# DOCTOR OF PHILOSOPHY

## Water relations of salt stressed wheat

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# WATER RELATIONS OF SALT STRESSED WHEAT

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# ABSTRACT

The present study was conducted to investigate the water relations of individual plant cells and the biophysical parameters controlling plant growth in the context of salt stress. Growth and water relations were studied in growing as well as in mature zones of the first emerged leaf of wheat seedlings (cv. Flanders, a British variety) in the context of NaCl stress. Various levels of NaCl (0, 25, 50, 75, 100, 125 and 150 mol $m^{-3}$) were used to salinize the media.

In the case of leaf elongation rate a two phase response was found i.e. an immediate decrease and then, a recovery in the elongation rate.

Leaf elongation rate decreased within 1-2 minutes of the onset of stress and, later, a recovery started 1-2 h after the salt addition. The time taken for the recovery was proportional to the levels of external salinity. After 24 h the elongation rate was almost fully recovered for all the NaCl concentrations. A similar response was observed when equi-osmolar concentrations (with NaCl) of mannitol were added to the media.

In control plants turgor pressure of the expanding cells was about 0.45 MPa while tissue osmotic pressure was equal to 1.1 MPa showing that the cell had a low water potential (-0.6 MPa). The transpiration tension was equivalent to 0.1 MPa. Turgor pressure in the growing cells did not change after the salt addition (0-150 mol $m^{-3}$ NaCl), however, the tissue osmotic pressure continuously increased with time. Turgor pressure dropped when more than 150 mol $m^{-3}$ NaCl were applied to the media i.e. 200 and 250 mol $m^{-3}$. This is presented as evidence that growing leaf

cells maintained their turgor pressure in response to the salt stress by taking up osmotically active solutes present in the cell wall. The salt stress had not any effect on Instron tensiometric measurements of elastic and plastic extension of the cell wall.

A different turgor pressure response was found in the mature cells. Turgor pressure was about 1.0 MPa, almost twice that in the growing cells, while tissue osmotic pressure was similar to that found in the growing cells i.e. 1.1 MPa. After the application of the stress the turgor pressure dropped within 15-20 min of the application of all the concentrations of NaCl. The osmotic pressure of osmotically active solutes present in the cell wall, $\pi_w$, was almost negligible i.e. $\leqslant$ 0.1 MPa, in mature cells and so could not contribute to turgor maintenance. The extent of decrease was proportional to the external stress of 25, 50 and 75 mol $m^{-3}$ NaCl only. Turgor pressure recovery, due to osmotic adjustment, started after about 10-12 h of the stress initiation. Complete turgor recovery was achieved after 24-48 h of the onset of stress depending on the applied NaCl concentration. Tissue osmotic pressure increased continuously with time. An increase in the $\pi_w$ was inferred during the whole experimental period and after 6 d of the stress application that appeared to correspond to the magnitude of external stress.

The concentrations of major ions and sugars were determined to measure their contribution towards the osmotic adjustment. Under control conditions $Na^+$, $Cl^-$, $PO_4^{3-}$, $SO_4^{2-}$, glucose, fructose and sucrose were present in small amounts, while, $K^+$ and $NO_3^-$ were

the major osmotica. Their concentrations were about 200 mol $m^{-3}$. After the stress a large increase in the concentrations of $Na^+$ and $Cl^-$ was observed, the sucrose concentration increased to a small extent. However, other osmotica remained uniform for whole of the experimental time. A small decrease was observed in $K^+$ concentration in response to higher salt levels.

Volumetric elastic modulus, $\varepsilon$, of mature cells was remained unchanged by the salt stress. However, the apparent resistance of the root cortex to osmotically driven water flow increased with the increase in stress level. No conclusion could be drawn about the contribution of these parameters to the control of growth and to leaf water relations in the context of salt stress.

The possible use of turgor pressure recovery in the mature cells was investigated for assessing the extent of salt tolerance of various Pakistani wheat varieties. These varieties were previously rated according to their performance in absolute grain yield in response to NaCl stress. No simple correlation was found.

# CRYNODEB

Gwnaed yr astudiaeth hon i astudio perthynas dŵr celloedd unigol planhigion a'r pharamedrau bioffisegol sy'n rheoli tyfiant planhigion mewn amgylchedd hallt. Astudiwyd tyfiant a pherthynas dŵr mewn mannau tŵf yn ogystal a mannau llawn dŵf deilen gwenith (cv Flanders, rhywoigaeth o Brydain) dan straen NaCl. Defnyddiwyd sawl lefel o NaCl (0, 25, 50, 75, 100, 125 a 150 mol $m^{-3}$) i halltu'r cyfrwng.

Cafwyd dau gam yng nghyflymder ymestyn y ddeilen hy. lleihad ar unwaith ac yna adferiad yn y cyflymder.

Dechreuai'r lleihad yn ymestyniad y ddeilen o fewn 1-2 funud i gyflwyno'r straen ac yna ceid adferiad ar ôl 1-2 awr wedi ychwanegu'r halen. Yr oedd yr amser a gymerid i adfer y tyfiant yn cyfateb i'r heli a gyflwynid. Ar ôl 24 awr yr oedd y cyflymder ymestyn wedi ei hadfer bron yn llwyr yn achos yr holl lefelau NaCl. Sylwyd mai'r un oedd yr adwaith pan ychwanegid grynodiadau osmolaidd cymesur o fanitol at y cyfrwng.

Yr oedd pwysedd twrgor celloedd y planhigion heb eu trin yn oddeutu 0.45 MPa tra 'roedd pwysedd osmotig y meinwe yn y planhigion yn cyfateb i 1.1 MPa sy'n dangos bod potensial dŵr y gell yn isel (-0.6 MPa). Yr oedd y tyndra trydarthu yn cyfateb i 0.1 MPa. Ni newidiodd pwysedd twrgor y celloedd tyfu ar ôl ychwanegu halen (0-150 mol $m^{-3}$ NaCl), fodd bynnag parhaodd pwysedd osmotig y meinwe i godi gydag amser. Gostyngodd pwysedd y twrgor pan gyflwynid mwy na 150 mol $m^{-3}$ NaCl i'r cyfrwng hy. 200 a 250 mol $m^{-3}$. Cyflwynir hyn fel tystiolaeth bod celloedd tyfu y ddeilen yn cynnal eu pwysedd twrgor dan straen heli drwy amsugno toddion sy'n osmotaidd weithredol yn wal y gell. Ni chafodd yr

heli unrhyw effaith ar allu wal y gell i ymestyn.

Cafwyd adwaith gwahanol mewn pwysedd twrgor celloedd wedi cyrraedd eu llawn dŵf. Yr oedd pwysedd y twrgor yn oddeutu 1.0 MPa, sef bron i ddwywaith yr hyn a geid mewn celloedd a oedd yn dal i dyfu, tra 'roedd pwysedd osmotig y meinwe yn debyg i'r hyn a geid mewn celloedd yn tyfu hy. 1.1 MPa. Ar ôl cyflwyno'r straen gostyngodd pwysedd y twrgor o fewn 15-20 munud i gyflwyno yr holl grynodiad NaCl. Yr oedd y gostyngiad yn cyfateb i 25, 50 a 75 mol $m^{-3}$ NaCl yn unig. Dechreuodd adferiad pwysedd y twrgor o ganlyniad i addasiad osmotig, oddeutu 10-12 awr ar ôl cyflwyno'r straen. Adferwyd pwysedd y twrgor yn llwyr 24-28 awr ar ôl cyflwyno'r straen gan ddibynnu ar grynodiadau'r NaCl. Cynyddodd pwysedd osmotig y meinwe yn gyson gydag amser. Gwelwyd bod lleihad ym motensial dŵr y gell a wal y gell dros gyfnod yr arbrawf ar ei hyd ac ar ôl 6 diwrnod ar ôl cyflwyno'r heli ymddangosai'r crynodiad o doddion yn wal y gell fel petai'n cyfateb i faint o straen a gyflwynid o'r tu allan.

Pennid beth fyddai toddion ionig ac osmotig gyda golwg ar fesur eu cyfraniad at yr ymaddasiad osmotig. Dan amodau arferol yr oedd ychydig o Na, Cl, Ffosffad, Sylffad, glwcos, ffrwctos a swcros yn bresennol. Fodd bynnag K a nitrad oedd y prif osmotica gan fod eu crynodiadau oddeutu 200 mol $m^{-3}$. Ar ôl y straen cafwyd bod cynnydd mawr yn lefelau Na a Cl, er mai cynnydd bychan a gafwyd yn lefel y swcros. Fodd bynnag, arhosodd yr osmotica eraill yr un fath drwy gydol yr arbrawf. Gwelwyd bod lleihad bychan yn y crynodiad o K fel y cynyddid yr heli.

Ni pharodd yr heli unrhyw newid yn y modwlws elastig foliwmetrig. Fodd bynnag, cynyddodd gwrthsafiad ymddangosiadol cortecs y gwraidd i lif dŵr a yrrid gan osmosis wrth i lefel y straen gynyddu. Ni ellid gweld unrhyw berthynas rhwng cyfraniad y paramedrau hyn at reoli tŵf ac at gysyllyiadau dŵr mewn dail pan gyflwynir heli.

Defnyddiwyd adferiad pwysedd twrgor i lunio techneg i fesur i ba radd y gallai gwahanol rywogaethau o wenith Pacistan wrthsefyll halen. Eisioes mesurwyd rhain yn ôl eu gallu i gynhyrchu grawn dan straen NaCl. Ni ellid dod i unrhyw gasgliad o'r profion hyn.

# CHAPTER 1

# *INTRODUCTION*

## 1.1 Salinity

The state and efficiency of agrarian practices define the prosperity level of an agriculture-based country. Agriculture, being an open industry, is exposed to the influence of different types of chemical, physical and environmental conditions. If any of these conditions exceeds the certain specified limit or level, the plants under such conditions will suffer from the stress. These conditions act collectively and under certain extremes can cause colossal damage to crops. Such circumstances are acute in arid and semi-arid regions of the world as a result of high temperature, high light intensity, low precipitation and low relative humidity. Further, the conditions are worsened by poor irrigation and drainage practices resulting in the origin and development of saline conditions in the soil. Soil salinity is a widespread phenomenon on the earth. It affects the substantial areas of the potentially productive lands and has been a serious threat particularly to arid-zone agriculture, since its beginning (Downton, 1984).

### 1.1.1 The global extent

About 30 % of the earth's surface is land area (149.7 million $km^2$) and about 30 % of this i.e. 49 million $km^2$, has been classified as arid and semi-arid (Reeve and Fireman, 1967). About 14.7 million $km^2$ of the land area on the earth is under cultivation (F.A.O., 1978) and 2.3 to 2.4 million $km^2$ is being irrigated for cultivation purposes (Kovda, 1980). It is difficult to know exactly the total area of the salt effected land due to

problems of soil mapping (Flowers and Yeo, 1988) and due to differences in the criteria set and techniques employed. The estimates of salt effected land area vary from less than 1 million $km^2$ i.e. 0.71 million $km^2$ (Maas and Hoffman, 1977a, 1977b) to more than 9 million $km^2$ (Ponnamperuma, 1984). Even the lower limit is approximately equal to the whole geographical area of Pakistan. Other estimates are based on criteria such as; about 13 % of the land under cultivation i.e. 1.94 million $km^2$ (Mudie, 1974) and about 50 % of the irrigated area i.e. 1.18 million $km^2$ (Kovda, 1980) have been classified as salt effected.

### 1.1.2 Pakistan

In Pakistan an area of 20.18 million ha out of the whole geographical area (79.61 million ha; since 100 ha = 1 $km^2$) is under cultivation. About 14.5 million ha is irrigated for agricultural practices; about 11.0 million ha by canals and only 3.5 million ha by tube-wells. However, irrigation is blamed to be responsible for the build-up of salinity as it adds 2 tonnes of salt a year to every hectare of farmland that is watered regularly (Stoner, 1988). The area comprising salt-effected soil is about 5.7 million ha, out of which 3.19 million ha is saline and sodic i.e. having excessive amounts of sodium on exchange sites on the clay (Muhammed, 1983). Therefore, salinity along with sodicity, waterlogging and aridity is a big menace to the crop production of Pakistan (Rashid, 1986).

### 1.1.3 Salt tolerance

Salt tolerance may be defined as the sustained growth of plants in an environment of NaCl and/or combination of mixed salts. Levitt (1980) has associated it with the absence of negative effects on growth in plants which accumulate salts within their tissues. Hence, plants have to exert considerable efforts to sustain their survival in saline environments. In other words, this empirical parameter is attributed to the resistance and survival of any living organism in the saline environment. However, the definition of salt tolerance is still a matter of controversy among the plant breeders (Shannon, 1984). This could be due to the lack of proper understanding of the mechanistic basis of this phenomenon.

#### a) Plant breeding for salt tolerance

In the past various efforts have been made to develop salt tolerant plant varieties in order to utilize the salt affected soils which are otherwise lethal to ordinary plants. These efforts include the selection and breeding of salt sensitive species with their salt tolerant wild relatives. Such attempts were made as early as in the early 40's. In 1941 Lyon studied the response of two species of tomato and their first generation to salinity. Further in 1954 Strogonov has suggested that the improved salt tolerance of plants can be achieved by crossing one selection from a saline field with a vigorous plant taken from non-saline locations and then screening the progeny of subsequent generation in saline medium. However, the hybrids produced by

such processes have an increased vigor more than salt tolerance and that will be lost in subsequent generations (Shannon, 1984). The use of 'wild' germplasm as suggested by Bernstein (1961) for breeding for improving the salt tolerance of plants has been quite popular among breeders.

**1) Flaws in breeding practices**

The fundamental selection criterion for barley, wheat and tomato has been the plant survival at high salt concentrations (Rush and Epstein, 1976, 1981; Epstein and Noryln, 1977). However, the mechanisms that plants adopt for their normal growth might not be the same which are necessary for the maintenance of high growth rates under saline conditions. Hence, the adaptive mechanisms of plants in response to salinity such as temporary dormancy, increased succulence or shortening of growth period (Levitt, 1980) contribute nothing to shoot and root lengths, dry weight, number of spikelets and number of tillers (Shannon, 1984) - the parameters commonly used by breeders (Ashraf et al. 1987, 1986a, 1986b). Because a number of criteria have been used for assessing the salt tolerance of plants, breeders do not seem to agree on a particular parameter to be considered as a criterion for selecting the salt tolerant species. The complexity of the phenomenon of salt tolerance and its lack of proper understanding apparently influences the plant breeding practices towards developing the salt tolerant varieties (Shannon, 1984; 1985; Epstein, 1985).

Study of salt tolerance is again complicated by various factors

such as variations in plant growth rate, growth pattern and the life history of plants. Further, the environmental and chemical factors such as relative humidity, temperature and ionic balance etc. also interfere, through changes in tolerance with the plant (Greenway et al. 1983). There is no clear-cut demarcation between the salt tolerant (halophyte) and salt sensitive (glycophyte) plants which are categorized on the basis of their ability to thrive under saline conditions. There is a continuous spectrum from highly salt tolerant to highly sensitive species (for further information see Wyn Jones, 1981).

In the case of wheat plants there a marked variations has been observed in the extent of their salt tolerance (Epstein et al. 1980; Qureshi et al. 1980). Recently, Rashid (1986) has classified the wheat varieties into 4 different categories depending on their performance in saline environment, as;

a) tolerant,

b) moderately tolerant,

c) moderately sensitive,

d) sensitive varieties.

Since this categorization is based on the study of various selection criteria such as weight of shoot and root, shoot height, number of tillers and/or green leaves and the plant mortality. It may not be truly representative of the adaptive physiological mechanisms of plant as the latter have no contribution to these selection criteria (Shannon, 1984).

b) Associated parameters

Most of the criteria used are without the understanding of plant mechanisms which enable them to survive and even to flourish in the environments which are fatal to other plants. Therefore, more work needs to be done to establish a strong relationship between breeding markers and the plant mechanisms (Shannon, 1984).

This project represents an attempt to asses certain well defined water relations parameters for their suitability as breeding parameters.

1) Compatible solutes

A similar approach has been used in the field of solute relations. One characteristic that has been considered by others in the laboratory of Prof. R.G. Wyn Jones at Bangor is the level of "compatible solutes". Under saline conditions a large amount of salt (mainly NaCl) accumulated in the leaf vacuoles and contribute towards the osmotic adjustment. It results in the tissue concentrations of about 200 mol $m^{-3}$, while the concentration of inorganic ions in cytosol is held in range of 100-200 mol $m^{-3}$ and the cytosol shows a strong selectivity for potassium over sodium, magnesium over calcium and phosphate over chloride or nitrate (Gorham et al. 1985). High concentrations of some cytosolic solutes can be lethal to the metabolic processes. However, in the vacuole even higher concentrations are benign to its biochemistry. Most of the metabolic enzymes and processes occur in the cytosol. A group of molecules known as the "compatible solutes" (Brown and Simpson, 1972; Borowitzka and

Brown, 1974) have been found in the cytosol. These includes two main groups of molecules;

a) polyhydric alcohols and their derivatives e.g. sorbitol, pinitol, sucrose etc.,
b) small zwitterions such as and similar to proline and glycinebetaine (N, N', N''-Trimethyl glycine) (Wyn Jones and Gorham, 1983; Wyn Jones, 1985).

The accumulation of the compatible solutes have been reported in different plant species e.g. proline accumulation in barley and wheat (Chauhan et al. 1980) and glycinebetaine deposition in wheat (Rashid, 1986). These solutes are quite non-inhibitory to the cytosolic metabolism (Storey and Wyn Jones, 1985) and even enhance enzyme activity e.g. malate dehydrogenase in the presence of monovalent salts (Pollard and Wyn Jones, 1979). Their presence also helps in the maintenance of the osmotic equilibrium across the tonoplast (Geenway and Munns, 1985; Gorham et al. 1985).

## 2) $K^+/Na^+$ ratio

Another character that has been successfully worked upon in the same laboratory is that of $K^+/Na^+$ uptake discrimination. Under salt stress plants start taking up more potassium ions than the sodium and the phenomenon of salt exclusion (mostly $Na^+$ and $Cl^-$) takes place in the leaves. Later these toxic ions are extruded out of the roots back into the external media. $Na^+$ exclusion has been studied in various plant species i.e. soybean (Abel, 1969), tomato (Rush and Epstein, 1981) and wheat grass (Shannon, 1978).

This selectivity can be estimated from the plant ion contents by the so-called 'selectivity ratio'. This is the ratio of $K^+/Na^+$ in the plant divided by $K^+/Na^+$ in the medium and given the symbol $S_{K,Na}$ (for details see Flowers and Yeo, 1988). The mechanism of ion discrimination increases the salt tolerance and results in increased $K^+/Na^+$ ratio in the leaves (Wyn Jones, 1981; Salim and Pitman, 1983).

In selecting a clan of potentially useful breeding characteristics we have available certain guidelines.

### 1.1.4 Transmission of salt effects to plant growth

The detrimental effects of salinity on plant growth are transmitted through the;

a) specific ion effects,

b) ion imbalance,

c) decreased water availability,

(Levitt, 1980; Wyn Jones, 1981). However, the relative importance of these stresses is quite controversial (Greenway and Munns, 1980; Munns and Termaat, 1986).

#### a) Specific ion effects

The study of effects of salts/ions on plant growth is quite complex due to the involvement of various fundamental processes of plant nutrition. However, the importance of such effects on plant growth is claimed to be quite dominant in mature and growing leaves after a long exposure to saline conditions (Munns

and Termaat, 1986). The excessive salt uptake and accumulation in plant is a specific and unavoidable consequence of high salt concentration present externally. Therefore, the "ion excess" has been designated as the main reason for decreased plant growth and increased plant susceptibility to the salt stress. Among those ions the $Na^+$ and $Cl^-$ are considered to be the most toxic to the plant metabolism under such conditions (Greenway and Munns, 1980).

b) Ion imbalance

The predominant accumulation of certain ions under salinity causes the disturbance in already existing ionic ratios in plant cells. Ion imbalance is the consequence of such disturbed ratios. Therefore, some ions are "preferred" by plants over others to avoid such imbalances and probably ions would also be competing to be taken up by the plants. The $Na^+$ and $Ca^{2+}$ are found to be quite antagonistic in the uptake of each other (Bernstein, 1975). A similar relationship has been observed between $K^+$ and $Na^+$, as well (Jeschke and Nassery, 1981). The effect of $NO_3^-$ concentration on the $Cl^-$ content in plant tissue has been found in some plant species (Deane-Drummond and Glass, 1982).

c) Decreased water availability

Salt presence in the root medium induces the lower water potential that ultimately results in reduced availability of water to plants. The resulting water potential gradient between plant and the medium causes water efflux from the plant roots

resulting in plant dehydration (Steponkus, 1980). Salt tolerant plants cope with this problem by adjusting their internal water potential as a result of the process of osmotic adjustment (Greenway and Munns, 1980). This process even reduces the requirement for increased cell wall extensibility, leaf thickness and water permeability that might otherwise be required for the maintenance of growth and turgor pressure in plants at low external water potential (Azhar, 1988). The dependence of growth on the external water potential has been studied in various plant species (for details see Rashid, 1986). The extent and duration of osmotic and salt stresses is determined mainly by the rate of ion uptake in roots, therefore, both of these stresses can be inter-related. Since we have chosen to asses the behaviour of plants to changes in their water relations a more detailed discussion of this aspect will now be made.

## 1.2 Water Relations

In this study the advantages of recent technical developments have been exploited to extend the study of salt tolerance to an understanding of the influence of salt on single cell water relations which will now be considered in further details.

### 1.2.1 Water potential

The chemical potential of a species represents the amount of free energy associated with it that is available for work (Nobel, 1983). It is equivalent to the work required for transporting a mole of a material from a system to a reference point in its

surroundings (Gibbs, 1951). The chemical potential of a species can be compared on two sides of a barrier to know whether the system is at equilibrium or not. If not, a net spontaneous movement of species would take place from a region of high chemical potential to one where it is low. It can be described by the equation;

$$\mu = \mu^* + RT\ln a_j + V_jP + Z_jFE + m_jgh \qquad (1.1)$$

$\mu$ = reference value + concentration/temperature + pressure component + electrical component + gravitational component

where $\mu$ = chemical potential of a species j in the solution, $\mu^*$ = $\mu$ at a reference point, R = gas constant, T = absolute temperature, $a_j$ = activity of j, $V_j$ = partial molar volume, P = pressure, $Z_j$ = the charge number of an ionic species, F = Faraday constant, E = electrical potential of the region, $m_j$ = mass of species per mole, g = the acceleration due to gravity, and h = height.

The electrical component of the equation becomes zero when water and uncharged solutes are taken into consideration. At the individual cell or tissue level, the difference of h becomes negligible when distance between two points in a system is smaller than the range of other components, it can therefore be safely ignored at cellular resolution. Under such conditions the equation 1.1 becomes;

$$\mu - \mu^* = RT\ln a_j + V_jP \qquad (1.2)$$

The activity of water is related to the osmotic pressure, $\pi$, of a solution by the fundamental definition of the latter, which describes, as well, the dependence of $\pi$ on solute concentration

$$RT\ln a_w = - V_w \pi \qquad (1.3)$$

where subscript, w, represents water and chemical potential difference between water at two points is represented as follows.

$$\Delta \mu_w = \Delta (-V_w \pi + V_w P) \qquad (1.4)$$

if $V_w$ is uniform in the system, then

$$\Delta \mu_w = V_w (P - \pi) \qquad (1.5)$$

This equation can be used for the description of two phases of an aqueous solution at equilibrium across a semi-permeable membrane. The water potential, $\psi$, of a solution can be derived by combining the equations 1.1 and 1.3 provided the electrical term is zero for neutral water. Therefore,

$$\mu_w = \mu_w^* - V_w \pi + V_w P \qquad (1.6)$$

if $V_w$ is constant in a system,

$$\frac{\mu_w - \mu_w^*}{V_w} = \frac{V_w P}{V_w} \quad \frac{V_w \pi}{V_w}$$

$$\psi = \frac{\mu_w - \mu_w^*}{V_w} = P - \pi \qquad (1.7)$$

It indicates that $\psi$ can be calculated from the value of P and $\pi$. The water potential is the chemical potential (free energy per unit volume) of matrically-bound, pressurized or osmotically-constrained water compared to the chemical potential of pure water at atmospheric pressure and at the same temperature. A spontaneous water movement takes place from a region of high $\psi$ to one with low $\psi$. An increase in cell turgor pressure raises the internal water potential and decreases simultaneously the water influx. While an increase in the cell osmotic pressure by solute accumulation lowers the internal $\psi$, raising the water influx which results in increased cell turgor pressure. At equilibrium no flow will take place across the cell membrane, as $\psi$ would be equal on either side of the plasma membrane.

The term '$\rho_w gh$' must be included in the equation 1.7 when water moves over an appreciable distance vertically in a gravitational field (movement in xylem tissue of a tall tree). The magnitude of '$\rho_w gh$' is 0.01 MPa $m^{-1}$ i.e. if water raises 10 m vertically of the xylem in a tree, the gravitational contribution to the water potential will be increased by 0.1 MPa (Nobel, 1983).

The equation 1.7 describes the two important components of water potential i.e. osmotic and turgor pressure which are described as follows.

### a) Osmotic pressure

When a solution is separated from water by a semi-permeable membrane a net flux of water takes place into the solution, as water potential is higher in the pure water than in the solution. This process is osmosis and the positive pressure applied to the solution necessary to prevent the net flow of water is the osmotic pressure, $\pi$. Osmotic pressure represents the amount of osmotically active solutes present in any solution. The presence of solutes in an aqueous solution tends to decrease the activity of water ($a_w$), that results in lowering of the chemical potential of water. An increase in the concentration of solutes raises the osmotic pressure indicating that $\pi$ and $a_w$ change in opposite direction, see equation 1.3.

There is much variation in the use of the terms "osmotic pressure" and "osmotic potential", as well as the algebric sign of the quantity (for details see Nobel, 1983). We chose the term osmotic pressure for this study.

### b) Turgor pressure

A semi-permeable membrane, the plasma membrane, surrounds the plant protoplast which can expand or contract in response to a change in the external osmotic pressure. The plasma membrane can maintain a higher concentration of solutes in the protoplast than in the cell wall and may result in the movement of water into the cell until the $\pi$ is balanced by the corresponding turgor pressure generated. Wall-less cells can not generate an appreciable turgor pressure and cell volume responds rapidly to osmotic pressure

fluctuations (Zimmermann and Steudle, 1978). Such cells would have developed transport systems (e.g. $Na^+$ extrusion pump; Gutknecht et al. 1978) to export osmotically active solutes out of the cells against their electrochemical gradients. Thus a volume equilibrium is attained at the expense of metabolic energy which helps in the maintenance of internal osmotic pressure.

The movement of water into the walled cells in response to osmotic gradient between cell and cell wall generates a positive pressure in the cell. Hence, the value of the pressure generated depends on the extent of the osmotic gradient since turgor pressure is the difference of the cell and cell wall osmotic pressures. Many metabolic processes taking place in plants are quite sensitive to the turgor pressure such as $K^+$ transport across plasma membrane in Valonia species (Gutknecht, 1968) and in higher plants (Zimmermann, 1978). The detailed description of such processes is presented in Zimmermann (1978) and Tomos (1988). Turgor pressure plays an important role in cell enlargement, guard cell movements and other processes dependent on changes in cell volume. Permeability to water and ions is affected by turgor pressure (Kramer, 1983). In the control of growth turgor pressure enjoys a central role as it is an integral part of Lockhart equation, when water conductivity to the expanding zone is not limiting (see equation 1.12).

## 1.3 Growth

Growth includes all the processes in an organism which result in

an increase in the volume, weight, cell number, amount of protoplast and complexity. In some systems, over a short period, growth can be represented by an increase in the tissue length only. This incease is generally the sum of the individual cell expansion taking place within the growing region of the tissue (Green et al. 1971; Pritchard, 1988).

Various growth models have been proposed to describe the biophysical parameters involved in control of growth such as a model for maize root growth (Grenetz and List, 1973), a membrane model (Hettiaratchi and O'Callaghan, 1974) and fluid dynamic model (Silk and Erickson, 1979). The most extensively used model for describing the physics of growth was developed by Lockhart (1965). This model was popularized later by Ray et al. (1972). The model is based on two equations; the first describes wall rheological properties and relates the wall pressure linearly, once the yield threshold is exceeded:

$$\frac{1}{V}\frac{dV}{dt} = \phi\,(P - Y) \qquad (1.8)$$

where $\phi$ = cell wall extensibility ($s^{-1}$ $MPa^{-1}$), P = turgor pressure (MPa), Y = yield threshold (MPa), and $\frac{1}{V}\frac{dV}{dP}$ = relative rate of volume increase.

The second equation describes the volumetric increase in cell size and relates it to the hydraulic conductivity of the water pathway into the cell.

$$J_v = L_p.A\,(\sigma\,\Delta\pi - P)$$

$$\frac{J_v}{V} = Lp \frac{A}{V} (\sigma \Delta\pi - P)$$

As $\frac{J_v}{V} = \frac{1}{V} \frac{dV}{dt}$, and $Lp \frac{A}{V} = L$

therefore,

$$\frac{1}{V} \frac{dV}{dt} = L (\sigma \Delta\pi - P) \qquad (1.9)$$

Where L = volumetric hydraulic conductivity ($MPa^{-1}$ $s^{-1}$), $\sigma$ = reflection coefficient (dimensionless), A and V are surface area and volume of the cell, respectively.

The ($\sigma \Delta\pi - P$) can be equal to the water potential gradient ($d\psi$) between two compartments provided the cell behave like an ideal osmometer, i.e. if $\sigma$ of the plasma membrane were unity.

In transpiring plants a hydrostatic tension is created in the cell wall which is a component of wall water potential (Tomos, 1985). Therefore, under transpiring conditions equation 1.9 can be modified as;

$$\frac{1}{V} \frac{dV}{dt} = L (\sigma \Delta\pi - [P - Pw]) \qquad (1.10)$$

where Pw = cell wall transpiration tension (MPa). A growth equation can be formulated by combining the equation 1.8 and

1.10.

$$\frac{1}{V}\frac{dV}{dt} = \frac{\phi . L}{\phi + L}(\sigma \Delta\pi - Y + Pw) \qquad (1.11)$$

This equation contains information about the effects of water flow, cell wall properties, transpiration tension and yield threshold on the cell expansion growth. If water supply to the cell is not limiting i.e. $L >> \phi$, the cell will be close to the osmotic equilibrium and turgor pressure will be equal to ($\sigma \Delta\pi$ + Pw) (Ray et al. 1972; Tomos, 1985). Equation 1.11 would change to equation 1.8.

$$\frac{1}{V}\frac{dV}{dt} = \phi (P - Y) \qquad (1.12)$$

Where, $\frac{1}{V}\frac{dV}{dt}$ = relative growth rate.

On the other hand if $L << \phi$ the turgor pressure of the cells will be equal to the yield threshold beyond which growth ceases (Tomos, 1985).

The growth rate can be modified by a change in any of the parameters i.e. $\phi$, P, Y and L. Under short duration of time where Y and $\phi$ are almost unchanged the relative growth rate changes with the change in turgor pressure. The ideal behaviour of cell wall growth as a function of cell turgor pressure is shown in Fig. 1.1. This is not always the case since growth rate is found

to be independent of turgor pressure in some instances e.g. in leaves of Lolium temulentum and soybean under temperature and water stress, respectively (Thomas et al. 1989; Nonami and Boyer, 1989). Nevertheless, turgor pressure is believed to provide a driving force in the start/initiation of the cell growth.

Plant growth is affected by many physical and chemical treatments e.g. light (Van Volkenburgh and Cleland, 1981), ions (see Taiz, 1984), hormones (see Cleland, 1986), solute supply (Cleland, 1967), anoxia (Smit et al., 1989) and genetic factors (Sakurai et al., 1984). Therefore, one basic parameter can not be considered being responsible for all these changes. For instance, in bean plants, leaf expansion is inhibited by salinity stress through the reduction in turgor pressure, whilst wall extensibility is found to be almost unchanged in this context (Neumann et al. 1988).

Expansion growth in plant cells can be described in physical terms (Tomos, 1985; Cosgrove, 1987; Tomos, 1988; Tomos et al., 1989) and can be outlined as a cycle of events (Figs. 1.2 and 1.3). These are as follows;

a) Wall tension,

b) Wall loosening,

c) Decreased turgor pressure,

d) Osmotic disequilibrium,

e) Water influx,

f) Turgid cell,

g) Solute influx.

All these events are dependent on each other and presumably take place simultaneously (Tomos, 1985), and each of the parameters may be a possible point for the regulation of growth (Cosgrove, 1987). Some measurable physical parameters are required for the quantitative determination of these processes as described above.

### 1.3.1 Plant growth in response to salt stress

Salinity affects plants at all stages of development and sensitivity varies from one growth stage to another. Wheat, barley and rice are quite sensitve at the seedling stage prior to tillering. Rice is also sensitive during pollination (Maas, 1984; Rashid, 1986). Beets are fairly sensitive during germination but their tolerance grows with time and results in highly tolerant plants (Bernstein, 1966). In the plant both leaf and root growth are influenced by salinity but leaf growth is more senitive to it this results in increased root : shoot ratio (Läuchli and Epstein, 1984; Munns and Termaat, 1986).

The basis of leaf growth decline under such conditions is not fully understood. In this context several hypotheses have been put forward e.g. some metabolic constraints resulting from adaptive changes necessary for salt tolerance can also be responsible for reduction in the growth such as reduction in the cell wall plasticity and cell elongation (Aceves-N et al. 1975; Yeo, 1983), an increase in respiration rates as a result of high energy requirements (Schwarz and Gale, 1981), increased resistance to water flow in the leaf growing zone (Munns et al.

1982), changes in metabolic processes i.e. enzyme activity, protein synthesis, nitrogen absorption and assimilation and photosynthesis (Poljakoff-Mayber, 1982; Flowers, 1985) reduced capacity for carbon assimilation by reducing area of photosynthetic surface (Papp et al. 1983). Water deficit in the elongating tissue due to saturation of ion uptake by individual cells (Delane et al. 1982) is also thought to be responsible for that along with certain osmotic effects on the roots and a subsequent message to the leaves (Munns and Termaat, 1986). All these rationales are based on whole plant basis and most of the studies are carried out in long term (weeks and months) exposure of plants to the salt stress.

For short term experiments, the measurements of growth of "linear" tissues can be conveniently characterized by the increase in tissue length (Pritchard, 1988). The growth inhibition could be due to osmotic or ionic components of salinity acting on biophysical and on metabolic components of expansive growth (Thiel et al. 1988). The biochemical mechanisms must always come down to biophysical components to affect growth. Experimental evidences has been shown that leaf growth rates fall within minutes of plant exposures to stress e.g. water stress (Acevedo et al. 1971; Nonami and Boyer, 1989) and salt stress (Matsuda and Riazi, 1981; Delane et al. 1982; Thiel et al. 1988). Likewise, the growth recovery in the same tissue on the removal of the stress was quite rapid (Acevedo et al. 1971; Matsuda and Riazi, 1981; Munns et al. 1982). The rapid recovery suggests inadequate turgor pressure in the growing cells to be the cause of reduced growth as it responds very quickly to the rise in

water potential on the removal of stress (Termaat et al. 1985). It could also be the result of stored growth as metabolic processes for cell wall relaxation might have proceeded unchecked during the stress period. The growth rate of leaves is quite sensitive to various environmental stresses (Terry et al. 1983) and it reduces in response to some other stresses as well i.e. drought (Clough and Milthorpe, 1975), nutrient deficiency (Schmalstig and Cosgrove, 1988), temperature (Thomas et al. 1989) and root hypoxia (Smit et al. 1989).

### 1.3.2 Cell wall: Its significance in growth

The cell wall is involved in growth processes of plants. As noted above growth rates may be controlled by either the membrane property, $L_p$ (hydraulic conductivity) or the wall properties ø and Y (Equation 1.11). Among these it has been argued that the predominant parameters in controlling growth are the wall properties, ø and Y (Tomos, 1988). Under stressed conditions changes in the gradients cause the growth inhibition initially but eventually cell walls loose extensibility and specific proteins accumulate so that metabolic changes in the wall probably limit later growth (Nonami and Boyer, 1989). Involvement of cell wall properties such as rheology (Cleland, 1981; Greenway and Munns, 1983; Pritchard et al. 1987), the physical state of cell wall and the solutes present in it (Oertli, 1969; Bernstein, 1971; Cosgrove and Cleland, 1983a; Clipson et al. 1985) has already been postulated. Cell wall properties as a major limiting parameter for growth has also been suggested by Tomos (1985). He

states in his review that the constancy of turgor pressure at different growth rates using tissue-averaged methods (Matsuda and Riazi, 1981; Michelena and Boyer, 1982; Matthews et al. 1984) and using pressure probe (Pritchard et al. 1987; Rich and Tomos, 1988; Thomas et al. 1989) implies the involvement of ø in control of growth. However, in saline conditions quite opposite has been observed as there is a decrease in turgor pressure but no change in ø in bean leaves (Neumann et al. 1988).

Keeping in mind the importance of ø in growth some experiments in our research work are designed to measure it in context of NaCl stress where ø will be measured using the Instron tensiometer (Van Volkenburgh et al. 1983).

## 1.4 Osmotic Adjustment

The term osmoregulation describes the behaviour of cell or tissue osmotic pressure in response to the external osmotic perturbations (Reed, 1984). Cram (1976) suggests that regulation represents the case of adjustment of a certain parameter to some already established level, standard or reference. Osmotic pressure is not a conserved parameter in most higher plants under such conditions, the term 'osmoregulation' should be replaced by a more appropriate term i.e. osmotic adjustment.

A change in the external water potential initiates two independent responses (Tomos, 1988) i.e.,

a) physical osmotic adjustment represented by the physical relaxation of the cell wall, this is a passive process,

b) biochemical osmotic adjustment namely the transport and/or metabolic generation of cell solutes to recover osmotic and turgor pressures, this would be active process.

### 1.4.1 Physical osmotic adjustment

The volumetric elastic modulus, ε, is an empirical parameter that describes the elastic properties of the cell wall that help in the process of osmotic adjustment. It represents the change in cell volume in response to a change in the turgor pressure to the corresponding change in volume and determines the slope of the pressure/volume curves. Cell volume is a function of the turgor pressure. This relation was first developed by Philip (1958).

$$\varepsilon = V \frac{dP}{dV} \simeq \frac{\Delta P}{\Delta V} \qquad (1.13)$$

where dV and dP are differential changes in the cell volume and cell turgor, respectively. It could not be measured for single higher plant cells in situ until the development of pressure probe (Hüsken et al. 1978). Many attempts were made to estimate it indirectly by two different techniques; vapour pressure equilibrium (Gardener and Ehling, 1965) and the pressure bomb or chamber (Scholander et al. 1964; Tyree and Hammel, 1972). Pressure volume/curves were made in both of the cases and weight averaged bulk values of P and ε of the tissue were obtained. A strong dependece of ε on the P was observed (Dainty, 1976). These techniques were criticised due to some assumptions made therein

such as considering the cell wall solutes as negligible and due to weight-averaged values and the longer time required for the individual measurements (Tyree and Jarvis, 1982; Jones, 1985). These techniques can be useful for the tissues having cells of the uniform volumetric elastic modulus and not for others (Steudle et al. 1977).

In addition to providing a means of direct turgor pressure measurements the pressure probe facilitates the measurements of ε in individual cells of giant algal cells (Steudle and Zimmermann, 1971; Frey et al. 1988) and in higher plants (Hüsken et al. 1978; Tomos et al. 1981; Steudle et al. 1982; Cosgrove, 1985; Shackel, 1987; Pritchard et al. 1987). The technique allows the quick measurements hopefully without the danger of irreversible plastic deformation that may take place on application of the previous methods. It has been confirmed with the technique, that ε is a function of turgor pressure and of the cell volume (Zimmermann and Steudle, 1978; Tomos and Zimmermann, 1982). The value of ε measured rapidly and at longer time span have been termed as instantaneous ($\varepsilon_i$) and stationary elastic moduli ($\varepsilon_s$), respectively (Zimmermann and Hüsken, 1980). They claim that the $\varepsilon_i$ rather than $\varepsilon_s$ determines the rate of volume change following a rapid water potential changes in H. pervula, that seems to be more appropriate.

Volumetric elastic modulus (ε) plays an important role in the relative proportion of osmotic and turgor pressure changes in response to external water potential perturbations. This parameter is thought to be the first under the plant's control

that responds during turgor and osmotic adjustment. Plant cells under stress conditions (water, osmotic and salt) are thought to maintain turgor pressure by exploiting the ε (Tomos, 1988). The role of ε in water stress tolerance has been advocated in higher plants (Bradford and Hsiao, 1982; Robichaux et al. 1986). The ε value decreases with the increase in stress magnitude and helps in turgor maintenance at the expense of loss in cell volume (Hsiao, 1987). Thinking that plants would adjust to osmotic and salt stress through the similar mechanism as observed in water stress, several attempts have been made to investigate the role of ε under such conditions. In response to osmotic stress the ε is reported to increase in single-celled alga Eremosphaera viridis (Frey et al. 1988). Whilst in salt stress the observations from experiments in the growing (Margritz and Cosgrove, 1987) and mature zone (Rygol et al. 1989) of leaves have been quite different. Margritz and Cosgrove (1987) report the growing cells of Pisum sativum stem to have unaltered ε under salt stress. The findings of Rygol et al. (1989) in Mesembryanthemum crystallinum in leaf mature cells show no decisive trend due to large data variations.

It is thought that in the leaf the turgor pressure maintenance at the expense of cell volume loss may be more important in the growing zone than in the mature zone as the critical turgor pressure needs to be maintained for the growth of cells (Tomos, 1988). In mature cells a decrease in tissue volume may have detrimental effects, due to reduced leaf surface, on various plant metabolic processes e.g. photosynthesis, photomorphogenesis etc. In the light of this, the role of ε in turgor pressure

maintenance of leaf mature zone in response to salt stress still needs to be investigated.

### 1.4.2 Biochemical osmotic adjustment

Plants can adjust their turgor and osmotic pressures biochemically following a change in the external water potential. This can be achieved either by transporting the ionic solutes into the cell/or by the metabolic generation of organic solutes within the cell. These mechanisms lead to two different types of adjustments (Tomos, 1988);

a) recovery of initial osmotic pressure i.e. change the turgor pressure to the same extent as that of the water potential i.e. osmoregulation,

b) recovery of initial turgor pressure i.e. modify the osmotic pressure to the same degree as the change in the water potential i.e. osmotic adjustment.

In the systems studied to date, with a few exceptions amongst the algae, osmotic adjustment is favoured (Tomos, 1988). Porphyra, however, exhibited large rhythmic changes in cell turgor pressure in response to the changes in external $\psi$, the turgor pressure values for sea water and fresh water were 0.2-0.3 MPa and 2.5 MPa, respectively (Wiencke et al., unpublished). Therefore, the turgor regulation is suggested for the plants growing in low water potential environments. However, in fresh water conditions external osmotic pressure is too low to bring about large changes in cell turgor pressure, therefore, osmoregulation will result in

effective turgor maintenance (Bisson and Barthlomew, 1984).

The degree of turgor maintenance in response to water and salt stress in higher plants was first reviewed by Hsiao et al. (1976). In response to water stress the maintenance of turgor, by increasing osmotic pressure, has been reported in maize roots (Acevedo et al. 1979) and the elongating regions of maize leaves (Michelena and Boyer, 1972). Recently Nonami and Boyer (1989) have studied complete turgor maintenance in soybean leaves using the pressure probe. They found that turgor decreased in a small number of cortical cells near the xylem which recovered within 30 minutes of the stress initiation. However, no change could be detected in most of the cells in the growing zone.

Under saline conditions partial or complete turgor maintenance has been recorded in various species. With indirect measurements of turgor pressure this phenomenon has been studied in barley leaves (Matsuda and Riazi, 1981; Munns and Passioura, 1984), in Acer pseudoplatanus (Pennarun and Maillot, 1988) and in wheat (Termaat et al. 1985). The pressure probe facilitated this study at the individual cell level in Mesembryanthemum crystallinum (Rygol et al. 1989), in Suaeda maritima (Clipson et al. 1985) and in barley leaves (Thiel et al. 1988).

Since cell wall plays a significant role in the process of biochemical osmotic adjustment (Tomos, 1988) a description of it as a compartment may help in better understanding of water relations of the cell as well as of the cell wall.

## a) Cell wall/apoplast

### 1) Role of cell wall in osmotic adjustment

Cell turgor pressure can be regulated by cell wall osmotic solutes. Oertli (1968) was the first to comment about the role of extracellular salt accumulation in imposing the deleterious effect on plant growth and metabolism under saline conditions. Two different types of solutes are present in cell wall;

a) those held by Donnan forces,
b) those present in the wall aqueous solution i.e. non-Donnan solutes.

Donnan solutes increase cell wall osmotic pressure ($\pi_w$) but their contribution is balanced by an exact value of Donnan hydrostatic pressure being produced as result of the former (Tomos, 1988). Therefore, the contribution of Donnan solutes, as a whole, to the net wall water potential is negligible. Therefore, these do not interfere with the cell turgor pressure. On the other hand osmotically active "free" solutes do. In the past it was quite difficult to assess the concentrations of these wall solutes, so it has been assumed that almost negligible amounts of such solutes occupy the cell wall (Tyree and Hammel, 1972). The assumption was based on the observations that the xylem sap expressed with a pressure bomb has low osmotic pressure. It could be however, that the apoplast is not uniform osmotically and that xylem sap is not representative of cell wall solutes.

## 2) Measurement of cell wall solutes

Various attempts have been made to measure the $\pi_w$, using techniques such as x-ray microanalysis (Storey et al. 1983a; 1983b; Harvey and Thorpe, 1986), elution techniques (Bernstein 1971; Jacobson, 1971) and various infiltration and perfusion techniques (Terry and Bonner, 1980; Cosgrove and Cleland, 1983a).

The pressure probe technique has provided another estimate of cell wall solutes. It appeared possible that the amount of cell wall solutes could be estimated from the difference of turgor pressure of cells and tissue osmotic pressure under non-transpiring conditions (for details see next chapter). Using the pressure probe cell wall osmotic pressure was found to be about 0.4-0.5 MPa in red beet tap root under non-transpiring and well watered conditions (Leigh and Tomos, 1983). It is suggested that halophytes may not be good models for general description of apoplast water relations as they have large amounts of osmotically active solutes in the cell walls (Clipson et al. 1985). In Suaeda maritima cell turgor pressure (0.1 MPa) was much lower than osmotic pressure of the same tissue (2.5-4.0 MPa, depending on the tissue) in response to 400 mol $m^{-3}$ NaCl present in external media, having wall hydrostatic tension equal to about 0.1 MPa. Cell wall osmotic pressure was estimated in the range of 2-3 MPa. Malone et al. (1989) have recently measured a small amount of osmotically active cell wall solutes in mature zone of wheat leaf, by measuring the cell osmotic pressure directly using nanoliter osmometer along with pressure probe for sap extraction and turgor pressure measurement. The difference between cell

turgor and osmotic pressure was about 0.1 MPa that is equal to the osmotic pressure of cell wall solutes under non-transpiring conditions.

The significance and potential role of cell wall solutes have been discussed by Tomos (1988). He suggests that under some circumstances very low wall water potential is unlikely to be maintained only by wall transpiration tension only. If it were so, then under the conditions of negligible rate of transpiration i.e. very high atmospheric humidity due to heavy rains etc., the cell turgor pressure would increase so much that cells would start bursting. Such a situation was observed in Suaeda maritima leaves, where after vacuum infiltration of tissue with dilute solutions or distilled water, the cells indeed burst (Tomos unpublished, quoted in Tomos, 1988). Under field conditions a thick wax cuticle prevents rain water getting into the leaves. The existence of an osmotic rather than a hydrostatic water potential gradient through the plant protects the leaf cell from excess pressure. In this context, therefore, the stability of osmotic potential gradients within the apoplast is of great interest.

Cell turgor pressure can be modulated more effectively by apoplast (wall) osmotic pressure than the protoplast osmotic pressure (Cram, 1980; Flowers and Yeo, 1986; 1988). The rate of change of solute concentration in any compartment will be the flux rate in or out divided by the compartment volume (Tomos, 1988). A solute flux across the plasma membrane will result in a faster change in wall osmotic pressure than that of the

protoplast, as the cell wall volume is much smaller than the protoplast. Cell turgor pressure is determined by differential osmotic pressure, therefore its behaviour will be determined by the wall osmotic pressure during a plasma membrane flux (Tomos, 1988).

b) Vacuole/protoplast

An osmotic pressure gradient probably does not exist between cytosol and vacuole since the reflection coefficient of the tonoplast is likely to be unity i.e. it is semi-permeable. Therefore, no pressure gradient can be sustained between these two compartments. However, in response to change in external water potential the vacuole and cytosol behave in different ways. During the process of osmotic adjustment the vacuole of higher plants is found to be quite non-selective in contrast of the cytosol which has crucial limits on the concentration of most osmotica (Wyn Jones, 1981).

1) Solutes involved in biochemical osmotic adjustment

Under saline conditions the plants can not survive without the absorption of inorganic solutes. Among the inorganic ions, $Na^+$ and $Cl^-$ are the most readily available in the medium. Plants can not resist the uptake of these monovalent ions in the root and subsequent transport to the leaves (Greenway and Munns, 1980). Wyn Jones et al. (1977) discussed a possible significance of the compartmentation of the ionic solutes between cytosol and vacuole. They suggested that $Na^+$ is mainly, but not exclusively,

sequestered in the vacuole resulting in relatively low ionic concentration in the cytosol. They also proposed that $K^+$ was maintained in the cytosol. $Cl^-$ is also accumulated in the vacuole. In the literature $Cl^-$ concentrations have not been discussed as much as those of $Na^+$ and $K^+$ since the $K^+/ Na^+$ ratio of selectivity is commonly used for assuming the salt tolerance of species (Pitman, 1965; Flowers et al. 1977; Wyn Jones and Gorham, 1983; Flowers and Yeo, 1988).

Potassium ion is a major osmoticum in glycophytes and is quite important for nutritional requirements of plants (Flowers and Läuchli, 1983). Its concentration, expressed on a tissue water basis, is often in the range of 100 to 200 mol $m^{-3}$ (Leigh and Johnston, 1983). A variety of organic solutes such as sugars, amino acids and other nitrogenous solutes complement $K^+$ for generation of osmotic pressure (Hsiao and Läuchli, 1986). Turgor pressure maintenance in the $K^+$ sufficient plants, the plants with enough $K^+$ concentration in the root media, is primarily generated by $K^+$ salts. When $K^+$ concentration in the medium is reduced the vacuolar potassium levels drop as low as 10 mol $m^{-3}$ and plants start using $Na^+$, $Mg^{2+}$, $Ca^{2+}$ and the organic solutes for maintaining the cell turgor pressure (Leigh and Wyn Jones, 1984).

Nitrates and sulphates are among the major anions absorbed by the plant but they are further metabolised to organic compounds. The charge balance so generated is counter-balanced by the accumulation of organic acids e.g. malate, citrate, and oxalate (Kirkby and Knight, 1977). However, nitrogen concentration may be lowered after exposure to NaCl as a result of inhibition by $Cl^-$

of the $NO_3^-$ uptake (Cram, 1963; Dean-Drummond and Glass, 1982). Nitrate deficiency can reduce plant growth rate to as low as 50 % as reported by Burns et al. (1981) in a study of lettuce growth. The response occurred within two days of the removal of $NO_3^-$ from the media.

The contribution of sugars to the osmotic adjustment in higher plants is not quite clear (Flowers et al. 1977; Gorham et al. 1981). Various plant responses have been studied in this respect. For instance, the increase in the levels of sucrose and reducing sugars was observed in the response of carrots (Bernstein and Ayers, 1953) and spinach leaves (Mehta et al., 1978) to salinity. Similarly, Albert and Popp (1977) studied the synthesis of soluble carbohydrates and found an increase in their concentrations in salt treated plants. In some halophytic species sucrose concentration increases in the leaves during the osmotic adjustment while glucose and fructose do not contribute to the same extent (Briens and Larher, 1982). The increase in sugar concentration could have been the result of either the decrease in growth or the lack of readily available ions in the environment, hence, causing the decreased utilization which ultimately results in increased carbohydrates level (Wyn Jones, 1981). At the other end of spectrum, a decrease in the free sugar level was observed in lettuce and spinach roots (Martar et al. 1978). Onions did not change their sugar contents in response to salt stress (Bernstein and Ayers, 1953).

In conclusion, there is no direct evidence that sugars have a special cytosolic role in the moderately salt tolerant

monocotyledonous species, nor to suggest that they are part of a specific mechanism for adaptation to the salt stress (Gorham et al. 1981).

## 1.5 Root Hydraulic Conductivity

The hydraulic conductivity of root from outside to fully conducting xylem, $L_{pr}$, can be crucial in the water relations of the leaf as the roots remain in close proximity of the external media. The anatomical complexities of root hinder the water and ion transport through it that affects the osmotic and hydrostatic components of leaf water potential. Therefore, in this study attempts have been made to study the probable role of root hydraulic conductivity in the control of leaf turgor pressure, a component of water potential.

The estimates of $L_{pr}$ are based on a simple approach in which roots are thought to be analogous to a membrane between an external and internal (xylem sap i.e. exudate) solution. $L_{pr}$ can be calculated using the equation (Pitman and Wellfare, 1978) as follows:

$$L_{pr} = \frac{J_v - J_{v,s}}{\sigma\ (\pi_{o,s} - \pi_s)} \qquad (1.14)$$

where $J_v$ and $J_{v,s}$ = rates of exudation of roots in control conditions and in response to osmotic stress, respectively, $\pi_s$ = osmotic pressure of external solution, $\pi_{s,o}$ = osmotic pressure of external solution after the addition of osmotic solute. The

reflection coefficient, $\sigma$, is assumed to be unity for the results.

The $\sigma$ gives an indication of the ideality of the pathway/solute couple in question. Each solute and pathway will have its own independent value of $\sigma$. A reflection coefficient of unity signifies that the solute is not crossing the membrane i.e. all of the solute is reflected from the membrane and the $\sigma$ has its maximum value. When a membrane, pathway or barrier is non-selective both water and the solute may cross it at the same velocity. This parameter along with hydraulic conductivity of cell membranes, $L_p$, and coefficient of solute permeability, $\omega$ are the basis of much current work on plant cell water relations (Tomos, 1988). The reflection coefficient is perhaps the most important parameter from irreversible thermodynamics relevant to the fluxes generally considered in plant physiology, as it is an integral part of the expression for volume flux density (Nobel, 1983), as follows,

$$J_v = L_p.A\ (\sigma\ \Delta\pi - P)$$

where P and $\Delta\pi$ are the turgor and osmotic pressure difference across the membrane, respectively.

Jones et al. (1988) compared the whole root and cellular hydraulic conductivity in wheat and maize roots. Lpr in wheat roots ranged from 1.6 to 5.5 $10^{-8}$ m $s^{-1}$ $MPa^{-1}$ with a mean value of $3.7 \pm 1.3$ (15) $\times 10^{-8}$ m $s^{-1}$ $MPa^{-1}$. For maize root it ranged from 0.9 to 4.8 $\times 10^{-8}$ m $s^{-1}$ $MPa^{-1}$ with a mean value of $2.1 \pm 1.3$

(8) x $10^{-8}$ m $s^{-1}$ $MPa^{-1}$. All of these measurements were carried out in single excised roots.

The detailed information about the various Lpr values for different plant roots measured by using various techniques/methods has been provided in Jones (1985) and Jones et al. (1988).

## 1.6 Preface to the Project

In this report the following aspects will be studied;

a) effect of NaCl on elongation rate of the first emerged leaf of wheat seedlings,
b) cellular water relations in the growing and in the mature zone of the same leaf,
c) the possibility of these parameters to be used as the selection criteria for breeding for salt tolerance.

In some of the experiments other osmotic solutes such as mannitol were used to study and compare its effects on plant physiology to that of the NaCl.

For the purpose the experiments of two different duration were carried out;

a) short term i.e. up to 24 h of the treatment application,
b) long term i.e. mostly for 6 d, unless otherwise mentioned.

Leaf elongation rate of the control and of the plants treated with osmotic solutes were measured using LVDT (Linear Variable Differential Transformer) displacement transducer which

facilitates continuous measurements with quite high accuracy.

Transverse leaf sections were made using plastic embedding technique to locate and study the epidermal cells in both types of the zones.

As far as the components of water potential are concerned, turgor pressure of the individual epidermal and mesophyll cells was determined by pressure probe (Hüsken et al. 1978), however, osmotic pressure was measured in expressed cell sap of the whole leaf tissue. The pressure probe was also used for determination of transpiration tension present in cell wall. The concentration of solutes in the cell wall can be estimated by the difference of the values of turgor pressure, osmotic pressure and the transpiration tension. Cell wall extensibility, ø, of the growing cells was also determined using Instron tensiometer (Van Volkenburgh et al. 1983; Clelend, 1984; Pritchard, 1988) to study its role in the control of growth. Root hydraulic conductivity, $L_{pr}$, was measured to study its effect on the leaf water relations.

The process of osmotic adjustment was studied after the salt stress application. For this purpose both of its types, physical and biochemical, were studied separately. Volumetric elastic modulus, ε, was also determined using pressure probe to study its contribution towards physical osmotic adjustment. The concentration of various ionic and osmotic solutes was determined to study their contribution in the process of biochemical osmotic adjustment. Cations and anions were

determined using flame photometry and H.P.L.C. (High Performance Liquid Chromatography), respectively. While, organic solutes i.e. sugars were measured using G.L.C. (Gas Liquid Chromatography).

The possibility of cellular water relations parameters to be used as selection criteria for the assessment of salt tolerance was also studied in different wheat cultivars with varying degrees of salt tolerance (already assessed on the basis of agronomic parameters).

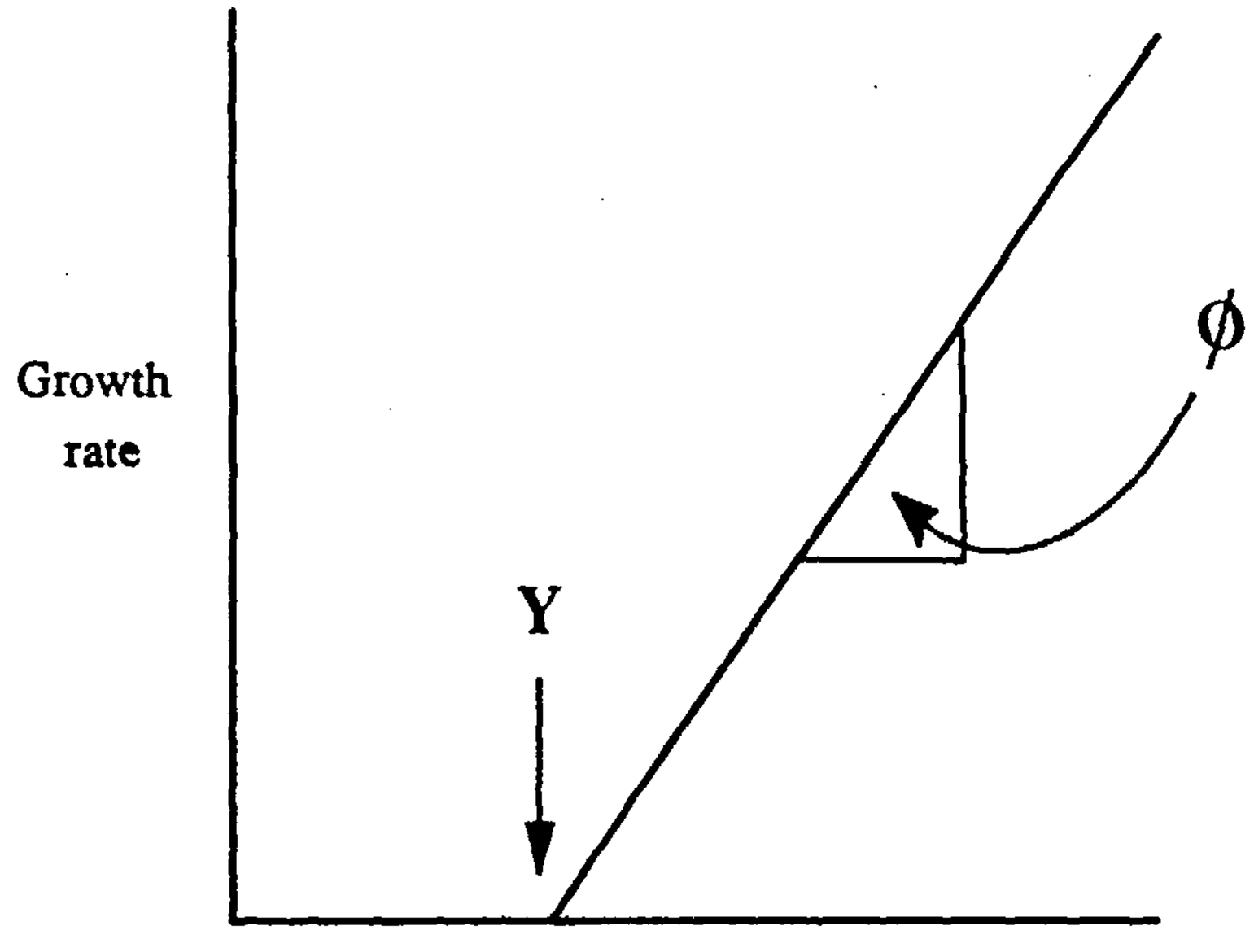



Fig. 1.1 Ideal behaviour of cell wall growth as a function of cell turgor pressure

# CHAPTER 2

# *MATERIALS AND METHODS*

## 2.1 Plant Material

Seeds of Triticum aestivum (five different cultivars) were soaked in aerated distilled water for 24 h and germinated on 2.0 mm plastic mesh suspended over aerated half strength (50 %) Hoagland solution (Hoagland and Arnon 1950) contained in black painted plastic vessels (0.5 $dm^3$). However, in some experiments the plants were germinated in 0.5 mol $m^{-3}$ $CaCl_2$ for certain period and were transferred later to 50 % Hoagland solution. The germinated seedlings were kept in the dark for 2 days and then in continuous light for the rest of the experimental period. After 7 days of germination different experimental treatments were applied to the plants. In most of the experiments NaCl concentrations (0, 25, 50, 75, 100, 125 and 150 mol $m^{-3}$) were added to salinize the root media, unless otherwise stated. All the NaCl concentrations were applied in one step to give a sudden shock to the plants.

## 2.2 Measurement of Leaf Growth Rate

Growth rate of the first leaf of the control and stressed plants was determined with a LVDT (Linear Variable Differential Transformer) displacement transducer (1353; Penny and Giles Potentiometers Limited, Someford Division, Someford Road, Christchurch, Dorset BH23 3RS U.K.). The transducer comprises of a body mounting and a shaft which suspends inside the body mounting. It requires a stabilized power supply of 9 V d.c. The principle of operation is a transformer having one primary and two secondary windings, coupled together by means of a ferro

magnetic core. Movement of the core couples one or the other of the secondaries, thus generating a voltage difference between the secondary windings which are arranged to provide a linear voltage relationship with the displacement of the core over a specified range, which is 10 mm for the model used as specified by the manufacturer. When the transducer was calibrated it showed a linear relationship between core displacement and voltage output up to 5 mm only. The body of the transducer was mounted on a stand and a shaft with ferro magnetic core was suspended inside the body. One end of the shaft was attached to the leaf tip via a steel rod mounted on a bearing (Fig. 2.1). During the measurements plants were kept in nutrient solution in a plastic beaker. A plastic pipe was fitted to the beaker that facilitated the solution replacement in about 12 seconds without causing any inconvenience to the plant. The leaf tip was held in a crocodile clip with a thread attached to the transducer by means of the steel rod. The tension on the thread was balanced with the help of a load (8 g) kept on the other end of the bar, without affecting the measurements (Fig. 2.1). The growth was recorded on a chart recorder (BD40; Kipp and Zonen, Mercuriusweg 1, 2624 BC Delft, Holland).

## 2.3 Pressure Probe Technique

### 2.3.1 Pressure probe

Turgor pressure in individual leaf epidermal cells was measured directly using the pressure probe originally developed by Hüsken et al. 1978 (Fig. 2.2). The pressure probe consists of a glass

microcapillary, a solid state pressure transducer (PDCR 200; Druck Limited, Fir Tree Lane, Groby, Leicestershire LE6 0FH U.K.), a motor-driven piston and one manually operated piston. All these are connected to a Perspex chamber. The whole equipment is mounted on a micromanipulator (Ernst Leitz Wetzler GMBH, D-6330 Wetzler 1, F.R.G.). The glass microcapillary was extended from 1 mm capillary (GC100-15; Clark electromedical Instruments, P.O. Box 8, Pangbourne, Reading RG8 7HU U.K.) to a tip diameter of 2-5 $\mu$m using a conventional electrical solenoid based puller (CFP Microelectrode puller, 230 V; Searle Bioscience, Harbour Estate, Sheerness, Kent ME12 1RZ U.K.), filled with low-viscosity silicon oil (AS4; Wacker-Chemi, München, F.R.G.) and sealed into the oil-filled Perspex chamber with a rubber seal. The turgor pressure measurements were performed under a zoom stereomicroscope (M8; Wild Heerbrugg Limited, 9435 Heerbrugg, Switzerland) under a cool light from a fibre optic glass source (Intralux 5000; Volpi AG, Wiesenstrasse 33, CH-8952, Schlieren, Switzerland). The careful positioning of light source helps in finding out the sharp sap/oil meniscus and ultimately in accurate measurements.

When tip of the microcapillary was inserted into the epidermal cell the turgor pressure pushed back (Fig. 2.3) the oil into the capillary forming a meniscus at the oil-cell sap boundary. Pressure in the chamber was manipulated using the piston (either motor-driven or manually operated) until the meniscus was pushed back and was stationary as near to the cell wall as practicable. The pressure was transmitted by the silicon oil to the pressure transducer which converted it into a proportional voltage.

Voltage output was sent to a chart recorder, via a control box, which helps in an immediate visual control and a permanent record of the measurements.

### 2.3.2 Measurement of turgor pressure

Turgor pressure was measured in individual epidermal cells of the growing zone as well as of the mature zone of the first emerged leaf. To observe the changes in cell turgor pressure on salination of the root media, the turgor pressure was first measured over shorter span of the time i.e. initially after every 15 min, then 30 min and finally at 1 h intervals after the addition of the NaCl to the roots of the 7 d old seedlings. Long term behaviour was also monitored by taking measurements at 24 h (1 d) intervals for 144 h (6 d).

#### a) Measurement in the growing zone

The position of the growing zone in the first leaf was determined by piercing fine holes through the stem of intact plant at regular intervals from the base (Kemp 1980; Thomas et al. 1989). The plant was then allowed to grow for 24 h, when the outer sheath was removed and the position of the growing zone was determined. It was confirmed that the zone of maximum growth was about 5–10 mm away from the base of the stem (root/shoot interface). The outer sheath was not removed completely due to possible loss of turgor pressure by excessive evapo-transpiration from the exposed surface of the growing zone. Instead, a window (2 x 2.5 mm) was cut carefully with a fresh scalpel blade through

the outer sheath about 5 mm away from the stem base. In the majority of experiment the procedure is believed not to interfere with the growth rate (Thomas et al. 1989). The growing zone was clamped horizontally (for better viewing of the meniscus) in a Perspex leaf holder mounted on a micromanipulator, for the measurements. The measurements were carried out in epidermal and mesophyll cells (see photographs of the transverse cross-section of the growing zone of leaf). The roots were retained in the bathing medium, covered with a paper 'wick' from the medium, with the result that the air around the growing zone was relatively humid.

**b) Measurement in the mature zone**

The expanded part of the leaf was also held in a Perspex leaf holder mounted on a micromanipulator. Initial measurements were made on both, abaxial and adaxial, surfaces of the leaf; no appreciable differences in turgor pressure from the two surfaces were seen (Table 2.1). Therefore, for all the subsequent experiments measurements were confined to the abaxial surface at 5-6 cm away from the leaf tip. At 7 and 13 days the control leaves were 8.4 $\pm$ 0.5 and 18.2 $\pm$ 1.25 cm long, respectively.

## 2.4 Estimation of Cell Wall Transpiration Tension

An attempt to measure the quantitative effect of cell wall transpiration tension on cell water potential was performed in both of the leaf zones. For the purpose, turgor pressure was measured (for each NaCl treatment) with the leaf kept in the air,

continuously transpiring, and then after immersing the leaf in the respective hydroponic root medium for at least 1 h. During this period turgor pressure was observed to rise and stabilize to a higher value (Fig. 2.4). It has been assumed that this represents the elimination of the transpiration tension as water is drawn into the capillaries of the apoplast. When cell wall transpiration tension was eliminated the cell wall water potential increased as the cell membrane is very permeable (Dainty 1963). The water potential of cell will also increase due to high permeability of the plasma membrane resulting in the observed increase in the cell turgor pressure. We propose that this increase in turgor pressure provides a quantative estimation of the cell wall transpiration tension.

## 2.5 Determination of the Osmotic Pressure of Cell Wall Solutes

The osmotic concentration of the cell wall solutes were determined by the difference of the values of turgor pressure (P), cell wall transpiration tension ($P_w$) and the tissue osmotic pressure ($\pi$). Cell osmotic pressure was not measured directly (since at the time of experiment this was not possible), it was assumed that osmotic pressure was equivalent to the cell osmotic pressure of expressed tissue sap. However, the cell sap would be expected to be slightly diluted by the extracellular water during the tissue crushing (Tomos et al. 1984).

The cell water potential and the cell wall water potential would be equal at water potential equilibrium between the two compartments. Equilibrium (or very near equilibrium) will be

expected in all cases here due to the high hydraulic conductivity of plasma membrane (Tomos, 1988).

$$\Psi_c = \Psi_w \qquad (2.1)$$

their components (see Equation 1.7),

$$P - \pi = P_w - \pi_w \qquad (2.2)$$

note $P_w$ is always a negative entity,

$$\pi_w = \pi - P + P_w \qquad (2.3)$$

$\pi_w$ exhibits the amount of osmotically active solutes present in the cell wall.

## 2.6 Measurement of Volumetric Elastic Modulus

Instantaneous volumetric elastic modulus ($\varepsilon$) was calculated by using the equation 1.13,

$$\varepsilon = \frac{\Delta P}{\Delta V} V$$

(Philip, 1958; Dainty, 1963; Zimmermann and Steudle, 1978). V, $\Delta V$ and $\Delta P$ are original cell volume, change in cell volume and instantaneous change in cell turgor pressure, respectively. For the purpose turgor pressure was measured using pressure probe as described earlier. The oil-cell sap boundary was moved quickly by

a defined amount in the microcapillary and "instantaneously" returned to its original position. The size of the resulting pressure change ($\Delta P$) was recorded on a chart recorder. $\Delta V$ was calculated from the volume swept by the oil/cell sap boundary. The inner diameter of microcapillary, cell length and cell diameter was measured using an eye-piece graticule (Graticules Ltd., Morley Road, Tonbridge, Kent TN9 1RN U.K) in the microscope and the cell volume was calculated from the formula, since the cell shape approximate a cylinder,

$$V = \pi r^2 l \qquad (2.4)$$

where r and l are cell radius and length, respectively. Measurements of cell diameter and length were sometimes facilitated by pumping the silicon oil into the cell when its different refractive index gave a sharp and clear image of the side- and the end-walls, after the completion of elasticity measurements.

## 2.7 Extraction of Tissue Sap

Tissue sap was extracted by using two different methods depending upon the kind and type of the tissue to be analysed. For the mature and expanded parts of the first leaf the method of Tomos et al. 1984 was followed with minor modifications (This method removes the problem of condensation reducing the osmotic pressure of the sample). A disc of Whatman GF/B glass microfibre paper was placed in the barrel of a 2.0 $cm^3$ plastic syringe so that it covered the outlet hole. Leaf tissue was then placed in the barrel. The plunger was inserted and the tip of the syringe was

sealed with Blu-Tack (Bostik Ltd., Ulverscraft Road, Leicester LE4 6BW U.K.). The syringe and contents were frozen in liquid $N_2$ and, still sealed, was thawed to ambient temperature. When temperature equilibration was complete, the Blu-Tack was removed and the barrel (with plunger still inserted) of the syringe was placed in a 1.5 $cm^3$ polypropylene tube (Eppendorf tube) (Fig. 2.5a). After centrifugation at 2500 g for 10 minutes clear sap was recovered in the Eppendorf tube whilst the tissue residue was retained in the barrel of the syringe.

For growing zone and sometimes for mature zone as well (when enough tissue was not available) another method was used. Leaf tissue was placed in an Eppendorf tube and sealed. It was then frozen by putting the tube in a freezer (at -18 $^{o}C$) for 24 h. After thawing to ambient temperature the tissue was crushed with a steel plunger and a hole was bored with a pin at the base of the tube. It was then placed inside a second tube with a hole on the side-wall (Fig. 2.5b). Both of the tubes were centrifuged at 2500 g for 10 minutes and sap was collected in the lower tube while the tissue residue was retained in the upper tube.

Sap was sealed in the lower Eppendorf tube and kept for later use. Frozen saps were thawed, mixed and re-centrifuged prior to the measurements.

### 2.7.1 Measurement of osmotic pressure

Osmotic pressure of the extracted sap was measured using a vapour pressure osmometer (Model 5100B; Wescor Inc., 459 South Main

Street, Logan, Utah 84321 U.S.A.), calibrated in mOsmol $kg^{-1}$ water using standard solutions (290 and 1000 mOsmol $kg^{-1}$ water) provided by the manufacturer. The values obtained in mOsmol $kg^{-1}$ were converted to pressure units (MPa) using the Van't Hoff relationship at 20 $^{o}C$ (Nobel 1983) i.e. by dividing by 407 mOsmol $kg^{-1}$ $MPa^{-1}$.

### 2.7.2 Measurement of cations

The monovalent cations, $Na^{+}$ and $K^{+}$, were measured by flame photometry at 589 and 766.5 nm, respectively, using a Pye Unicam SP-90 atomic absorption spectrophotometer (Pye Unicam Instrument Ltd., York Street, Cambridge CB1 9PX U.K.), operated in emission mode. Extracted sap was diluted with distilled water and the standard solutions were prepared using NaCl and KCl.

### 2.7.3 Measurement of anions

Four different anions, $Cl^{-}$, $PO_4^{3-}$, $SO_4^{2-}$ and $NO_3^{-}$, were determined in the mature zone of the first leaf by using automated ion chromatography, HPLC (High Performance Liquid Chromatography). The equipment consists of a Dionex 2010i ion chromatograph linked to a Pye 4700 autoinjector and a Pye DP88 integrator (Gorham 1987).

Analysis was performed on an HPIC-AS4A column and the conductivity of the eluent (2 mol $m^{-3}$ $Na_2CO_3$ + 0.7 mol $m^{-3}$ $NaHCO_3$ in 2 % (v/v) propan-2-ol) was suppressed in an anion fibre suppressor regenerated with 25 mol $m^{-3}$ $H_2SO_4$. Small changes in the amount of $NaHCO_3$ in the eluent had little effect on overall

retention times, but altered the position of the phosphate peak relative to the peaks of other anions. The small amount (2 % v/v) of propan-2-ol was added to the eluent to inhibit microbial contamination. Only high purity water (>18 Mohm $cm^{-1}$, from an Elga Spectrum ROI; Elga Ltd., Lane End, High Wycombe, Bucks HP14 3JH U.K.) was used in the preparation of eluent and regenerants.

The leaf sap was treated with 4 parts of 25 % (v/v) propan-2-ol, centrifuged at 2000 g for 1 minute and was diluted again to give a final dilution of 250 times. The samples were poured into the glass vials to be injected automatically. Quantitative analysis was carried out at 30 $\mu$S FSD and was based on peak height measurements. Standard curves for all anions were straight lines (concentrations between 0.01 and 1 mol $m^{-3}$).

### 2.7.4 Measurement of sugars

The organic compounds glucose, fructose and sucrose were measured in mature zone of the leaf using Gas-liquid chromatography, G.L.C. (Gorham 1981).

Leaf samples were weighed immediately after the harvest, placed in glass vials (10 $cm^3$), then frozen in liquid $N_2$ and crushed. Methanol (10 $cm^3$) was added, the vials were sealed and extracted for 1 h at 80 $^oC$ to destroy the invertase activity. The samples were concentrated to 60 % of the volume on a sample concentrator (Dri-Block DB 3; Techne (Cambridge) Limited, Duxford, Cambridge, U.K.) filtered through cottonwool and made up to a volume of 10 $cm^3$ with distilled water. The vials were again placed in the

sample concentrator with aeration needles in at 80 $^{o}$C till about 1.5 ml of the solution was left. The extract was poured into 2 ml volumetric flask and volume was made by distilled water. 1 ml of the extract was put in an Eppendorf tube and stored in freezer. The other half was ready for further procedure. To remove unwanted cations in the extract a freshly regenerated and surface-dried strong cation exchanger, (Dowex-50-X8 (H); BDH Limited, Broom Road, Poole, Dorset BH12 4NN U.K.) was added to the extract together with 100 $\mu$l of phenyl-$\beta$-glucopyranoside (1 mg ml$^{-1}$) as internal standard. The tube was put on a rotator and mixed for 15 minutes. Samples were centrifuged in a bench centrifuge (Centra-M Centrifuge; International Equipment Company, 300 Second Avenue, Needham Hts., MA 02194 U.S.A.) for 2 minutes and decanted into small glass vials (4 cm$^{3}$). The samples were then freeze-dried overnight to complete dryness.

For chemical derivatization of the extract 100 $\mu$l of hydroxylamine hydrochloride (15 mg ml$^{-1}$ in pyridine) was added to the vials to convert the reducing sugars to their oximes. For this purpose vials were capped and kept at 80 $^{o}$C for 30 minutes. The samples were cooled and 100 $\mu$l of Bis(trimethylsilyl)-trifluoroacetamide (BSTFA; Sigma Chemical Company, P.O. Box 14508, St. Louis, MO 63178 U.S.A.) was added to the vials, which were capped and kept at 80 $^{o}$C for 15 minutes for silylation.

Gas-liquid chromatography was performed on a Pye 104 gas chromatograph fitted with flame-ionization detectors and 1.5 m X 4 mm i.d. glass column of 1.5 % SE-52 on chromosorb-G. The initial oven temperature was 170 $^{o}$C and was programmed to rise by

24 $^{o}C$ $min^{-1}$ to 300 $^{o}C$ and then kept there for 2 minutes. The gas flow rate was 40, 40 and 240 ml $min^{-1}$ for $N_2$, $H_2$ and air, respectively. The peak heights were measured with a ruler. To correct for variation in sample injection volume and performance of the gas chromatograph, the peak heights were corrected by comparing with the internal standard peak height. The concentrations of sugars were calculated from the ratio of the sample peak height to that of the standard solutions' peak heights.

## 2.8 Measurement of Cell Wall Extensibility

Cell wall extensibility of the leaf growing zone was measured using the tensiometer (Instron) (Van Volkenburgh et al. 1983; Cleland 1984; Pritchard et al. 1989). Leaf samples were cut 16 mm long from the base of the stem with two new razor blades fixed together 16 mm apart. Samples were boiled in methanol to release turgor stress from the cell walls, rehydrated in distilled water for two minutes and then inserted into the clamps of the tensiometer which were 10 mm apart. The tissue was extended by moving the lower clamp downward, facilitated by a constant speed reversible motor and gear box (Synchronous a.c. motor (336-393) and Gear box (336-400); RS Components Limited, 13-17 Epworth Street, London, U.K.) connected to a moveable shaft upon which lower clamp was mounted. The clamp was displaced at a rate of 2.3 mm $min^{-1}$ and its position was monitored by a position transducer (LVDT, D402-01; Electro Mechanisms Limited, Slough, Bucks, U.K.) attached the moveable shaft. When sample was extended tension developed and the load was measured by a strain gauge (Isometric

Transducer 220 g; Bioscience, Harbour Estate, Sheerness, Kent ME12 1RZ U.K.) from which the upper clamp was suspended. The output from the strain gauge was amplified by a coupling device (Coupler Unit A100; Bioscience, Harbour Estate, Sheerness, Kent ME12 1RZ U.K.) and it was displayed alongside the unamplified output from the LVDT on a linear speed chart recorder.

Tissue samples were extended twice to a load of 15g. Extensibility was calculated as the reciprocal slope of each load-extension relationship,

$$\text{percentage change in length per 100 g} = \left(\frac{l_f - l_i}{l_i} \times 100\right)/2$$

where $l_f$ and $l_i$ were the final and initial lengths of the samples. Plasticity was calculated from the difference between first (plastic and elastic) and second (elastic) extensibilities. For most of the samples a certain amount of the slack was present indicating that initial length of the tissue was longer in the gap between the clamps (10 mm). The actual length of each sample was measured by drawing a line tangent to the load-extension relationship and extrapolating back to the x-axis.

