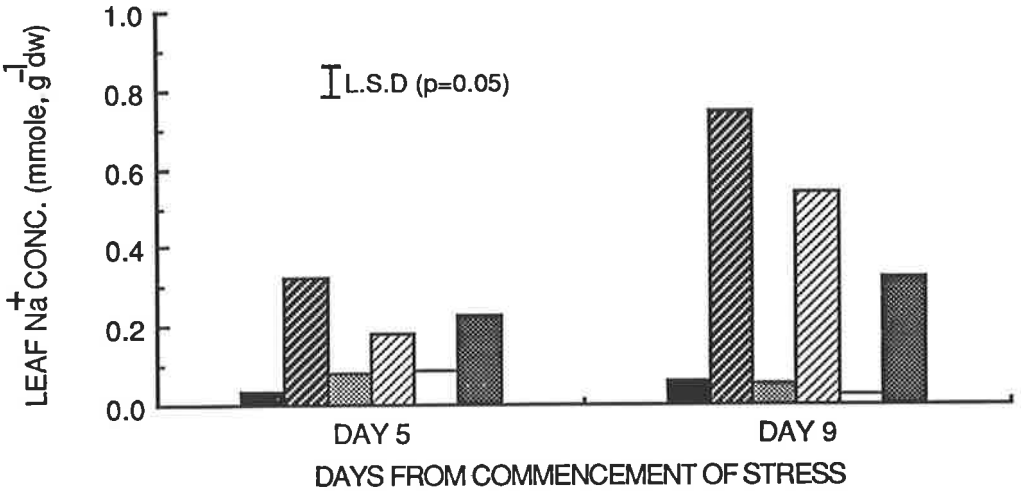
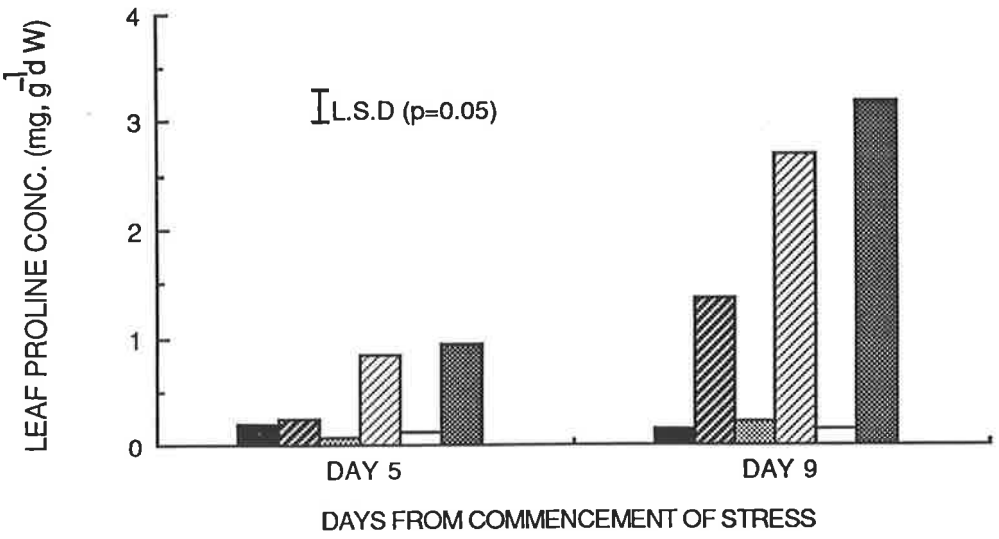


Fig. 86 Effect of varying levels of Ca^{++} (mM) at the rooting medium on leaf proline concentration (mg, g^{-1}dw) of NaCl stressed tomato


LEAF Na^+ CONC. (mmole, g^{-1}dw)
1.0
0.8
0.6
0.4
0.2
0.0
L.S.D (p=0.05)
DAY 5
DAY 9
DAYS FROM COMMENCEMENT OF STRESS


LEAF Cl^- CONC. (mmole, g^{-1}dW)
0.5
0.4
0.3
0.2
0.1
0.0
L.S.D (p=0.05)
DAY 5
DAY 9
DAYS FROM COMMENCEMENT OF STRESS


LEAF PROLINE CONC. (mg, g^{-1}d W)
4
3
2
1
0
L.S.D (p=0.05)
DAY 5
DAY 9
DAYS FROM COMMENCEMENT OF STRESS

Stewart, 1984) appear not to be causal relationships but due to parallel changes occurring during salt stress. The accumulation of proline in significant amounts despite no increase in leaf Na^+ and Cl^- also rules out the possibility of leaf enzyme responses (Boggess *et al.*, 1975; Huber, 1974) as the sole cause of proline accumulation.

The parallel increase in leaf proline and $Ca(NO_3)_2$ concentration in the saline rooting medium suggests the possible involvement of Ca^{++} in proline accumulation. Ca^{++}-sequestering and antagonistic agents have been shown to prevent ABA, KCl, NaCl and moisture stress induced proline accumulation (Uma Shaanker *et al.*, 1988). This led to the proposal that enhancement of cytosolic Ca^{++} concentration may be the mechanism causing proline accumulation, mediated through calcium-binding proteins. However, an increase in salinity in the rooting medium resulted in a proportionate decrease in leaf Ca^{++} concentration accompanying a large increase in proline concentration in tomato (Rajasekaran, 1979). Ion micrographs of leaf cells of NaCl stressed tomato also show a reduction in cytosolic Ca^{++} concentration (Sacher *et al.*, 1983 cf. Sacher and Staples, 1984). This evidence suggests that the trigger for initiation of proline accumulation in salt stress is unlikely to be an increase in cytosolic Ca^{++} concentration. The increase in proline concentration in NaCl stressed plants is also unlikely to be due to an increase in calcium concentration in the rooting medium alone, as a similar increase in calcium concentration in unstressed control plants did not lead to proline accumulation. The inhibition of proline accumulation observed in the studies of Uma Shaankar *et al.*, (1988) may be due to ruthenium red, $LaCl_3$, and EGTA which were used as calcium sequestering and antagonistic agents as nothing is known about the effects of these compounds on proline metabolism.

The increase in proline concentration in NaCl salinity could be due to the higher NO_3^- availability but control plants that received similar amounts of NO_3^- showed no increase in proline concentration. $Ca(NO_3)_2$ level could modulate proline accumulation when proline accumulation is triggered by another factor (eg. salinity, Chu *et al.*, 1981). The observed inverse relationship between leaf Na^+ and proline

concentration suggests that high internal Na^+ concentration suppresses full expression of proline accumulation and disproves the proposal that the increase in proline compensates for osmotic disequilibrium within the cell following an increase in leaf Na^+ (Voetberg and Stewart ,1984).

SECTION 4.5 ABA AND ACCUMULATION OF PROLINE AND QUATERNARY AMMONIUM COMPOUNDS DURING SALT STRESS

4.5.1 Introduction

The present study suggests that the accumulation of proline (Sections 4.3.2; 4.3.5; 4.4), trigonelline (Section 4.3.2) and glycinebetaine (Section 4.3.5) in response to salt stress is triggered by a factor from the roots. Variation in concentrations of these compounds in low and high humidity (Sections 4.3.2; 4.3.5) have been attributed to variation in transport of this root derived factor. ABA is synthesised in roots (Creelman *et al.*, 1987; Zhang and Davis, 1987) and chloroplasts (Milborrow, 1974), but the accumulation of ABA in response to salt stress is not fully established. Walton *et al.*, (1977) reported a rapid increase in foliar ABA concentration in bean within 2 h of imposing stress but Mizrahi *et al.*, (1972), found no increase in tobacco exposed to salt stress for 4 days. Similarly, the studies of Walker and Dumbrooff (1981) with tomato show no correlation between external salinity and endogenous ABA concentration.

The application of ABA induces proline accumulation in *Hordeum* and *Lolium* leaves (Aspinall *et al.*, 1973; Stewart, 1980) but this response is not universal (Aspinall *et al.*, 1973; Mc Donnell *et al.*, 1983). Moreover, in barley leaf segments exposed to salinity, proline accumulation was not preceded by an increase in ABA (Stewart and Voetberg,1985) suggesting that ABA may not be necessarily involved in proline accumulation. Moreover, the ABA -deficient mutant tomato, *flacca*, accumulates proline in response to wilting although there is no increase in ABA (Stewart and Voetberg, 1987). In intact water -stressed barley plants, however, ABA accumulation preceded proline accumulation and, when the plants rehydrated, a decline in ABA preceded the disappearance of proline (Aspinall, 1980) suggesting that ABA and proline accumulation may be linked. The two undisputed facts are : (1) In some

species, ABA will cause proline accumulation in the absence of any stress and (2), in some cases, stress will cause proline accumulation in the absence of any increase in endogenous ABA. There is no doubt, therefore, that ABA is not a universal intermediary between stress and proline accumulation. However, it remains unclear whether (1) ABA acts as an intermediary in some systems or (2) ABA modifies stress-induced accumulation.

Relationships between ABA and glycinebetaine accumulation also remain controversial. Huber and Sankhla (1980) reported an increase in glycinebetaine concentration in response to exogenous ABA whereas the studies of Mc Donnell *et al.*, (1983) show no such effect. Trigonelline has been shown to accumulate along with an increase in ABA concentration (Parameshwara, 1984), although a lack of time course experiments does not permit us to extend the relationship between these two solutes.

These various reports suggest that there is no critical evidence for either NaCl induction of ABA accumulation or the obligatory ABA induction of the accumulation of proline, trigonelline or glycinebetaine. The experiment in this section was therefore planned to (1) investigate the effect of NaCl on endogenous ABA concentration at high and low humidity (2) elucidate the relationship between the accumulation of ABA, proline and quaternary ammonium compounds.

4.5.2 Materials and Methods

Seedlings of tomato cv. Duke and wheat cv. Sun-9-E were grown at 20±1°C with a 16 h photoperiod and a photon flux density of 350-420 μEin sec^{-1} (Materials and Methods, 3.2.1;3.2.2). They were supplied with Hoagland nutrient solution until 25 (tomato) or 10(wheat) days after emergence of the seedlings. On the 25th(tomato) or 10th(wheat) day, these seedlings were supplied with full strength Hoaglands nutrient solution or full strength Hoagland's solution containing NaCl. A day prior to stress imposition these plants were placed in an environment of either low:70 (tomato),51(wheat) or high: 94 (tomato),90 (wheat) per cent humidity, simulating that in sections 4.3.2 and 4.3.5. The specific relative humidity in the growth cabinet

was achieved as described in section 4.3.2.1. Salt stress was imposed on the plants in both relative humidities at a rate of increase of 0.1 MPa day^{-1} for 10 days reaching -1.065 MPa. The youngest fully expanded leaves were harvested on 0, 5, 9 and 11 days from commencement of stress for measurement of ABA (Section 3.2.10), proline (Section 3.2.9.2), trigonelline (Section 3.2.9.3) and glycinebetaine (Section 3.2.9.3). Log(e) transformation was performed on proline, trigonelline and glycinebetaine data before statistical analysis to distribute the error homogeneously. The design was a factorial randomised block with three replications of each treatment.

4.5.3 Results

4.5.3.1 ABA concentration

Salinity caused no increase in ABA concentration in tomato or wheat plants at either humidity during the entire period of the experiment (Figs. 87, 88). The main effect in the data was a very slight but statistically significant increase in ABA concentration in wheat stressed at the high humidity, but this appears to be physiologically negligible(Table 41)

4.5.3.2 Proline concentration

Leaf proline concentrations in the control plants of both tomato (Fig. 89) and wheat (Fig. 90) were low in both low and high humidity throughout the experiment but in the salinised plants increased substantially by day 5 and continued to increase until day 11 (tomato) or 9(wheat). Proline concentrations were generally lower in high humidity than in low humidity in both salinized tomato and wheat.

4.5.3.3 Quaternary Ammonium Compounds

4.5.3.3.1 Trigonelline concentration

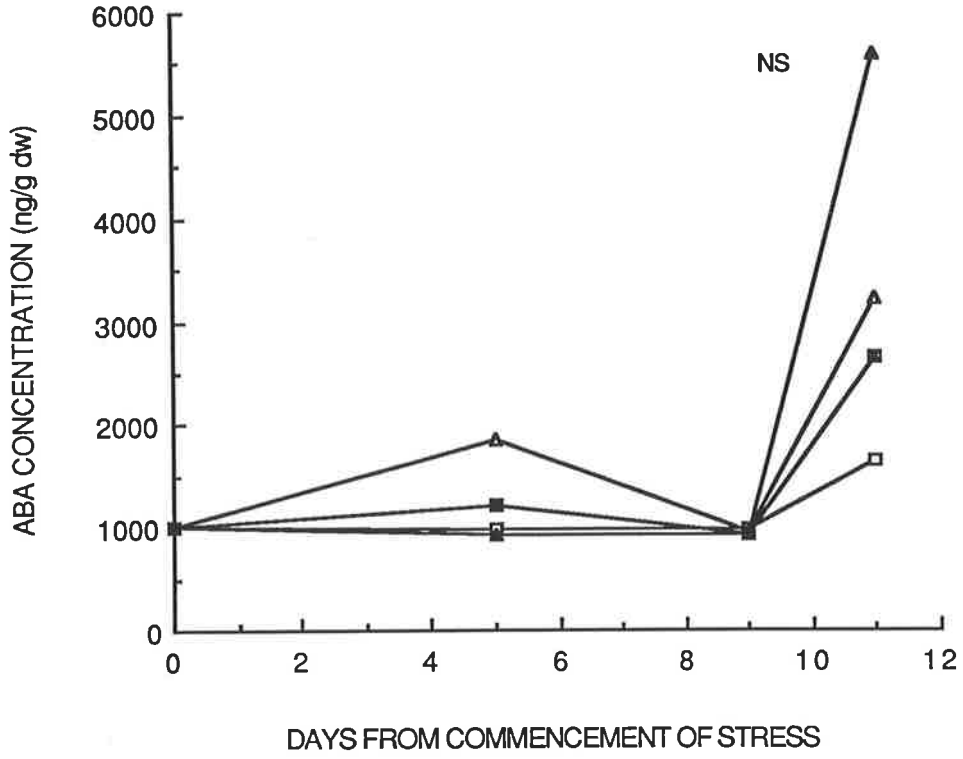
The trigonelline concentrations in control tomato plants at both humidities were similar (Fig. 91;Table 42) but increased significantly when plants were stressed at low humidity alone.

Fig. 87 Leaf ABA concentration (ng, g^{-1} dw) of NaCl stressed tomato at low (LH) and high (HH) humidities

- □ LH control
- ■ LH NaCl
- △ HH control
- ▲ HH NaCl

Fig. 88 Leaf ABA concentration (ng,g^{-1} dw) of NaCl stressed wheat at low (LH) and high (HH) humidities

- □ LH control
- ■ LH NaCl
- △ HH control
- ▲ HH NaCl


6000
5000
4000
3000
2000
1000
0
ABA CONCENTRATION (ng/g dw)
NS
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

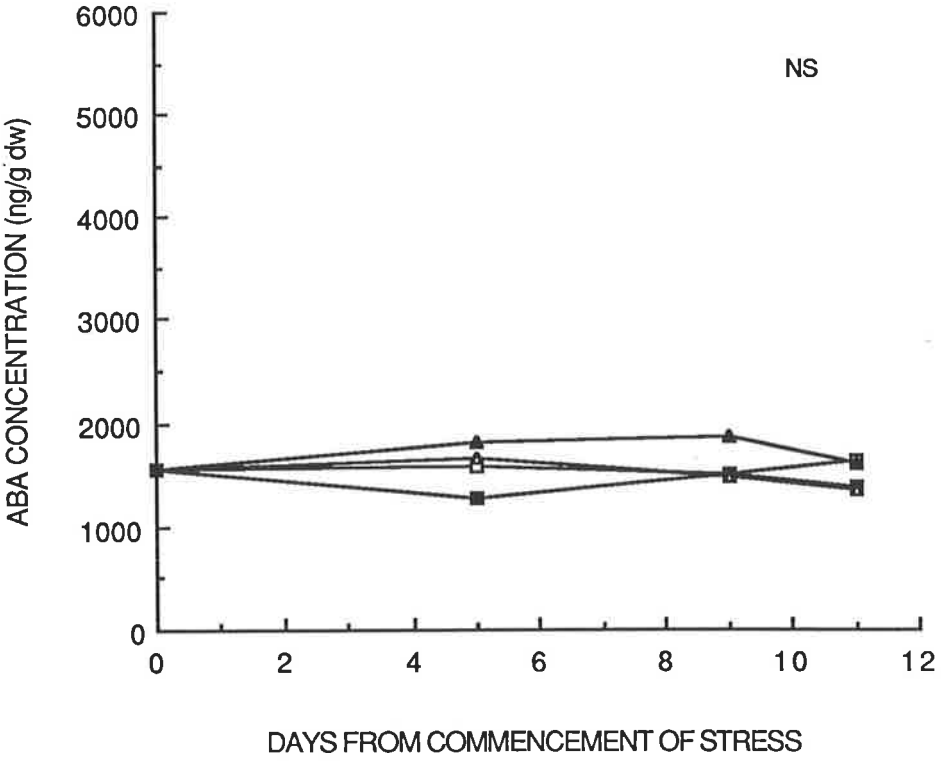

6000
5000
4000
3000
2000
1000
0
ABA CONCENTRATION (ng/g dw)
NS
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Table 41 Effect of NaCl on ABA concentration of wheat in different humidities (Other interactions were not significant) (ng,g^{-1} dw)

HUMIDITY	TREATMENTS			
	CONTROL	STRESS	MEAN	L.S.D(p=0.05)
LOW	1572	1385	1478	
HIGH	1497	1756	1626	177.95

Fig. 89 Log (e) leaf proline concentration (μg g^{-1} dw) of NaCl stressed tomato at low (LH) and high (HH) humidities

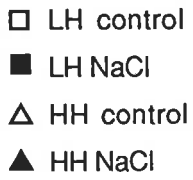
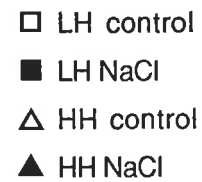
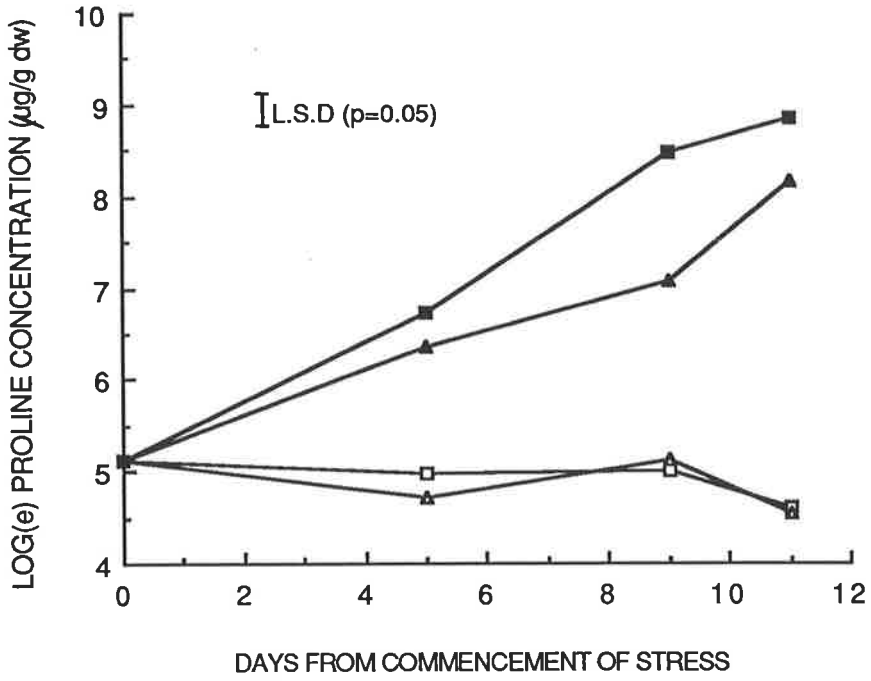


Fig. 90 Log (e) leaf proline concentration (μg,g^{-1} dw) of NaCl stressed wheat at low (LH) and high (HH) humidities




LOG(e) PROLINE CONCENTRATION (µg/g dw)
10
9
8
7
6
5
4
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

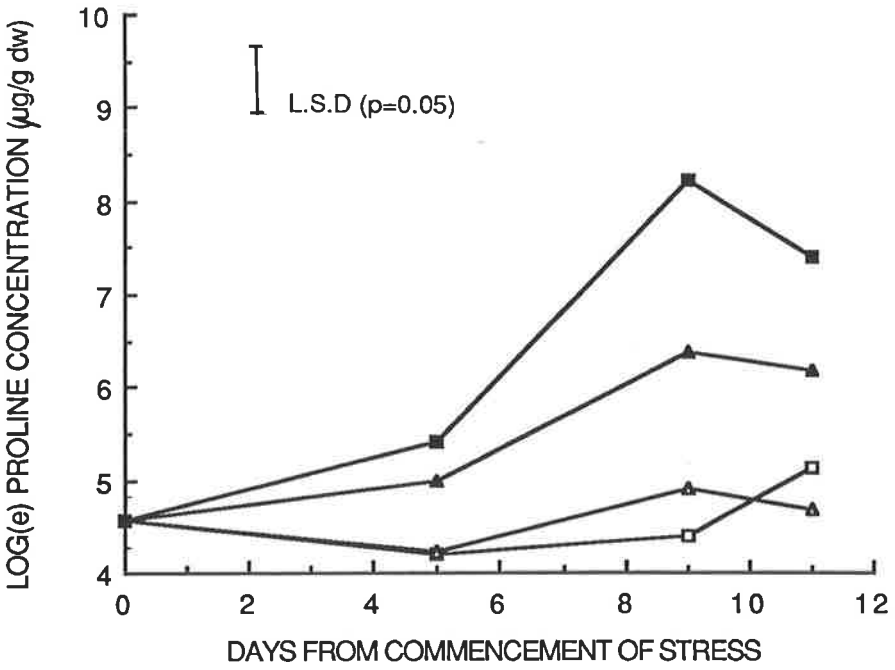
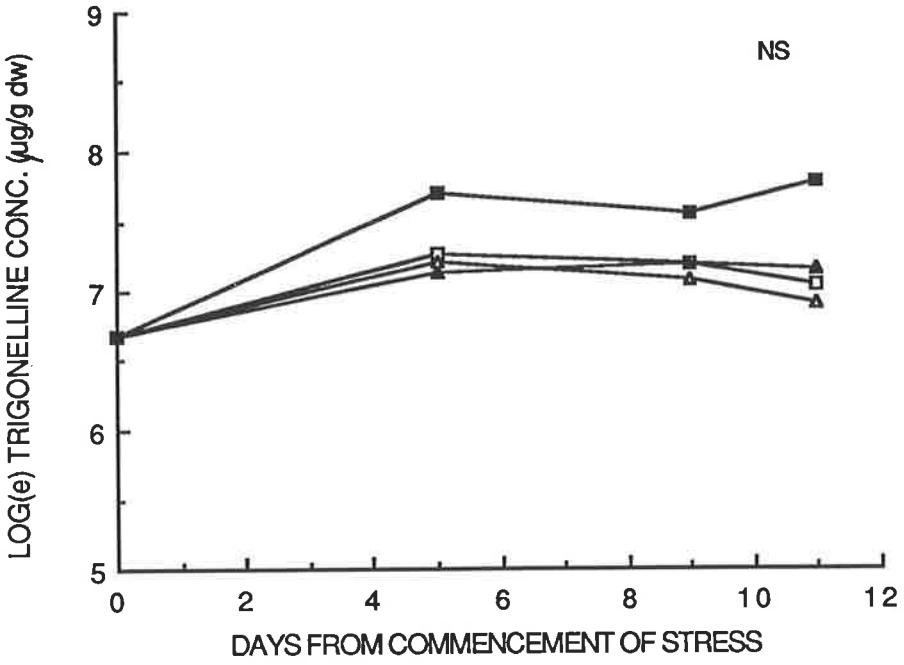

LOG(e) PROLINE CONCENTRATION (µg/g dw)
10
9
8
7
6
5
4
L.S.D (p=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Fig. 91 Log (e) leaf trigonelline concentration (μg g^{-1} dw) of NaCl stressed tomato at low (LH) and high (HH) humidities

□ LH. control
■ LH. NaCl
△ HH. control
▲ HH. NaCl

Fig. 92 Leaf glycinebetaine concentration (Log(e) μg g^{-1} dw) of NaCl stressed wheat at low (LH) and high (HH) humidities

□ LH control
■ LH NaCl
△ HH control
▲ HH NaCl


LOG(e) TRIGONELLINE CONC. (µg/g dw)
9
8
7
6
5
NS
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

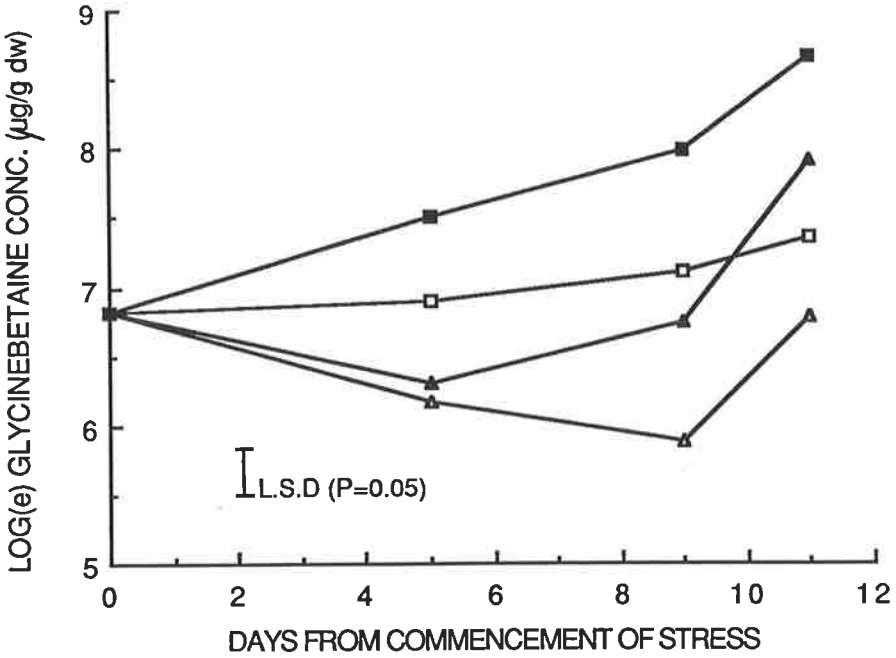

LOG(e) GLYCINEBETAINE CONC. (µg/g dw)
9
8
7
6
5
L.S.D (P=0.05)
0
2
4
6
8
10
12
DAYS FROM COMMENCEMENT OF STRESS

Table 42 Effect of NaCl on Log(e) trigonelline concentration of tomato in different humidities (Other interactions were not significant) ($\mu g, g^{-1}$ dw)

HUMIDITY	TREATMENTS			
	CONTROL	STRESS	MEAN	L.S.D(p=0.05)
LOW	7.136	7.623	7.380	
HIGH	7.048	7.146	7.097	
MEAN	7.093	7.384		0.477

4.5.3.3.2 Glycinebetaine concentration

The glycinebetaine concentration of the control wheat plants at high humidity decreased significantly by day 5, decreasing further to day 9,remaining below the levels of the controls in low humidity (Fig. 92). Glycinebetaine concentration increased significantly in response to salinity in both low (day 5) and high (day 9) humidity, however, the levels in plants in high humidity was below that of those in the controls in low humidity until day 9. Further increase to day 11, however, was significantly higher than the controls at the low humidity. These responses were consistent with those observed in section 4.3.5.

4.5.4 Discussion

NaCl in the rooting medium did not cause ABA accumulation either in tomato or in wheat supporting the observations of Mizrahi *et al.*, (1972) who found no increase in ABA in bean stressed for 12 days, and Walker and Dumbroff (1981) who found no correlation between endogenous ABA concentration and an increase in salt concentration in the rooting medium. It has been reported that ABA accumulates in water stressed plants only when the turgor potential of the tissues approaches zero (Pierce and Raschke, 1980) and it has been demonstrated (Creelman and Zeevaart, 1985) in spinach leaf tissues that loss of turgor is essential for ABA to accumulate. Maintenance of turgor in salt stressed tomato (Section 4.3.2) and wheat (Section 4.3.5) may be the cause for the lack of accumulation of ABA.

Proline accumulated in response to salt stress in both humidities as found in sections 4.3.2 and 4.3.5 despite the lack of increase in endogenous ABA, suggesting that ABA is not the causal factor in proline accumulation and that the variation in proline accumulation at different humidities is not due to variation in ABA concentration. Lack of correlation between endogenous ABA and proline accumulation supports the views of Stewart and Voetberg (1985).

As glycinebetaine and trigonelline accumulated without an increase in endogenous ABA,

ABA cannot be the triggering factor in their accumulation. These results concur with those of Mc Donnell *et al* (1983) who reported no increase in glycinebetaine concentration in response to exogenous ABA. Parameshwara (1984) reported a significant increase in both ABA and trigonelline concentration in salt-shocked alfalfa, but here trigonelline increased without ABA. ABA probably accumulated in response to turgor loss as there was no correlation between ABA and trigonelline concentration in alfalfa subjected to various environmental stresses.

SECTION 4.6 INTERSPECIFIC VARIATION IN ACCUMULATION OF PROLINE AND TRIGONELLINE AND MECHANISMS OF TOLERANCE OF *Lycopersicon* spp. TO SALT STRESS

4.6.1 Introduction

The *Lycopersicon* genus includes an array of species which have evolved in varying ecological habitats (Table 43) including saline-coastal areas. The physiological mechanisms of salt tolerance have been investigated in a few species (Tal, 1971, Tal and Shannon, 1983; Rush and Epstein, 1976), but there is a considerable difference of opinion on the relative tolerance of different species. Lyon (1941) reported that *L. pimpinellifolium* was more tolerant than *L. esculentum*, Tal and Shannon (1983) claimed *L. pennillii* to be the most tolerant of the species they tested whereas Rush and Epstein (1976) believed *L. cheesmanii* to be the most salt tolerant. Many species remain untested (Rick, personal communication) and no decisive conclusions can be drawn from the above studies as to relative tolerances as the workers adopted different experimental procedures. Relative tolerances of all the species have yet to be established using the same methodology.

Various mechanisms have been suggested to be involved in the ability of a plant to tolerate salt (see Greenway and Munns, 1980). Salt tolerance in *L. pennillii* has been attributed to the ability to osmoregulate (Tal, 1971; Tal and Shannon, 1983) and in *L. cheesmanii* to high Na^+ absoption (Rush and Epstein, 1976). Arguments on plant tolerance based on such mechanisms alone are highly contentious, as sensitive cultivars also osmoregulate and accumulate high concentrations of Na^+ (Greenway and Munns, 1980; Greenway, 1962 a). In this context, identifying a suitable physiological parameter which correlates with tolerance would be ideal. Proline accumulation when exposed to salinity is a general response of many plant species from various families (Aspinall and Paleg, 1981; Stewart and Lee, 1974) including plants from saline

Table 43 Distribution and salient features of selected wild *Lycopersicon* spp.

S.No.	Species	Distribution	Breeding system	Genetic variability	Ecological preferences
1.	*Lycopersicon cheesmanii* Riely	Endemic to Galapagos archipelago	Highly autogamous	Uniformity within populations; differences between populations	Sub-species minor prefers low elevations extends into saline littoral zone
2.	*L.chilense* Dun.	Southern Peru and northernmost Chile	Entirely self-incompatible	Relatively variable	Prefers very dry environments
3.	*L. chmielewskii*	Central Peru; inter-Andean region	Self-compatible	Moderately variable	Moist habitats although well-drained, rocky environments
4.	*L.esculentum* var. *cerasiforme*	Tropical regions; Native area: Ecuador/Peru	Wide range Self-pollination	Eastern Peru variable; other regions monomorphic	Wide range wet conditions. Weed in newly open ground
5.	*L. hirsutum* Humb. & Bonpl.	From central Peru to northern Ecuador, 500-3,300 m	Regional differences of self-pollination	Regional differences	Prefers moist well-drained areas; ascends to high altitudes
6.	*L. parviflorum*	Northern and central Peru; southern Ecuador; inter-Andean region	Highly autogamous	Relatively uniform	Well-drained rocky environments
7.	*L. pennillii* Corr.	Mid-elevations in western drainage central Peru	Self-incompatible; few exceptions	Highly variable	Adapted to xeric areas; subject to dew and fog

Table 43 continued

S.No.	Species	Distribution	Breeding system	Genetic variability	Ecological preferences
8.	*L. peruvianum* (L) Mill.	Distributed widely in Peru, extending into northern Chile	Strict self-incompatible	The most variable of *Lycopersicon* spp; many 'races'	
9.	*L. pimpinellifolium* (Jusl.) Mill.	Coastal Peru inter-Andean northern Peru Ecuador	Regional differences in self-pollination	Regional differences; correlation with self-pollination	Weed in cultivated fields of Peruvian coastal valleys. Dry inter-Andean washes

SOURCE: Esquinas-Aleazar, J.T.,(1981) Genetic resources of tomatoes and wild relatives. IBPGR, Rome. 1981.

habitats (Poljakoff-Mayber *et al.*, 1987). The increase in tissue proline concentration has been claimed to be correlated with the salt tolerance of both higher plants and algae (Levitt, 1972; Hsiao, 1973; Stewart and Lee, 1974; Stewart *et al.*, 1979; Cavalieri and Huang, 1979; Rajasekaran, 1979; Sampathkumar, 1980) but there is evidence against this proposal (Chu, 1974; Tal *et al.*, 1979). A correlation has also been found between salt tolerance and tissue glycinebetaine concentration (Storey and Wyn Jones, 1977; Wyn Jones and Storey, 1981), but there is no similar evidence for such a correlation for trigonelline, although it is found in many species from saline and arid-saline habitats (Wyn Jones and Storey, 1981; Blaim, 1962; Tramontano *et al.*, 1986).

Since the ability to accumulate such compounds under salt stress has survived through evolution it is reasonable to hypothesise that this response may have some role in plant tolerance and/ or adaptation to salinity. However, there is no conclusive evidence as to the mechanism by which the accumulation of these low molecular weight solutes may increase plant tolerance or resistance to salt stress. Two hypotheses have been put forward, either that these substances, especially proline and glycinebetaine, acts as compatible osmotica (Wyn Jones *et al.*, 1977; Borowitzka, 1981; Wyn Jones, 1984), or that these compounds have the additional function of protecting cellular enzymes and membrane proteins against dehydration and conformational changes (Paleg *et al.*, 1981; Ahmad *et al.*, 1982; Jolivet *et al.*, 1983; Paleg *et al.*, 1985). As accumulation of these compounds occurs is a result of enzymatic reactions which are gene controlled, if they are involved in salt tolerance, it may be possible to genetically engineer commercial species with enhanced salt tolerance.

This experiment planned to (1) evaluate the salt tolerance of a group of *Lycopersicon* spp. (2) investigate mechanisms involved in tolerance among these species and (3) study the relationship between accumulation of proline and quaternary ammonium compounds and the salt tolerance of these species.

4.6.2 Materials and Methods

Seedlings of *Lycopersicon cheesmanii*,Riely, *L. chilense*, *L. chmieleswskii*, *L.esculentum var cerasiforme*, *L. esculentum cv. Duke*, *L. hirsutum* Humb., *L. parviflorum*, *L. pennillii* Corr., *L. peruvianum*, (L) Mill., and *L. pimpinellifolium* (Jusl) were germinated as described in section 3.2.2 and grown at 20±1°C day and 17±1°C night temperature, with a 16 hour photoperiod and a photon flux density of 350-420 µEin sec^{-1} (Section 3.2.1), and supplied with Hoagland solution (Section 3.2.2) until 25 days after emergence. On the 25th day, stress was imposed with NaCl, dissolved in full strength Hoagland solution. The water potential of the rooting medium was decreased gradually by -0.10 MPa day^{-1} for 10 days. On the final day the plants were experiencing -1.065 MPa (221.4 mM). Salinization continued at this level for a further period of 20 days. The control plants received full strength Hoagland solution throughout. 55 days after emergence(31days from commencement of stress), the plants were sampled for water, osmotic and turgor potential of the expanded and expanding leaves (Section 3.2.6). Root, stem, expanded and expanding leaves and meristematic tissues were sampled separately for the measurement of proline (Section 3.2.9.2), quaternary ammonium compounds (Section 3.2.9.3) and ion concentrations (Section 3.2.11). Shoot and root dry weights were also measured (Section 3.2.4).

4.6.3 Results

4.6.3.1 Growth

4.6.3.1.1 Shoot dry weight

Significant variation in shoot growth was found between unstressed *Lycopersicon* spp. (Table 44). The commercial cultivar, *L. esculentum* cv. Duke and *L. chmieleswskii* were found to be the most vigorous and *L. hirsutum* the least. Salinity produced a significant decrease in shoot growth in all species except *L. cheesmanii*. The slow growing *L. hirsutum* experienced less reduction in shoot growth than all other species. In decreasing salt sensitivity, *L. pennillii* > *L. esculentum var ceresiforme* > *L.*

Table 44 Growth responses of *Lycopersicon* spp. under NaCl stress

S.No.	SPECIES	SHOOT DRY WEIGHT (g)		ROOT DRY WEIGHT (g)		TOTAL DRY WEIGHT (g)		SHOOT/ROOT RATIO	
		control	stress	control	stress	control	stress	control	stress
1.	*Lycopersicon esculentum* Mill. cv.Duke	12.21	4.66	1.49	0.56	13.71	5.26	8.13	8.26
2.	*L. cheesmanii* Riely	4.58	4.70	0.78	0.89	5.36	5.59	6.02	5.52
3.	*L. pennillii* Corr.	8.70	1.20	2.01	0.31	10.76	1.50	4.53	3.87
4.	*L. hirsutum* Humb.	1.41	0.97	0.68	0.38	2.10	1.34	2.25	2.99
5.	*L. parviflorum*	14.43	8.26	8.19	2.77	24.07	11.03	1.76	3.12
6.	*L.pimpinellifolium*(Jusl.)	6.18	4.21	2.11	1.11	8.29	5.32	3.01	4.60
7.	*L. peruvianum*(L.)Mill.	9.24	5.83	1.99	2.18	11.23	8.01	5.37	2.81
8.	*L. chmieleswski*	10.67	2.42	3.40	1.06	14.07	3.48	3.46	2.29
9.	*L. esculentum* var. *cerasiforme*	2.58	0.53	0.62	0.32	3.20	0.85	4.19	1.69
10.	*L. chilense* Dun.	5.48	2.80	2.58	1.56	8.06	4.36	2.17	1.95
	source of variation	**VR**	**L.S.D (p=0.05)**	**VR**	**L.S.D (p=0.05)**	**VR**	**L.S.D (p=0.05)**	**VR**	**L.S.D (p=0.05)**
	Species	19.26***	2.00	22.69***	0.99	48.85***	1.84	10.25***	1.66
	Treatment	81.34***	0.89	40.91***	0.44	131.37***	0.82	1.05ns	-
	Species* Treatment	4.96***	2.83	8.65***	1.40	17.41***	2.60	1.51ns	-

*** (p=0.01) NS = not significant

chmieleswskii > *L. esculentum* cv. Duke all experienced a more than 50% reduction in shoot growth whereas *L. chilense, L. peruvianum, L. pimpinellifolium* and *L. parviflorum* suffered less than 50% reduction.

4.6.3.1.2 Root dry weight

As with shoot growth, the root growth of the unstressed *Lycopersicon* species also varied significantly with *L. parviflorum* recording the maximum and *L.esculentum var cerasiforme* , the least (Table 44). Exposure to salinity significantly reduced root growth in all species except *L. cheesmanii* and *L. peruvianum* . As with shoot growth, root growth of *L. pennillii* was the most sensitive to salinity at only 15.4 per cent of control root weight.

4.6.3.1.4 Shoot : root ratio

The shoot/root ratios were not significantly influenced by salinity but there were considerable differences between species with *L. esculentum* cv. Duke highest and *L. chilense* least (Table 44). There were three distinct groups: Group 1: apportioning more dry matter to the root than to the shoot (*L. chilense, L.chmieleswskii, L. esculentum var cerasiforme, L. hirsutum, L. parviflorum, L. pimpinellifolium*) Group 2: apportioning dry matter equally to shoot and root (*L. cheesmanii, L. peruvianum, L. pennillii*) and Group 3: apportioning more dry matter to the shoot than the root (*L. esculentum* cv. Duke).

4.6.3.1.4 Salt tolerance Index

The *Lycopersicon* species exposed to salt were indexed for salt tolerance based on their total dry matter production in relation to control(Table 45). The most tolerant, *L. cheesmanii* , exhibited complete tolerance to this level of exposure to salt. In contrast, *L. pennillii* ranked last in the order with growth reduced by 86%. *L.chilense* > *L. hirsutum* > *L. pimpinellifolium* > *L. peruvianum* all experienced less than 50% growth reduction whereas, *L. parviflorum* < *L. esculentum* cv. Duke < *L. esculentum var cerasiforme* < *L. chmieleswskii* suffered a growth reduction of more than 50%.

Table 45 Tolerance of *Lycopersicon* spp. to salinity

S.No.	SPECIES	SALT TOLERANCE INDEX (% control)	RANK
1.	*Lycopersicon cheesmanii* Riely	104.3	1
2.	*L. peruvianum* (L.)Mill.	71.3	2
3.	*L. pimpinellifolium* (Jusl.)	64.2	3
4.	*L. hirsutum* (Humb.)	63.9	4
5.	*L. chilense* Dun.	56.1	5
6.	*L. parviflorum*	45.8	6
7.	*L. esculentum* Mill. cv.Duke	38.4	7
8.	*L. esculentum* var. *cerasiforme*	26.6	8
9.	*L. chmielewskii*	24.7	9
10.	*L. pennillii* Corr.	13.9	10

4.6.3.2 Water relations

The water potential of expanded (Table 46) and expanding leaves (Table 47) of unstressed control plants of the *Lycopersicon* species varied between 0.16 and 0.65 MPa. In all species the leaf water potential in salinized plants decreased significantly below $\psi_{ext.,}$ with *L. esculentum var cerasiforme* falling furthest and *L. cheesmanii* least.

As with water potential, the osmotic potential of both expanded and expanding leaves of these species differed markedly without stress, with *L. cheesmanii* recording the lowest and *L. pennillii* the highest. Variation was also evident in salinized plants, with *L. esculentum var cerasiforme* recording the lowest osmotic potential followed by *L. cheesmanii*. Although the osmotic potential of *L. esculentum var cerasiforme* fell furthest of all species, the water potential fell even further resulting in a loss of turgor in both expanded and expanding leaves. Variation in water and osmotic potential of the expanded and expanding leaves of these species was reflected in the derived turgor potential, with *L. cheesmanii* the highest and *L. esculentum var cerasiforme* the least. Apart from *L. chilense* , which experienced turgor loss in expanded leaves, but maintained turgor in the expanding leaves, and *L.esculentum var cerasiforme*, all species maintained turgor during salt stress.

4.6.3.4 Proline

Proline accumulated in response to salt stress in all organs of all *Lycopersicon* spp. (Table 48). Proline accumulation varied between 2.9 (*L. esculentum* cv. Duke) and 180.9 (*L. peruvianum*) fold. The response of individual tissues also differed markedly between species, *L. peruvianum* accumulated most in the root and meristematic tissues, *L. pimpinellifolium* and *L. hirsutum* in the stem and *L. esculentum var ceresiforme* in the expanding and expanded leaf tissues. The domestic cultivar*L. esculentum* cv. Duke accumulated the least in all organs. The variability in proline accumulation in these species in the absence of stress warranted development of a realistic measure to assess the capacity to accumulate proline. Hence the proline accumulation index (PAI) was

Table 46 Water relations of the expanded leaves of *Lycopersicon* spp. under NaCl stress

S.No.	SPECIES	WATER POTENTIAL (-MPa)		OSMOTIC POTENTIAL (-MPa)		TURGOR POTENTIAL (MPa)	
		control	stress	control	stress	control	stress
1.	*L. cheesmanii* Riely	0.38	1.06	1.35	1.98	0.99	0.92
2.	*L. chilense* Dun.	0.25	1.54	0.70	1.59	0.45	0.05
3.	*L. chmieleswskii*	0.57	1.12	0.77	1.61	0.20	0.49
4.	*L. esculentum* var. *cerasiforme*	0.65	2.53	0.91	2.41	0.26	-0.11
5.	*L. esculentum* Mill. cv.Duke	0.33	1.26	0.73	1.75	0.40	0.49
6.	*L. hirsutum* Humb.	0.58	1.82	0.66	2.02	0.20	0.19
7.	*L. parviflorum*	0.49	1.31	0.75	1.88	0.25	0.41
8.	*L. pennillii* Corr.	0.38	1.71	0.55	1.83	0.18	0.12
9.	*L. peruvianum*(L.)Mill.	0.25	1.13	0.59	1.36	0.35	0.24
10.	*L.pimpinellifolium*(Jusl.)	0.42	1.32	0.80	1.63	0.38	0.32

source of variation	**VR**	**L.S.D (p=0.05)**	**VR**	**L.S.D (p=0.05)**	**VR**	**L.S.D (p=0.05)**
Species	19.39***	0.17	9.875***	0.20	28.712***	0.13
Treatment	737.34***	0.08	546.163***	0.01	0.063ns	---
Species x Treatment	10.22***	0.25	4.298***	0.28	5.576***	0.18

*** (p=0.01) NS = not significant

Table 47 Water relations of the expanding leaves of *Lycopersicon* spp. under NaCl stress

S.No.	SPECIES	WATER POTENTIAL (-MPa)		OSMOTIC POTENTIAL (-MPa)		TURGOR POTENTIAL (MPa)	
		control	stress	control	stress	control	stress
1.	*L. cheesmanii* Riley	0.16	0.82	1.40	2.08	1.23	1.26
2.	*L. chilense* Dun.	0.42	1.15	0.79	1.41	0.37	0.27
3.	*L. chmieleswskii*	0.50	1.03	0.82	1.32	0.32	0.30
4.	*L. esculentum* var. *cerasiforme*	0.46	2.87	0.69	2.46	0.23	-0.41
5.	*L.esculentum* Mill. cv.Duke	0.31	1.39	0.71	1.82	0.40	0.42
6.	*L. hirsutum* Humb.	0.47	1.27	0.52	1.47	0.05	0.22
7.	*L. parviflorum*	0.41	1.14	0.62	1.35	0.22	0.22
8.	*L. pennillii* Corr.	0.46	1.36	0.51	1.52	0.06	0.16
9.	*L. peruvianum*(L.)Mill.	0.27	0.95	0.58	1.26	0.31	0.30
10.	*L.pimpinellifolium*(Jusl.)	0.39	1.14	0.62	1.54	0.24	0.42

source of variation	**VR**	**L.S.D (p=0.05)**	**VR**	**L.S.D (p=0.05)**	**VR**	**L.S.D (p=0.05)**
Species	26.78***	0.17	15.47***	0.20	75.82***	0.12
Treatment	594.16***	0.08	417.60***	0.09	1.16ns	- - - -
Species x Treatment	20.38***	0.24	6.75***	0.28	8.29***	0.16

Table 48 Proline concentration of various organs of *Lycopersicon* spp. under NaCl stress (mg,g^{-1} dw)

SPECIES	ROOT		STEM		EXD.LEAF		EXG.LEAF		MERISTEM	
	control	stress	control	stress	control	stress	control	stress	control	stress
Lycopersicon cheesmanii Riely	0.22	8.30	1.87	18.17	0.72	10.81	0.29	16.59	0.75	18.23
L. chilense Dun.	0.20	6.95	0.56	13.70	0.17	19.59	1.69	30.38	2.05	23.94
L. chmieleswskii	0.12	7.99	1.04	9.87	0.60	7.91	1.16	9.25	2.95	17.61
L. esculentum var. *cerasiforme*	0.29	1.85	0.19	9.97	0.70	25.41	0.88	31.48	0.70	34.97
L. esculentum Mill. cv.Duke	0.25	0.72	0.23	1.65	0.10	6.99	0.08	4.47	0.11	2.86
L. hirsutum Humb.	0.23	8.57	0.96	14.89	1.01	16.47	0.91	16.80	0.97	35.47
L. parviflorum	0.10	8.88	1.92	10.32	0.28	10.02	0.62	13.87	4.58	20.71
L. pennillii Corr.	0.03	10.61	0.06	17.47	0.12	8.65	0.22	12.49	0.61	21.12
L. peruvianum(L.)Mill.	0.07	12.66	11.82	13.82	2.85	12.48	2.48	14.94	4.21	24.33
L.pimpinellifolium(Jusl.)	0.15	10.50	0.96	21.15	1.28	14.54	1.22	19.55	2.39	24.88

source of variation	VR	L.S.D (p=0.05)
Species	58.84***	0.994
Treatment	3426.03***	0.445
Species x Treatment	50.45***	1.403
Parts	111.43***	0.782

source of variation (contd.)	VR	L.S.D (p=0.05)
species x parts	10.00***	2.43
treatment x parts	76.61***	1.08
species x treatment x parts	9.73***	3.43

*** (p=0.01)

calculated as the ratio of concentration in control and stressed tissues (Table 49). PAI also demonstrated considerable differences in proline accumulating ability between species, in general salt sensitive species had higher PAI than tolerant species. PAI also differed among organs, with *L. pinnellii* recording the highest for stem and root tissues, *L. chilense* for the expanded leaf, *L. esculentum* cv. Duke for the expanding leaves and *L. hirsutum* the meristematic tissues.