## 2.9 Measurement of Root Hydraulic Conductivity

An osmotically induced back-flow technique (Pitman and Wellfare 1978; Jones et al. 1988) was used. A precision-bore glass capillary (1 mm and 0.5 mm diameter for whole and single root system, respectively) was attached to either a whole root system or a single excised root and the roots were immersed in

vigorously aerated 50 % Hoagland's nutrient solution. Xylem fluid was allowed to exude into the capillaries for a certain period of time i.e. 45-60 min, after which the rate of exudation was measured by following the rise of the meniscus in the capillary. An efflux of water from the surface of the root was induced by the addition of the various concentrations of NaCl (see Section 2.1) and equi-osmolal concentrations of mannitol in the root media, which was monitored by measuring the lowering of the meniscus in the capillary. The osmotic coefficient of NaCl was considered to be 1 for all the concentrations used, however, its value is about 0.925 for 150 mol $m^{-3}$, the highest concentration used (Wyn Jones and Gorham, 1983). Since the time course of the efflux appeared to be exponential, curves were fitted to the data using the exponential fit program of the GENSTAT 5 package. The initial rate of efflux was then calculated from the slope at zero time. The surface area of the root was determined by measuring the root diameter and length using a stereomicroscope. The hydraulic conductuvity of the root ($Lp_r$) was calculated using the equation 1.14 (Pitman and Wellfare, 1978) as follows;

$$Lp_r = \frac{J_v + J_{v,s}}{\sigma\ (\pi_{o,s} - \pi_s)}$$

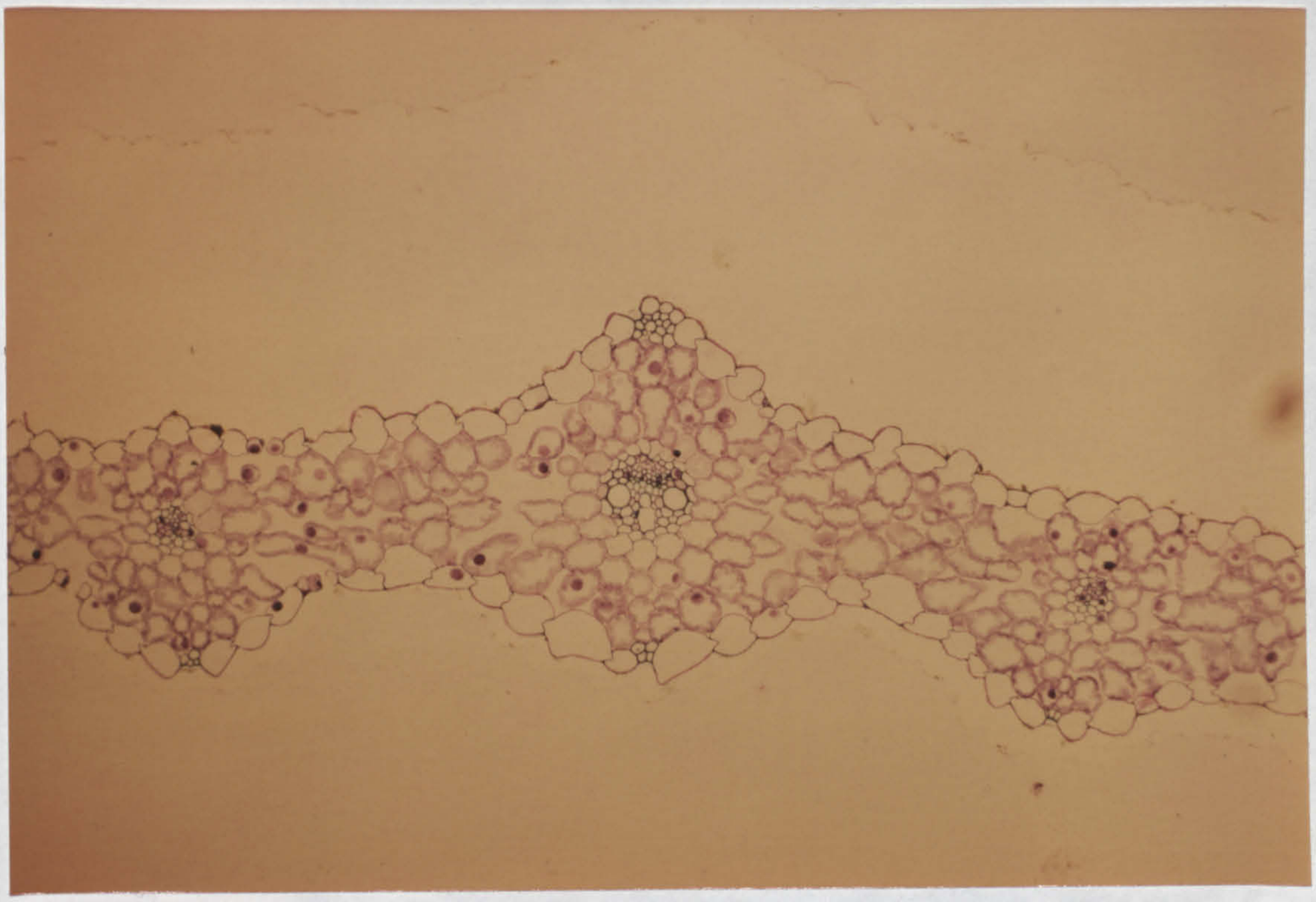
where Jv is the rate of water efflux in 50 % Hoagland solution, Jv,s is the rate of efflux induced by the NaCl or mannitol, $\pi o,s$ is the osmotic pressure of the nutrient solution plus the osmotic solutes, $\pi s$ is the osmotic pressure of 50 % Hoagland's solution, $\sigma$ is the root reflection coefficient which was assumed to be 1 for both of the solutes. All experiments were performed at

ambient temperature of 24 ± 1 $^{o}$C.

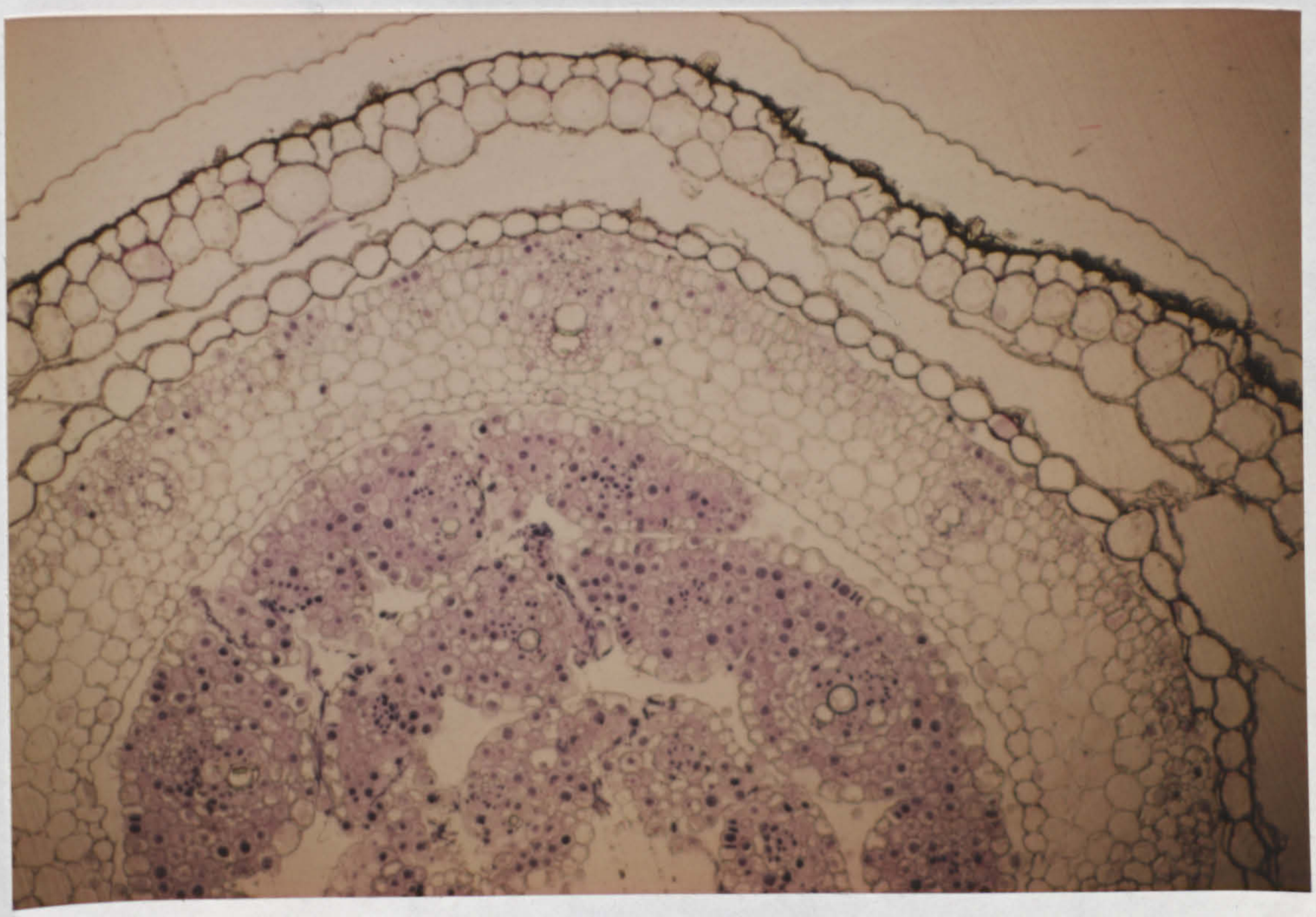
## 2.10 Leaf Sectioning

A plastic embedding technique was employed for making transverse sections of the first leaf at varying distances from the tip. Leaf tissue was fixed in 3 % glutaraldehyde in 50 mol $m^{-3}$ $Na^{+}$ cocodylate buffer for 24 h under vacuum at room temperature. The tissue was dehydrated in graded series of ethanol (25, 50, 75 and 100 %, 12 h each) and placed in a 1:1 ethanol and LKB infiltration solution (LKB Produckter AB, P.O. Box 305, S16126, Bromma, Sweden) on a rotator overnight. The bathing solution was replaced with pure LKB infiltration solution and kept on the rotator for 4 days. Specimens were placed in plastic moulds and covered with LKB embedding medium for 3 h at room temperature to polymerise. The solidified resin blocks were removed from the moulds and mounted on wooden blocks in order to facilitate section cutting. The leaf tissue was sectioned 4 $\mu$m thick on a very precise microtome (Supercut 2050; Reichert-Jung, G.m.b.h., D6907, Nubloch, F.R.G.).

Infiltration solution was prepared by mixing 50 ml of Basic resin and 1 packet (0.5 g) of activator with continuous stirring until dissolved by using mechanical stirrer at low speed to avoid bubble formation. Embedding medium was made by adding 1 ml of hardener to 15 ml of infiltration solution and was used immediately.



A) The mature zone



B) The growing zone

**Transverse cross–sections of two different zones of the first emerged wheat leaf.**

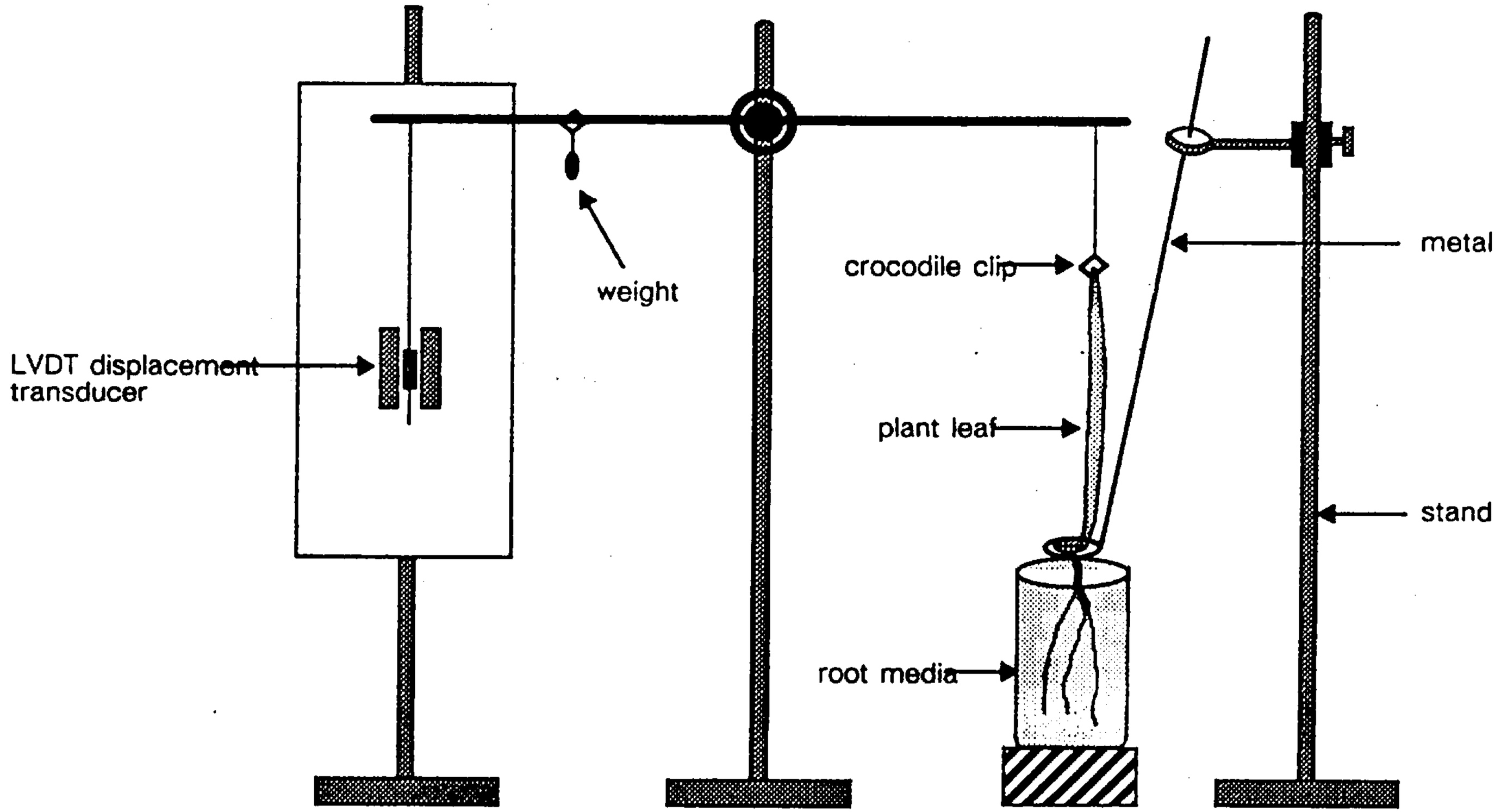



Fig. 2.1 The arrangement for the continuous measurement of leaf elongation rate.

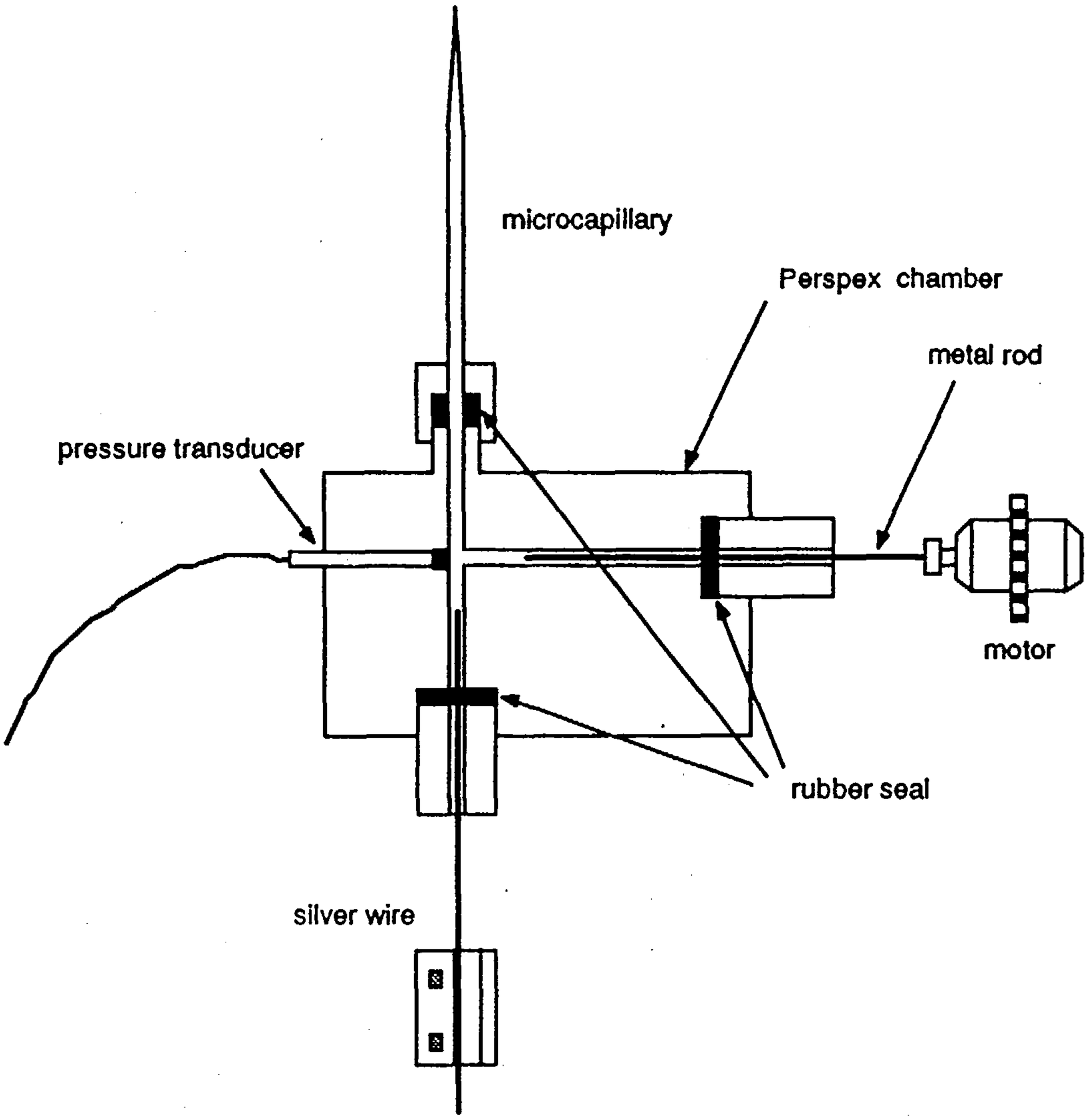



Fig. 2.2 The pressure probe.

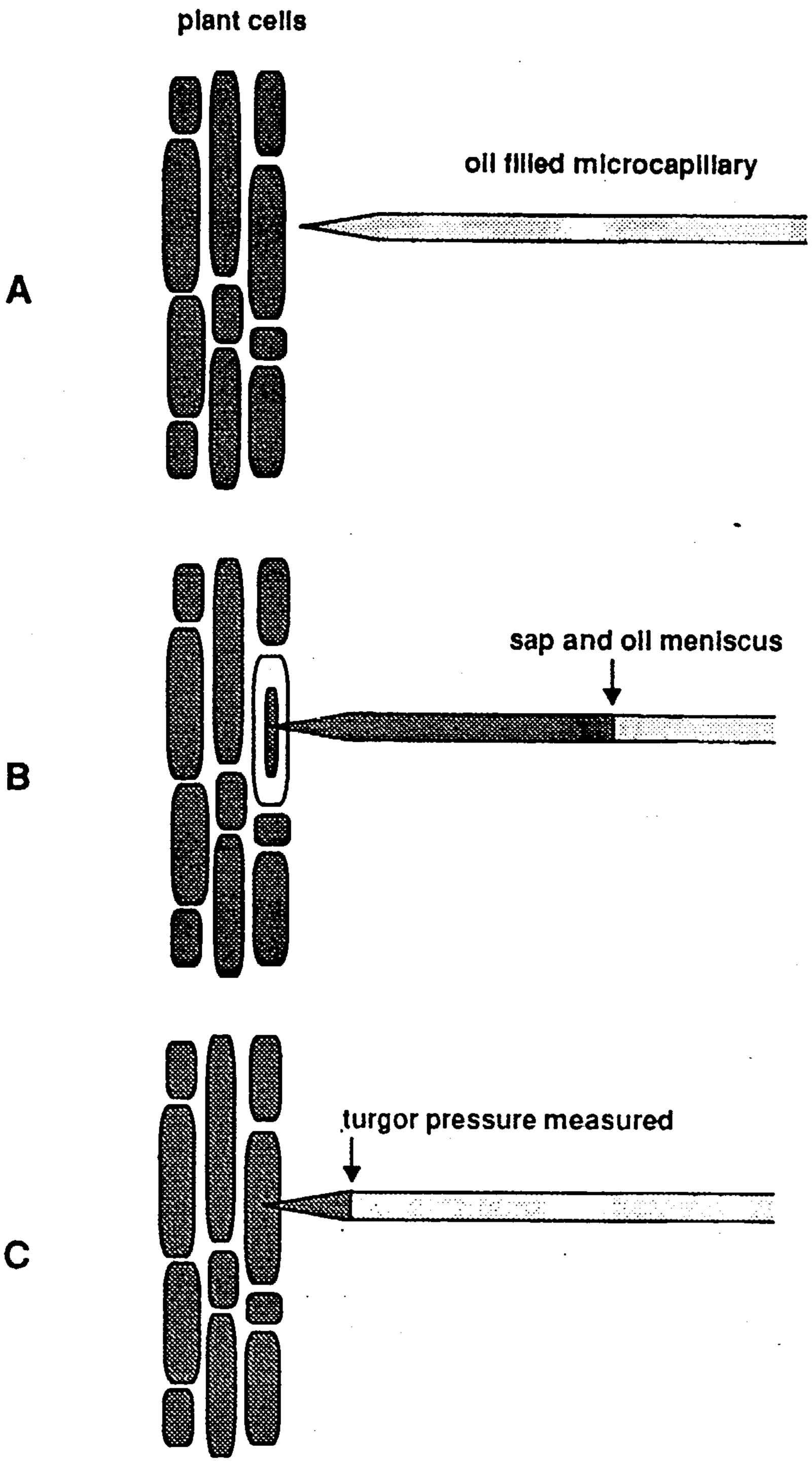


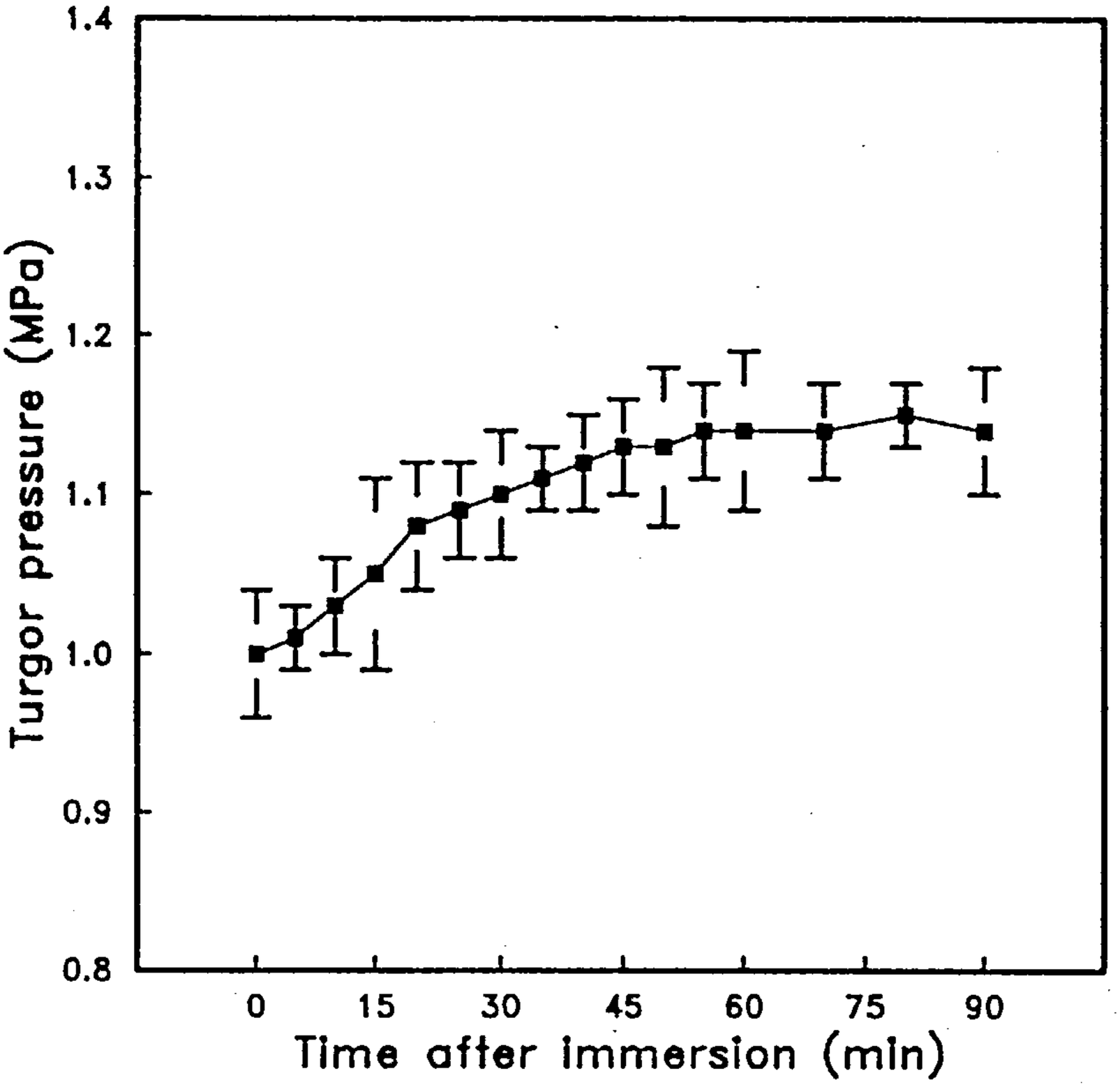

Fig. 2.3 The measurement of turgor pressure in single cell using pressure probe;

A) silicon oil filled microcapillary and the cell,

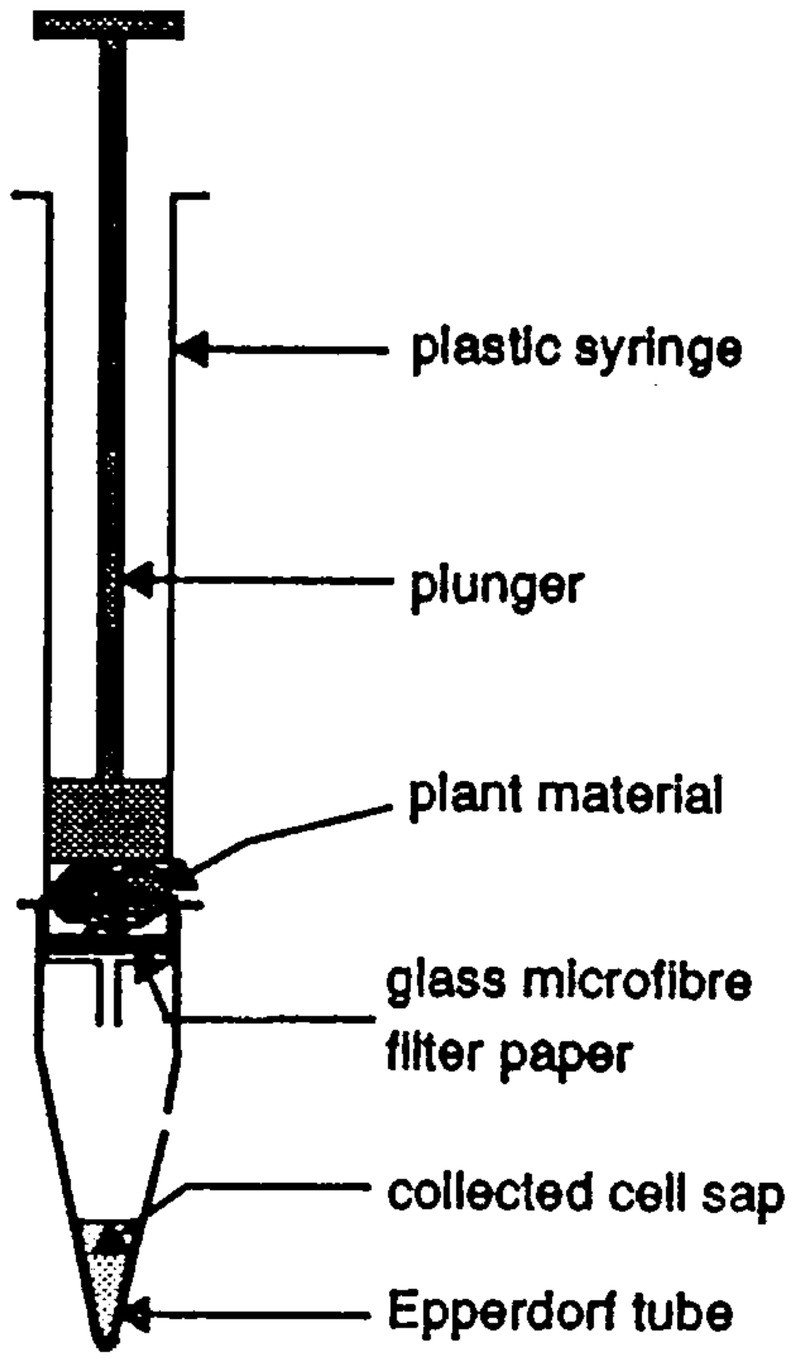
B) on insertion of microcapillary into the cell, turgor pressure pushes the sap/oil meniscus back,

C) pressure in the equipment is increased using piston (either motor–driven or manually operated) to push the meniscus back and keep it stationary as near to cell wall as practicable.

**Fig. 2.4** The response of cell turgor pressure after the plant immersion under the hydroponic medium. Turgor pressure increases due to inhibition of cell wall transpiration tension as water is drawn into the capillaries of the apoplast. This increase provides a quantitative estimation of the cell wall transpiration tension and it was stabilized after 1 h of the immersion.


1.4
1.3
1.2
1.1
1.0
0.9
0.8
Turgor pressure (MPa)
0
15
30
45
60
75
90
Time after immersion (min)

A




B

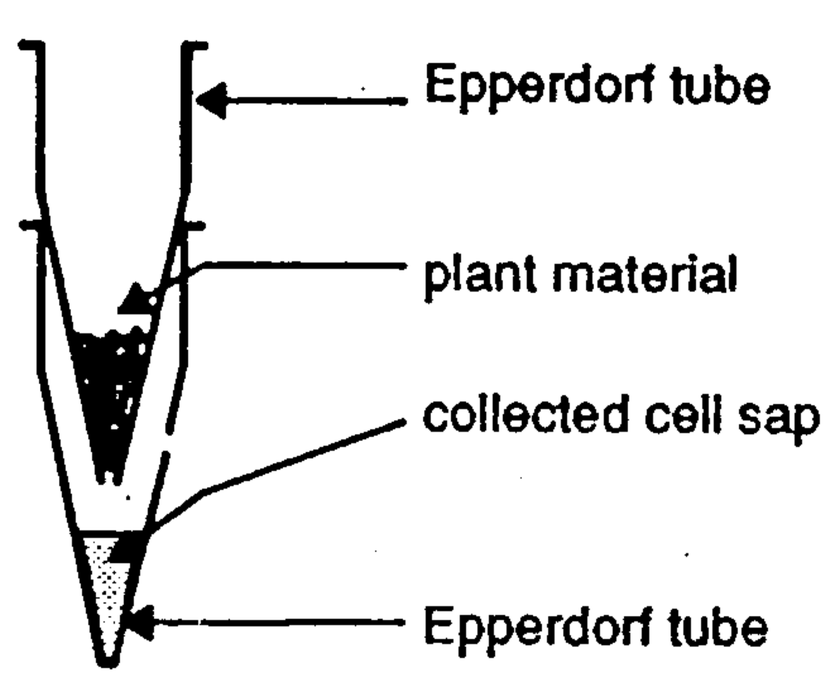



Fig. 2.5 The arrangement for sap extraction from growing as well as from mature zone of the wheat leaf used;

A) for the mature zone tissue, where enough tissue was available,

B) for the growing zone as well as for the mature zone where enough tissue was not available.

**Table 2.1 Turgor pressure values measured in epidermal cells of mature zone on both sides of the first emerged leaf of wheat seedlings.**

| | Turgor pressure (MPa) | |
|---|---|---|
| Time (d) | Abaxial surface | Adaxial surface |
| 0 | 1.02 ± 0.03 (4) | 1.03 ± 0.04 (4) |
| 1 | 1.01 ± 0.06 (7) | 0.98 ± 0.03 (7) |
| 2 | 1.12 ± 0.09 (6) | 1.03 ± 0.01 (6) |
| 3 | 1.04 ± 0.04 (8) | 1.10 ± 0.09 (8) |
| 4 | 1.04 ± 0.08 (6) | 1.09 ± 0.09 (6) |
| 5 | 1.01 ± 0.07 (8) | 1.03 ± 0.04 (8) |
| 6 | 1.01 ± 0.05 (5) | 0.99 ± 0.04 (5) |

# CHAPTER 3

# *GROWTH*

Initial experiments were designed to allow familiarity with and quantification of the influence of various treatments on leaf extension growth rate.

## 3.1 Effect of Nutrient Status of Root Media on Growth

The effect of nutrient status of the root medium on leaf growth rate was studied by measuring the elongation rate of leaf and root of the plant. For this purpose, plants were grown in three different types of medium and were kept there for certain time. The details are as shown below;

a) $CaCl_2$ (0.5 mol $m^{-3}$) - low nutrient,
(144 h)

b) $CaCl_2$ (0.5 mol $m^{-3}$) + 50 % Hoagland solution - late nutrient,
(120 h) (24 h)

c) $CaCl_2$ (0.5 mol $m^{-3}$) + 50 % Hoagland solution - late nutrient,
(72 h) (72 h)

d) 50 % Hoagland solution - High nutrient.
(144 h)

All the measurements of leaf length were performed with a ruler and the units were converted into $\mu m\ min^{-1}$ to make them compatible with the units of other experiments where LVDT displacement transducer was used for the measurements. These measurements were done for the final 24 h following the 144 h period of all the treatments. However, the variation in growth rate with time was not measured.

For the roots, the elongation rate (Table 3.1) was found to be similar for all types of nutrient status and treatments. On the other hand, in case of leaf, elongation rates were quite

different for different media and treatments. The measured lowest values were for the 'low nutrient' media where sufficient nutrients were apparently not provided to the roots. The leaf tissue osmotic pressure of 'low nutrient' plants was also not very high (0.73 ± 0.03 MPa) compared to all the other treatments (Table 3.2). For 'late nutrient' plants (b), where Hoagland solution was available for 24 h, the measured elongation rates were slightly higher which could be due to higher osmotic pressure (1.2 ± 0.02 MPa) being generated in the last 24 h of the experiment, that results in higher turgor pressure. A big boost in the growth rate was measured for both of the last treatments, i.e. 'late nutrient' with 72 h in Hoagland solution (c) and the 'high nutrient' treatments (d). The respective osmotic pressures were 1.18 ± 0.02 and 1.25 ± 0.08 MPa. These values were identical to the osmotic pressure values for 'late nutrient' with 24 h for Hoagland solution (b). The growth rates were quite similar for both of these treatments (c and d). However, the high nutrient treatment (d) seems more appropriate in terms of uniform growth rate from the very beginning. The high growth rate in case of 'late nutrient' media (c) were observed after their transfer to Hoagland solution. In 'late nutrient' experiments two variables were involved; 1) different status of nutrients and 2) NaCl stress, hence, to maintain uniform conditions during the experiment the former variable was eliminated. Therefore, the 'high nutrient' medium (d) was thought to be more suitable for our purpose, hence, it was decided to use this medium throughout future experiments. In this medium plants continue growing at an almost uniform growth rate.

## 3.2 Effect of Osmotic Stress on Leaf Growth

The growth rate of first leaf was measured at intervals of 5 min. In control conditions the leaf was growing at a rate of 17.5 ± 2.5 $\mu$m min $^{-1}$ (Fig. 3.1). To study the deleterious effects of salt stress on leaf growth a range of NaCl concentrations (see section 2.1) were applied to the root media. On addition of the salt, a decline in the growth rate was observed in 1 to 2 minutes (Fig. 3.1), which continued decreasing for all the NaCl concentrations. The growth rate was curtailed more quickly for higher NaCl concentrations than the lower ones. For 25 mol m$^{-3}$ growth did not stop completely and reduced to 8.2 $\mu$m min$^{-1}$ (about half of the control values). However, the growth was almost stopped completely for all other concentrations, though, the time taken was quite different.

After 60 minutes of the stress onset the salt was washed from the root media. Instantaneously (within 1 to 2 minutes) a big jump in the growth rate was recorded resulting in very high growth rate i.e. in most cases approximately double of the control rates (Fig. 3.1). However, the increase was temporary and the growth rate declined gradually in about 30 minutes to the normal values.

For testing the hypothesis, that the response could be specific to the NaCl only another experiment was carried out in which equi-osmolal (with NaCl) concentrations of mannitol were applied (Fig. 3.2). The osmotic coefficient of $Na^+$, $Cl^-$ and mannitol was considered to be equal to 1 for all the concentrations applied. [In fact, its magnitude is 0.925 for 150 mol m$^{-3}$ NaCl, the

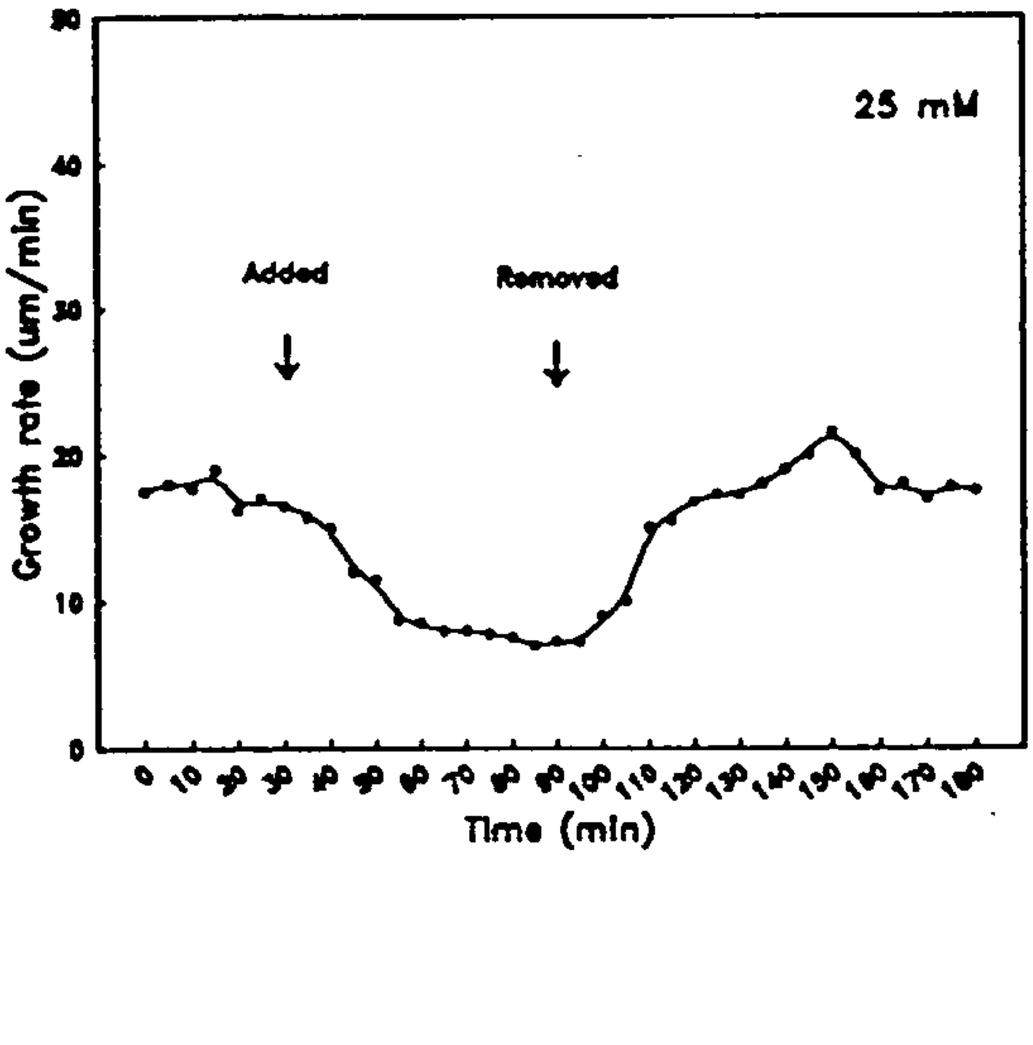
highest concentration used (Wyn Jones and Gorham, 1983)]. The overall trend of growth rate is similar to that observed in case of NaCl treatments except for 100 mol $m^{-3}$ mannitol where growth did not decline to the extent of 50 mol $m^{-3}$ NaCl. Curiously, the increase in growth rate on subsequent removal of mannitol was not as sharp as in case of NaCl.

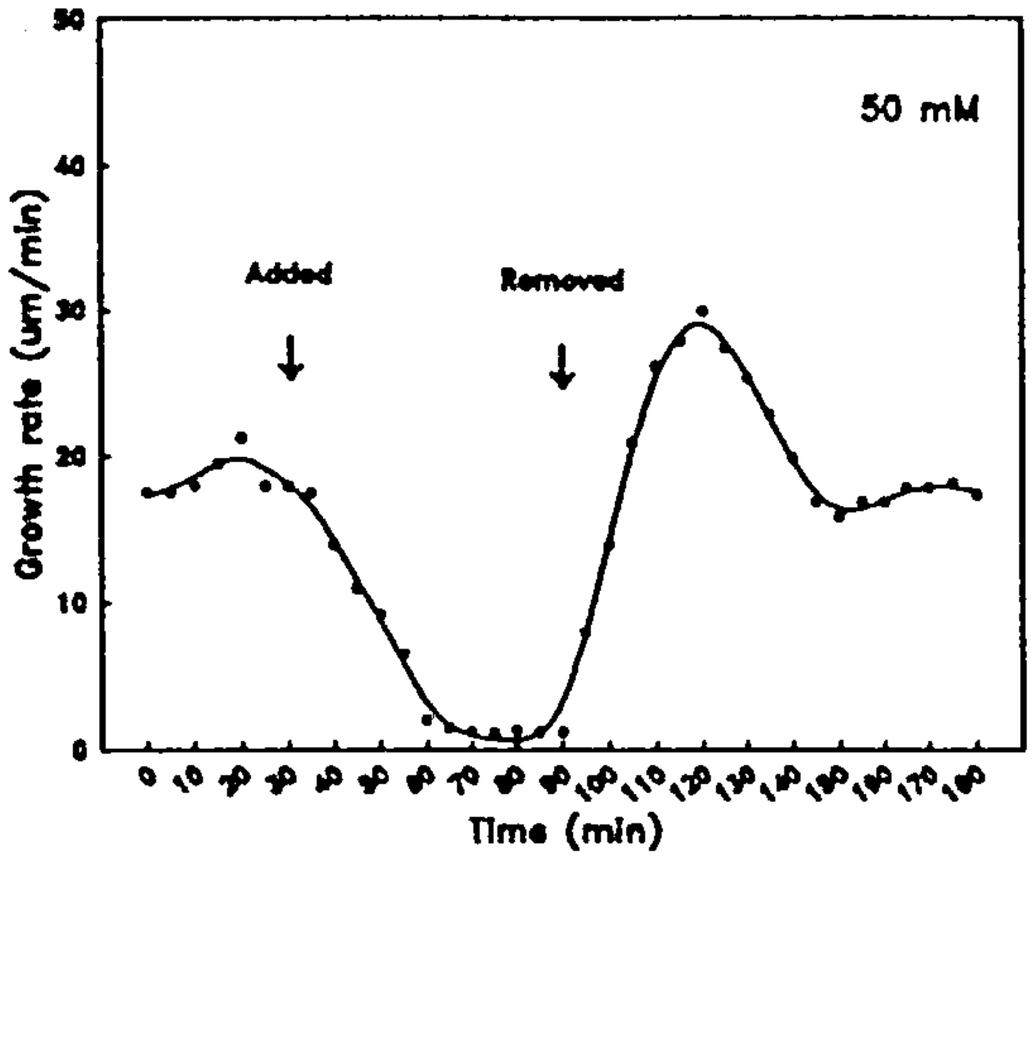
The next experiment was carried out for longer span of time i.e. 24 h and individual measurements were performed at 1 h interval after the onset of NaCl stress (Fig. 3.3). In this, and in all subsequent experiments, the plants were kept under stress for the whole of the experimental period. The response of growth rate to the salt stress was in two phases; an immediate decline and a subsequent gradual recovery. The growth rate declined rapidly after the stress application (as shown in the previous experiment, Fig. 3.1) for all the concentrations. After 2 to 3 h of the media salination a gradual and steady increase in the growth rate was recorded which continued until the previous rate of elongation (prior to stress application) was attained. The speed of growth rate recovery roughly corresponded to the respective NaCl concentrations as it recovered more rapidly for lower concentrations than the higher ones. Therefore, the data were consistent to the hypothesis that the time taken for growth recovery corresponds to the NaCl concentration present in the medium.

The same experiment was continued for another period of 5 d. The rate of elongation in control leaves was unchanged for the first 3 d, later it declined gradually and became almost negligible

after 6 d (Fig. 3.4). This is presumably due to the leaf reaching its mature length as at the same time second leaf was emerging very rapidly (visual observation). Similar trends were found for 25 and 50 mol $m^{-3}$ NaCl. Higher concentrations might have contributed towards earlier slowing of growth of the leaf because elongation rate started decreasing even after 2 d of NaCl application.

Fig. 3.1 Effect of NaCl stress on growth rate of the first emerged leaf of 7 days old wheat seedlings. The elongation rate was measured continuously using LVDT displacement transducer over a period of 3 h of the stress application. Various NaCl concentrations i.e. 25, 50, 75, 100, 125 and 150 mol $m^{-3}$, as indicated in top right corner of every graph, were applied to salinize the root media. All the NaCl concentrations were applied in one step to give sudden shock to plants. Each point is the mean of 4-10 replicates taken from two experiments.


25 mM
Added
Removed
Growth rate (um/min)
Time (min)


50 mM
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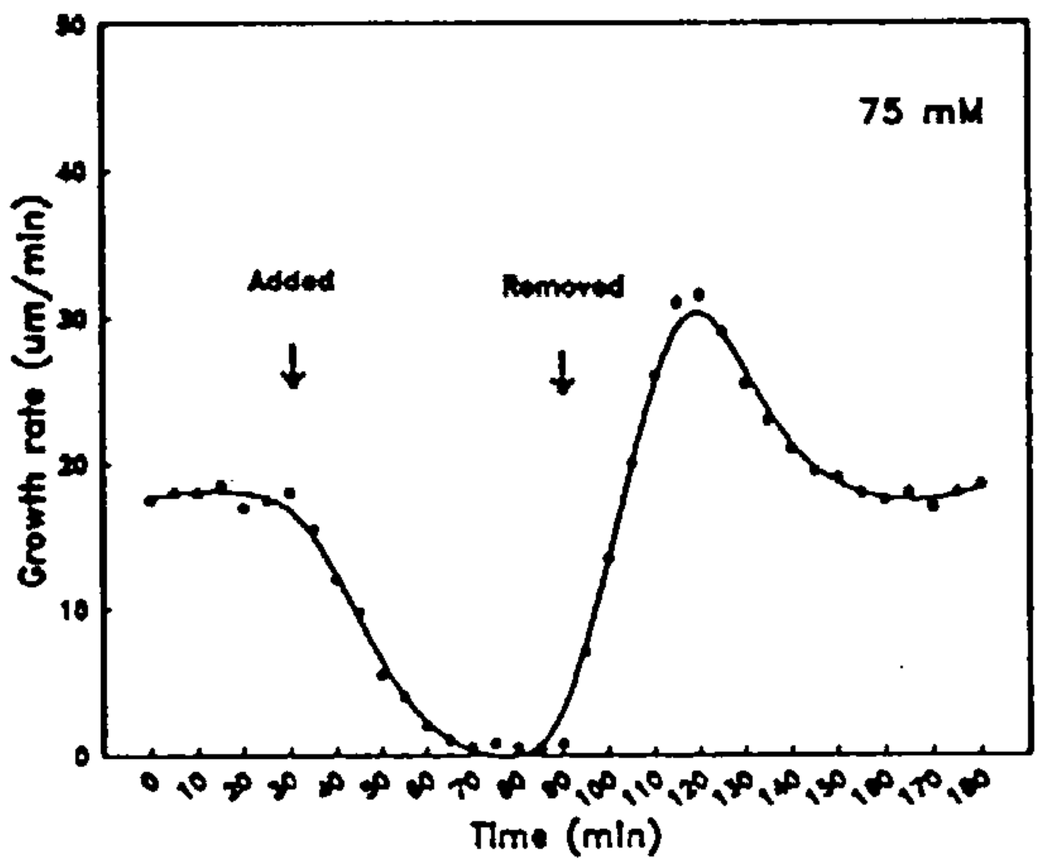
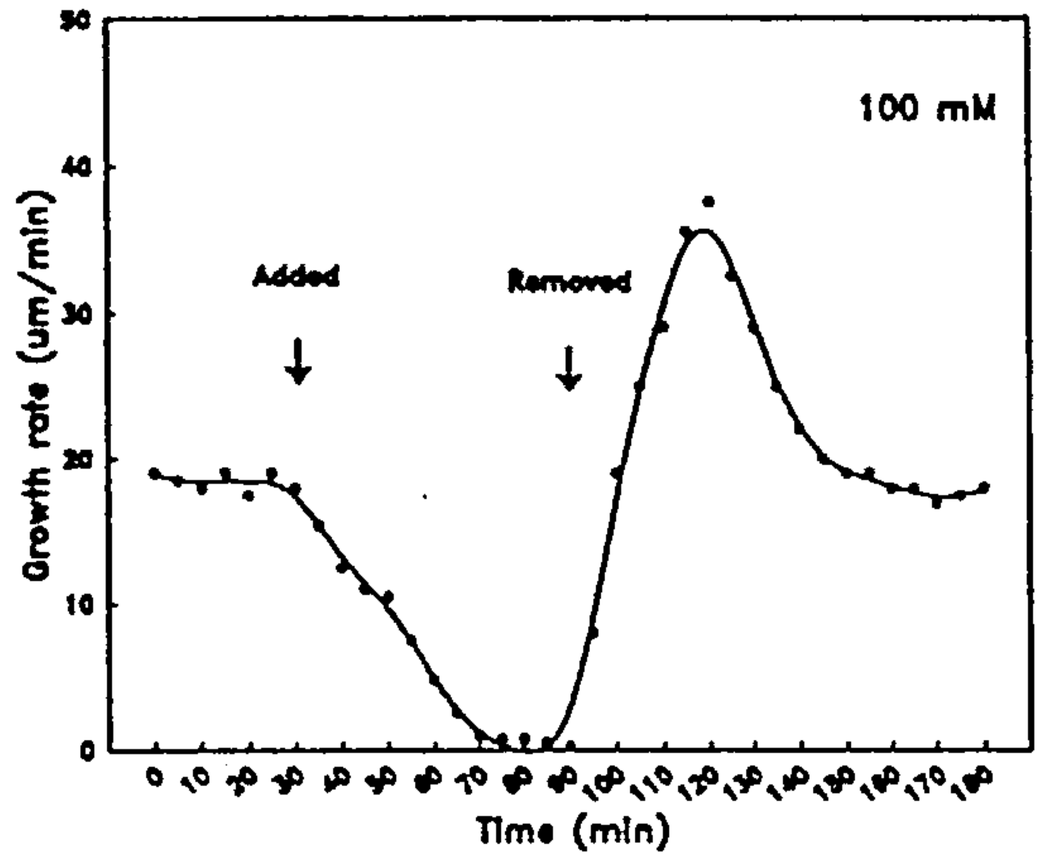
Removed
Growth rate (um/min)
Time (min)


75 mM
Added
Removed
Growth rate (um/min)
Time (min)


100 mM
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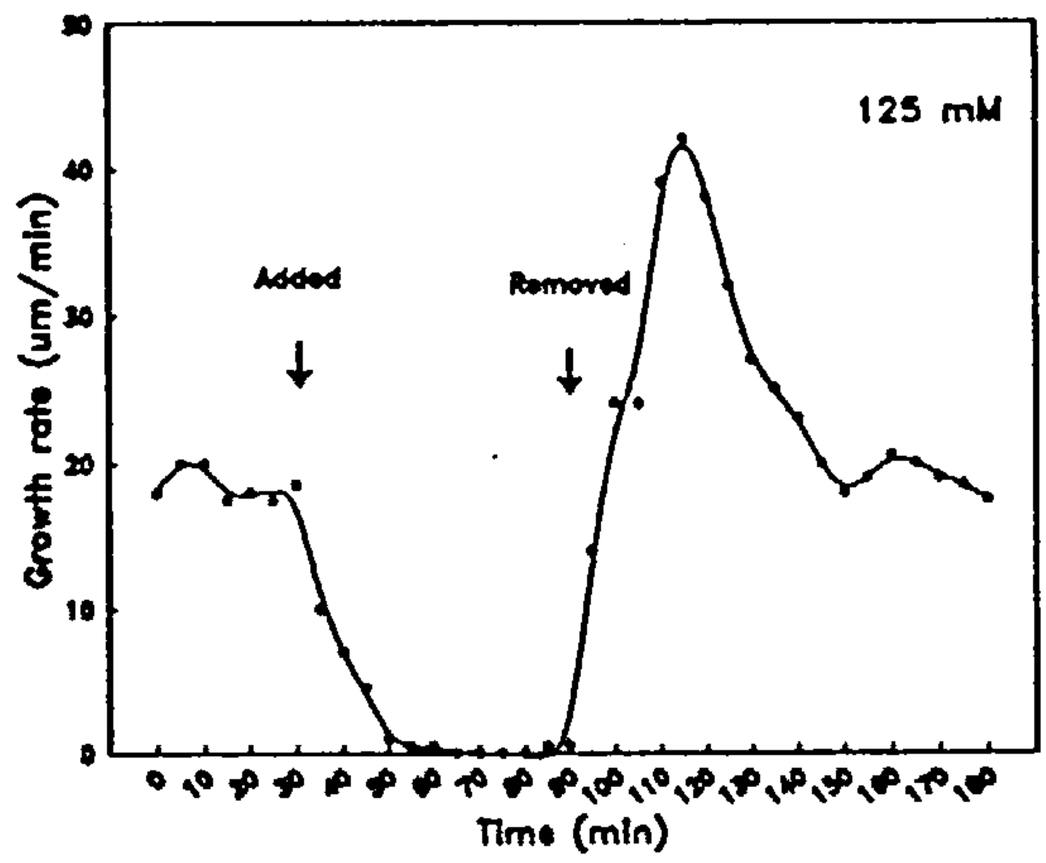
Removed
Growth rate (um/min)
Time (min)


125 mM
Added
Removed
Growth rate (um/min)
Time (min)

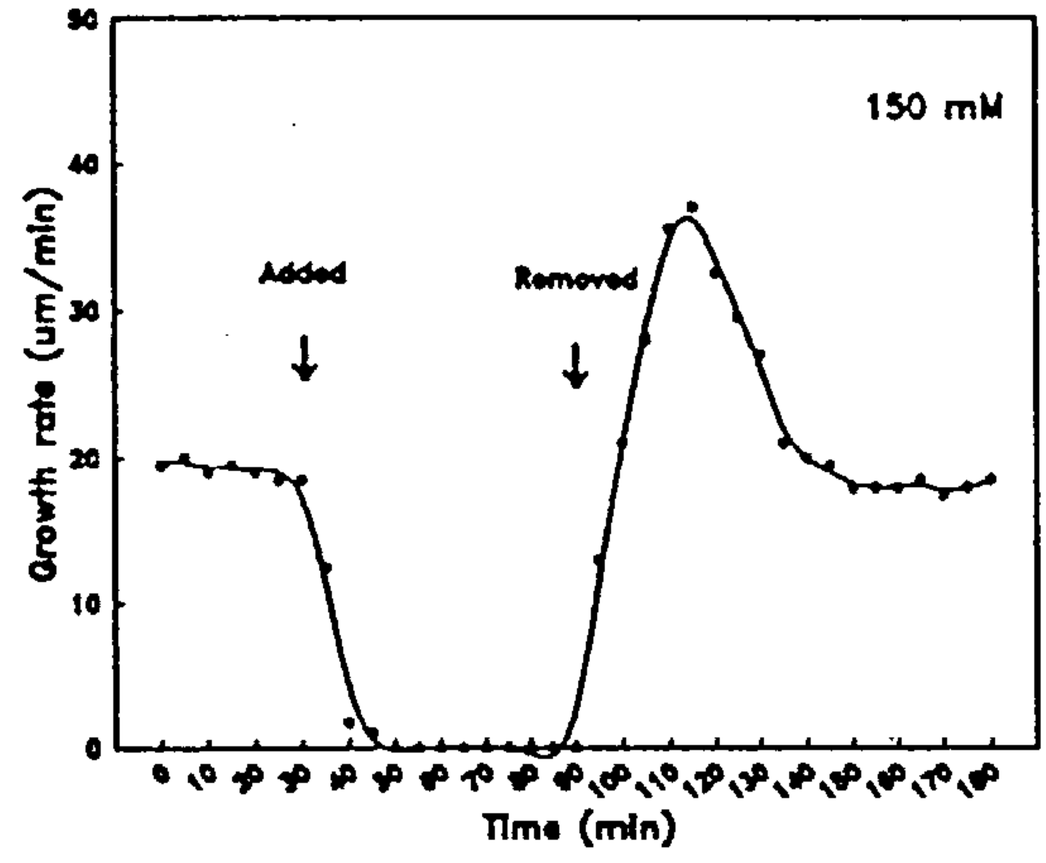
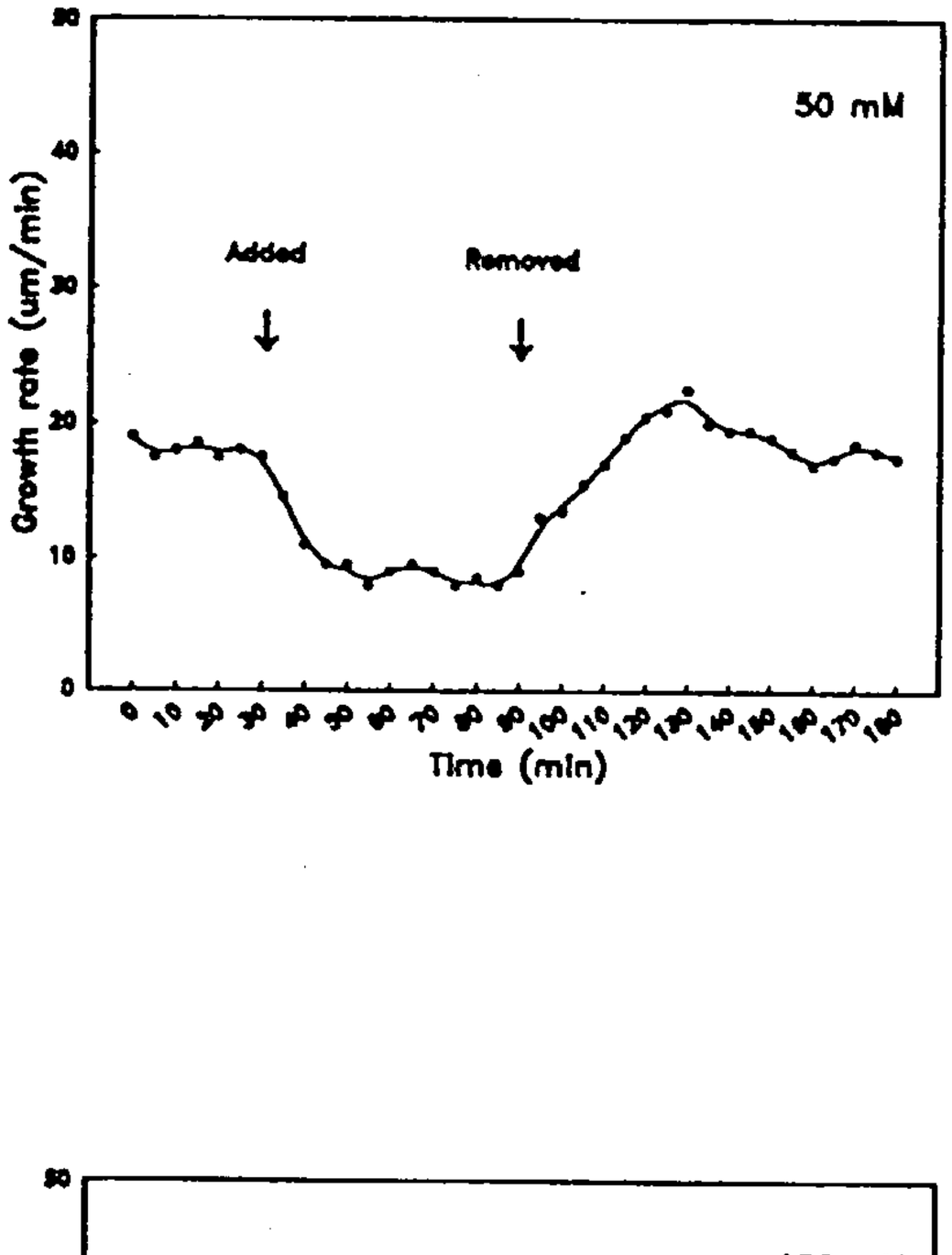

150 mM
Added
Removed
Growth rate (um/min)
Time (min)

**Fig. 3.2** Effect of mannitol stress on growth rate of the first emerged leaf of 7 days old wheat seedlings. Conditions as in Fig. 3.1. Mannitol concentrations are approximately equi-osmolar with NaCl in the previous figure. Each point is the mean of 4-6 replicates taken from one experiment.


50 mM
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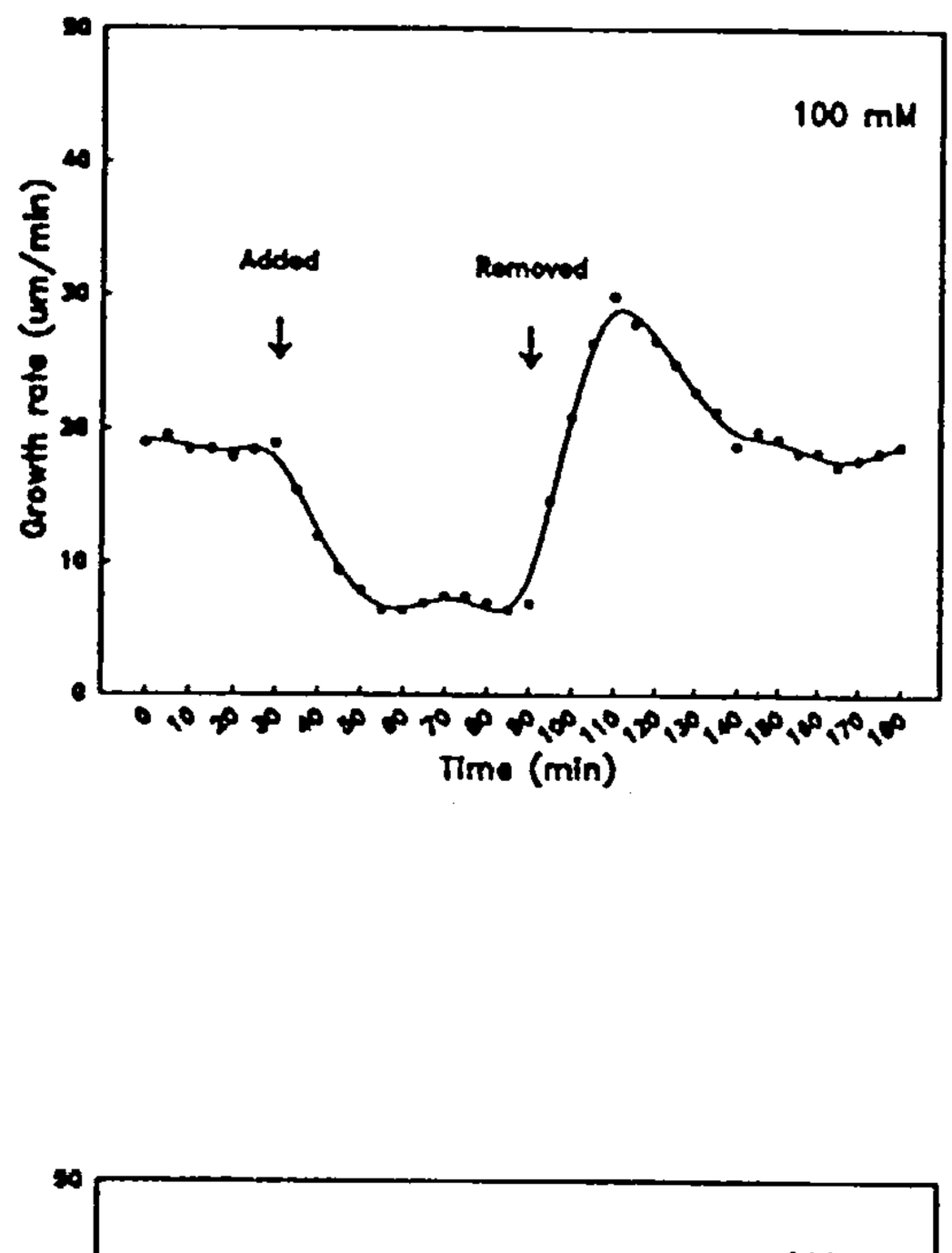
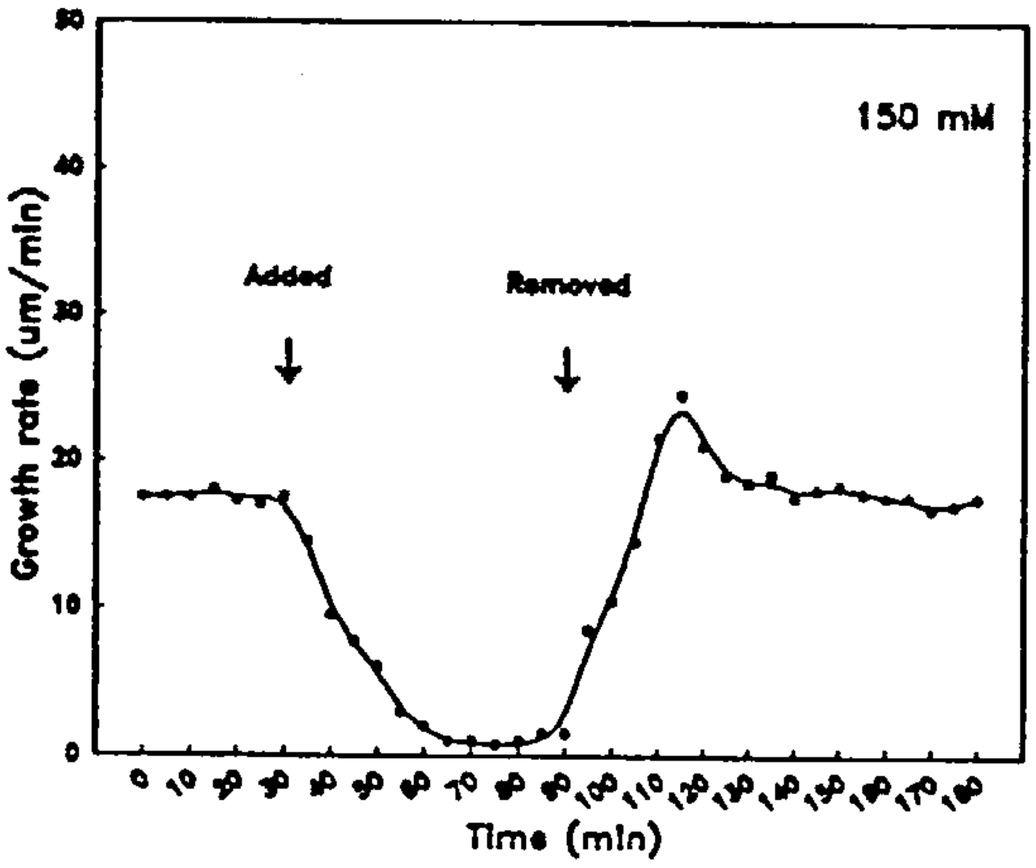
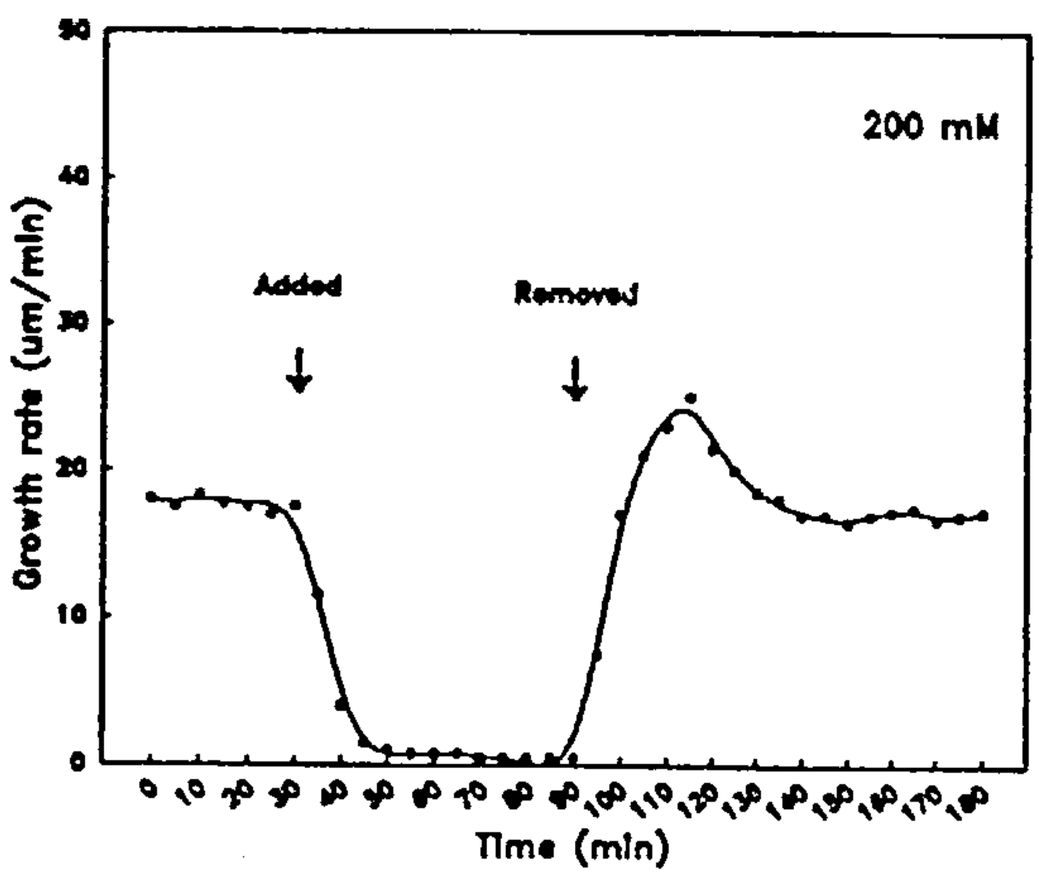
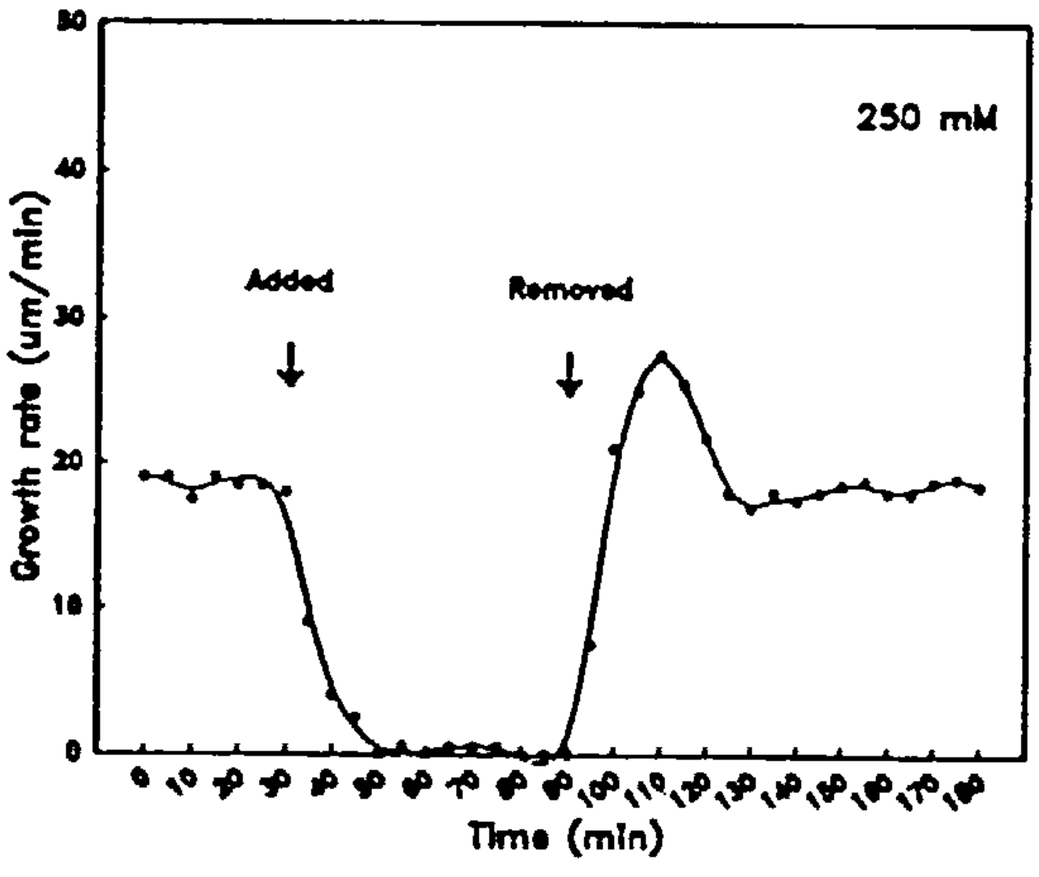
Removed
Growth rate (um/min)
Time (min)


100 mM
Added
Removed
Growth rate (um/min)
Time (min)


150 mM
Added
Removed
Growth rate (um/min)
Time (min)


200 mM
Added
Removed
Growth rate (um/min)
Time (min)


250 mM
Added
Removed
Growth rate (um/min)
Time (min)

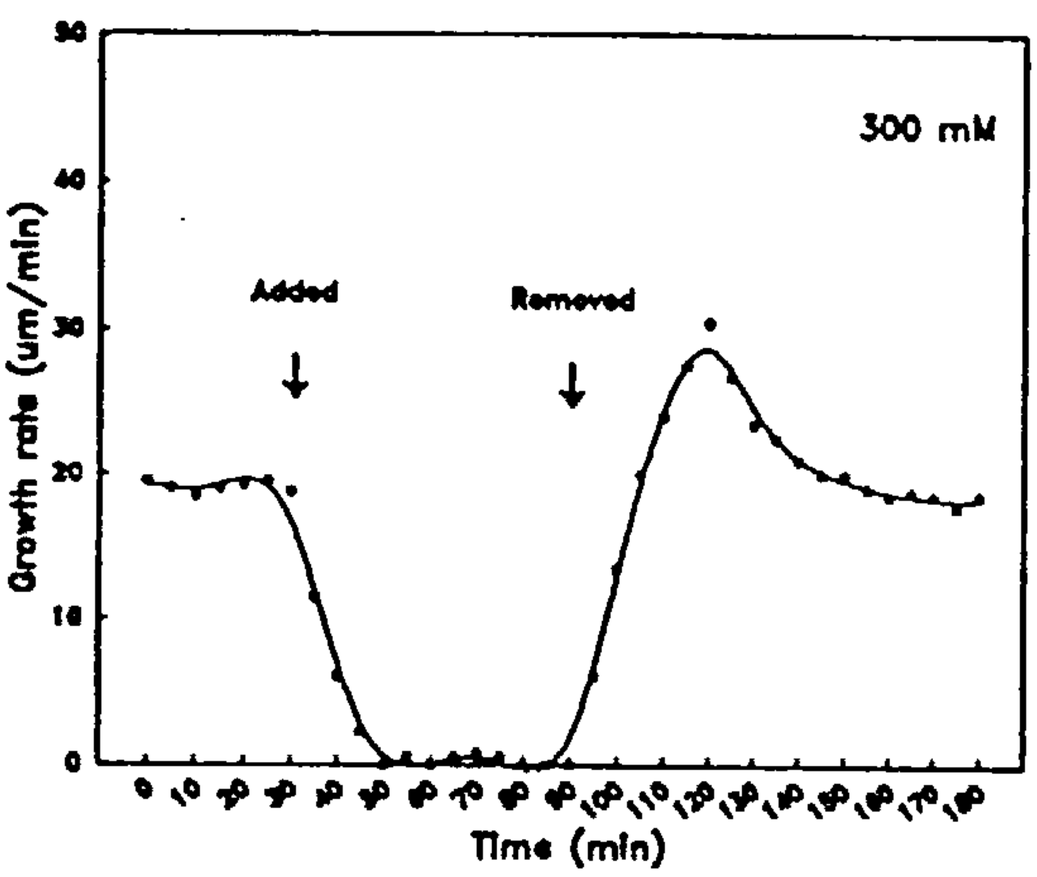
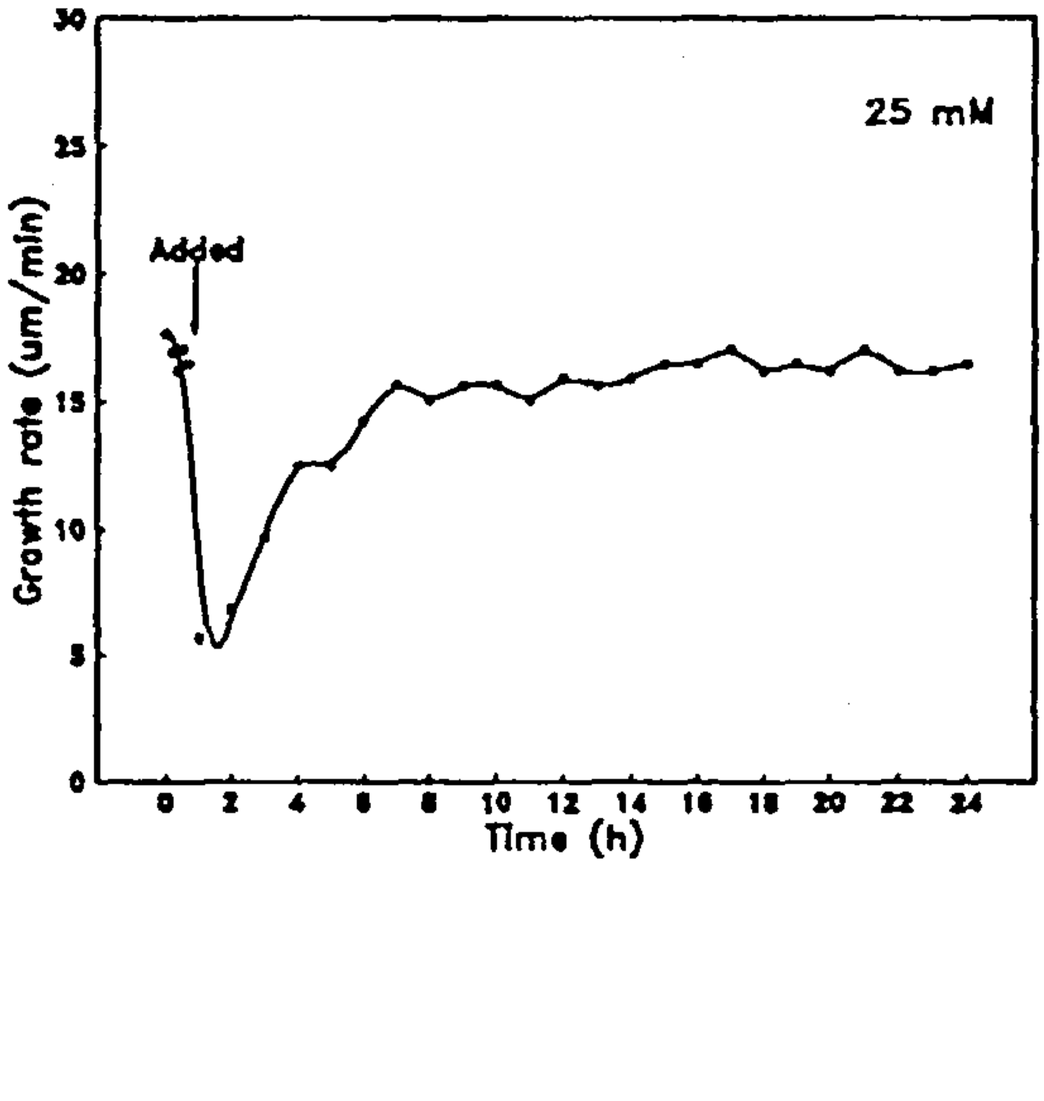
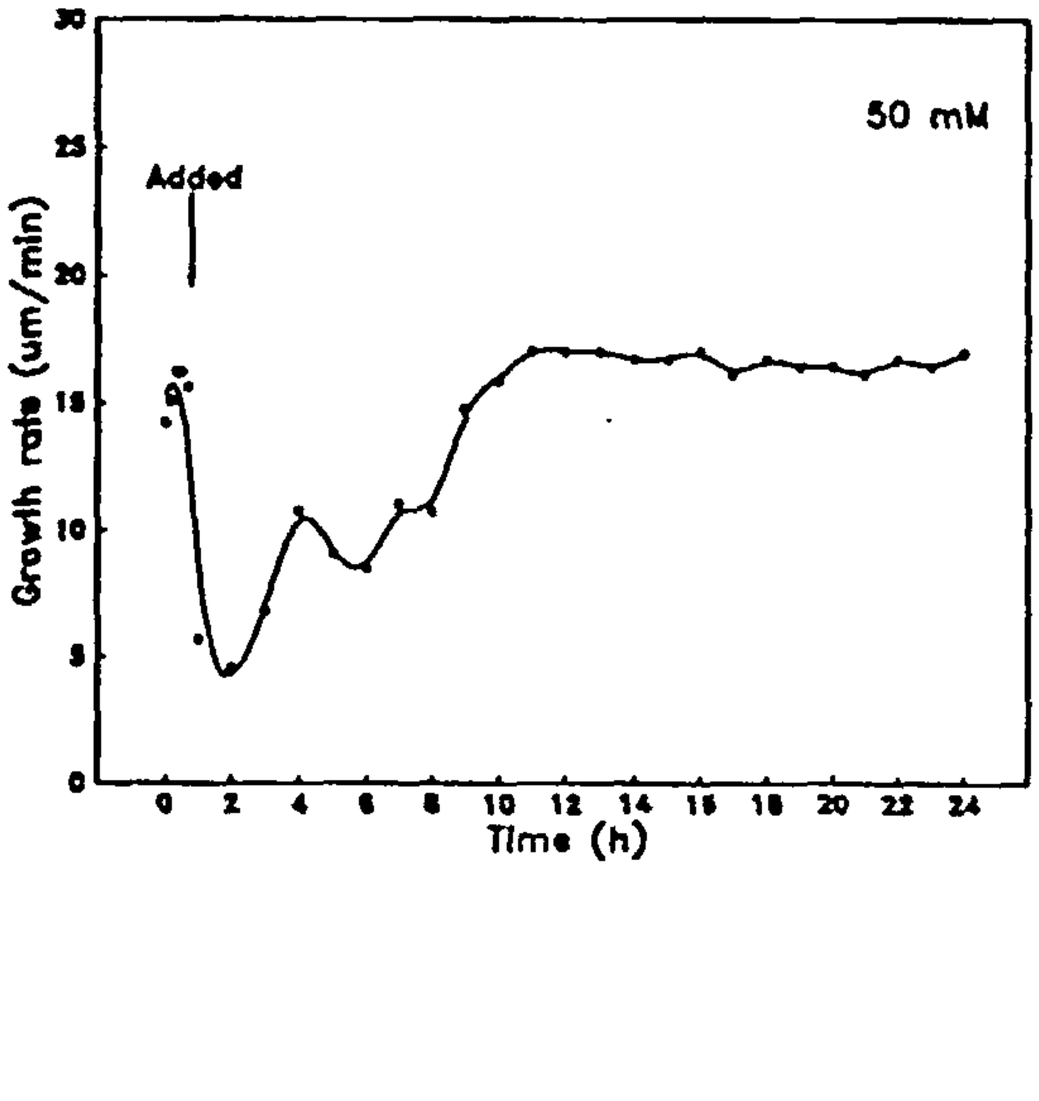
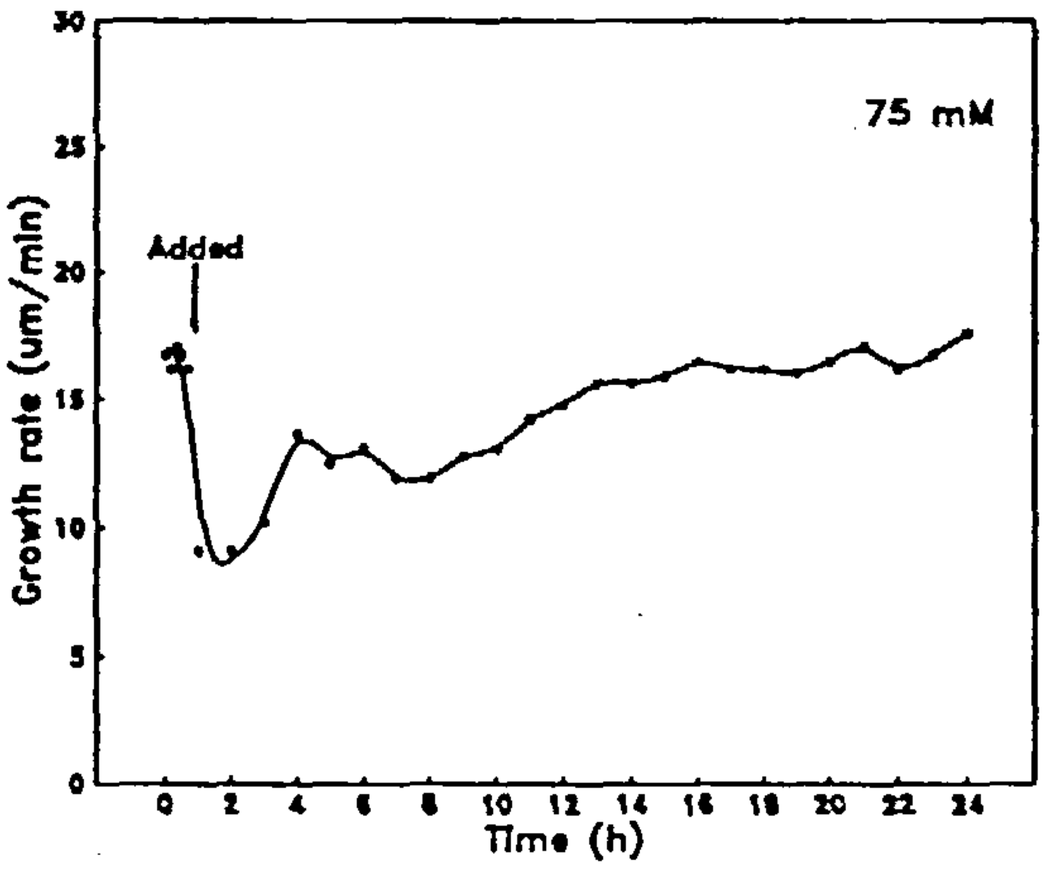
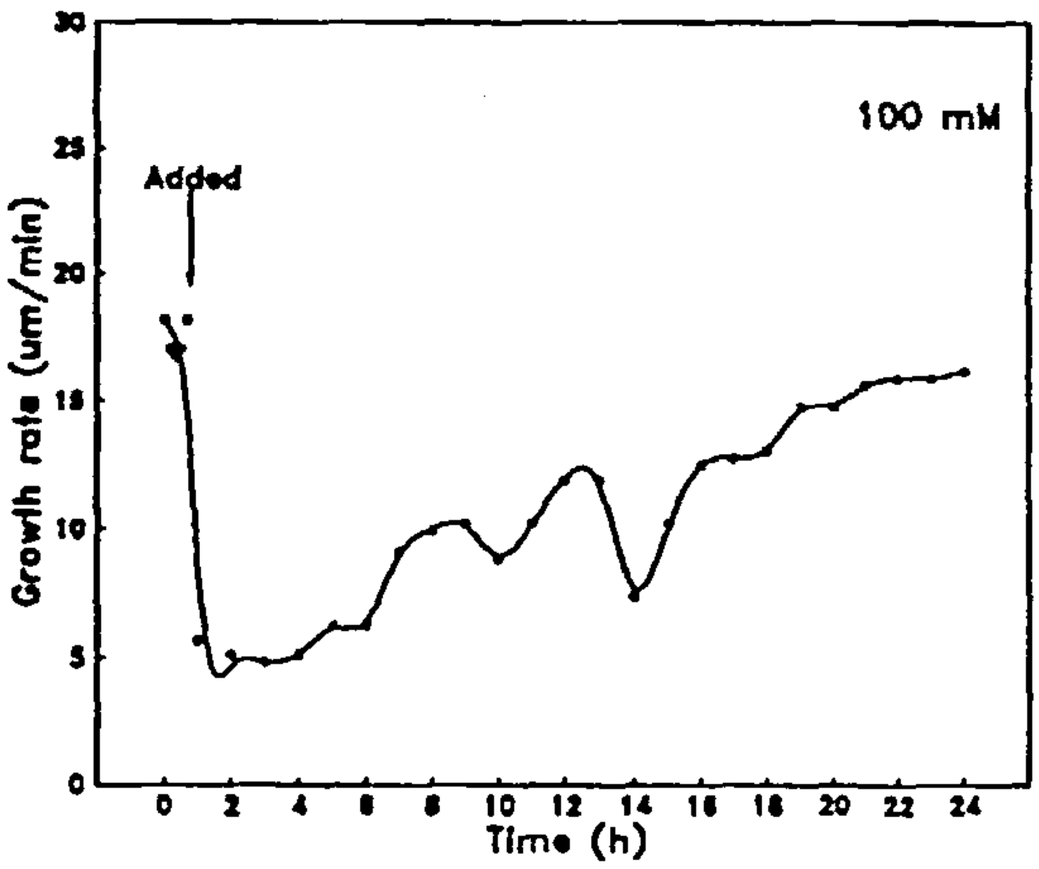

300 mM
Added
Removed
Growth rate (um/min)
Time (min)

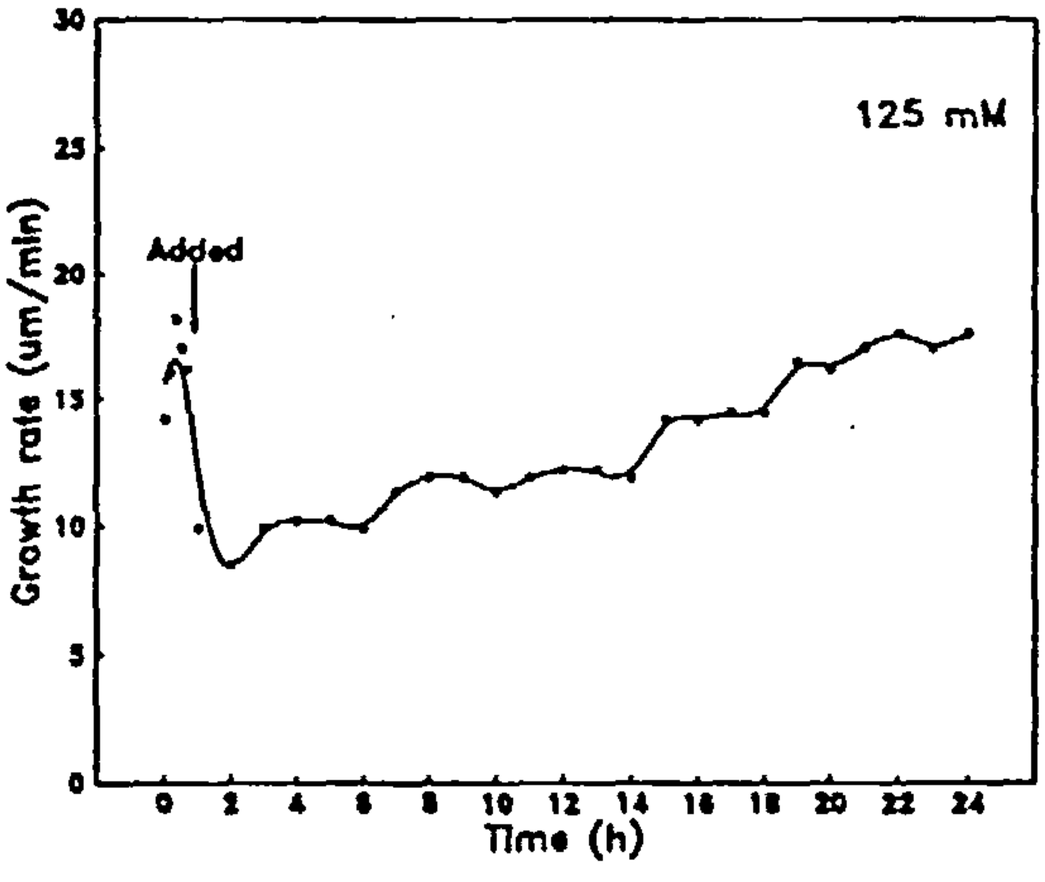
Fig. 3.3 The time course of leaf elongation rate recovery following the decrease in response to the NaCl stress. (Fig. 3.1). The growth rate was measured continuously using LVDT displacement transducer over a period of 24 h of the stress application. Various NaCl concentrations i.e. 25, 50, 75, 100, 125 and 150 mol $m^{-3}$, as indicated in top right corner of every graph, were applied to salinize the root media. All the NaCl concentrations were applied in one step to give sudden shock to plants. Each point is the mean of 4-8 replicates taken from two experiments. Arrow indicates the time at which the plants were introduced to the stress.