4.6.3.5 Trigonelline

The quaternary ammonium compound, trigonelline was present in all *Lycopersicon* species tested (Table 50). In the unstressed plants of most species, the roots contained the lowest concentration and the apical meristem had the highest concentration. The trigonelline concentration in the expanding and meristematic tissues of control plants varied significantly between species. Subjecting these species to stress increased the trigonelline concentration in all except *L. chemeleswskii* and *L. pennillii.* However, the response differed between different organs of these species. Root tissues responded in *L. cheesmanii*; stem tissues in *L. esculentum* cv. Duke, *L. hirsutum* and *L. peruvianum*; expanded leaf tissues in *L. esculentum var cerasiforme*, cv.Duke, and *L. peruvianum* ; expanding leaf in *L. cheesmanii, L. chilense,* and *L. esculentum* cv. Duke, *L. peruvianum* and meristematic tissues in *L. cheesmanii, L. chilense L. parviflorum,* and *L. pimpinellifolium.* The salt tolerant species, *L. cheesmanii* accumulated the highest amount of trigonelline in the root and meristematic tissues and the salt sensitive *L. pennillii* did not respond to salt stress at all. The accumulation index for trigonelline(TAI) was high in the root and expanding leaf tissues of *L. cheesmanii* (Table 51).

4.6.3.6 Sodium concentration

There was some variation between species in Na^+ concentration in unstressed plants but there was a significant increase in all organs of all species when they were subjected to salinity (Table 52). In most species, the highest Na^+ concentration was in root or stem tissues and the lowest in meristematic tissues. *L. pennillii* accumulated

Table 49 Proline Accumulation Index(PAI) of various organs of *Lycopersicon* spp. under NaCl stress (data obtained from table 48)

SPECIES	ROOT	STEM	EXD.LEAF	EXG.LEAF	MERISTEMATIC TISSUE
Lycopersicon cheesmanii Riely	37.72	9.72	15.01	57.21	24.31
L. chilense Dun.	34.75	24.11	115.93	17.93	11.65
L. chmieleswskii	66.58	9.51	13.16	7.95	5.99
L. esculentum var. *cerasiforme*	6.38	51.48	36.09	35.71	49.76
L. esculentum Mill. cv.Duke	2.84	6.88	69.87	58.01	25.31
L. hirsutum Humb.	37.59	15.49	16.31	18.40	36.65
L. parviflorum	88.89	5.38	22.67	22.56	4.52
L. pennillii Corr.	353.67	277.30	73.93	56.76	34.62
L. peruvianum(L.)Mill.	180.86	1.17	36.16	5.63	5.78
L.pimpinellifolium(Jusl.)	70.00	22.03	11.39	16.07	10.42

Table 50 Trigonelline concentration of various organs of *Lycopersicon* spp. under NaCl stress (mg, g^{-1} dw)

SPECIES	ROOT		STEM		EXD.LEAF		EXG.LEAF		MERISTEM	
	control	stress	control	stress	control	stress	control	stress	control	stress
Lycopersicon cheesmanii Riely	0.33	2.74	1.53	1.57	1.63	2.41	2.22	5.66	2.61	6.55
L. chilense Dun.	ND	2.63	2.08	2.51	4.99	5.63	3.99	8.14	5.35	9.40
L. chmieleswskii	ND	ND	1.59	1.31	1.43	2.11	1.98	2.73	4.89	4.24
L. esculentum var. *cerasiforme*	ND	ND	0.90	ND	2.58	4.52	3.46	6.02	4.38	5.59
L. esculentum Mill. cv.Duke	0.89	1.81	0.98	3.27	1.13	2.59	0.80	1.13	1.49	1.70
L. hirsutum Humb.	ND	ND	1.41	4.54	1.25	1.02	1.56	2.28	1.93	4.38
L. parviflorum	ND	ND	0.93	ND	1.27	2.35	1.47	3.08	2.23	6.69
L. pennillii Corr.	ND	1.88	ND	ND	2.01	ND	ND	ND	2.82	ND
L. peruvianum(L.)Mill.	ND	ND	1.58	7.00	1.79	6.42	3.26	4.39	3.36	6.27
L.pimpinellifolium(Jusl.)	ND	1.63	1.58	2.80	1.74	3.01	2.29	1.92	2.19	5.96

source of variation	**VR**	**L.S.D (p=0.05)**
Species	76.06***	0.331
Treatment	291.00***	0.148
Species x Treatment	18.69***	0.469
Parts	114.59***	0.335

source of variation (contd.)	**VR**	**L.S.D (p=0.05)**
species x parts	8.12***	1.01
treatment x parts	2.930**	0.45
species x treatment x parts	4.620***	1.42

Table 51 Trigonelline Accumulation Index(TAI) of various organs of *Lycopersicon* spp. under NaCl stress (data obtained from table 50)

SPECIES	ROOT	STEM	EXD.LEAF	EXG.LEAF	MERISTEMATIC TISSUE
Lycopersicon cheesmanii Riely	8.28	1.03	1.48	2.55	2.51
L. chilense Dun.	-	1.20	1.25	2.04	1.76
L. chmieleswskii	-	0.82	1.48	1.38	0.87
L. esculentum var. *cerasiforme*	-	-	1.75	1.74	1.28
L. esculentum Mill. cv.Duke	2.03	3.34	2.29	1.41	1.14
L. hirsutum Humb.	-	3.22	0.82	1.46	2.27
L. parviflorum	-	-	1.85	2.10	3.00
L. pennillii Corr.	-	-	-	-	-
L. peruvianum(L.)Mill.	-	4.43	3.59	1.35	1.87
L.pimpinellifolium(Jusl.)	-	1.77	1.73	0.84	2.72

Table 52 Sodium concentration of various organs of *Lycopersicon* spp. under NaCl stress (mmole,g^{-1} dw)

SPECIES	ROOT		STEM		EXD.LEAF		EXG.LEAF		MERISTEM	
	control	stress	control	stress	control	stress	control	stress	control	stress
Lycopersicon cheesmanii Riely	0.070	0.842	0.131	1.433	0.040	0.889	0.037	0.603	0.032	0.360
L. chilense Dun.	0.022	1.101	0.007	1.063	0.016	0.878	0.006	0.236	0.005	0.152
L. chmieleswskii	0.034	1.178	0.005	0.145	0.008	0.913	0.005	0.503	0.004	0.044
L. esculentum var. *cerasiforme*	0.217	0.332	0.028	2.148	0.014	1.479	0.097	1.327	0.004	0.678
L. esculentum Mill. cv.Duke	0.045	1.333	0.005	1.633	0.009	0.540	0.002	0.638	0.002	0.620
L. hirsutum Humb.	0.016	2.430	0.010	3.137	0.088	2.000	0.115	0.897	0.129	0.723
L. parviflorum	0.353	2.590	0.024	0.643	0.006	0.449	0.045	0.257	0.069	0.165
L. pennillii Corr.	0.129	1.553	0.085	4.250	0.014	3.137	0.035	2.710	0.067	1.477
L. peruvianum(L.)Mill.	0.075	0.815	0.043	0.530	0.027	0.510	0.009	0.171	0.008	0.603
L.pimpinellifolium(Jusl.)	0.220	1.507	0.177	1.833	0.100	2.470	0.243	1.383	0.205	0.300

source of variation	VR	L.S.D (p=0.05)
Species	125.42***	0.085
Treatment	3119.54***	0.038
Species x Treatment	109.82***	0.121
Parts	75.03***	0.075

source of variation (contd.)	VR	L.S.D (p=0.05)
species x parts	13.13***	0.228
treatment x parts	71.67***	0.102
species x treatment x parts	12.67***	0.322

most Na^+in all organs and *L. chmieleswskii* the least in stem and meristematic tissues, *L. parviflorum* in the expanded leaf and *L. peruvianum* in the expanding leaf tissues. When Na^+concentration is calculated on tissue water content, shoot Na^+ concentration was also highest in *L. pennillii* and lowest in *L. cheesmanii* (Table 53). In root tissues, however, *L. parviflorum* accumulated the highest Na^+ concentration and *L. esculentum var cerasiforme* the least. The sodium concentration of stressed tissues relative to control concentration (sodium regulation index, SRI), shows that these species have varying abilities to regulate tissue Na^+ concentration in different tissues (Table 54), *L. pimpinellifolium* being the best regulator in all except root tissues, whereas *L. esculentum* cv. Duke (expanded leaf and meristematic tissues), *L. hirsutum and L. pimpinellifolium* (root and expanded leaf tissues) the worst.

4.3.6.7 Potassium concentration

Salinity affected organ K^+ concentration differently in different species (Table 55). K^+ concentration was reduced in the roots of *L parviflorum* and increases in the stem and expanded leaf tissues. This response was reversed in *L. pennillii* . K^+ concentration in various organs was also reduced in *L. hirsutum, L. pimpinellifolium* (stem); *L. esculentum var cerasiforme, L.pennillii* (expanded leaf); *L. chilense, L.esculentum* cv. Duke and *L. peruvianum* (meristem) K^+ concentration expressed on tissue water content showed no significant change in response to salinity in the root tissues of any species but there were significant changes in shoot K^+ concentration with reductions in *L. esculentum var cerasiforme* and *L. pimpinellifolium* and accumulation in *L. parvifloum* (Table 56). The salt tolerant *L. cheesmanii* suffered no change in K^+ concentration in any organ.

4.6.3.8 Chloride concentration

A significant increase in tissue Cl^- concentration was observed in all species subjected to salinity but the response of different tissues differed (Table 57). In most species, stem tissues accumulated most $Cl^-$and least was accumulated in the meristematic tissues. *L. esculentum var cerasiforme* accumulated most in expanded, expanding and

Table 53 Shoot and root sodium concentration of *Lycopersicon* spp. under NaCl stress (mM)

SPECIES	SHOOT		ROOT	
	CONTROL	STRESS	CONTROL	STRESS
Lycopersicon cheesmanii Riely	3.00	51.80	12.90	148.20
L. chilense Dun	1.00	74.90	2.00	142.40
L. chmieleswskii	1.10	66.80	5.80	138.20
L.esculentum Var. *cerasiforme*	4.00	105.50	24.10	59.50
L. esculentum Mill.cv.Duke	0.40	100.20	4.70	113.70
L. hirsutum Humb.	9.70	213.00	1.40	175.30
L. parviflorum	7.10	76.90	92.40	226.30
L.pennillii Corr.	4.90	398.90	26.80	114.60
L. peruvianum(L) Mill.	3.20	75.70	7.20	122.60
L. pimpinellifolium (Jusl.)	40.60	310.50	28.00	170.00

SOURCE OF VARIATION	**VR**	**L.S.D** (p=0.05)	**VR**	**L.S.D** (p=0.05)
Species	7.10***	68.01	5.08***	40.22
Treatments	86.42***	30.42	183.67***	17.99
Species x treatments	5.70***	96.18	1.79ns	--

*** (p=0.01) NS = not significant

Table 54 Sodium Regulation Index(SRI) of various organs of *Lycopersicon* spp. under NaCl stress (data obtained from table 52)

SPECIES	ROOT	STEM	EXD.LEAF	EXG.LEAF	MERISTEMATIC TISSUE
Lycopersicon cheesmanii Riely	12.03	10.94	22.25	16.39	11.25
L. chilense Dun.	50.05	149.72	54.50	39.25	18.76
L. chmieleswskii	34.67	30.15	139.68	100.60	12.11
L. esculentum var. *cerasiforme*	1.53	76.73	102.71	13.68	169.50
L. esculentum Mill. cv.Duke	29.62	326.60	60.00	319.00	310.00
L. hirsutum Humb.	151.88	313.70	22.72	7.80	5.61
L. parviflorum	7.34	26.79	74.83	5.71	2.39
L. pennillii Corr.	12.04	50.00	224.07	77.43	22.05
L. peruvianum(L.)Mill.	10.86	12.34	19.25	20.00	75.38
L.pimpinellifolium(Jusl.)	6.85	10.36	13.83	5.70	1.46

Table 55 Potassium concentration of various organs of *Lycopersicon* spp. under NaCl stress (mmole, $g^{-1}dw$)

SPECIES	ROOT		STEM		EXD.LEAF		EXG.LEAF		MERISTEM	
	control	stress	control	stress	control	stress	control	stress	control	stress
Lycopersicon cheesmanii Riely	0.943	1.307	1.560	1.247	0.733	0.730	0.817	0.707	0.820	0.517
L. chilense Dun.	0.152	0.416	0.993	0.811	0.319	0.239	0.302	0.218	1.391	0.418
L. chmieleswskii	0.396	0.298	0.583	0.736	0.224	0.341	0.171	0.272	0.181	0.151
L. esculentum var. *cerasiforme*	0.363	0.275	1.412	1.415	0.752	0.163	0.342	0.150	0.168	0.107
L. esculentum Mill. cv.Duke	0.407	0.483	1.573	0.807	0.654	0.957	0.333	0.453	0.237	0.617
L. hirsutum Humb.	0.550	0.256	1.170	0.687	0.623	0.357	0.530	0.313	0.450	0.447
L. parviflorum	0.951	0.461	0.397	1.510	0.360	0.993	0.500	0.579	0.708	0.390
L. pennillii Corr.	0.600	1.064	1.413	0.843	0.669	0.283	0.272	0.447	0.955	0.647
L. peruvianum(L.)Mill.	0.459	0.277	0.378	0.178	0.349	0.264	0.343	0.241	0.433	0.057
L.pimpinellifolium(Jusl.)	0.299	0.487	0.613	0.257	0.410	0.250	0.450	0.247	0.507	0.337

source of variation	VR	L.S.D (p=0.05)
Species	12.216***	0.155
Treatment	6.480***	0.069
Species x Treatment	1.610ns	- - - -
Parts	58.97***	0.077

source of variation (contd.)	VR	L.S.D (p=0.05)
species x parts	5.223***	0.267
treatment x parts	2.984***	0.120
species x treatment x parts	4.284***	0.378

*** (p=0.01) NS = not significant

Table 56 Shoot and root potassium concentration of *Lycopersicon* spp. under NaCl stress (mM)

SPECIES	SHOOT		ROOT	
	CONTROL	STRESS	CONTROL	STRESS
Lycopersicon cheesmanii Reily	49.20	50.30	90.60	123.50
L. chilense Dun	76.80	54.10	13.60	53.80
L. chmieleswskii	58.00	60.60	63.10	35.00
L.esculentum Var. *cerasiforme*	76.10	34.30	41.00	49.40
L. esculentum Mill.cv.Duke	66.60	87.40	42.10	41.10
L. hirsutum Humb.	79.90	53.60	46.60	18.90
L. parviflorum	96.30	173.80	97.20	39.60
L.pennillii Corr.	86.00	79.30	128.30	75.30
L. peruvianum(L) Mill.	55.80	27.10	42.60	40.70
L. pimpinellifolium (Jusl.)	98.90	57.00	37.90	54.80

SOURCE OF VARIATION	**VR**	**L.S.D** (p=0.05)	**VR**	**L.S.D** (p=0.05)
Species	8.39***	25.65	3.28**	42.58
Treatments	1.35ns	--	0.57ns	--
Species x treatments	3.99**	36.28	1.28ns	--

*** (p=0.01)
** (p=0.1)
NS = not significant

Table 57 Chloride concentration of various organs of *Lycopersicon* spp. under NaCl stress (mmole, $g^{-1}dw$)

SPECIES	ROOT		STEM		EXD.LEAF		EXG.LEAF		MERISTEM	
	control	stress	control	stress	control	stress	control	stress	control	stress
Lycopersicon cheesmanii Riely	0.129	0.594	0.146	0.640	0.046	0.685	0.047	0.284	0.073	0.153
L. chilense Dun.	0.232	0.654	0.028	1.324	0.059	0.098	0.023	0.755	0.032	0.458
L. chmieleswskii	0.009	0.585	0.039	0.790	0.042	1.205	0.051	0.850	0.045	0.160
L. esculentum var. *cerasiforme*	0.051	0.735	0.046	1.708	0.032	1.951	0.060	1.789	0.083	1.493
L. esculentum Mill. cv.Duke	0.014	0.628	0.012	1.196	0.003	0.226	0.004	0.338	0.004	0.336
L. hirsutum Humb.	0.046	0.673	0.010	1.276	0.018	0.871	0.035	0.426	0.035	0.357
L. parviflorum	0.600	0.683	0.006	1.030	0.010	0.597	0.003	0.290	0.043	0.150
L. pennillii Corr.	0.007	0.857	0.009	1.956	0.003	1.005	0.002	0.902	0.018	0.548
L. peruvianum(L.)Mill.	0.070	0.805	0.080	0.886	0.044	0.699	0.063	0.490	0.048	0.847
L.pimpinellifolium(Jusl.)	0.023	0.697	0.003	0.673	0.010	0.910	0.009	0.740	0.030	0.200

source of variation	VR	L.S.D (p=0.05)
Species	37.227***	0.069
Treatment	2025.528***	0.114
Species x Treatment	41.731***	0.098
Parts	67.39***	0.042

source of variation (contd.)	VR	L.S.D (p=0.05)
species x parts	11.278***	0.137
treatment x parts	74.902***	0.061
species x treatment x parts	9.955***	0.193

*** (p=0.01)

meristematic tissues, followed by *L. pennillii* which accumulated most Cl^- in the root and shoot. *L. cheesmanii* had the lowest chloride concentration in nearly all tissues. Similarly, the shoot chloride concentration expressed on a tissue water content basis revealed *L. pennillii* accumulated the highest amount and the least was accumulated by *L. cheesmanii* whereas root chloride concentration was highest in *L. esculentum var cerasiforme* and low concentrations were found in *L. hirsutum, L. cheesmanii, L. esculentum,* and *L parviflorum* (Table 58). The chloride concentration in different tissues in relation to the control, (chloride regulation index, CRI) showed considerable differences between organs and species (Table 59). The salt sensitive *L. pennillii* had the least regulation (high value) in all but the meristem (*L. esculentum* cv. Duke) whereas the most regulation (lowest CRI) was recorded for *L. cheesmanii* in stem, expanding and meristematic tissues. The salt tolerant *L. cheesmanii* excludes Cl^- from nearly all organs whereas most of the salt sensitive species allow Cl^- to enter.

4.6.3.9 K^+/Na^+ ratio

There were significant differences in K^+/Na^+ ratio between different organs of *Lycopersicon* spp. (Table 60). Most salt sensitive species had lower K^+/Na^+ ratios than the salt tolerant species, *L. cheesmanii.* Root tissues (*L. chemieleswskii, L. esculentum* cv. Duke, *L. hirsutum, L. parviflorum* and *L. peruvianum*) and expanded leaves (*L. chilense, L. esculentum var cerasiforme, L. hirsutum, L. peruvianum, L. pimpinellifolium*) experienced low K^+/ Na^+ ratios.

4.6.3.10 Cl^-/Na^+ ratio

The Cl^-/Na^+ ratio of salinity-stressed plants also differed greatly between species (Table 61). There were no significant differences in Cl^-/Na^+ between tissues within species however, except *L. chilense* (expanding leaf and meristematic tissues), *L. chmeleswskii* (stem and meristem) and *L. esculentum var cerasiforme* (root and meristem). *L. peruvianum* maintained the ratio closest to 1 in all the tissues studied.

Table 58 Shoot and root chloride concentration of *Lycopersicon* spp. under NaCl stress (mM)

SPECIES	SHOOT		ROOT	
	CONTROL	STRESS	CONTROL	STRESS
Lycopersicon cheesmanii Reily	3.90	27.70	12.90	56.40
L. chilense Dun	4.30	84.90	12.90	84.60
L. chmieleswskii	9.00	120.60	1.40	69.80
L.esculentum Var. *cerasiforme*	6.20	129.20	5.40	119.90
L. esculentum Mill.cv.Duke	0.60	69.50	1.50	53.50
L. hirsutum Humb.	2.80	90.90	3.90	49.30
L. parviflorum	3.00	115.50	14.32	58.90
L.pennillii Corr.	0.80	154.40	1.10	62.70
L. peruvianum(L) Mill.	10.00	112.40	7.30	120.80
L. pimpinellifolium (Jusl.)	2.60	131.80	2.90	78.10
SOURCE OF VARIATION	**VR**	**L.S.D (p=0.05)**	**VR**	**L.S.D (p=0.05)**
Species	2.79**	31.61	4.74***	30.34
Treatments	201.94***	14.13	69.97***	13.56
Species x treatments	2.69**	44.68	6.78***	42.91

*** (p=0.01)
** (p=0.1)

Table 59 Chloride Regulation Index(CRI) of various organs of *Lycopersicon* spp.under NaCl stress (data obtained from table 57)

SPECIES	ROOT	STEM	EXD.LEAF	EXG.LEAf	MERISTEMATIC TISSUE
L. cheesmanii Reily	4.61	4.39	14.79	6.08	2.10
L. chilense Dun.	2.80	47.32	1.66	32.13	14.36
L. chmieleswskii	65.00	20.26	28.69	16.67	3.56
L. esculentum var. *cerasiforme*	14.27	36.73	60.97	147.08	17.49
L. esculentum Mill. cv.Duke	43.31	98.01	75.33	78.61	78.21
L. hirsutum Humb.	41.31	128.86	47.61	12.06	10.11
L. parviflorum	1.14	171.67	59.67	85.88	3.46
L. pennillii Corr.	125.98	205.89	372.33	563.56	30.42
L. peruvianum(L.)Mill.	11.45	11.08	16.07	7.78	17.92
L.pimpinellifolium(Jusl.)	29.90	224.43	88.37	85.06	6.67

Table 60 K:Na ratio of the various organs of NaCl stressed *Lycopersicon* spp.