25 mM
Added
Growth rate (um/min)
Time (h)


50 mM
Added
Growth rate (um/min)
Time (h)


75 mM
Added
Growth rate (um/min)
Time (h)


100 mM
Added
Growth rate (um/min)
Time (h)


125 mM
Added
Growth rate (um/min)
Time (h)

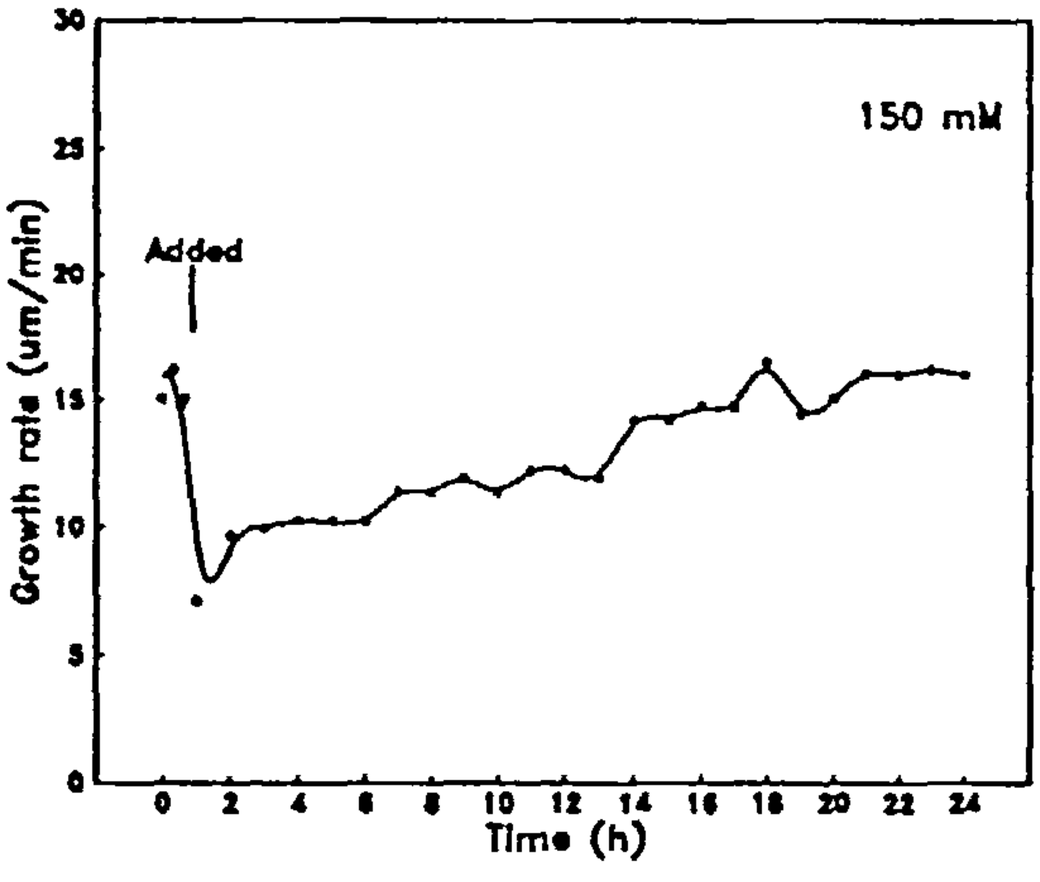
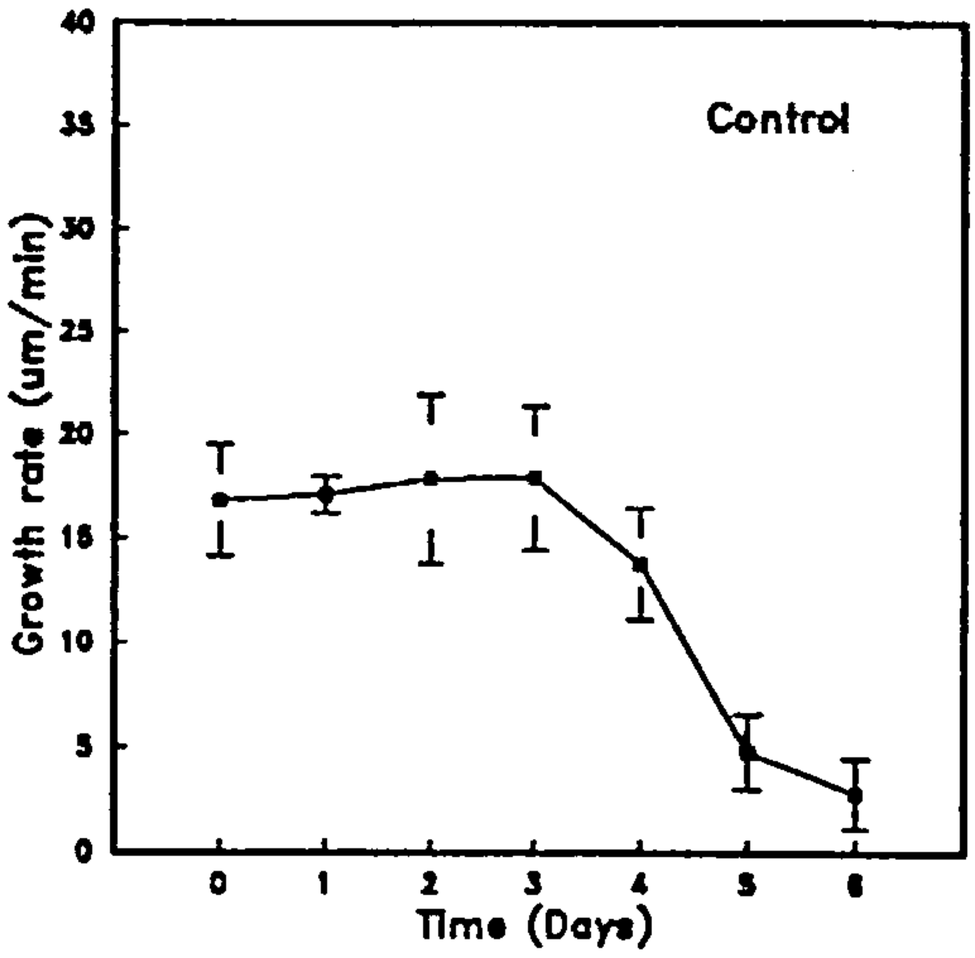
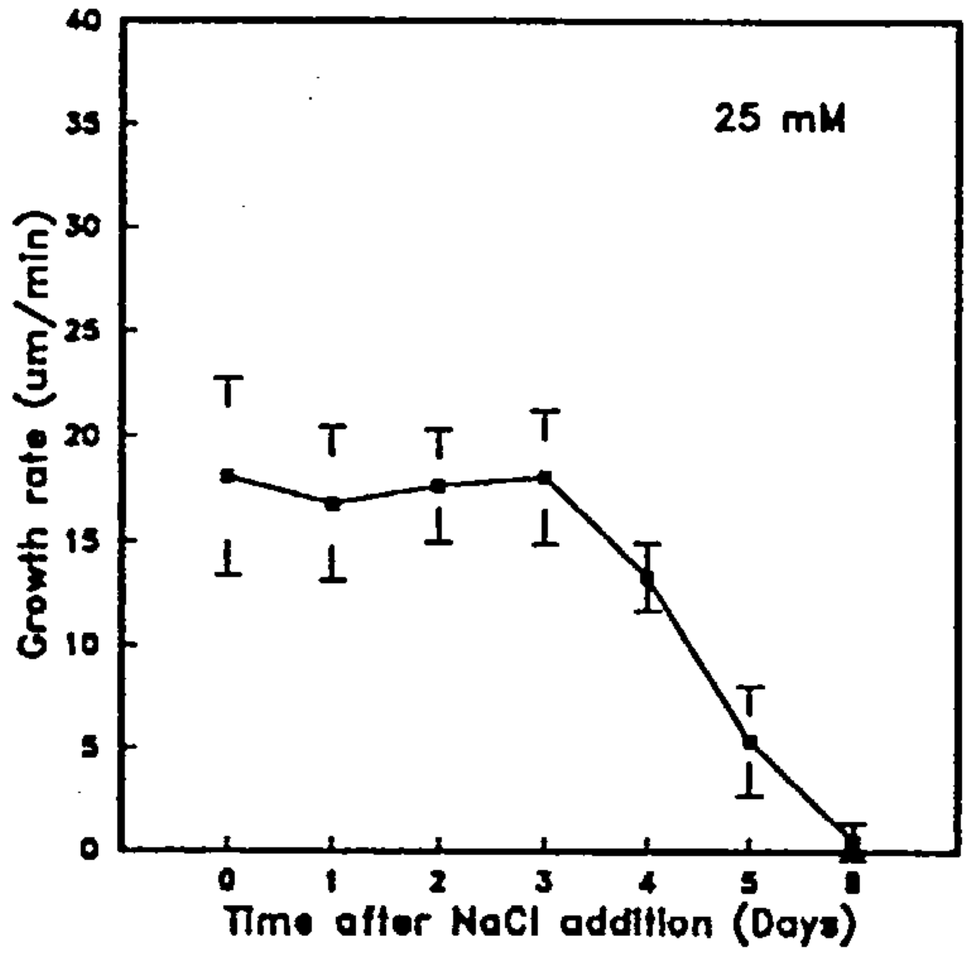
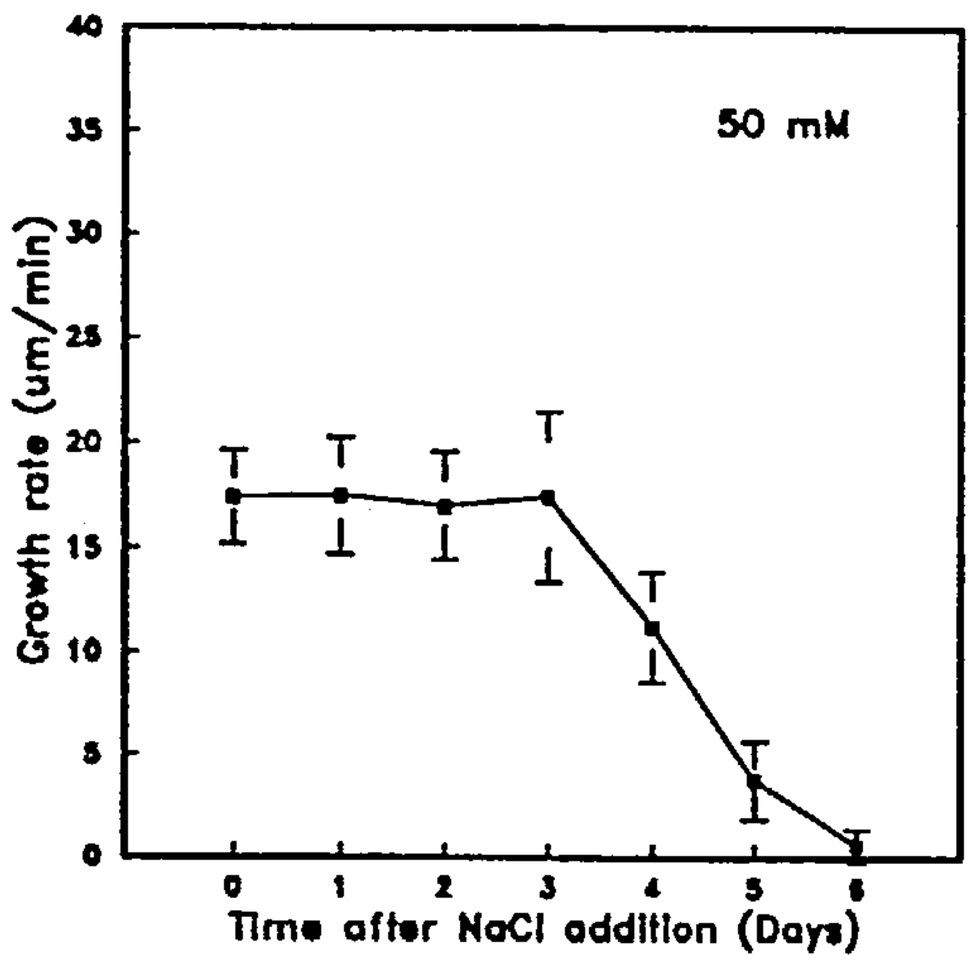
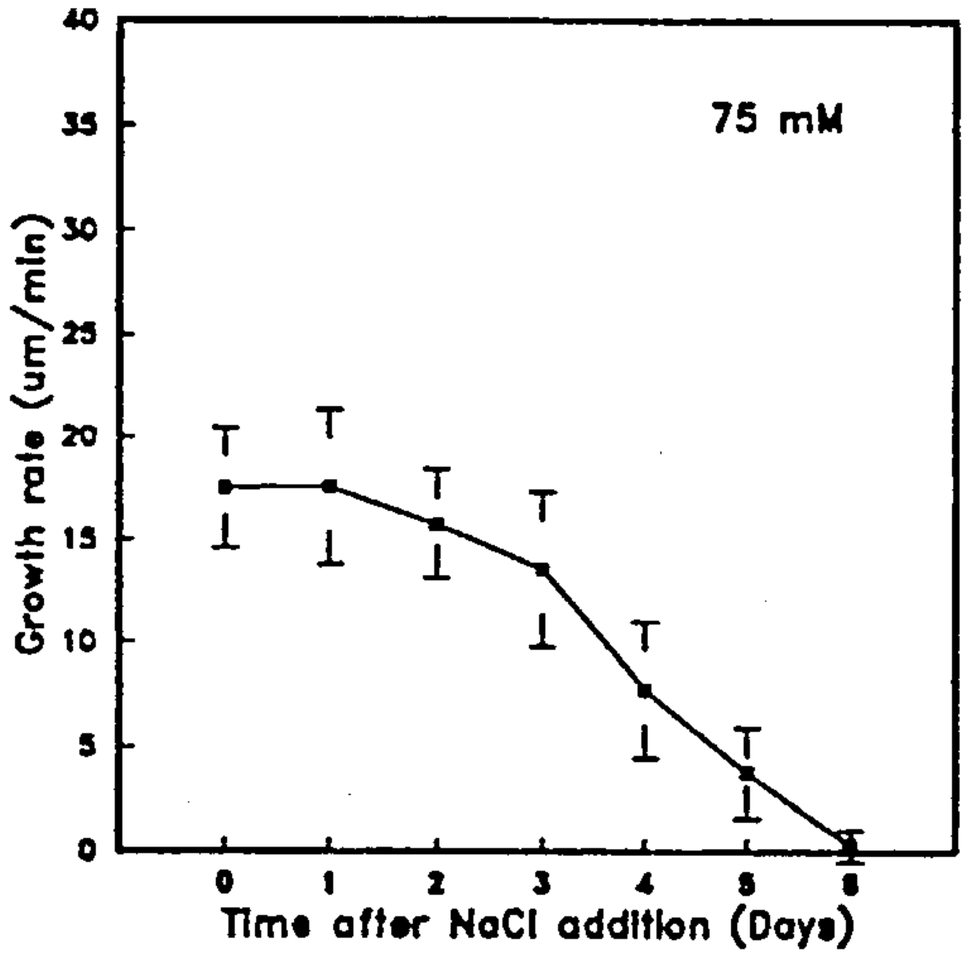

150 mM
Added
Growth rate (um/min)
Time (h)

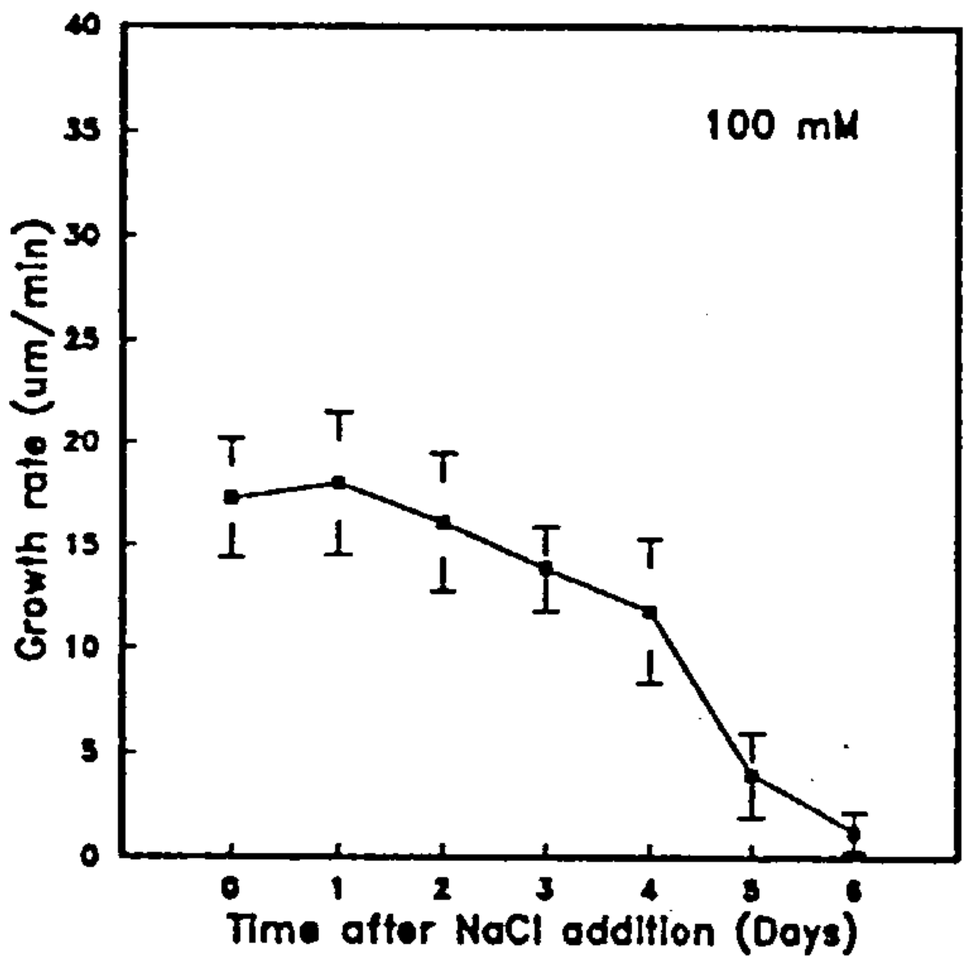
**Fig. 3.4** The response of leaf elongation rate to the salt stress over a period of 6 days. The growth rate was measured continuously using LVDT displacement transducer. Conditions as in Fig. 3.3. Each point is the mean of 5-15 replicates taken from two experiments. Error bars indicate the standard deviation of the mean of all replicates.

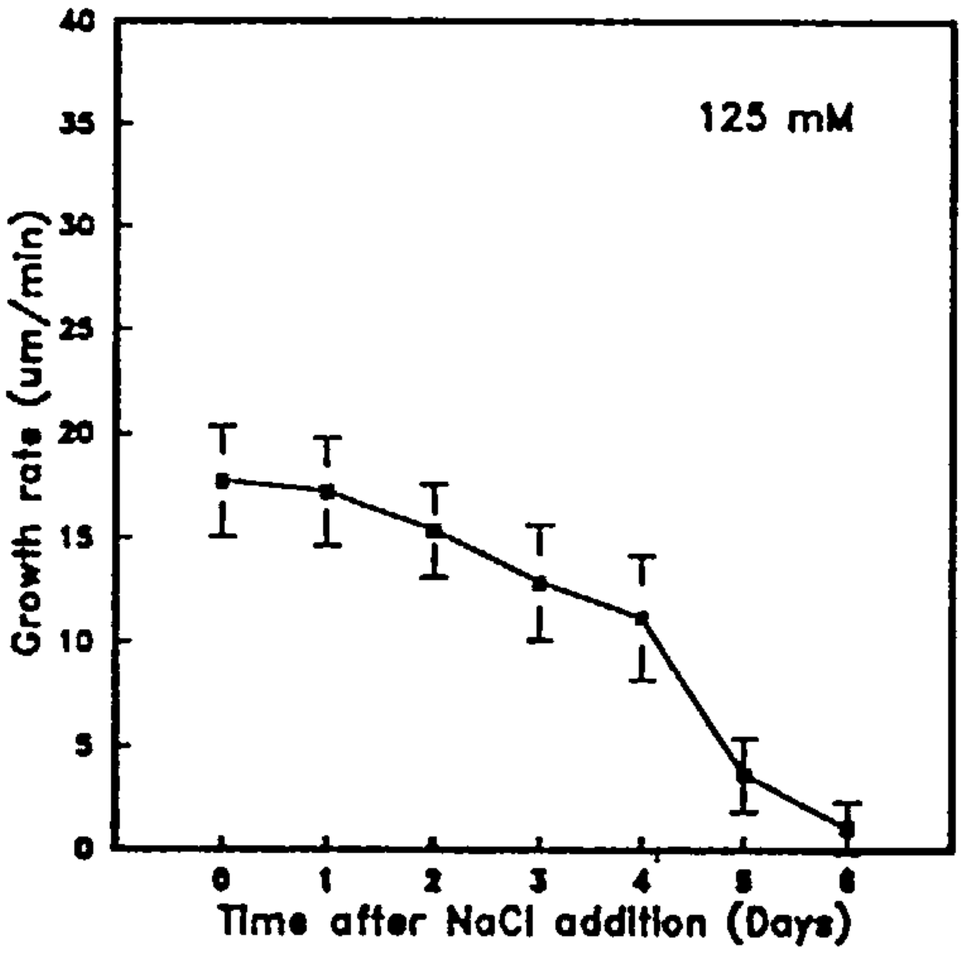

Control
Growth rate (um/min)
Time (Days)


25 mM
Growth rate (um/min)
Time after NaCl addition (Days)


50 mM
Growth rate (um/min)
Time after NaCl addition (Days)


75 mM
Growth rate (um/min)
Time after NaCl addition (Days)


100 mM
Growth rate (um/min)
Time after NaCl addition (Days)


125 mM
Growth rate (um/min)
Time after NaCl addition (Days)

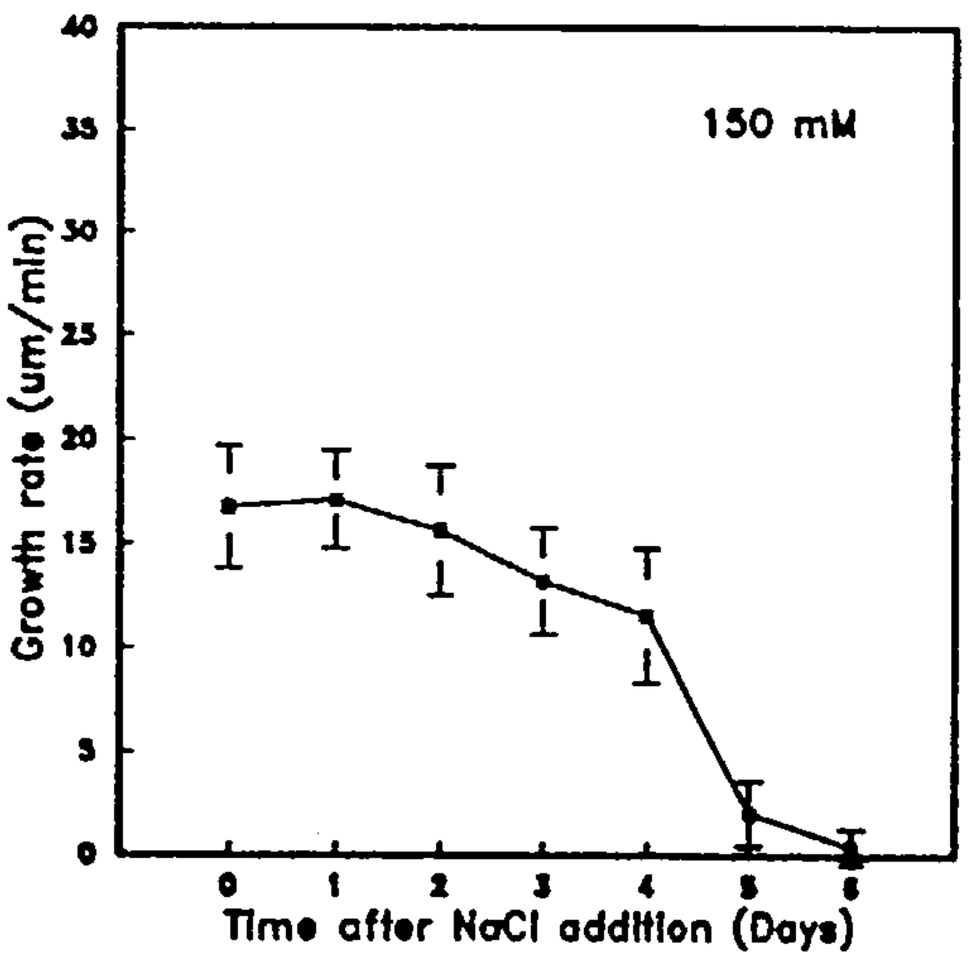

150 mM
Growth rate (um/min)
Time after NaCl addition (Days)

**Table 3.1 The effect of nutrient status of different types of root medium on leaf elongation rate ($\mu m\ min^{-1}$). The measurements were taken with a ruler and were converted into units compatible with the units of same type of other experiments. Results ± s.d.**

| | Leaf | Root |
|---|---|---|
| $CaCl_2$ (0.5 mol $m^{-3}$) (144 h) | 12.12 ± 1.13 (8) | 21.87 ± 2.92 (8) |
| $CaCl_2$ + Hoagland solution (50 %) (120 h) (24 h) | 12.23 ± 0.98 (8) | 22.54 ± 0.54 (8) |
| $CaCl_2$ + Hoagland solution (120 h) (24 h) | 17.24 ± 0.96 (8) | 20.51 ± 1.47 (8) |
| Hoagland solution (144 h) | 17.82 ± 0.84 (8) | 20.35 ± 1.75 (8) |

**Table 3.2 The effect of nutrient status of different types of root medium on tissue osmotic pressure in the mature zone of leaf (MPa).**

| | Leaf | Root |
|---|---|---|
| $CaCl_2$ (0.5 mol $m^{-3}$) (144 h) | 0.73 ± 0.1 (10) | 0.45 ± 0.08 (10) |
| $CaCl_2$ + Hoagland solution (50 %) (120 h) (24 h) | 1.21 ± 0.14 (10) | 0.52 ± 0.07 (10) |
| $CaCl_2$ + Hoagland solution (120 h) (24 h) | 1.36 ± 0.13 (10) | 0.48 ± 0.06 (10) |
| Hoagland solution (144 h) | 1.20 ± 0.06 (10) | 0.52 ± 0.08 (10) |

# CHAPTER 4

# *WATER RELATIONS OF LEAF GROWING ZONE*

## 4.1 Short Term Changes in Turgor Pressure

In the previous experiments on NaCl addition to root media the leaf growth rate declined immediately. Taking into consideration the Lockhart equation, when water conductivity to the expanding cells is not limiting (equation 1.12);

Growth rate = ø (P - Y)

Where ø, P and Y are the cell wall extensibility, turgor pressure and the yield threshold, respectively. Experiments were designed to see whether drop in turgor pressure was responsible for the growth rate decline. Therefore, turgor pressure in epidermal and mesophyll cells of the growing zone of the leaf (recognized by the technique of Kemp, 1980; see section 2.3.2) was measured. The average value for control plants was 0.45 $\pm$ 0.08 MPa. The turgor pressure was monitored for a 6 h period following the transfer of the plants to different external NaCl concentrations. Surprisingly and in marked contrast to the mature tissue (see section 5.1.1), the turgor pressure did not change in response to any of the added NaCl concentrations up to 150 mol $m^{-3}$ (Fig. 4.1).

## 4.2 Long Term Changes in Turgor Pressure

Similar turgor pressure responses were observed in the long term experiments also where the turgor pressure was monitored for 6 d following the stress onset (Fig. 4.2). Individual measurements were performed at 1 d intervals. From these data, it was concluded that turgor pressure was not responsible for the

decline in the growth rate, or that (P-Y) value is so small that it could not be measured (P-Y would have to be lower than 0.01 MPa which is the practical limit of turgor pressure measurement described in this experiment).

## 4.3 Measurement of Cell Wall Extensibility

The next experiment was an attempt to investigate the effect of the second parameter in the equation 1.12, the cell wall extensibility. Since we were unable to measure growth rate over a range of turgor pressure, as recently achieved for roots by Pritchard et al. (1987; 1989), direct measurement of the Lockhart parameters did not prove possible. The Instron technique used does not measure the parameter directly (Cosgrove, 1987; Pritchard et al. 1989) but has been shown in the past to correspond qualitatively with growth rates. Both of the components measured by the method; elastic and plastic, were measured with tensiometer after 5 and 144 h of the stress onset. The elastic extensibility did not change for any of the NaCl concentrations applied and it was similar to that of the control values (7.42 $\pm$ 0.49 % extension per 15 g load, Table 4.1). The elastic extensibility decreased after 144 h of stress application for all the NaCl concentrations (Table 4.1). However, the similar decrease was observed in case of control plants after 144 h. Therefore, this decrease must be due to some other reasons than the salt stress, such as tissue maturity.

For the control plants, the measured irreversible plastic extensibility of the cell wall was 3.80 $\pm$ 0.55 % extension per 15

g load and that is about one half of the elastic extensibility values. Responses similar to that of the elastic extensibility were observed for the plastic extensibility i.e. it did not change after 5 h but changed (decreased) after 144 h for both the control plants (Table 4.1) and all the plants under salt stress. Therefore, this decline is also presumably due to the aging of the tissue.

## 4.4 Estimation of Cell Wall Transpiration Tension

The cell wall transpiration tension was estimated by the difference of the turgor pressure measurements performed before and after immersing the whole plant under the respective root medium (see section 2.4). It was found to be about 0.11 $\pm$ 0.04 MPa for the growing region of the leaf i.e. when the plant was immersed under the root medium the turgor pressure increased by about 0.11 MPa (Table 4.2).

## 4.5 Other Experiments on Growth

Following the increase in turgor pressure upon immersing the whole plant in the growing media (see section 3.2) it was decided to investigate the response of leaf growth rate under the similar conditions. Surprisingly, the leaf growth rate remained uniform throughout the experiment i.e. no change could be detected in leaf growth rate after the immersion (Table 4.3). This observation was unexpected. If P-Y is smaller than 0.11 MPa the increase in turgor pressure should have a large effect on growth rate.

## 4.6 Tissue Osmotic Pressure

The tissue osmotic pressure of the leaf growing zone was measured by osmometry. For control plants the osmotic pressure value was nearly 1.1 MPa and it remained almost unchanged for all the experimental period i.e. 6 d. When NaCl was added to the root media it increased gradually with time for 25, 50 and 75 mol $m^{-3}$ concentrations. Whilst for the higher concentrations (100, 125, 150 mol $m^{-3}$) the value rose more drastically after the NaCl addition (Fig. 4.3). From the same figure it becomes obvious that this increase was observed only within 48 h of the stress onset after which the value remained almost constant. The increase in tissue osmotic pressure corresponded to the amount of the external NaCl concentrations in the media.

## 4.7 Cell Wall Solutes

The magnitude of osmotic pressure was much higher than the turgor pressure for the same tissue. For instance the osmotic pressure value (refer to Fig. 4.3, control) was 0.65 MPa higher than the turgor pressure value i.e. 0.45 MPa (refer to Fig. 4.1, control). Since the transpiration tension was about 0.11 MPa in the cell wall (Table 4.2) and hence, on deduction $P_w$ from the difference in turgor pressure and osmotic pressure a value of 0.54 MPa is obtained for the apparent osmotic pressure of the cell wall. This value is thought to be osmotic pressure due to the presence of osmotically active solutes in the cell wall. The estimated cell wall solutes for different NaCl treatments are given in Fig. 4.4 where it seems that the increase in cell wall solutes corresponds

to the amount of external salt stress. In addition, the estimated $\pi_w$ value in growing zone was almost 5 times higher than that in the mature zone (see Fig. 4.4 and 4.14, control). The presence of so much osmotic pressure in the cell wall provided evidence for the possible involvement of the cell wall solutes in turgor pressure adjustment, as discussed in the introduction 0.54 MPa corresponds to some 220 mOsmol $kg^{-1}$. Turgor adjustment by this process would be impossible for changes in water potential greater than that. It was therefore, decided to extend the range of salt stress above the 150 mol $m^{-3}$ in order to test the hypothesis.

## 4.8 Turgor Pressure Response at High Salt Concentrations

In the previous experiments the external salt concentrations applied were only up to 150 mol $m^{-3}$ and the measured turgor pressure did not show any change. Therefore, to investigate if still higher salt concentrations affect the turgor pressure the higher external NaCl concentrations (200 and 250 mol $m^{-3}$) were applied in the root media. In sharp contrast to NaCl level up to 150 mol $m^{-3}$ turgor pressure declined immediately after the addition of salt in the root medium. After 30 minutes of salt addition the turgor pressure was about 0.2 MPa for both of the concentrations applied (Fig. 4.5). This initial decline was small to correspond to the amount of the NaCl applied. After 2 h following the salt addition the turgor pressure was about 0.2 and 0.12 MPa for 200 and 250 mol $m^{-3}$, respectively. Therefore, the differences in turgor pressure became slightly more obvious after 2 h of the stress commencement. However, after 1 to 2 h the

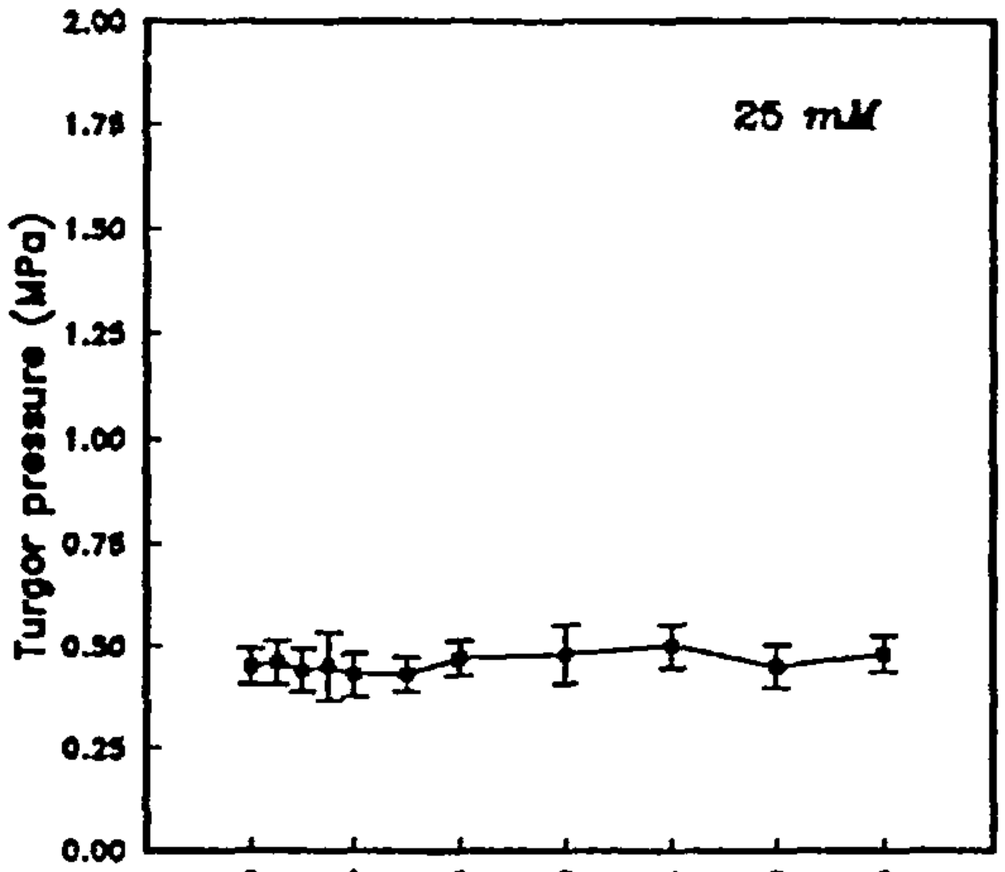
change in turgor pressure showed a plateau (Fig. 4.5).

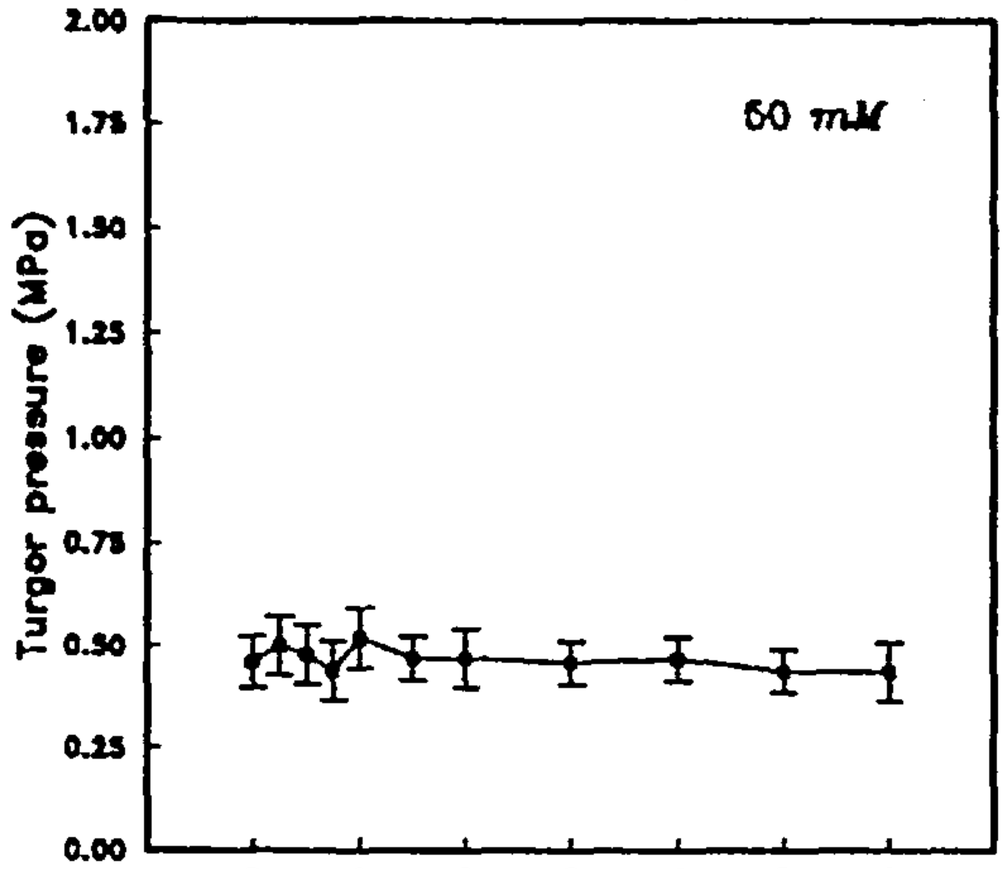
These observations are consistent that the cells are taking up all the solutes present in the cell walls to maintain their turgor pressure up to the level of 150 mol $m^{-3}$. Above this there were no solutes left behind in the cell wall to maintain the turgor pressure such as in case of 200 and 250 mol $m^{-3}$ NaCl.

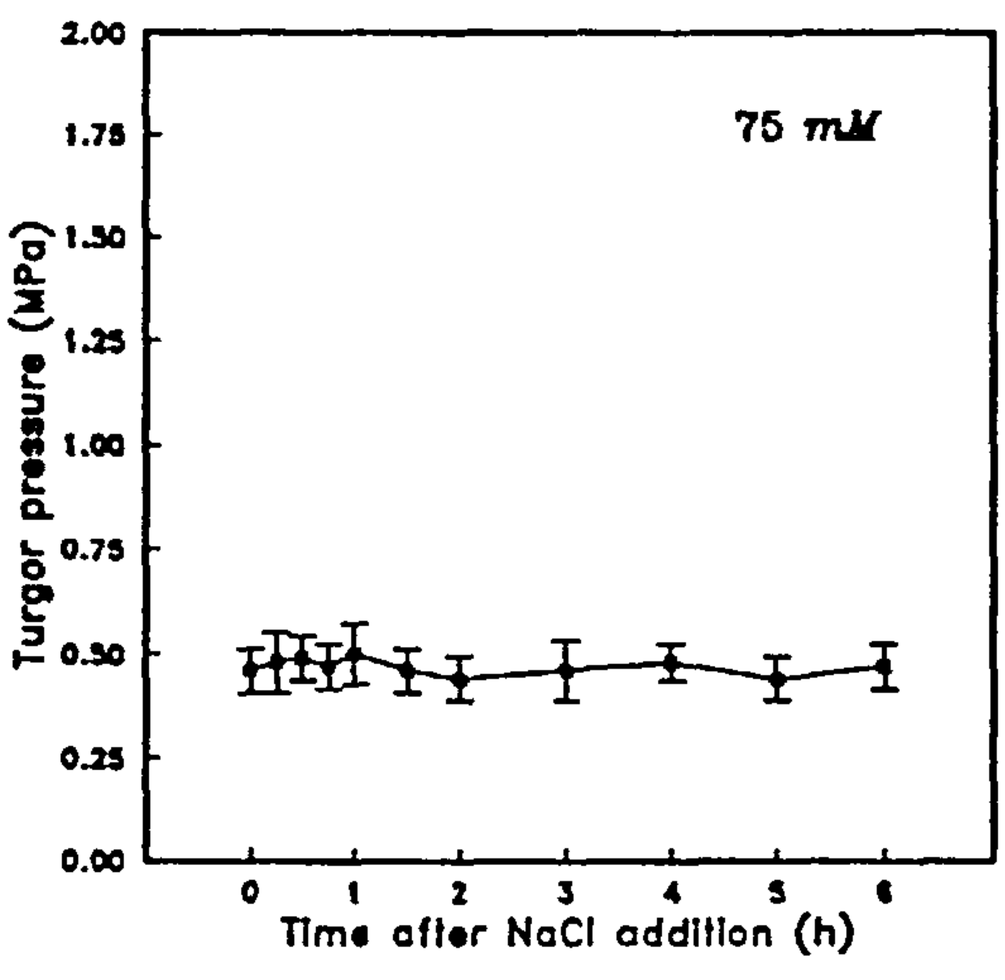
The next experiments were planned to investigate whether the proposed mechanism could 'reset' the wall solutes level i.e. does the wall solutes concentrations rise again as the cell osmotic pressure rises by classic osmotic adjustment (Fig. 4.3). The turgor pressure response was followed after applying 150 mol $m^{-3}$ and 100 mol $m^{-3}$ NaCl in two different doses. The turgor pressure was first measured after 3 (a) and 36 h (b) following the first dose of the salt (150 mol $m^{-3}$) where it did not show any differences with that of the previous experiments (Fig. 4.1). However, upon adding a further increment of 100 mol $m^{-3}$ there was an instantaneous decline in turgor pressure for 3 h treatment (a) attaining a value of 0.12 MPa which afterwards became stable. For 36 h treatment (b) also an instantaneous drop similar to that in the 3 h treatment was observed. The difference between 3 and 36 h was not statistically significant. Again the turgor pressure became stable after 1 h of 100 mol $m^{-3}$ increment addition and the level of turgor pressure was almost similar at the point of stability (Fig. 4.6; a and b). In this set of experiments the turgor pressure drop in response to the second increment of 100 mol $m^{-3}$ NaCl (Fig. 4.6) supports the assumption that cells could not cope with the excessive external salts and that the putative

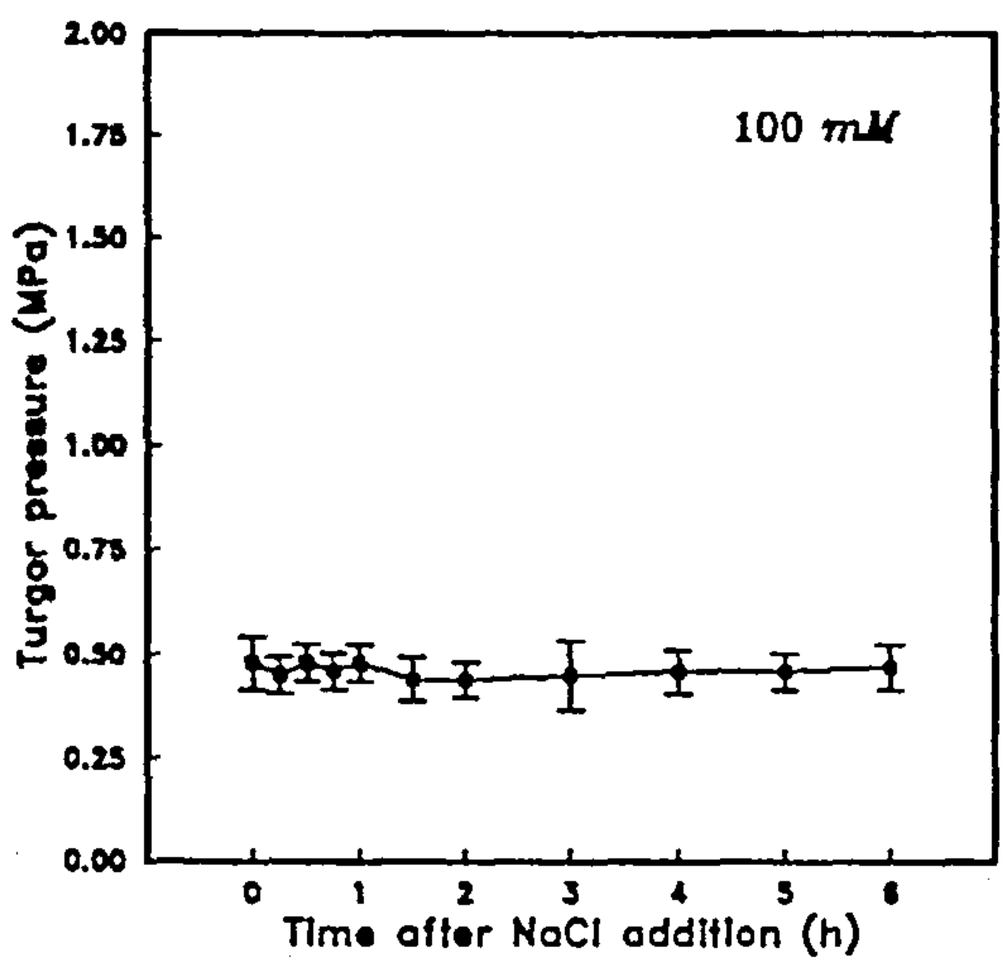
cell wall osmotic pressure is not 'reset' in the 36 h following turgor regulation.

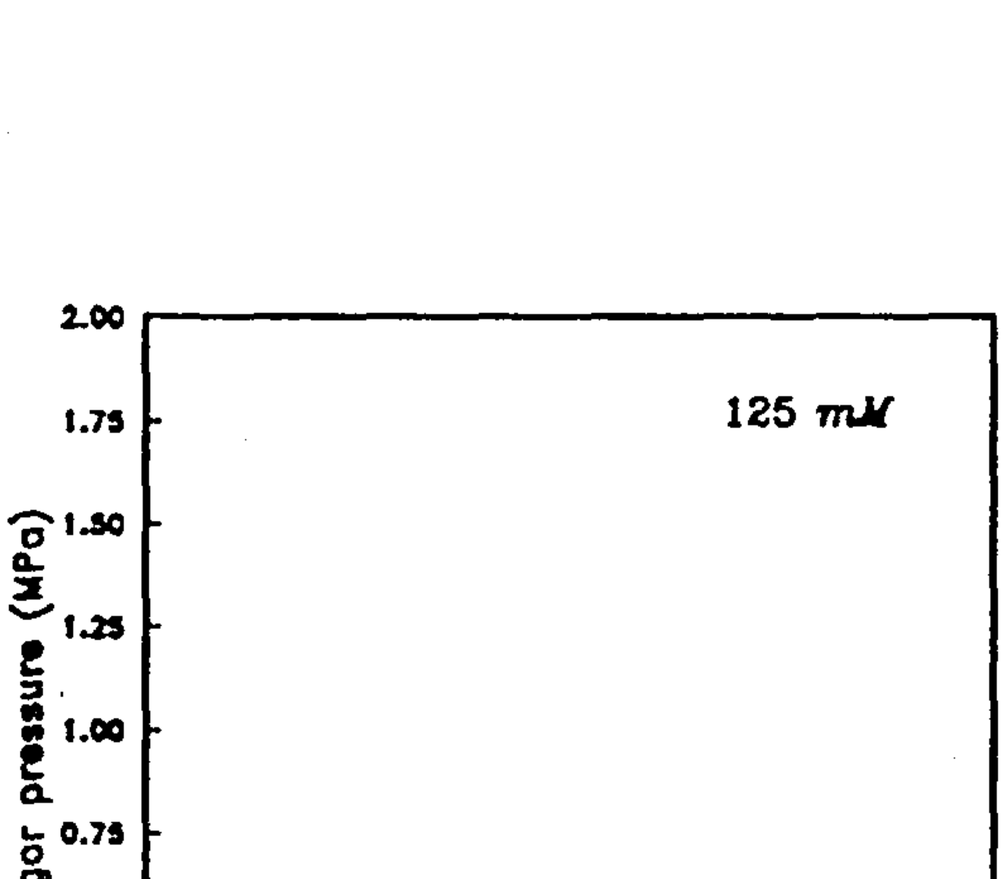
**Fig. 4.1** Time course of turgor pressure in the expanding cells of leaves in response to NaCl stress studied in a short term experiment i.e. 6 h. Conditions as in Fig. 3.3. Turgor pressure was measured using pressure probe. Each point is the mean of 5–25 replicates taken from three experiments. Error bars indicate the standard deviation of the mean of all replicates.


25 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)


50 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)


75 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)


100 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)


125 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)

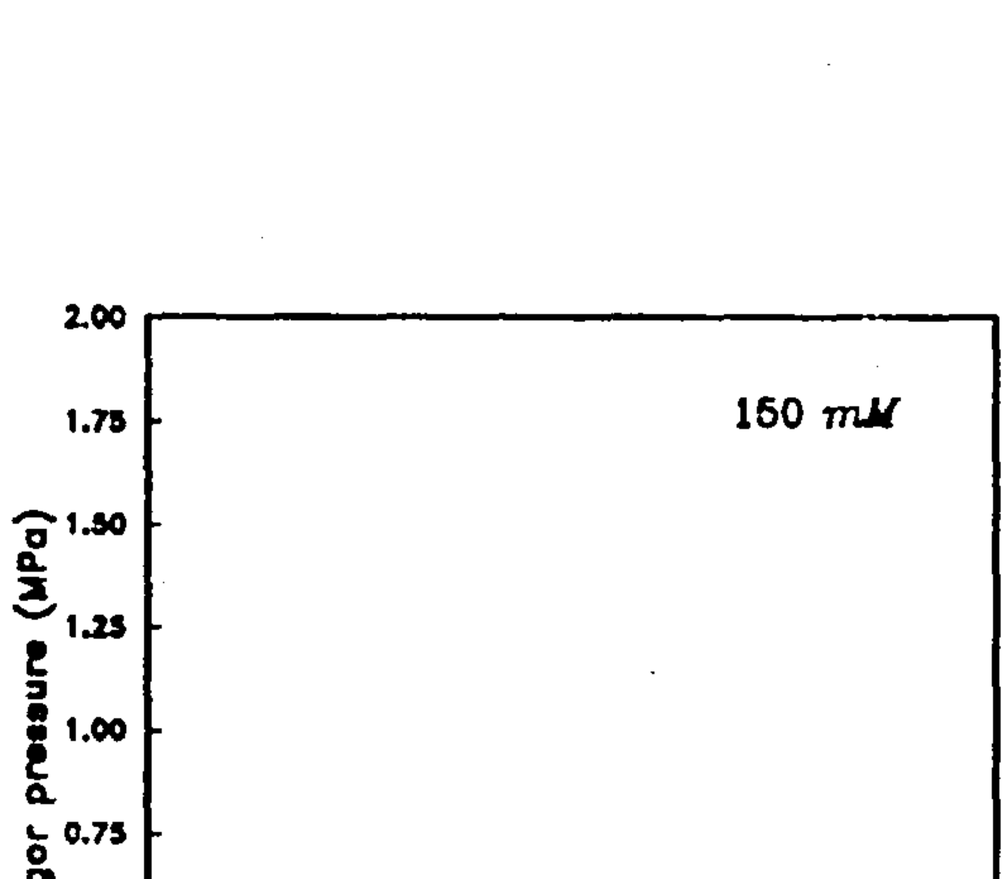
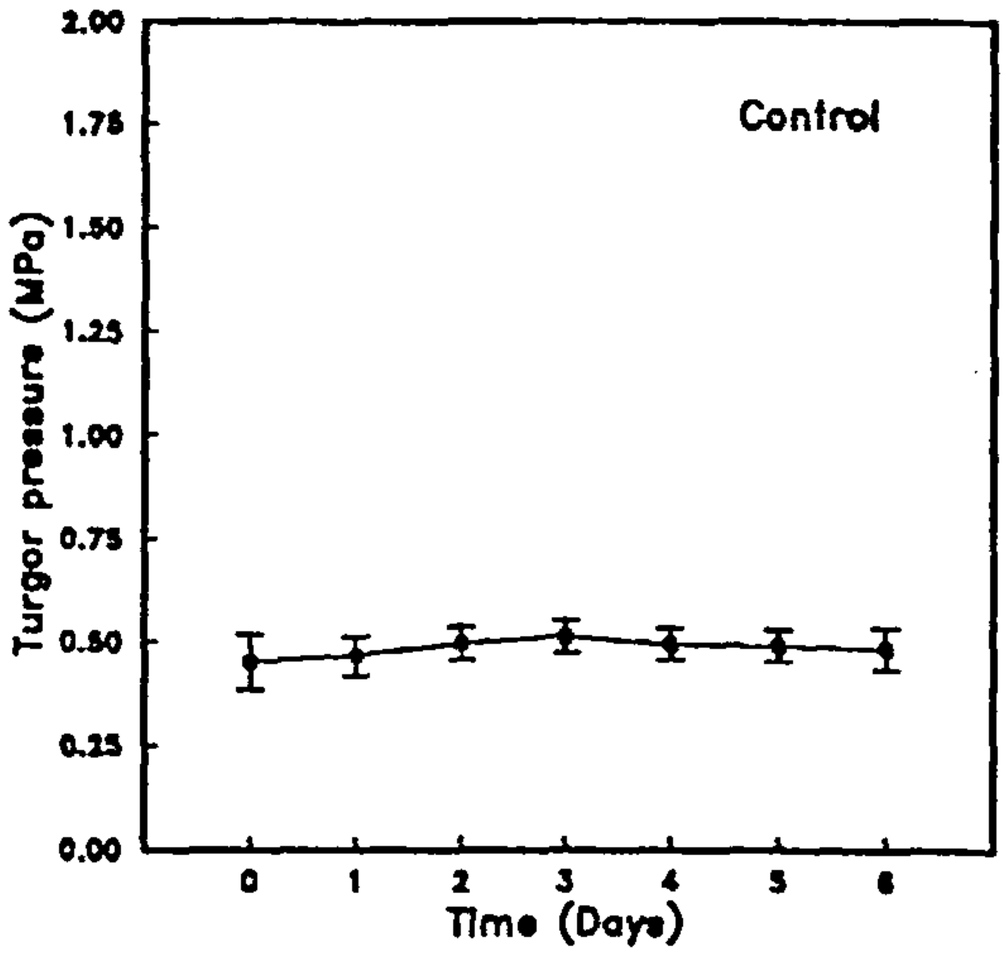
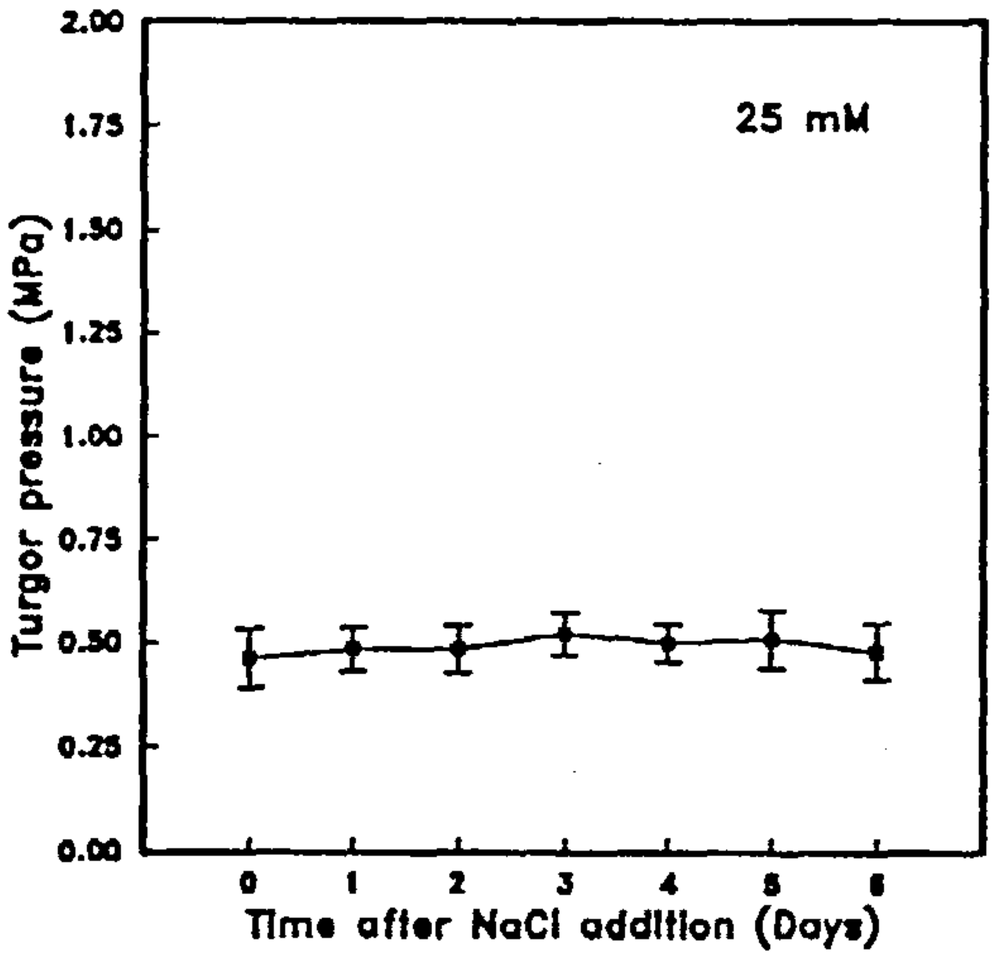
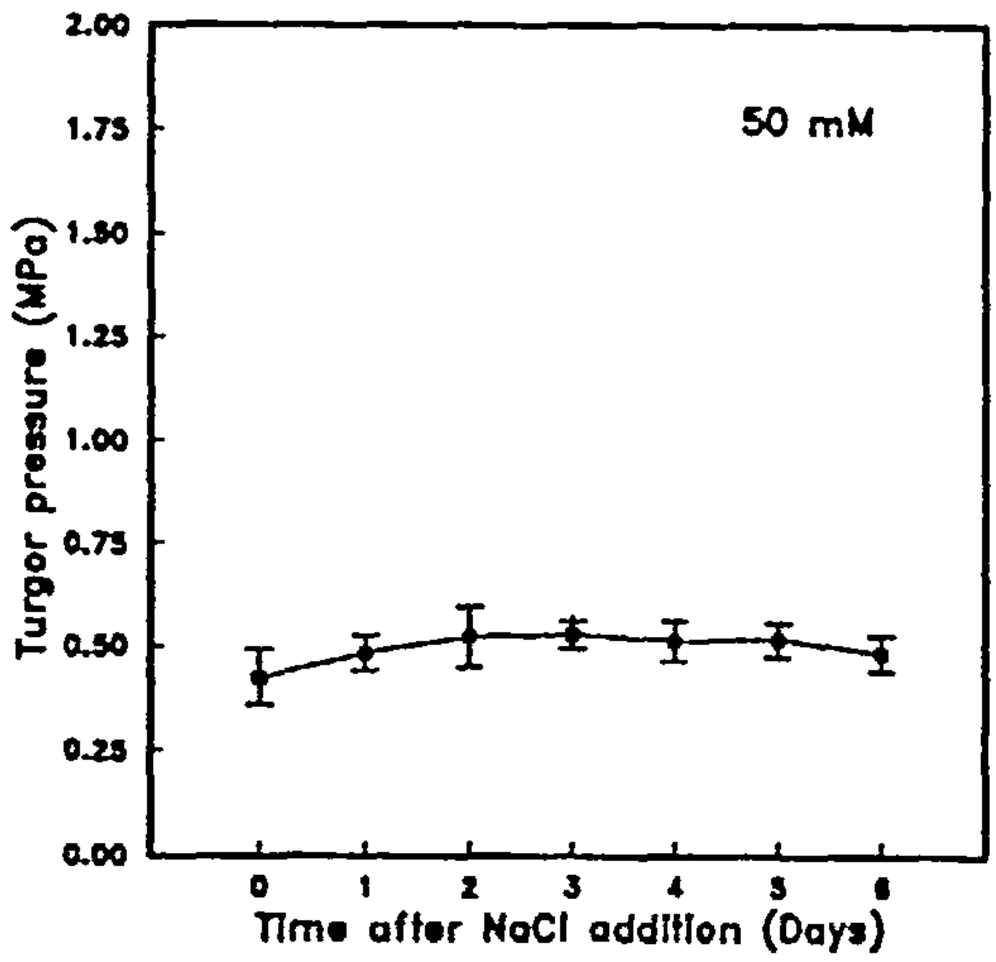
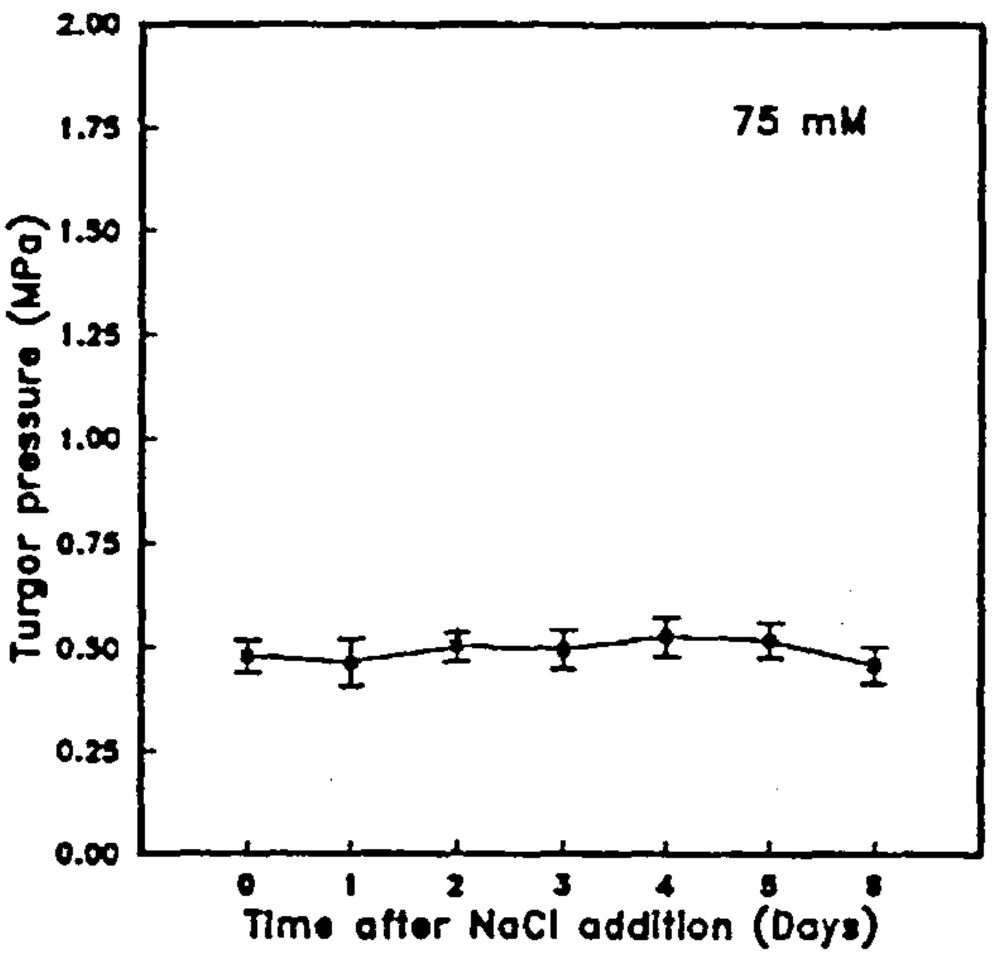

150 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)

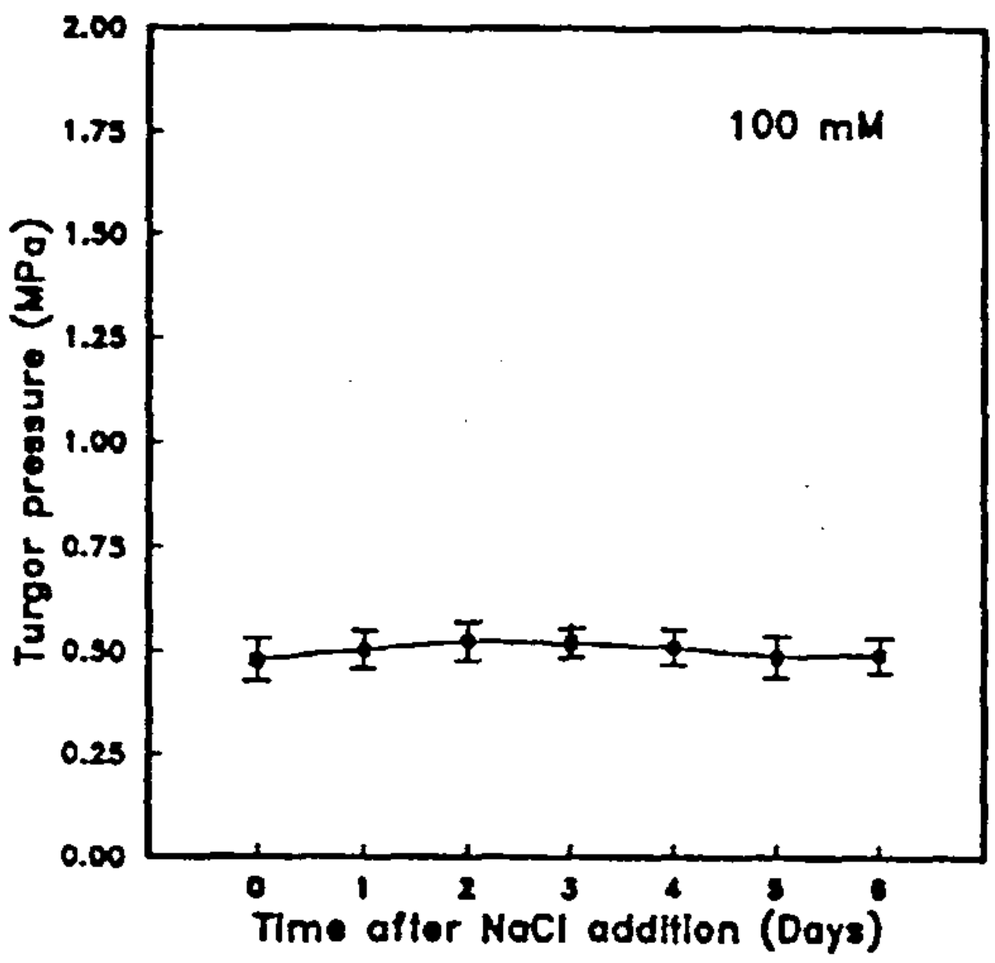
**Fig. 4.2** The response of turgor pressure in the expanding cells of leaves to the NaCl stress studied in a long term experiment i.e. 6 days. Details as in Fig. 4.1.

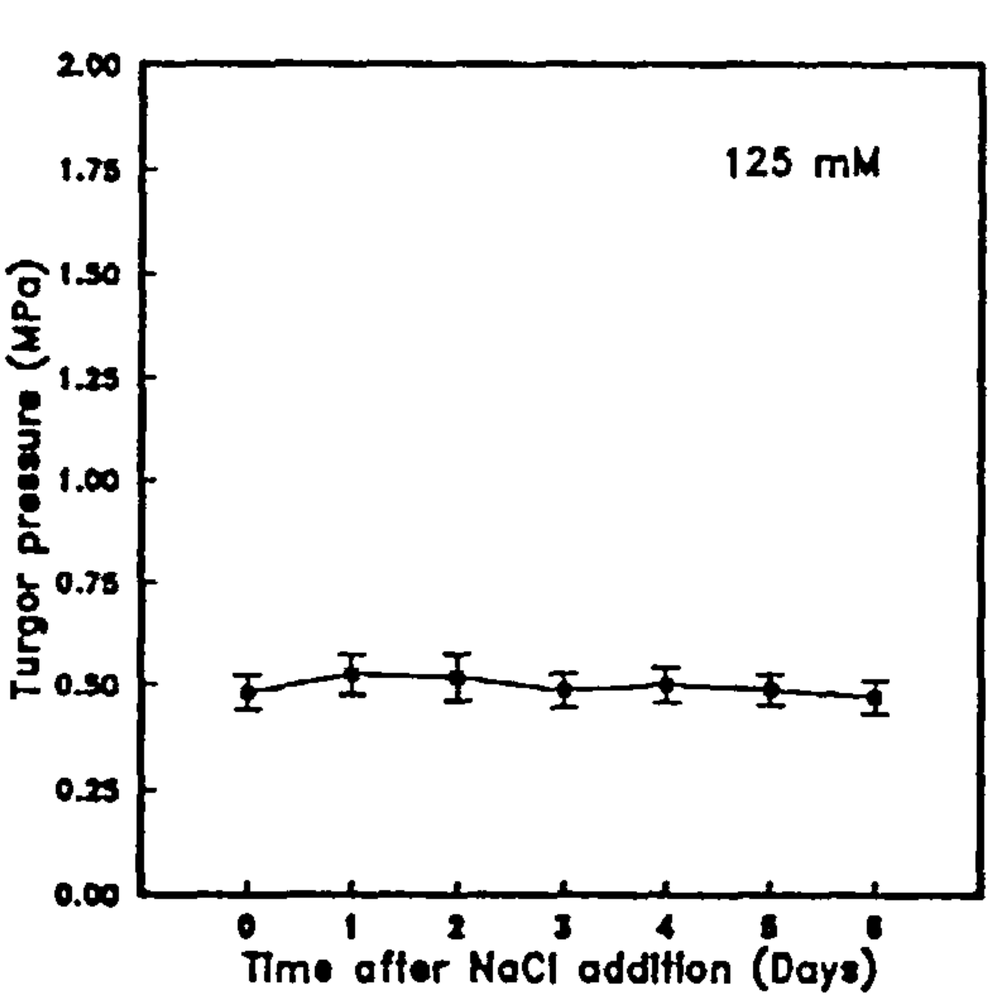

Control
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time (Days)


25 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

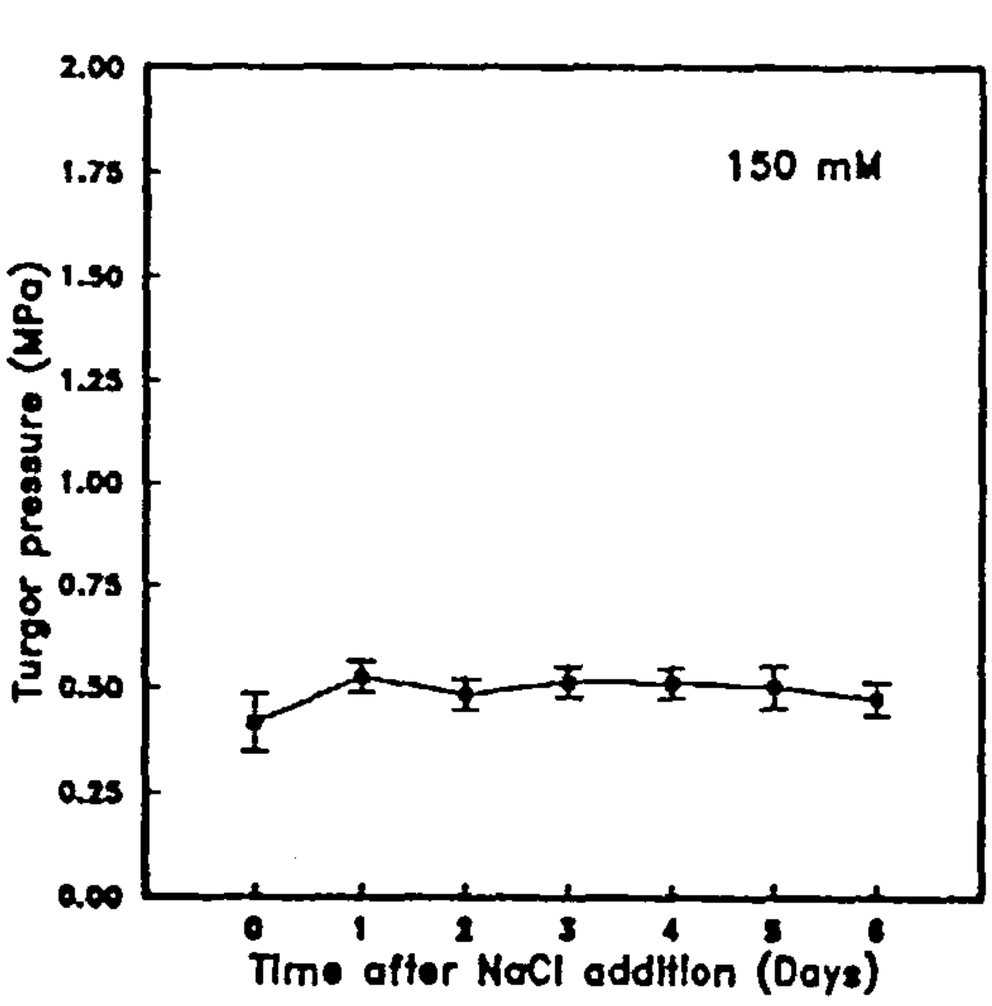
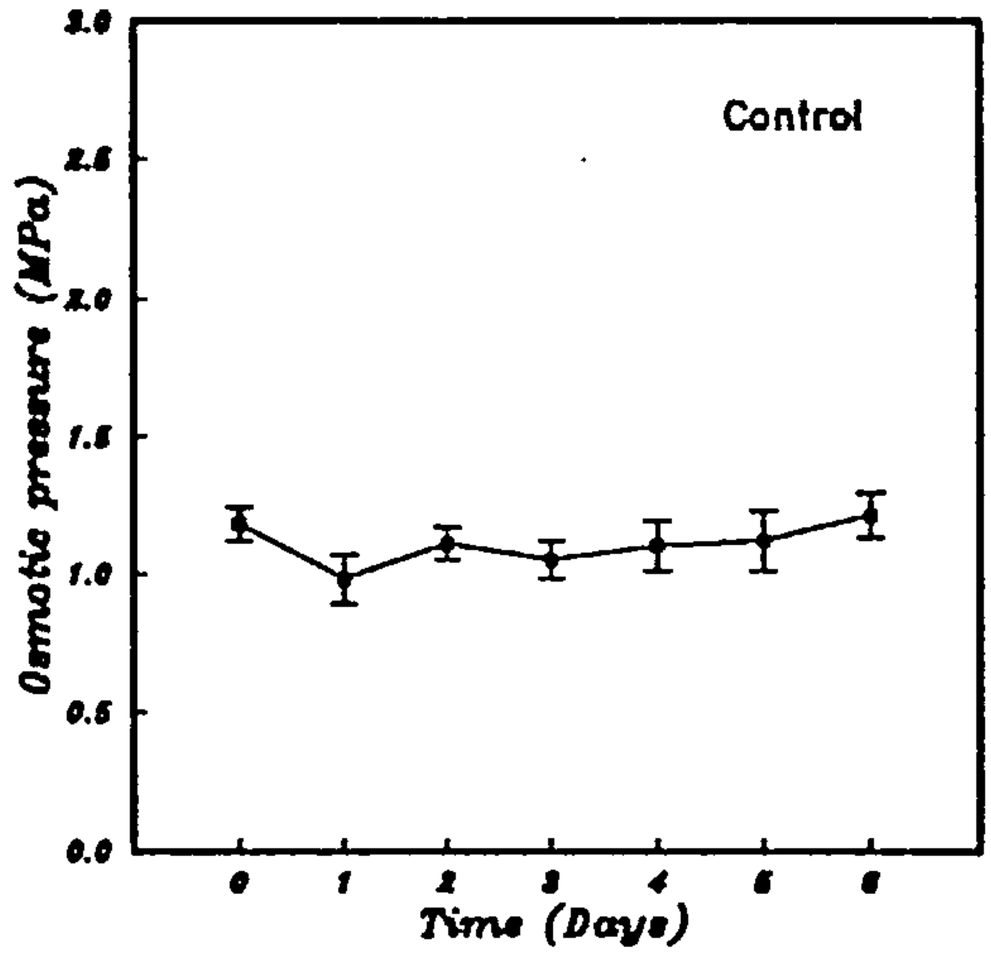
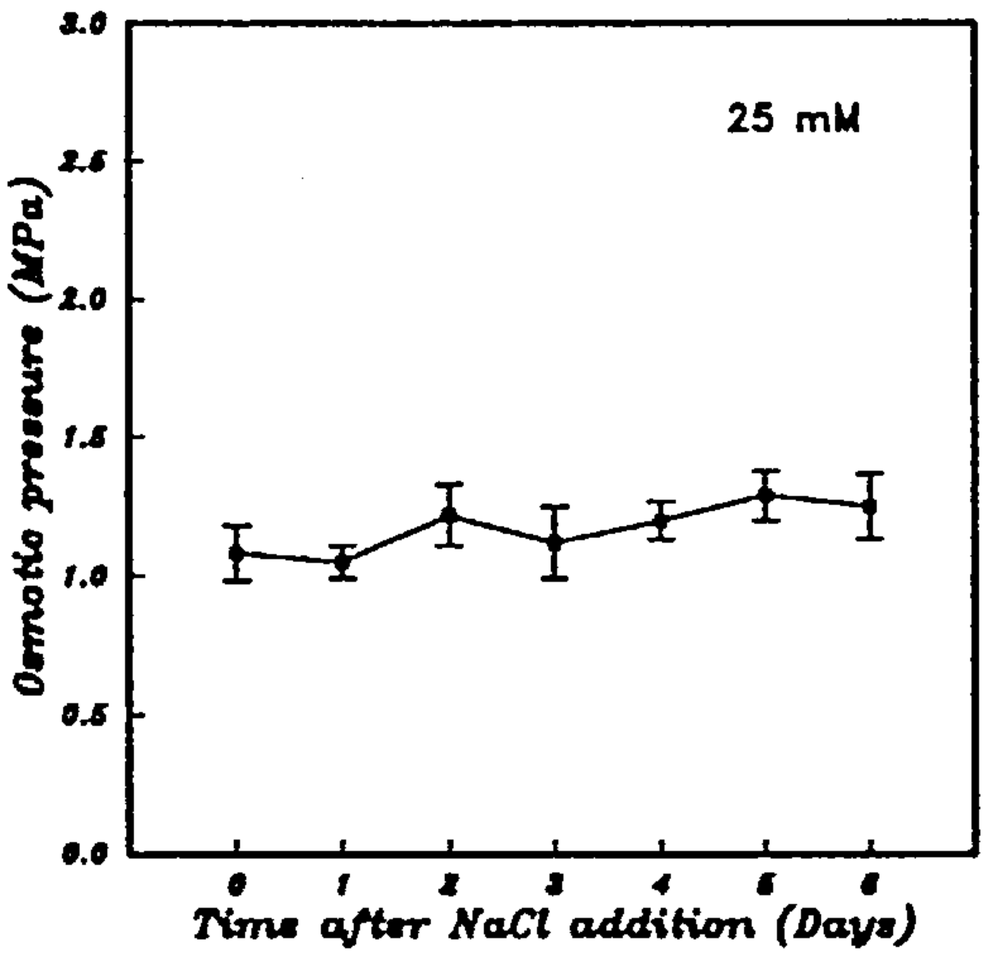
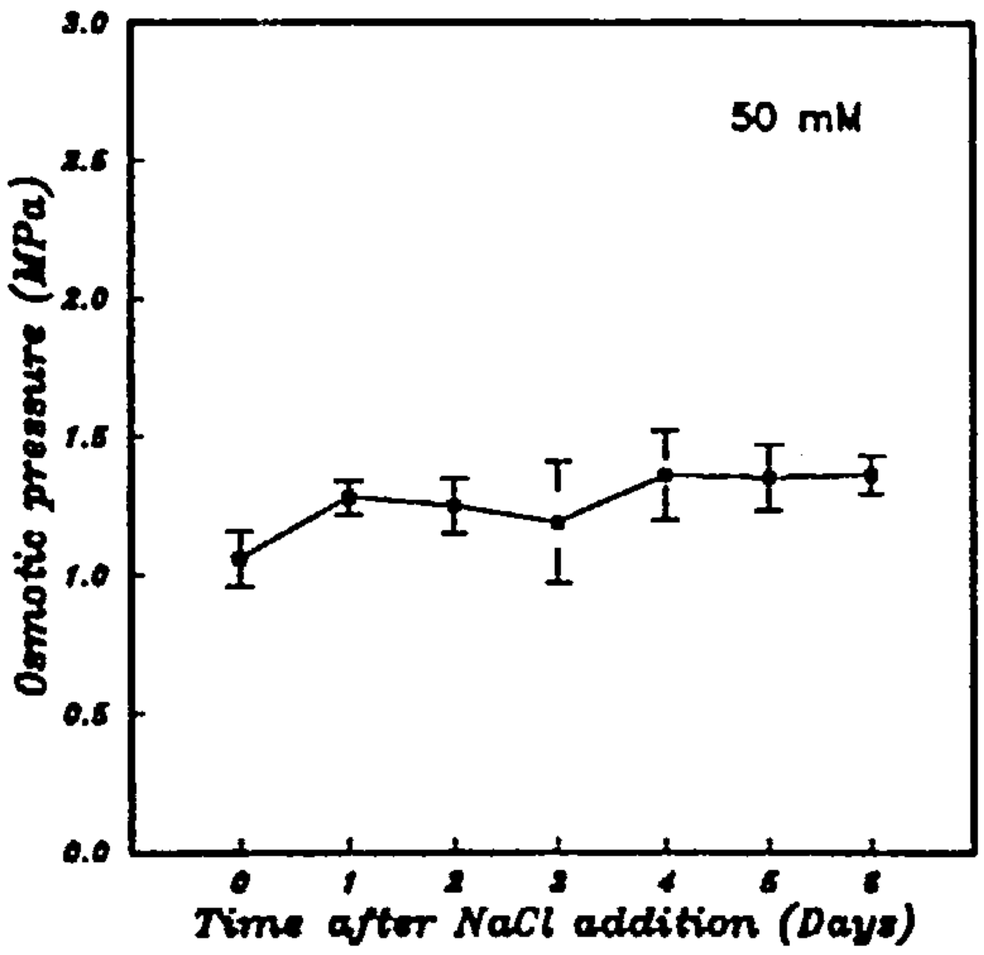
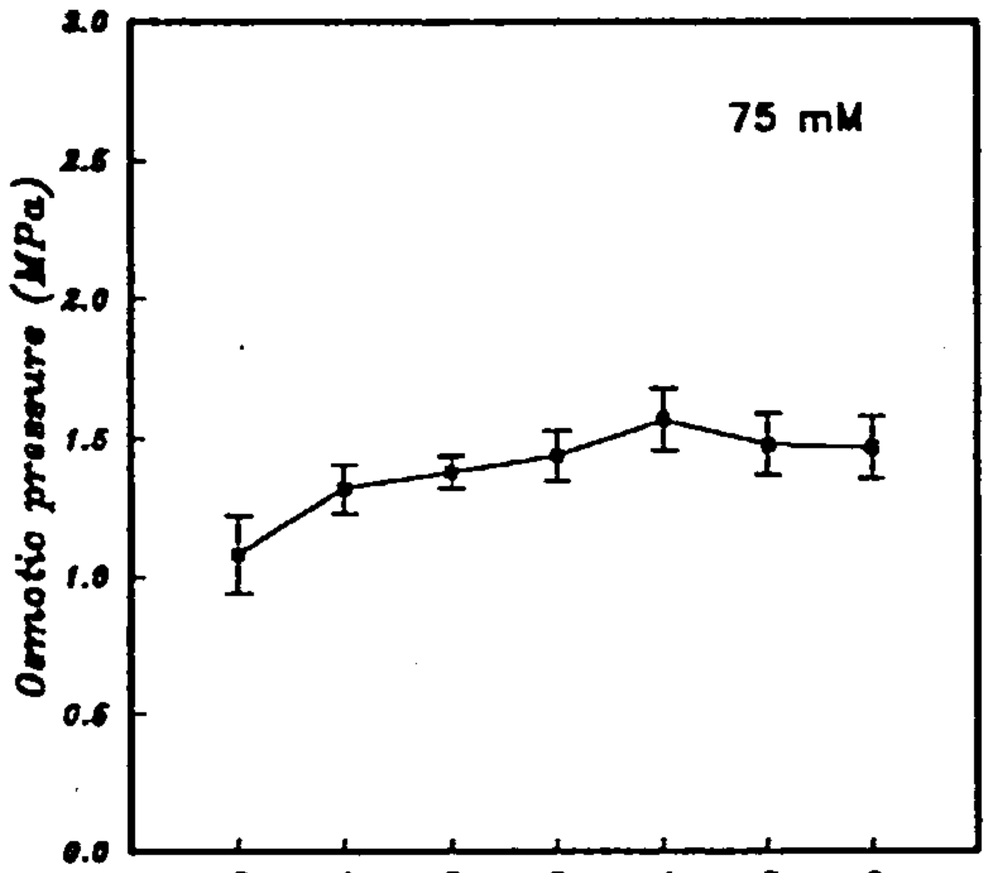

150 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

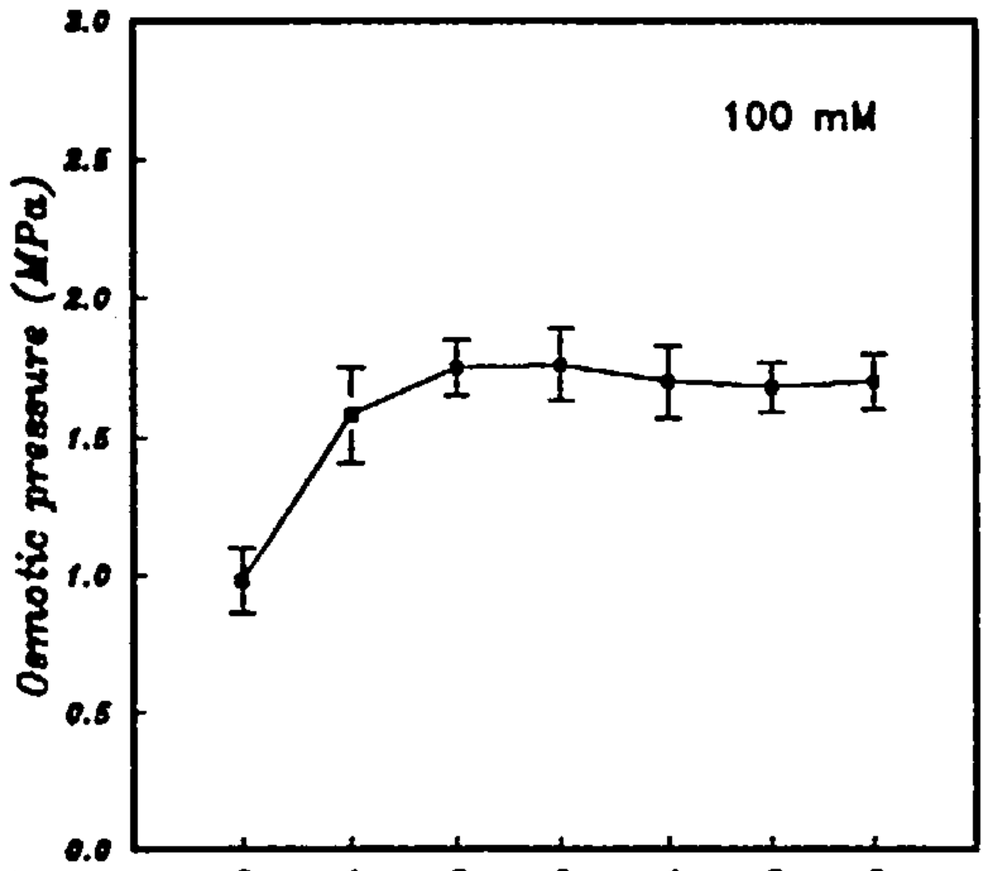
Fig. 4.3 The response of osmotic pressure of the entire expanding zone of the first emerged leaf to the NaCl stress studied in a long term experiment i.e. 6 days. Osmotic pressure was measured using vapour pressure osmometer on the whole tissue basis. Details as in Fig. 4.1.

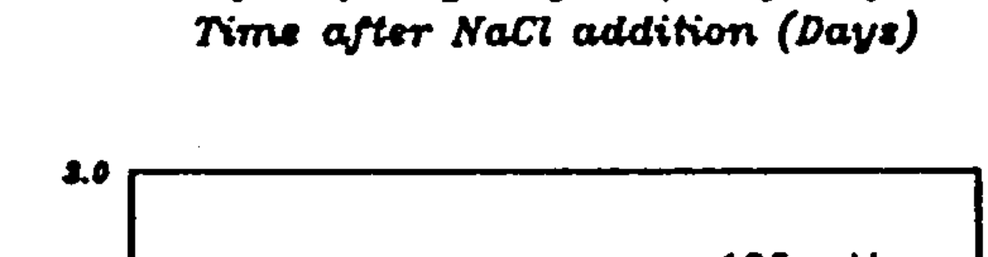

Control
Osmotic pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time (Days)


25 mM
Osmotic pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Osmotic pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Osmotic pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Osmotic pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Osmotic pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

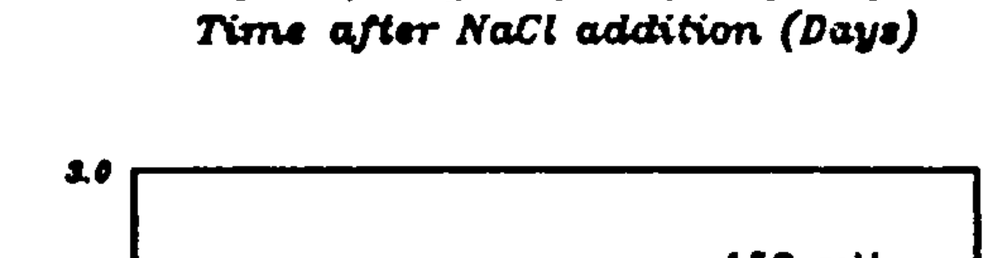

150 mM
Osmotic pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

**Fig. 4.4** An estimation of the concentration of osmotically active solutes present in the cell wall of leaf growing zone measured after 6 days of the stress application. The cell wall solutes were estimated by the difference of cell turgor pressure, tissue osmotic pressure and the cell wall transpiration tension. It corresponds to the amount of the salts present externally indicating the deposition of all the taken up solutes in the cell wall.

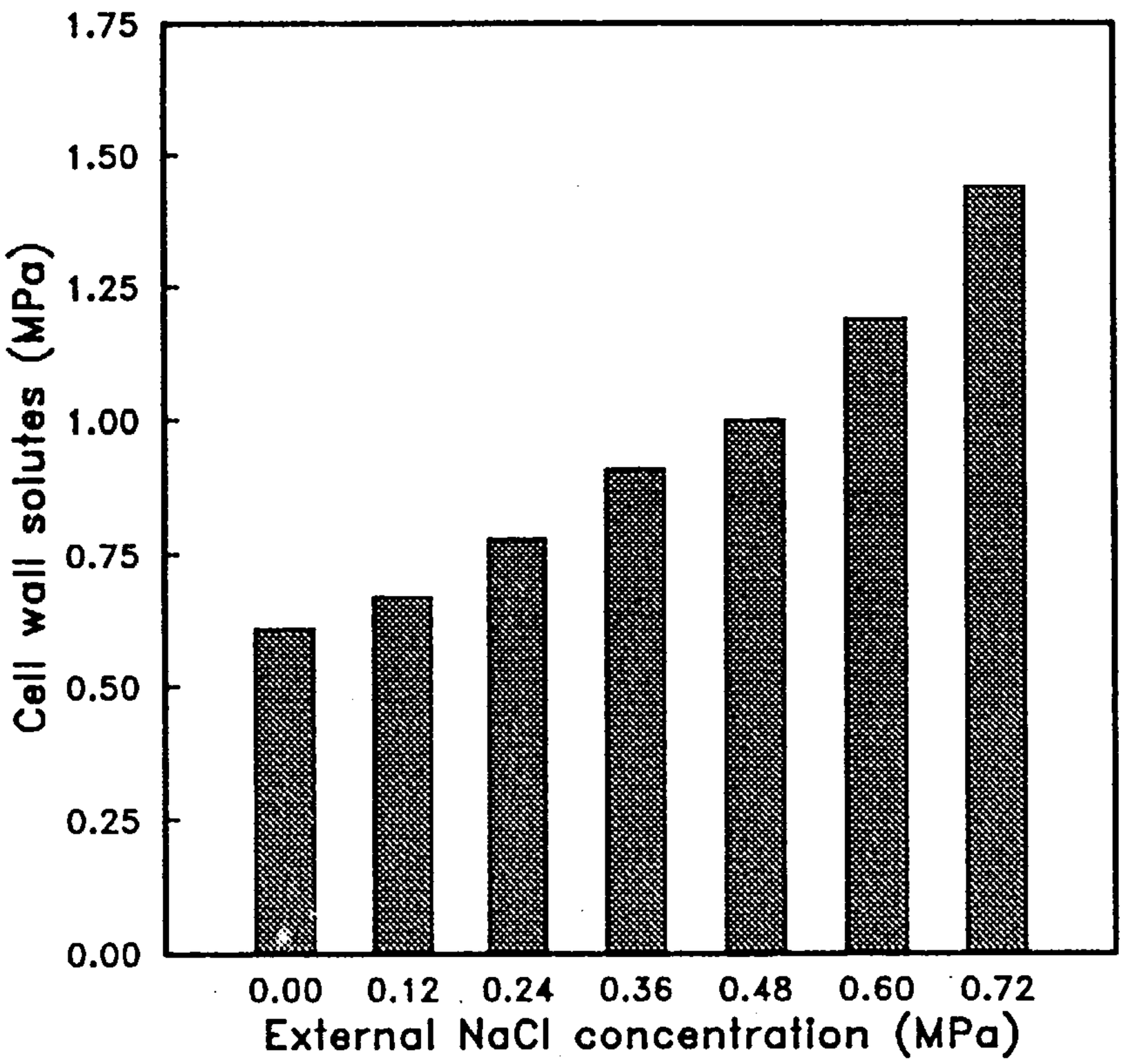
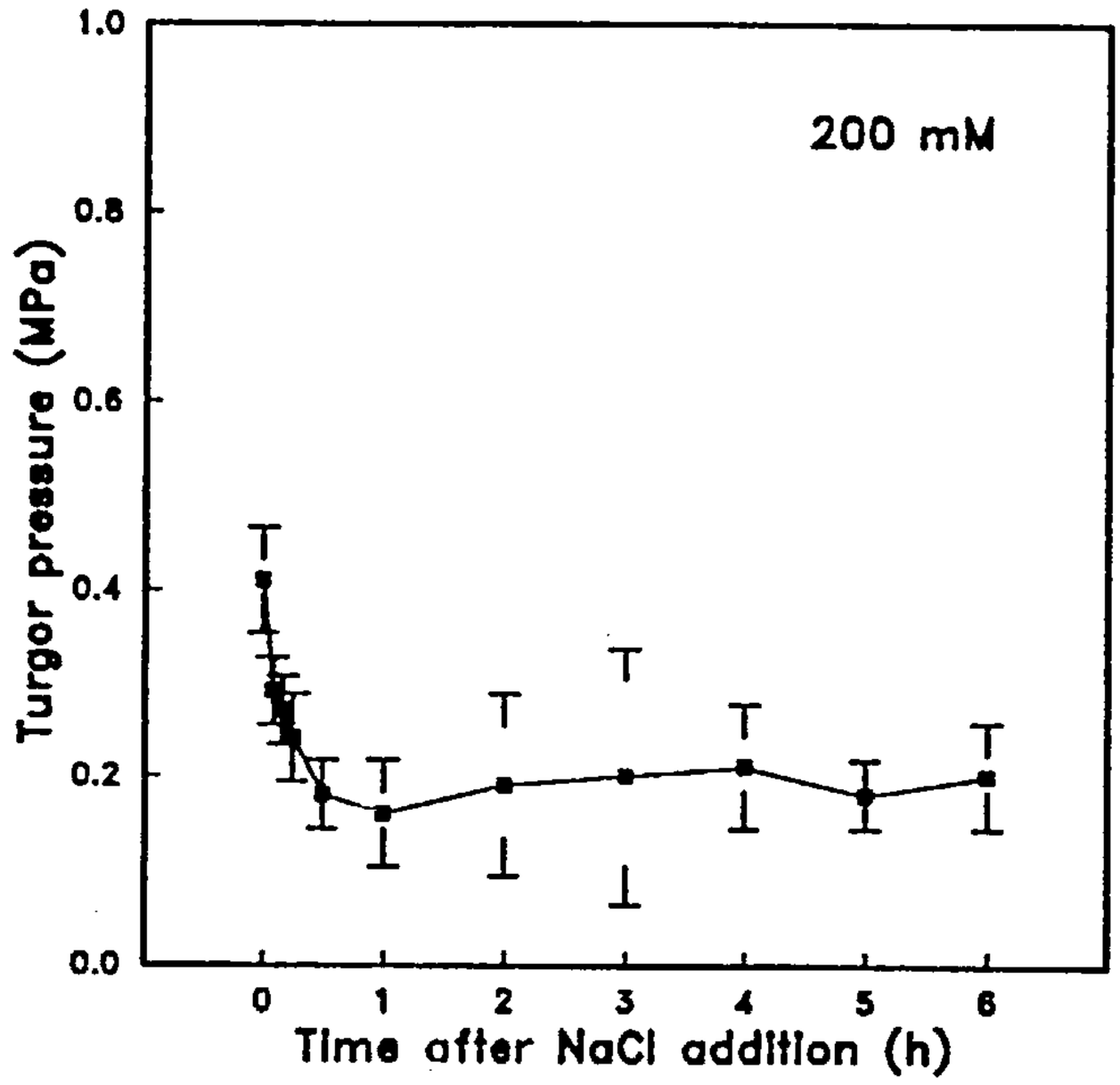

1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Cell wall solutes (MPa)
0.00
0.12
0.24
0.36
0.48
0.60
0.72
External NaCl concentration (MPa)

**Fig. 4.5** The behaviour of turgor pressure of expanding cells of leaves in response to the NaCl stress studied in a short term experiment i.e. 6 h. (An experiment to overload turgor regulation). Two NaCl concentrations i.e. 200 (a) and 250 (b) mol $m^{-3}$, were applied to salinize the root media. Each point is the mean of 5-12 replicates taken from two experiments. Error bars indicate the standard deviation of the mean of all replicates.


200 mM
Turgor pressure (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

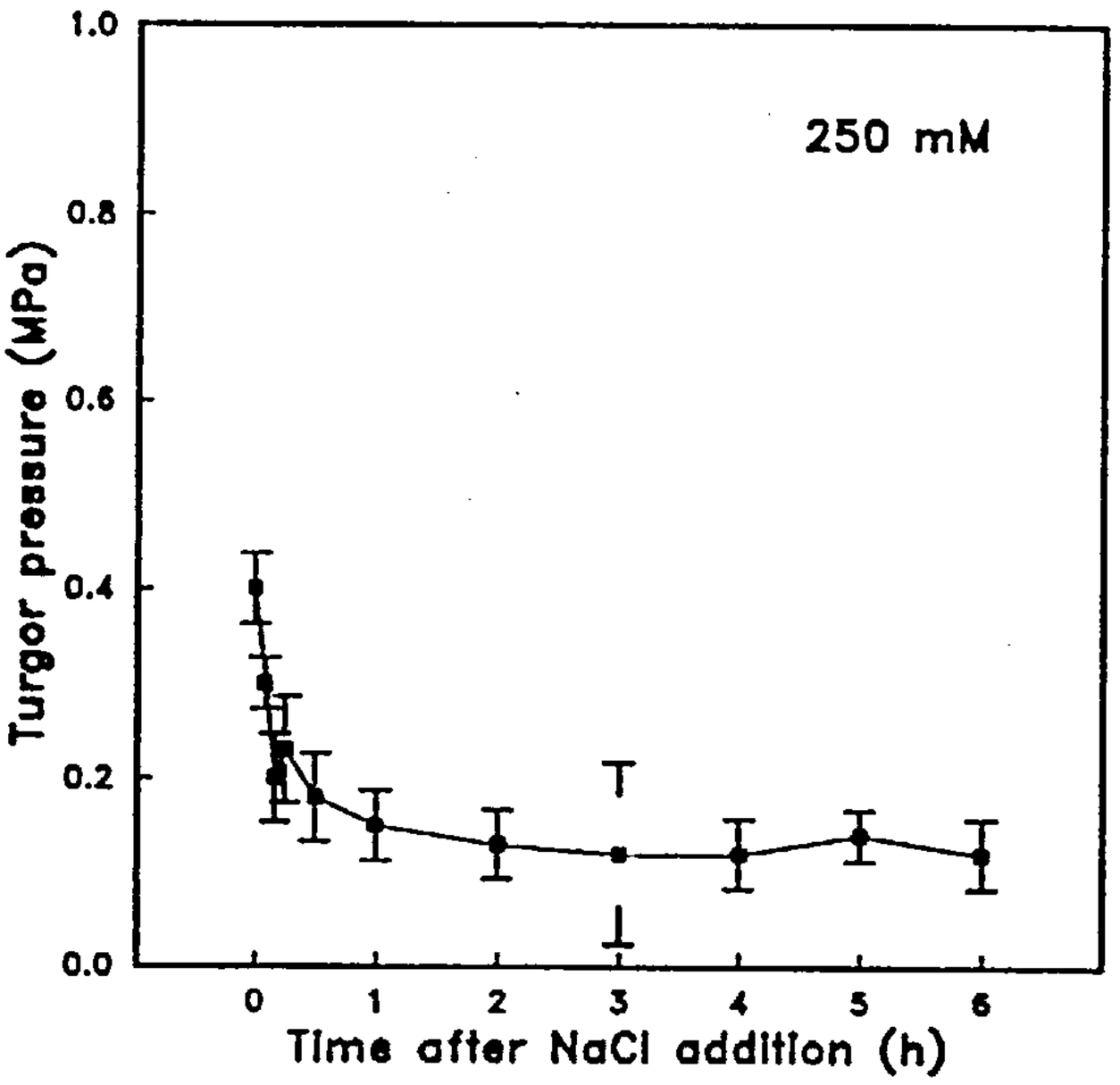
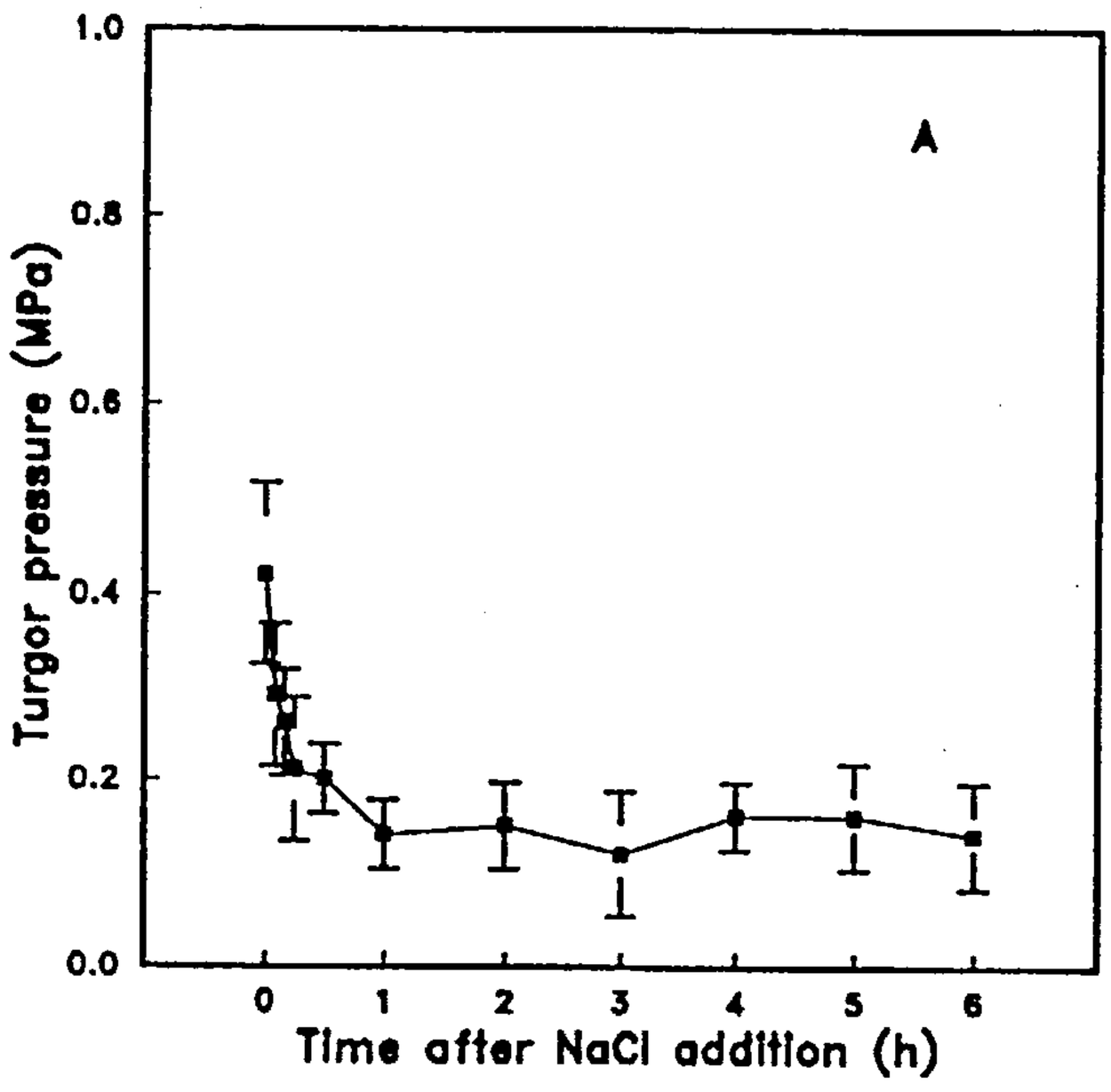

250 mM
Turgor pressure (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

**Fig. 4.6** The behaviour of turgor pressure of expanding cells of leaves in response to the NaCl stress studied in a short term experiment i.e. 6 h. An experiment to test for cell acclimitization. One NaCl concentration 250 mol $m^{-3}$, was applied to salinize the root media in two doses i.e. the first dose of 150 mol $m^{-3}$ and the second of 100 mol $m^{-3}$. The second dose was added after interval of 3 h (a) and 36 h (b). Each point is the mean of 5-12 replicates taken from two experiments.


A
Turgor pressure (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

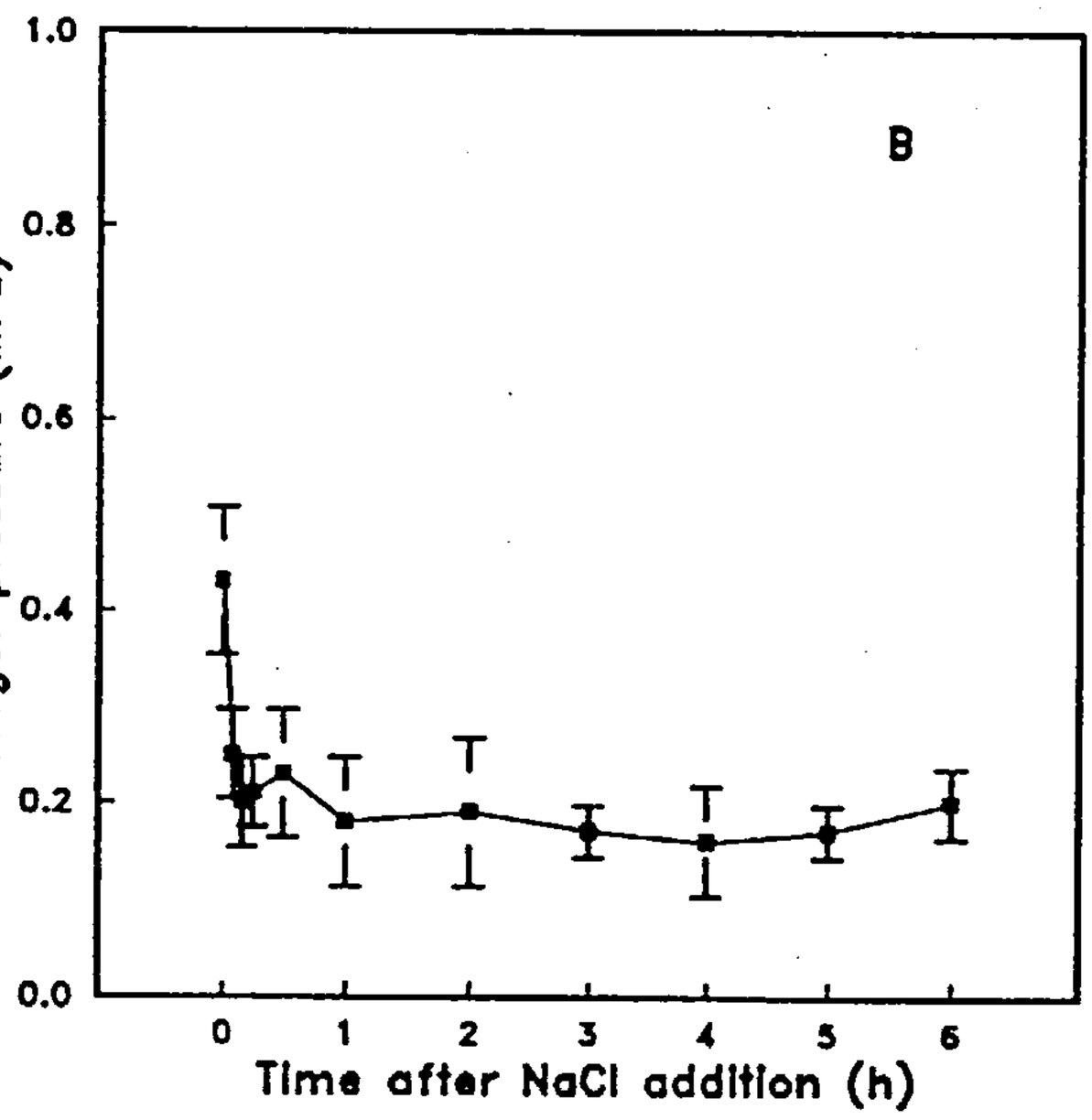

B
Turgor pressure (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

Table 4.1 : Effect of NaCl stress on the (Instron) cell wall extensibility (elastic and plastic)

A – Elastic extensibility (% / 15 g load)

| NaCl concentration (mM) | Time after stress onset (h) | | |
|---|---|---|---|
| | 0 | 5 | 144 |
| 0 | 7.42 ± 0.49 | 7.62 ± 0.78 | 3.49 ± 1.7 |
| 25 | 7.08 ± 1.08 | 6.48 ± 0.90 | 2.82 ± 1.09 |
| 50 | 6.97 ± 0.88 | 6.76 ± 1.50 | 2.05 ± 0.64 |
| 75 | 6.41 ± 1.15 | 6.05 ± 2.36 | 2.04 ± 0.74 |
| 100 | 7.09 ± 0.98 | 6.27 ± 1.10 | 2.03 ± 0.85 |
| 125 | 6.86 ± 1.50 | 6.34 ± 1.09 | 2.13 ± 0.60 |
| 150 | 7.62 ± 0.99 | 6.72 ± 1.39 | 2.27 ± 0.70 |

B – Plastic extensibility (% / 15 g load)

| NaCl concentration (mM) | Time after stress onset (h) | | |
|---|---|---|---|
| | 0 | 5 | 144 |
| 0 | 3.82 ± 0.56 | 3.18 ± 1.22 | 1.23 ± 0.96 |
| 25 | 3.60 ± 2.60 | 2.20 ± 0.94 | 1.51 ± 0.95 |
| 50 | 3.10 ± 0.60 | 2.91 ± 0.60 | 0.59 ± 0.35 |
| 75 | 3.10 ± 1.68 | 2.13 ± 0.74 | 1.23 ± 1.14 |
| 100 | 2.61 ± 0.78 | 2.17 ± 0.91 | 0.65 ± 0.43 |
| 125 | 2.06 ± 0.81 | 2.15 ± 1.03 | 1.08 ± 0.52 |
| 150 | 3.34 ± 1.90 | 2.15 ± 1.03 | 0.91 ± 0.45 |

**Table 4.2** The effect of plant immersion under the hydroponic medium on turgor pressure in epidermal cells of leaf growing zone. The turgor pressure increased after the plant immersion and was stabilized after 1 h of the immersion. This increase provides a quanttitative estimation of the transpiration tension in the cell wall. Results ± standard deviation.

| Turgor pressure (MPa) | | Transpiration tension (MPa) |
|---|---|---|
| In the air | Under the medium | |
| 0.45 ± 0.05 | 0..56 ± 0.09 | 0.11 |

**Table 4.3 The effect of plant immersion under the hydroponic medium on the leaf growth rate. The measurements were carried out after 1 h of the plant immersion.**

| Leaf Growth Rate ($\mu m\ min^{-1}$) | |
|---|---|
| **In the air** | **Under the medium** |
| 17.4 ± 0.06 | 17.8 ± 0.08 |

# CHAPTER 5

# *WATER RELATIONS OF LEAF MATURE ZONE*

In parallel to studying the water relations of the growing zone the attention was also aimed towards the mature, expanded zone of the leaf. Experiments were carried out to find out changes in the water relations which might have occured after the NaCl stress. It was also thought that plants did not have to maintain turgor pressure in this zone contrary to those of the growing zone, since the cells were not growing.

## 5.1 Short Term Changes in Turgor Pressure

To investigate this turgor pressure was measured in the mature zone of the control as well as of the NaCl-treated plants for 6 h. The individual measurements were performed initially at 15 min, then 30 min and later at 1 h intervals (Fig. 5.1). The average turgor pressure value was 1.02 $\pm$ 0.08 MPa for the control plants, which kept uniform for the whole of the experimental period. In contrast to the expanding zone a gradual decrease in the turgor pressure was observed immediately after the NaCl addition to the root media, for all the salt concentrations. The time span for the pressure drop varied with external salt concentrations between 10 to 20 min. The decline continued and a low and stable value was obtained after about 1 h of the stress onset. The magnitude of this drop was in accordance with the level of NaCl treatment for 25, 50 and 75 mol $m^{-3}$. However, in the case of higher concentrations the decline was not as high as anticipated from the salt applied i.e. it did not correspond to the higher salt level in the media. The turgor pressure did not decline more than the drop for 75 mol $m^{-3}$ NaCl for then higher NaCl concentrations. At NaCl concentrations above 75 mol $m^{-3}$ the

epidermal cells behaved qualitatively not quantitatively like osmometers for the external medium. Owing to the complexity of the tissue this was not unexpected. However, since they apparently behaved as ideal osmometer at and below 75 mol $m^{-3}$ NaCl. Various experiments were performed in order to investigate this apparently anomalous behaviour.

## 5.2 Volumetric Elastic Modulus

One possible explanation could be due to some behaviour of the volumetric elastic modulus (ε) acting to maintain turgor pressure at the expense of loosing cell volume. Therefore, ε was determined with the pressure probe in the same tissue and for the same time span as the experiment described in Fig. 5.1. The ε value for the plants in control conditions was found to be about 4.5 MPa (Fig. 5.2). No apparent changes in the ε were observed in response to 25, 75, 100 and 150 mol $m^{-3}$. These measurements show a large scatter possibly due to the difficulty of measuring cell volume with accuracy (Tomos et al. 1981). The regression line fitted to the data was almost horizontal for each of the concentrations applied and it was concluded that ε was irrespective of the extent of turgor pressure decline after the stress application.

## 5.3 Root Hydraulic Conductivity

The next experiment was carried out to find out any relationship between the leaf turgor pressure and the root hydraulic conductivity ($L_{pr}$) in order to determine whether the tissue was

in some way protected from the larger changes in water potential. The $L_{pr}$ was measured for the whole root system in response to both NaCl and mannitol as osmotica (Fig. 5.3). For the NaCl stress the $L_{pr}$ value was found to be slightly higher (31 x $10^{-8}$ m $s^{-1}$ $MPa^{-1}$) for the lowest NaCl concentration jump (0 to 25 mol $m^{-3}$) than other salt treatments. The value of $L_{pr}$ decreased with the increase in the salt content following the jump up to 75 mol $m^{-3}$. Further, no appreciable change was observed for the larger salt treatments. However, the differences between $L_{pr}$ for all the NaCl concentration jumps were not statistically significant. Slightly different results were obtained for the equi-osmolal mannitol concentrations i.e. higher values were determined for 0 to 25 (x 2) mol $m^{-3}$ which decreased for 0 to 100 (x 2) mol $m^{-3}$ and then increased in case of 0 to 125 (x 2) and 0 to 150 (x 2) mol $m^{-3}$ (Fig. 5.3). $L_{pr}$ for the excised individual roots was also determined and found to be much higher than the whole root system. The values in excised roots were about 25 to 225 x $10^{-8}$ cm $s^{-1}$ $MPa^{-1}$ and for the whole root system was about 10 to 30 x $10^{-8}$ cm $s^{-1}$ $MPa^{-1}$ for the NaCl and about 10 to 25 x $10^{-8}$ cm $s^{-1}$ $MPa^{-1}$ for the mannitol as osmoticum. It can therefore be concluded that the changes in turgor pressure in leaf cells is irrespective of the root hydraulic conductivity.

## 5.4 Long Term Changes in Turgor Pressure

The turgor pressure response was monitored in another experiment carried out over longer time span i.e. 6 d with the individual measurements at 1 d intervals (Fig. 5.4). In the previous

experiments (Fig. 5.1) the process of turgor pressure recovery seemed to start after 6-8 h of the salt addition only for 25 and 50 mol $m^{-3}$ NaCl. The data obtained in the longer term experiment exhibited the almost complete turgor recovery after 24 h (1 d) of the stress application for up to 100 mol $m^{-3}$ NaCl. After that no obvious changes were observed in the turgor pressure value i.e. it remained uniform for rest of the experimental duration. In case of 125 and 150 mol $m^{-3}$ turgor pressure took longer to recover from the stressed position. The process of recovery was found to be completed after 2 to 3 d of the stress application.

The turgor pressure was also measured after 11 d of the stress onset (Table 5.1). No obvious differences could be detected in turgor pressures of control as well as in the plants treated with lower NaCl concentrations between them and the 6 d values. However, lower pressure values were determined in the plants exposed to higher salt levels. These lower values may will be an indicator of early senescence of the leaf brought about by these concentrations.

### 5.4.1 Effect of nutrient status of root media on turgor pressure

During the investigation of the effect of nutrient status on growth patterns an experiment was carried out to study the response of leaf turgor pressure to the nutrient status of the root media. For the purpose the plants were exposed to nutrients after a delay (see section 3.1). They were grown in 0.5 mol $m^{-3}$ $CaCl_2$ for 120 h and then transferred to half strength Hoagland solution for 24 h prior to the addition of various NaCl

concentrations to salinize the root medium.

In the control plants (in 0 mol $m^{-3}$) a large increase was measured in the leaf cell turgor pressure, this increment later declined towards the normal value (Fig. 5.5). The extent of the increase declined with the increase of NaCl level in the root media. Original turgor pressure was fully recovered for all the NaCl concentrations after 24 h of the stress application except for the 150 mol $m^{-3}$ NaCl as turgor pressure was appeared to be lowered at 1 d of the stress onset. However, it increased at 2 d and a small and delayed increase was observed at 3 d which lasted for a day only.

The increase in turgor pressure was presumably due to uptake of $K^+$ ions into the cells (Hsiao and Läuchli, 1986) and it was superimposed on the decrease in turgor pressure due to salt stress (as described previously).

## 5.5 Short Term Changes in Osmotic Pressure

The osmotic pressure ($\pi$) of the same mature zone was determined in a short duration experiment i.e. 6 h (Fig. 5.6). The magnitude of $\pi$ was about 1.02 $\pm$ 0.06 MPa for the control plants. After the stress onset the $\pi$ did not seem to change for the lower NaCl concentrations i.e. 25, 50 and 75 mol $m^{-3}$. The response was persistent even after 6 h of the stress onset. The $\pi$ did not react to the 100 mol $m^{-3}$ for the first 2 h and later it continued increasing gradually (the start of the osmotic adjustment). For the final two concentrations i.e. 125 and 150 mol $m^{-3}$ the $\pi$ increased after 1 h of the stress initiation. The

increase is evidence for the start of the long term osmotic adjustment possibly due to salt uptake in roots and their subsequent transport to leaves for helping in the turgor pressure recovery.

## 5.6 Long Term Changes in Osmotic Pressure

The subsequent changes in $\pi$ were followed over a longer experimental time (Fig. 5.7) when the plants were exposed to the NaCl stress for 6 d. A large boost in the tissue $\pi$ level was observed over the first 1 to 2 d of the stress initiation which became stable later at a level depending upon the external NaCl concentration. For the lower salt concentrations the increase took place at a moderate rate resulting in higher $\pi$ levels than the control values. The process of osmotic adjustment took place at a much faster rate for the higher salt concentrations. The $\pi$ values after 6 d were twice the magnitude of the control levels. These $\pi$ levels were equivalent to the amount of osmotically active solutes present in the root surroundings.

The tissue osmotic pressure was also determined after 11 d of the salt addition. The process of osmotic adjustment continued and resulted in very higher osmotic pressure levels in leaves after 11 d of the salt application (Table 5.2). The $\pi$ levels were corresponding to the external salt stress, however, in case of 150 mol $m^{-3}$ NaCl a very large increase in $\pi$ level was observed resulting in about 4 times higher tissue $\pi$ than the external salinity.

## 5.7 Cell Wall Transpiration Tension

The cell wall transpiration tension ($P_w$) was estimated in the same zone in a long term experiment. The measurements were performed in the control and NaCl-treated plants (Fig. 5.8). The average value for the control plants was 0.1 $\pm$ 0.05 MPa. No obvious changes were determined in its magnitude in response to the NaCl stress, within the resolution of the experiment (which depends on measuring the difference between two larger values each with an associated experimental error). The extent of the fluctuations was in any case not pronounced. Therefore, salt stress appears to have no profound effects on the cell wall transpiration tension.

## 5.8 Cell Wall Solutes

The osmotic pressure of cell wall solutes ($\pi_w$) for plants for 6 d of NaCl treatment was calculated by the difference of the values of turgor pressure, tissue osmotic pressure and the $P_w$ (Fig. 5.9). The estimated amount of $\pi_w$ was found to be 0.1 MPa in the control plant leaves. Whilst, a gradual and continuous increase was observed in the NaCl stressed plants. For example, the $\pi_w$ of plants under 150 mol $m^{-3}$ NaCl treatment was 0.85 MPa. After 6 days of the stress application the amount of the $\pi_w$ was equivalent to the amount of the osmotically active solutes in the external solution (Fig. 5.9). A similar trend was observed after 11 d of the stress onset, however, the values of $\pi_w$ were much higher than those at 6 d for all the NaCl concentrations (Table 5.3).

## 5.9 Solutes Used for Osmotic Adjustment

### 5.9.1 Ionic Solutes

The concentrations of various cations, anions and organic solutes were measured in the expressed tissue sap from the mature region of leaf to estimate their contribution towards the whole tissue osmotic pressure. All the measurements were performed in the long duration experiments i.e. over 6 d.

**a) Cations**

**1) Sodium**

Sodium concentration was determined and was found to be less than 10 mol $m^{-3}$ in the control plants (Fig. 5.10). On salination of the media, the leaf $Na^+$ level increased gradually with time for all the NaCl concentrations. This gradual increase was observed to be lasting for all the time and resulted in manifold increase in the leaf $Na^+$ content. In case of the 25 mol $m^{-3}$ only, a small boost in the $Na^+$ concentration was observed. In case of 50, 75 and 100 mol $m^{-3}$ NaCl treated plants the increase was more or less linear, however, for 75 mol $m^{-3}$ lower $Na^+$ value were measured at day 4 and 5 day data. A rapid and large increase was observed after addition of 125 and 150 mol $m^{-3}$ NaCl. After the 6 day period a massive deposition of the $Na^+$ had taken place in the leaf mature zone.

**2) Potassium**

The potassium ion ($K^+$) concentration was measured in the same cell sap and under the similar experimental conditions (Fig. 5.11). It was found to be in large quantities (200-250 mol $m^{-3}$) in the control plants, and that is almost 40-50 times higher than the $Na^+$ content. Immediately after the stress the potassium concentration was found to be slightly increased but it attained the normal value after certain time (2 to 3 d) and remained almost unchanged for the rest of the experimental period. The NaCl stress could not bring about any appreciable variation in the leaf $K^+$ content. This was true probably for all the NaCl concentrations.

**b) Anions**

The concentrations of four anions; chloride ($Cl^-$), nitrate ($NO_3^-$), phosphate ($PO_4^{3-}$) and sulphate ($SO_4^{2-}$) were also determined.

**1) Chloride**

The tissue level of $Cl^-$ in control conditions was about 15 to 20 mol $m^{-3}$ (Fig. 5.12). The response of leaf $Cl^-$ content to the external salination was qualitatively similar to that of the $Na^+$. However, the magnitude of $Cl^-$ level in the leaf was approximately twice the $Na^+$ concentration. A steady and gradual increase in the $Cl^-$ concentration took place in salt treated plants. The extent of increase was approximately proportional to the osmotic pressure of external media. A 4 to 5 fold increase was observed

after 6 d of the stress onset in case of lower NaCl concentrations. This response was almost 9 to 13 times for the higher values of NaCl present in external media.

**2) Nitrate**

The $NO_3^-$ concentration was about 175 to 200 mol $m^{-3}$ for the control conditions with a gradual increase in its content with time (Fig. 5.13). The increase in the $NO_3^-$ concentration appeared to be suppressed by the increase in the NaCl concentration in the root media, with time.

**3) Phosphate**

The $PO_4^{3-}$ concentration was found to be 20 to 30 mol $m^{-3}$ in the control plant leaves. It was uniform without any distinct change with the time (Fig. 5.14). Almost similar tendency was exhibited for the stressed plants indicating that the NaCl stress did not have any apparent effects on the $PO_4^{3-}$ uptake in the roots and their subsequent transport to the leaves.

**4) Sulphate**

Among all the anions the $SO_4^{2-}$ was determined in the lowest concentrations i.e. 4 to 8 mol $m^{-3}$ (Fig. 5.15). Its magnitude was almost unchanged in the control as well as in the salt treated plants for whole of the experimental time. However, a small but proportionately large increase appeared in the presence of 125 and 150 mol $m^{-3}$ NaCl. This increment was gradual and continued

with the tissue ageing.

### 5.9.2) Sugars

Sugars were also determined to investigate the extent of their contribution towards the tissue osmotic pressure and their possible role in osmotic adjustment.

#### a) Glucose and Fructose

Glucose and Fructose were present in quite small amounts in the control and in the NaCl treated plants (Figs. 5.16 & 5.17). No apparent changes were detected in their concentrations neither with the increase in the salt level nor with the time.

#### b) Sucrose

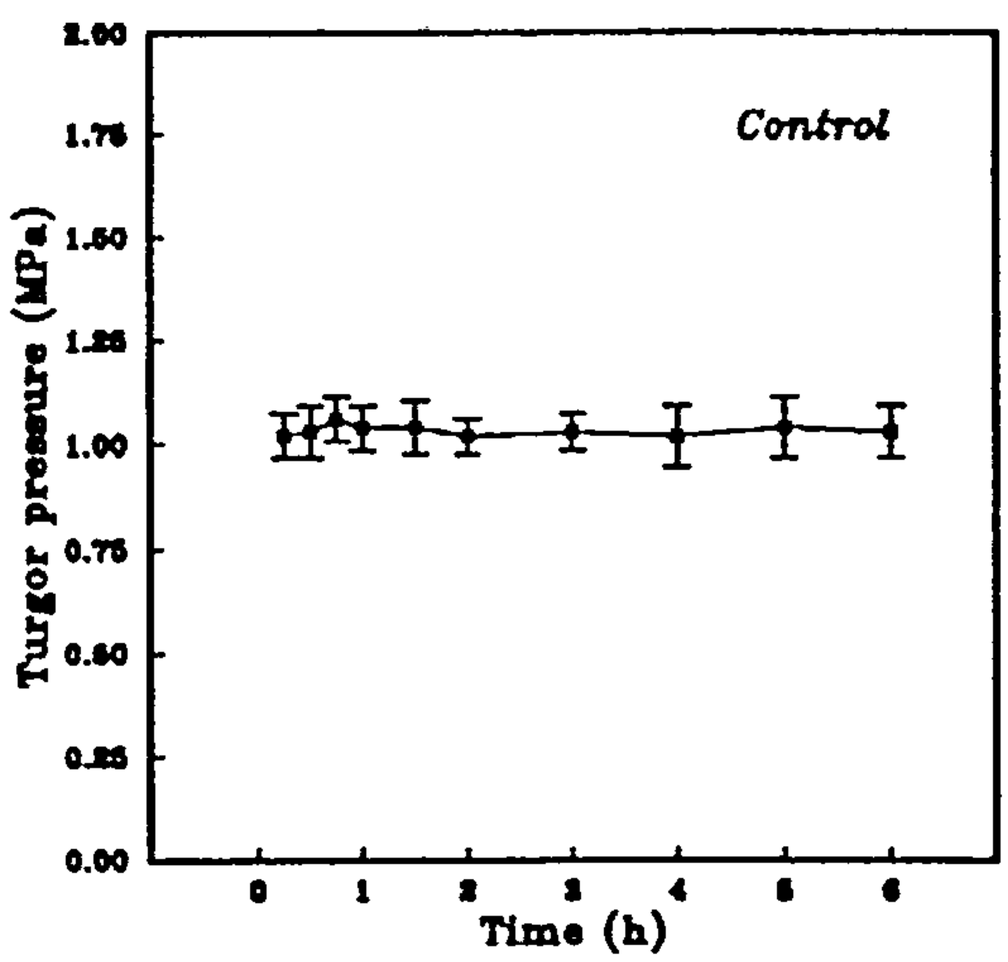
The sucrose, however, was found to be in slightly higher concentrations than both of the non-reducing sugars. In the control plants it was present in about 2 to 5 mmol $kg^{-1}$ fresh weight and was quite uniform with the tissue age (Fig. 5.18). A late increase in its amount was observed in the plants treated with 25 and 50 mol $m^{-3}$, which resulted in a two fold increase in sucrose concentration, depending upon the salt content present in the external media. An immediate and gradual response was observed after the addition of the four higher salt concentrations. It caused an increase of three times in the sucrose level at 6 d of the experiment.

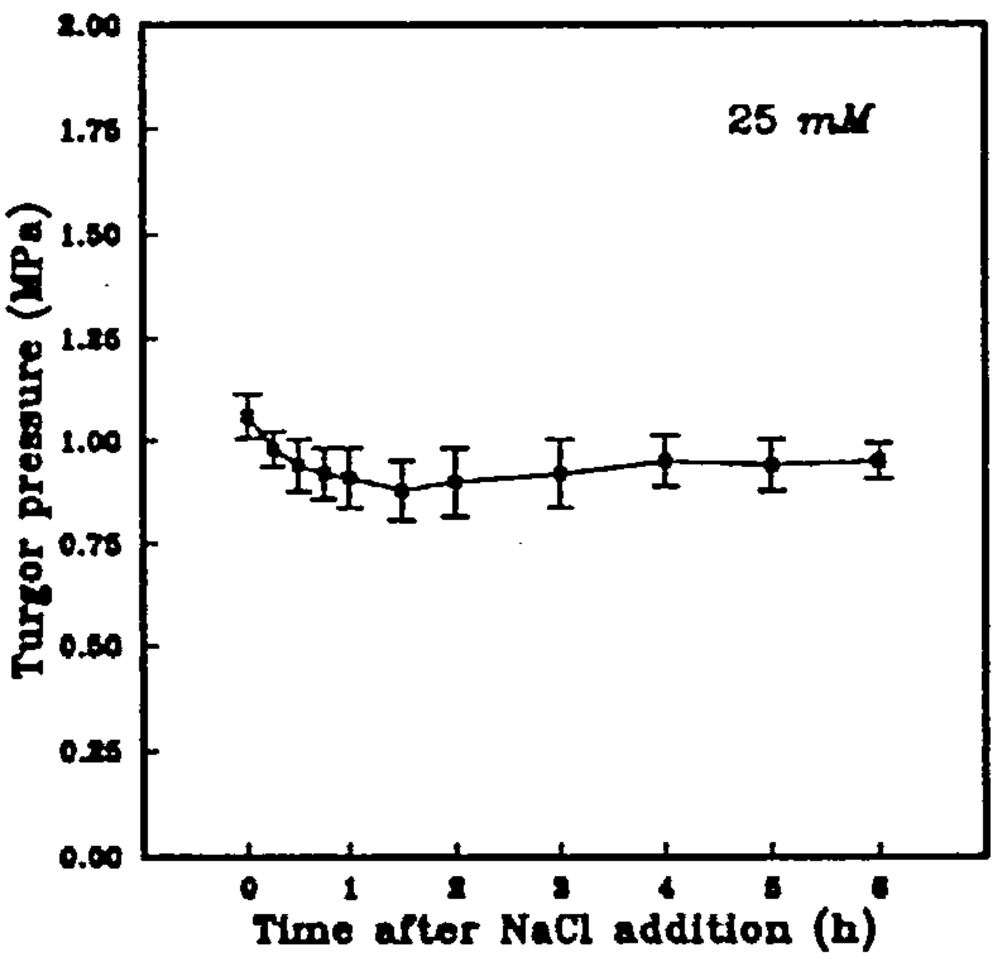
## 5.10 Comparison of Measured and Calculated Osmotic Pressure

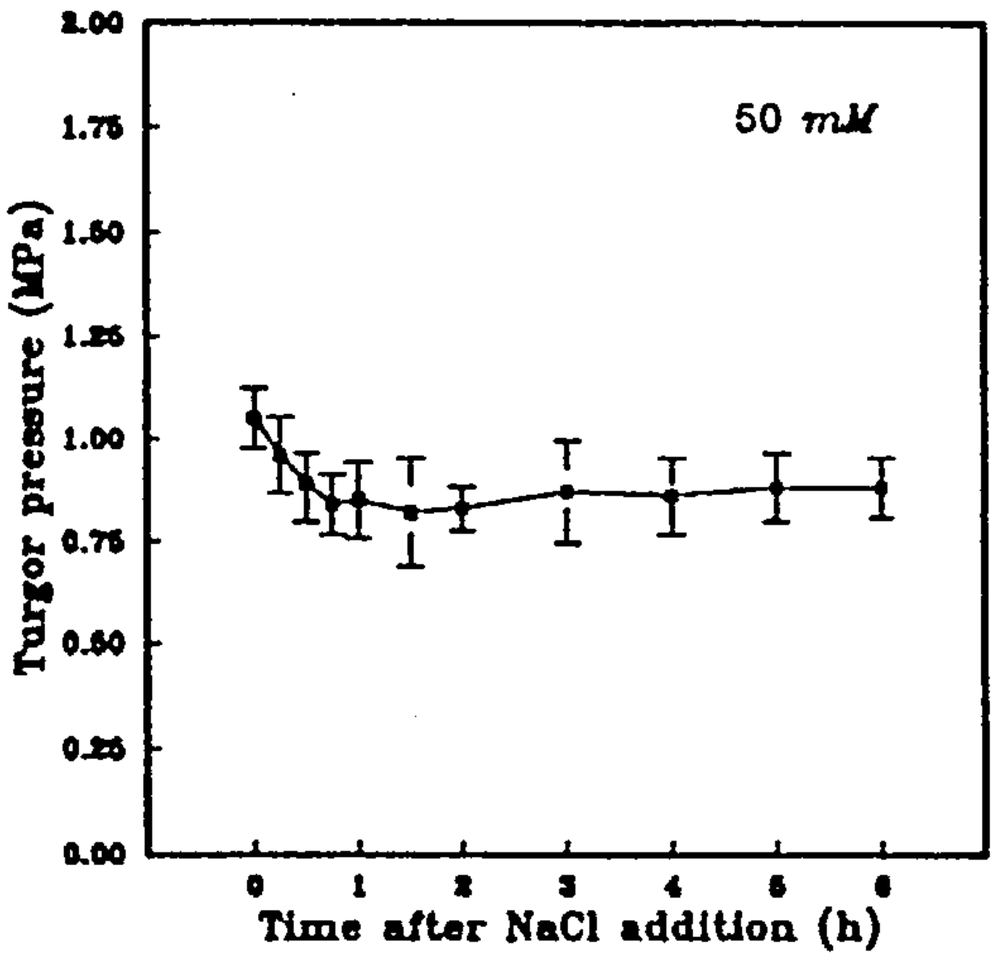
The osmotic pressure that could be accounted for was calculated by the summation of the values of all the ionic and the organic solutes measured in the experiments described in section 5.1.9. It was concluded that $K^+$ and $NO_3^-$ contributed about 80 % of the calculated osmotic pressure in the control plants.

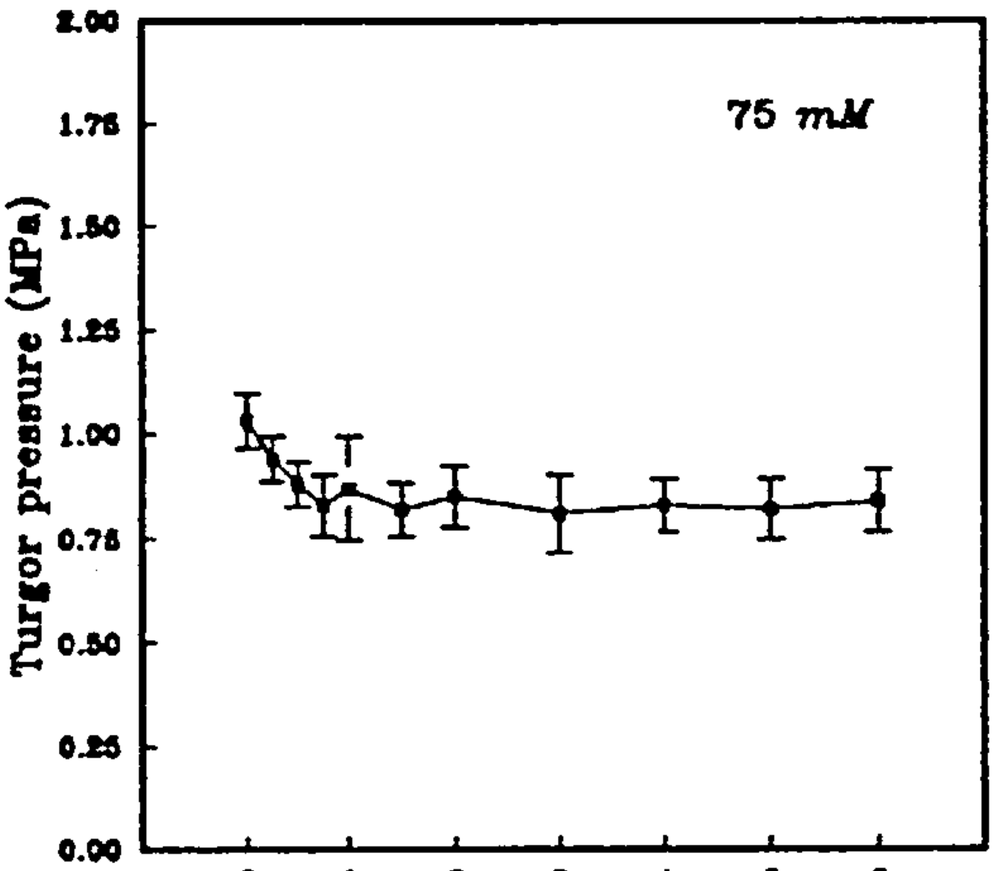
A comparison was made between the values of the calculated and the measured (see section 5.1.6) osmotic pressure (Fig. 5.19). Both of these parameters were based on the whole tissue basis. No appreciable differences were found in their magnitudes in the control as well as in the plants suffering from a stress of 25 mol $m^{-3}$ NaCl. However, for the higher NaCl concentrations the differences between these two parameters became more conspicuous with the increase in the stress level and with the tissue ageing.

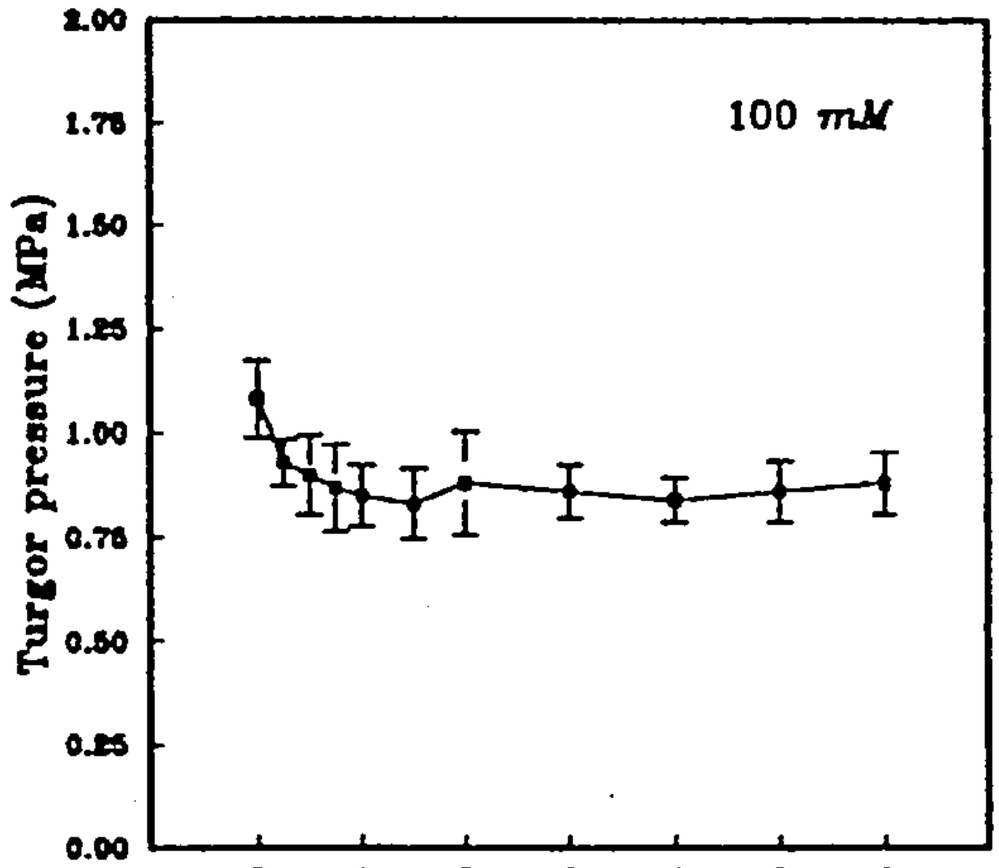
Fig. 5.1 Time course of turgor pressure response of mature (expanded) cells of leaves to the NaCl stress studied in a short term experiment i.e. 6 h. Conditions as in Fig. 3.3.

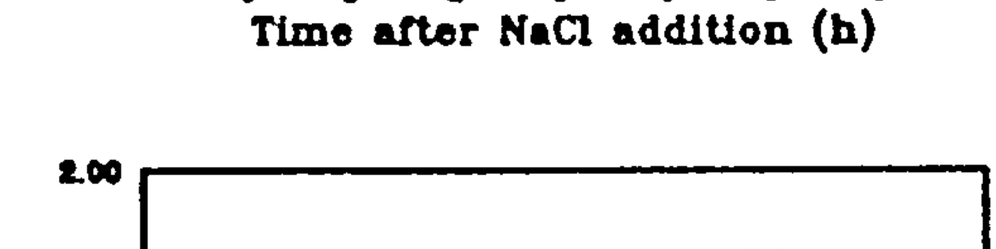

Control
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time (h)


25 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)


50 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)


75 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)


100 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)


125 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)

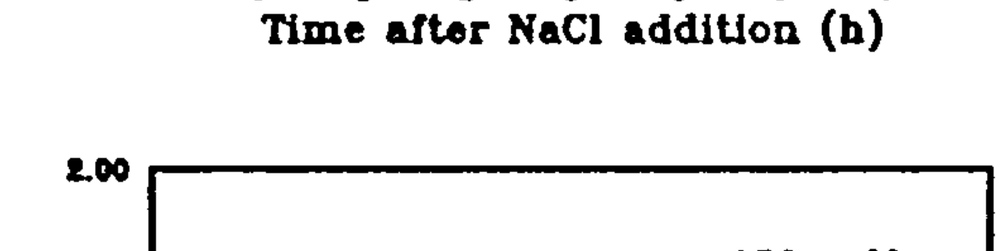
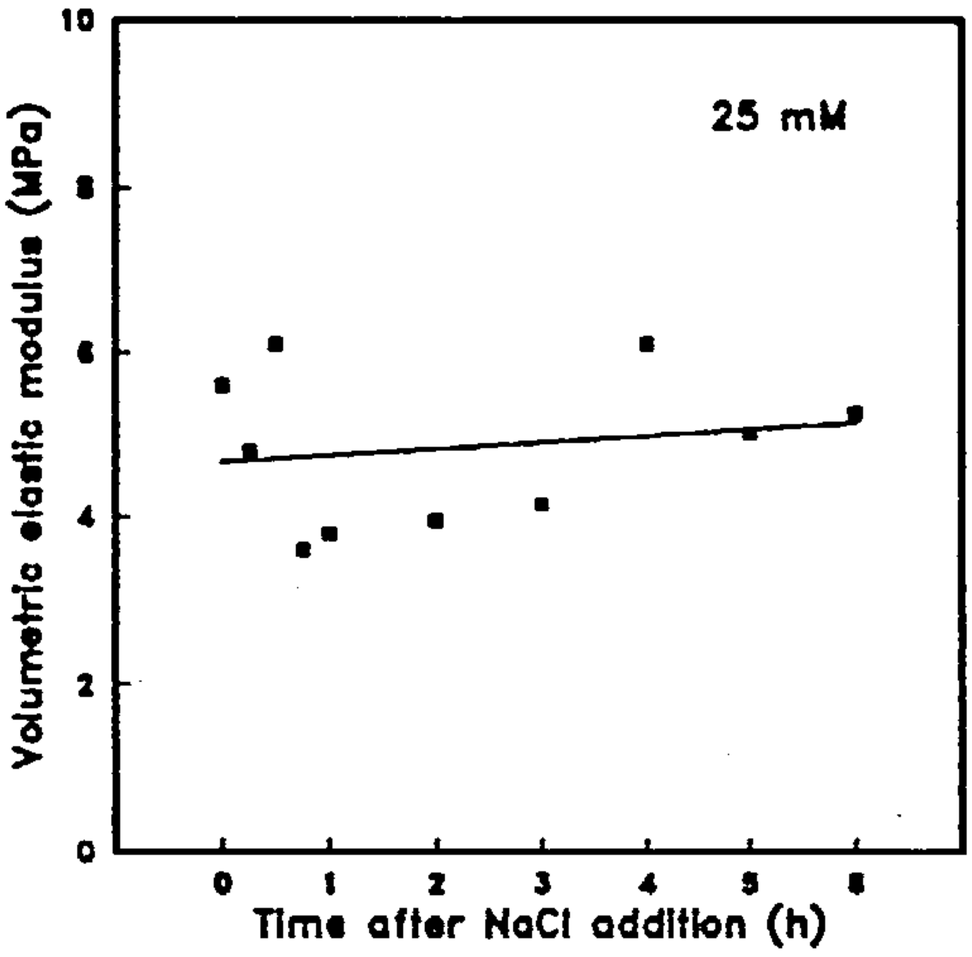
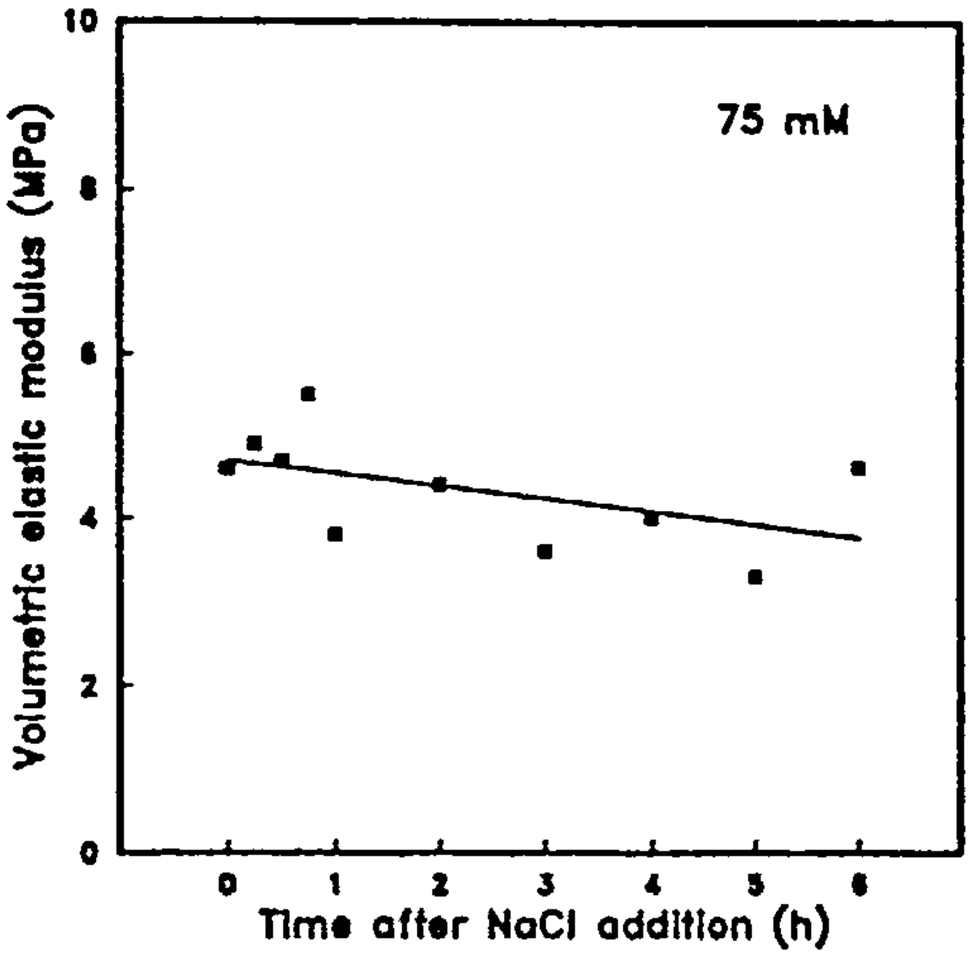
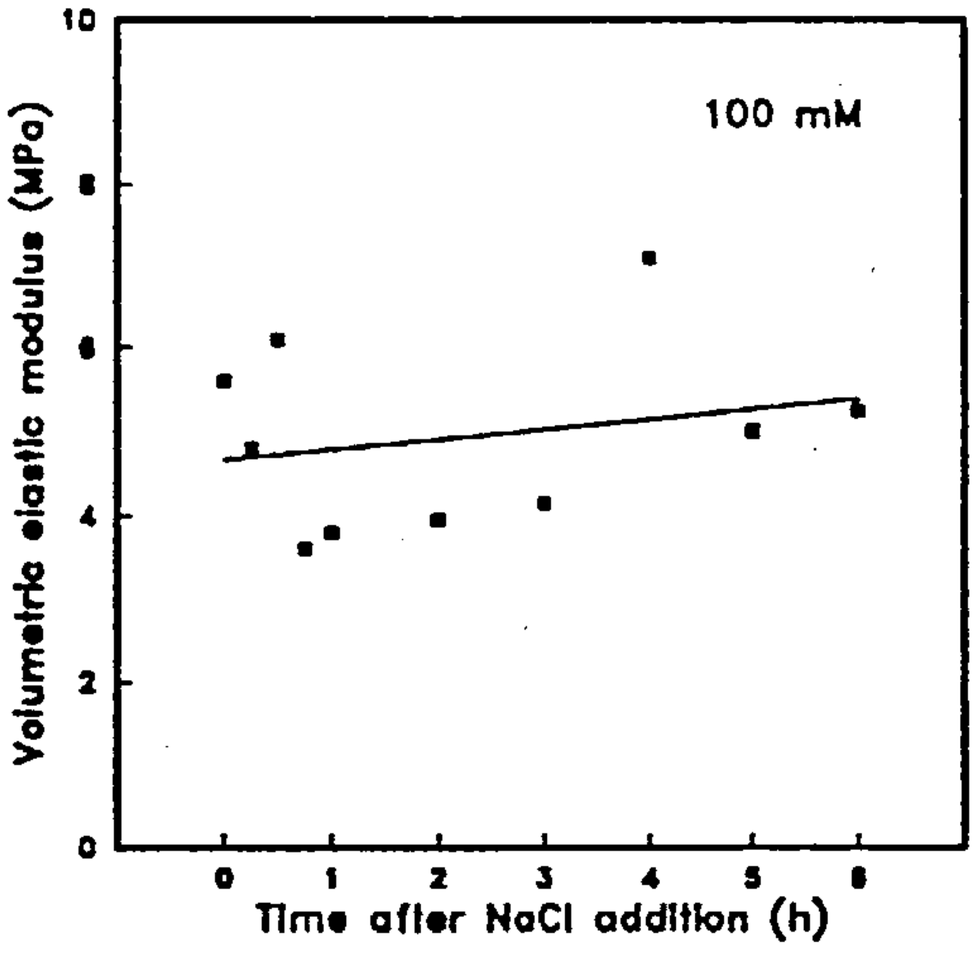

150 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (h)

**Fig. 5.2** The response of volumetric elastic modulus studied in context of the NaCl stress, measured in the mature zone of leaf using pressure probe in a short term experiment i.e. 6 h. The regression line fitted to the data. Various NaCl concentrations i.e. 50, 75, 100, and 150 mol $m^{-3}$, as indicated in top right corner of every graph, were applied to salinize the root media. All the NaCl concentrations were applied in one step to give sudden shock to plants. Each point is the mean of 5-8 replicates taken from two experiments. Error bars indicate the standard deviation of the mean of all replicates.