SPECIES	ROOT	STEM	EXD.LEAF	EXG.LEAF	MERISTEMATIC TISSUE
Lycopersicon cheesmanii Reily	1.55	0.87	0.85	1.17	1.45
L. chilense Dun.	0.38	0.79	0.28	0.96	3.19
L. chmieleswskii	0.25	8.24	0.32	0.54	3.50
L. esculentum var. *cerasiforme*	0.83	0.78	0.11	0.11	0.16
L. esculentum Mill. cv.Duke	0.37	0.50	1.76	0.73	1.14
L. hirsutum Humb.	0.11	0.26	0.17	0.38	0.62
L. parviflorum	0.18	2.39	2.47	3.25	2.38
L. pennillii Corr.	0.86	0.20	0.09	0.17	0.44
L. peruvianum(L.)Mill.	0.34	0.42	0.49	1.41	0.10
L.pimpinellifolium(Jusl.)	0.31	0.14	0.10	0.18	1.20

source of variation	**VR**	**L.S.D.(p=0.05)**
species	6.609***	0.85
parts	3.904**	0.60
species x parts	3.266***	1.91

*** (p=0.01) ** (p=0.1)

Table 61 Cl:Na ratio of various organs of NaCl stressed *Lycopersicon* spp.

SPECIES	ROOT	STEM	EXD.LEAF	EXG.LEAF	MERISTEMATIC TISSUE
Lycopersicon cheesmanii Reily	0.71	0.45	0.81	0.47	0.43
L. chilense Dun.	0.59	1.27	0.11	3.17	3.08
L. chmieleswskii	0.50	5.45	1.08	1.84	3.66
L. esculentum var. *cerasiforme*	2.13	0.85	1.32	1.35	2.14
L. esculentum Mill. cv.Duke	0.47	0.73	0.42	0.52	0.52
L. hirsutum Humb.	0.28	0.43	0.43	0.47	0.50
L. parviflorum	0.26	1.65	1.49	1.39	0.91
L. pennillii Corr.	0.55	0.46	0.32	0.33	0.37
L. peruvianum(L.)Mill.	0.99	1.79	1.42	2.87	1.41
L.pimpinellifolium(Jusl.)	0.53	0.37	0.36	0.54	0.71

source of variation	**VR**	**L.S.D.(p=0.05)**
species	21.44***	0.64
parts	10.80***	0.45
species x parts	10.05***	1.44

*** (p=0.01)

4.6.4 Discussion

Growth of these species in saline conditions in relation to that of control plants (Salt Tolerance Index) has been taken as the criterion of salt tolerance. On this basis *L. cheesmanii*, Riely, a coastal ecotype is the most tolerant and *L. pennillii*, adapted to xeric areas, the least tolerant to salt. *L. peruvianum* and *L. pimpinellifolium* ranked next to *L. cheesmanii*. The commercial cultivar *L. esculentum* cv. Duke,ranked 7th in the order. These results support the views of Rush and Epstein (1976)and contradict the claims of Tal (1971) and Tal and Shannon (1983) who reported that *Solanum pennillii* (presently classified as *L. pennillii*, Rick, personal communication) was a tolerant species. The relative salt sensitivity of *L. pennillii* is also apparent in the reports of Tal and Shannon (1983) as it experienced a greater reduction in elongation rate than did *L. cheesmanii* when exposed to salinity. Nevertheless, *L. pennillii* has continued to be claimed to be tolerant (Tal, 1971; Talkeisnik-Gertel *et al.*, 1983). It is possible that this is due to the experiments being conducted at a lower concentration range for a relatively shorter period of time (14 days), or to the seeds of *L. pennillii* used in the present study and in that of Tal (1971) coming from different seed batches although originating from the same source. *L. pennillii* is a self-incompatible species (Table 43) so variation in genotype between seed lots is not unexpected.

Several mechanisms seem possible for the enhanced salt tolerance of *L. cheesmanii* in contrast to *L. pennillii* and other salt sensitive species. Hayward and Wadleigh (1949) maintain that salinity tolerance depends on the ability of the plants to (1) compensate for the increase in osmotic potential of the rooting medium by increasing internal osmotic potential, (2) avoid excessive accumulation of ions, which is achieved by regulating their uptake and distribution and (3) prevent deleterious effects of the accumulated ions in the cytoplasm. Ability to decrease the internal osmotic potential alone is not sufficient to attain salt tolerance (Table 46 and 47), ψ_s fell in all species with no correlation between ψ_s of expanded or expanding leaves and salt tolerance. In fact, *L. esculentum var cerasiforme*, in which ψ_s fell furtherest suffered the most

growth reduction. Bernstein and Hayward (1958) and Greenway and Munns (1980) are of a similar view and salt sensitive varieties of grape, wheat and cotton have been found to maintain a higher osmotic potential than salt tolerant varieties (Greenway,1962).

Turgor or cell volume maintenance, especially in expanding leaf tissues, has been suggested to be essential for sustained growth and development, however (Greenway and Munns, 1980; Munns and Termaat, 1986; Barlow, 1986) and a significant correlation between turgor potential of expanding tissues and salt tolerance index has been found in the present study (Fig. 93). The salt tolerant *L. cheesmanii* regulated turgor effectively and efficiently without significant compromise to the decreased leaf water potential. In contrast, in *L. esculentum var cerasiforme*, although ψ_s fell further, there was little control of decreasing ψ_{leaf} , resulting in loss of turgor and growth.

Organic and inorganic solutes constitute a proportion of the osmotic adjustment leading to turgor maintenance. Such a metabolic response has been found to be correlated with the salt tolerance of halophytes and has been suggested as a marker for establishing tests for salt tolerance (Stewart and Lee, 1974). In cultivars of tomato (Rajasekaran, 1979) and bhendi, *Abelmoschus esculentus* (Sampathkumar, 1980), similar correlations were found. Here, however, no such correlation existed for any tissue, although the proline concentration in *L. cheesmanii* was higher than that in the commercial cultivar *L. esculentum* cv. Duke. No general correlation was found between the salt tolerance index and proline concentration or proline accumulation index among the species in the present experiments. However, (1) these species may have different mechanisms to tolerate salt. Both salt includers such as *L. pennillii, L. esculentum var cerasifome, L. parviflorum* and salt excluders such as *L. cheesmanii, L. hirsutum, L. chilense, L. peruvianum* accumulated proline, presumably helping to combat the deleterious effects of salt or perhaps balancing the cell osmotic potential (2) proline biosythesis and metabolism has been found to be influenced by many factors: (A) differences in photosynthetic leaf area, as observed among these species

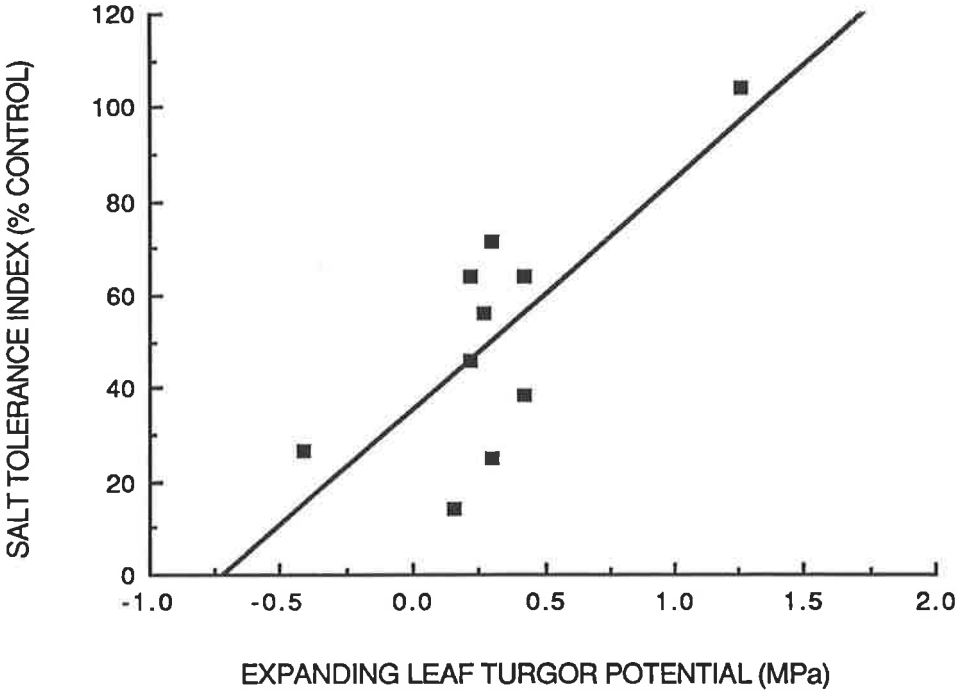
Fig. 93 Relationship between expanding leaf turgor potential and salt tolerance of *Lycopersicon* spp. (Data obtained from Table 45 and 47)

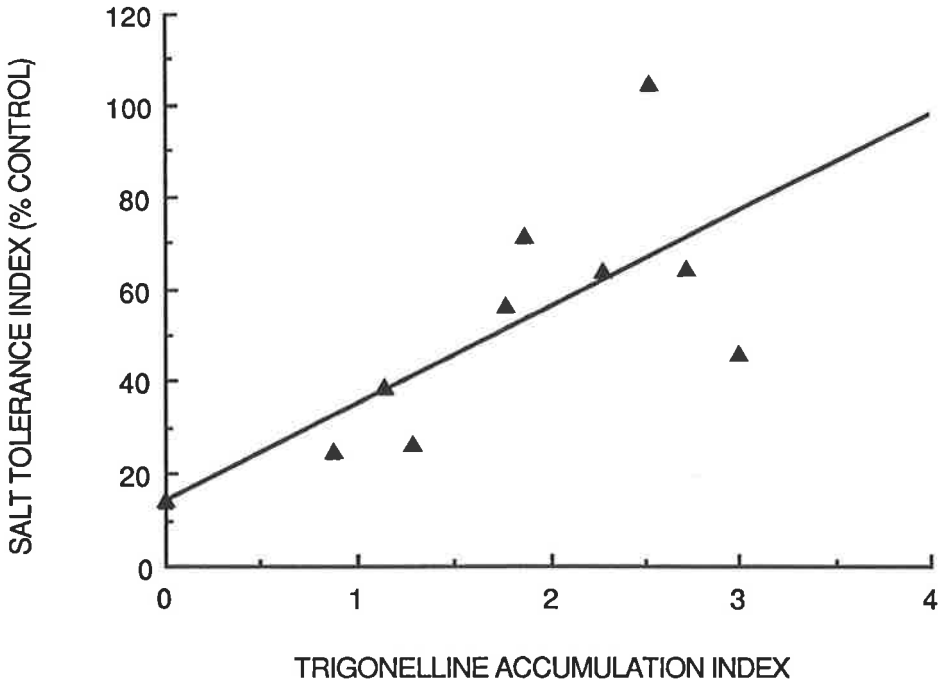
Y= 35.3939 + 49.258x R=0.75*

Fig. 94 Relationship between trigonelline accumulation in the meristematic tissues and salt tolerance of *Lycopersicon* spp. (Data obtained from Table 45 and 51)

Y= 14.4592 + 20.9304x R= 0.73*

** R values significant at 5%


SALT TOLERANCE INDEX (% CONTROL)
120
100
80
60
40
20
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
EXPANDING LEAF TURGOR POTENTIAL (MPa)


SALT TOLERANCE INDEX (% CONTROL)
120
100
80
60
40
20
0
0
1
2
3
4
TRIGONELLINE ACCUMULATION INDEX

could vary the amount of precursors synthesised (Aiyar, 1981),(B). variation in root growth could influence the amount of causal factor synthesised and translocated (Section 4.3;4.4). (C). differing levels of Na^+ in the leaf tissues in these species, could also vary proline accumulation (Secion 4.4).

Trigonelline, a QAC, was found in these *Lycopersicon* species and accumulated with salinity stress, as measured by the Trigonelline Accumulation Index (TAI). There were considerable differences in the ability of these species to accumulate trigonelline under stress and the ability to accumulate trigonelline in the meristematic tissues was significantly correlated with the salt tolerance of these species (Fig. 94). Although trigonelline has been found in many plant species of arid and arid-saline habitats (Wyn Jones and Storey, 1981; Blaim, 1962; Tramontano *et al* ., 1986) no relationship with salt tolerance has been reported previously. The physiological significance, if any of this relationship is unknown, but it is possible that trigonelline may protect the meristematic tissues from salt-induced perturbations. It is also of interest to note that *L. cheesmanii* accumulated the highest amount of trigonelline in the root tissues. Evans and Tramontano (1981) postulated a hormonal role for trigonelline, promoting cell arrest in G2 of the cell cycle. Karstein (1966) showed that trigonelline acts in a similar manner to cytokinins and it is possible that the trigonelline accumulated in root and apical meristems may have such functions.

A major proportion of turgor maintenance is achieved by the uptake of ions, however, increased uptake of ions does not necessarily lead to tolerance. As seen from Tables 53&58, *L. pennillii*, although accumulating Na^+ and Cl^- in the shoot and regulating turgor, still suffered growth reduction when exposed to salinity. A key factor may be a synchronization of ion compartmentation in the leaf cells with the rate of ion transport to the shoot, which *L. pennillii* and other salt sensitive ecotypes presumably lack. Salt tolerant *L. cheesmanii* accumulated less Na^+ and Cl^- in the shoot than *L. pennillii* and *L. esculentum*. Rush and Epstein (1981) reported that *L. cheesmanii* tolerates salinity through a greater Na^+ uptake than *L. esculentum.* This may be true when the concentrations are expressed on a dry weight basis, but there is a considerable

difference in the succulence of these species, with *L. cheesmanii* recording the highest succulence. This would dilute the ion concentration and may be the mechanism by which *L. cheesmanii* tolerates an apparent high internal ion concentration. An increase in succulence is a halophytic adaptation (Luttge and Smith, 1984), but there was no correlation between absolute Na^+ or Cl^- concentration in the various tissues and salt tolerance of these species.

Regulation of the ion contents is generally understood to be important (Rains, 1972) but it is difficult to measure the relative contribution of this property to mesophytic salt tolerance. For this purpose, the regulation indexes (SRI,CRI) are used here. Such an index is distinctive for a component mechanism involved in salt tolerance and is useful with any ion regardless of whether there is an increase or decrease. The regulation indices rely on data from plant responses to control and salinity treatments and not upon comparisons with other plant species or the overall range found in the plant kingdom. This dimensionless index may be legitimately compared/correlated across species if used within a given range of salinities. Comparing salt tolerance (as measured by the salt tolerance index) with SRI and CRI of various organs produced significant correlations for SRI of the expanded leaf tissues (Fig. 95) and for CRI of root tissues (Fig. 96). The salt tolerance of *L. cheesmanii* may be due to its ability to regulate ions in these organs as successful regulation is evidence of homeostatic control in the face of differing environmental conditions. Ion homeostatis has been found necessary for salt tolerance (Sacher *et al.*, 1981; Greenway, 1962a; Gates *et al.*, 1970) and regulation of Na^+ in expanded leaf tissue may be important for sustained photosynthetic activity as high levels of Na^+ have been shown to inhibit various aspects of the photosynthetic process (Seeman and Critchley, 1985; Section 4.7). Similarly, Cl^- regulation in root tissues may be an important mechanism in achieving salt tolerance. Most salt sensitive legumes exclude Cl^- from the leaves and accumulate large amounts in the roots (Lauchli, 1984). Cl^- regulation at the root level may be associated with sustained root growth and the supply of nutrients and root-derived factors such as cytokinins to the shoot (Itai *et al.*, 1968; Walker and Dumbroff,

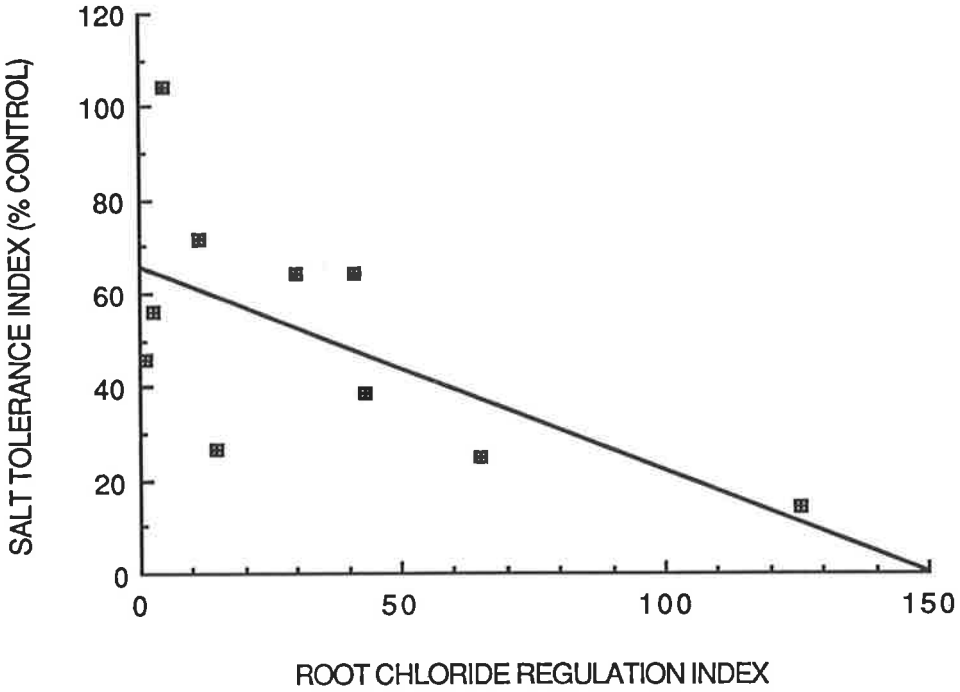
Fig. 95 Relationship between sodium regulation in the expanded leaf and salt tolerance of *Lycopersicon* spp. (Data obtained from Tables 45 and 54)

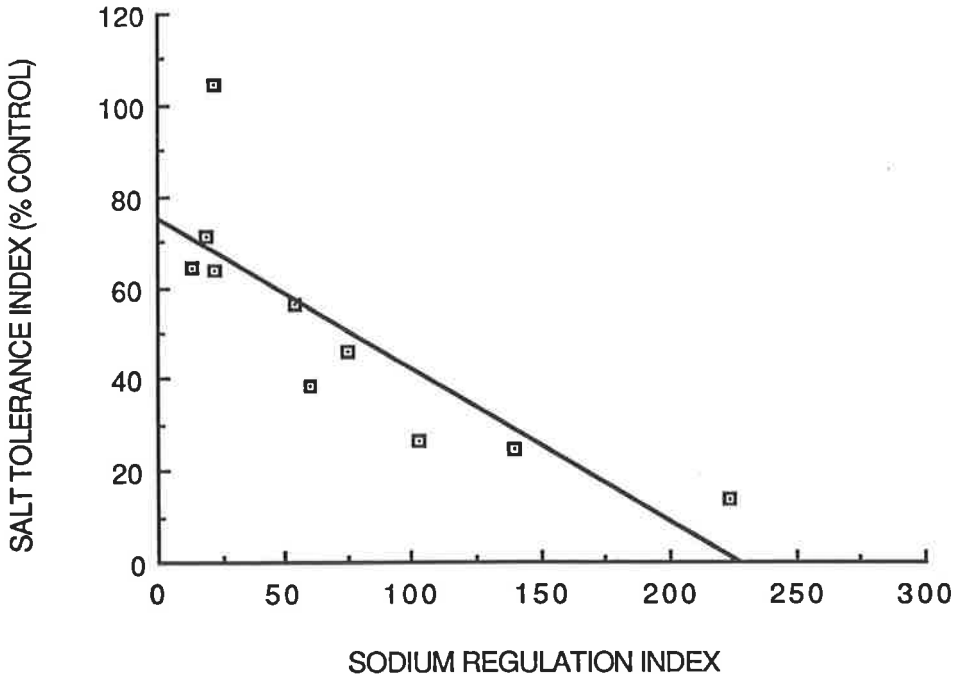
Y= 75.1158 - 0.3297x R=0.82***

Fig. 96 Relationship between chloride regulation in the root tissues and salt tolerance of *Lycopersicon* spp. (Data obtained from Tables 45 and 59)

Y= 65.714 - 0.4354x R=0.63*

*, *** R values significant at 5 and 0.1% respectively


SALT TOLERANCE INDEX (% CONTROL)
120
100
80
60
40
20
0
0
50
100
150
ROOT CHLORIDE REGULATION INDEX


SALT TOLERANCE INDEX (% CONTROL)
120
100
80
60
40
20
0
0
50
100
150
200
250
300
SODIUM REGULATION INDEX

1981). Hence, salt tolerance in *L. cheesmanii* may be due to sustained root growth achieved by the regulation of Cl^- at the root level.

The K^+/Na^+ ratio (Pitman, 1984; Jeschke, 1984) and the Cl^-/Na^+ ratio (Greenway and Munns, 1980) were suggested to be important in determining selectivity and electroneutrality in the cells. The importance of these ratios in relation to salt tolerance has been reviewed by Rains (1972), Pitman (1984) and Jeschke (1984). In the present study, although, no significant overall correlation between K^+/Na^+ ratio and salt tolerance of these species was found, the salt tolerant *L. cheesmanii* maintained the ratio closer to 1in expanded and expanding tissues and >1 in root and meristematic tissues, whereas *L. pennillii* recorded the lowest ratio and lacked K^+ selectivity. This differs from the report by Rush and Epstein (1981) of a low ratio for *L. cheesmanii*, and their suggestion that *L. cheesmanii* substitutes Na^+ for K^+. This discrepancy may be due to the short term (30 min.) nature of their experiment with plants subjected only to a concentration of 50 mM NaCl.

Data on Cl^-/Na^+ shows that most species suffered from disturbances in electroneutrality including *L. cheesmanii* but the effect may not have been large enough to cause growth reduction. Moreover, the zwitterions, such as proline, trigonelline and other amino acids and organic acids contribute to the electroneutrality of the cells and may account for the lack of correlation between the Cl^-/Na^+ ratio and salt tolerance.

In conclusion, *L. cheesmanii* is the most tolerant species to salt. The mechanisms by which it attains this capability include (1) maintaining high turgor in the expanding tissues (2) higher trigonelline accumulation in root tissues and apical meristem (3) effective regulation of Na^+ in the expanded leaves and Cl^- in root tissues.

SECTION 4.7 PHYSIOLOGICAL SIGNIFICANCE OF PROLINE AND GLYCINEBETAINE: MAINTAINING PHOTOSYNTHESIS DURING NaCl STRESS IN WHEAT

4.7.1 Introduction

Proline and glycinebetaine accumulate in wheat in response to salt stress and also in halophytes (Stewart and Lee, 1974; Coughlan and Wyn Jones, 1980; Wyn Jones and Storey, 1981). They have been postulated to act in osmoregulation (Stewart and Lee, 1974; Storey and Wyn Jones, 1977; Jagels, 1983; Jolivet *et al.*, 1983; Grumet and Hanson, 1986) in protecting proteins against biologically unfavourable consequences of dehydration -induced thermodynamic perturbation (Nash *et al.*, 1982; Paleg *et al.*, 1984) and in protecting enzyme activity (Pollard and Wyn Jones, 1979). Evidence from the present study indicates that proline and glycinebetaine may have roles additional to osmoregulation. A major proportion of the accumulated proline and glycinebetaine is found in chloroplasts (Noguchi *et al.*, 1966; Stewart and Lee, 1974; Hanson *et al.*, 1985; Robinson and Jones, 1986) suggesting that these compounds may have specific role(s) in this organelle. An *in vivo* role for proline and glycinebetaine has yet to be established for salt-stressed glycophytes. The experiments in this section were planned to explore the possible role(s) of these compounds in sustaining photosynthesis in wheat during NaCl stress, *in vivo* .

4.7.2 Methods

Seedlings of wheat cv. Sun-9-E were grown in a glass house at 20°C as described in sections 3.2.1and 3.2.2 for 70 days and supplied with full strength Hoagland nutrient solution. CO_2 gas exchange measurements were made on the flag leaf on the 71st day. Measurements were started usually at 09:00 h and continued until 17:00 h. NaCl (0, 100, 150 or 200 mM) was fed to the penultimate flag leaf through the cut end of the

leaf tip and the leaf was equlibrated in the gas exchange chamber for 30 min. (Section 3.2.5.5) before the gas exchange measurements commenced.

In experiments to investigate the effects of solutes on photosynthesis, solutes proline[20mM], glycinebetaine [5, 10mM], or 3-O-methyl glucopyranose[5mM] were pre-fed to the penultimate flag leaf through the cut end of the leaf tip which were then equilibrated in the gas exchange chamber for 30 min. After this period, these solutions were replaced with 200 mM NaCl and the leaf tips were again equilibrated for a further period of 30 min. before the gas exchange measurements began. The distal end of the leaf tip remained in salt solution until the measurements were completed. Assimilation (A) and evaporation rate, leaf temperature, conductance and intercellular CO_2 partial pressure (Pi) were measured simultaneously (section 3.2.5.4) over a range of external CO_2 partial pressures (Pa). These measurements were made on a single leaf for each treatment.

4.7.3 The effect of a range of NaCl concentrations on photosynthesis

4.7.3.1 Results

4.7.3.1.1 Stomatal limitations

Increases in the NaCl concentration in the solution bathing the cut end of the leaf tip resulted in a decline in stomatal conductance (expressed as H_2O conductance),compared to the control (Fig. 97; Table 62). However, the intercellular CO2 concentration (Pi) at ambient CO_2 concentration did not show any decline with stomatal conductance except at 200mM concentration. In the leaf fed with 200 mM NaCl the value of Pa/Pi declined from 0.77 to 0.69 at ambient CO_2 concentration, indicating that stomatal limitation of photosynthesis increased due to NaCl treatment to 9.9 per cent (Table 62). Parallel to this response, the evaporation rate declined accompanied by an increase in leaf temperature (Table 62).

Fig. 97 Effect of a range of NaCl concentrations on stomatal conductance (mol m^{-2} sec^{-1}) of wheat flag leaf at varying intercellular CO_2 concentrations

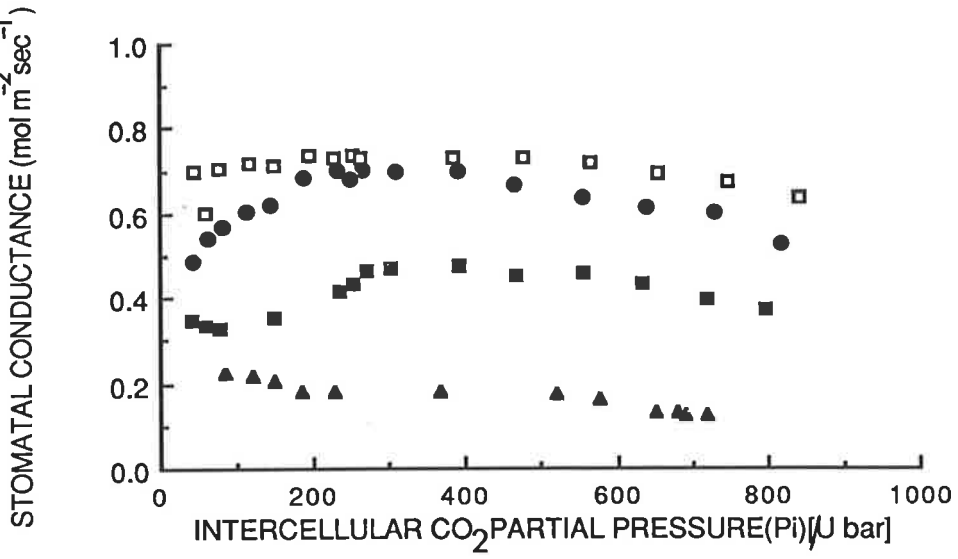
□ Control
● NaCl (100 mM)
■ NaCl (150 mM)
▲ NaCl (200 mM)

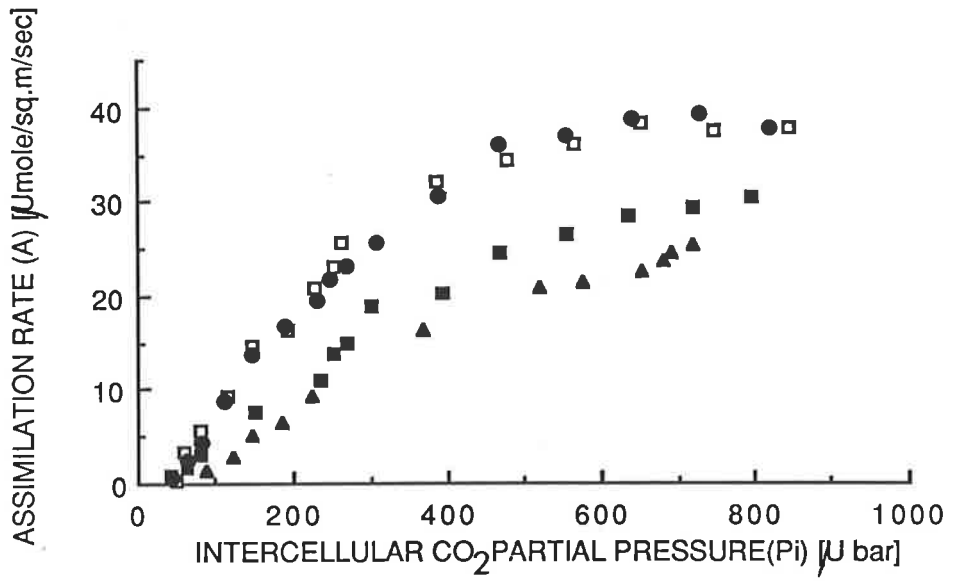
Fig. 98 Effect of a range of NaCl concentrations on assimilation rate (μmole m^{-2} sec^{-1}) of wheat flag leaf at varying intercellular CO_2 concentration

□ Control
● NaCl (100 mM)
■ NaCl (150 mM)
▲ NaCl (200 mM)

Fig. 98A Relationship between intercellular CO_2 partial pressure and assimilation rate of wheat flag leaf fed with a range of NaCl concentrations (Data obtained from the linear portions of A/Pi curve of Fig. 98)

□ Control
● NaCl (100 mM)
◢ NaCl (150 mM)
▲ NaCl (200 mM)
*** R value significant at 0.1%


STOMATAL CONDUCTANCE (mol m^{-2} sec^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
INTERCELLULAR CO_2 PARTIAL PRESSURE(Pi)[U bar]


ASSIMILATION RATE (A) [Umole/sq.m/sec]
40
30
20
10
0
0
200
400
600
800
1000
INTERCELLULAR CO_2 PARTIAL PRESSURE(Pi) [U bar]

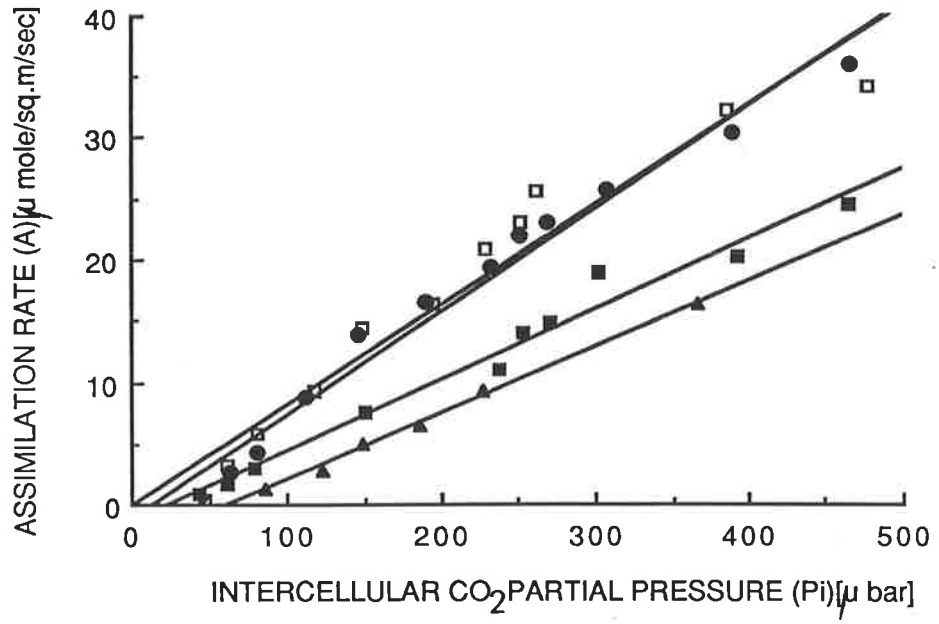

ASSIMILATION RATE (A)[μ mole/sq.m/sec]
40
30
20
10
0
0
100
200
300
400
500
INTERCELLULAR CO_2 PARTIAL PRESSURE (Pi)[μ bar]

Table 62 The effects of range of NaCl concentrations on various gas exchange parameters at ambient CO_2 partial pressure (350 µbar) (data obtained from Figs. 96 and 97)

TREATMENTS	Assimilation (µmole m^{-2} sec^{-1})	Stomatal conductance (mole m^{-2} sec^{-1})	Pa/Pi	Leaf temp. (°C)	Evaporation (mole m^{-2} sec^{-1})
CONTROL	23.0	0.737	0.77	23.1	6.18
NaCl (100mM)	23.0	0.705	0.78	23.2	6.05
NaCl (150mM)	14.8	0.465	0.78	23.9	4.50
NaCl (200mM)	9.2	0.178	0.69	25.6	2.68

Table 63 Test of significance of slopes of the regression between intercellular CO_2 partial pressure and assimilation rates (Data obtained from Fig. 97)

TREATMENT	REGRESSION EQUATION	R
T1 CONTROL	Y= 0.1018+0.0818X	0.97***
T2 NaCl(100mM)	Y=-0.9927+0.0842X	0.99***
T3 NaCl(150mM)	Y=-1.3441+0.0576X	0.99***
T4 NaCl(200mM)	Y=-3.3321+0.0539X	1.00***

Standard deviation(SD)	0.0158
Standard error of mean difference of slopes	0.0079
L.S.D ($t_{0.05,n-4}$)	0.0161

T4 T3 T1 T2

*** R values significant at .0.01%

4.7.3.1.2. Capacity and efficiency

To determine the effect of salt on the intrinsic photosynthetic capacity (A) of the leaf to varying intercellular CO_2, CO_2 partial pressure (Pi) was measured for the leaf exposed to varying NaCl concentrations. Fig. 98 illustrates an A:Pi curve for control and NaCl treated leaves. The arrows indicate the ambient (Pa 350 µbar) operating Pi for the leaves. The initial slope of the response Pi< 50 µbar has been used as a measure of the RuBP-carboxylase-limited CO_2 fixation capacity of the leaves (Von Caemmerer and Farquhar, 1981; Seeman and Berry, 1982). Determination of initial slope permits an assessment of photosynthetic capacity independent of stomatal aperture, since calculation of Pi includes differences in stomatal conductance. An increase in NaCl concentration above 100 mM resulted in a decline in assimilation rate at an ambient CO_2 partial pressure of 350 µbar (Table 62). The reduction being 35.7 (150) and 60 (200 mM) per cent. Furthermore, NaCl inhibited CO_2-limited (initial slope of A:Pi curve)rate of photosynthesis. Test of significance of the slopes of the regressions of A:Pi at the linear region of the curve (Fig. 98A; Table 63) showed a significant difference in the slopes between control and NaCl(150; 200mM) treated leaves indicating that NaCl influenced RuBP Carboxylase activity significantly. NaCl also influenced the CO_2-saturated (upper portion of A:Pi curve) rates of photosynthesis (Fig. 98) at 150 and 200 mM concentration.

4.7.4 The effects of pre-treatment with proline on NaCl-inhibited photosynthesis

4.7.4.1 Results

4.7.4.1.1. Stomatal limitations

Stomatal conductance of control leaves was about 0.74 µmole m^{-2} sec^{-1} and was greatly reduced on exposure to NaCl (0.18 µmole m^{-2} sec^{-1}). Pre-treatment with proline alleviated the NaCl induced effect to a small extent only(17 per cent at ambient Pa) [Fig.99].

Fig. 99 Effect of proline pretreatment on stomatal conductance (mol m^{-2} sec^{-1}) of NaCl treated wheat flag leaf at varying intercellular CO_2 concentrations

□ Control
■ [Water]-NaCl
▲ [Proline]-NaCl

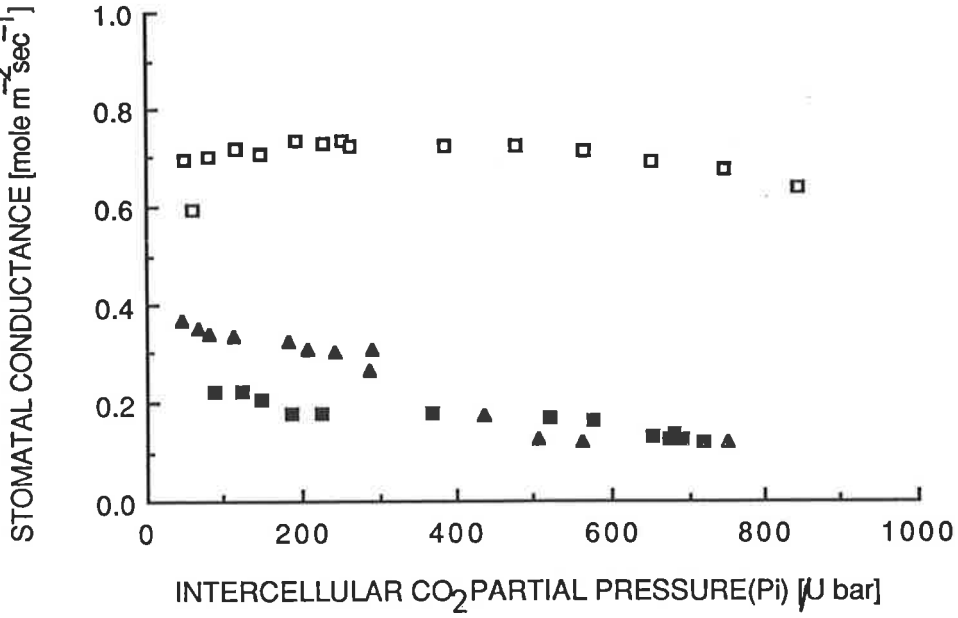
Fig. 100 Effect of proline pretreatment on assimilation rate (μmole m^{-2} sec^{-1}) of NaCl treated wheat flag leaf at varying intercellular CO_2 concentration

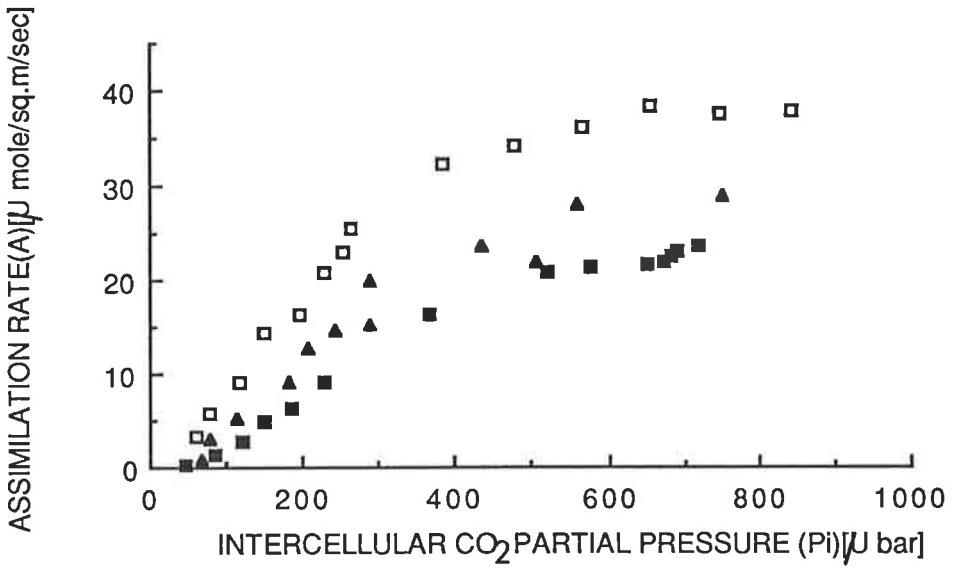
□ Control
■ [water]-NaCl
▲ [Proline]-NaCl

Fig. 100A Relationship between intercellular CO_2 partial pressures and assimilation rates of proline pretreated and non-pretreated, NaCl fed wheat flag leaves (Data obtained from the linear portions of A/Pi curve of Fig. 100)

□ Control
■ [Water]-NaCl
▲ [Proline]-NaCl

*** R value significant at 0.1%


STOMATAL CONDUCTANCE [mole m^{-2}sec^{-1}]
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
INTERCELLULAR CO_2 PARTIAL PRESSURE(Pi) [μ bar]


ASSIMILATION RATE(A)[μ mole/sq.m/sec]
40
30
20
10
0
0
200
400
600
800
1000
INTERCELLULAR CO_2 PARTIAL PRESSURE (Pi)[μ bar]

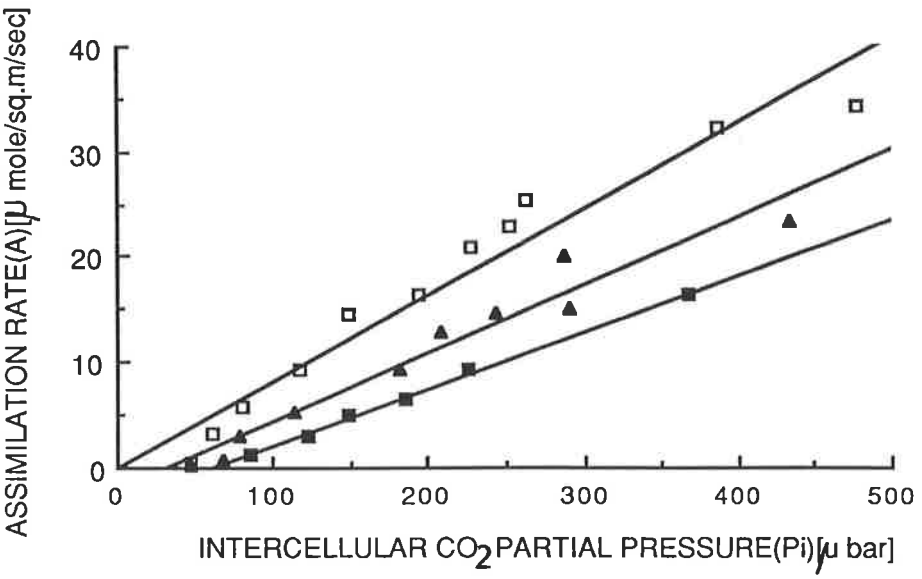

ASSIMILATION RATE(A)[μ mole/sq.m/sec]
40
30
20
10
0
0
100
200
300
400
500
INTERCELLULAR CO_2 PARTIAL PRESSURE(Pi)[μ bar]

Table 64 Test of significance of slopes of the regression between intercellular CO_2 partial pressure and assimilation rates (Data obtained from Fig. 99)

TREATMENT	REGRESSION EQUATION	R
T1 CONTROL	Y= 0.1018+0.0818X	0.97***
T2 NaCl(200mM)	Y=-3.3321+0.0539X	1.00***
T3 [Proline]+ NaCl(200mM)	Y=-2.0963+0.0645X	0.98***

Standard deviation(SD)	0.0141
Standard error of mean difference of slopes	0.0081
L.S.D ($t_{0.05,n-4}$)	0.0219

T2 T3 T1

*** R values significant at 0.01%

4.7.4.1.2. Capacity and efficiency

As with the previous experiment, feeding NaCl (200mM) to the leaf reduced both CO_2-limited and CO_2-saturated rates of photosynthesis substantially compared to the control (Fig.100). Although proline pre-treatment appeared to alleviate NaCl-inhibited photosynthesis in both regions of the A:Pi curve to some extent, the test of significance of the slopes of the regressions for the CO_2-limited region showed no significant alleviation of NaCl inhibition (Fig. 100A; Table 64).

4.7.5 The effect of pre-treatment with glycinebetaine on NaCl-inhibited photosynthesis

4.7.5.1 Results

4.7.5.1.1 Stomatal limitations

A substantial reduction in stomatal conductance due to exposure to NaCl, as observed in the previous experiment, was found. Here, however, pre-treatment with glycinebetaine substantially alleviated this response, a 10mM concentration giving conductances similar to control values (Fig. 101).

4.7.5.1.2. Capacity and efficiency

As with previous experiments, NaCl substantially reduced the CO_2-limited and CO_2-saturated rates of photosynthesis compared to the control. Pre-treatment with glycinebetaine completely alleviated this inhibition in both regions of the A:P_i curve irrespective of the concentration range tested (Fig 102). Test of significance of the slopes of the regressions of the linearly related regions of the A:Pi curve showed no significant difference between control and glycinebetaine pre-treated leaves (Fig.