25 mM
Volumetric elastic modulus (MPa)
Time after NaCl addition (h)
0
2
4
6
8
10
0
1
2
3
4
5
6


75 mM
Volumetric elastic modulus (MPa)
Time after NaCl addition (h)
0
2
4
6
8
10
0
1
2
3
4
5
6


100 mM
Volumetric elastic modulus (MPa)
Time after NaCl addition (h)
0
2
4
6
8
10
0
1
2
3
4
5
6

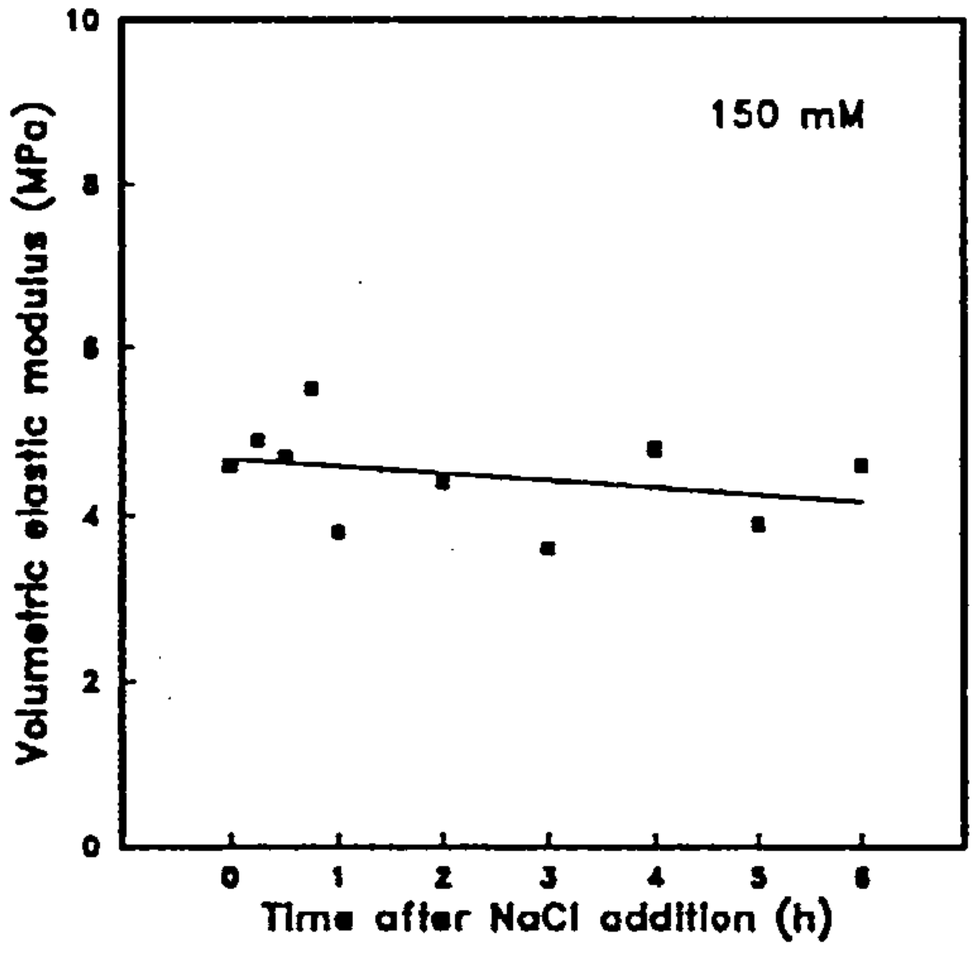
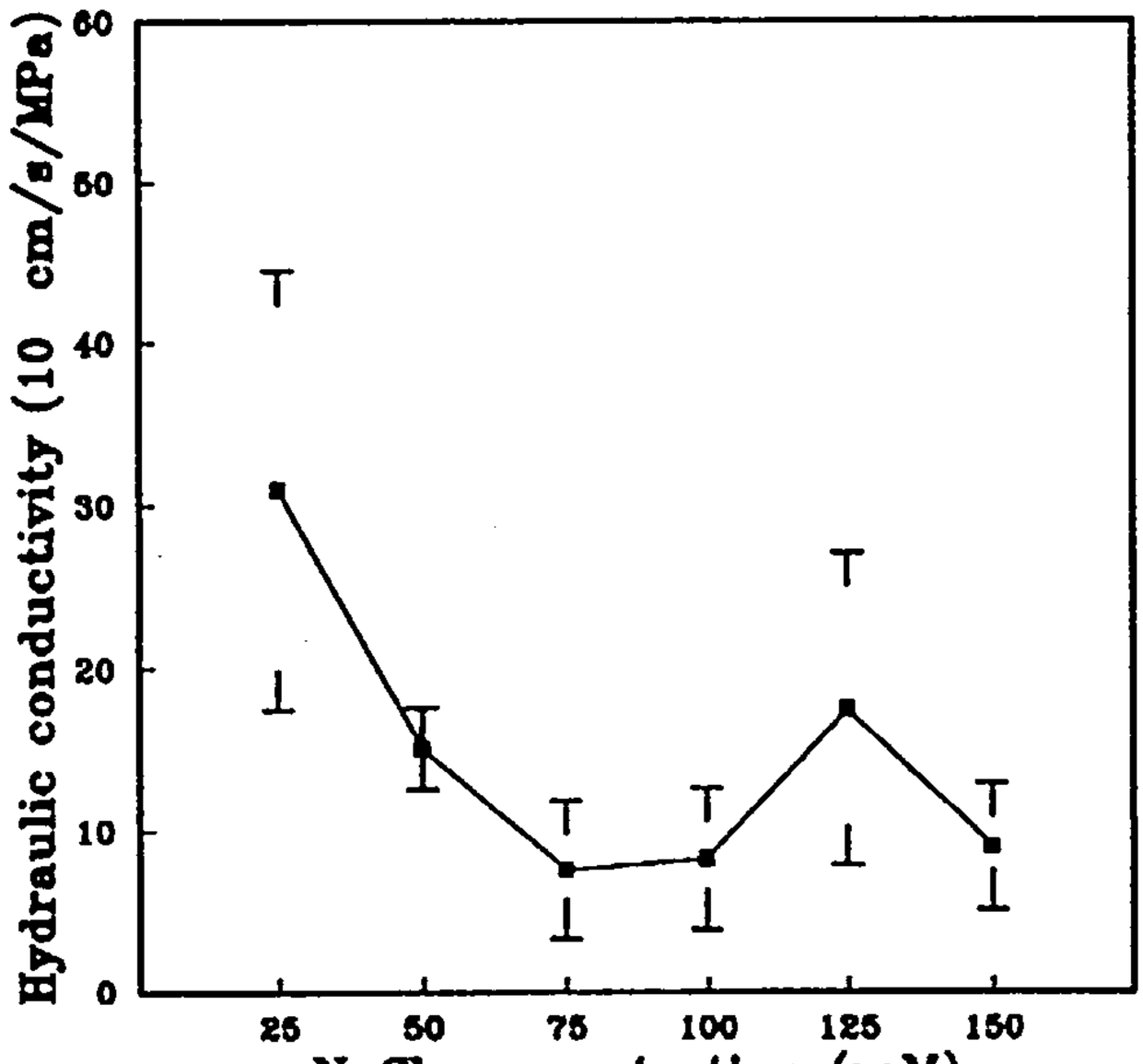

150 mM
Volumetric elastic modulus (MPa)
Time after NaCl addition (h)
0
2
4
6
8
10
0
1
2
3
4
5
6

**Fig. 5.3** The response of root hydraulic conductivity to NaCl and mannitol stresses as studied using a osmotically induced back-flow technique. 25, 50, 75, 100, 125 and 150 mol $m^{-3}$ of NaCl and equi-osmolar concentrations of mannitol i.e. 50, 100, 150, 200, 250 and 300 mol $m^{-3}$ were applied. All the NaCl and mannitol concentrations were applied in one step to give sudden osmotic shock to plants. Each point is the mean of 8-15 replicates taken from three experiments. Error bars indicate the standard deviation of the mean of all replicates.


Hydraulic conductivity (10 cm/s/MPa)
60
50
40
30
20
10
0
25
50
75
100
125
150
NaCl concentration (mM)

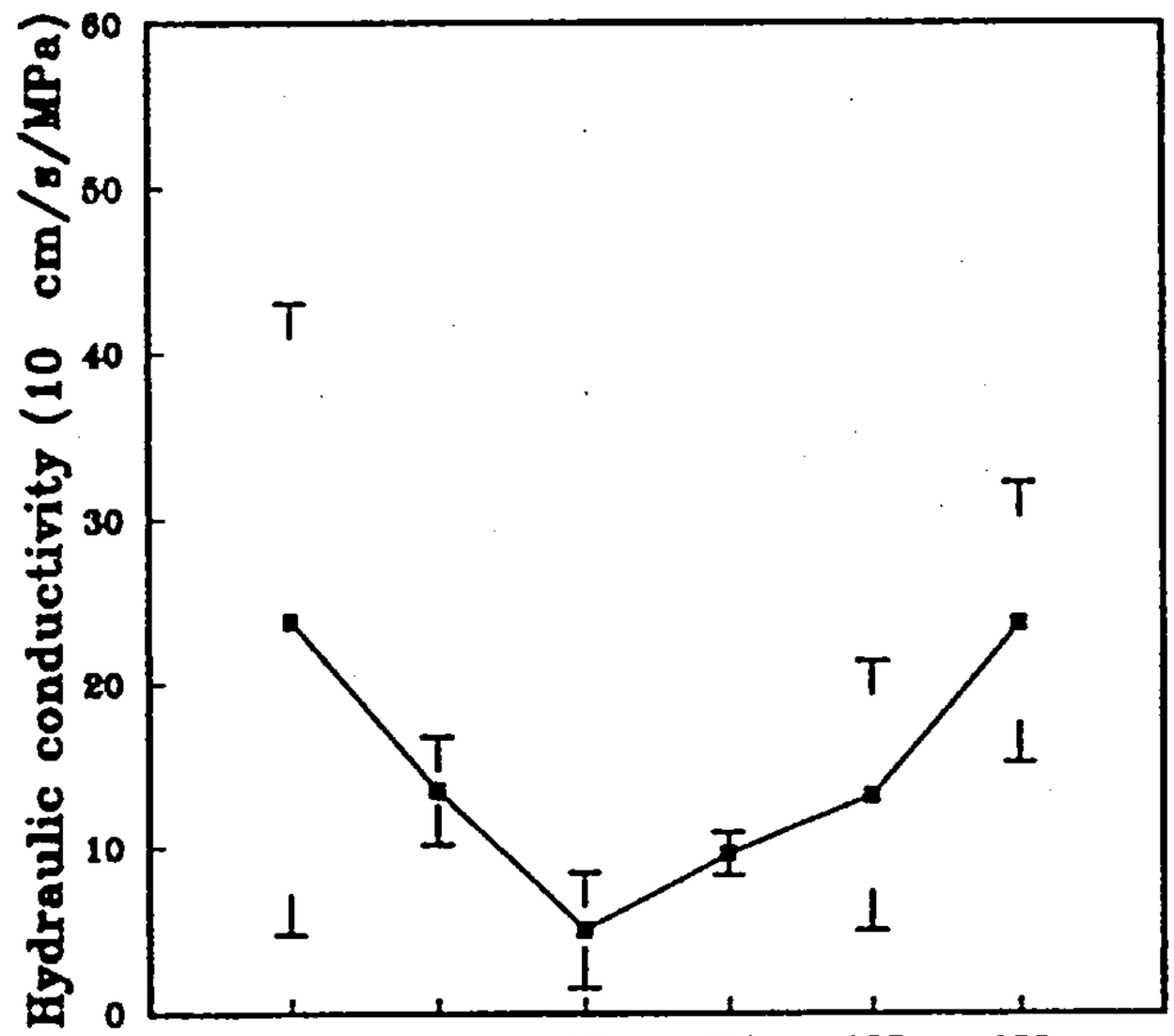
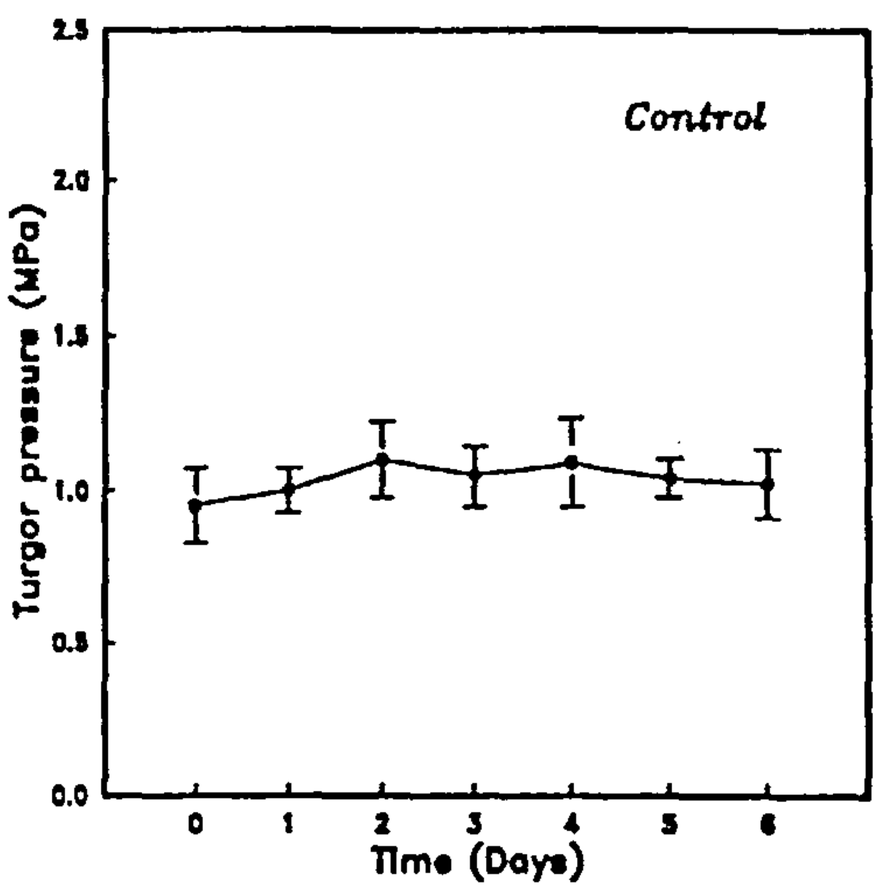
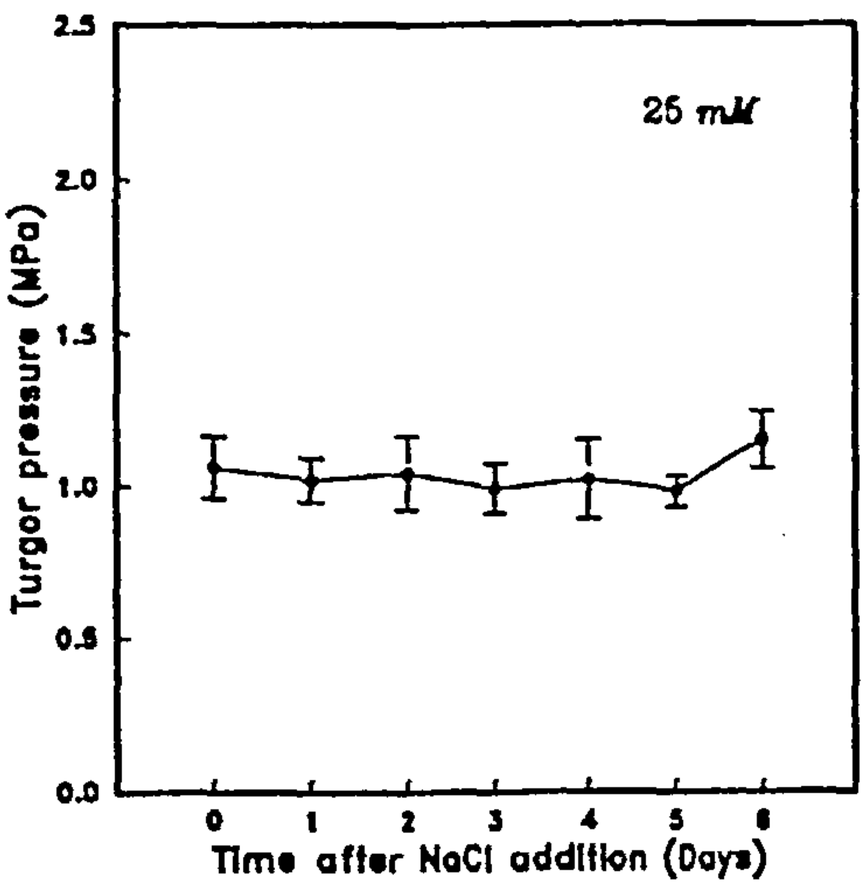
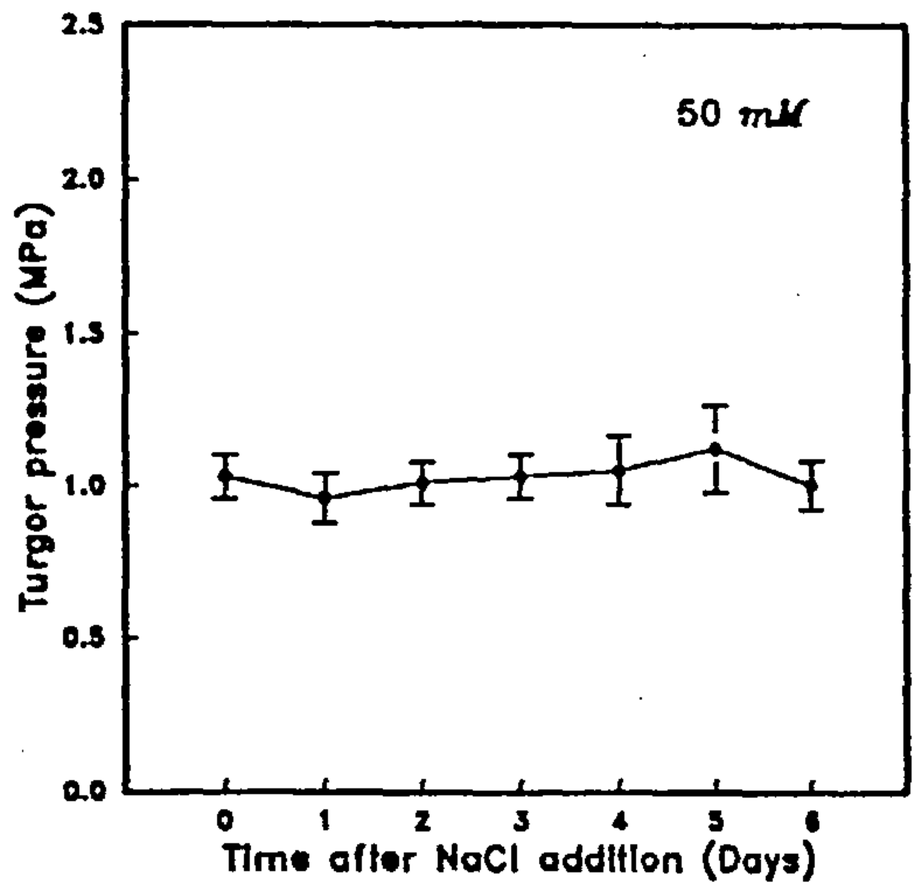
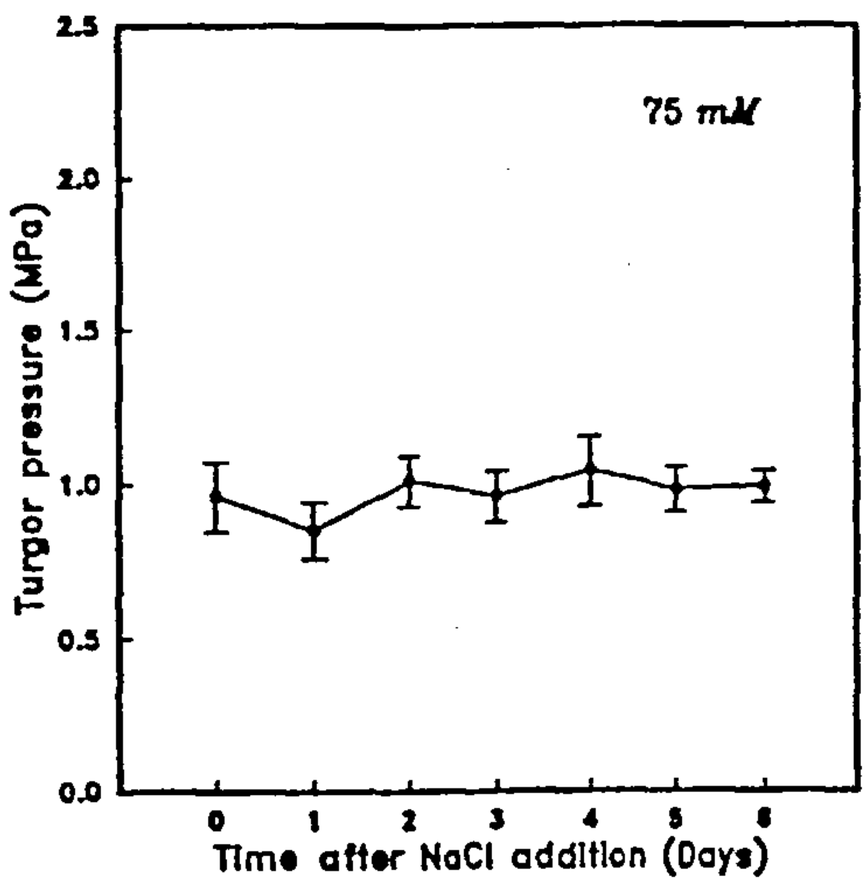

Hydraulic conductivity (10 cm/s/MPa)
60
50
40
30
20
10
0
25
50
75
100
125
150
Mannitol concentration (2 x mM)

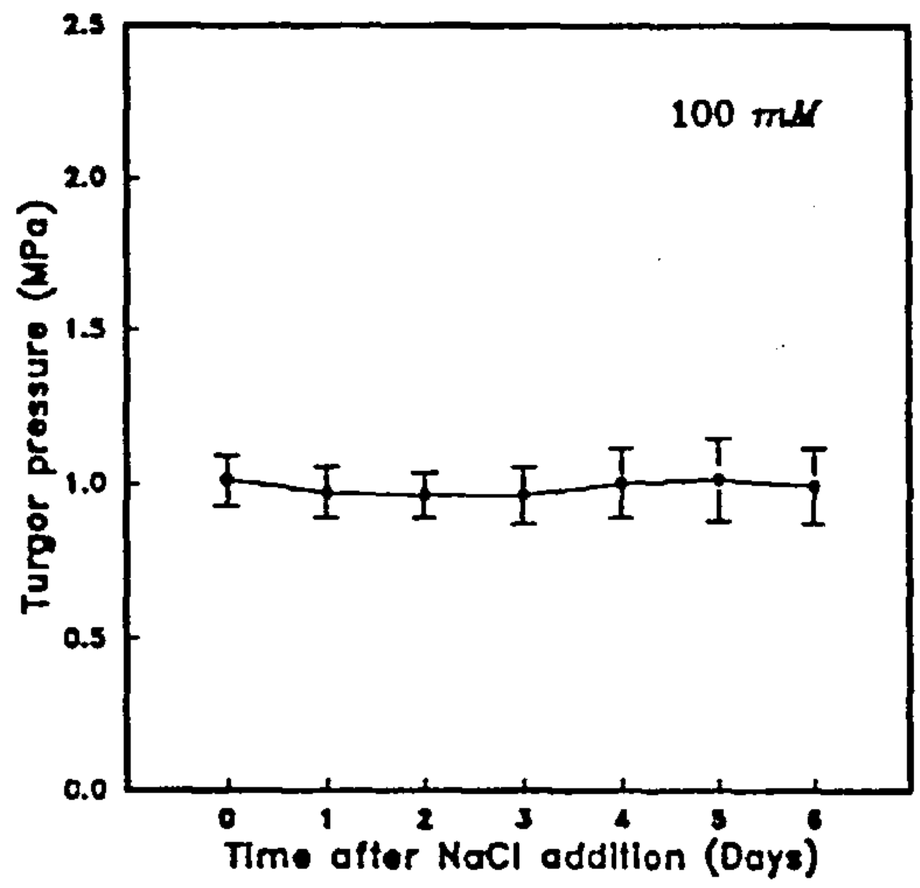
Fig. 5.4 The response of turgor pressure of mature (expanded) cells of leaves to the NaCl stress studied in a long term experiment i.e. 6 days. Each point is the mean of 5-25 replicates taken from three experiments.

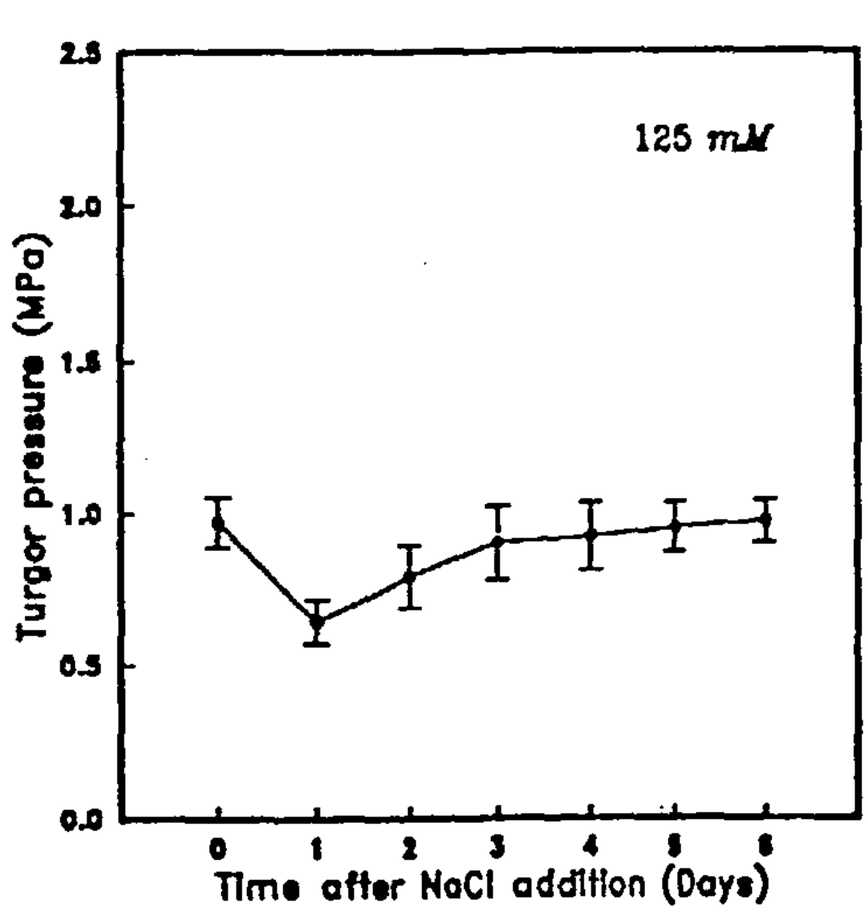

Control
Turgor pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time (Days)


25 mM
Turgor pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


50 mM
Turgor pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


75 mM
Turgor pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


100 mM
Turgor pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


125 mM
Turgor pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)

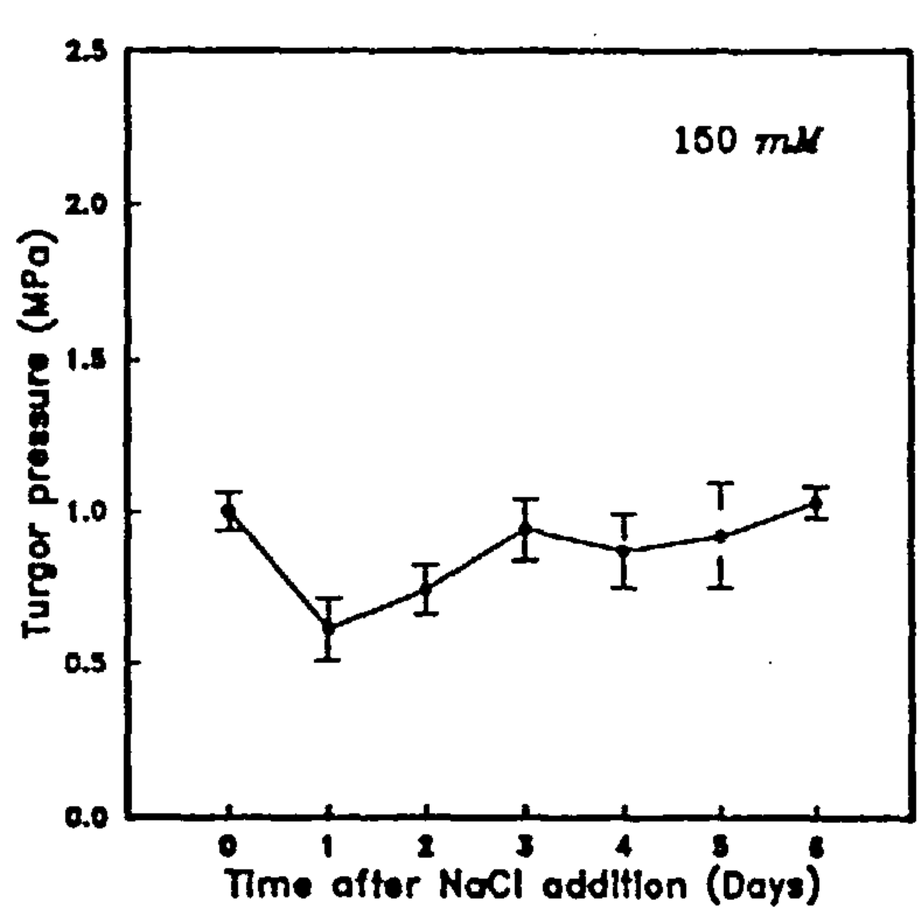
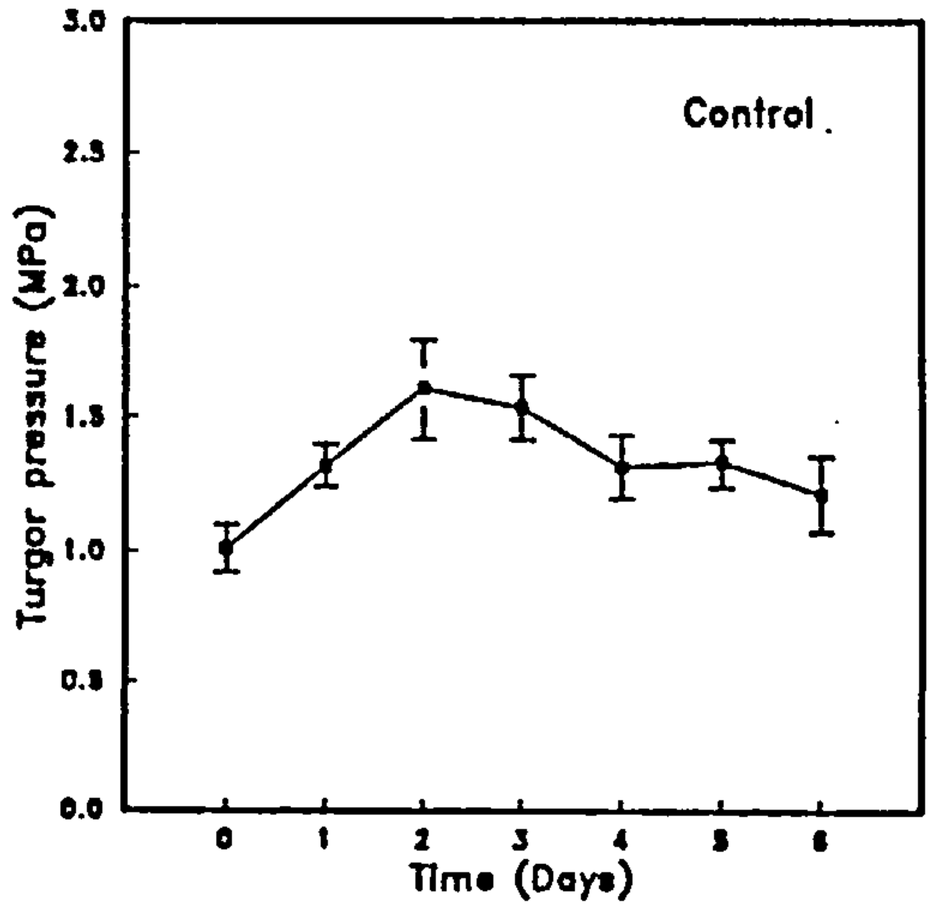
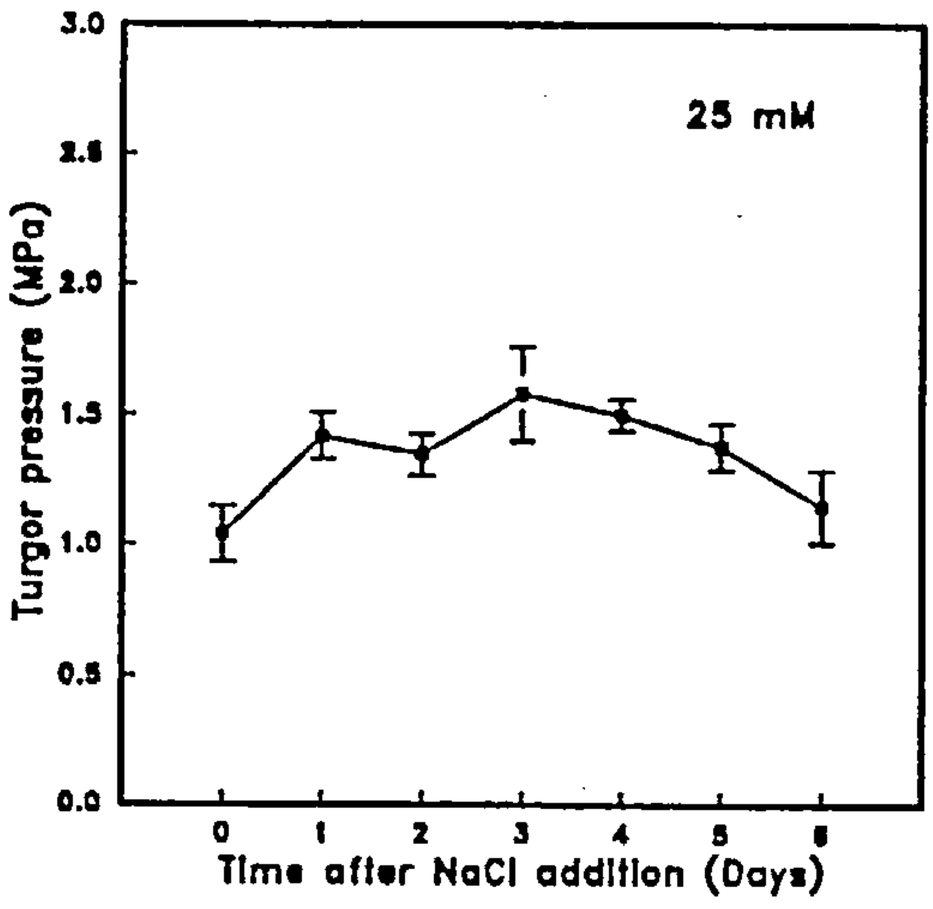
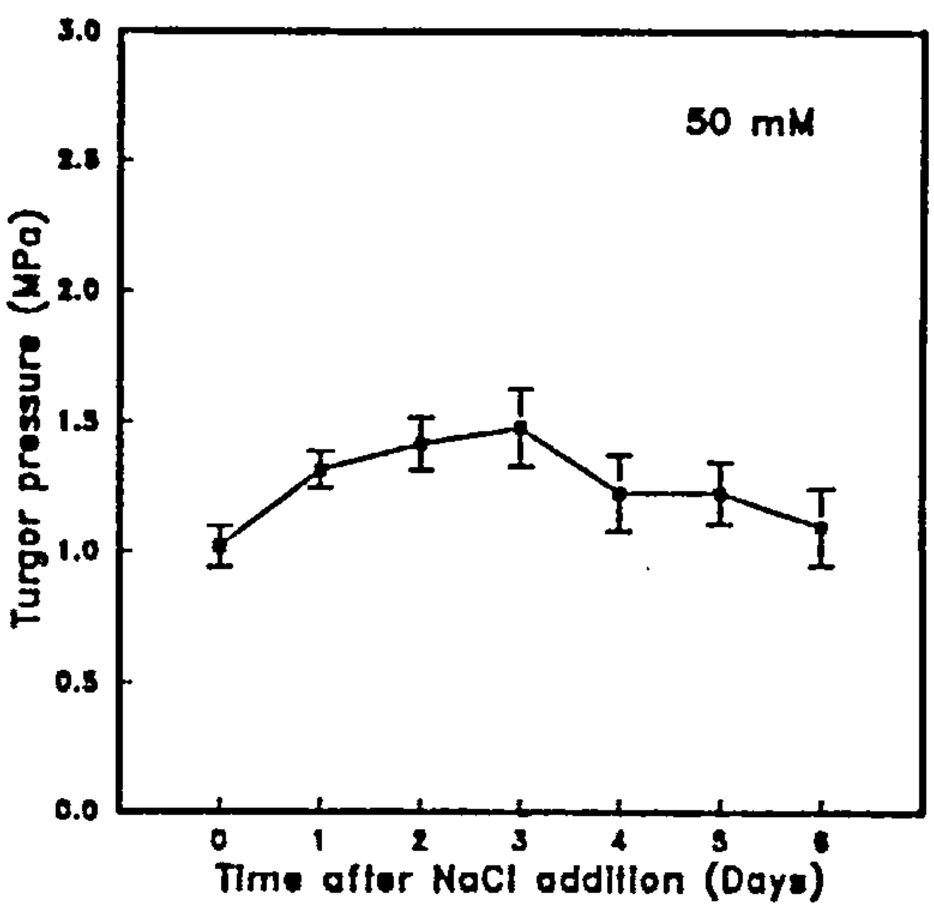
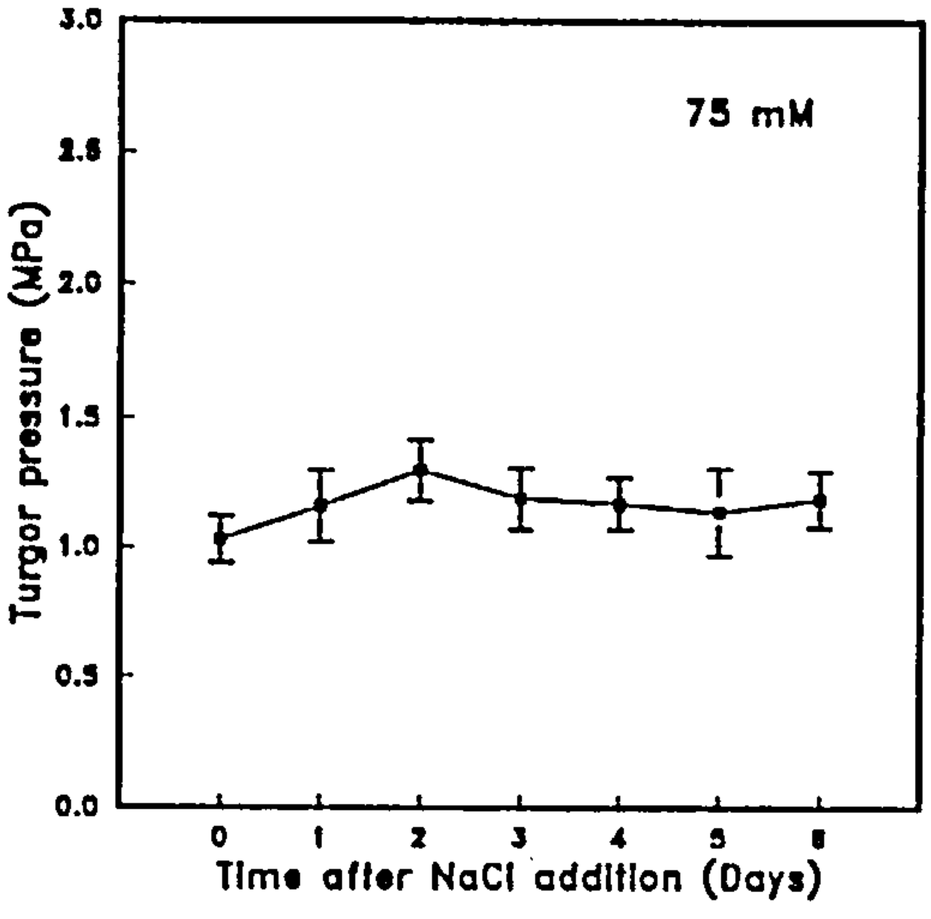

150 mM
Turgor pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)

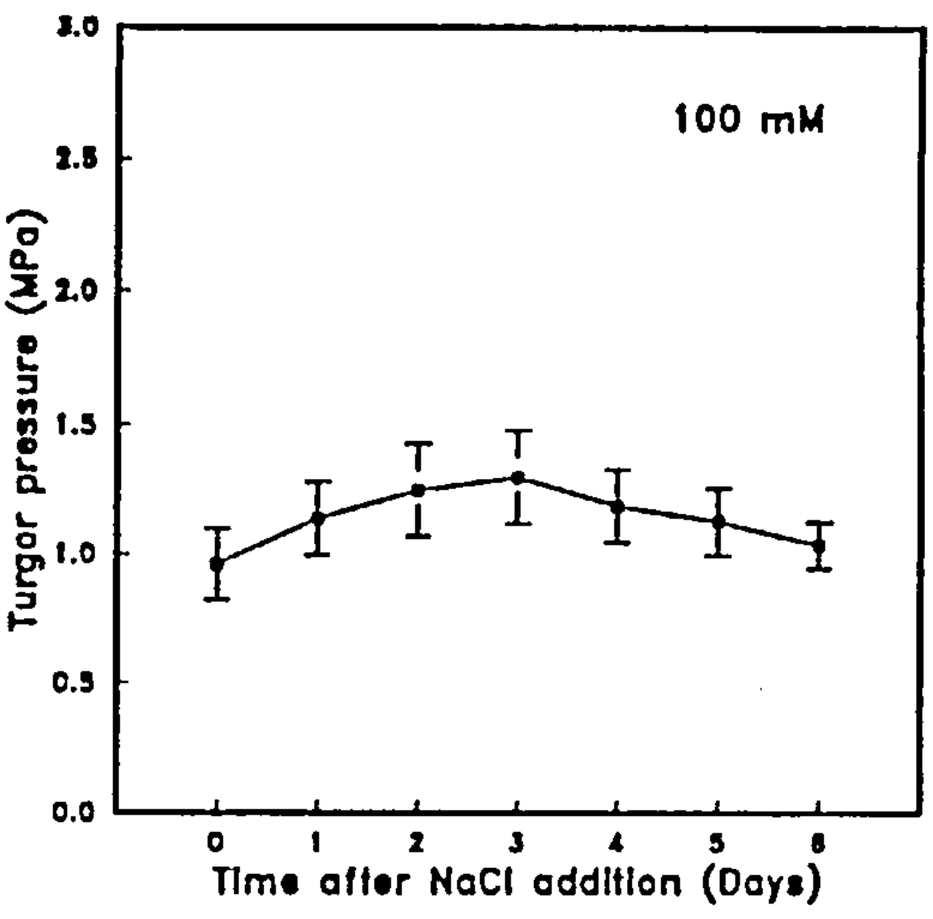
**Fig. 5.5** The effect of nutrient status of root media on turgor pressure of individual mature cells of leaf. Plants were grown in 0.5 mol $m^{-3}$ $CaCl_2/CaSO_4$ for 6 days and were transferred to 50 % Hoagland solution for 1 day prior to the stress application. Subsequent conditions as in Fig. 3.3. Each point is the mean of 5-25 replicates taken from three experiments.

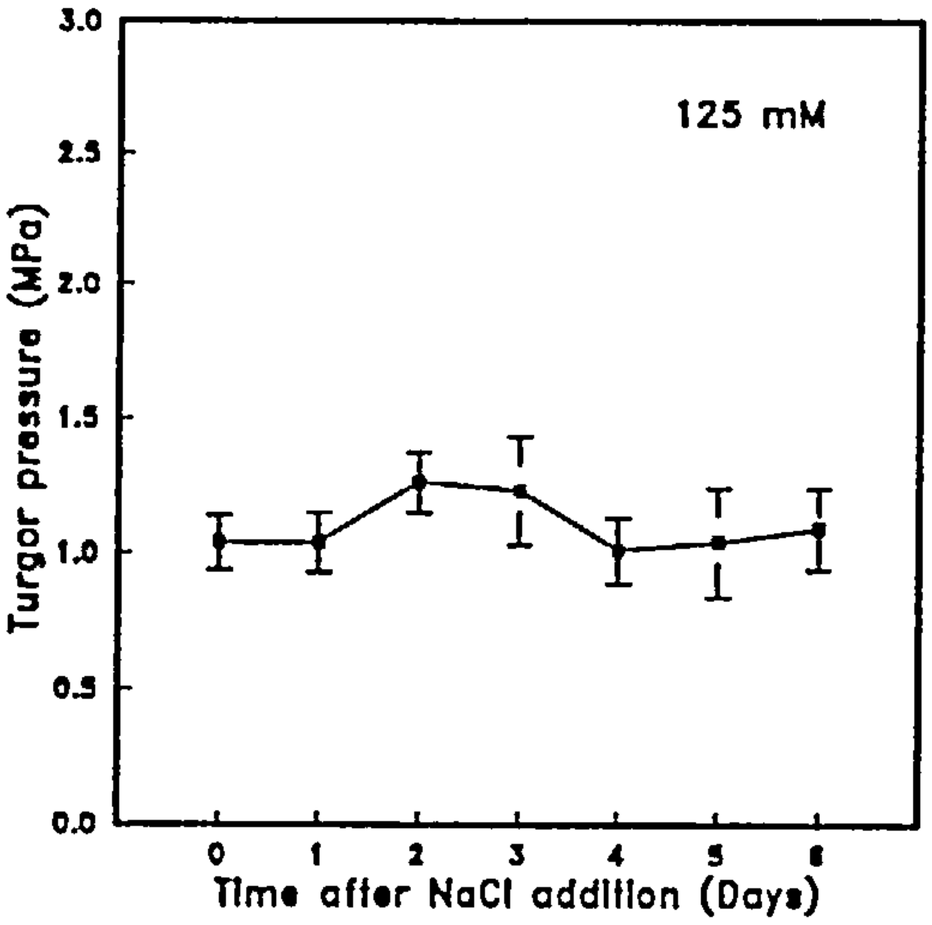

Control
Turgor pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time (Days)


25 mM
Turgor pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


50 mM
Turgor pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


75 mM
Turgor pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


100 mM
Turgor pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


125 mM
Turgor pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)

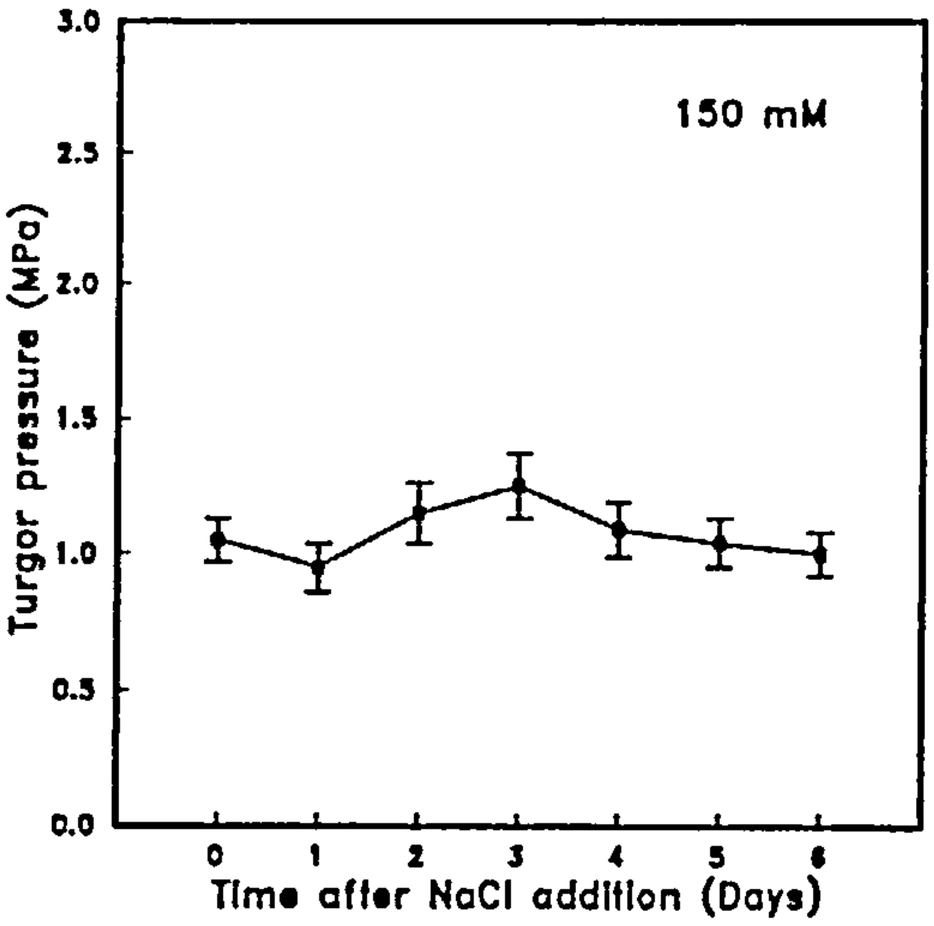
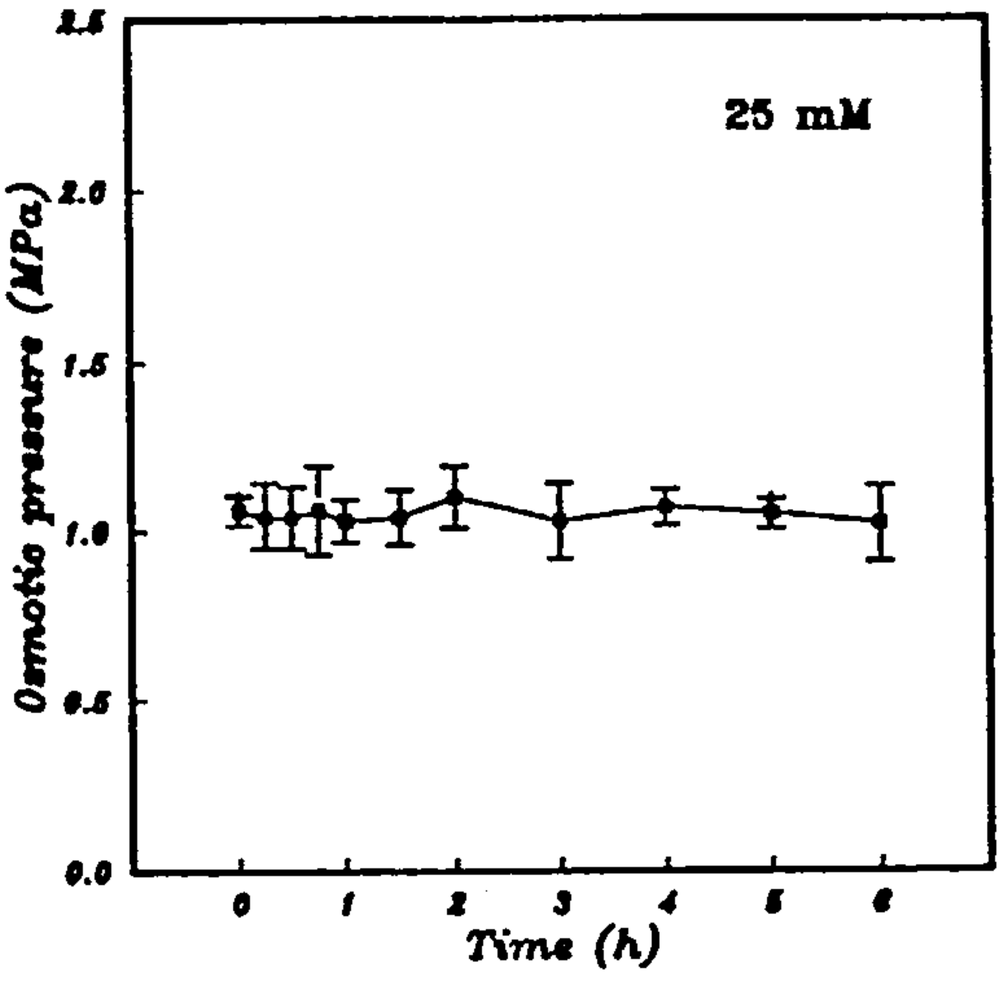
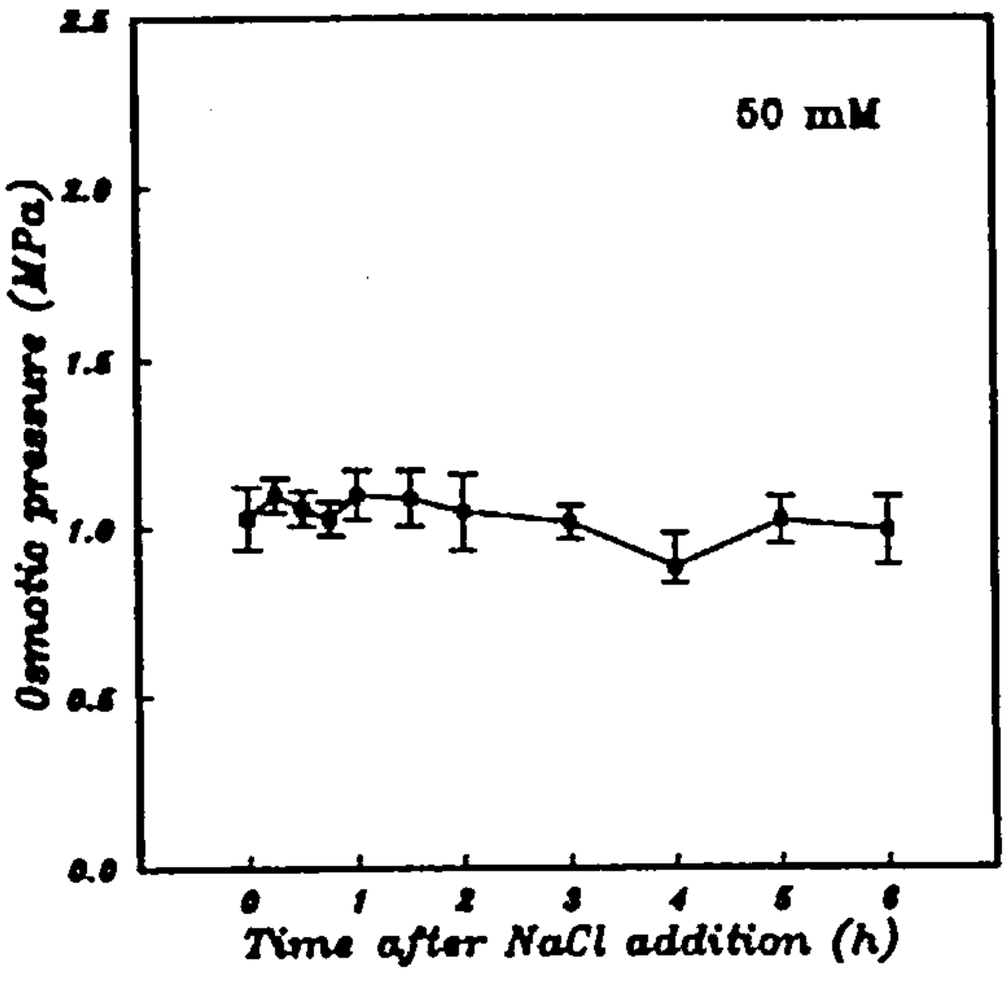
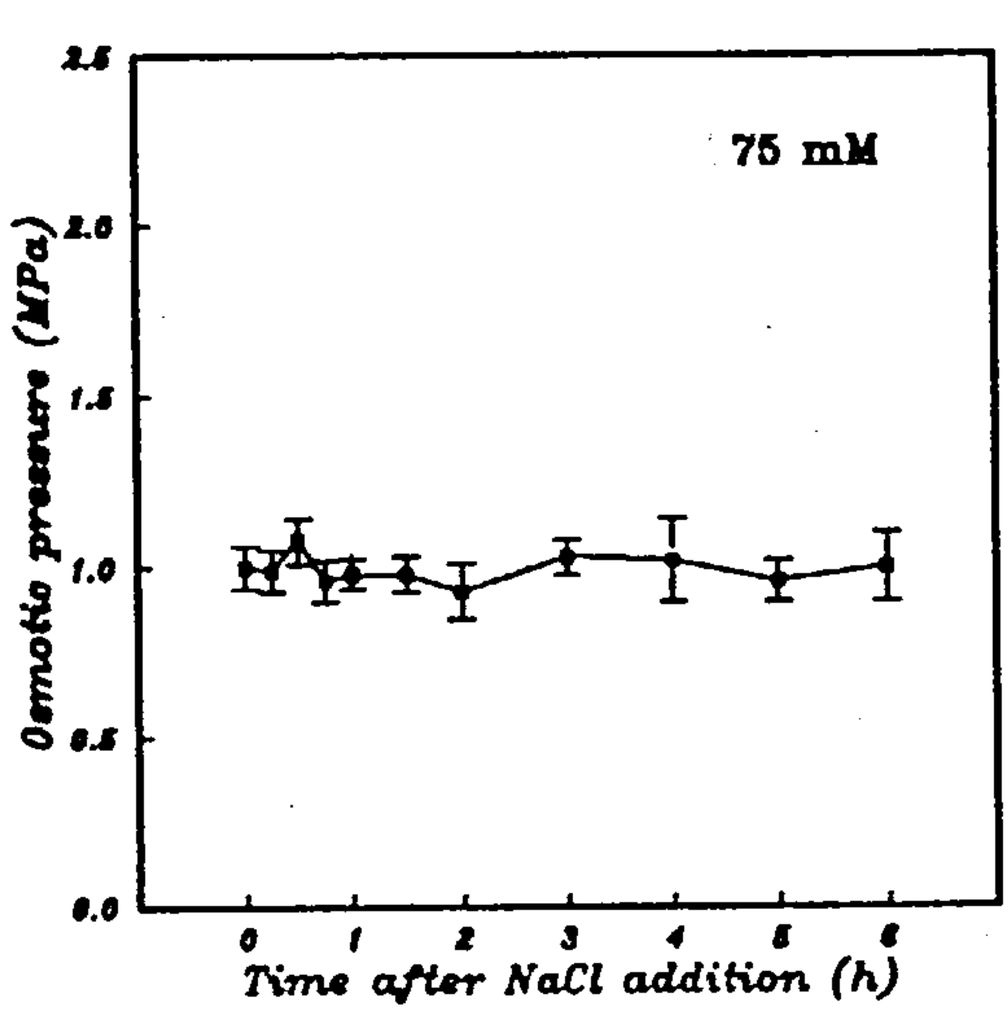
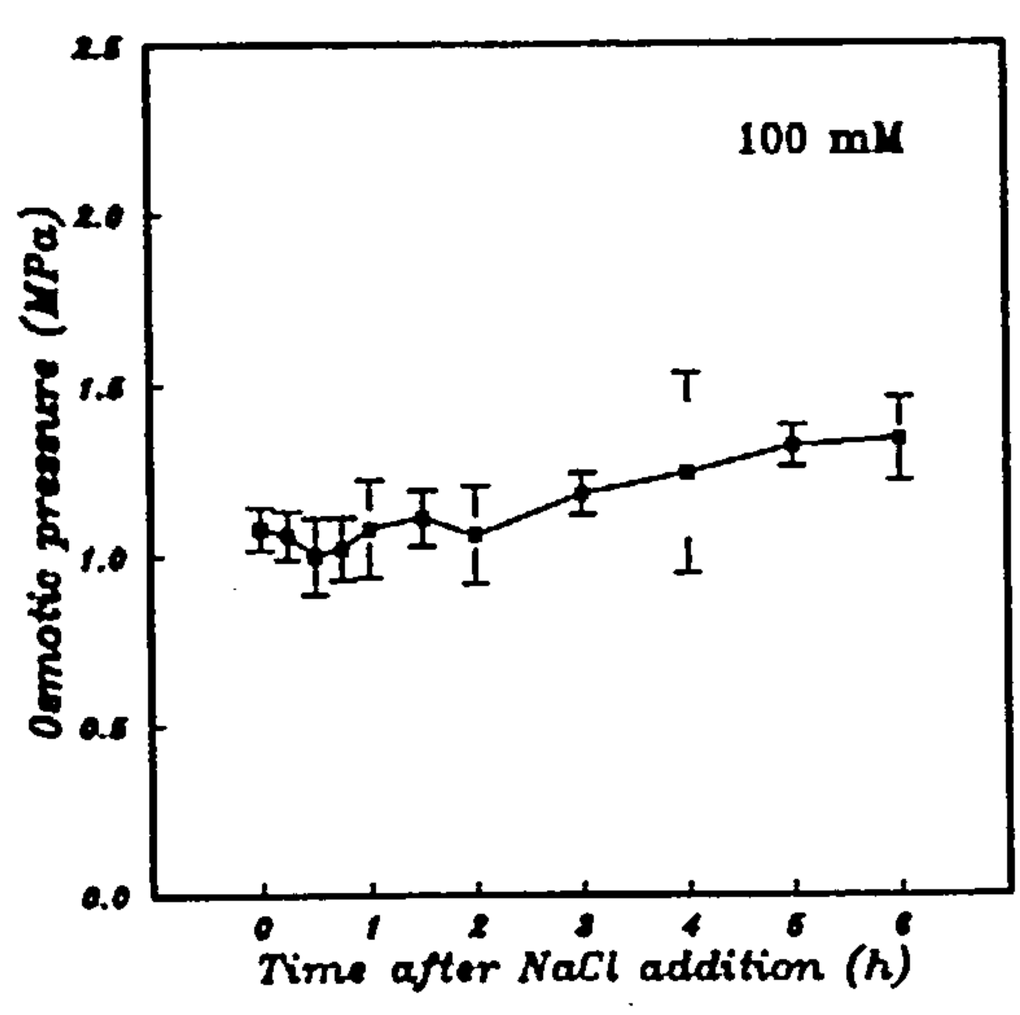

150 mM
Turgor pressure (MPa)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)

**Fig. 5.6** Time course of osmotic pressure response to the NaCl stress studied in a short term experiment i.e. 6 h. Osmotic pressure was measured using vapour pressure osmometry on the whole tissue basis. Conditions as in Fig. 3.3. Each point is the mean of 5-25 replicates taken from three experiments.


25 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time (h)


50 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

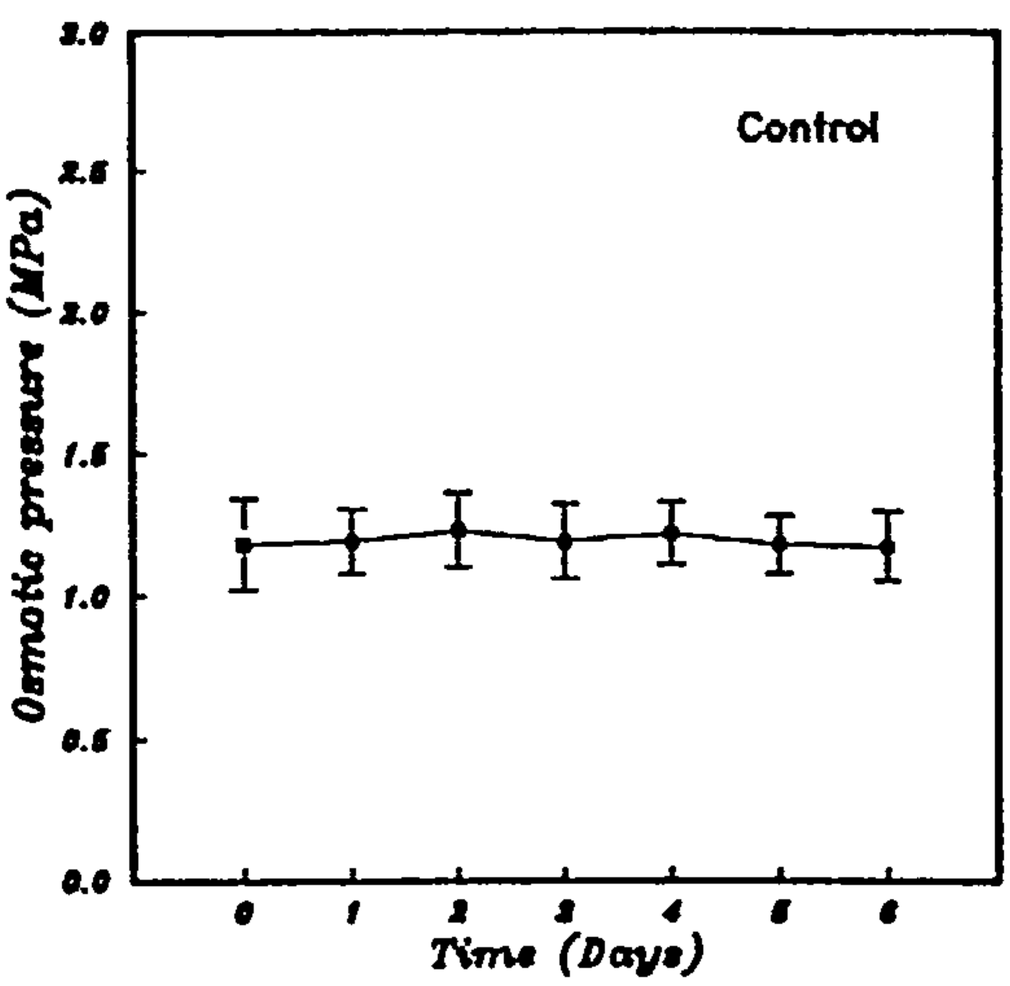

75 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

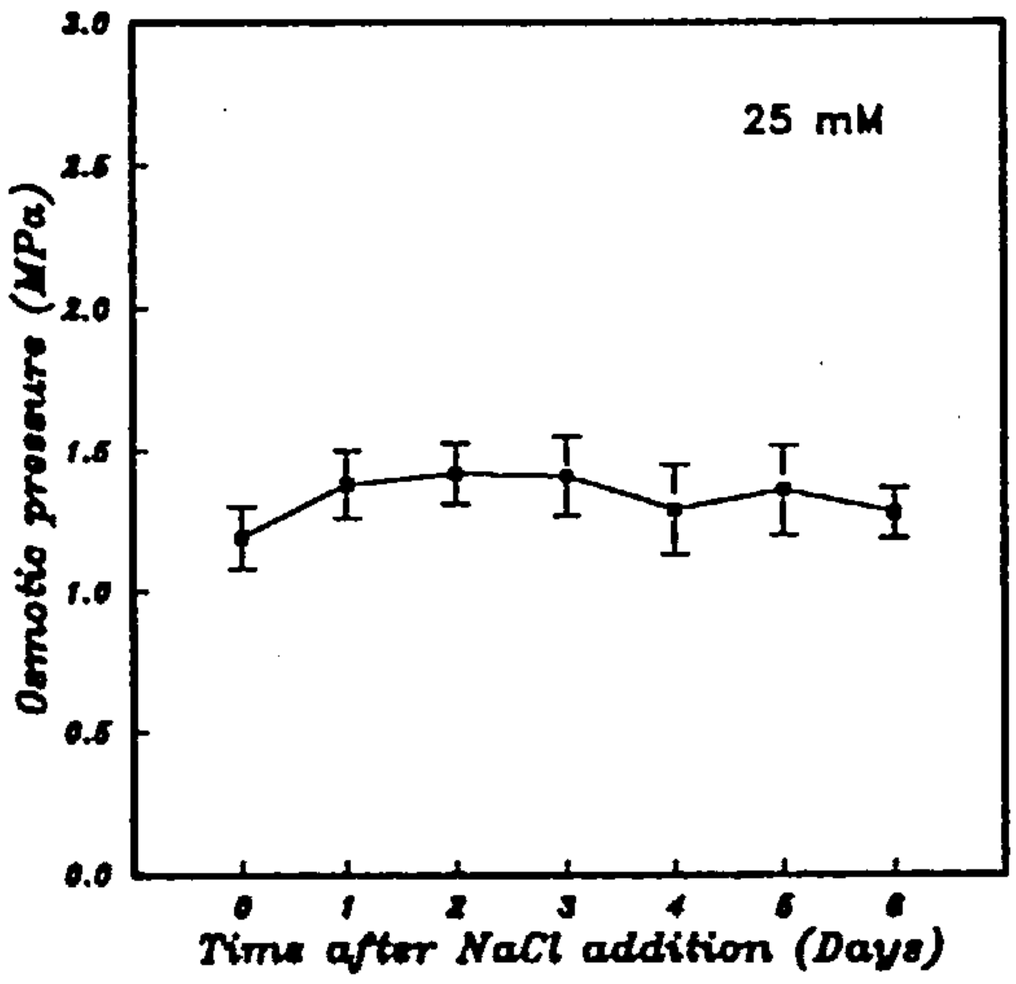

100 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

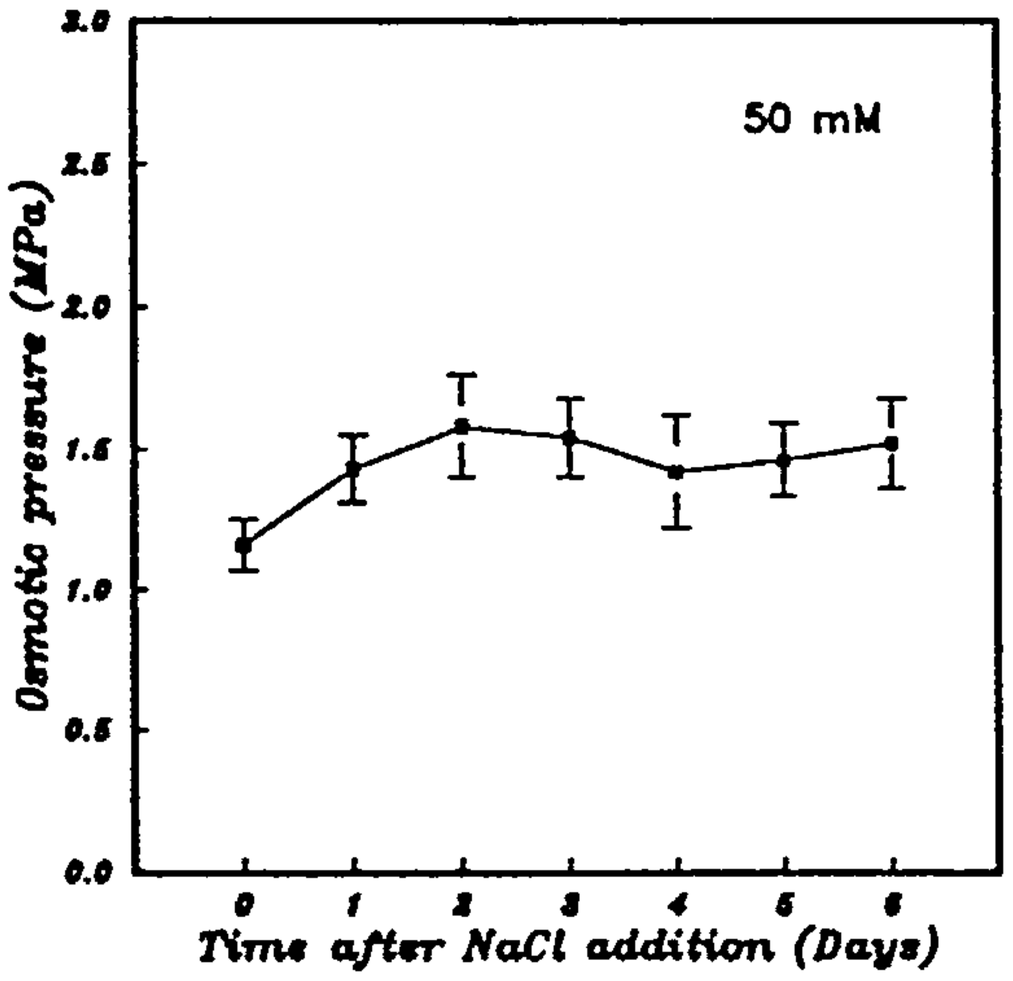

125 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

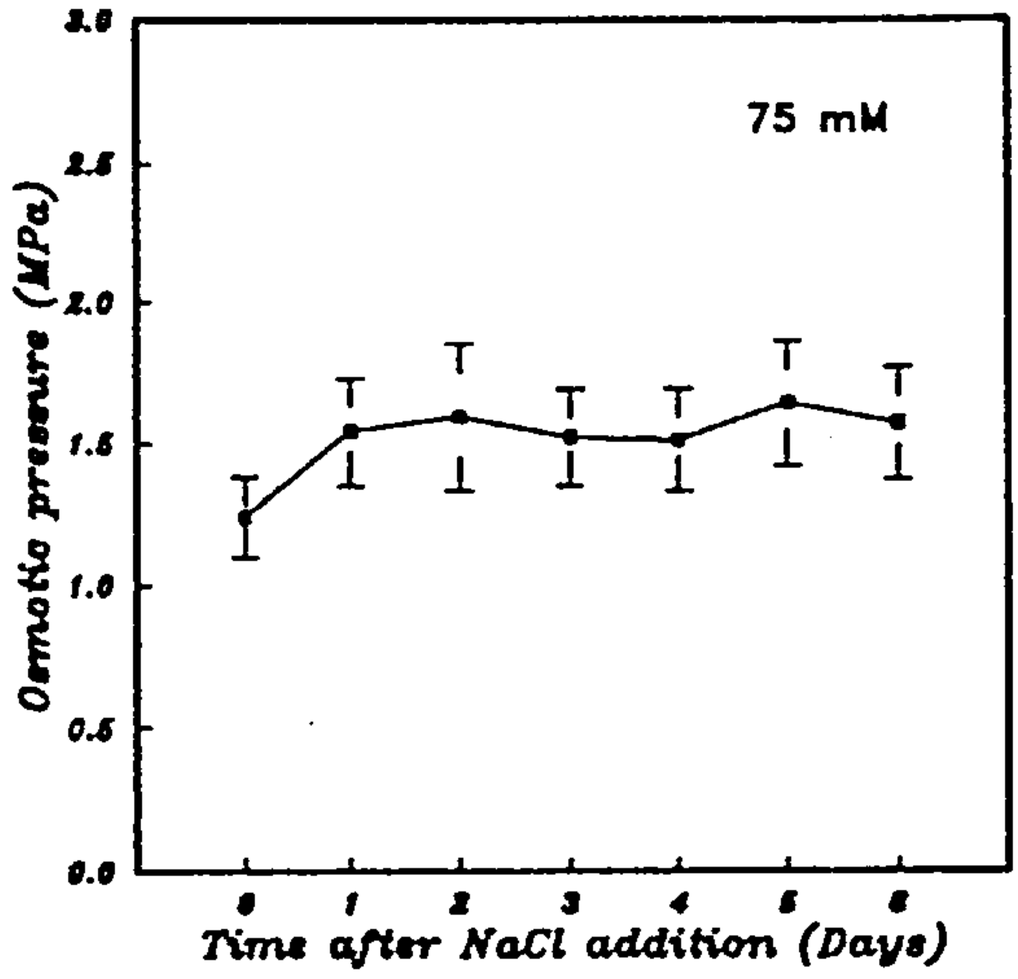

150 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

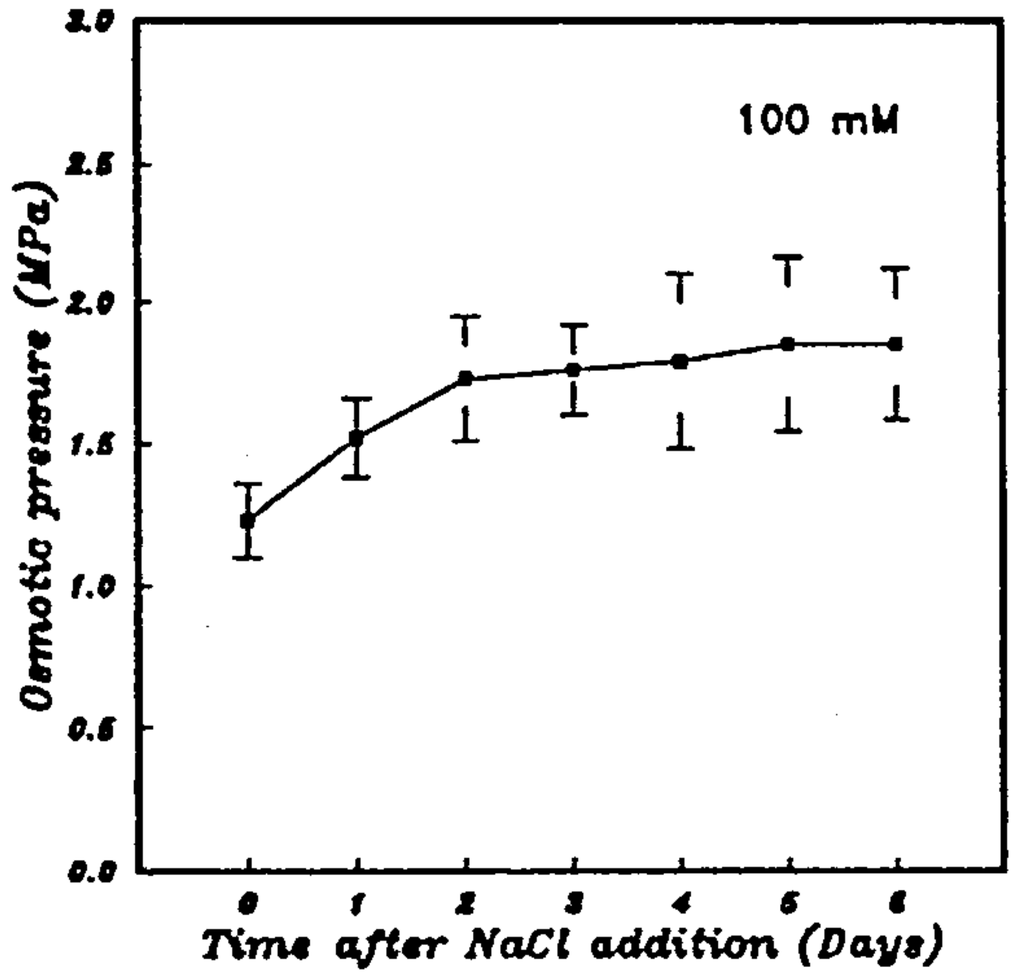
Fig. 5.7 The response of osmotic pressure to the NaCl stress studied in a long term experiment i.e. over 6 days. Osmotic pressure was measured using vapour pressure osmometry on the whole tissue basis. NaCl treatments as in Fig. 3.3. Each point is the mean of 10-25 replicates taken from three experiments.