Fig. 101 Effect of glycinebetaine (GB) pretreatment on stomatal conductance (mol m^2 sec^{-1}) of NaCl treated wheat flag leaf at varying intercellular CO_2 concentrations

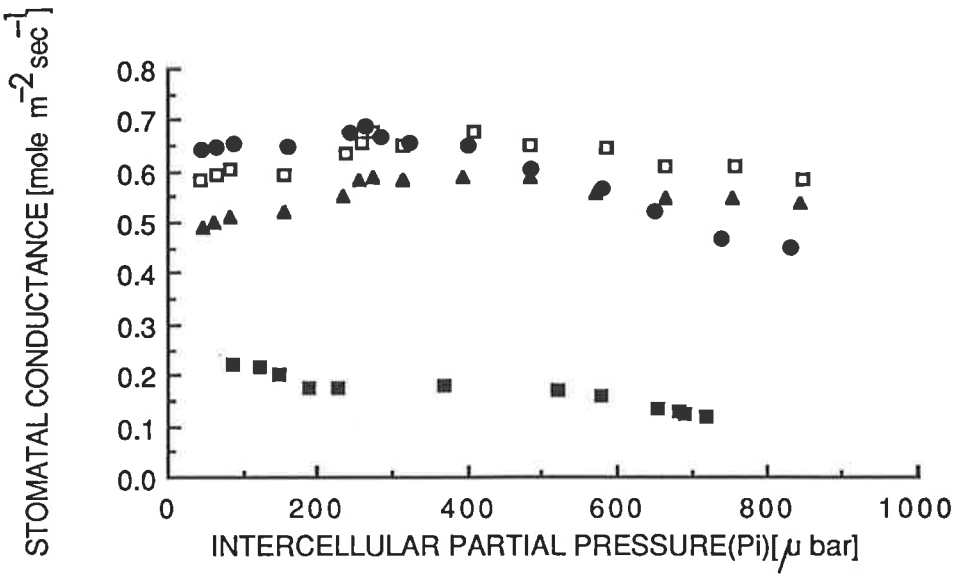
□ Control
■ [Water]-NaCl
▲ [GB 5 mM]-NaCl
● [GB 10mM]-NaCl

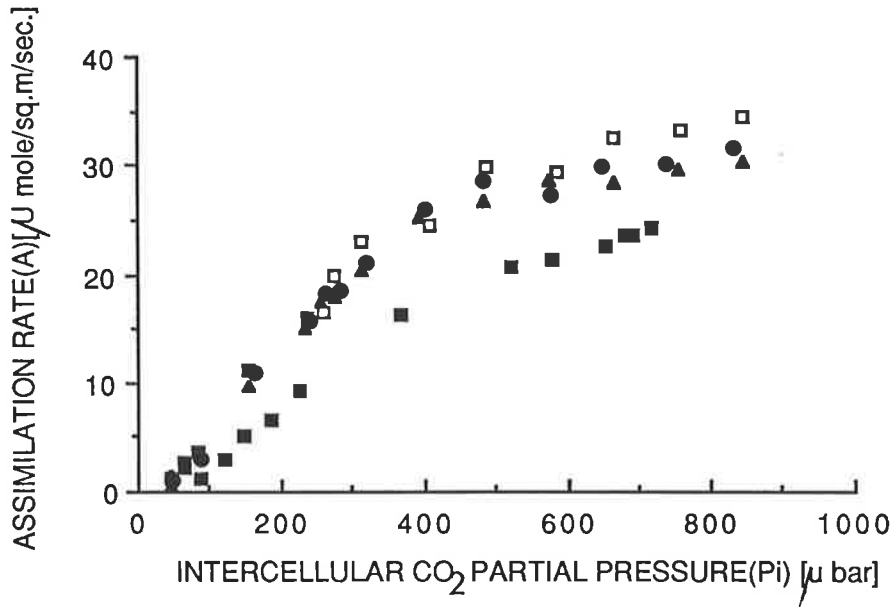
Fig. 102 Effect of glycinebetaine (GB) pretreatment on assimilation rate (µmole m^{-2} sec^{-1}) of NaCl treated wheat flag leaf at varying intercellular CO_2 concentration

□ Control
■ [water]-NaCl
▲ [GB 5mM]-NaCl
● [GB 10mM]-NaCl

Fig. 102A Relationship between intercellular CO_2 partial pressures and assimilation rates of glycinebetaine pretreated and non-pretreated, NaCl fed wheat flag leaves (Data obtained from the linear portions of A/Pi curve of Fig. 102)

□ Control
■ [Water]-NaCl
▲ [GB 5mM]-NaCl
● [GB 10mM]-NaCl
*** R value significant at 0.1%


STOMATAL CONDUCTANCE [mole m^{-2} sec^{-1}]
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
200
400
600
800
1000
INTERCELLULAR PARTIAL PRESSURE(Pi)[μ bar]


ASSIMILATION RATE(A)[μ mole/sq.m/sec.]
40
30
20
10
0
0
200
400
600
800
1000
INTERCELLULAR CO_2 PARTIAL PRESSURE(Pi) [μ bar]

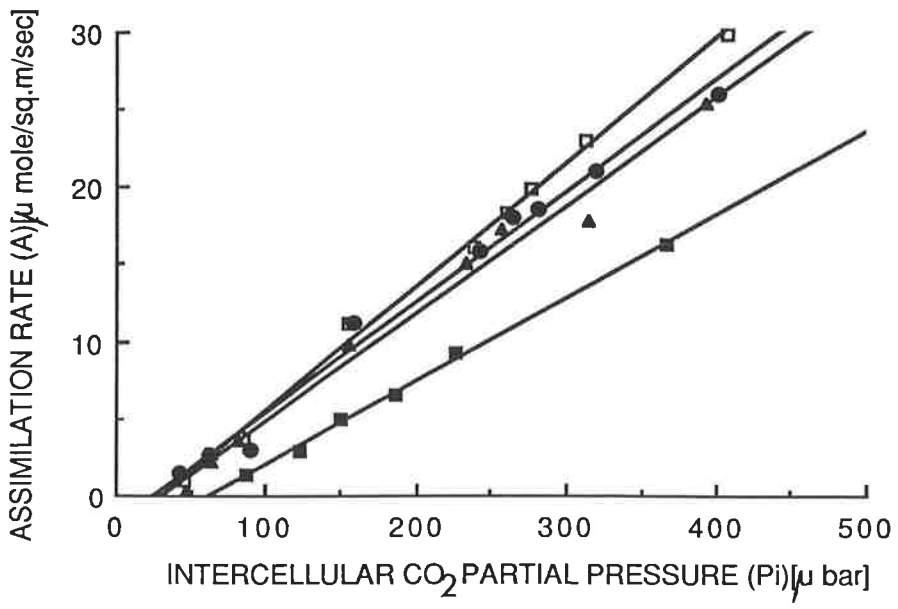

ASSIMILATION RATE (A)[μ mole/sq.m/sec]
30
20
10
0
0
100
200
300
400
500
INTERCELLULAR CO_2 PARTIAL PRESSURE (Pi)[μ bar]

Table 65 Test of significance of slopes of the regression between intercellular CO_2 partial pressure and assimilation rates (Data obtained from Fig. 101)

TREATMENT	REGRESSION EQUATION	R
T1 CONTROL	Y= -2.404 +0.0801X	1.00***
T2 NaCl(200mM)	Y=-3.3321+0.0539X	1.00***
T3 [Glycinebetaine 5mM]+ NaCl(200mM)	Y=-1.9732+0.0696X	0.99***
T4 [Glycinebetaine 10mM]+ NaCl(200mM)	Y=-1.7037+0.0716X	0.99***

Standard deviation(SD)	0.0109
Standard error of mean difference of slopes	0.0055
L.S.D ($t_{0.05,n-4}$)	0.0113

T2 T3 T4 T1

*** R values significant at 0.01%

102A; Table 65), indicating that glycinebetaine protected RuBP Carboxylase activity from NaCl inhibition.

4.7.6 The effects of pre-treatment with 3-O-methyl-∞-glucopyranose (3-OMG) on NaCl-inhibited photosynthesis

The previous experiment showed that glycinebetaine completely alleviated NaCl - induced inhibition of photosynthesis. However, it is difficult to ascertain whether this response is due to the maintenance of chloroplast volume (Robinson and Jones, 1986) or to a more specific effect on photosynthetic processes. Although such a distinction is difficult to disentangle, pre-treatment with a permeating, non-dissociating osmoticum, 3-O-methylglucopyranose (Greenway *et al.*, 1982) may help in arriving at such a distinction. The present experiment has this aim.

4.7.6.1 Results

4.7.6.1.1. Stomatal limitations

The effect of NaCl on stomatal conductance was consistent with the results of the previous experiment. Pre-treatment with 3-OMG completely eliminated the stomatal barrier consequent upon exposure to NaCl, as did glycinebetaine (Fig. 103).

4.7.6.1.2. Capacity and efficiency

The responses of the NaCl exposed leaf in photosynthesis were consistent with the previous experiments. Pre-treatment with 3-OMG alleviated NaCl - induced inhibition of both CO_2-limited and CO_2-saturated rates of photosynthesis substantially as did glycinebetaine (Fig. 104) and the test of significance of slopes of the regressions (Fig. 104A; Table. 66) for the linear region of the A:Pi curves showed that 3-OMG also produced complete recovery from NaCl inhibited photosynthesis .

Fig. 103 Effect of 3-orthomethylglucose (3-OMG) pretreatment on stomatal conductance (mol m^2 sec^{-1}) of NaCl treated wheat flag leaf at varying intercellular CO_2 concentrations

□ Control
■ [Water]-NaCl
▲ [3-OMG]-NaCl

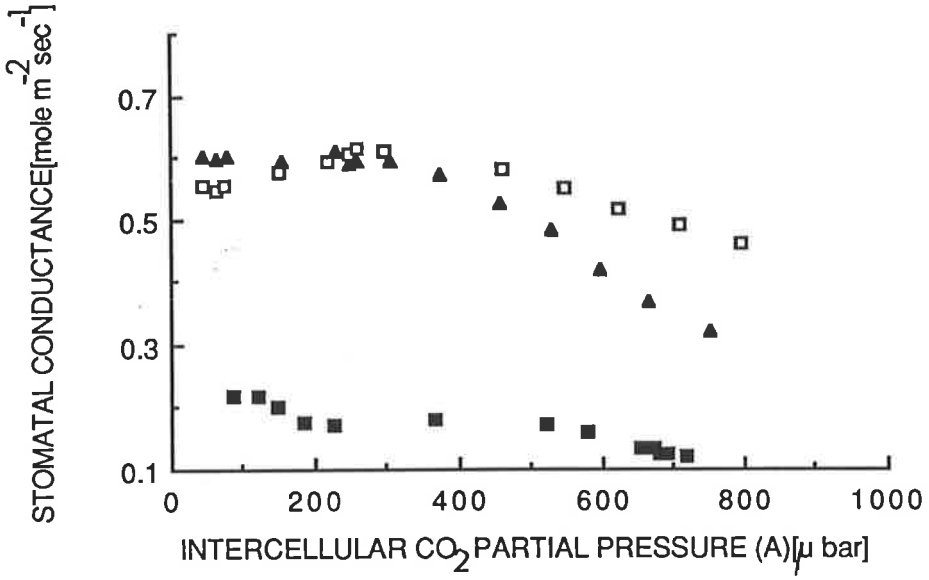
Fig. 104 Effect of 3-orthomethylglucose (3-OMG) pretreatment on assimilation rate (μmole m^{-2} sec^{-1}) of NaCl treated wheat flag leaf at varying intercellular CO_2 concentration

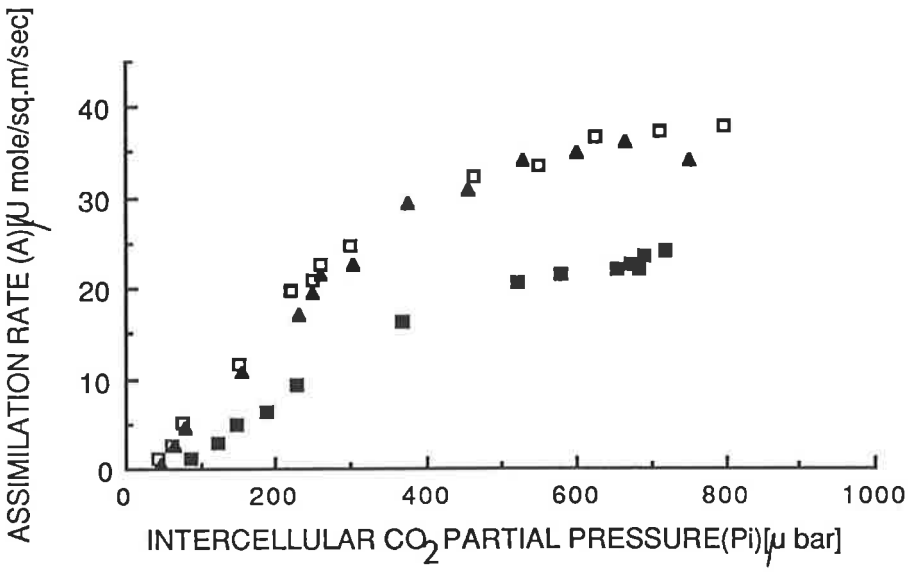
□ Control
■ [water]-NaCl
▲ [3-OMG]-NaCl

Fig. 104A Relationship between intercellular CO_2 partial pressures and assimilation rates of 3-orthomethylglucose pretreated and non-pretreated, NaCl fed wheat flag leaves (Data obtained from the linear portions of A/Pi curve of Fig. 104)

□ Control
■ [Water]-NaCl
▲ [3-OMG]-NaCl

*** R value significant at 0.1%


STOMATAL CONDUCTANCE[mole m-2 sec-1]
0.7
0.5
0.3
0.1
0
200
400
600
800
1000
INTERCELLULAR CO2 PARTIAL PRESSURE (A)[μ bar]


ASSIMILATION RATE (A)[μ mole/sq.m/sec]
40
30
20
10
0
0
200
400
600
800
1000
INTERCELLULAR CO2 PARTIAL PRESSURE(Pi)[μ bar]

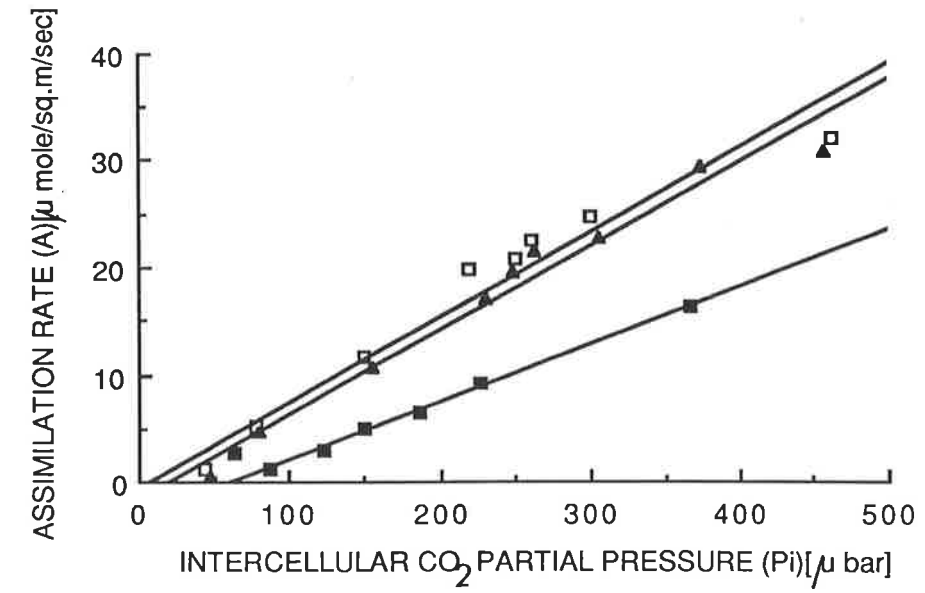

ASSIMILATION RATE (A)[μ mole/sq.m/sec]
40
30
20
10
0
0
100
200
300
400
500
INTERCELLULAR CO2 PARTIAL PRESSURE (Pi)[μ bar]

Table 66 Test of significance of slopes of the regression between intercellular CO_2 partial pressure and assimilation rates (Data obtained from Fig. 103)

TREATMENT	REGRESSION EQUATION	R
T1 CONTROL	Y= -0.4175+0.0791X	0.98***
T2 NaCl(200mM)	Y=-3.3321+0.0539X	1.00***
T3 [3-OMG]+ NaCl(200mM)	Y=-1.5668+0.0788X	0.99***

Standard deviation(SD) 0.0145

Standard error of mean difference of slopes 0.0084

L.S.D ($t_{0.05,n-4}$) 0.0174

T2 T3 T1

*** R values significant at 0.01%

4.7.7 Discussion

Salt effects on photosynthesis have been reported for many plant species (Gale *et al.*, 1967; Downton and Milhouse, 1983; Seeman and Critchely, 1985; Yeo *et al.*, 1985; West *et al.*, 1986). However, in all these studies, the direct effects of salt were indistinguishable from perturbations in root metabolism-induced alteration in photosynthesis. It is known (Itai and Vaadia, 1965, Walker and Dumbroof, 1981) that salt influences root metabolism and particularly that of cytokinins and ABA. An increase in ABA concentration and a reduction in cytokinin have been reported to influence both stomatal and non-stomatal factors controlling photosynthesis (Jones and Mansfield, 1972; Raschke, 1979; Davis *et al.*, 1986). Feeding salt through the leaf tip facilitated investigation of the effects of salt on photosynthesis with less likelihood of perturbations at the root level. The responses observed in the present study indicate that solutes fed through the cut leaf tip reach the site of measurement.

Salt effects on photosynthetic processes fall into two major categories;(1) response of stomates to salinization and (2) effects of salt on the capacity of the plant for CO_2 fixation, independent of altered diffusion limitations. Stomatal closure is generally associated with salinization of glycophytes (Downton, 1977, Gale *et al.*, 1967, Walker *et al.*, 1983). The data presented in the present study show that stomatal conductance is reduced by salt; however, the extent to which stomatal closure affects photosynthetic capacity is indicated by the magnitude of the reduction in Pi (Berry and Downton, 1982; Farquhar and Sharkey, 1982). The results of the present study show no decline in Pi (at an ambient CO_2 concentration of 350 µbar) up to 150 mM concentration of NaCl and a reduction of only 9.9 % at 200 mM (Table 62). This indicates that the decline in assimilation is not solely due to limitation in CO_2 diffusion.

The extent of non-stomatal limitations on photosynthesis can be assessed *in vivo* by analysing the response of photosynthetic CO_2 fixation to varying intercellular CO_2 concentrations since the initial slope at CO_2 limiting region is dependent on the

kinetics and concentration of RuBP Carboxylase and the response at high CO_2 concentration at CO_2-saturating region is a function of the capacity for the regeneration of RuBP (Badger *et al.*, 1984, Von Caemmerer and Farquhar, 1981; Seeman and Barry, 1982). Using such an approach Ball and Farquhar, (1984) found salinity brought about a reduction in both the initial slope and the CO_2-saturated portions of the A:Pi curve. The response of photosynthesis in wheat, in the present study, was the same (Figs.98, 98A, Table 63) as has been found in a number of salt - sensitive glycophytes (Downton, 1977; Gale *et al.*, 1967; Walker *et al.*, 1983). The biochemical basis for such alterations in photosynthetic capacity as a result of stress is yet to be understood. Changes in the apparent photosynthetic capacity of the leaf that are not a consequence of increased diffusional limitations, may be the result of either reduction in capacity or efficiency of RuBPCarboxylase or reduction in regeneration capacity. This conclusion, however, rests on uniformity of response in stomatal aperture. Recently, Terashima *et al*(1988) with *Vicia faba* (L), found that the apparent non-stomatal inhibition of photosynthesis by ABA, deduced from the depression of A;Pi relationship, is an artefact which they have attributed to the non-uniform distribution of transpiration and photosynthesis over the leaves. However, no such variations in wheat has been reported so for.

Reduction in CO_2-saturated non-linear region of photosynthesis suggest that NaCl influenced either the capacity to regenerate RuBP (Farquhar and Von Caemmerer, 1982) or to utilise triose phosphate (Badger *et al.*, 1984). Reduction in regeneration capacity indicates limiations in rate of photosynthetic electron transport (Farquhar and Von Caemmerer, 1982). However, electron transport processes appear not to be as sensitive as enzyme reactions. In another glycophyte, tomato, subjected to NaCl there was no reduction in electron transport (data not presented). In fact, in halophytes, electron transport is considerably enhanced *invitro* during salt stress (Crichley, 1982), while the enzymes in halophytes are as salt sensitive as glycophyte enzymes *in vitro* (Flowers *et al.*, 1977). It is easier to envisage how triose phosphate utilization (TPU) could limit photosynthesis of NaCl-treated leaves. TPU involves the

conversion of triose phosphates, products of phosphorylation of 3-phosphoglycerate, into starch and sucrose, with a resultant release of inorganic phosphate. If TPU proceeds at a rate less than the rate of CO_2 fixation the inorganic phosphates released will be insufficient to maintain phosphorylation (Walker and Herold, 1977) and CO_2 assimilation would decline.

Pre-treatment with proline in the present study showed some effect in alleviating NaCl-inhibited photosynthesis (Figs. 100, 100A; Table 64), but a complete recovery was not observed. Incharoensakdi *et al.*, (1986) have also observed in effectiveness of proline in alleviating KCl-induced inhibition of RuBP Carboxylase. However this may be due to the proline being rapidly metabolised. Exogenous labelled proline is oxidised in turgid leaves (Oaks *et al.*, 1970; Stewart, 1972), resulting in carbon being fed into Kreb's cycle and respired.

Pre-treatment with glycinebetaine virtually abolished the NaCl-induced decline in photosynthesis in both the CO_2-limited and saturated regions (Figs.102; 102A; Table 65), suggesting that glycinebetaine acts as a compatible osmoticum in protecting photofuction during salt stress. Many such protective roles of glycinebetaine have been established *in vitro* (Paleg *et al.*, 1981, 1984; Incharoensakdi *et al.*, 1986). Pre-treatment with glycinebetaine also ameliorated NaCl effects on stomatal conductance. The alleviation of NaCl-inhibited photosynthesis was not solely due to the alleviated stomatal response as Pi was not greatly limited by stomatal conductance, suggesting that protection of the activity of RuBP Carboxylase and capacity to regenerate RuBP were involved. A similar effect of glycinebetaine in protecting RuBPCarboxylase activity, against inhibition by KCl, has been demonstrated in the cynobacteria *Aphanothece halophytica* (Incharoensakdi *et al.*, 1986).

These ameliorative effects of glycinebetaine may be due simply to the maintenance of the osmotic volume of the chloroplast as 3-O-methylglucopyranose, a permeating, non-dissociating osmoticum also alleviated NaCl-inhibited photosynthesis (Figs.104; 104A: Table 66). In spinach, a major proportion (30-40%) of glycinebetaine

accumulated in the chloroplasts, in response to NaCl stress, contributing 64% of the decrease in ψ_s of the chloroplast. There was no decline in photosynthesis (Robinson and Jones, 1986). Alternatively, Incharoensakadi *et al.*(1986) claim that the alleviating nature of glycinebetaine comes from the methyl group of this compound. They have shown many methylated compounds such as glycerol, sucrose and sarcosine protect RuBP Carboxylase activity as effectively as glycinebetaine and suggest that these methylated compounds protect the enzyme-protein activity by acting at the protein-water surface, decreasing the effects of concentrated salts on the enzyme-proteins and other macro-molecules. However, as similar protective effects were observed (Incharoensakadi *et al.*, 1986) with 300 mM KCl on heat and cold inactivated RUBP-Carboxylase activity the possibility that a specific effect of the methyl group of the glycinebetaine molecule is involved would seem to be ruled out.

CHAPTER-5

General Discussion

CHAPTER 5 GENERAL DISCUSSION

The present study demonstrates that neither leaf water status nor leaf ion concentration trigger accumulation of proline or the quaternary ammonium compounds, trigonelline and glycinebetaine. The correlations between various physical effects of salt stress and accumulation of these compatible solutes observed in Section 4.1.2, and by others (Chu *et al.*, 1976; Greenway *et al.*,1982; Munns *et al.*, 1982; Buhl and Stewart, 1983; Wyn Jones and Storey, 1978; Storey and Wyn Jones, 1978) demonstrate only apparent not absolute relationships which may be due to other effects of exposure to salinity. It can be suggested that accumulation of proline and QACs is controlled by (1) a factor (unmeasured) originating in the sampled leaves (2) a factor originating elsewhere (a) in other leaves (b) in other parts of the plant. Change in water status and increase in ion concentration are the two primary effects in leaves of plants exposed to salinity (Greenway and Munns, 1980),but these have been discounted as the cause of accumulation (Section 4.3 and 4.4). No other direct effect of salinity on the leaf has been recorded. It is unlikely that changes in other leaves would control accumulation of proline and QACs in the sampled leaf, and there is no evidence for such control in the literature. Accumulation of proline and QACs without a change in these primary leaf responses to salt stress suggest that accumulation may result from a factor(s) of root origin. Evidence for a signal from the roots regulating stomata and affecting assimilation in the leaves during drought has been produced (Schulze, 1986) and Munns and Termaat (1986) suggest that a root-derived factor is responsible for the reduction of leaf growth in salinity. It is possible that the same signal induces accumulation of proline and QACs . The 'signal' could either be a chemical (NO_3^-, hormone) factor or a factor yet to be described.

The nitrogen supply to the shoot is reduced after exposure to NaCl, as Cl^- inhibits NO_3^- uptake (Cram, 1973; Deane-Drummond and Glass, 1982). Trewavas (1985) proposed that NO_3^- was a plant growth regulator, affecting plant metabolism and

development, but a salt-induced reduction in NO_3^- uptake cannot be causal since proline and QACs also accumulated in plants exposed to KNO_3 (section 4.2.2).

The roots communicate with the shoot via growth regulators (Torrey, 1976). The roots synthesise hormones such as auxin, gibberellins, abscisic acid, cytokinins and ethylene (Torrey, 1976). These root-derived hormones are known to influence various metabolic processes leading to growth and differentiation (Torrey, 1976). There is no direct evidence for effects of salinity on auxin or gibberellin metabolism in plants nor any information on a role for these hormones in solute accumulation triggered by salinity. Ethylene, although increasing the salt tolerance of rice did not respond to salinity (Khan *et al.*, 1987). ABA and cytokinins, however, play major roles in regulating the adaptive response of plants exposed to various forms of osmotic stress (Vaadia, 1976). An increase in ABA in plants exposed to salinity (Mizrahi *et al.*, 1970, 1972; Hartung *et al.*, 1983) and lack of such a response (Stewart and Voetberg, 1985) have both been reported for salinised plants. In the present study, however, there was no increase in ABA with salinity in either tomato or wheat (Section 4.5). Reported increases in ABA concentration with salinity (Mizrahi *et al.*, 1970, 1972; Hartung *et al.*, 1983) may have been due to salt shock resulting in turgor loss, as turgor loss has been reported to be essential for ABA accumulation (Creelman and Zeevaart, 1985; Pierce and Raschke, 1980). It is also possible that this discrepancy could be due to a difference in the ABA extraction method. The workers (Mizrahi *et al.*, 1970, 1971, 1972; Hartung *et al.*, 1983) who reported increases in ABA concentration after salinity stress used methanol for extraction. It has been found (Loveys and VanDijk, personal communication) that extracting ABA with any organic solvent results in an over-estimation of free ABA since endogenous ABA conjugates are hydrolysed during such extraction procedures. In order to prevent this, the tissues have to be boiled in water before extraction, the method used in the present study. If this were the cause of the differences in response it would suggest that salinity caused an increase in the concentration of ABA-conjugates, but there is no evidence for this.

Although exogenous application of ABA leads to accumulation of proline in some species (Aspinall, 1980, Aspinall *et al.*, 1973) ABA is evidently not the trigger for proline accumulation in salt stressed tomato or wheat (Section 4.5) and an increase in ABA is not an obligatory requirement for accumulation. Stewart and Voetberg, (1987) also found that ABA accumulation is not a prerequisite for wilt-induced proline accumulation. Similarly, glycinebetaine and trigonelline accumulation occurred despite a lack of increase in ABA suggesting that ABA neither causes nor modulates accumulation of these QACs.

Cytokinin is a root-derived hormone, transported from the root to the shoot in many plants (Henson and Wareing, 1976) where it influences many aspects of shoot physiology (Horgan, 1984) including stomatal behaviour, leaf growth, senescence and abscission (Davis *et al.*, 1986). Changes in the root environment modify hormone production in the root, change the export of the hormone via the xylem sap to the shoot and thereby elicit changes in the shoot. Roots subjected to salinity stress show reduction in endogenous cytokinins, especially *trans* and *cis*-zeatins, in salt stressed tomato (Walker and Dumbroff,1981). Reduction in cytokinin during salt stress alters salt and water balance (Livne and Vaadia, 1965), decreases hydraulic conductance (Tal and Imbar, 1971), inhibits protein synthesis and increases proteolysis (Itai *et al.*, 1968). Although, there has been no detailed investigation on the relationship between such a reduction in endogenous cytokinin and accumulation of proline or QACs, preliminary experiments with excised tomato leaf sections show that proline accumulation is responsive to cytokinin, the accumulation of proline in response to salt stress being reduced by BA applied exogenously (data not presented). A similar response has been observed in intact salt stressed tomato and wheat (data not presented). BA has been shown to prevent wilt, salt and ABA-induced proline accumulation in excised barley leaf tissues (Stewart *et al.*, 1986) and water stress-induced accumulation of proline was inhibited by application of kinetin (Aspinall, 1980). As with proline, salt-induced glycinebetaine or trigonelline accumulation is prevented by application of BA (data not presented). This suggest that a salt-induced

reduction in cytokinin supply may be the signal for the accumulation of proline and QACs in the leaves.

Another possible interpretation of the results of the present study is that salt stressed roots produce some as yet unidentified substance. Several substances have been demonstrated to have anti-cytokinin effect on stomata (Jung and Rademacher, 1983) however, nothing is known about their response to environmental stress or their effects on the accumulation of proline or QACs.

The concept of plant growth substances acting as signals within the plant is currently a contentious one (Trewavas, 1981) but, it seems unnecessary to seek a more complicated explanation for the accumulation of these compatible solutes, at least until good quantitative measurements of cytokinin synthesis and transport or identification of some other root derived factor force us to do otherwise.

If reduction in cytokinin is the signal for accumulation of proline, trigonelline and glycinebetaine in salt-stressed tomato and wheat, one would expect a common cause for the accumulation of these compounds in plants under other environmental stresses since these compounds also accumulate in response to water stress (Chu, 1974; Wyn Jones and Storey, 1981; Parameshwara, 1984), low and high temperature (Chu, 1974; Naidu, 1988) and flooding (Dubinina, 1961). It is interesting to note that root systems subjected to water stress (Itai and Vaadia, 1965; Mizrahi and Richmond, 1972), heat stress (Itai *et al.*, 1973), low temperature (Itai and Benzioni, 1974), and flooding (Reid and Railton, 1974), all export less cytokinin. Hence, it is possible that reduction in cytokinin may be the common signal affecting accumulation of these compounds under all environmental stresses.

Speculation on the mechanisms of accumulation of proline and QAC

A mechanism for an effect of cytokinin on the accumulation of these compounds is not known, but it is possible that accumulation of proline may be due to (1) reduced incorporation of proline into protein (2) proteolysis (3) an inhibition of protein

synthesis (4) a reduction in proline oxidation (5) an increase in *de nova* synthesis (6) a combination of one or more of these . A marked reduction in proline incorporation into protein has been observed for salt-stressed barley (Buhl and Stewart, 1983). A reduction in cytokinin supply during salt stress has been found to reduce incorporation of amino acids into protein (Itai *et al.*, 1968) and kinetin treatment alleviates reduced incorporation of ^{14}C-leucine into protein. It is also known that a reduction in cytokinin supply under salt stress leads to proteolysis (Shibaoka and Thimann, 1970; Vaadia and Waisal, 1967). This would also increase the proline levels in salt stressed plants. If the increase in proline pool is considered to be due to proteolysis caused by a reduction in cytokinin, this would also increase other protein amino acids. However, no increase comparable to that in proline has been reported for any amino acids in salinised plants (Chu, 1974). Further, the amount of free proline accumulated greatly exceeds that which could be accounted for by protein breakdown in rye grass (Kemble and Mac Pherson, 1954) and turnip (Thompson *et al.*, 1966) and stressed cells of *Cyclotella cryptica* showed no loss of activity, yet the cells accumulated significant amounts of proline (Liu and Hellebust, 1976). Moreover, proteolysis would lead to growth reduction but the lack of growth reduction with continued increase in proline concentration in salinised tomato and wheat (Section 4.1.3) suggests that the increase in proline concentration is unlikely to be due to proteolysis. It is also unlikely that impaired protein synthesis is the sole mechanism of proline accumulation since inhibiting protein synthesis is not sufficient to cause proline accumulation (Boggess and Stewart, 1980).

Salt stress causes a marked reduction in proline oxidation in barley (Boggess and Stewart, 1980) and this would also increase the proline pool. Although evidence for ions inhibiting Δ^1-P-5-C dehydrogenase, *in vitro*, is available (Boggess *et al.*, 1975), the significant proline accumulation found without a change in tissue ion concentration (Section 4.4), eliminates the possibility that proline accumulation in salt-stressed plants is solely due to this mechanism. It is not known whether a reduction in cytokinin concentration inhibits the activity of this enzyme. Proline

concentration would also increase following a promotion of *de nova* synthesis. Although evidence for NaCl promoting the *in vitro* activity of Δ^1P-5-C reductase is available (Huber, 1974), the significant proline accumulation which occured without a change in tissue ion concentration (Section 4.4) also rules out this possibility. It is not known whether a reduction in cytokinin concentration increases the activity of this enzyme.

Glycinebetaine is synthesised from choline (Hanson *et al.*, 1985). According to the scheme (Plate 3) in wheat, the choline which is later oxidised to glycinebetaine is produced *via* a turnover of phosphatidylcholine (PC). There appears to be a link between PC turnover, membrane and /or chloroplast development, and glycinebetaine biosynthesis. Wheat uses the so-called eukaryotic pathway for biosynthesis of unsaturated fatty acids (Heemskerk and Wintermans, 1987). In this pathway PC is essential for the production of unsaturated diacylglycerol in the biosynthesis of monogalactosyldiacylglycerol (MGDG), a major glycolipid of photosynthetic membranes. Breakdown of PC to diacylglycerol would also release choline which would be available for glycinebetaine synthesis. On liberation of choline, choline oxidase acts on this substrate forming glycinebetaine by a two-step oxidation. The link between glycinebetaine biosynthesis and production of unsaturated fatty acids for MGDG synthesis indicates that glycinebetaine biosynthesis may be a consequence of another metabolic pathway rather than an end in itself. An alteration in lipid metabolism would be expected to influence glycinebetaine accumulation. Cytokinins have been shown to be associated with enhanced methylation of neutral and polar lipids, especially phosphatidylcholine (Schaeffer *et al.*, 1972). It has been shown that a decrease in kinetin concentration alters lipid metabolism substantially (ref. Letham *et al.*, 1978). Exogenous application of kinetin prevents senescence of detached wheat leaves and lowers lipase activity (Sodek and Wright, 1969). Changes in the composition of saturated and unsaturated fatty acids of heat-stressed plants has also been shown to be reversed by kinetin (Kull and Buxenstein, 1974). This evidence suggests that a reduction in tissue cytokinin concentration during salt stress is likely to increase

lipase activity, releasing phosphatidylcholine for the synthesis of glycinebetaine. It also appears that a reduction in cytokinin concentration may effect choline oxidase which is involved in the conversion of choline to glycinebetaine. The substrates glycine and serine are in continuous supply for glycinebetaine synthesis (ref. Letham *et al.*, 1978) as the glycolysis associated with photorespiration, in which the precursors of glycinebetaine synthesis are involved (Keys, 1980), increases with a reduction in cytokinin concentration, resulting in the synthesis of more glycine.

Although it is known that trigonelline is synthesised from nicotinic acid by nicotinic acid methylpherase (Cantoni, 1951), there is no evidence for a reduction in cytokinin concentration influencing accumulation of this compound or the activity of the enzyme. It is generally considered that trigonelline acts as a reservoir for nicotinic acid and, therefore, of pyridine nucleotides (Willeke *et al.*, 1979) as a high concentration of nicotinic acid is considered to be toxic.

Physiological significance of proline and QACs

The general response of accumulation of these compounds under various environmental stresses, including salt stress, suggests a role(s) in plants under stress. Accumulation of proline in halophytes (Stewart and Lee, 1974) and a few glycophytes (Rajasekaran, 1979; Sampathkumar, 1980) has been shown to relate to salt tolerance. Genetic evidence for a link between glycinebetaine accumulation and salt tolerance has also been produced in barley (Grumet and Hanson, 1986). Such a relationship is of particular interest with tomato as the *Lycopersicon* genus includes an array of species which have evolved in varying ecological habitats. A relationship between salt tolerance and accumulation of solutes would be of advantage in any attempt to genetically engineer the commercial species to enhance salt tolerance. Many traits such as high tissue Na^+ or Cl^- concentrations, ion exclusion, and greater osmoregulation etc., have been suggested as selection criteria for salt tolerance. Any selection made using these traits alone could be misleading as salt sensitive species frequently have similar attributes (Greenway *et al.*, 1962; Aswathappa and Bachelard,1986). There is a wide range of

tolerance in *Lycopersicon* species, *L. cheesmanii*, a saline-coastal inhabitant, being the most tolerant and *L.pennillii* the most sensitive. The commercial cultivar of *L. esculentum* ranked 7th in the order of salt tolerance. All species accumulate proline in all organs and there is no general relationship between proline accumulation potential and relative salt tolerance. This suggest that although genetic differences in salt tolerance within species may be correlated with proline accumulation potential(Rajasekaran, 1979; Sampathkumar, 1980; Stewart and Lee, 1974) this does not extend across specific boundaries. This lack of correlation could be due to the various factors discussed in Section 4.6. Trigonelline accumulation in the meristematic tissues of salt-stressed plants (Trigonelline Accumulation Index, TAI) did show a significant correlation with relative salt tolerance, however. A feasible physiological explanation for this relationship is not known.

Alternatively, salt tolerance in any species may not be due to a single factor and many requirements, including effective turgor maintenance in expanding tissues, efficient compartmentation of ions, continued root growth for acquisition of water and nutrients and supply of growth factors need to be satisfied (Greenway and Munns, 1980; Mass and Neiman, 1977). In the present study, the salt tolerance of these species was demonstrated to be related to the turgor potential of expanding leaves, Na^+ exclusion in the expanded leaf and Cl^- exclusion in the roots, and *L. cheesmanii*, the most tolerant species, apparently achieves salt tolerance through a combination of these complementary traits.

Many further roles have been postulated for accumulated proline and glycinebetaine and they satisfy the definition of a 'compatible solute' (Borowitzka, 1981) in that they cause minimal disruption of metabolism. Accumulation of these solutes has been shown to protect enzymes from salt (Pollard and Wyn Jones, 1979), heat (Paleg *et al.*, 1981), p^H (Ahmad *et al* 1982) and chemical (Bowlus and Somero, 1979) perturbations, *in vitro*. No similar protective function in the plant has been demonstrated for plants *in vivo*. Accumulation of glycinebetaine to large concentration in chloroplasts (Robinson and Jones, 1986) indicates that this compound may have

some specific role in the function of that organelle. Glycinebetaine, but not proline, alleviates NaCl-induced inhibition of stomatal and non-stomatal limited photosynthesis. This function mimics that of cytokinins (Milborrow, 1981). The mechanism is unknown but alleviation of the non-stomatal limitation may be achieved by maintaining RUBISCO activity. A similar protective role for glycinebetaine has been demonstrated in a cyanobacterium (Incharoensakdi *et al.*, 1986). Glycinebetaine also alleviates NaCl-induced disruption of regeneration capacity, reflecting sustained electron flow from photosystem II to I during salt stress. The mechanism of this effect is also unknown, but it is hard to conceive that this compound acts in a specific manner on every photosynthetic process. Rather, each of these processes would suffer if chloroplast volume were to be reduced (Kaiser *et al.*, 1983; Kaiser and Heber, 1981). A reduction in chloroplast volume correlated with a reduction in photosynthetic capacity has been demonstrated in chloroplasts equilibrated in various osmotica (Robinson, 1986). Under conditions of salinity, cytoplasm solute potential decreases and sub-cellular organelles accumulate solutes which decreases organelle osmotic potential in parallel with the changes in the cytoplasm and thereby avoid damage and dysfunction (Kaiser *et al.*, 1983). Accumulation of glycinebetaine in the chloroplast in salinised spinach contributes to 36% of the osmotic potential of that organelle (Robinson and Jones, 1986) and glycinebetaine may protect photofunction solely by maintaining chloroplast volume. If the sustained photofunction during salinity is due to volume maintenance alone, then feeding a permeating non-dissociating solute should also alleviate salt stress-induced inhibition of photosynthesis. This was tested with 3-orthomethyl glucose (3-OMG) which was shown to alleviate NaCl-induced inhibition of photosynthesis in a similar manner to glycinebetaine.

CONCLUSION

It can be concluded that the accumulation of proline and QACs, (trigonelline and glycinebetaine in salt stressed tomato and wheat respectively) is induced by a root-derived factor other than ABA. The nature of the mode of salinization, the specific ions in the rooting medium, humidity and wind velocity of the environment all modify the

accumulation potential for these compounds. Accumulation of these compounds has some physiological significance in plant adaptation and in maintaining photofunction during salt stress.

CHAPTER-6

Appendix

CHAPTER-6

APPENDIX-I Confirmation of QACs in tomato and wheat by NMR spectroscopy

Trigonelline, choline in tomato and glycinebetaine and choline in wheat were identified by TLC using Dragondoff reagent (Section 3.2.9.3). However, it was essential that the identity of these compounds was confirmed by NMR spectroscopy as this method was chosen to measure QACs.

Materials and Methods:

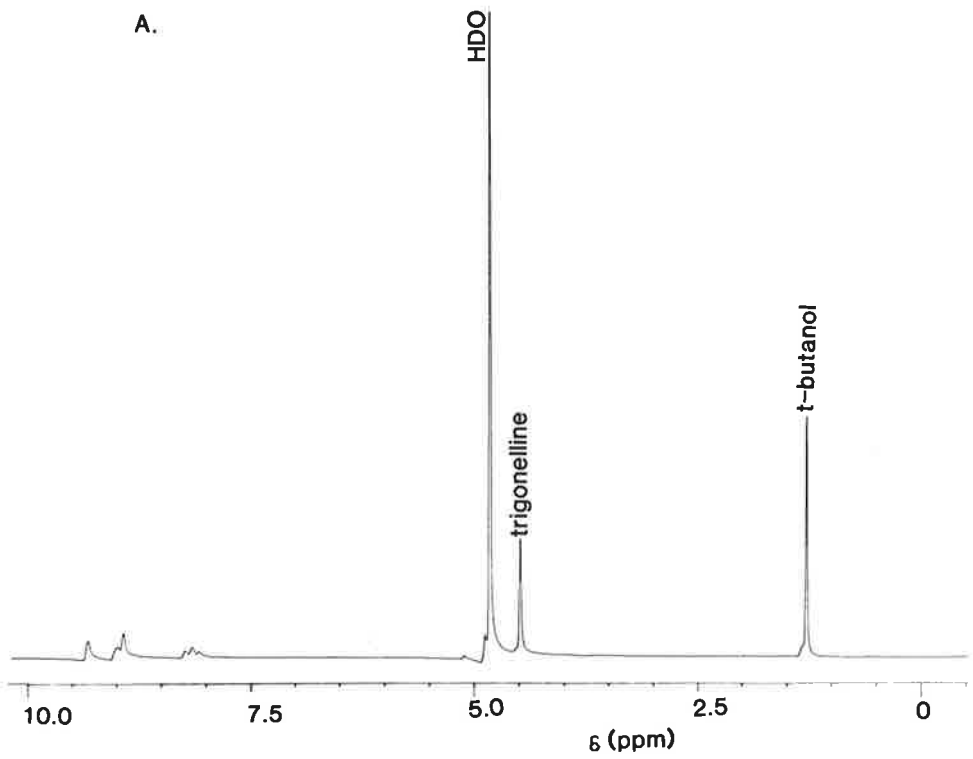
Tomato and wheat samples were chromatographed as described in section 3.2.9.3. Authentic standards were co-chromatographed at both the ends of the TLC plates. The authentic QACs alone were sprayed with dragrondoff reagent. The sample areas corresponding to the authentic standards were eluted . These eluted silica gel samples containing QACs were extracted 4 times with 15 ml of hot methanol each time. The methanol fractions of QAC obtained from tomato and wheat were pooled and dried *in vacuo* at 55°C. The dried extracts were dissolved in 0.8 ml of D_2O. 0.6ml of these were transferred to NMR tubes and 1µl of t-butanol was added. These were sujected to NMR spectroscopy (Section 3.9.2.3). The peaks in the spectra of tomato and wheat were further confirmed by spiking with 1mg of authentic trigonelline, choline or glycinebetaine.

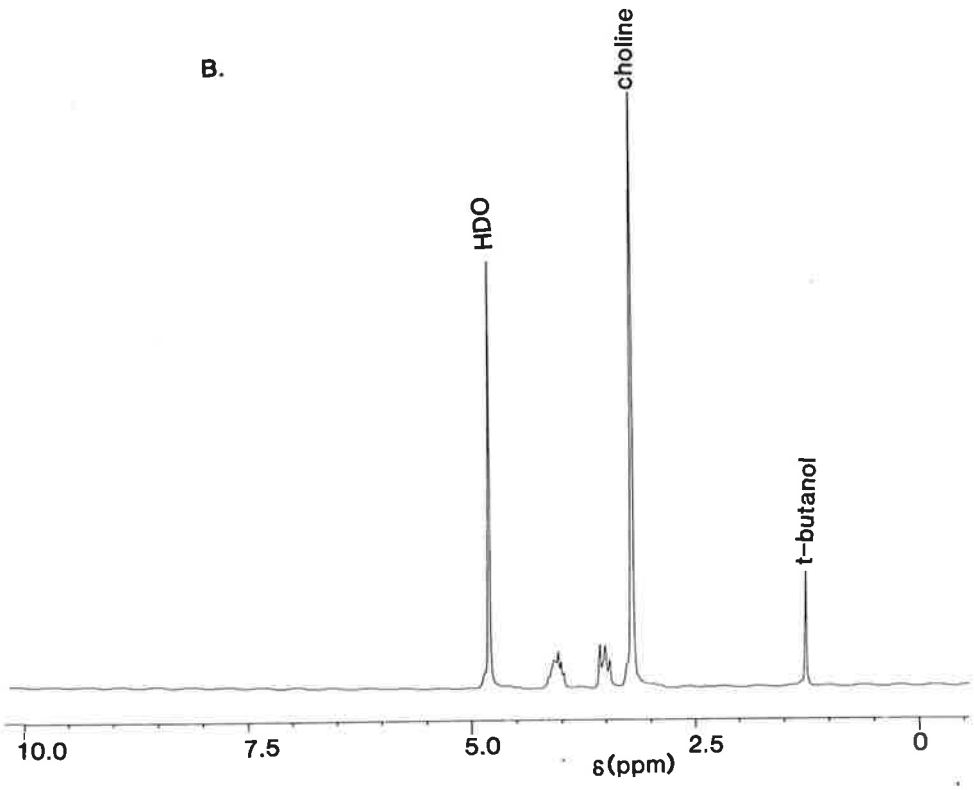
Results:

H^1NMR spectrum of the authentic trigonelline (Plate A.1.A) consisted of peaks at 9.332 ppm (S, 1H), two overlapping doublets centered at 9.010 ppm (2H), 8.253 ppm (t, 1H) and 4.577 ppm (S,3H). The above signals were assigned to C-2, C-4, C-6, C-5 and N-CH_3 respectively. The authentic choline spectrum (Plate. A.1.B) consisted of a primary peak at 3.212 ppm and two multiplets centered at 3.588 and

Plate A.1.A ^{1}H NMR spectrum of authentic trigonelline

Plate A.1.B ^{1}H NMR spectrum of authentic choline


A.
HDO
trigonelline
t-butanol
10.0
7.5
5.0
2.5
0
δ (ppm)

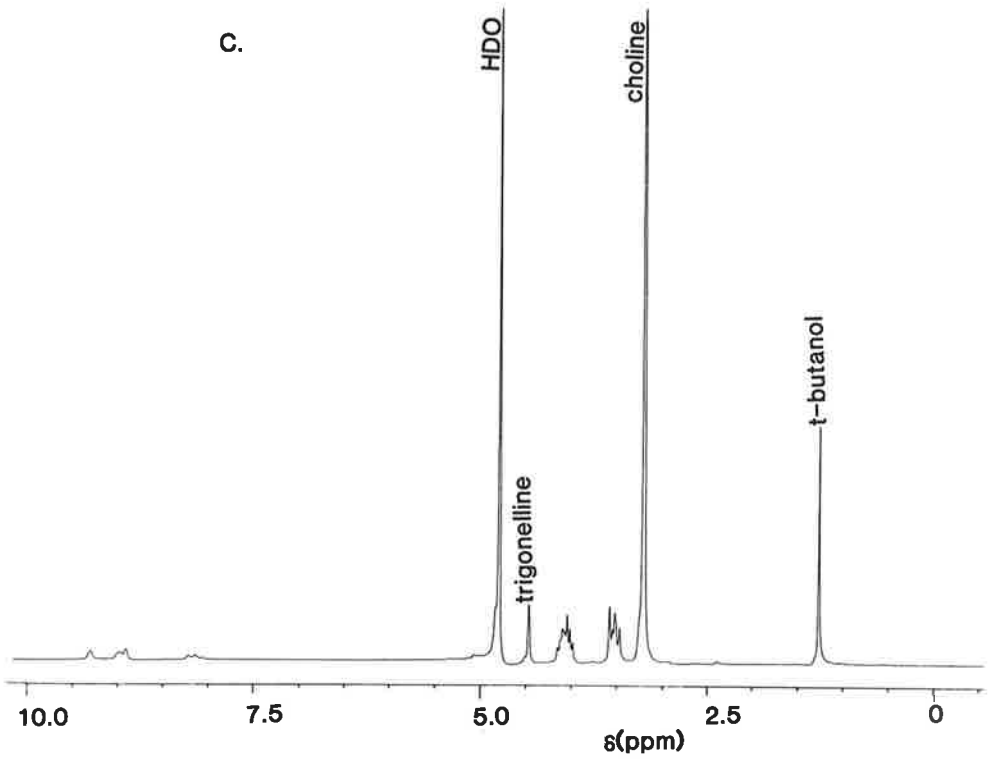

B.
choline
HDO
t-butanol
10.0
7.5
5.0
2.5
0
δ(ppm)

4.076 ppm. The spectrum obtained for the tomato sample (Plate. A.1.D) contained all these peaks as in authentic trigonelline and choline. Spiked spectra (Plate. A.1.E; A.1.F) showed a corresponding increase in the signal intensity of the related peaks of trigonelline and choline confirming that trigonelline and choline are the QACs present in tomato.