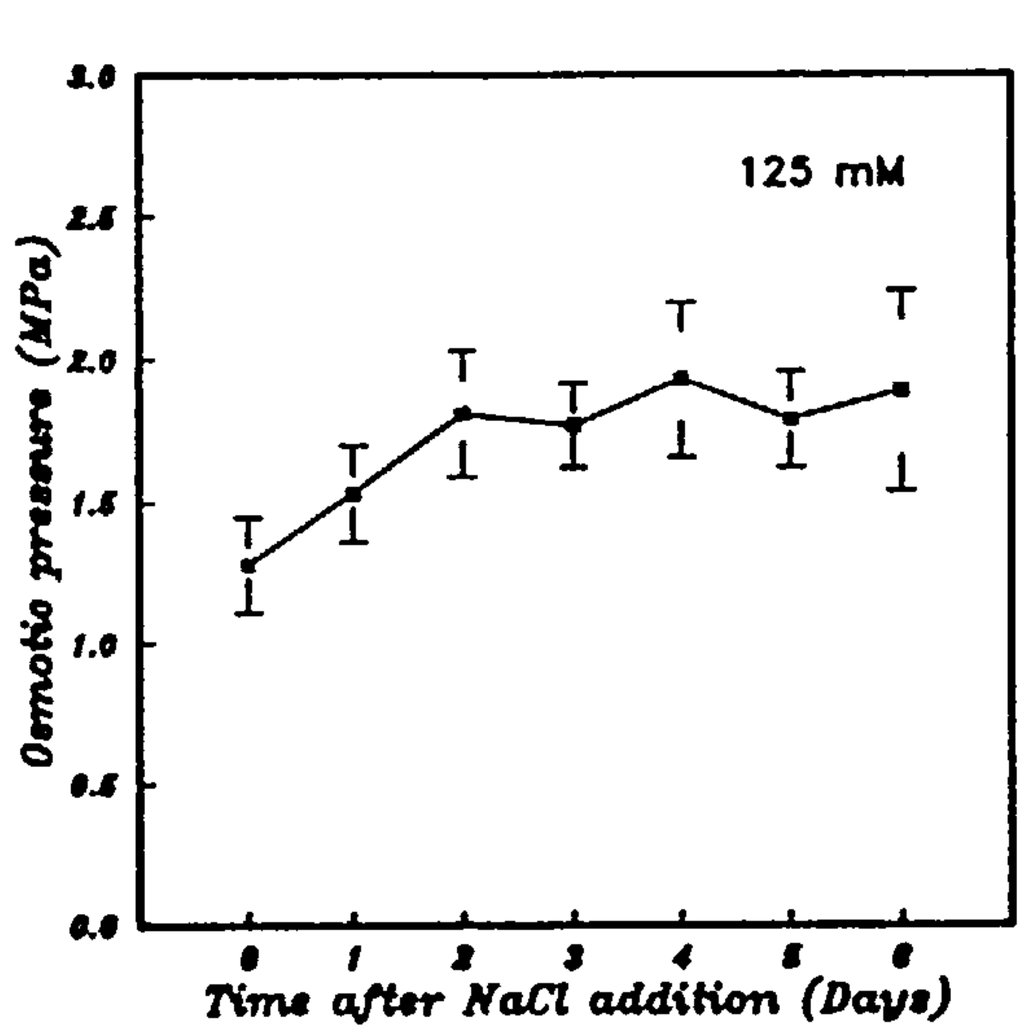

Control
Osmotic pressure (MPa)
Time (Days)


25 mM
Osmotic pressure (MPa)
Time after NaCl addition (Days)


50 mM
Osmotic pressure (MPa)
Time after NaCl addition (Days)


75 mM
Osmotic pressure (MPa)
Time after NaCl addition (Days)


100 mM
Osmotic pressure (MPa)
Time after NaCl addition (Days)


125 mM
Osmotic pressure (MPa)
Time after NaCl addition (Days)

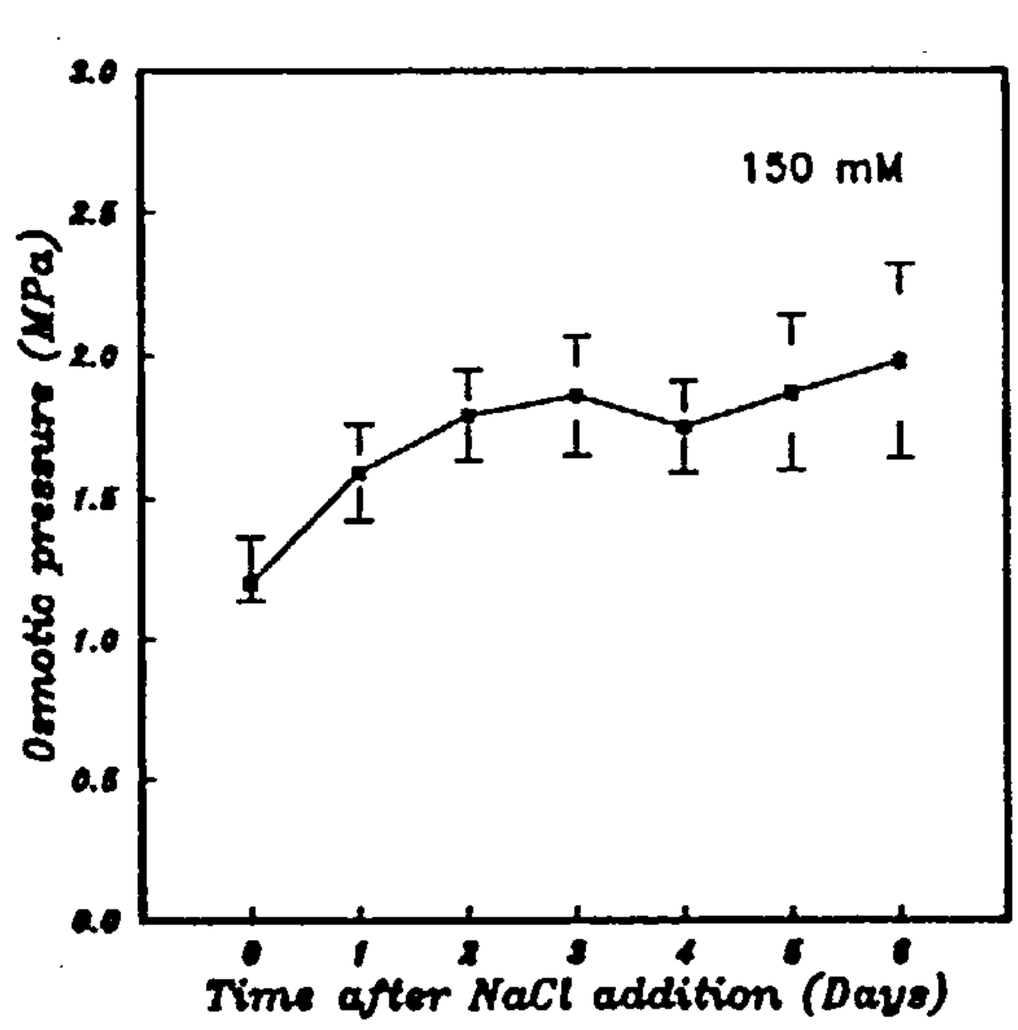
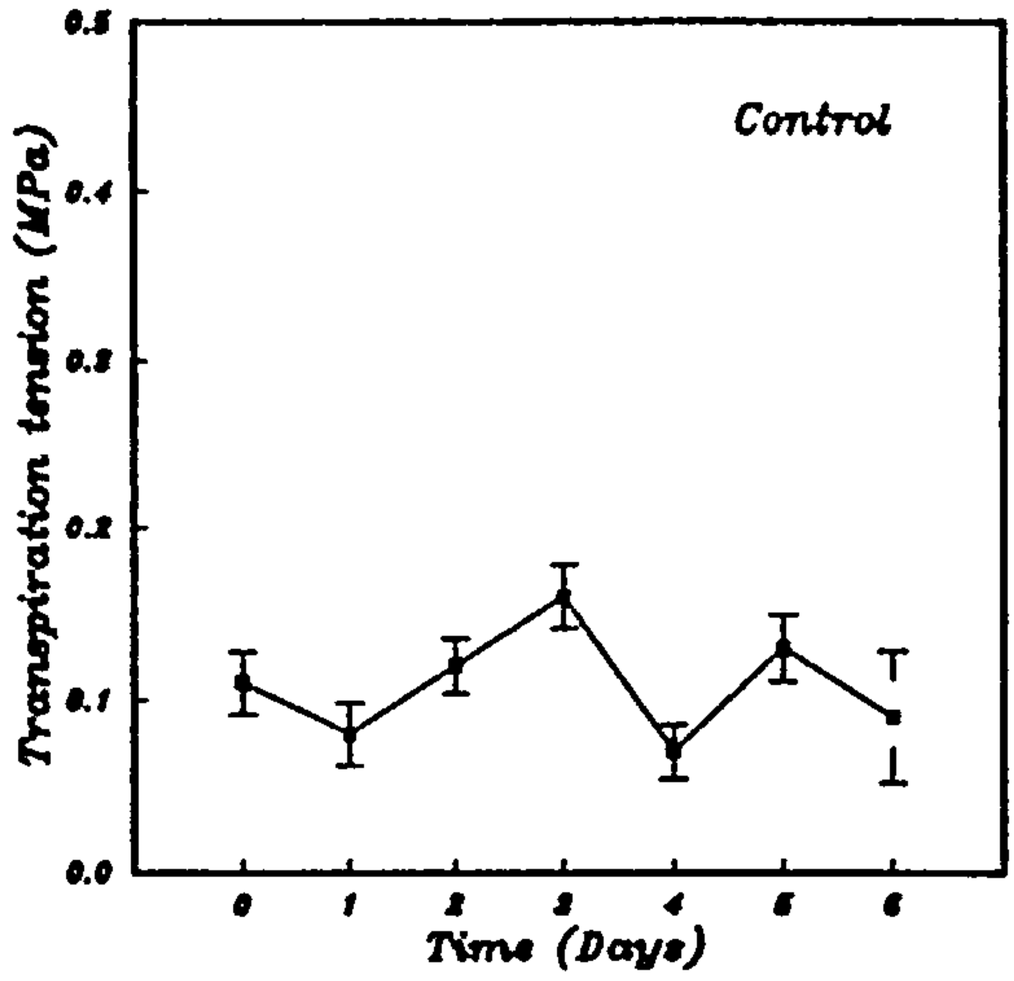
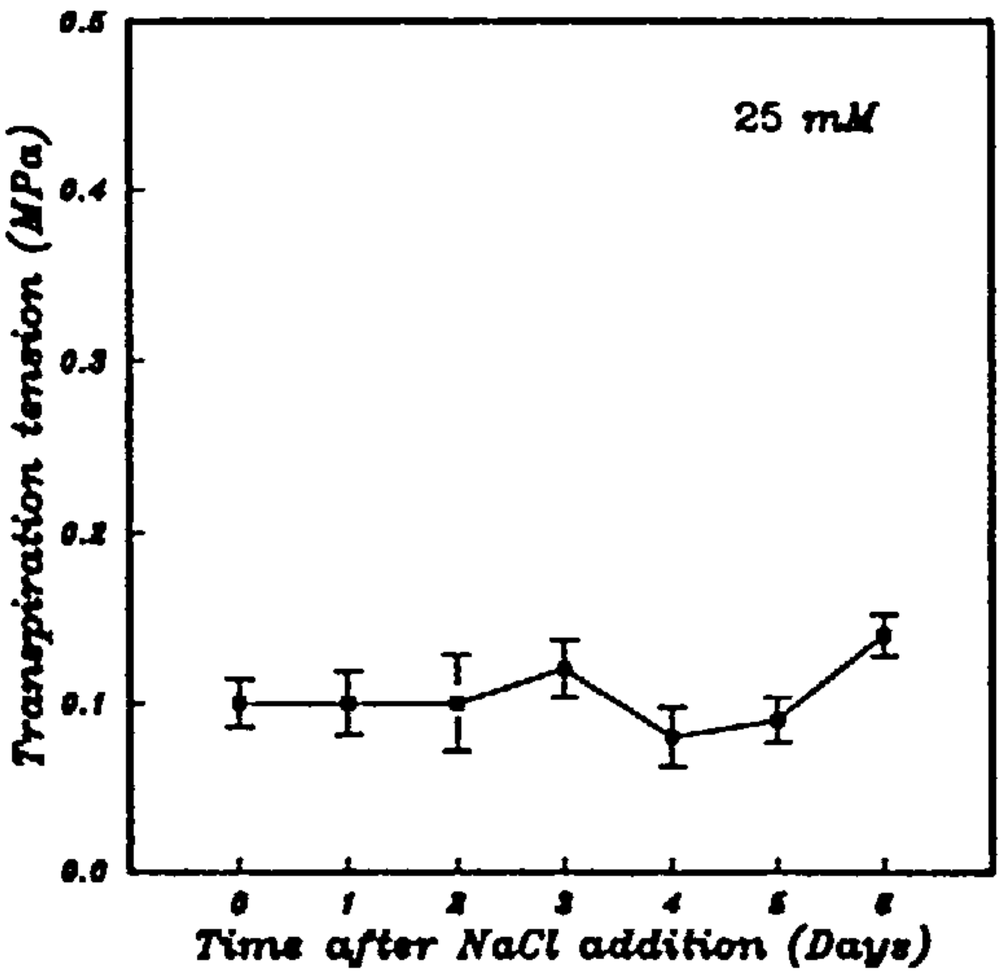
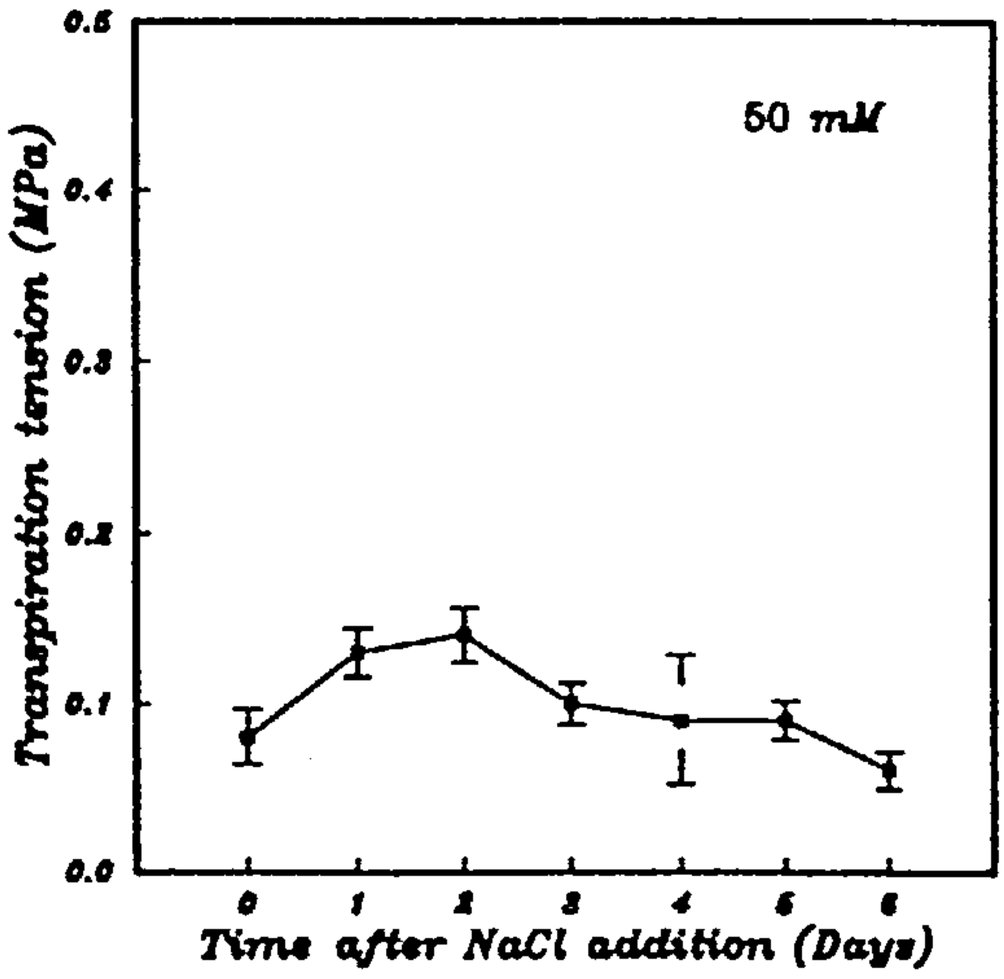
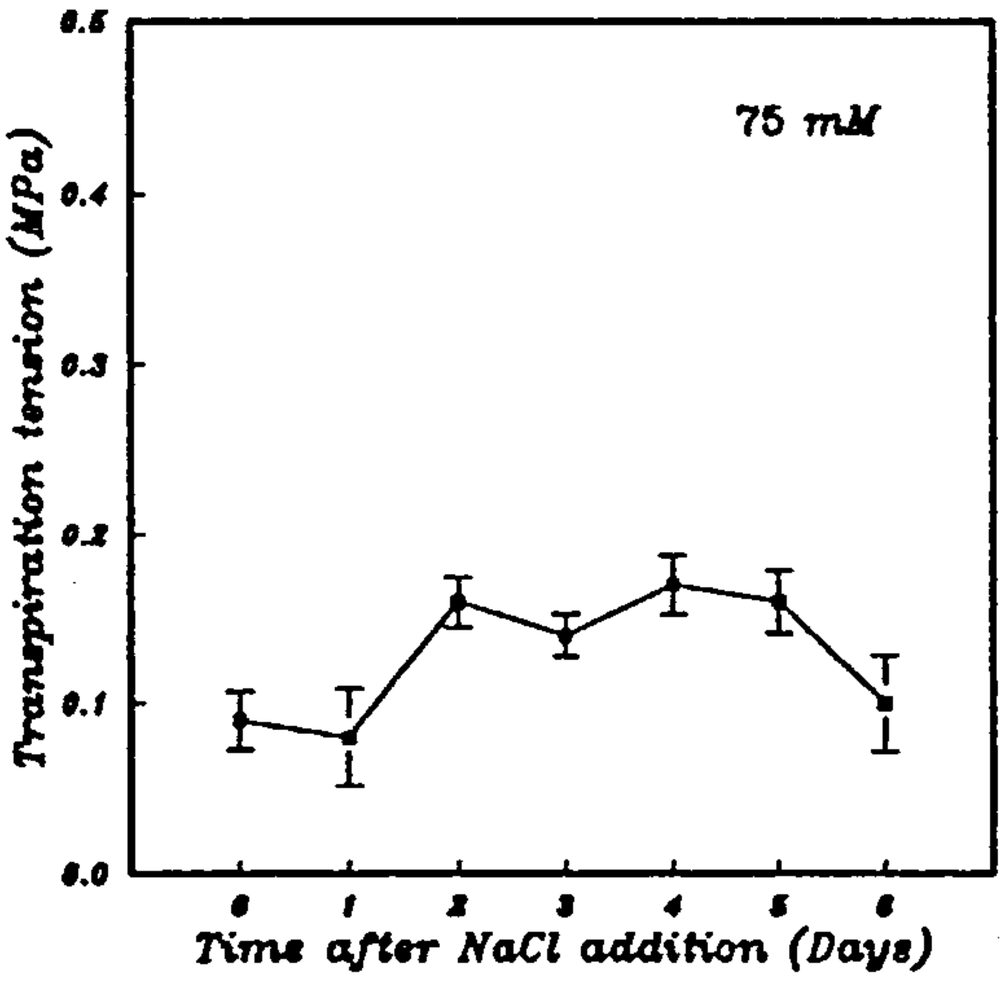

150 mM
Osmotic pressure (MPa)
Time after NaCl addition (Days)

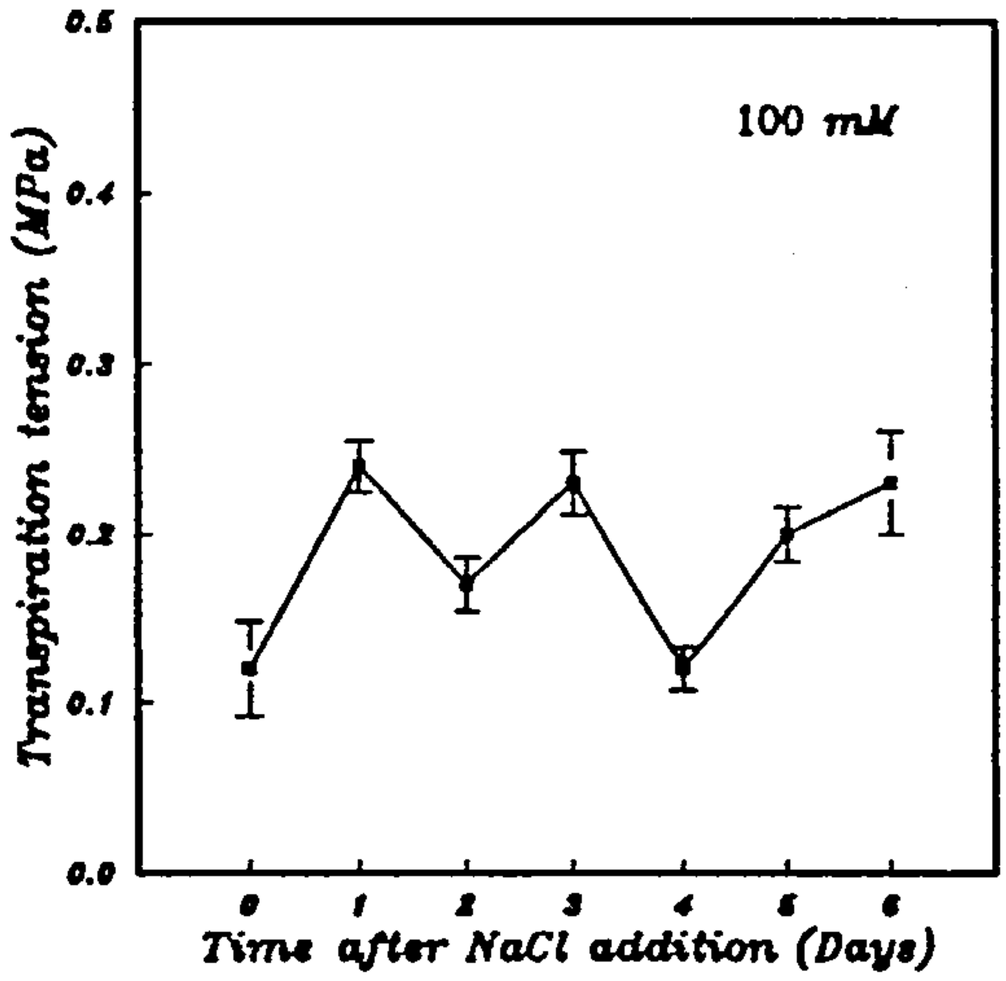
**Fig. 5.8** The estimation of transpiration tension in the cell wall of leaf mature zone. Cell wall transpiration tension was estimated by the difference of turgor pressure of transpiring and the non-transpiring plant leaves. NaCl treatments as in Fig. 3.3. Each point is the mean of 5–8 replicates taken from two experiments.

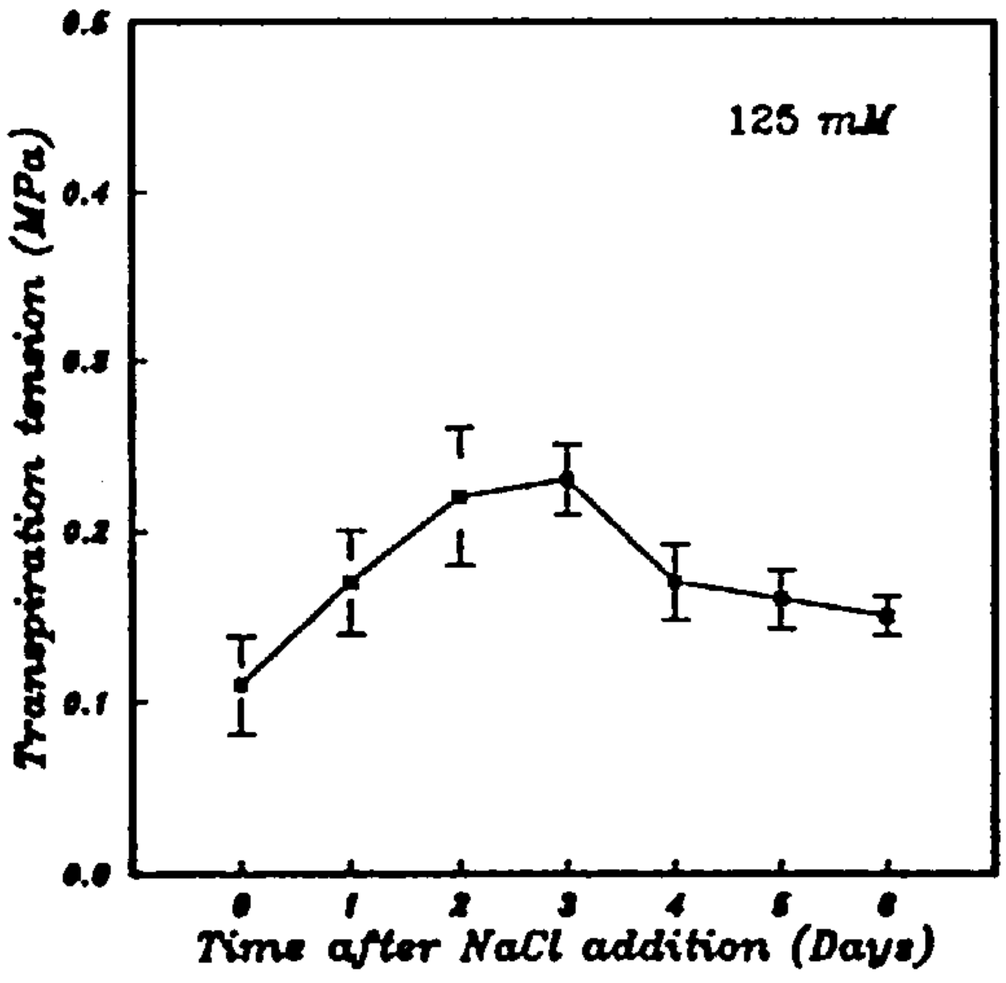

Control
Transpiration tension (MPa)
0.0
0.1
0.2
0.4
0.5
0 1 2 3 4 5 6
Time (Days)


25 mM
Transpiration tension (MPa)
0.0
0.1
0.2
0.3
0.4
0.5
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Transpiration tension (MPa)
0.0
0.1
0.2
0.3
0.4
0.5
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Transpiration tension (MPa)
0.0
0.1
0.2
0.3
0.4
0.5
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Transpiration tension (MPa)
0.0
0.1
0.2
0.3
0.4
0.5
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Transpiration tension (MPa)
0.0
0.1
0.2
0.3
0.4
0.5
0 1 2 3 4 5 6
Time after NaCl addition (Days)

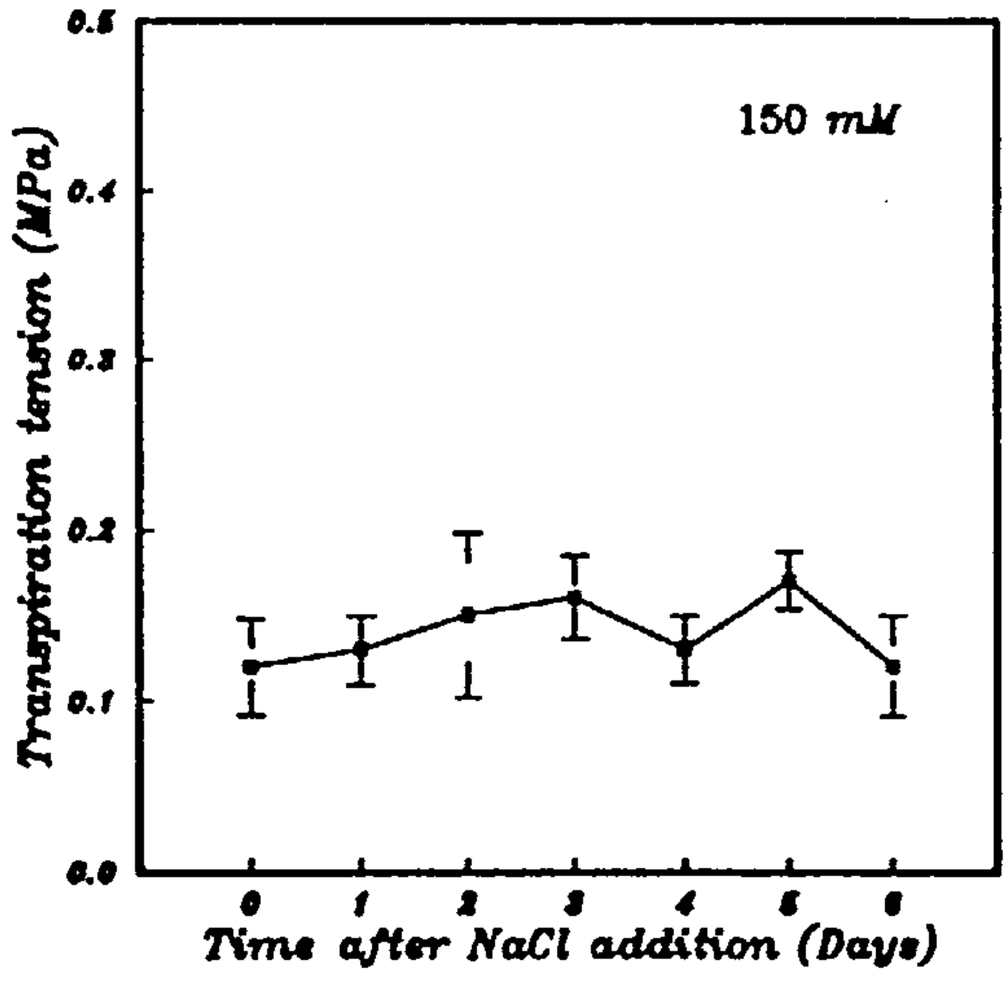

150 mM
Transpiration tension (MPa)
0.0
0.1
0.2
0.3
0.4
0.5
0 1 2 3 4 5 6
Time after NaCl addition (Days)

**Fig. 5.9** The estimated concentration of osmotically active solutes present in the cell wall of leaf mature zone measured after 6 days of the stress application. The cell wall solutes were estimated by the difference of cell turgor pressure, tissue osmotic pressure and the cell wall transpiration tension. It corresponds to the amount of all the taken up solutes in the cell wall. (Concentrations expressed as osmotic pressure).

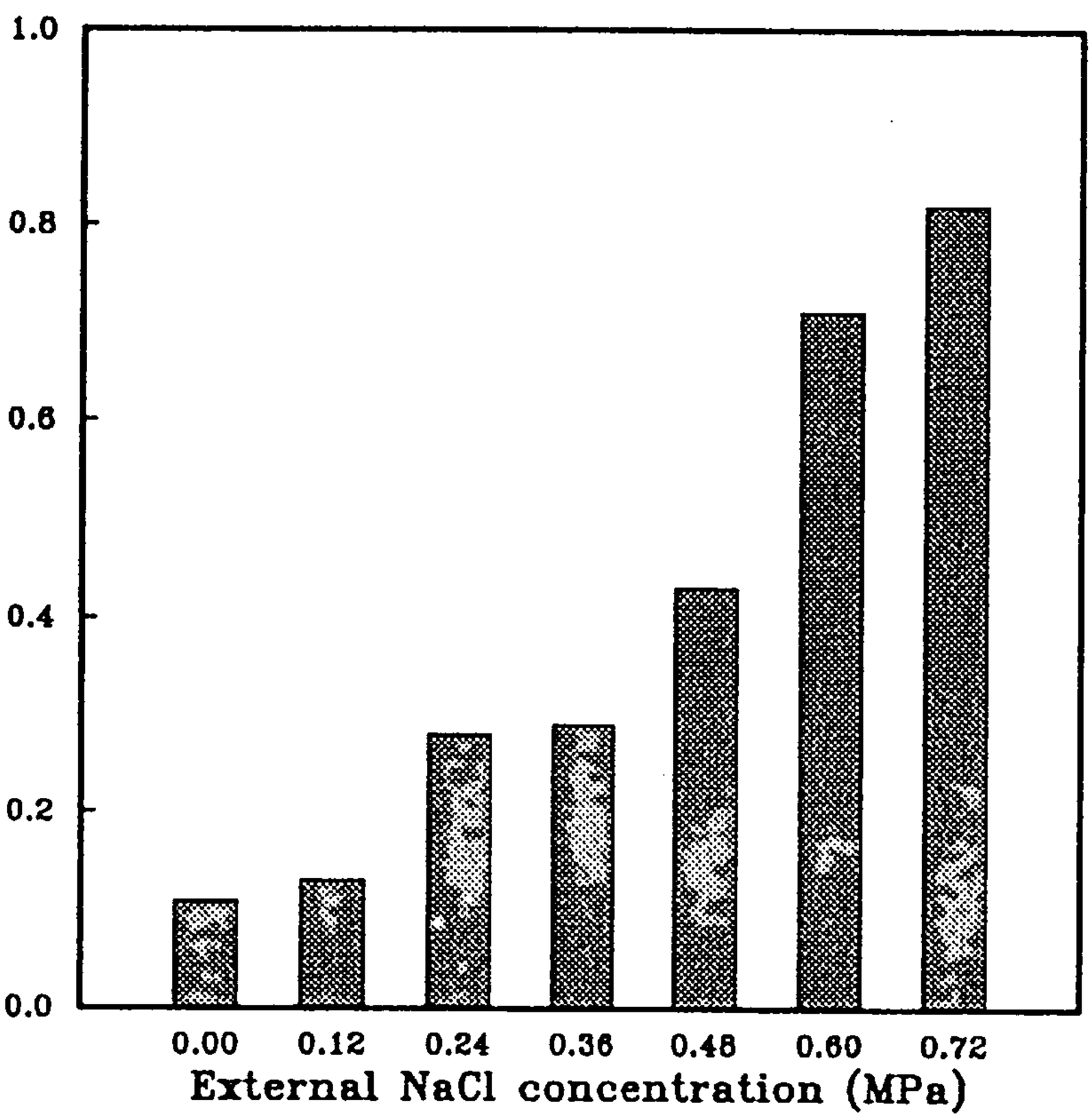
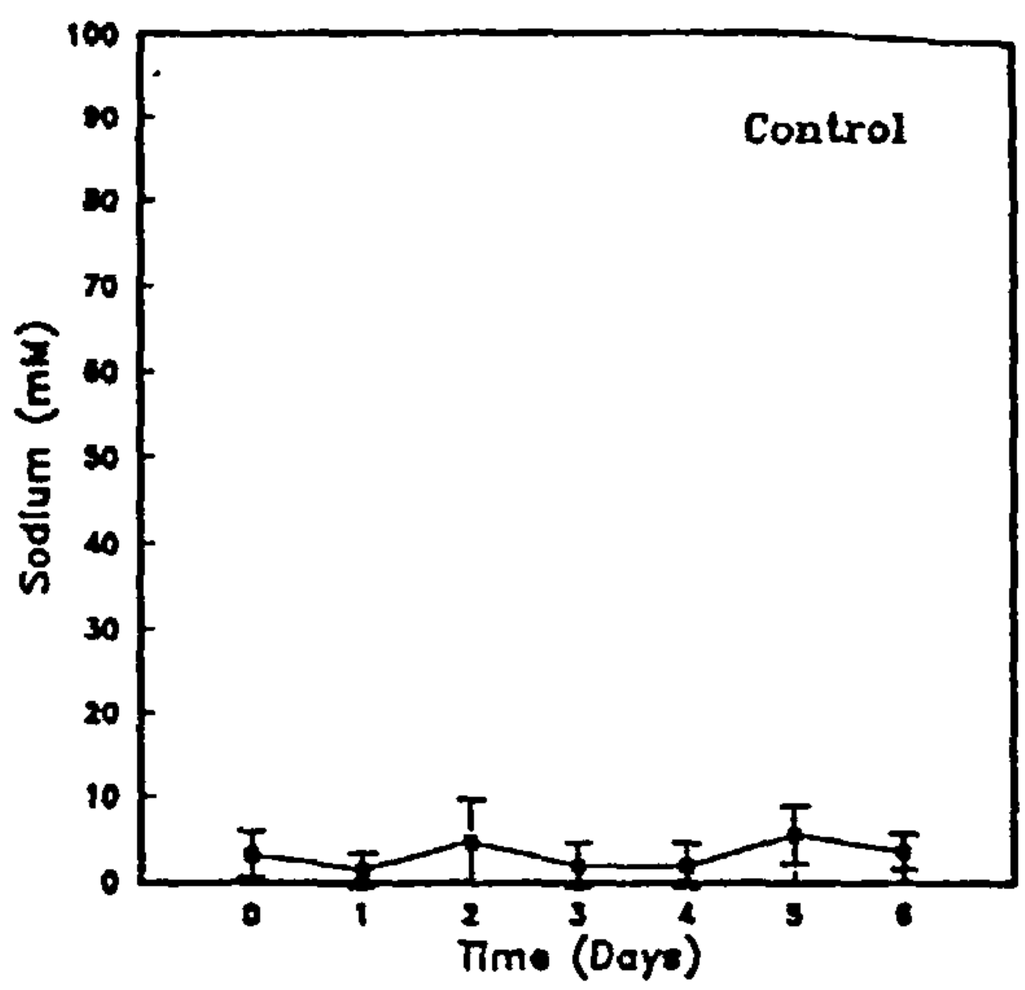
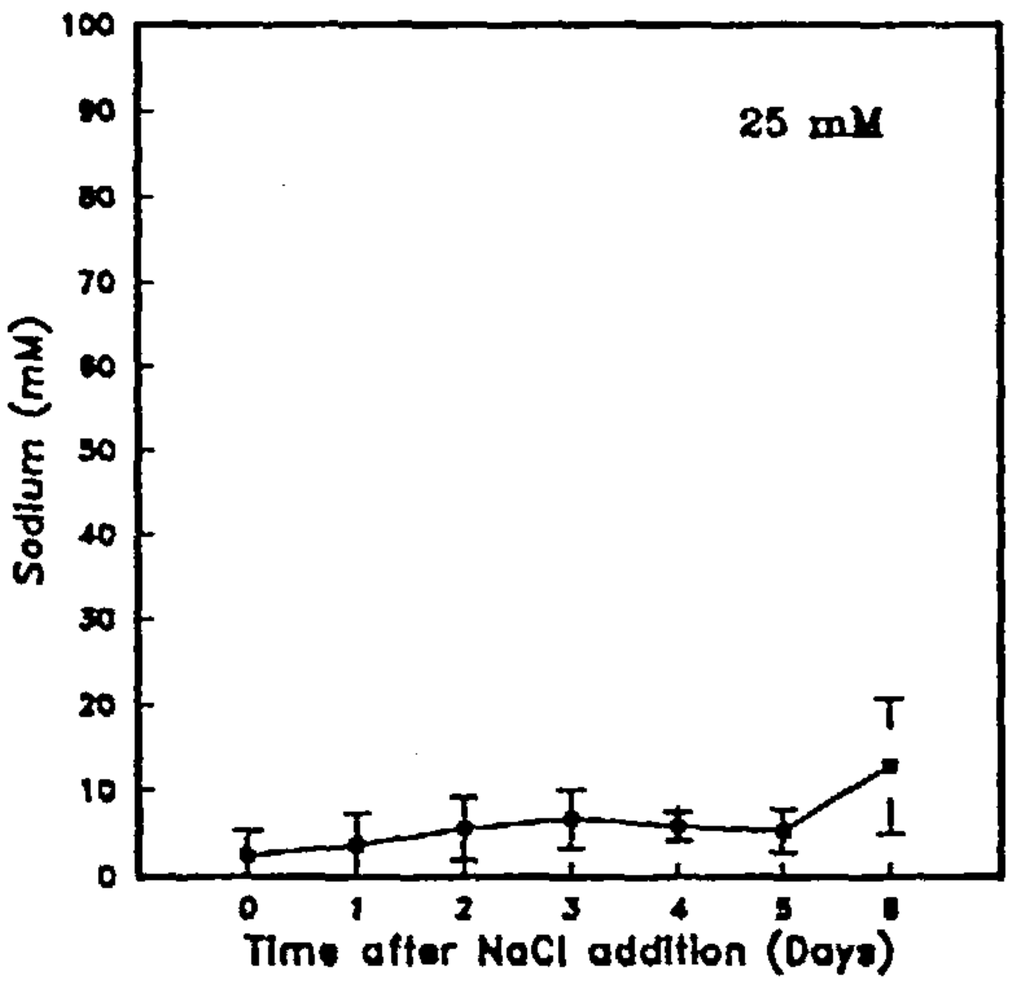
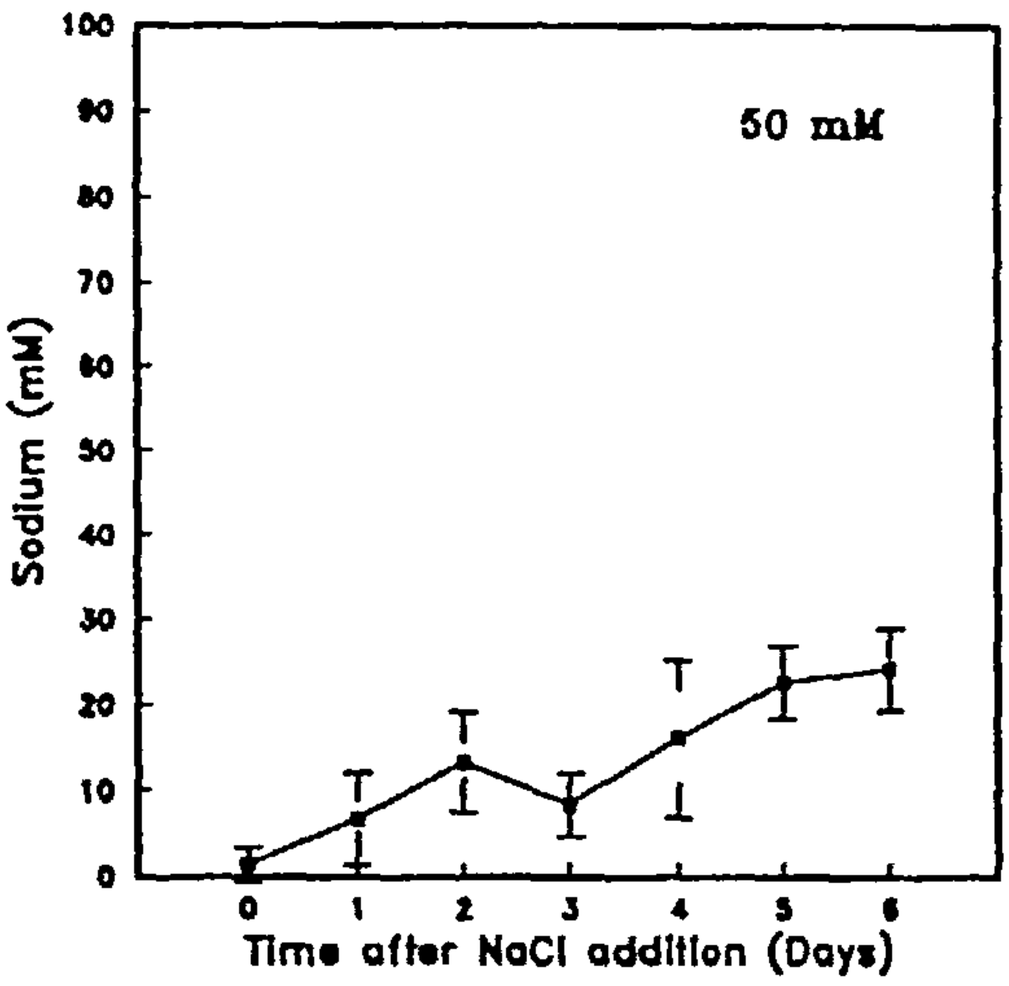
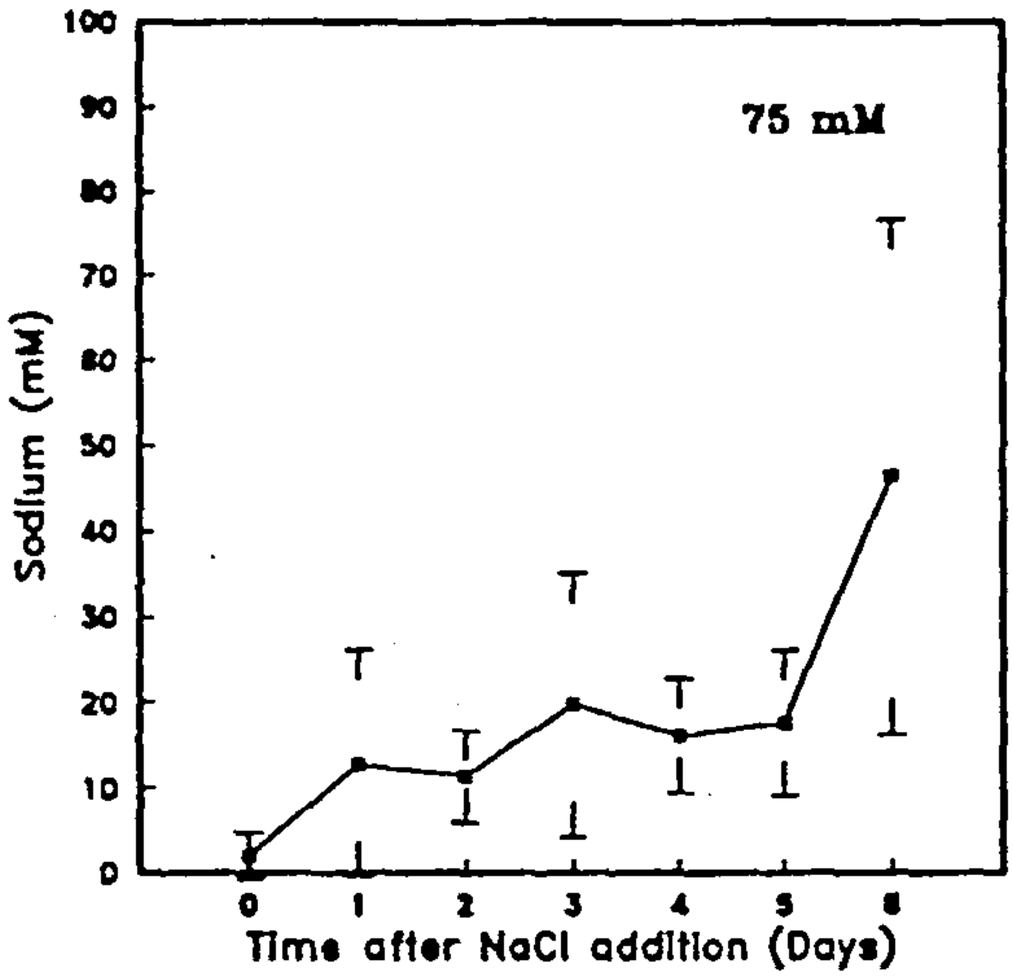

1.0
0.8
0.6
0.4
0.2
0.0
Cell wall solutes (MPa)
0.00
0.12
0.24
0.36
0.48
0.60
0.72
External NaCl concentration (MPa)

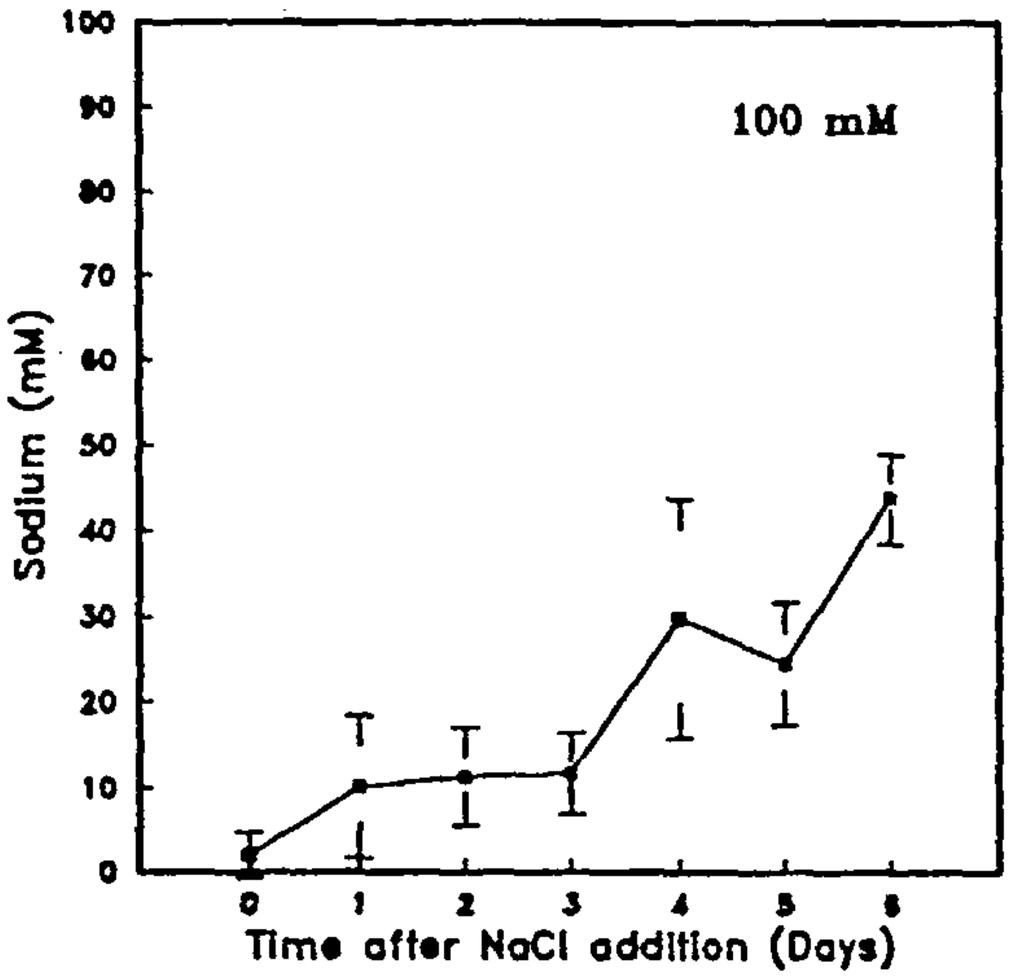
Fig. 5.10 The concentration of sodium ion in the expressed cell sap taken from leaf mature zone following NaCl treatment, studied in a long term experiment i.e. for 6 days. NaCl treatment as in Fig. 3.3. Sodium content was measured using flame photometry. Each point is the mean of three replicates.

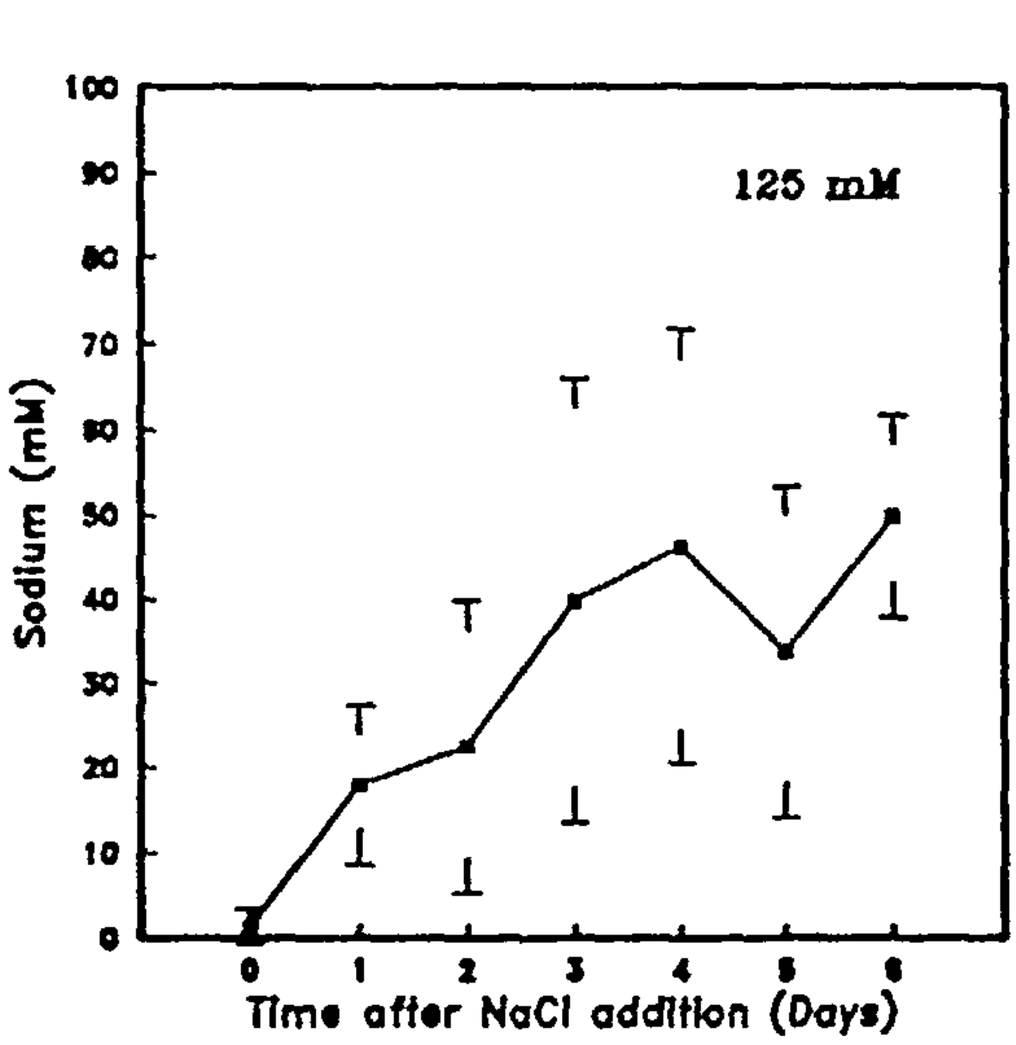

Control
Sodium (mM)
Time (Days)


25 mM
Sodium (mM)
Time after NaCl addition (Days)


50 mM
Sodium (mM)
Time after NaCl addition (Days)


75 mM
Sodium (mM)
Time after NaCl addition (Days)


100 mM
Sodium (mM)
Time after NaCl addition (Days)


125 mM
Sodium (mM)
Time after NaCl addition (Days)

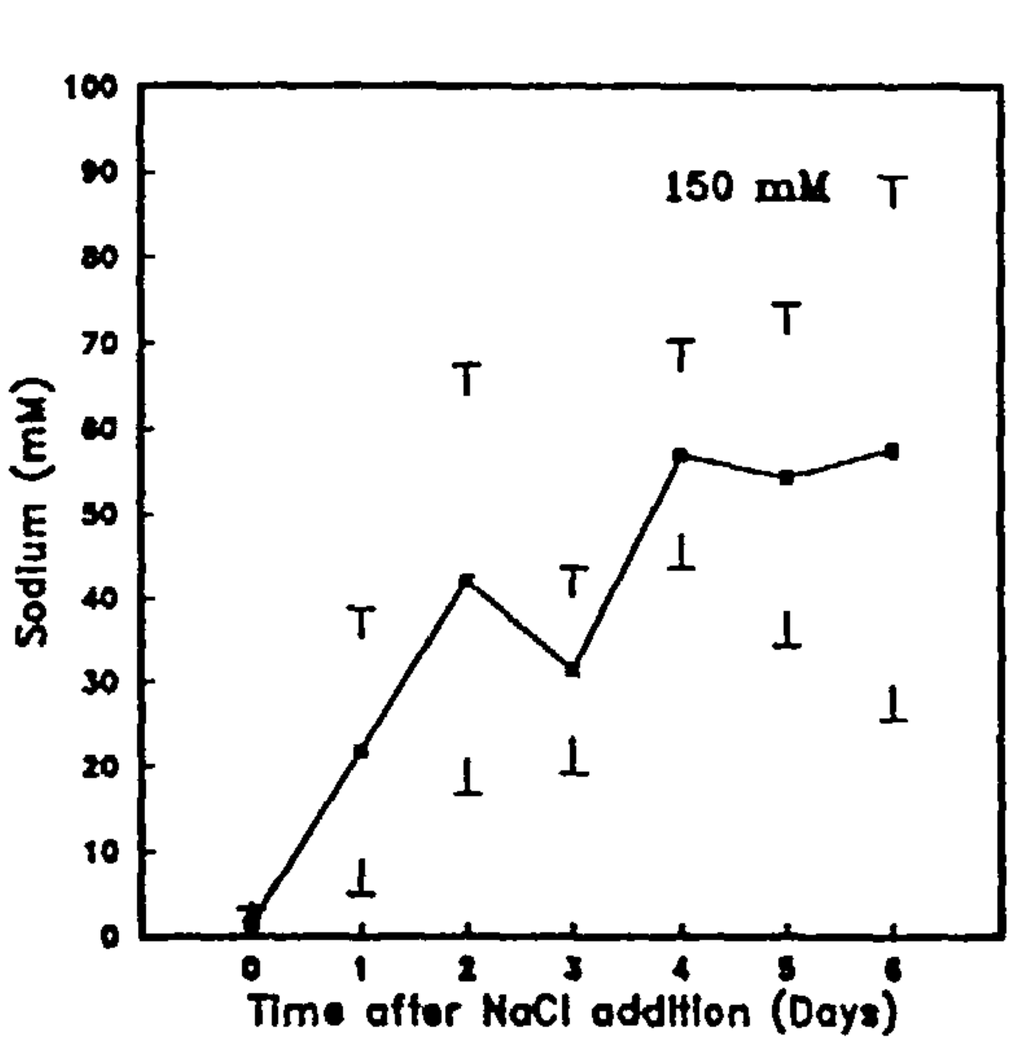
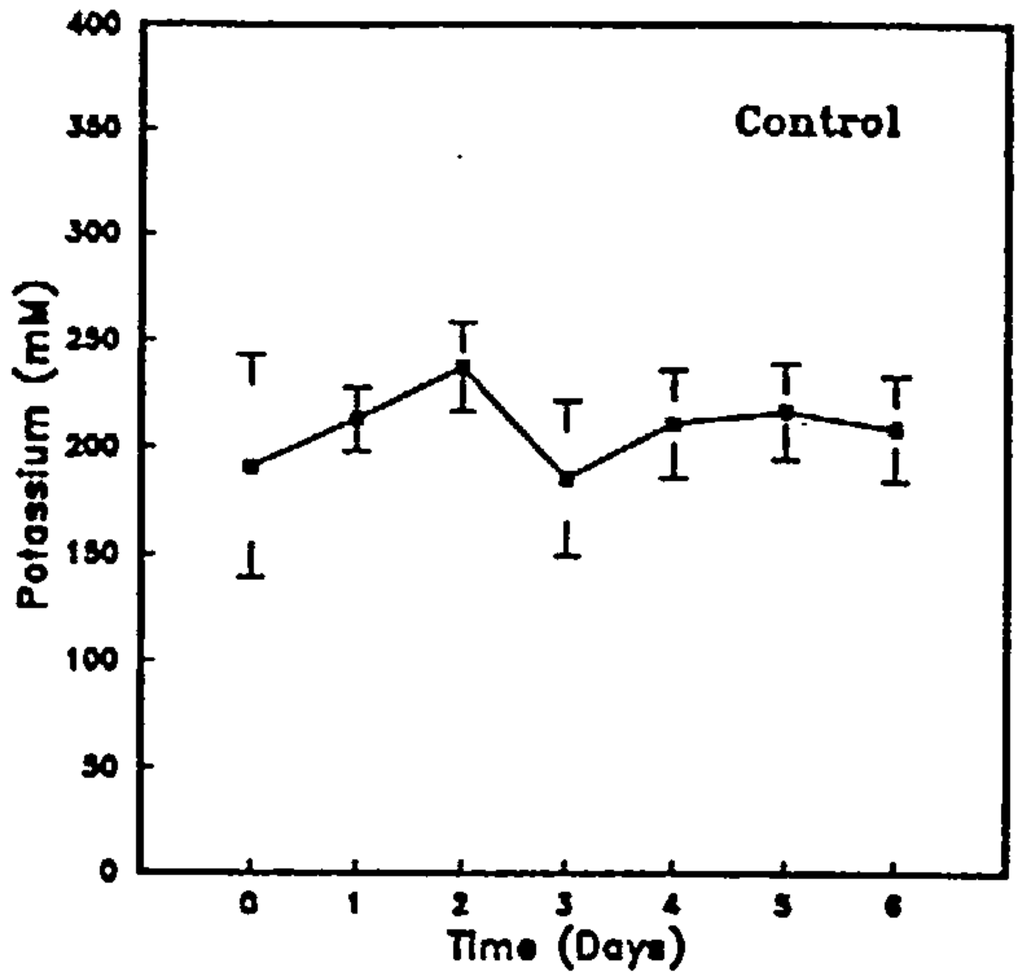
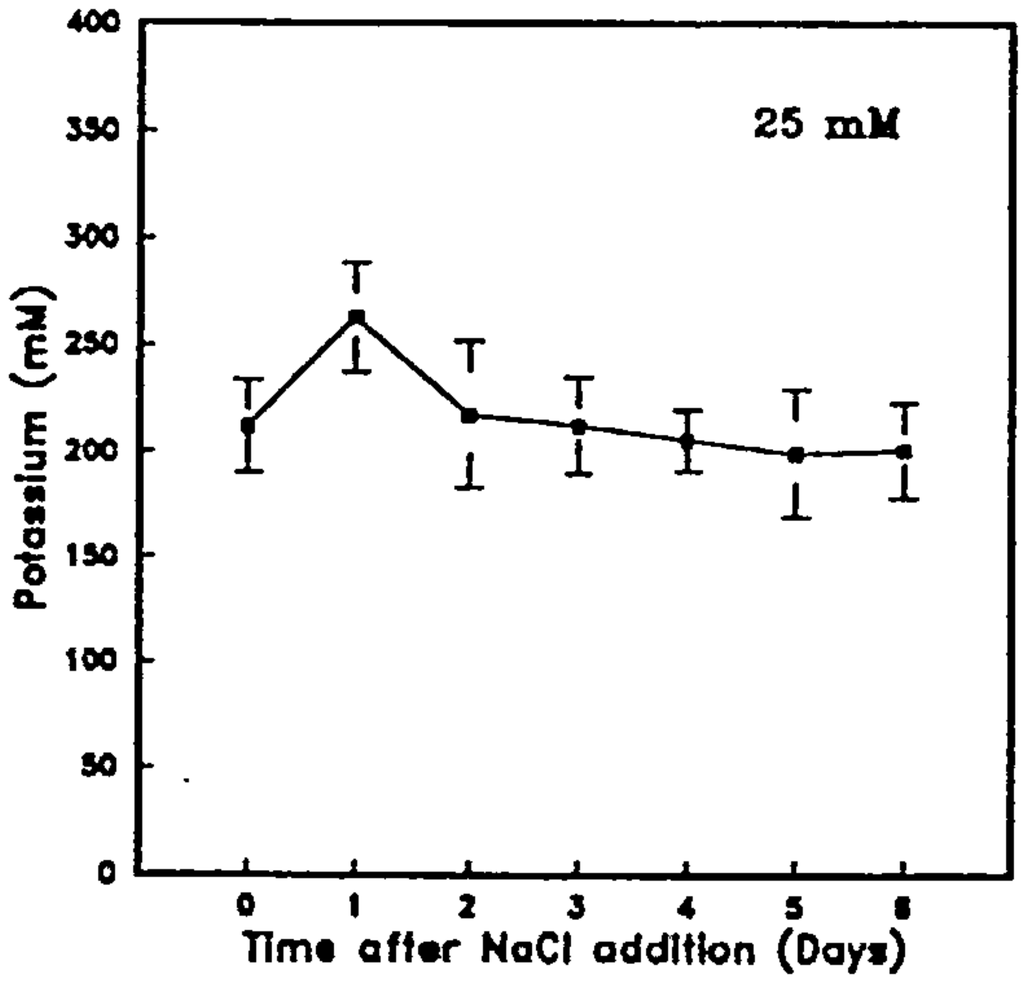
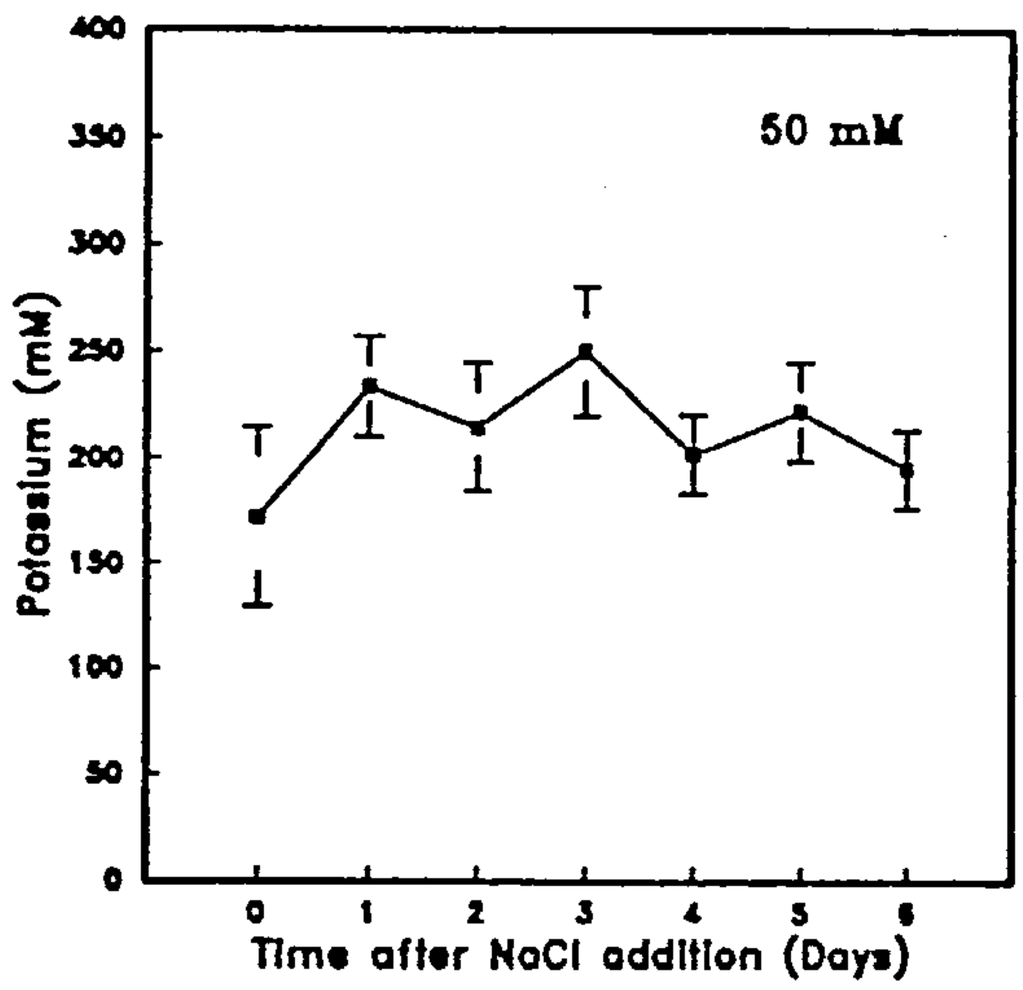
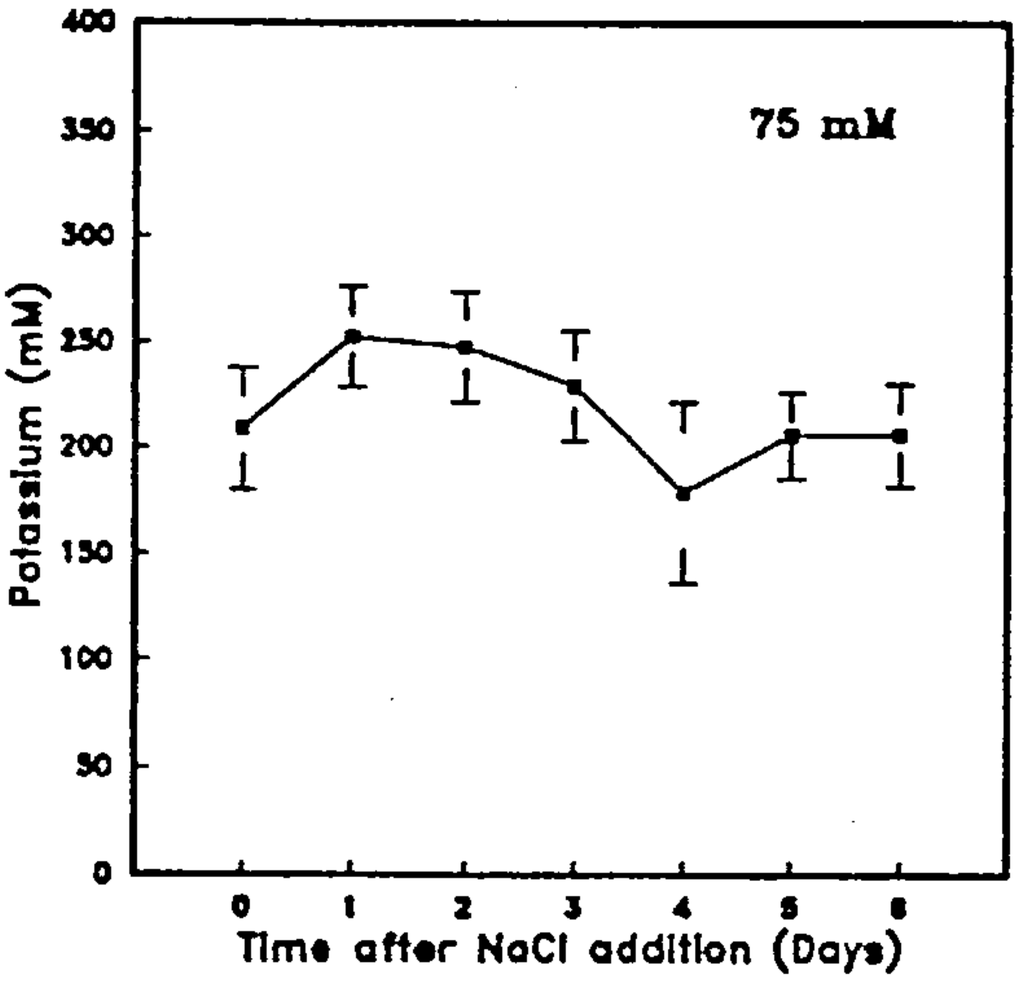

150 mM
Sodium (mM)
Time after NaCl addition (Days)

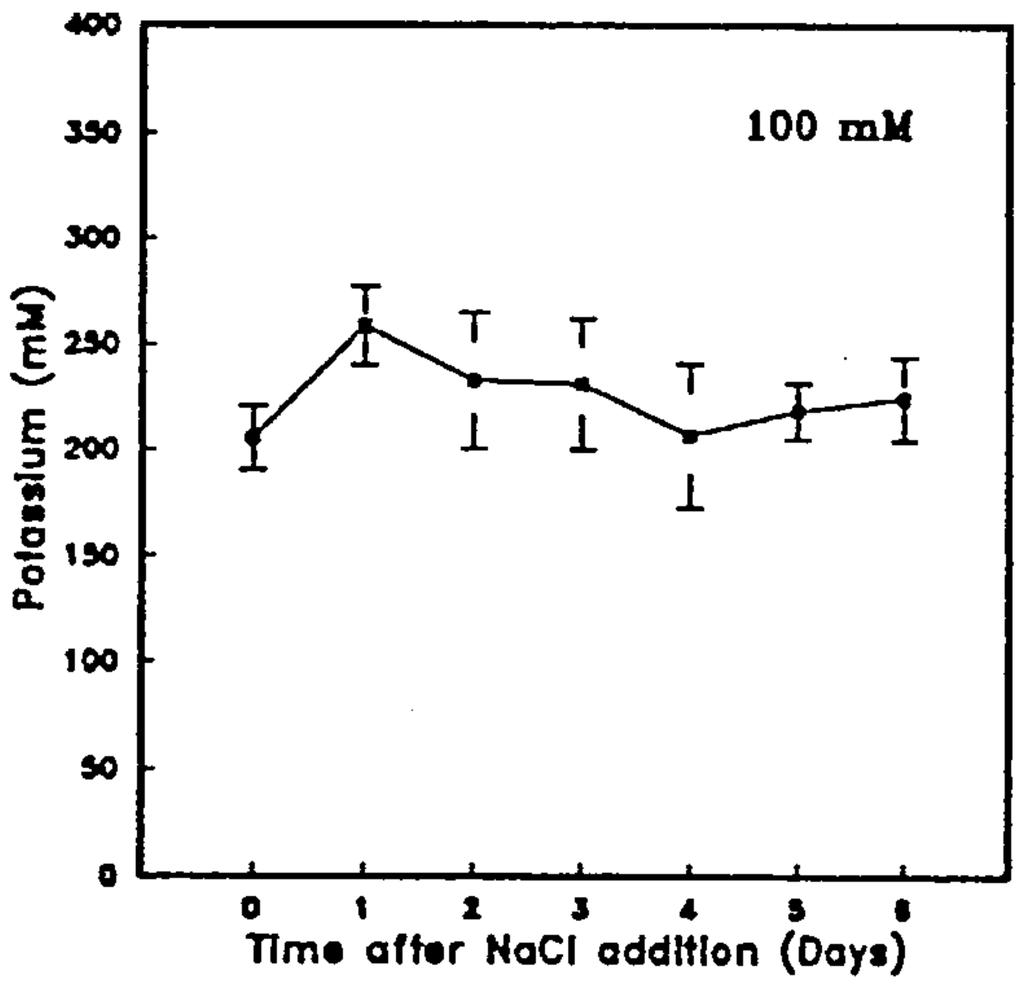
**Fig. 5.11** The concentration of potassium ion in the expressed cell sap taken from leaf mature zone following NaCl treatment, studied in a long term experiment i.e. for 6 days. Parallel experiment to Fig. 5.10 ($Na^+$).

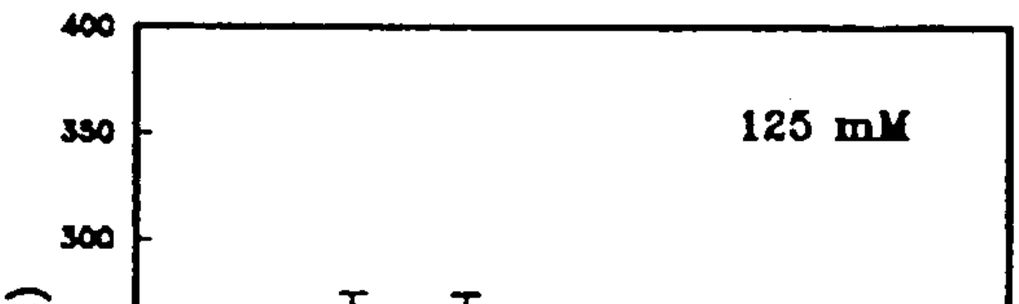

Control
Potassium (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time (Days)


25 mM
Potassium (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Potassium (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

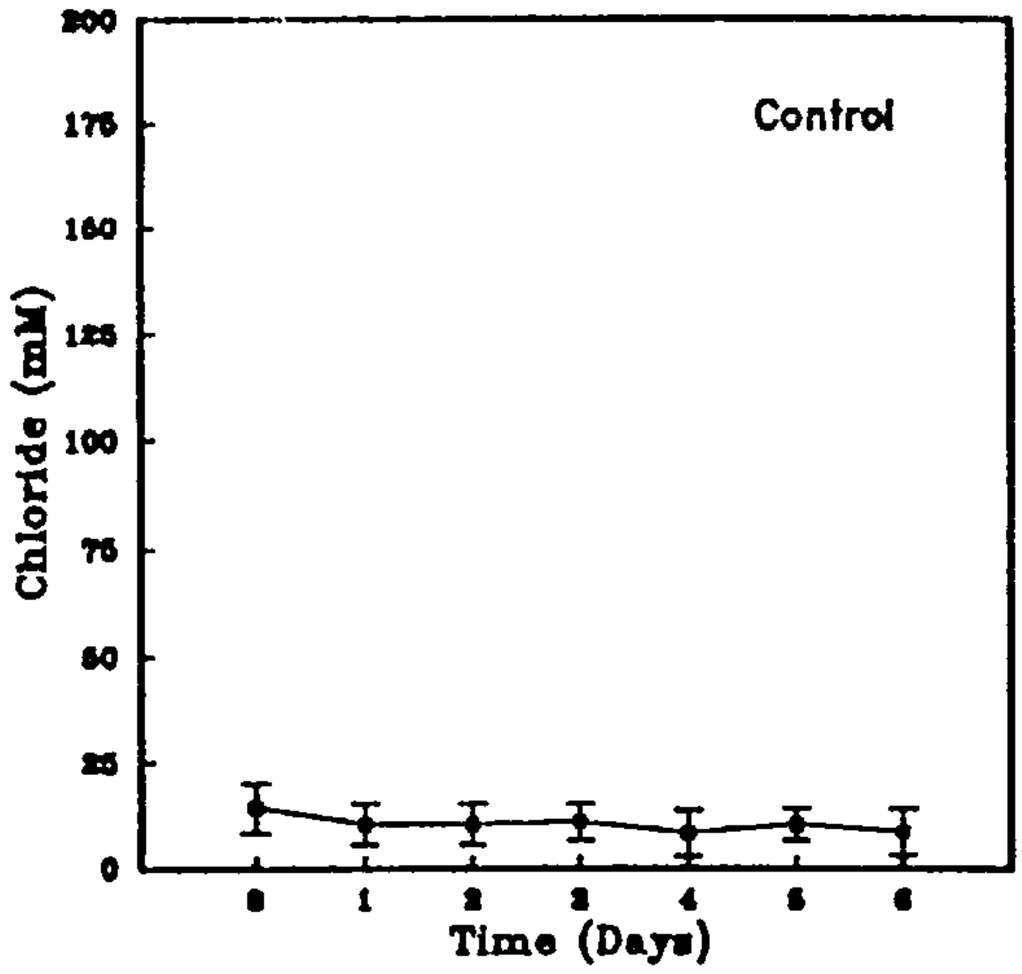

75 mM
Potassium (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

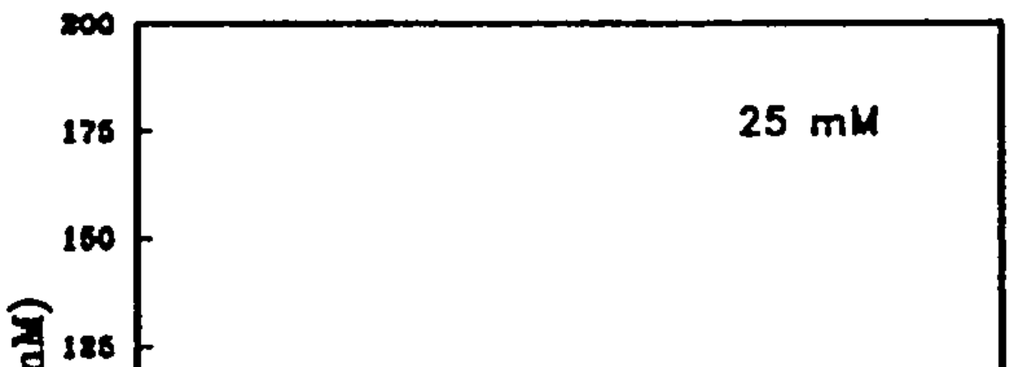

100 mM
Potassium (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Potassium (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

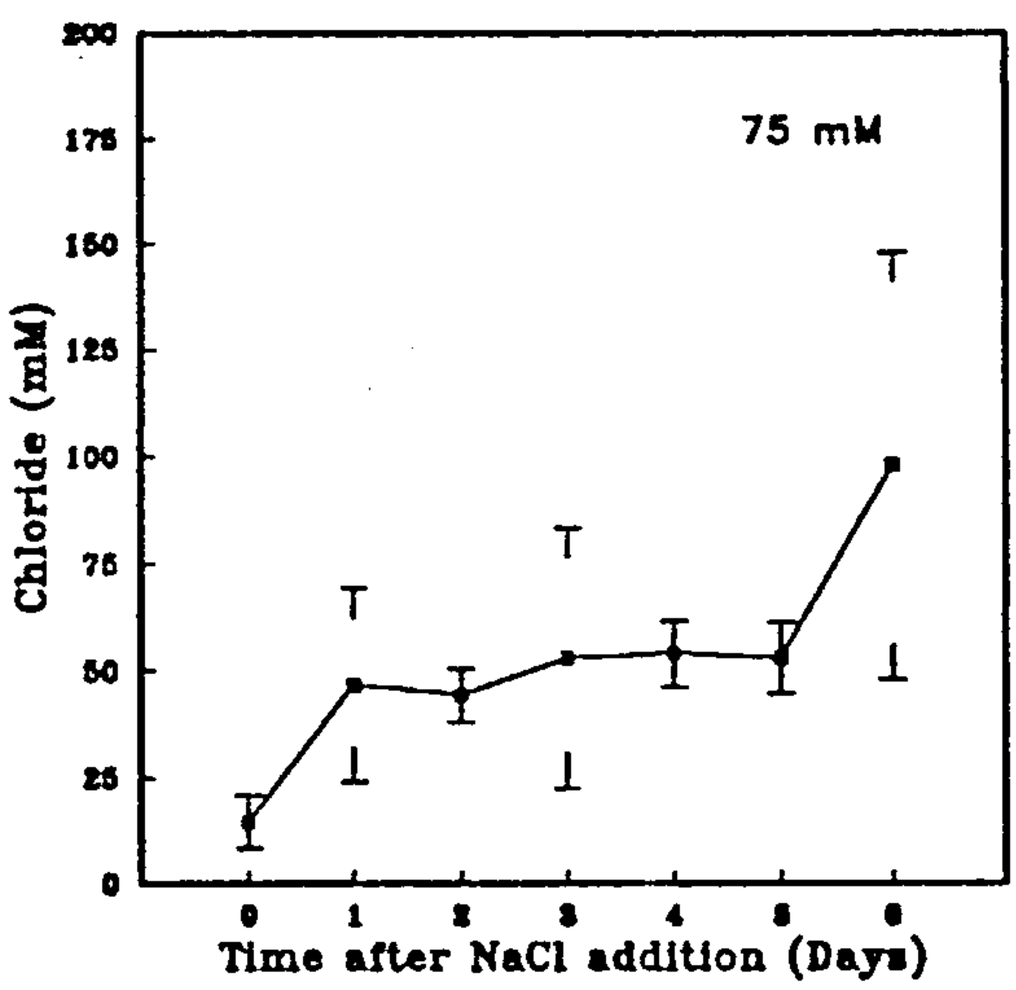

150 mM
Potassium (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

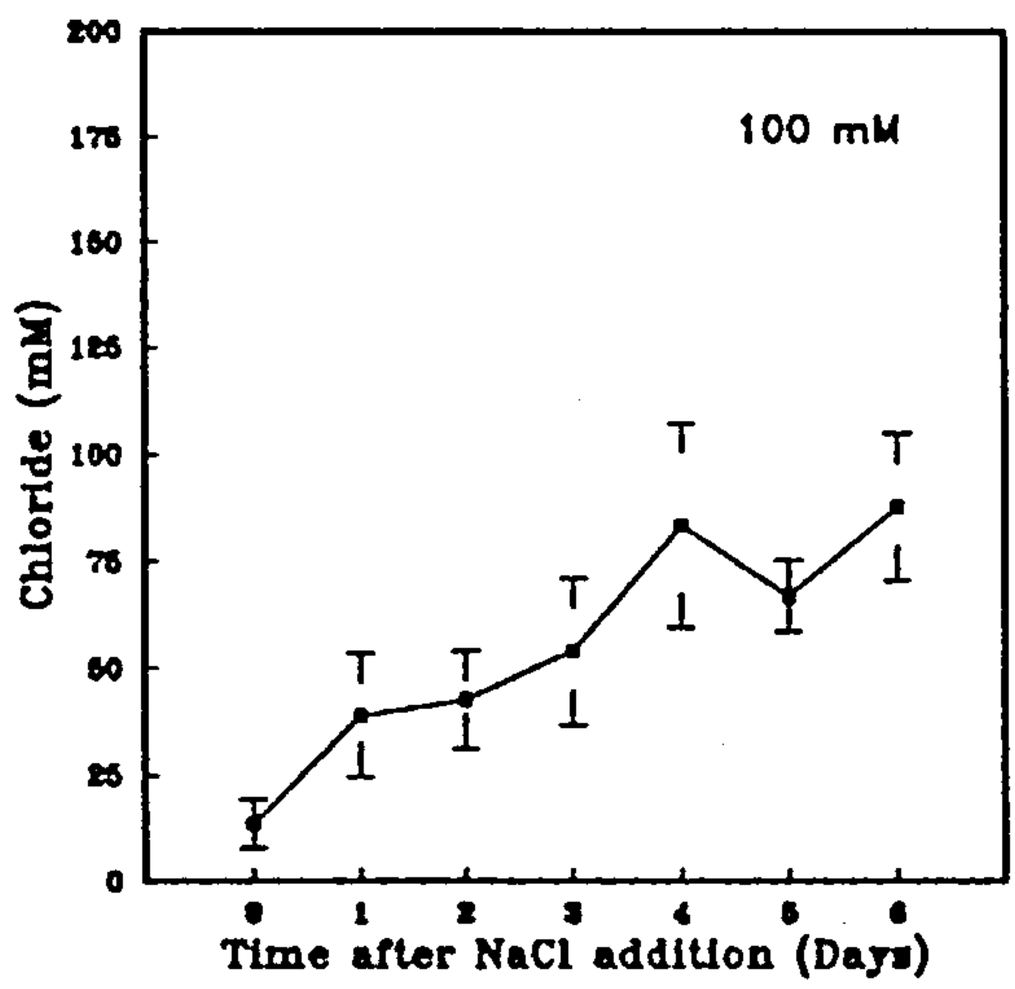
**Fig. 5.12** The concentration of chloride ion in the expressed cell sap taken from leaf mature zone after NaCl treatment, studied in a long term experiment i.e. for 6 days. Parallel experiment to Fig. 5.10 (for $Na^+$). Chloride content was measured using HPLC.