The authentic glycinebetaine spectrum (Plate. A.2.A) contained a primary peak at 3.31ppm and a secondary peak at 4.182 ppm. The spectrum obtained for wheat (Plate. A.2.D) contained peaks corresponding to authentic glycinebetaine. Another peak at 3.212 ppm and two multiplets at 3.588 and 4.076 ppm corresponded to the peaks of authentic choline (Plate. A.2.B; A.2.D). On spiking wheat sample with authentic glycinebetaine or choline (Plate. A.2.E) a corresponding increase in the signal intensity of the respective peaks were observed confirming that wheat contains glycinebetaine and choline.

Plate A.1.C ^{1}H NMR spectrum of authentic trigonelline and choline

Plate A.1.D ^{1}H NMR spectrum of tomato sample


C.
HDO
choline
t-butanol
trigonelline
10.0
7.5
5.0
2.5
0
δ(ppm)

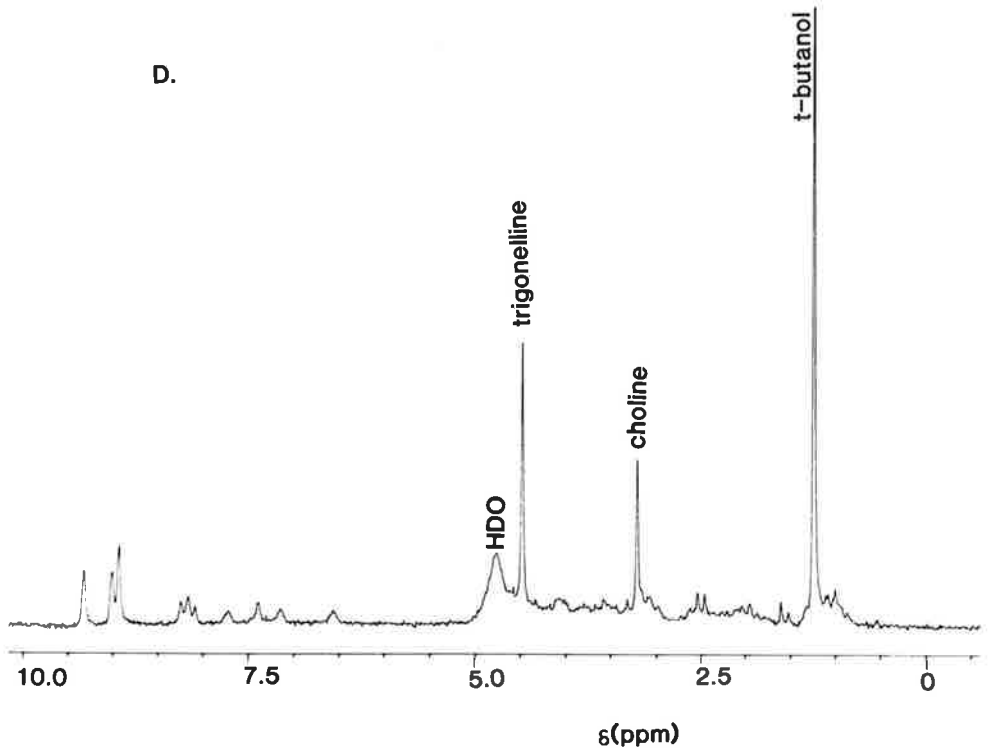
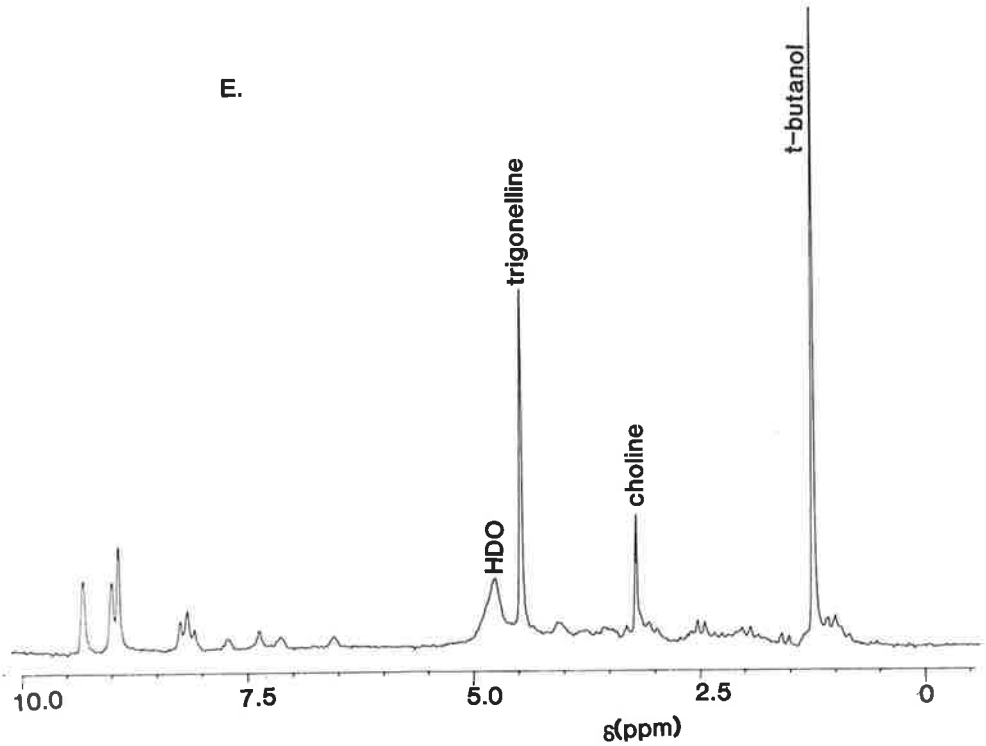

D.
t-butanol
trigonelline
choline
HDO
10.0
7.5
5.0
2.5
0
δ(ppm)

Plate A.1.E ^{1}H NMR spectrum of tomato sample spiked with 1mg of authentic trigonelline

Plate A.1.F ^{1}H NMR spectrum of tomato sample spiked with 1mg of authentic 1mg of authentic trigonelline and choline

E.


t-butanol
trigonelline
choline
HDO
10.0
7.5
5.0
2.5
0
δ(ppm)

F.

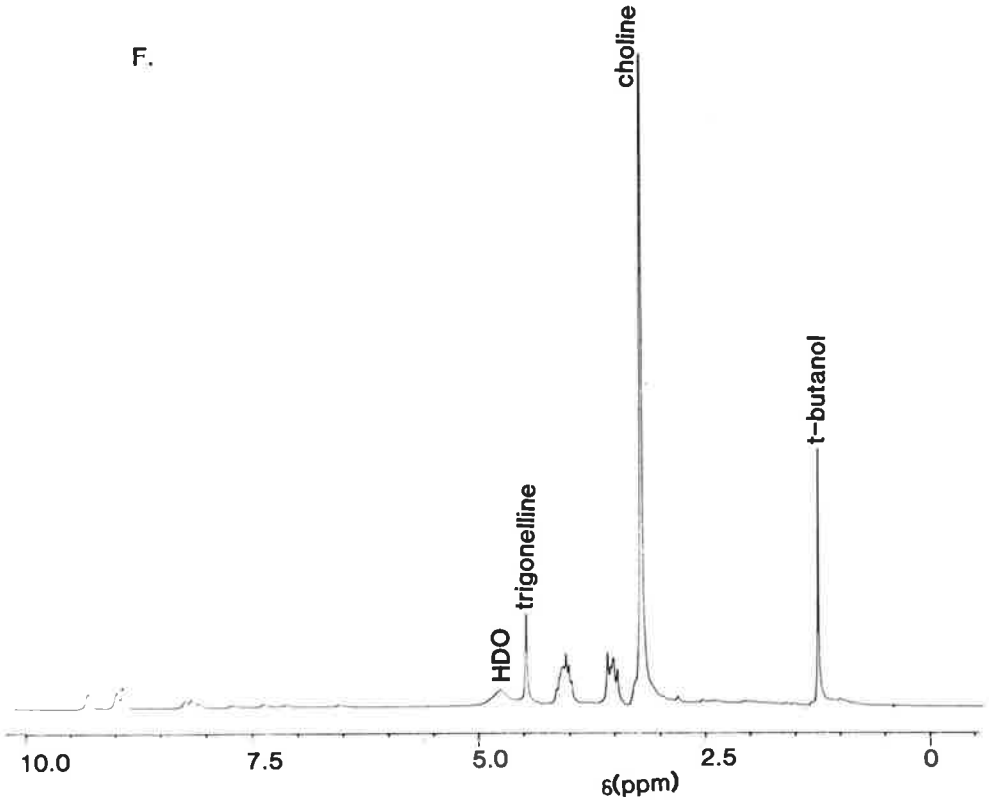
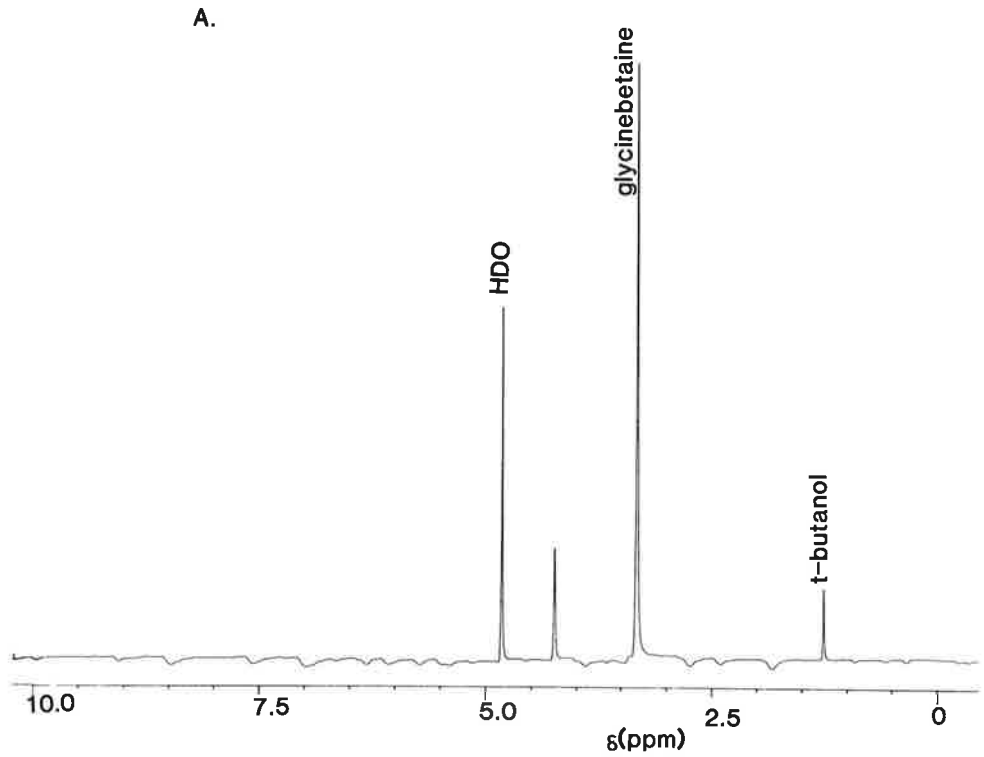

choline
t-butanol
trigonelline
HDO
10.0
7.5
5.0
2.5
0
δ(ppm)

Plate A.2.A ^{1}H NMR spectrum of authentic glycinebetaine

Plate A.2.B ^{1}H NMR spectrum of authentic choline


A.
glycinebetaine
HDO
t-butanol
10.0
7.5
5.0
2.5
0
δ(ppm)

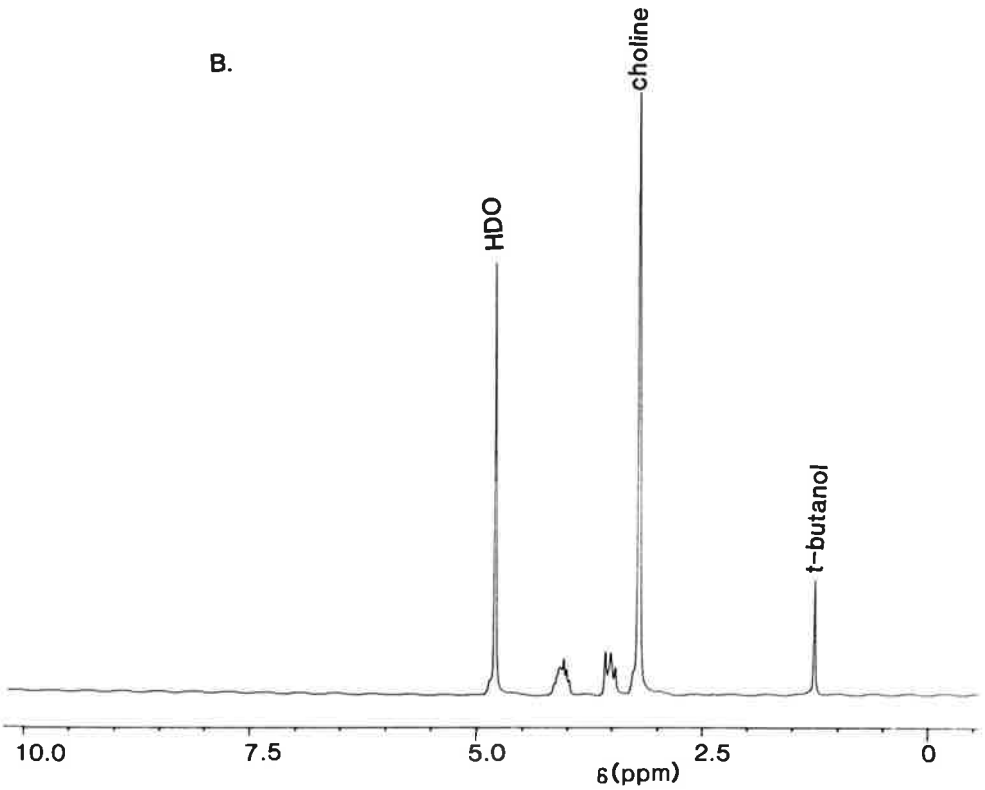
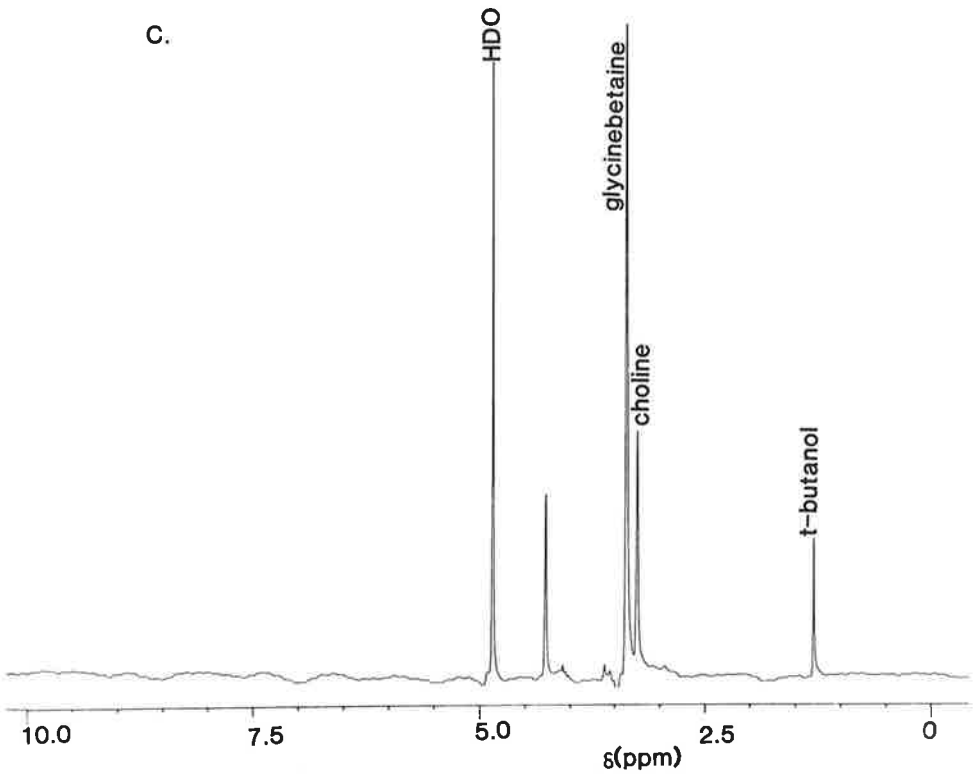

B.
choline
HDO
t-butanol
10.0
7.5
5.0
2.5
0
δ(ppm)

Plate A.2.C ^{1}H NMR spectrum of authentic glycinebetaine and choline

Plate A.2.D ^{1}H NMR spectrum of wheat sample


C.
HDO
glycinebetaine
choline
t-butanol
10.0
7.5
5.0
2.5
0
δ(ppm)

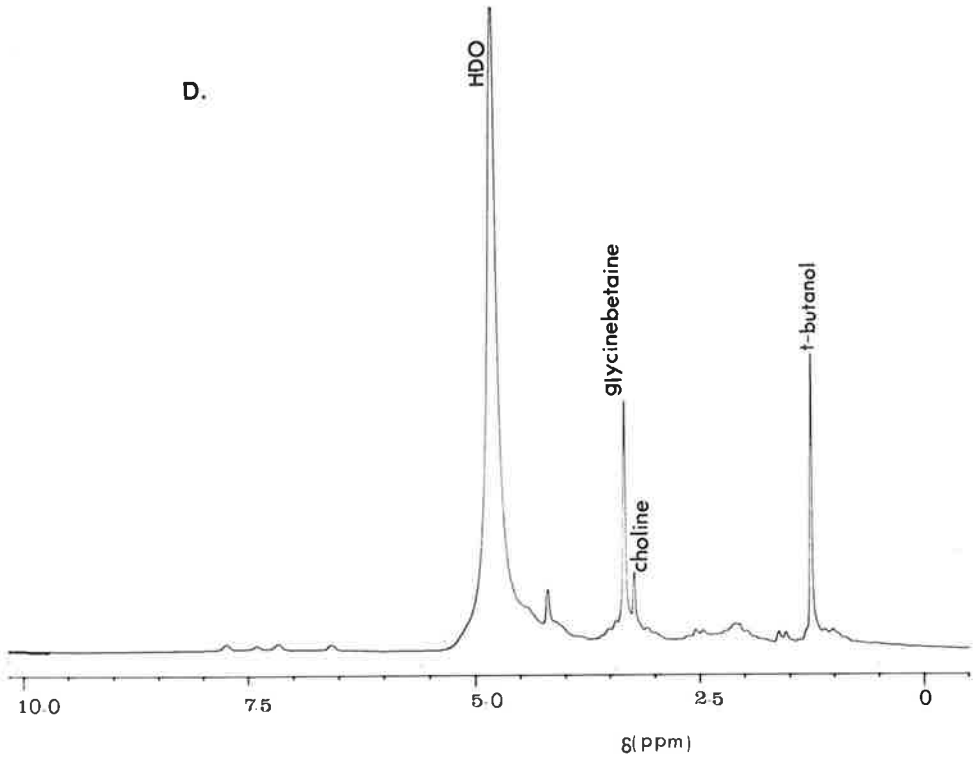
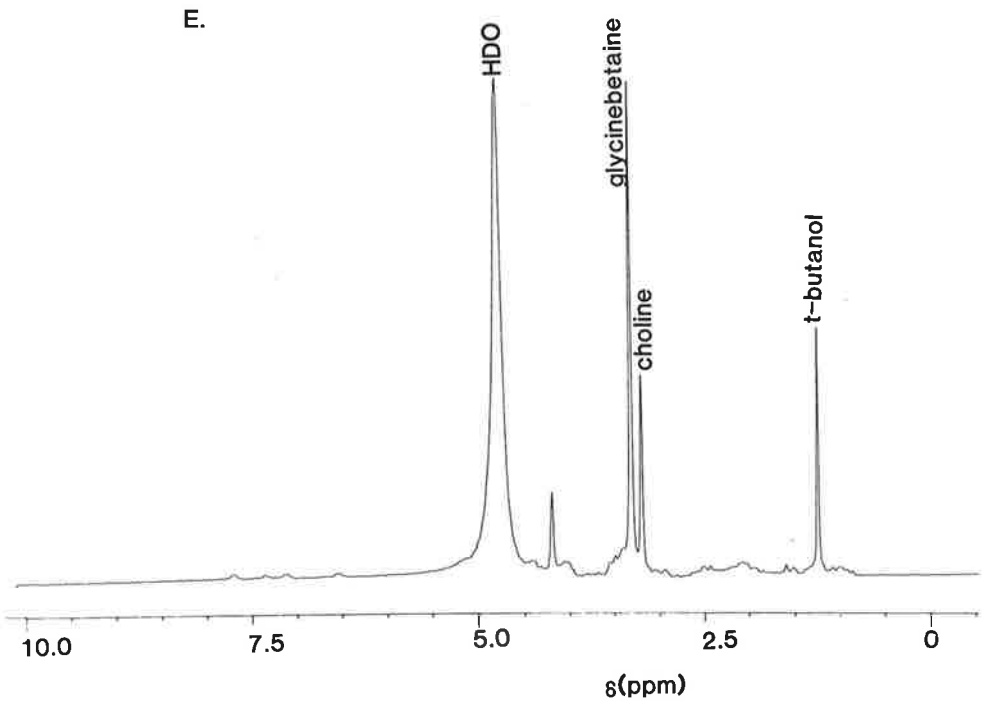

D.
HDO
glycinebetaine
choline
t-butanol
10.0
7.5
5.0
2.5
0
δ(ppm)

Plate A.2.E ^{1}H NMR spectrum of wheat sample spiked with 1mg of authentic glycinebetaine and choline


E.
HDO
glycinebetaine
choline
t-butanol
10.0
7.5
5.0
2.5
0
δ(ppm)

CHAPTER-7

Bibliography

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