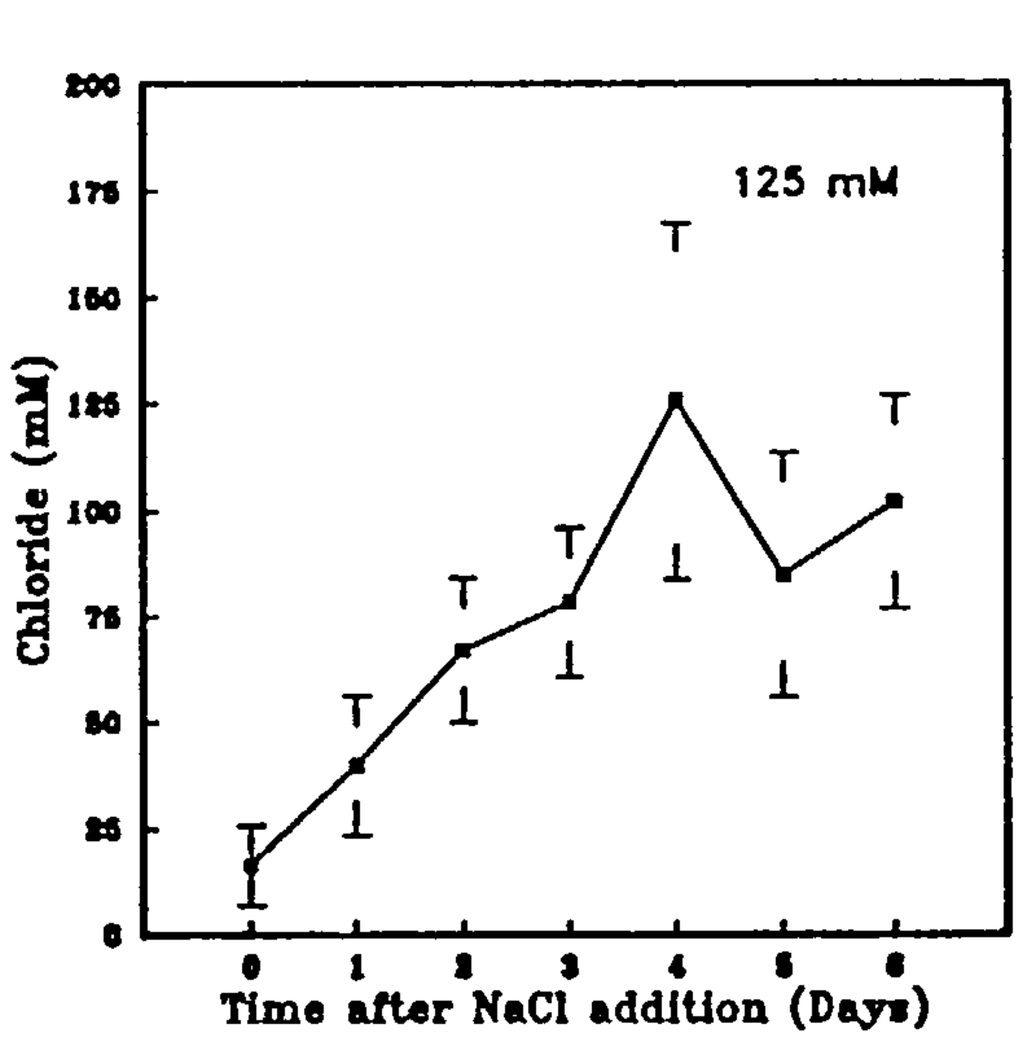

Control
Chloride (mM)
Time (Days)


25 mM
Chloride (mM)
Time after NaCl addition (Days)


50 mM
Chloride (mM)
Time after NaCl addition (Days)


75 mM
Chloride (mM)
Time after NaCl addition (Days)


100 mM
Chloride (mM)
Time after NaCl addition (Days)


125 mM
Chloride (mM)
Time after NaCl addition (Days)

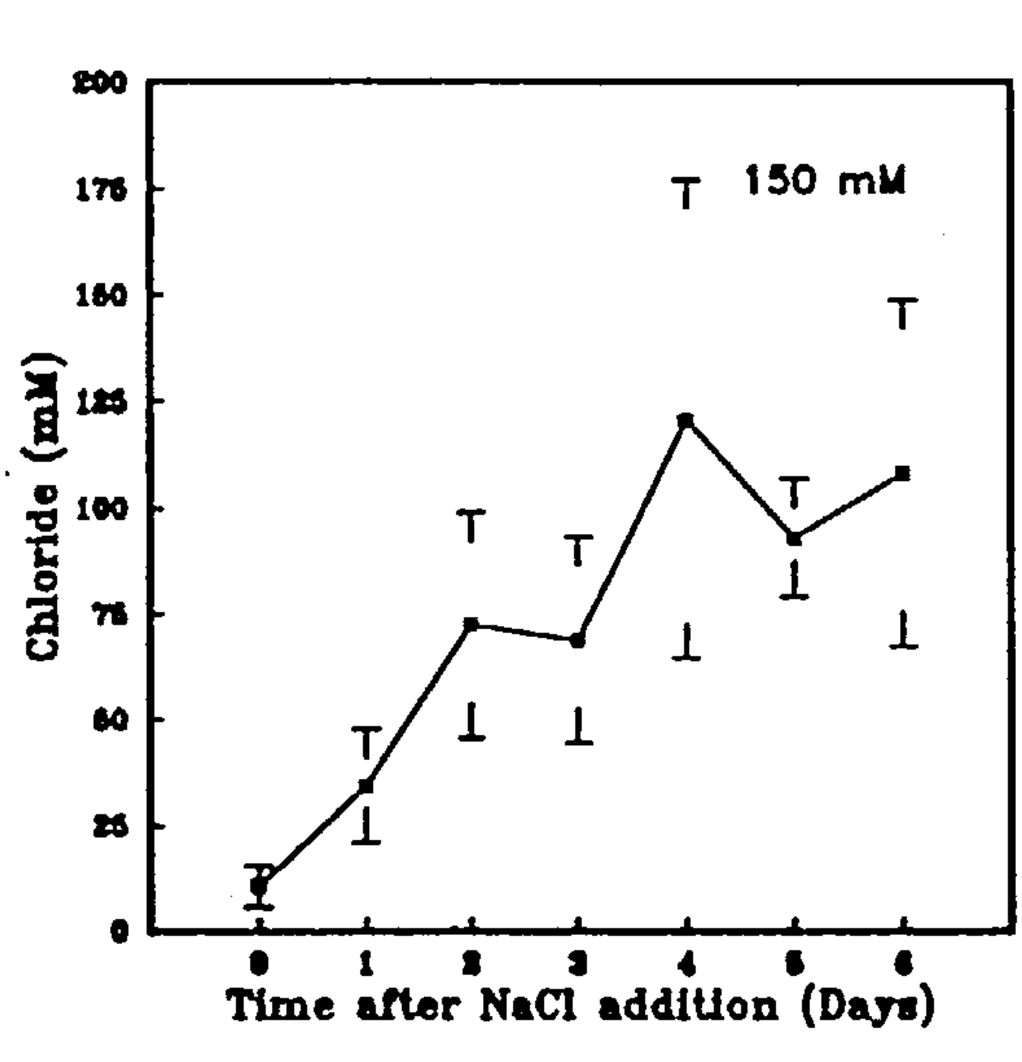
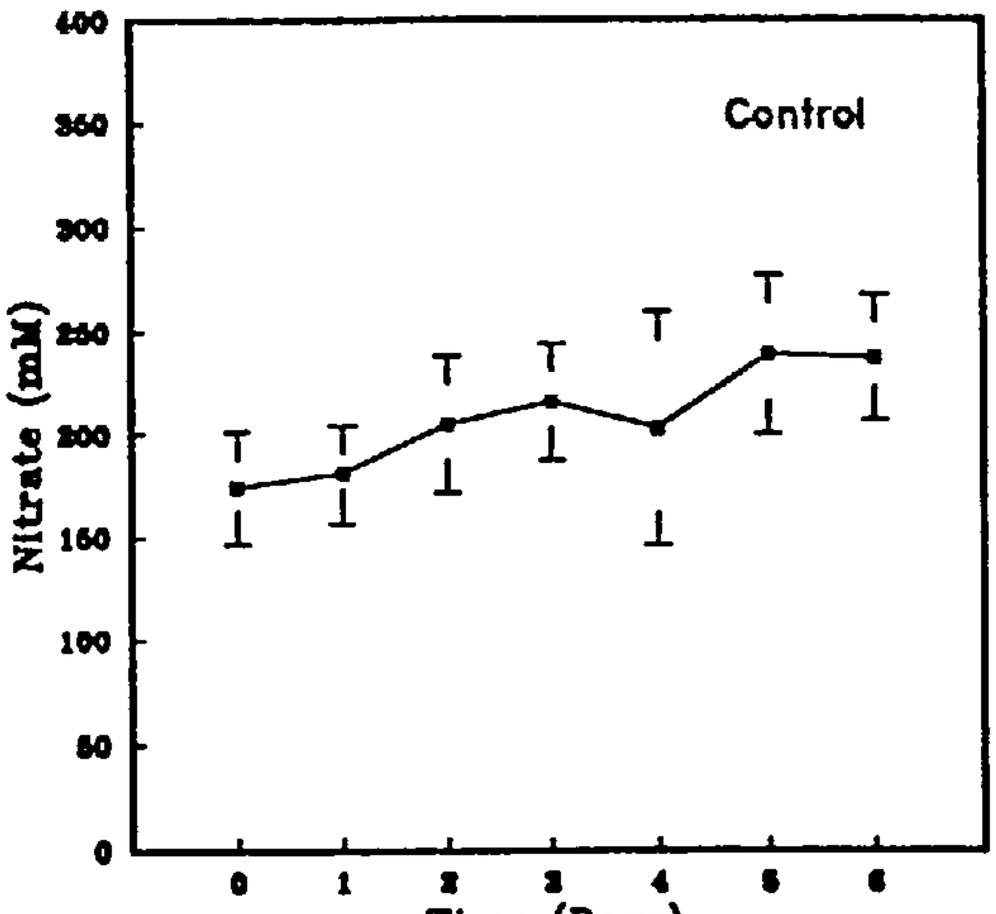
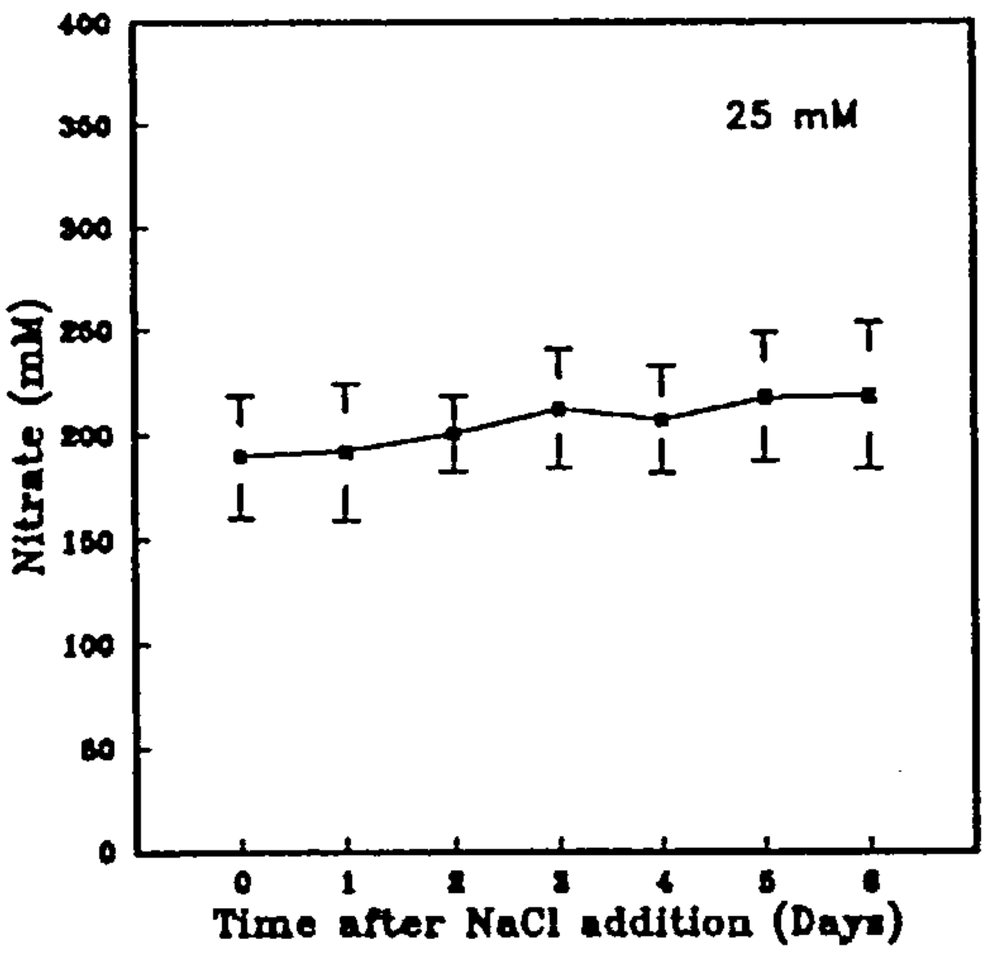
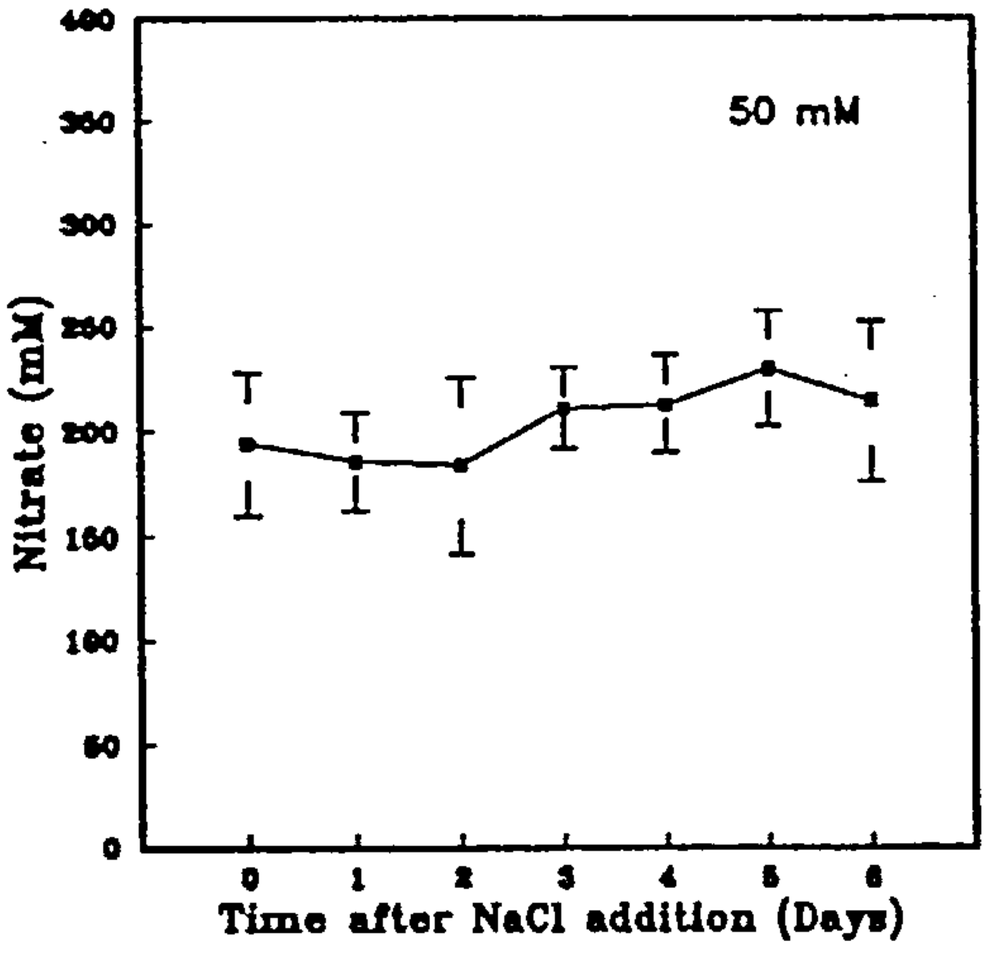
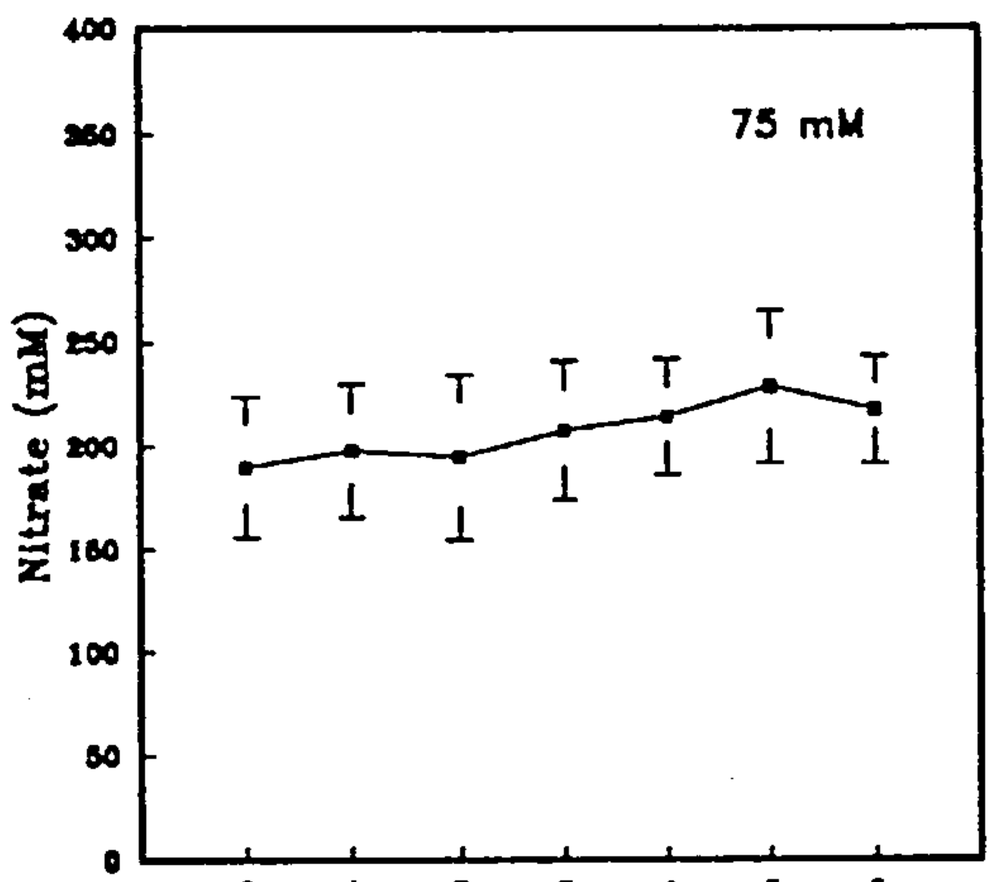

150 mM
Chloride (mM)
Time after NaCl addition (Days)

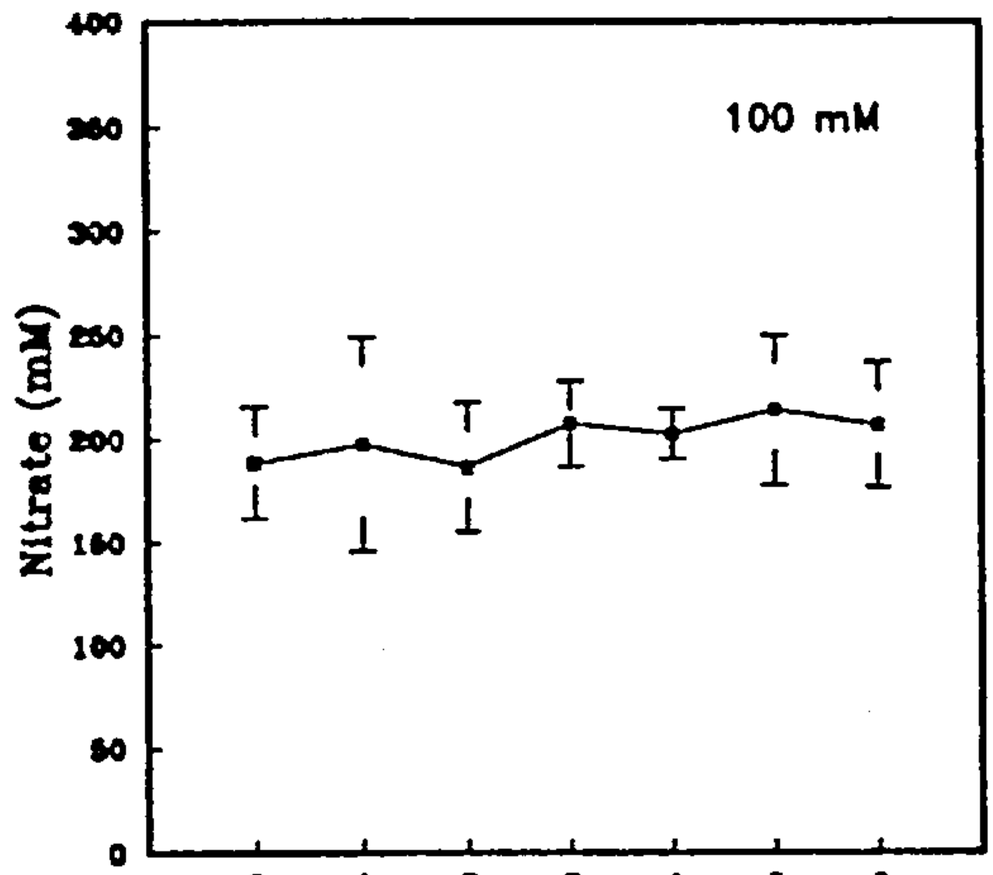
Fig. 5.13 The concentration of nitrate ion in the expressed cell sap taken from leaf mature zone after NaCl treatment, studied in a long term experiment i.e. for 6 days. Parallel experiment to Fig. 5.10 (for $Na^+$). Nitrate content was measured using HPLC.

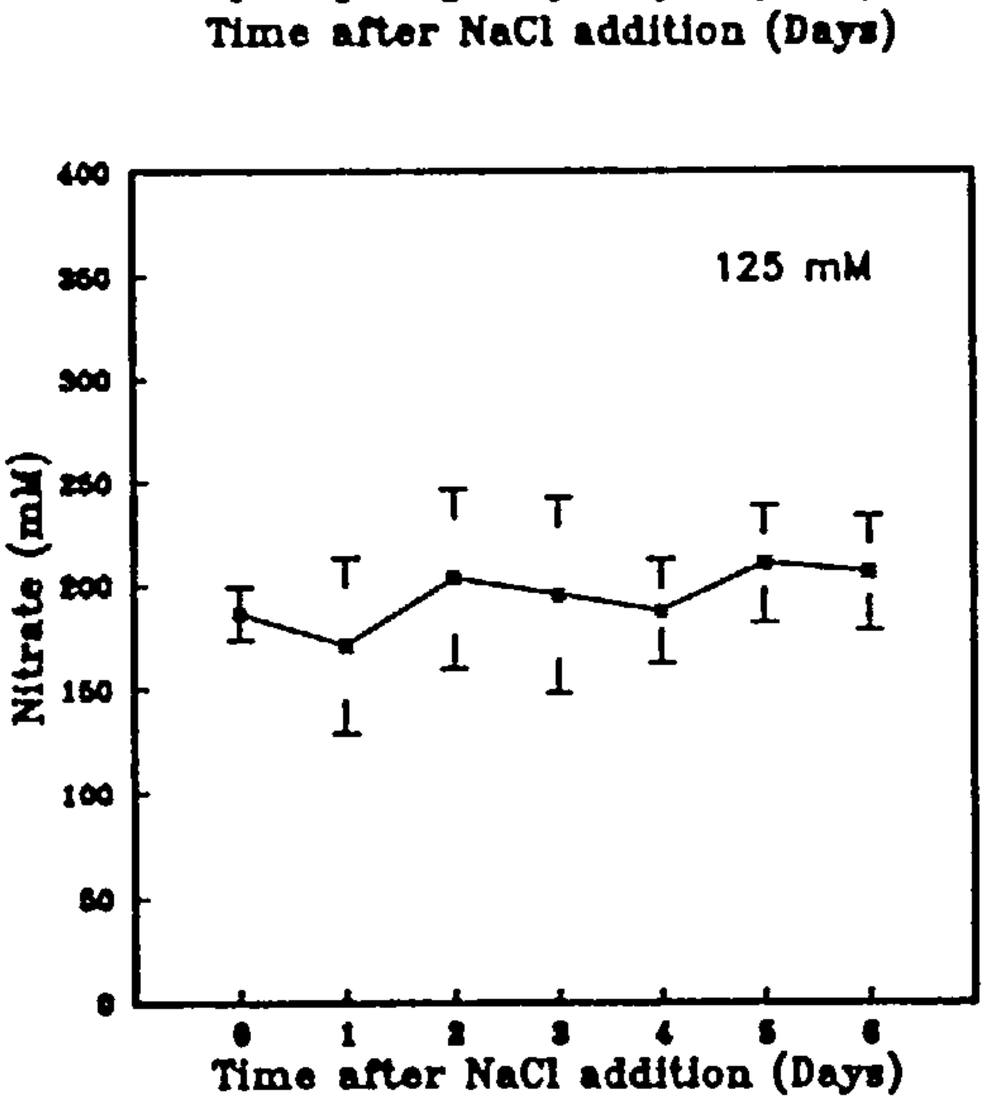

Control
Nitrate (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time (Days)


25 mM
Nitrate (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Nitrate (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Nitrate (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Nitrate (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Nitrate (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

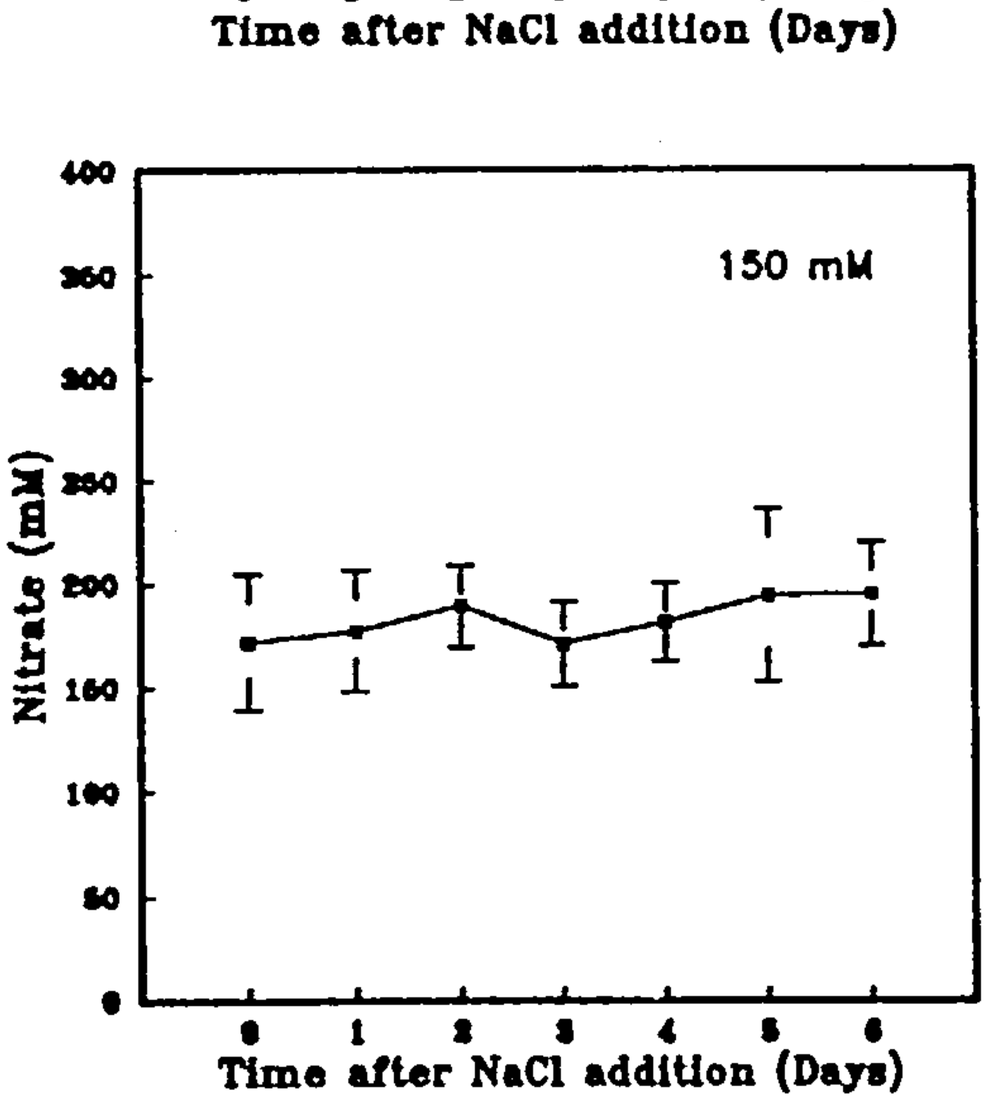
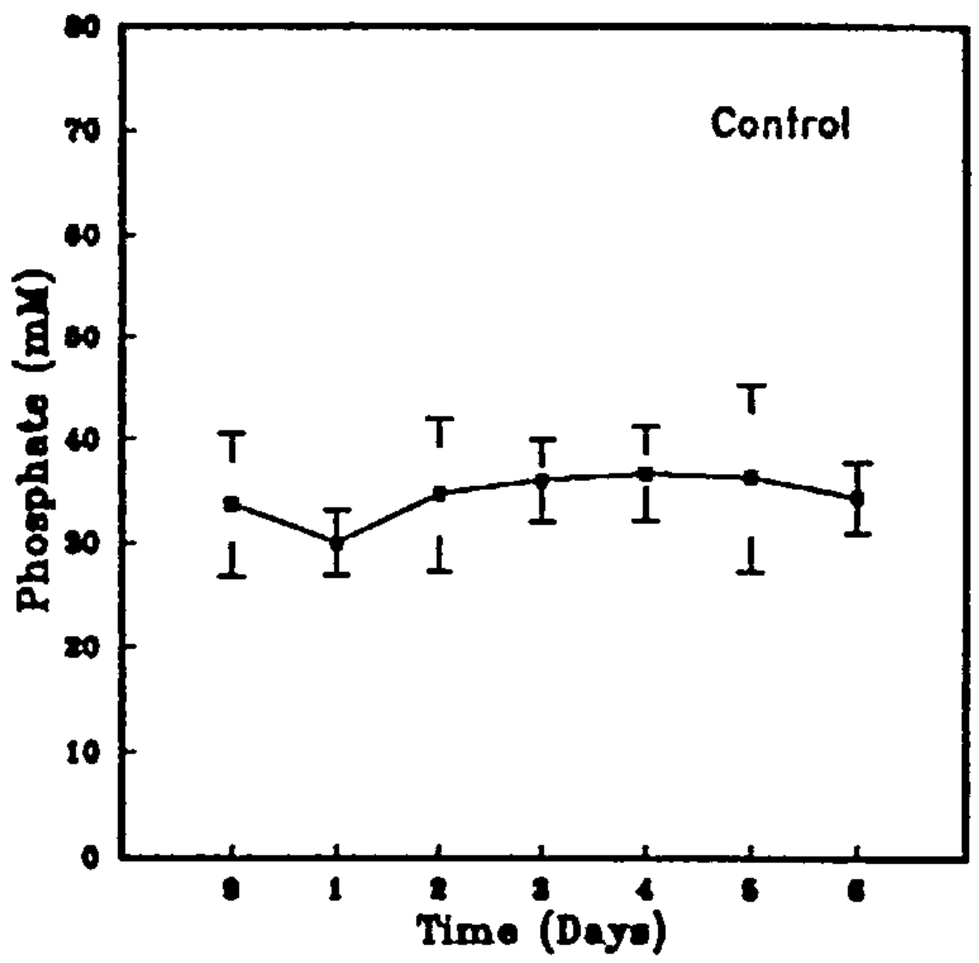
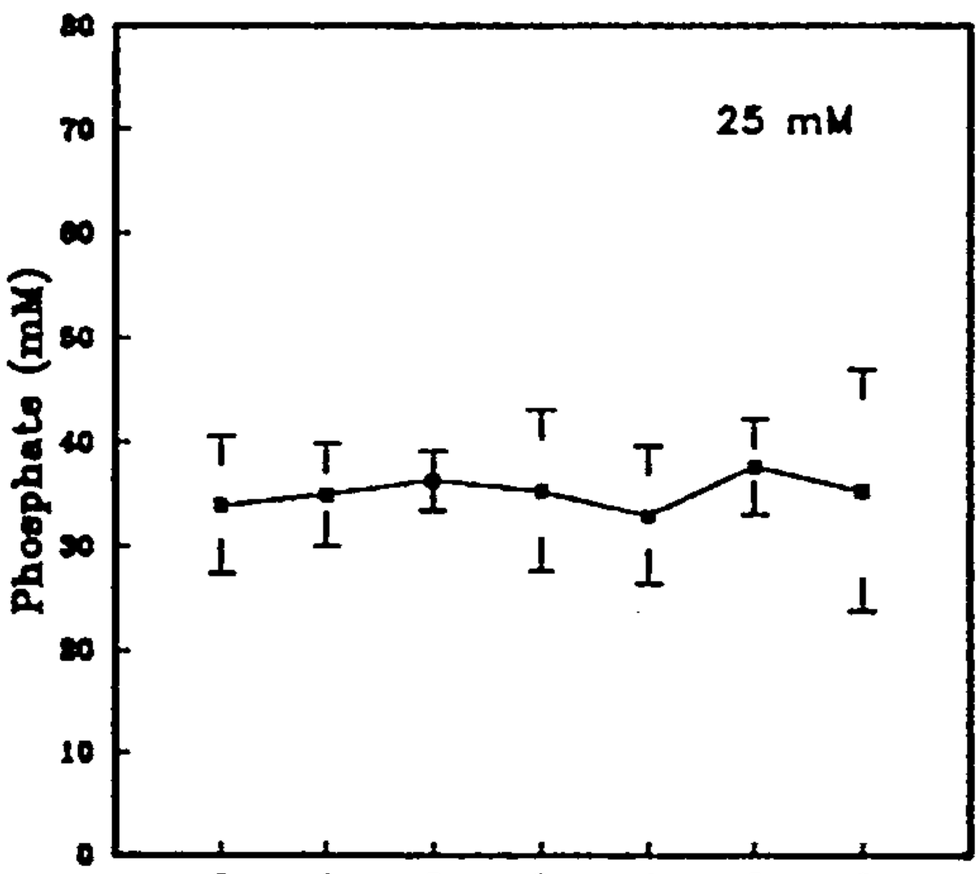
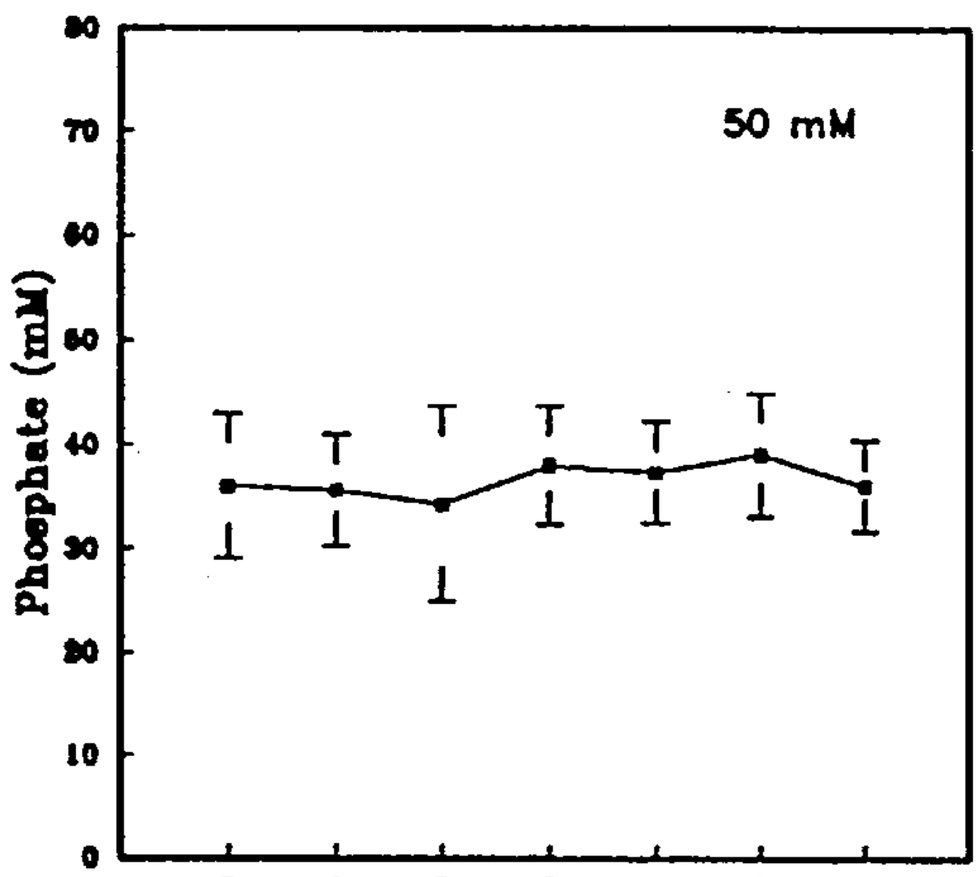
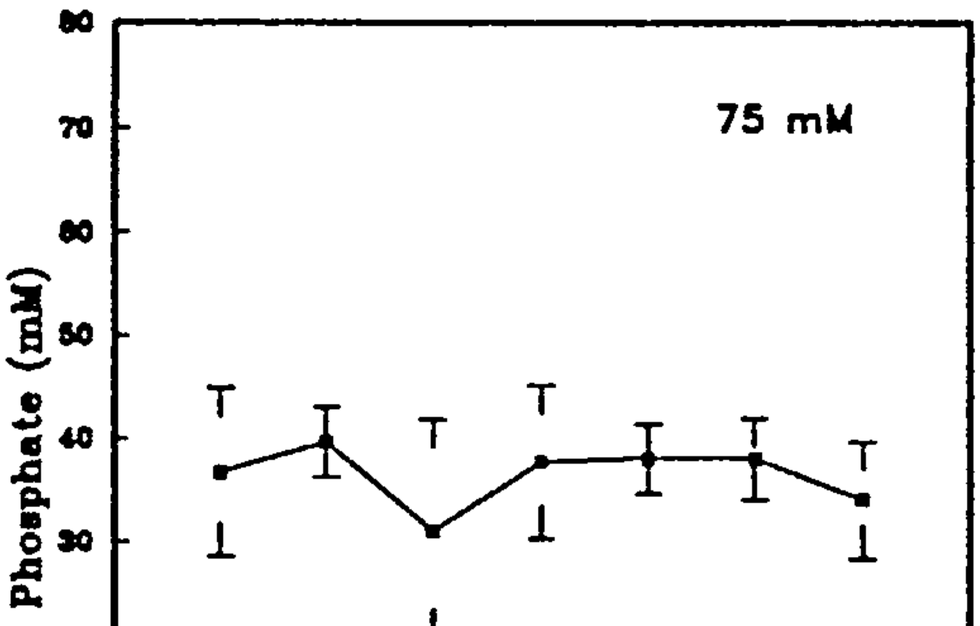

150 mM
Nitrate (mM)
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

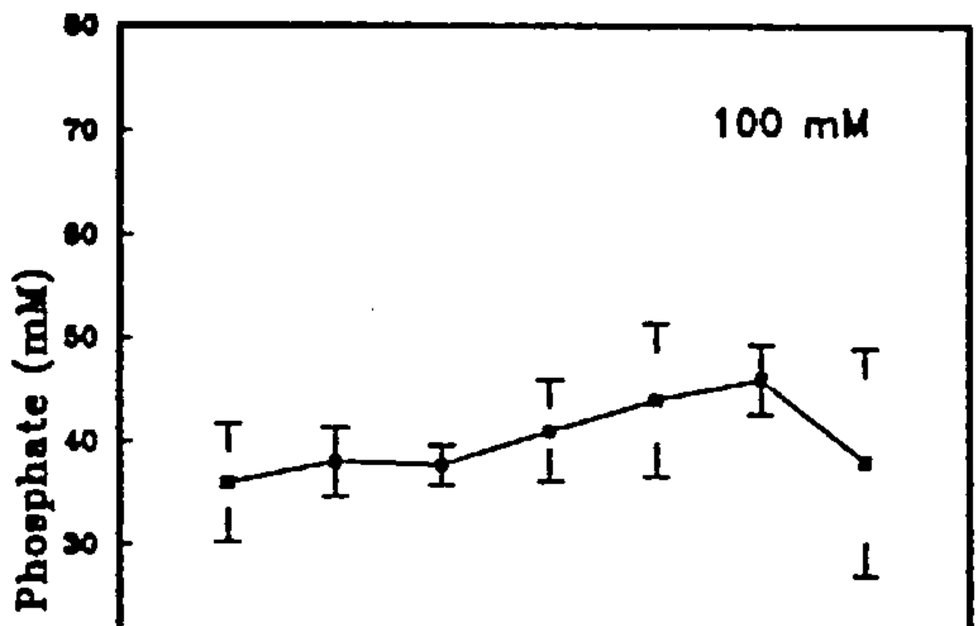
**Fig. 5.14** The concentration of phosphate ion in the expressed cell sap taken from leaf mature zone after NaCl treatment, studied in a long term experiment i.e. for 6 days. Parallel experiment to Fig. 5.10 (for $Na^+$). Phosphate content was measured using HPLC.

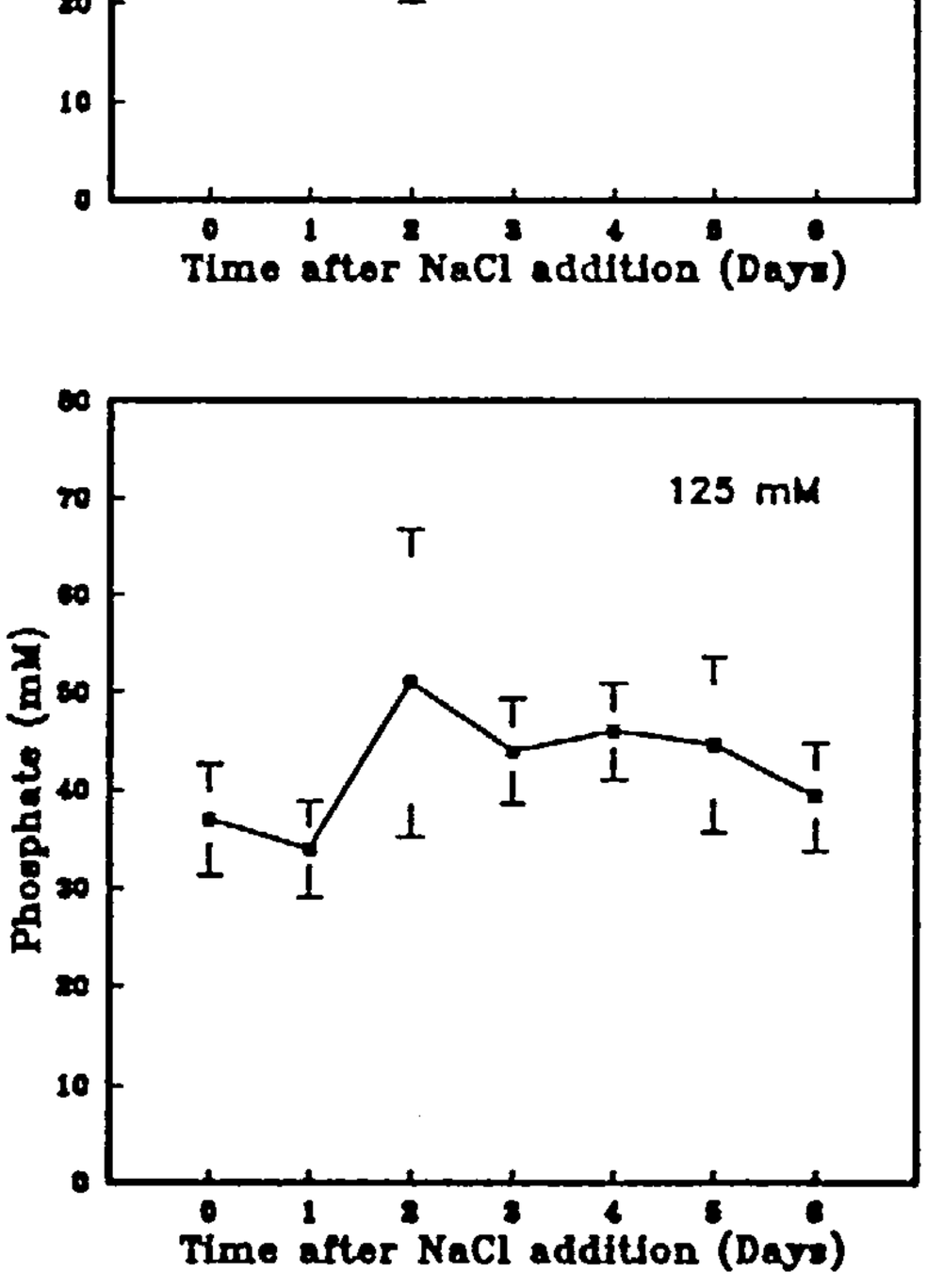

Control
Phosphate (mM)
0
10
20
30
40
50
60
70
80
0 1 2 3 4 5 6
Time (Days)

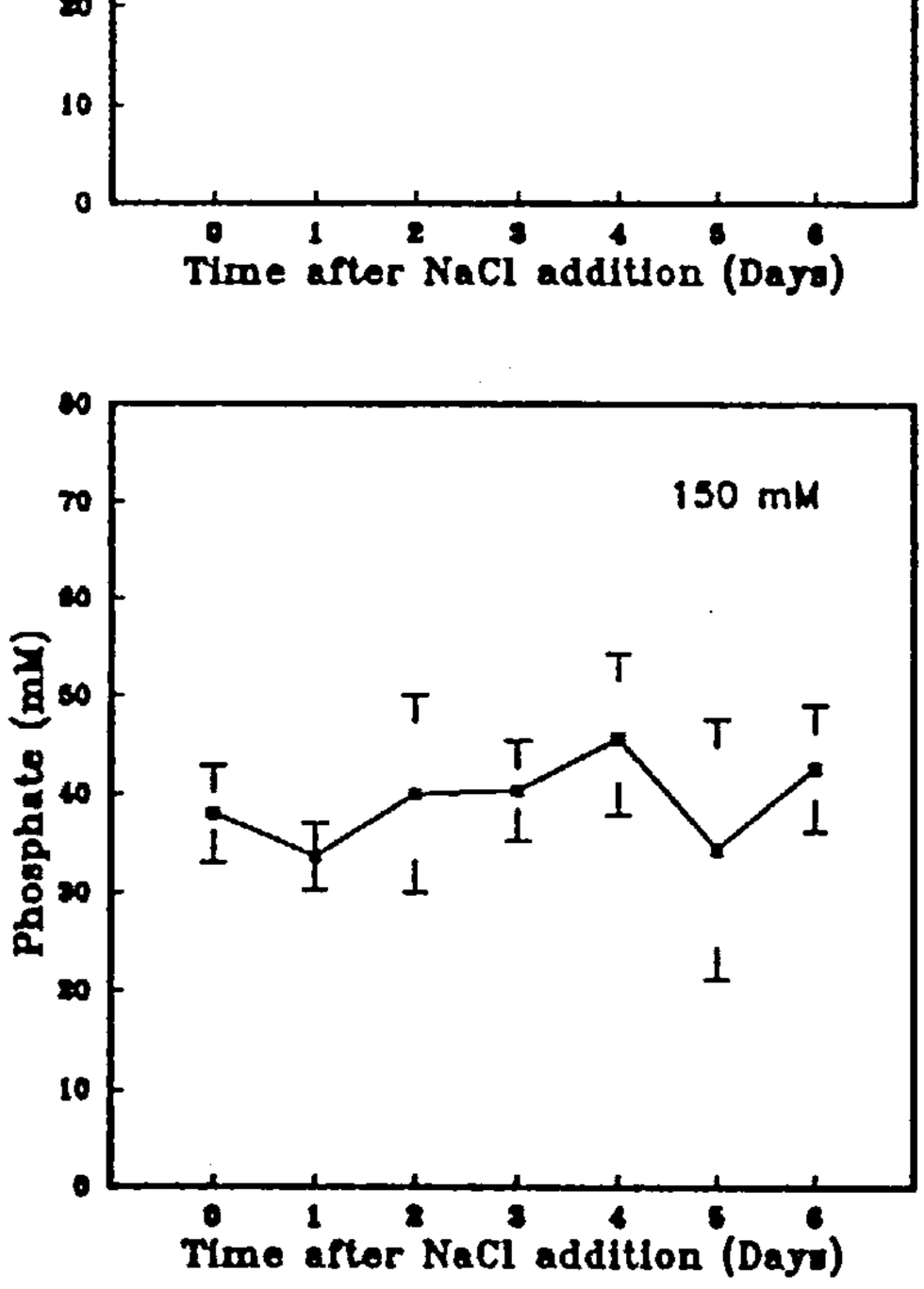

25 mM
Phosphate (mM)
0
10
20
30
40
50
60
70
80
0 1 2 3 4 5 6
Time after NaCl addition (Days)

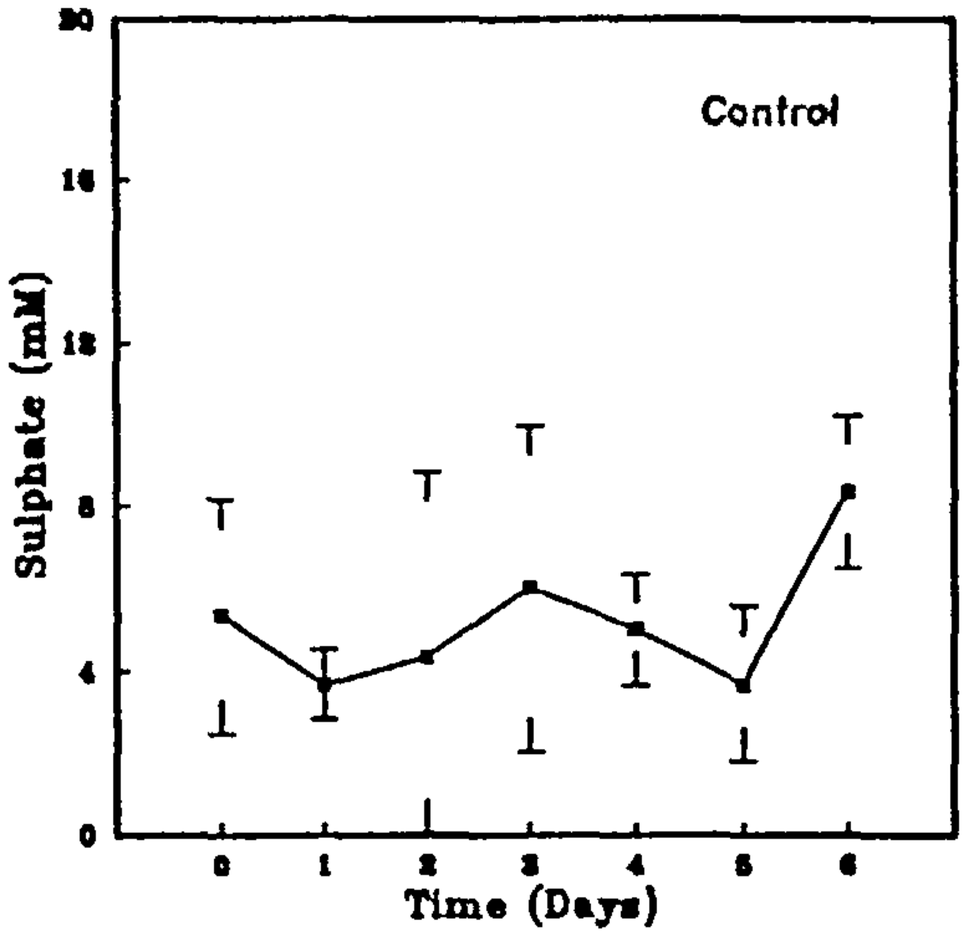

50 mM
Phosphate (mM)
0
10
20
30
40
50
60
70
80
0 1 2 3 4 5 6
Time after NaCl addition (Days)

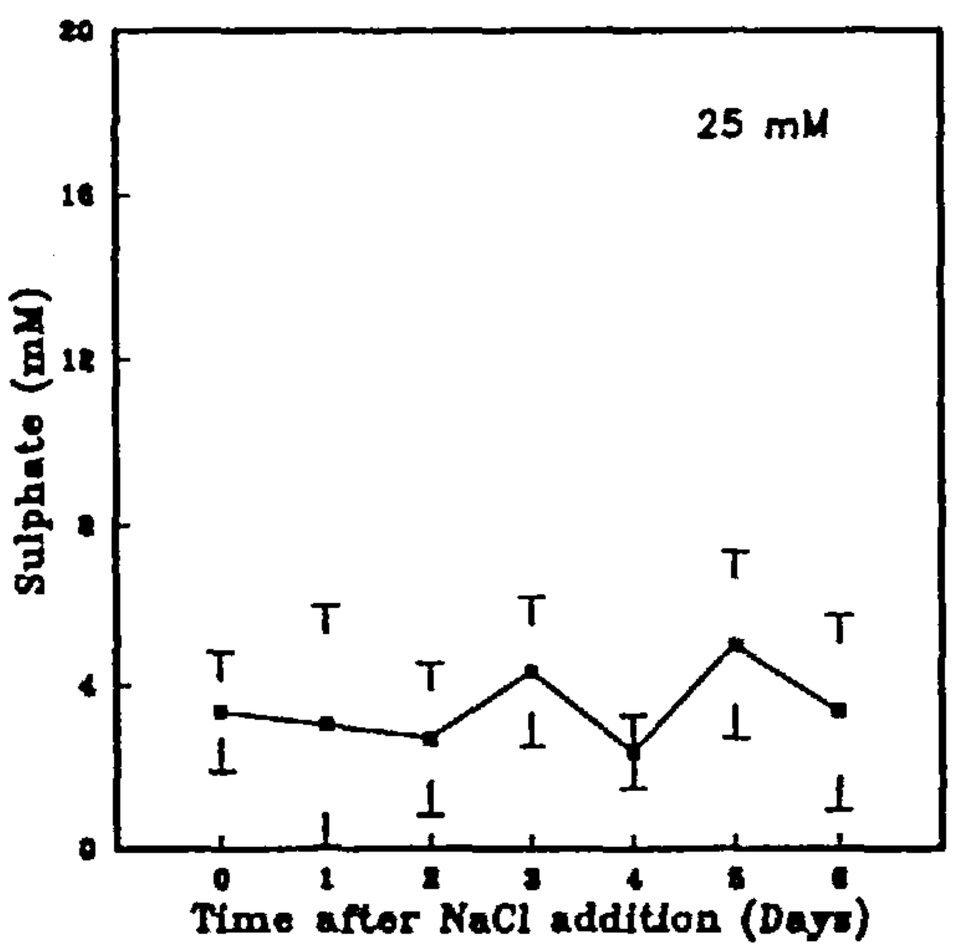

75 mM
Phosphate (mM)
0
10
20
30
40
50
60
70
80
0 1 2 3 4 5 6
Time after NaCl addition (Days)

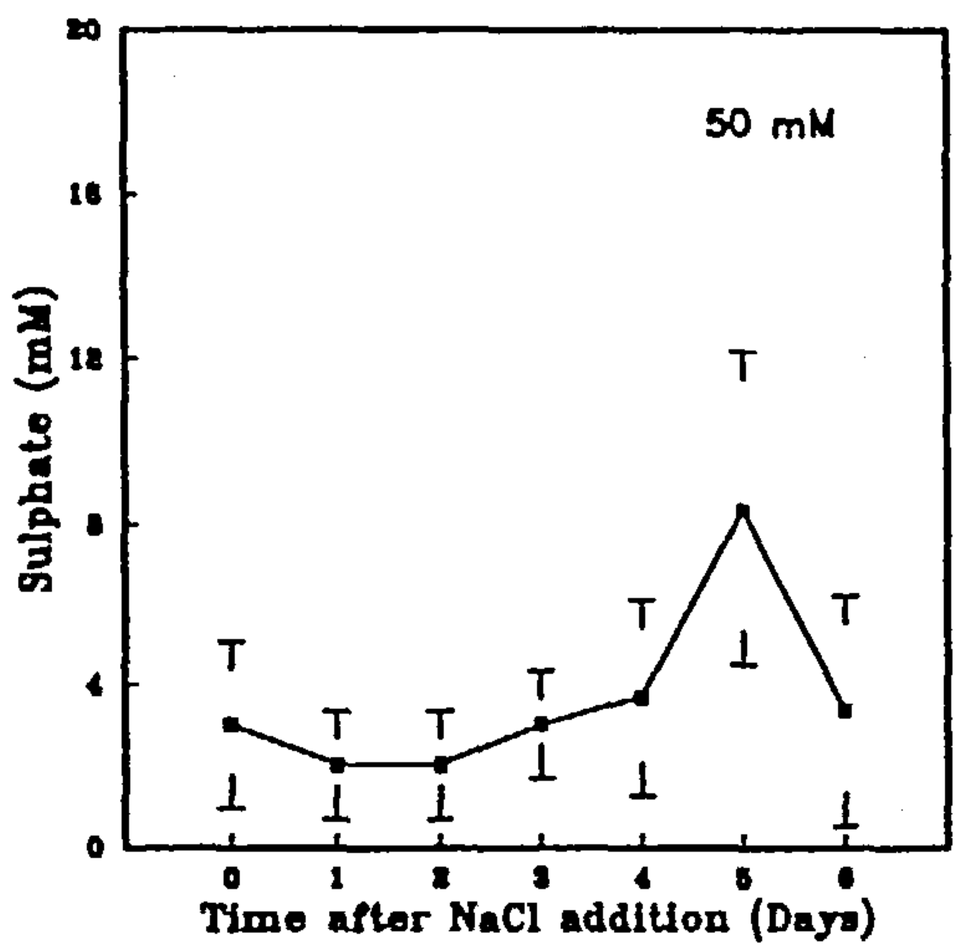

100 mM
Phosphate (mM)
0
10
20
30
40
50
60
70
80
0 1 2 3 4 5 6
Time after NaCl addition (Days)

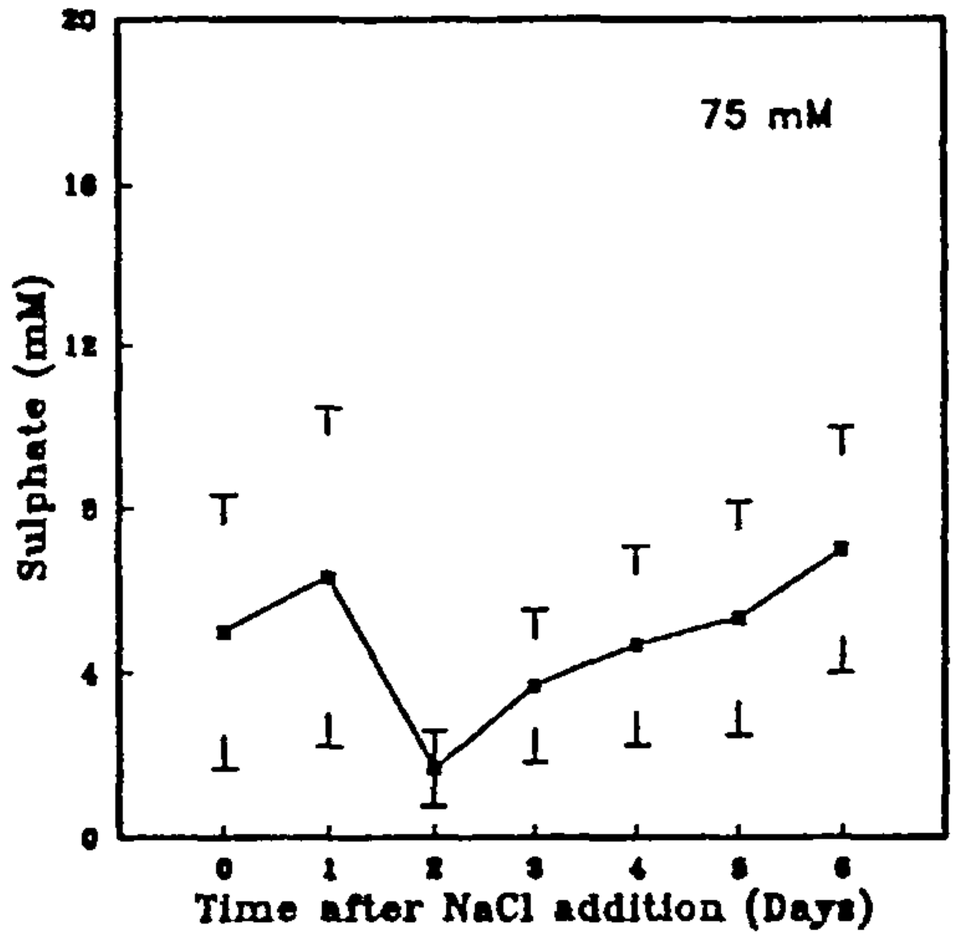

125 mM
Phosphate (mM)
0
10
20
30
40
50
60
70
80
0 1 2 3 4 5 6
Time after NaCl addition (Days)

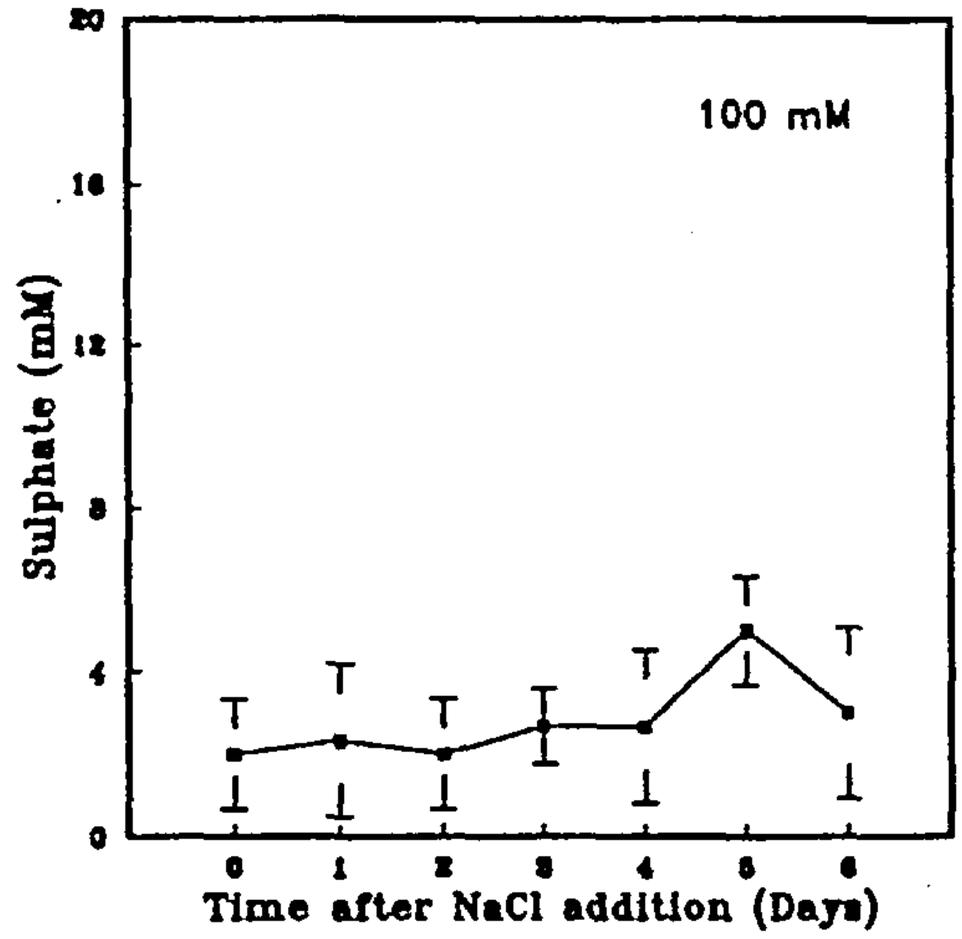

150 mM
Phosphate (mM)
0
10
20
30
40
50
60
70
80
0 1 2 3 4 5 6
Time after NaCl addition (Days)

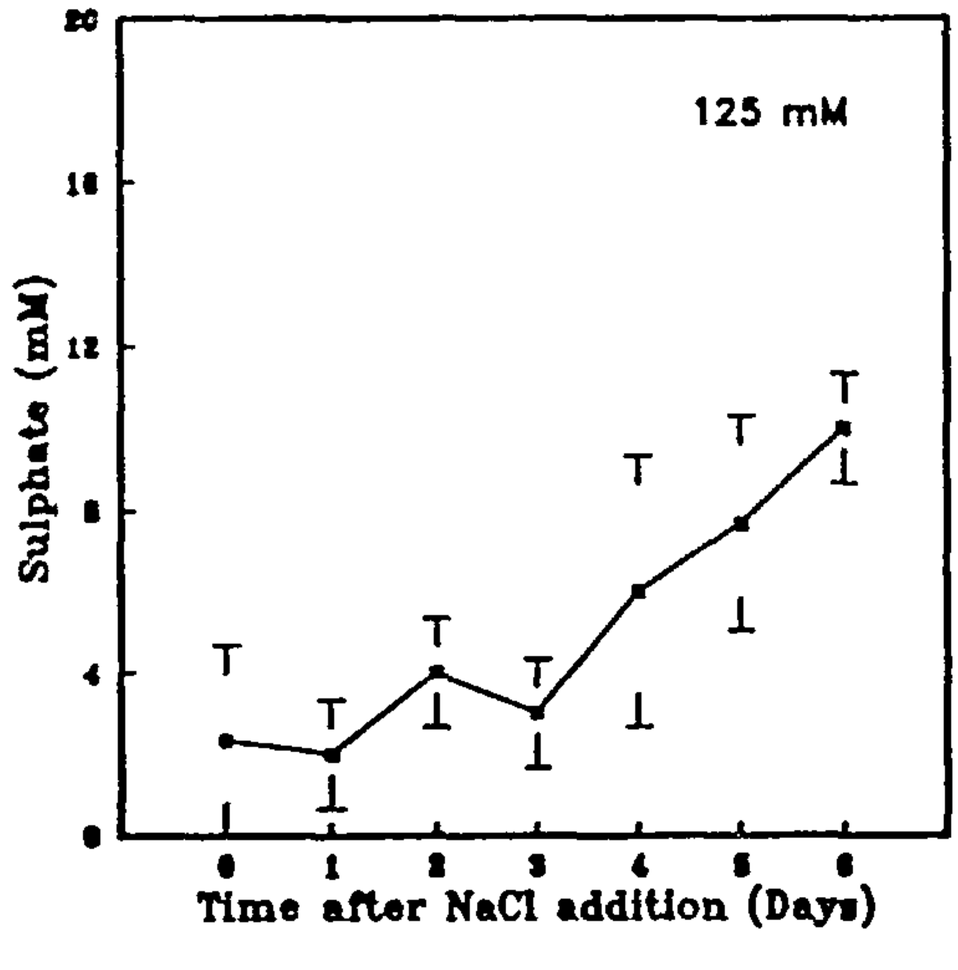

Control
Sulphate (mM)
Time (Days)


25 mM
Sulphate (mM)
Time after NaCl addition (Days)


50 mM
Sulphate (mM)
Time after NaCl addition (Days)


75 mM
Sulphate (mM)
Time after NaCl addition (Days)


100 mM
Sulphate (mM)
Time after NaCl addition (Days)


125 mM
Sulphate (mM)
Time after NaCl addition (Days)

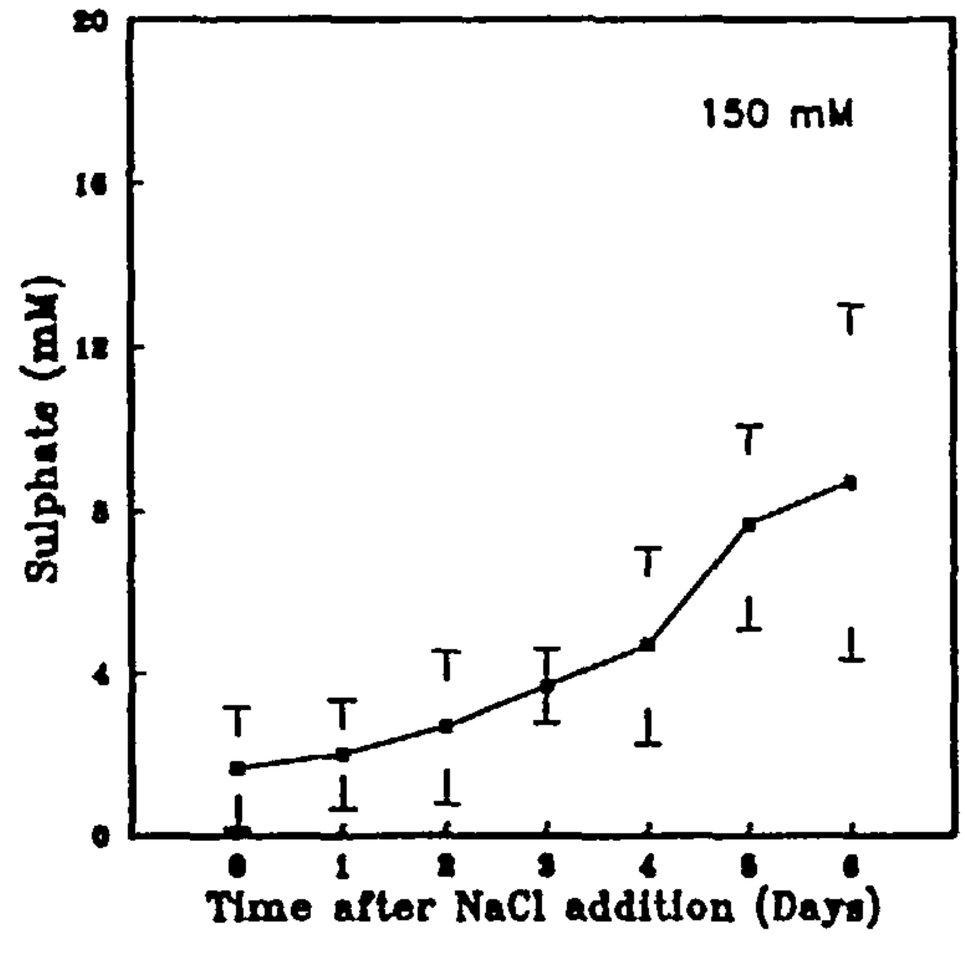
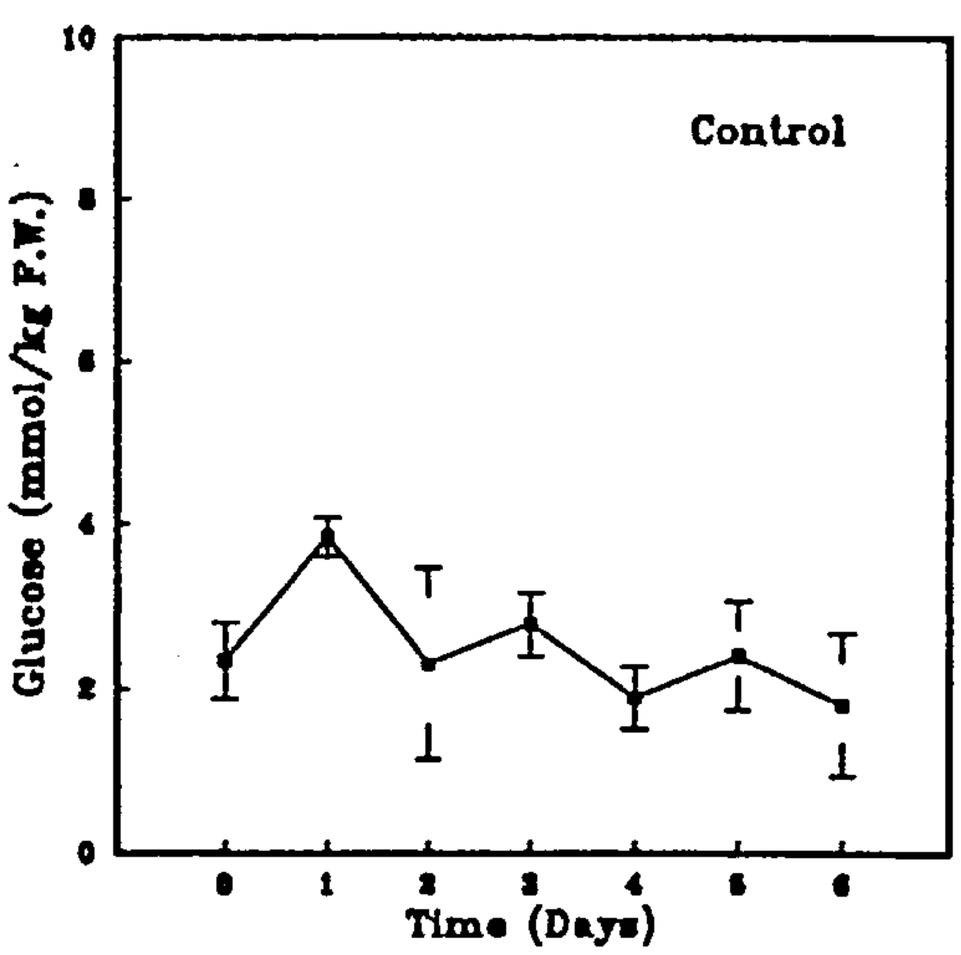
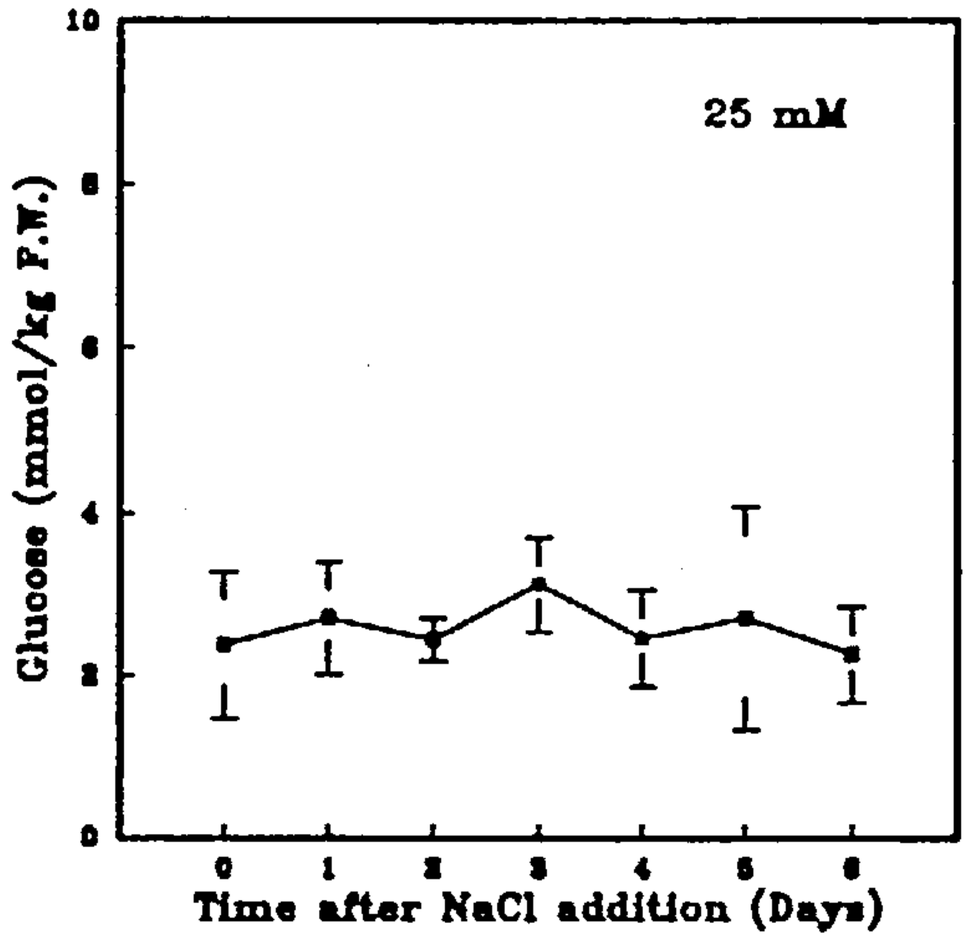
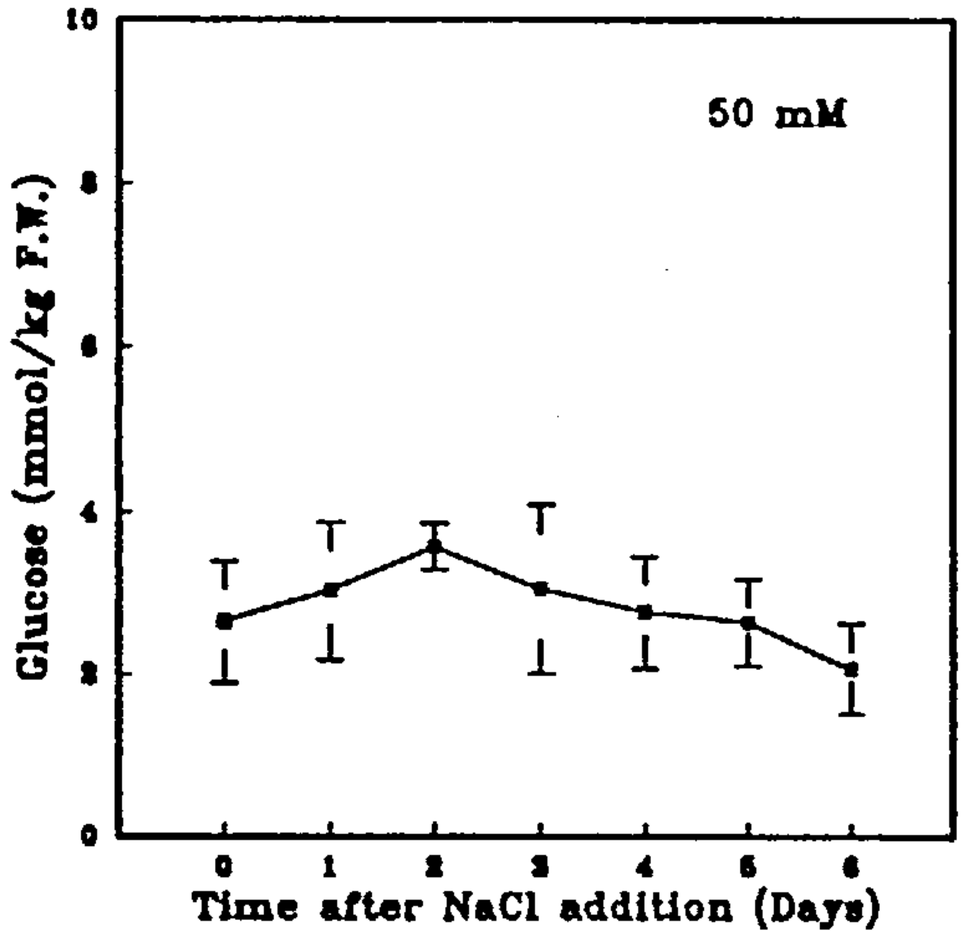
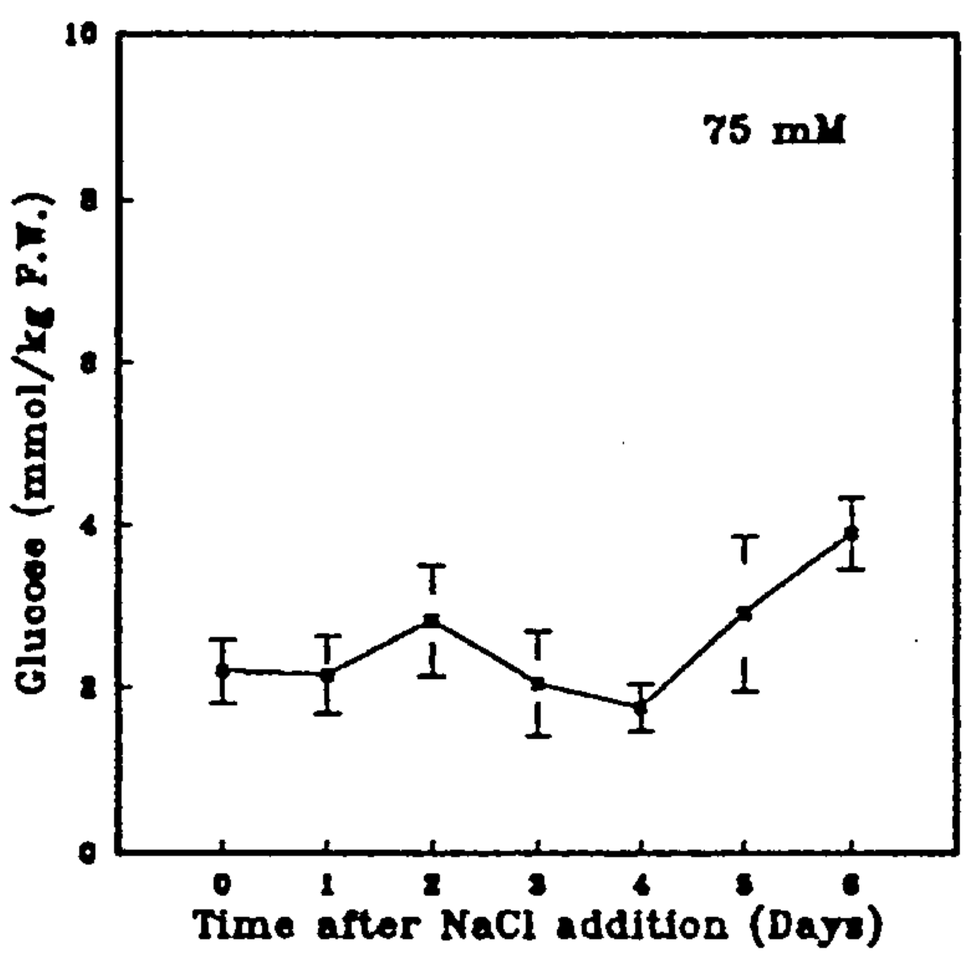

150 mM
Sulphate (mM)
Time after NaCl addition (Days)

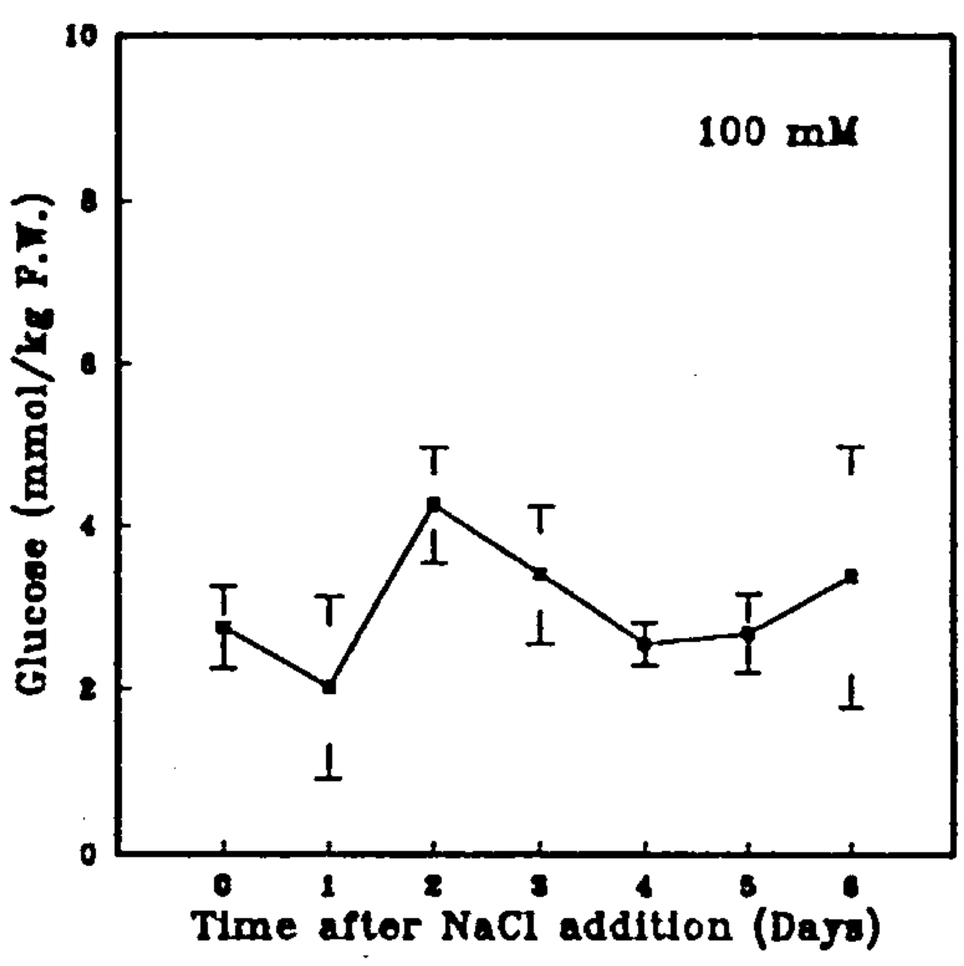
**Fig. 5.16** The concentration of glucose in the leaf mature zone following NaCl treatment, studied in a long term experiment i.e. for 6 days. Parallel experiment to Fig. 5.10 (for $Na^+$). Glucose content was measured using G.L.C.

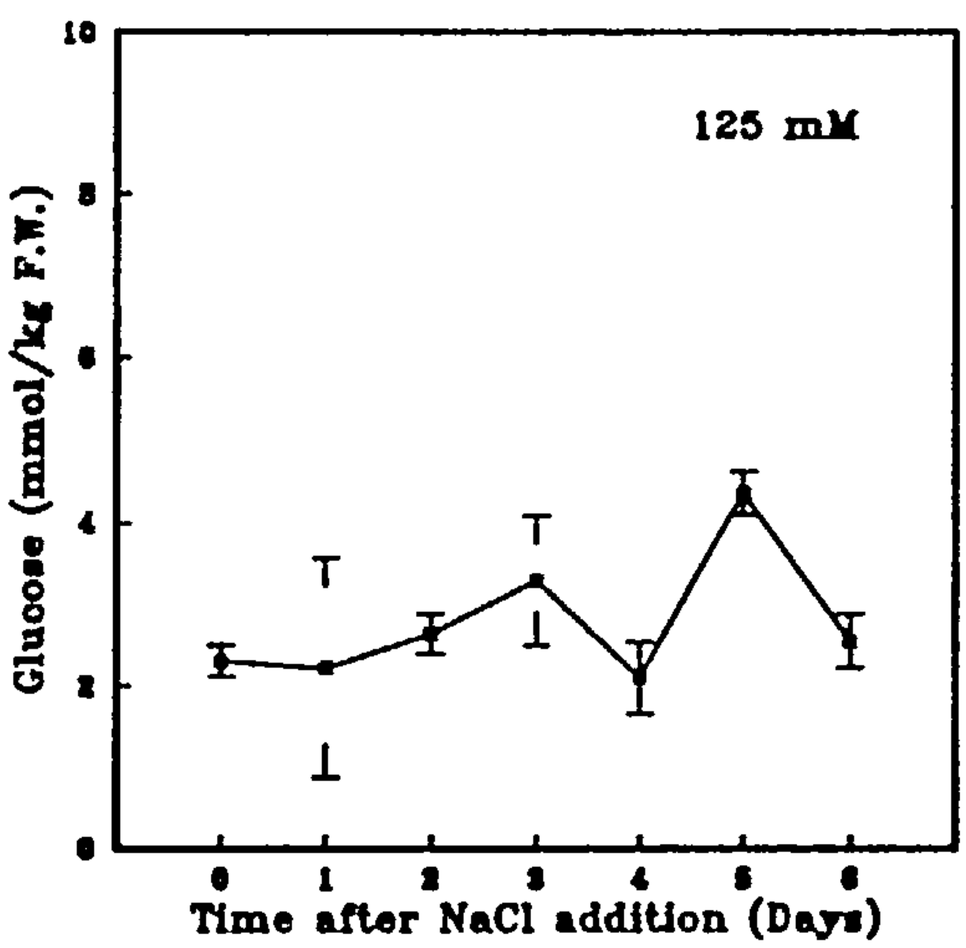

Control
Glucose (mmol/kg F.W.)
Time (Days)


25 mM
Glucose (mmol/kg F.W.)
Time after NaCl addition (Days)


50 mM
Glucose (mmol/kg F.W.)
Time after NaCl addition (Days)


75 mM
Glucose (mmol/kg F.W.)
Time after NaCl addition (Days)


100 mM
Glucose (mmol/kg F.W.)
Time after NaCl addition (Days)


125 mM
Glucose (mmol/kg F.W.)
Time after NaCl addition (Days)

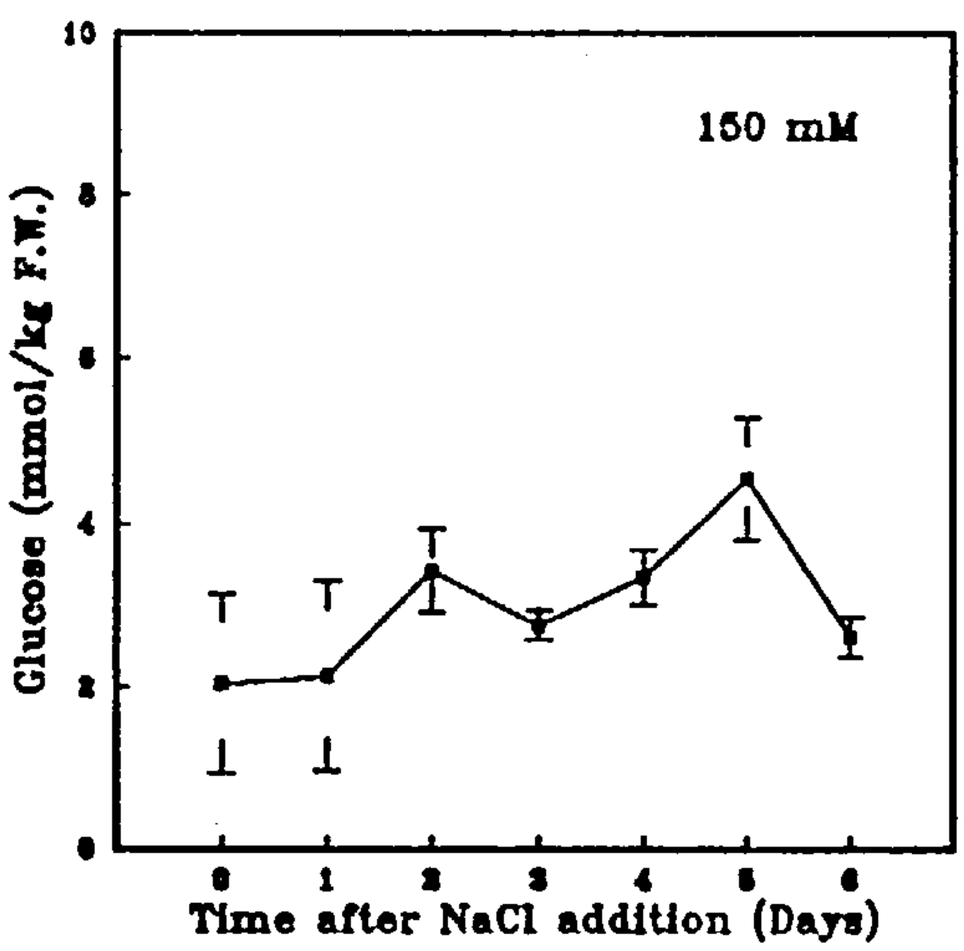
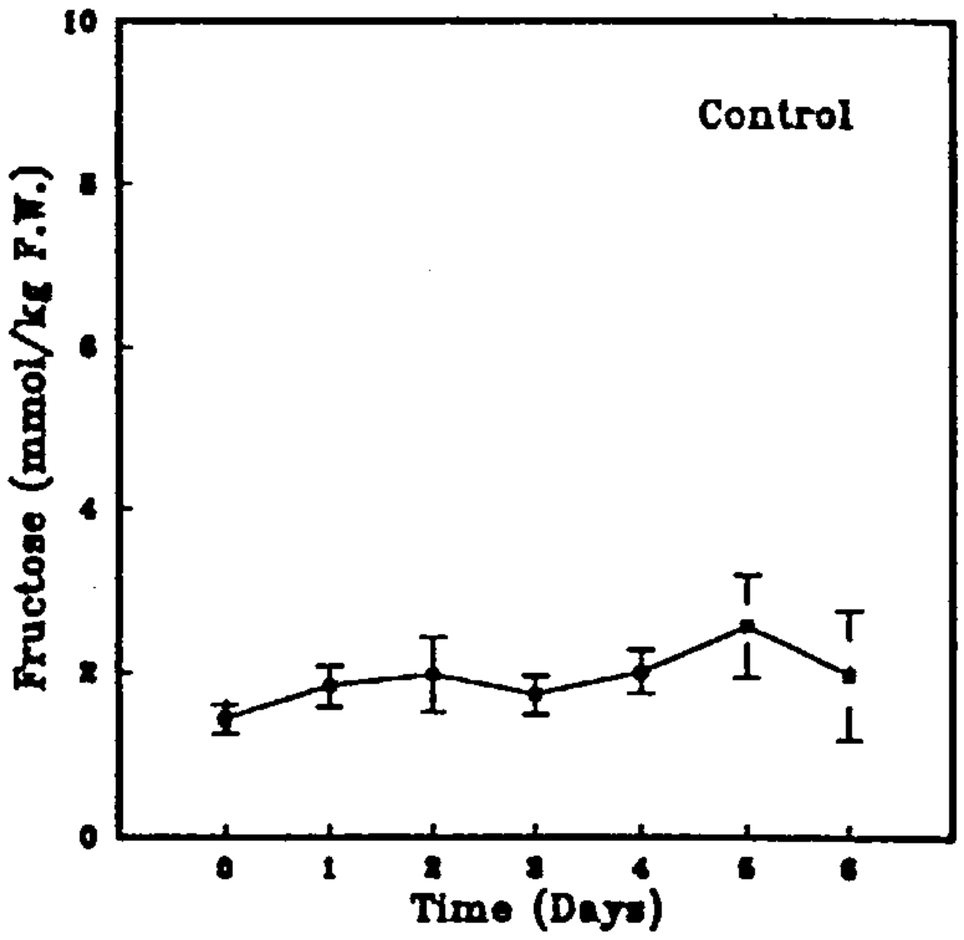
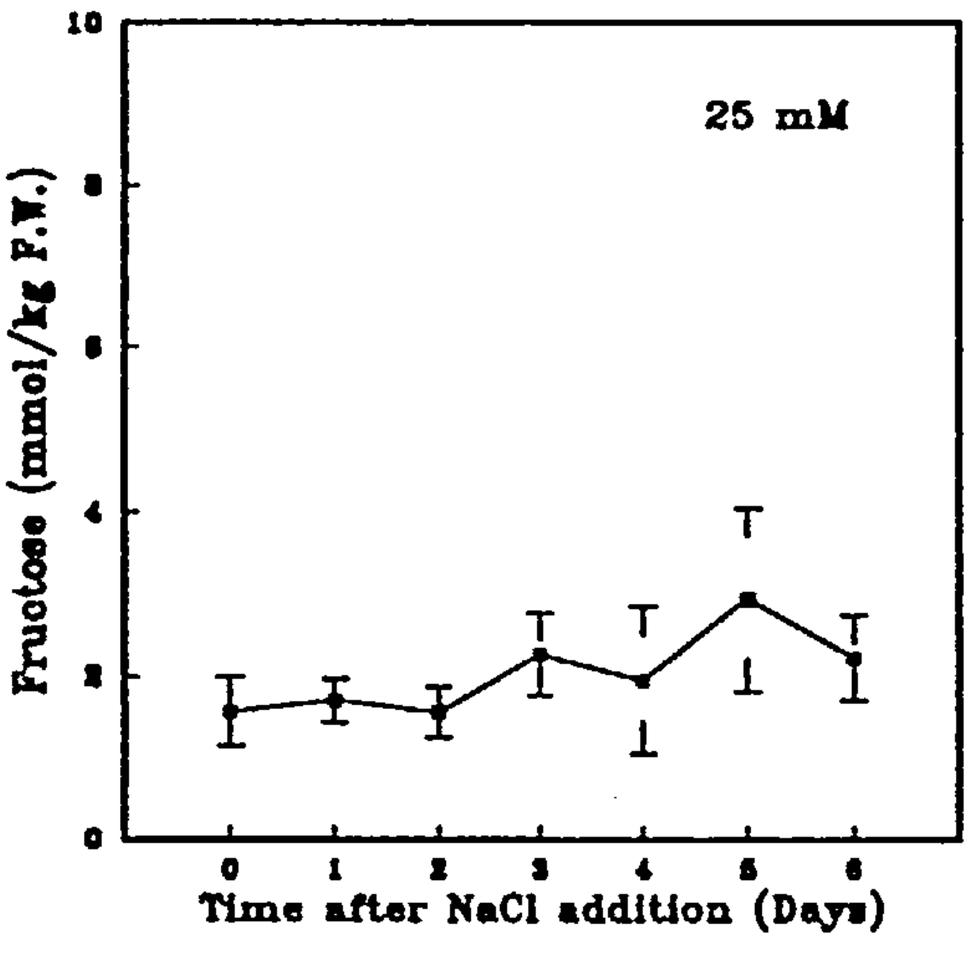
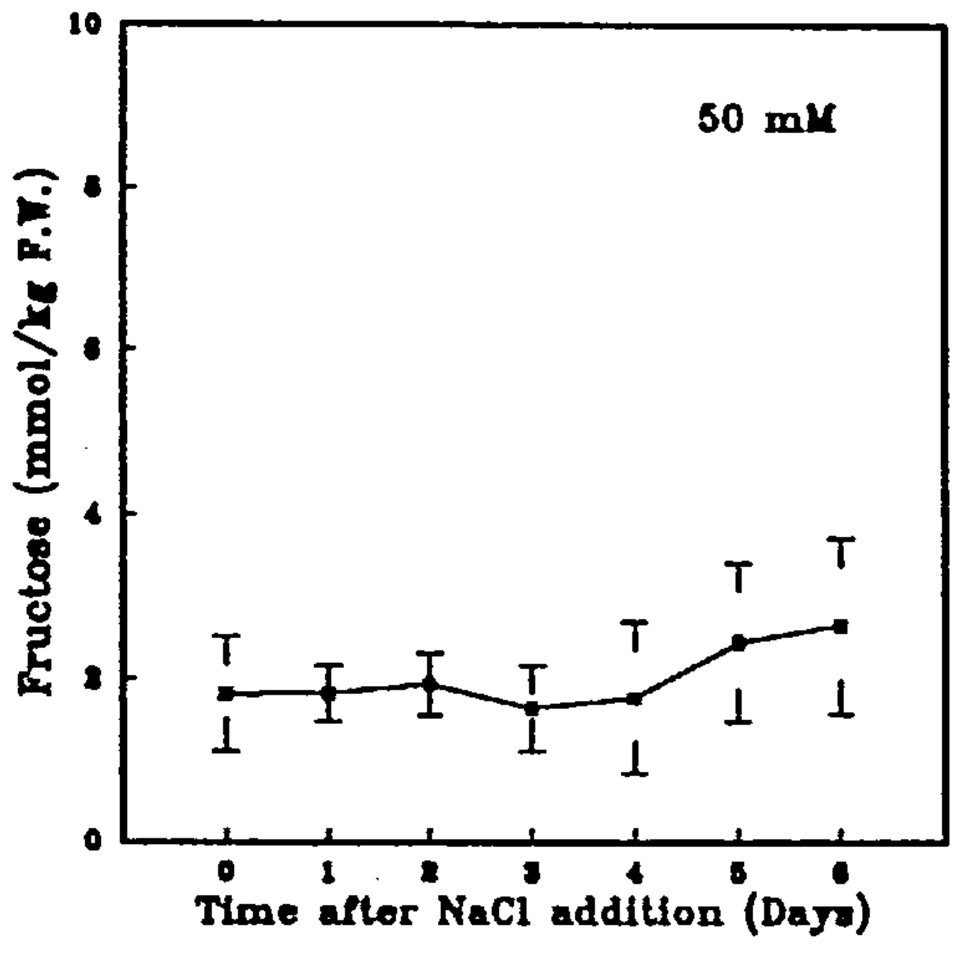
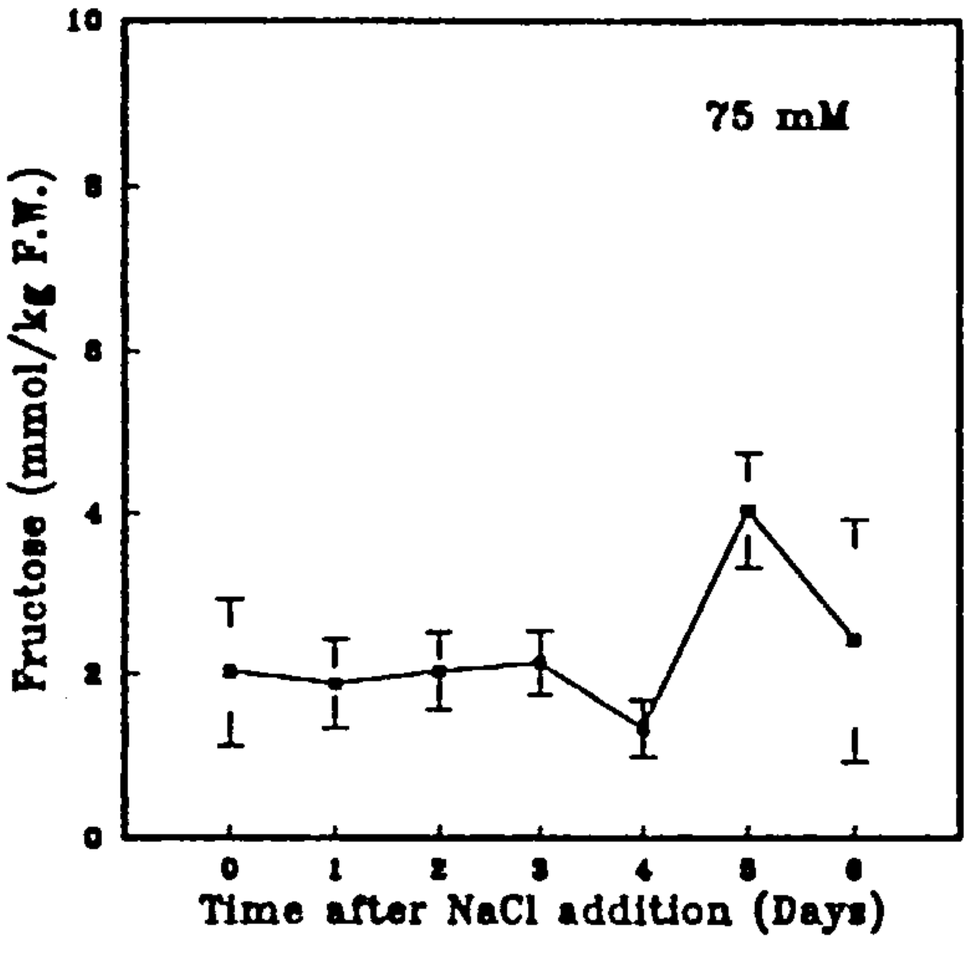

150 mM
Glucose (mmol/kg F.W.)
Time after NaCl addition (Days)

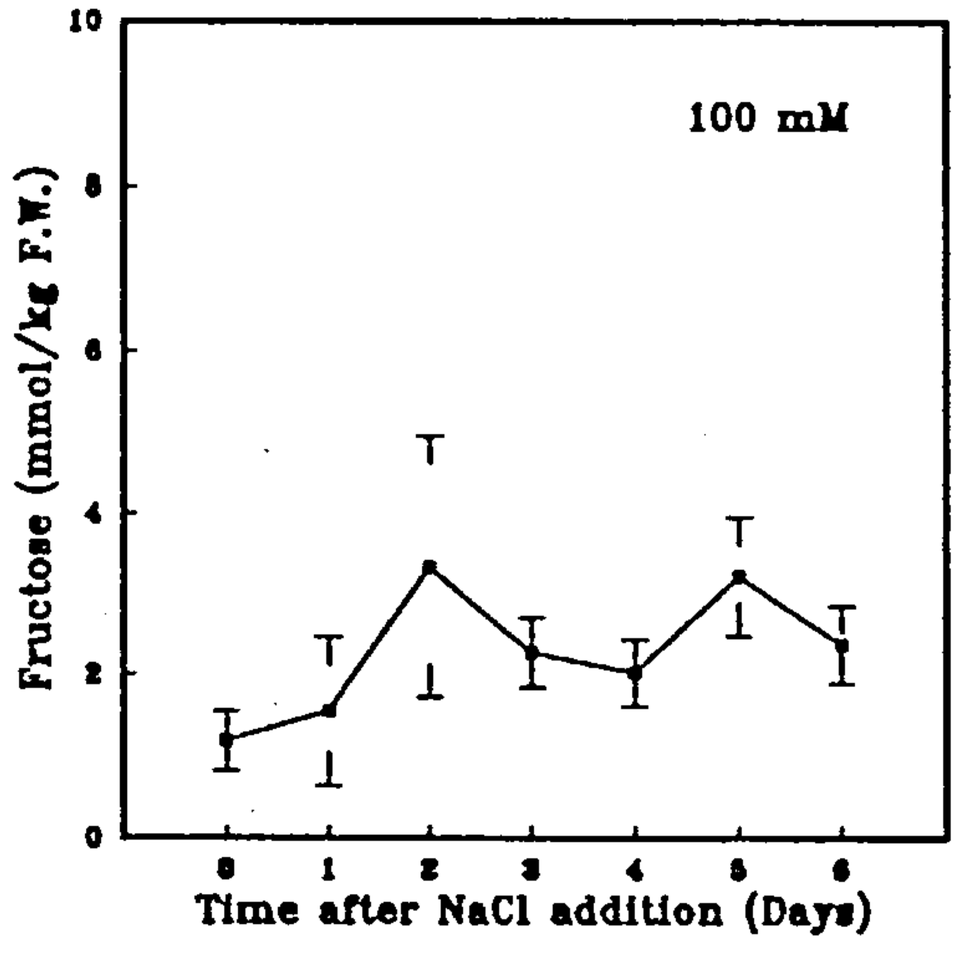
**Fig. 5.17** The concentration of fructose in the leaf mature zone following NaCl treatment, studied in a long term experiment i.e. for 6 days. Parallel experiment to Fig. 5.10 (for $Na^+$). Fructose content was measured using G.L.C.

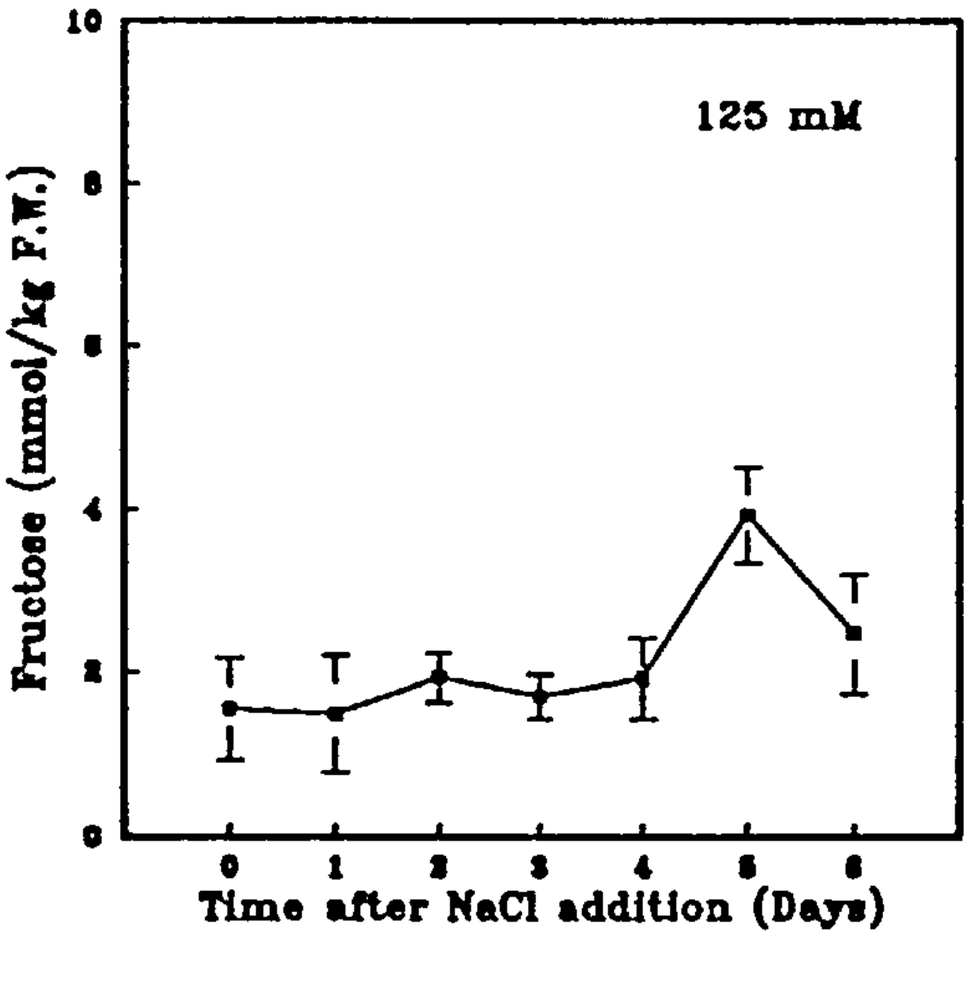

Control
Fructose (mmol/kg F.W.)
Time (Days)


25 mM
Fructose (mmol/kg F.W.)
Time after NaCl addition (Days)


50 mM
Fructose (mmol/kg F.W.)
Time after NaCl addition (Days)


75 mM
Fructose (mmol/kg F.W.)
Time after NaCl addition (Days)


100 mM
Fructose (mmol/kg F.W.)
Time after NaCl addition (Days)


125 mM
Fructose (mmol/kg F.W.)
Time after NaCl addition (Days)

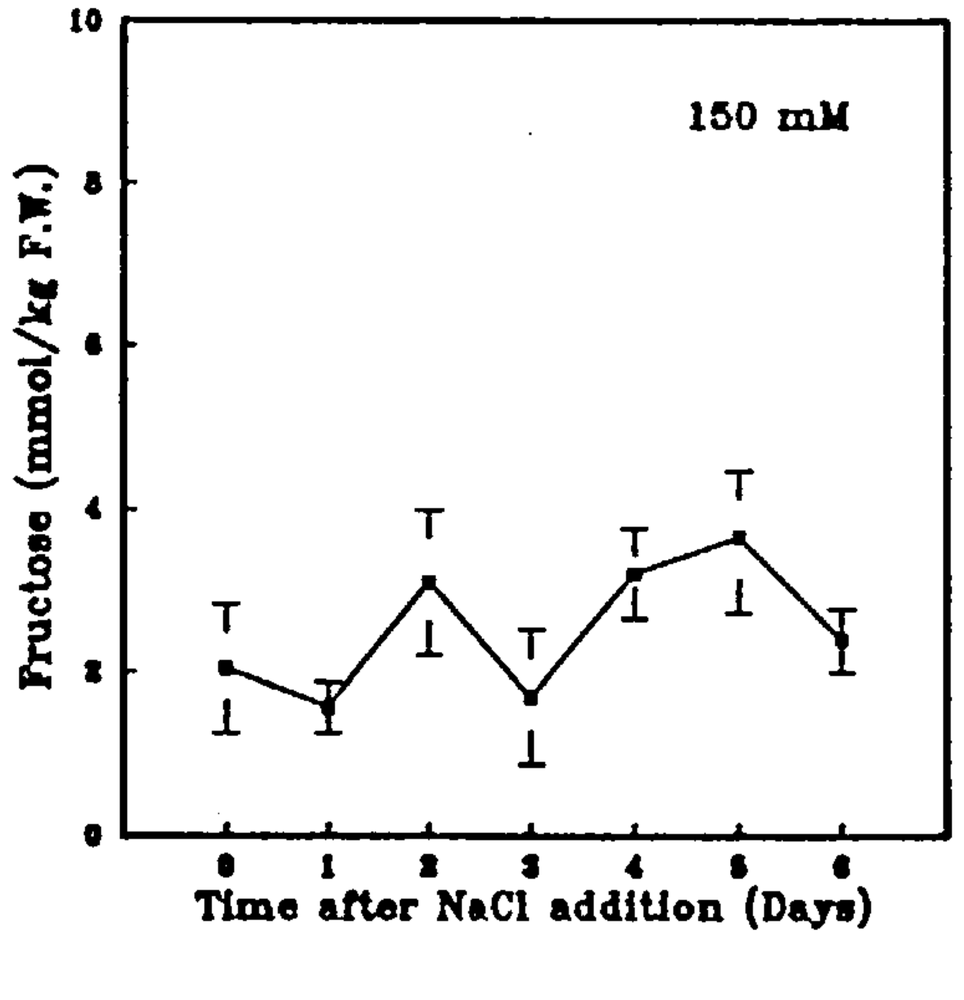
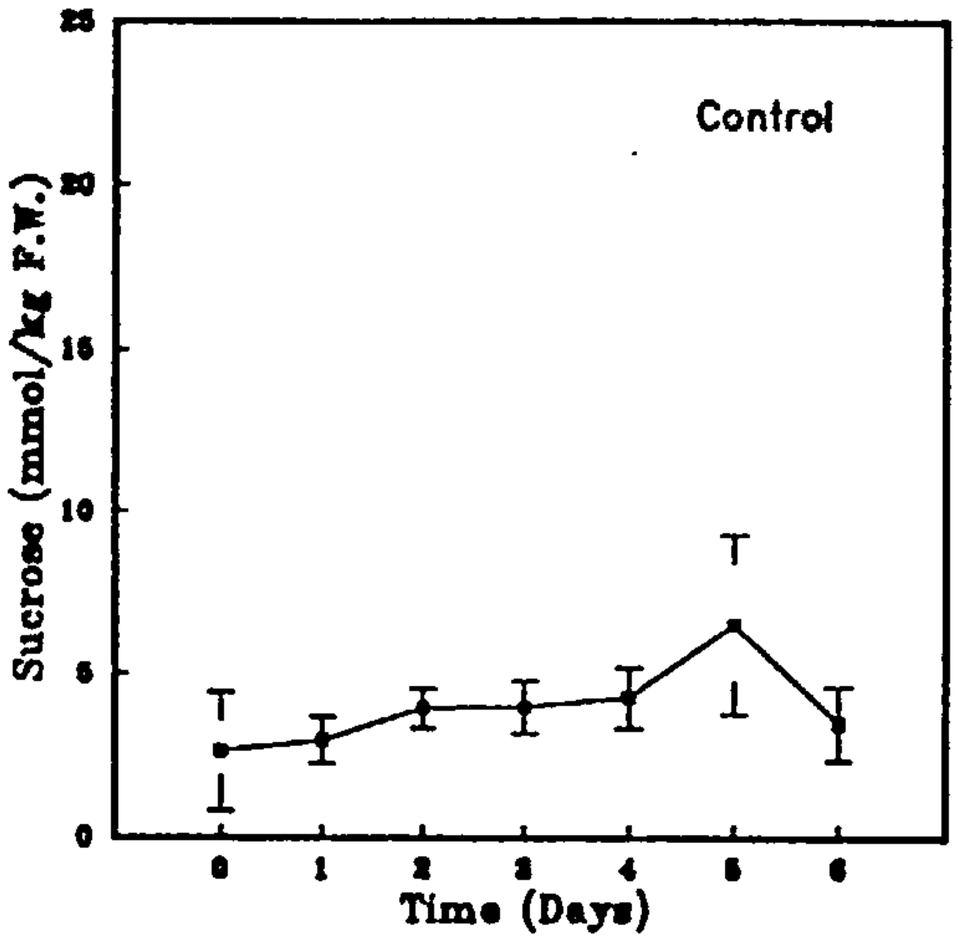
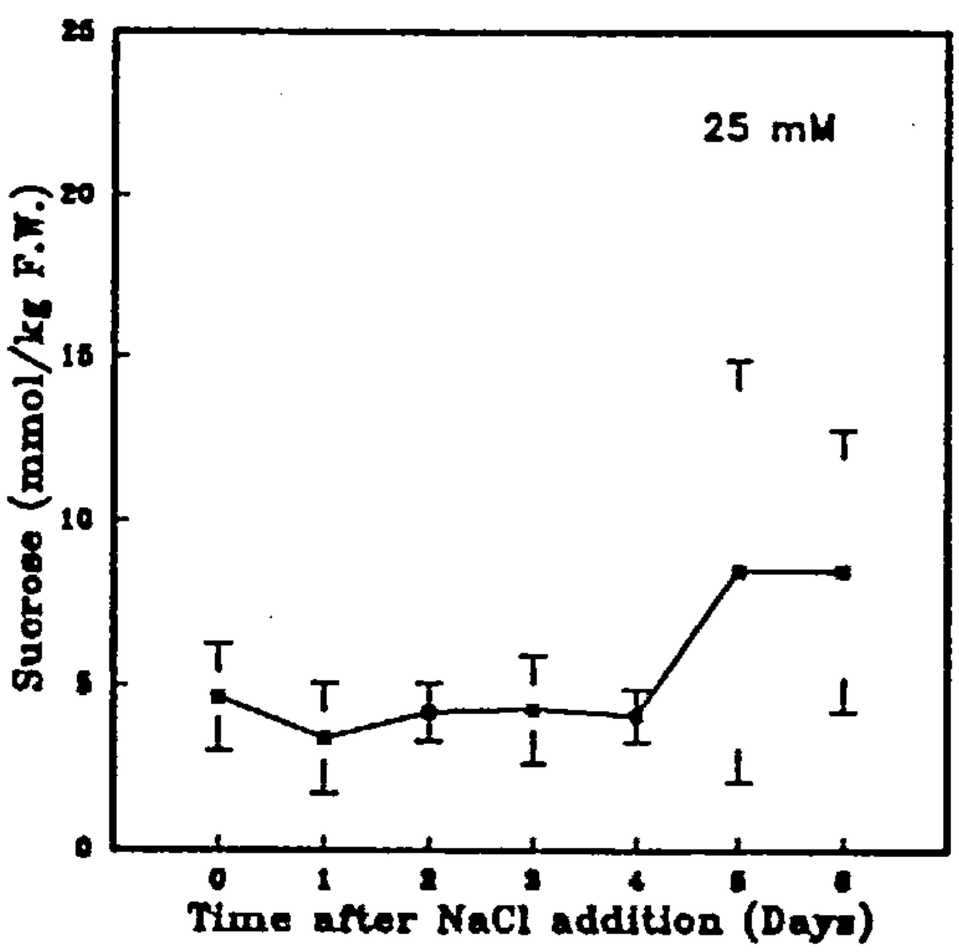
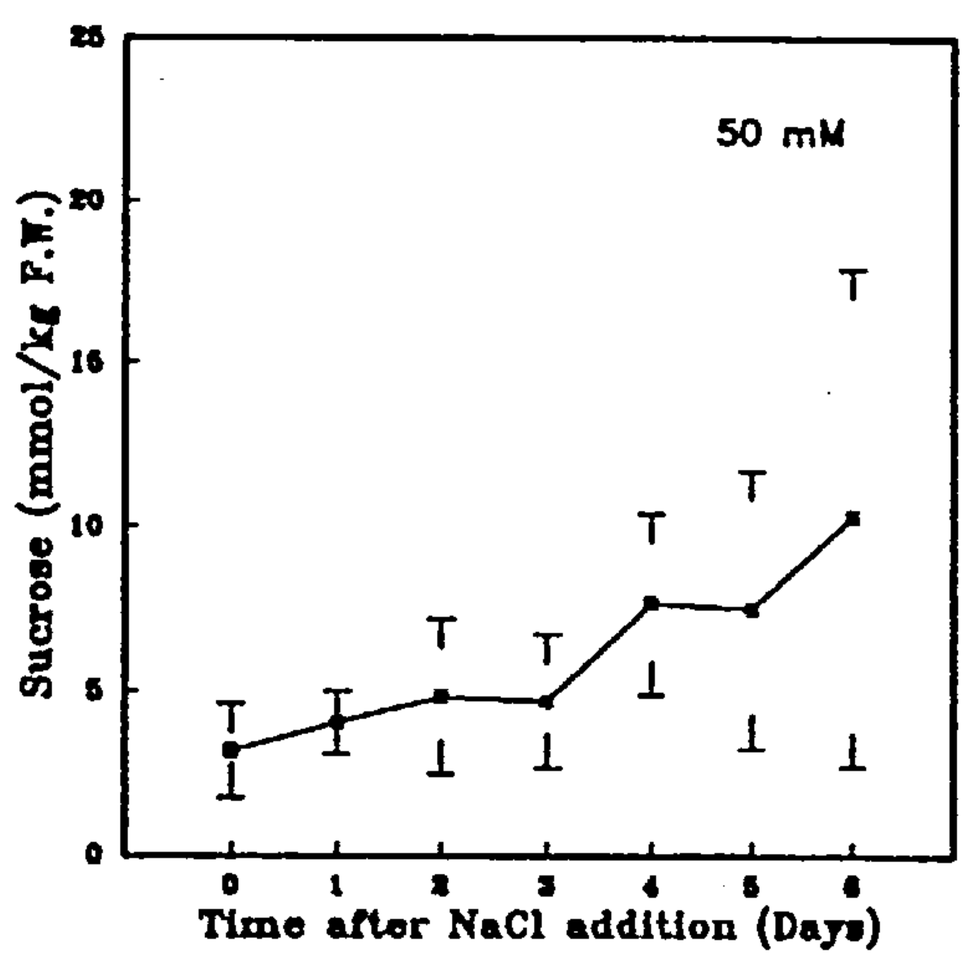
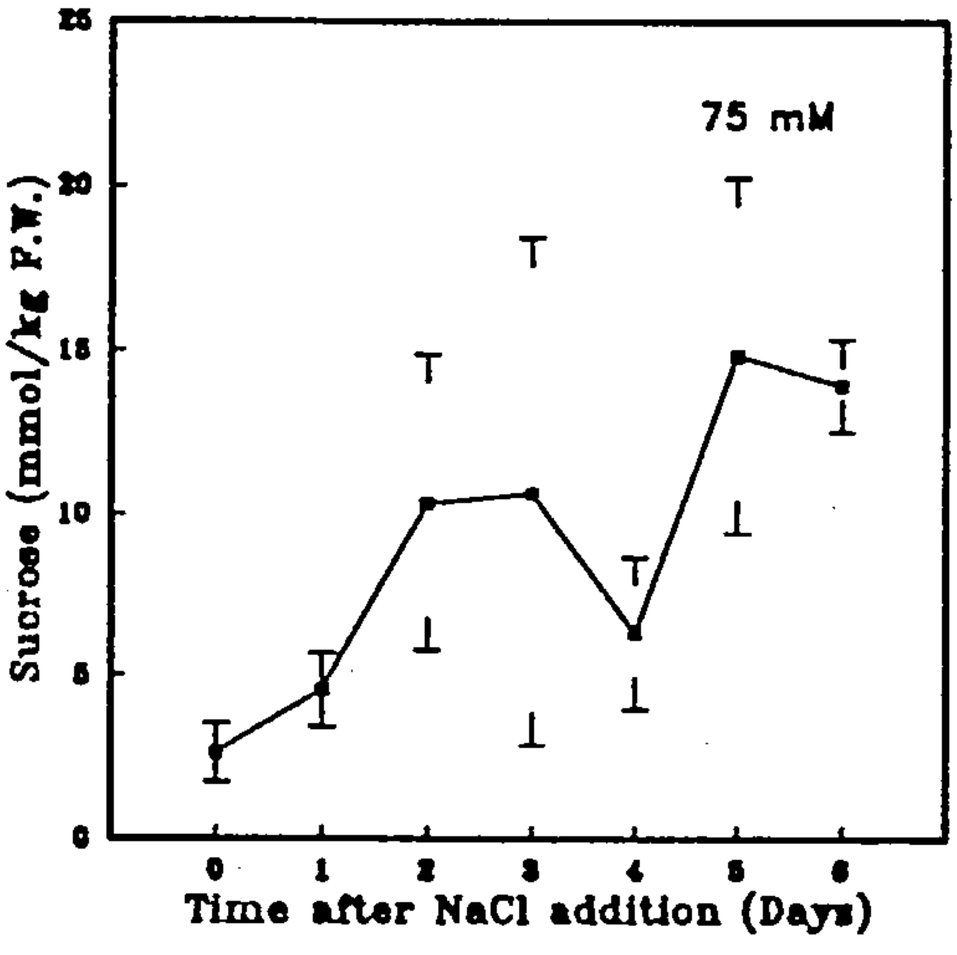

150 mM
Fructose (mmol/kg F.W.)
Time after NaCl addition (Days)

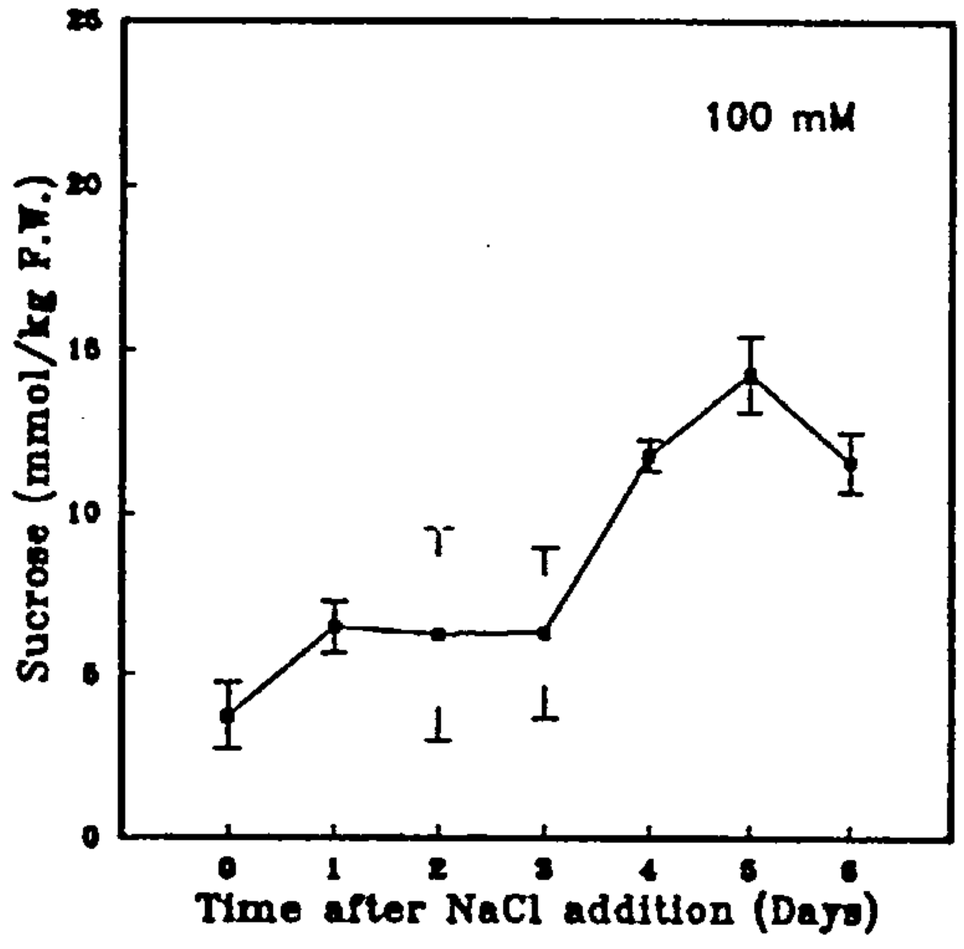
**Fig. 5.18** The concentration of sucrose in the leaf mature zone following NaCl treatment, studied in a long term experiment i.e. for 6 days. Parallel experiment to Fig. 5.10 (for $Na^+$). Sucrose content was measured using G.L.C.

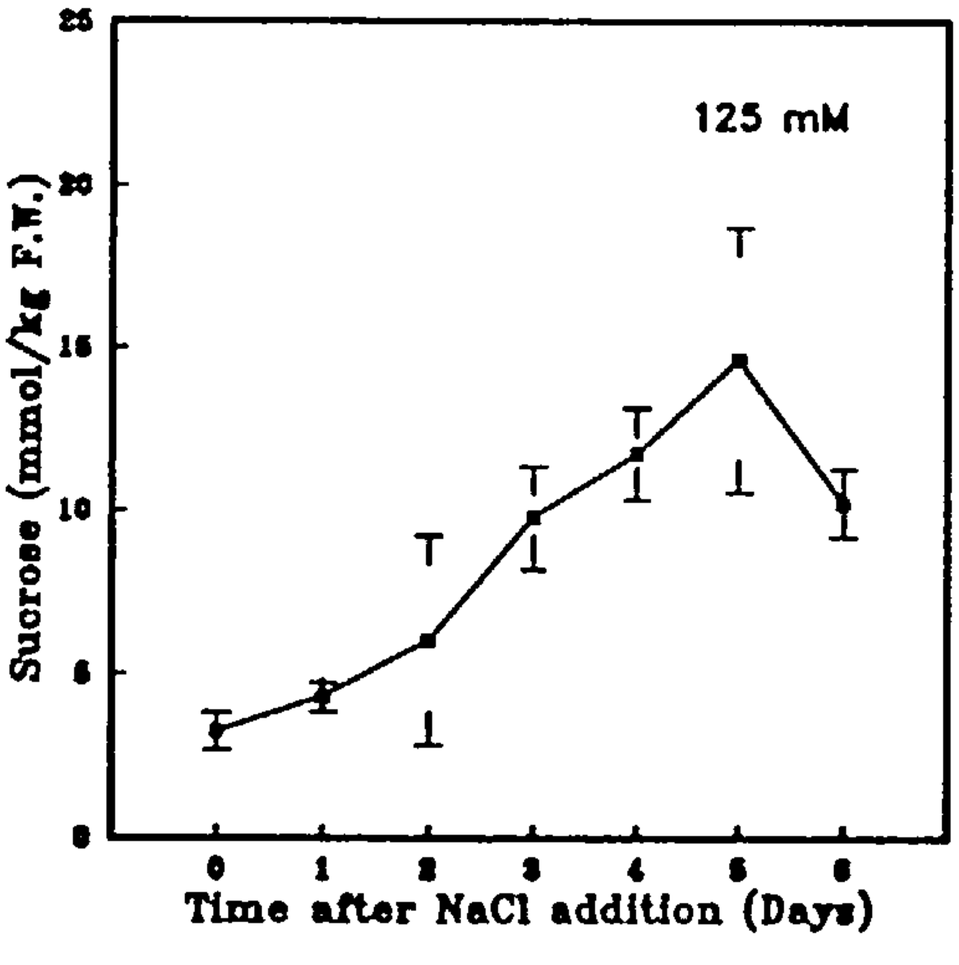

Control
Sucrose (mmol/kg F.W.)
Time (Days)


25 mM
Sucrose (mmol/kg F.W.)
Time after NaCl addition (Days)


50 mM
Sucrose (mmol/kg F.W.)
Time after NaCl addition (Days)


75 mM
Sucrose (mmol/kg F.W.)
Time after NaCl addition (Days)


100 mM
Sucrose (mmol/kg F.W.)
Time after NaCl addition (Days)


125 mM
Sucrose (mmol/kg F.W.)
Time after NaCl addition (Days)

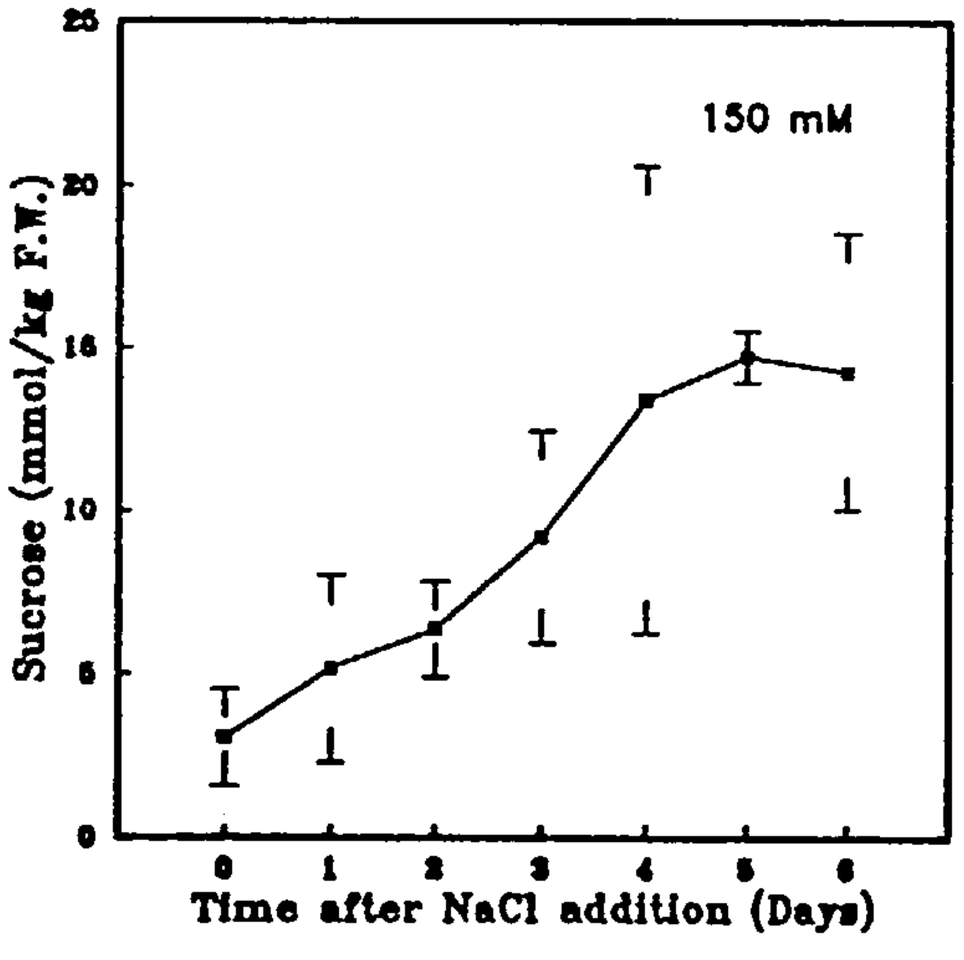
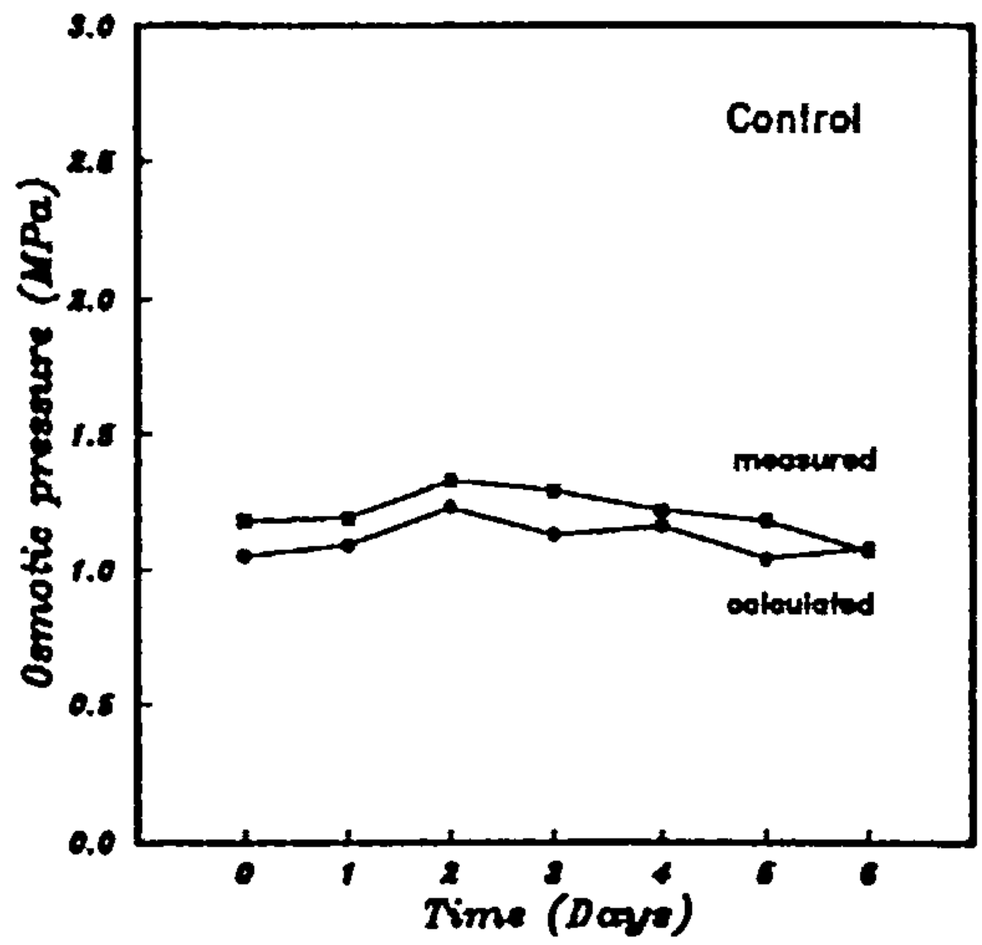
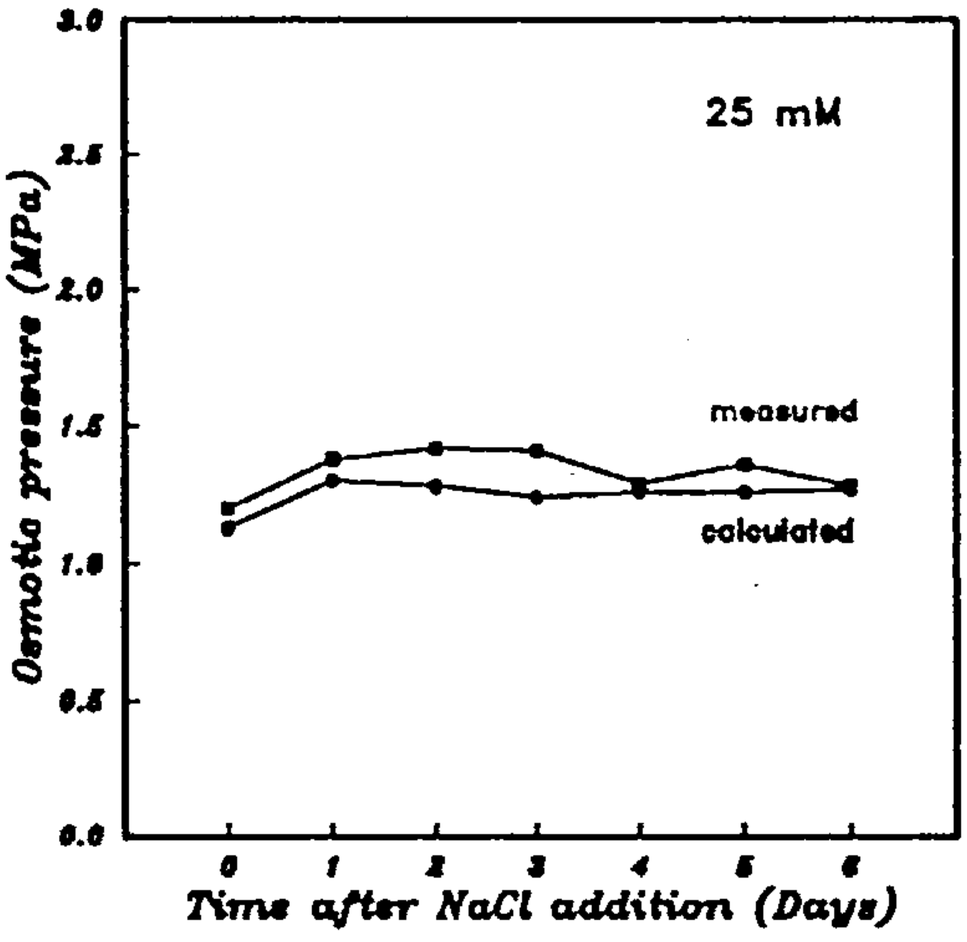
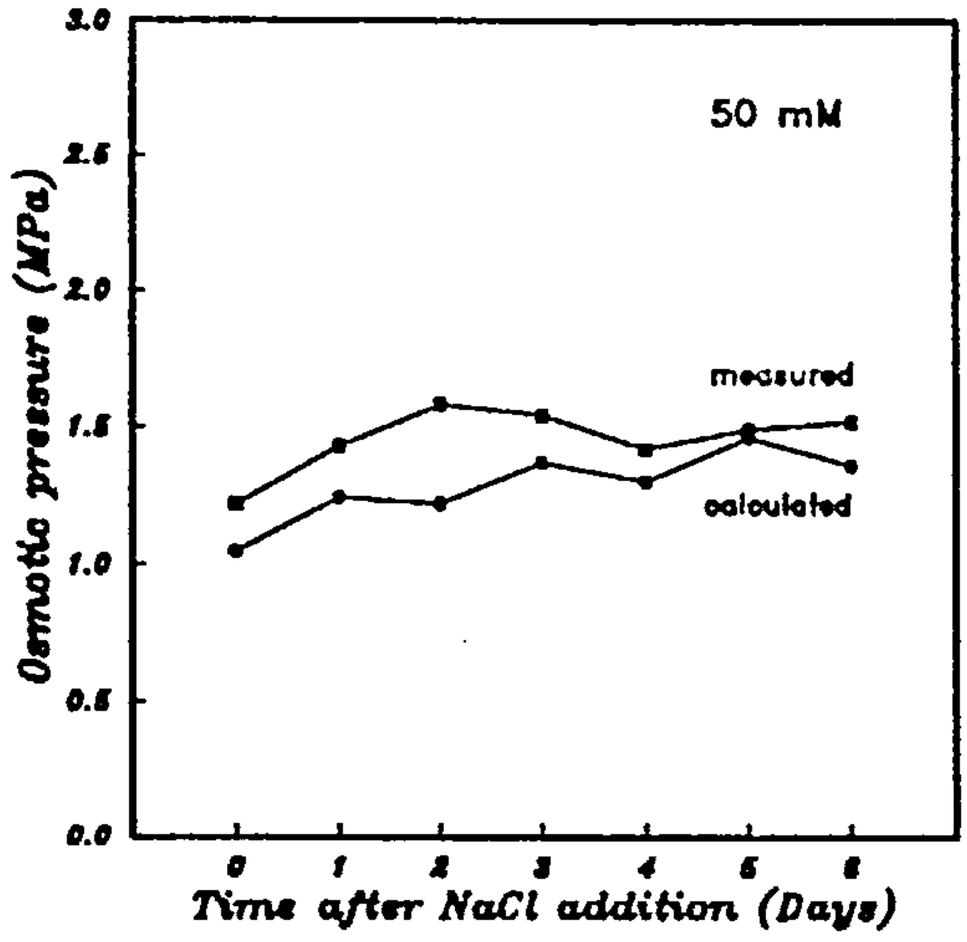
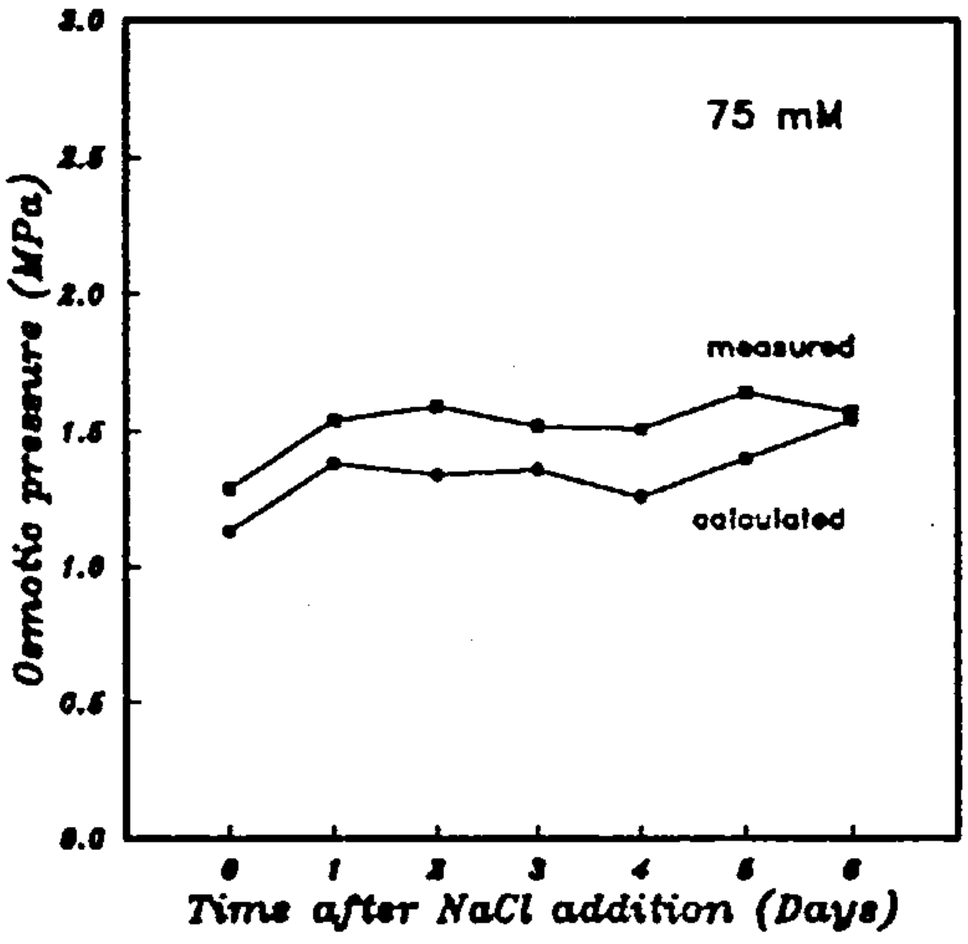

150 mM
Sucrose (mmol/kg F.W.)
Time after NaCl addition (Days)

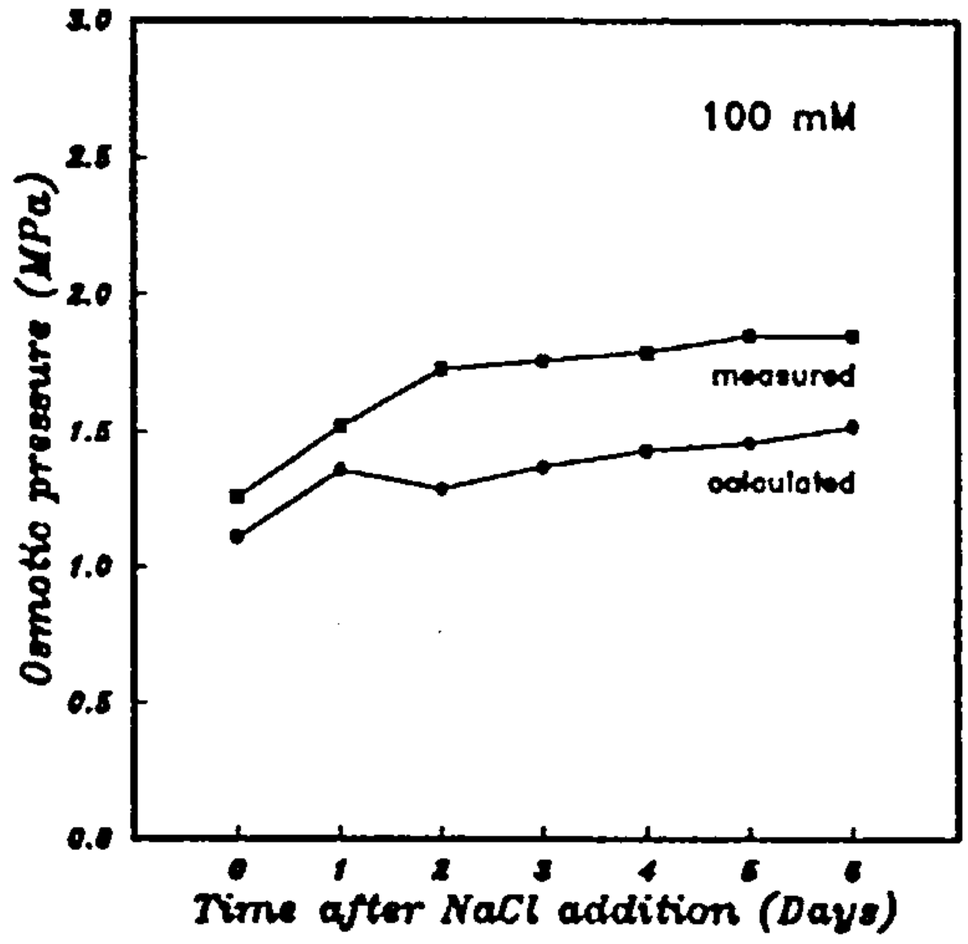
**Fig. 5.19** Comparison of calculated osmotic pressure ($\pi_c$) (sum of concentrations of all the ions and osmotic solutes measured following NaCl treatment, see Figs. 5.10 to 5.18) with the values of osmotic pressure measured ($\pi_m$) in a long term experiment i.e. for 6 days of the stress application (for details see Fig. 5.7).

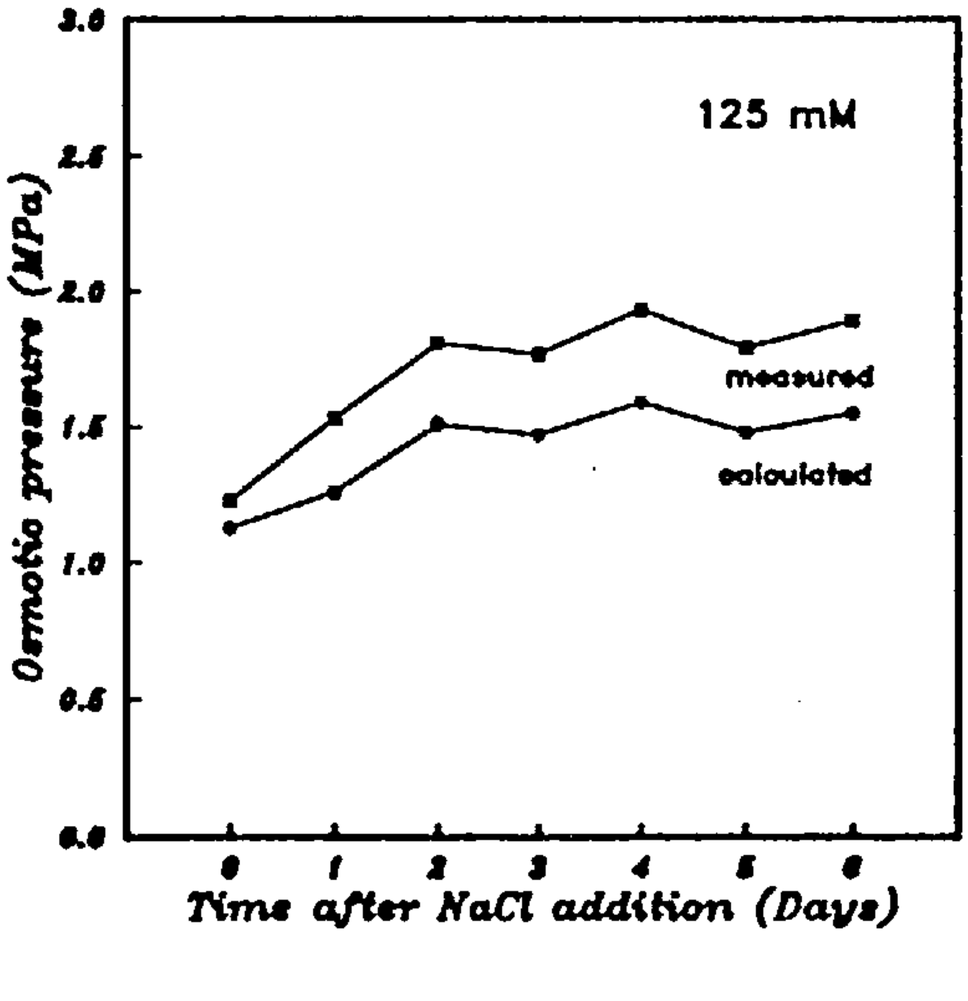

Control
measured
calculated
Osmotic pressure (MPa)
Time (Days)


25 mM
measured
calculated
Osmotic pressure (MPa)
Time after NaCl addition (Days)


50 mM
measured
calculated
Osmotic pressure (MPa)
Time after NaCl addition (Days)


75 mM
measured
calculated
Osmotic pressure (MPa)
Time after NaCl addition (Days)


100 mM
measured
calculated
Osmotic pressure (MPa)
Time after NaCl addition (Days)


125 mM
measured
calculated
Osmotic pressure (MPa)
Time after NaCl addition (Days)

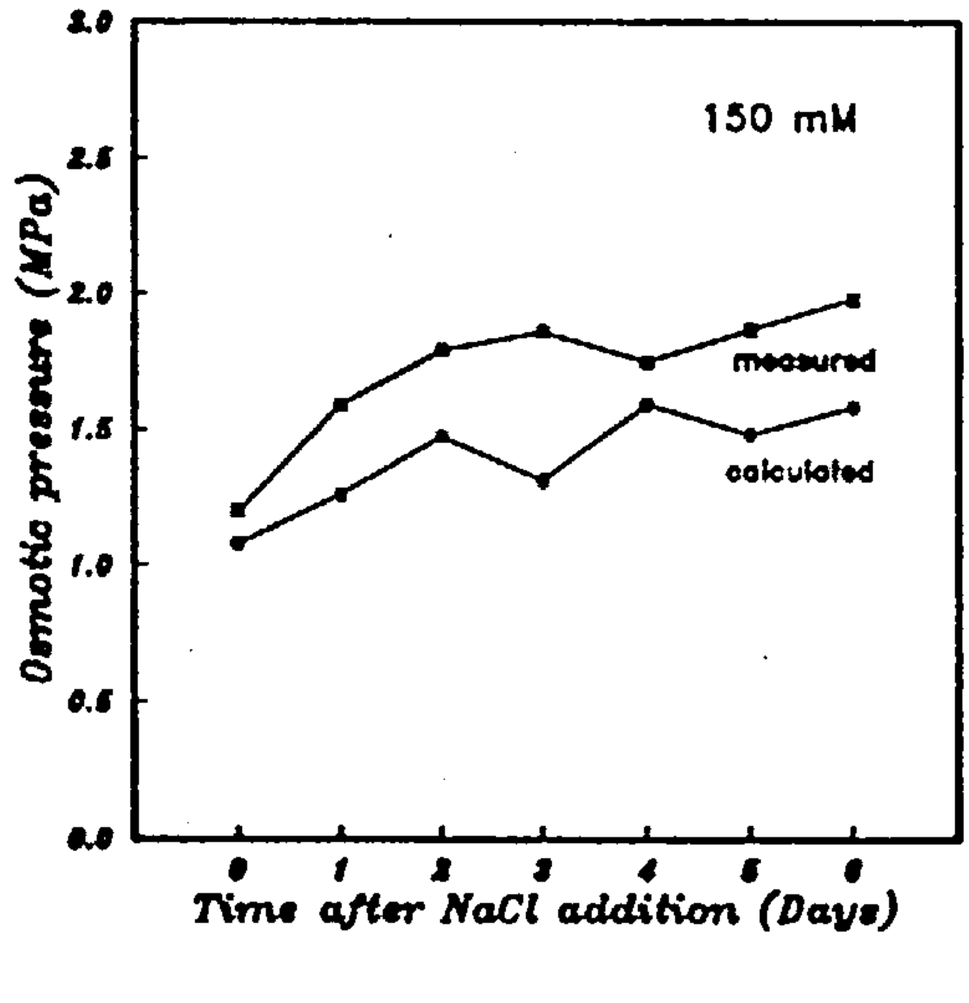

150 mM
measured
calculated
Osmotic pressure (MPa)
Time after NaCl addition (Days)

**Table 5.1 Turgor pressure levels in cells of mature zone of leaf after 11 days of salt stress initiation.**

| NaCl concentration (mM) | Turgor pressure (MPa) |
|---|---|
| 0 | 1.02 ± 0.01 (3) |
| 25 | 1.0 ± 0.04 (3) |
| 50 | 0.98 ± 0.05 (3) |
| 75 | 1.02 ± 0.09 (3) |
| 100 | 0.81 ± 0.04 (3) |
| 125 | 0.79 ± 0.07 (3) |
| 150 | 0.72 ± 0.06 (3) |

**Table 5.2 Osmotic pressure levels in mature zone of leaf after 11 days of salt stress application.**

| NaCl concentration (mM) | O.P. of NaCl levels (MPa) | Osmotic pressure (MPa) |
|---|---|---|
| 0 | 0.00 | 1.18 ± 0.06 (6) |
| 25 | 0.12 | 1.36 ± 0.08 (6) |
| 50 | 0.24 | 1.58 ± 0.08 (6) |
| 75 | 0.36 | 1.81 ± 0.15 (6) |
| 100 | 0.48 | 1.74 ± 0.27 (6) |
| 125 | 0.60 | 1.81 ± 0.26 (6) |
| 150 | 0.72 | 2.54 ± 0.27 (6) |

**Table 5.3 Levels of cell wall solutes in mature zone of leaf after 11 days of salt stress initiation.**

| NaCl concentration (mM) | Cell wall solutes (MPa) |
|---|---|
| 0 | 0.06 |
| 25 | 0.16 |
| 50 | 0.50 |
| 75 | 0.69 |
| 100 | 0.63 |
| 125 | 0.92 |
| 150 | 1.72 |

# CHAPTER 6

# *VARIATION IN SALT TOLERANCE IN DIFFERENT CULTIVARS*

In attempt to assess the suitability of several of the parameters described for Flanders a potential breeding trait for salt tolerance at the seedling stage, the seeds of four different varieties i.e. LU26S, LYP-73, Pb-81 and Indus-79 were grown and their water relations were studied in context of the salt stress. All the varieties exhibit a varying degree of salt tolerance and originated from the Pakistan. These are rated on the basis of their absolute grain yields under salt stress and are classified as tolerant, moderately tolerant, moderately sensitive and sensitive, respectively. The behaviour and recovery of turgor pressure in the leaf mature zone was thought to be a particularly suitable candidate for the purpose, as it occured in previous experiments (see section 5.1.4). All the experiments were performed on long term basis i.e. for 6 d with individual measurements at 1 d intervals. The turgor pressure value under the control conditions was about 0.7 to 0.85 MPa for all the varieties.

## 6.1 Water Relations of the Cultivars

### 6.1.1 Turgor pressure

#### a) Salt tolerant variety

Turgor pressure of mature cells was 0.83 $\pm$ 0.04 MPa in control plants of LU26S, a salt tolerant variety (Fig. 6.1). No drop in response to 25 mol $m^{-3}$ NaCl was observed. Presumably it would have dropped immediately in response to the salt stress (as it was observed in the Flanders, see section 3.3.1) but it recovered before the first measurement that was after 1 d. A slight decline

was observed in 50 mol $m^{-3}$ NaCl but it seemed to be temporary as turgor pressure was almost recovered at the 6 d. In contrast to the behaviour of Flanders in case of the final four concentrations turgor pressure once dropped did not recover completely even by 6 d.

b) Moderately salt tolerant variety

A quite different turgor pressure response was studied for the moderately salt tolerant variety, LYP-73. Its value was almost equal to 0.8 ± 0.05 MPa in the control plants (Fig. 6.2). On the addition of 25 and 50 mol $m^{-3}$ NaCl any turgor pressure recovery was completed before the first measurement and remained almost uniform for all the experimental period. The recovery process took longer for the final four concentrations and the time taken was proportional to the amount of the salt present externally. However, in contrast to the situation with the previous cultivar (LU26S) the turgor pressure was almost completely recovered at the 6 d of the stress application, although, this was slower than the response with Flanders.

c) Moderately salt sensitive variety

The moderately salt sensitive variety, Pb-81, showed different response. The control value of turgor pressure was 0.75 ± 0.06 MPa (Fig. 6.3). The turgor pressure recovery was completed within the first 24 h and no subsequent variations could be detected in turgor pressure, level for remainder of the experiment. This response was studied for 25, 50, 75 and 100 mol $m^{-3}$ NaCl present

in the root media. The recovery process started after 2 d for 125 mol $m^{-3}$ and was almost completed by the 6 d. In case of the 150 mol $m^{-3}$ turgor pressure declined gradually for 3 d and later it started increasing steadily but had not recovered fully by the end of the experiment. If the experiment was prolonged the complete turgor recovery might possibly have observed.

**d) Salt sensitive variety**

In salt sensitive variety, Indus-79, slightly higher turgor pressure was measured for the control plants i.e. 0.8 ± 0.05 MPa (Fig. 6.4). In the presence of lower salt concentrations (25, 50 and 75 mol $m^{-3}$) any decrease in turgor pressure recovered within 24 h and no subsequent changes could be observed in its level. For 100 mol $m^{-3}$ turgor pressure was recovered within 2 d of the stress onset. Turgor pressure did not recover completely in case of 125 and 150 mol $m^{-3}$ NaCl for whole of the experimental time. This behaviour was remarkably similar to that of the most salt tolerant variety, LU26S.

### 6.1.2 Osmotic pressure

The osmotic pressure of all the varieties was also measured under the same conditions as in the previous experiments (section 6.1.1). The measurements were carried out on whole tissue basis in the mature zone of leaf.

The osmotic pressure values were about 1.0 ± 0.02 MPa in the leaf mature zone of LU26S (Fig. 6.5). This value was slightly lower

for LYP-73 than all other varieties i.e. 0.85 ± 0.08 MPa (Fig. 6.6). The other two varieties Pb-81 and Indus-79 were quite similar in their osmotic pressure levels which was approximately 0.9 ± 0.1 MPa (Figs. 6.7 and 6.8). Under the stressed conditions all these varieties (together with Flanders) showed similar responses in terms of continued increase in tissue osmotic pressure with the time. In case of lower NaCl concentrations a small and steady increase in $\pi$ was observed. This increase was, however, more sharp in the same context. Anyhow, the extent and magnitude of the increase in $\pi$ was almost proportional to the external salt concentration and to the time for which plants were kept in the salinized media.

### 6.1.3 Cell wall transpiration tension

The cell wall transpiration tension ($P_w$) in the leaf mature zone was not determined for these varieties. However, in order to allow an estimation of the cell wall osmotic pressure it was assumed that the magnitude of $P_w$ is similar to that in the same zone of the Flanders i.e. about 0.1 MPa (Fig. 5.8).

### 6.1.4 Cell wall solutes

The osmotic pressure of the cell wall solutes was determined by the difference of the values of turgor pressure, osmotic pressure and assumed $P_w$ present in the leaf mature zone (as described in section 5.1.8). All the varieties exhibited similar trends regarding the contents of cell wall solutes, $\pi_w$. After the 6 d of the stress application the $\pi_w$ levels corresponded to the NaCl

concentration present in the external media (Fig. 6.9). Thus, the magnitude of $\pi_w$ in all the four cultivars was approximately similar to the $\pi_w$ level of the Flanders (Fig. 5.9).

## 6.2 Water Relations in Growing Zone of Salt Tolerant Variety

The same parameters were studied in the growing zone of salt tolerant variety, LU26S, in short and long term experiments. The behaviour of turgor pressure was observed in the salt stressed plants. Only 4 different treatments were applied to the plants i.e. control, 50, 100 and 150 mol $m^{-3}$ NaCl.

### 6.2.1 Turgor pressure

The turgor pressure value for the control plants was 0.35 ± 0.01 MPa (Fig. 6.10). It is slightly lower than the turgor pressure of the Flanders. As with Flanders no obvious changes in the turgor pressure could be measured in response to the salt stress, over the whole experimental period.

When same experiments were carried out for longer time span i.e. 6 d, the results obtained (Fig. 6.11) were quite uniform and they were again similar to those of Flanders (Fig. 5.1). The turgor pressure was unchanged for all the NaCl concentrations with time.

### 6.2.2 Osmotic pressure

The osmotic pressure of whole tissue was also determined under the similar experimental conditions in long term experiments. The control plants' growing zone had osmotic pressure equivalent to

the 1.15 ± 0.1 MPa (Fig. 6.12). In the salt stressed plants the osmotic pressure level increased linearly with increasing the external media osmotic pressure and with the time, as well. A sudden increase was observed in it within 2 d of the addition of 100 and 150 mol $m^{-3}$ NaCl to the root media. Later, no obvious fluctuations could be detected in its behaviour . The extent of osmotic pressure adjustment in the leaf growing zone was higher than that of the mature zone. (Figs. 6.5 and 6.12).

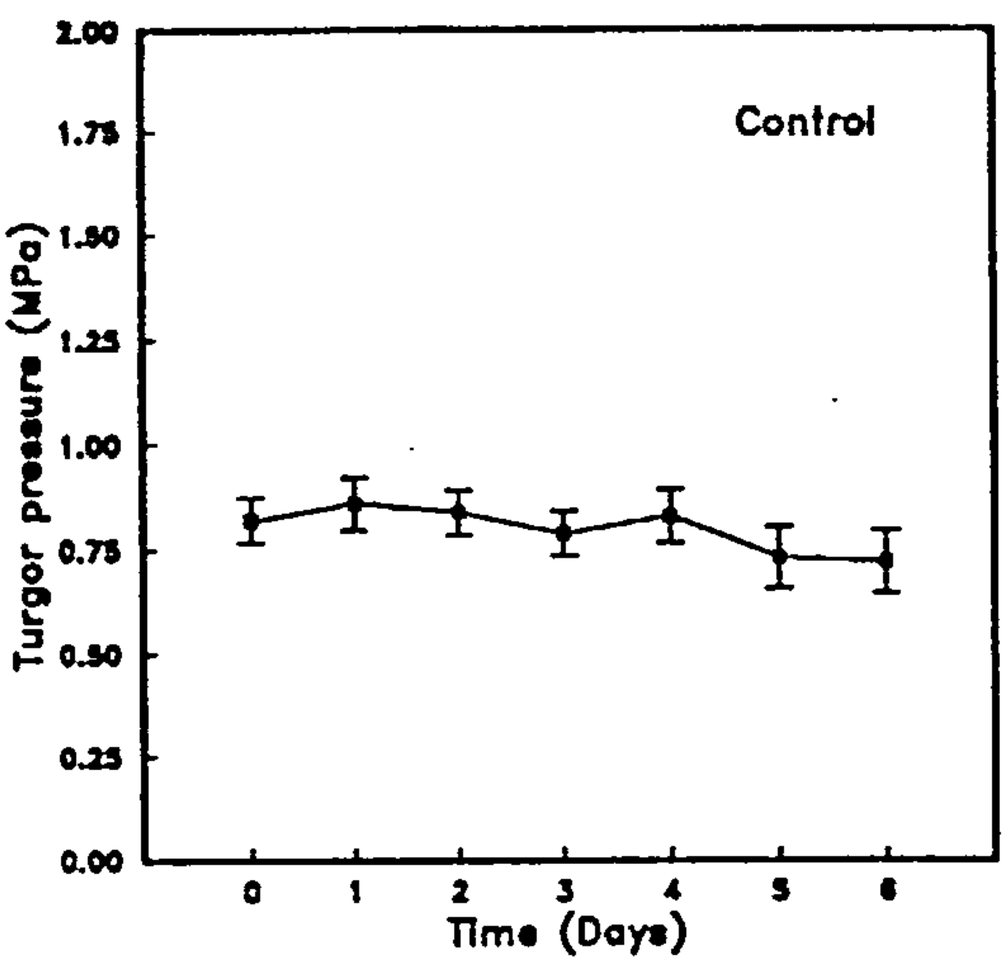
### 6.2.3 Cell wall transpiration tension

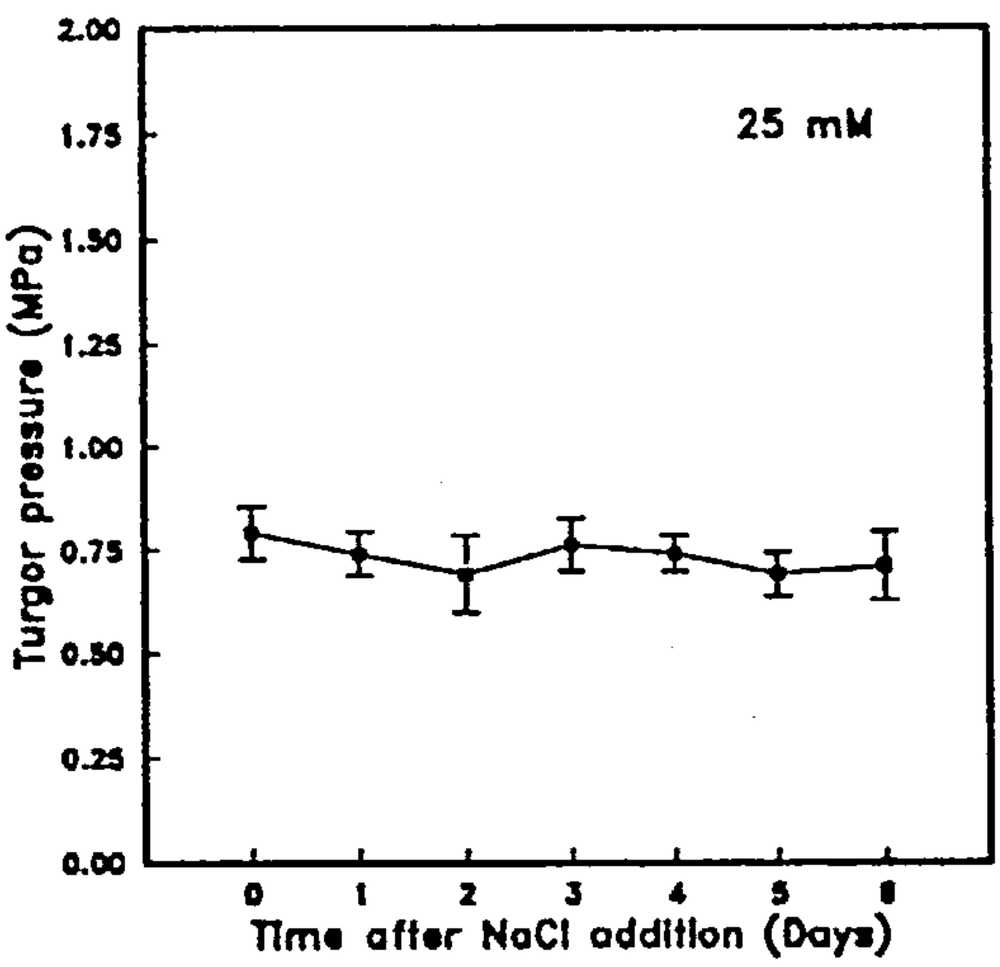
The cell wall transpiration tension in the growing zone, $P_w$, was assumed to be the same as for Flanders (Fig. 5.8) to allow an estimate of the wall solute osmotic pressure to be made.

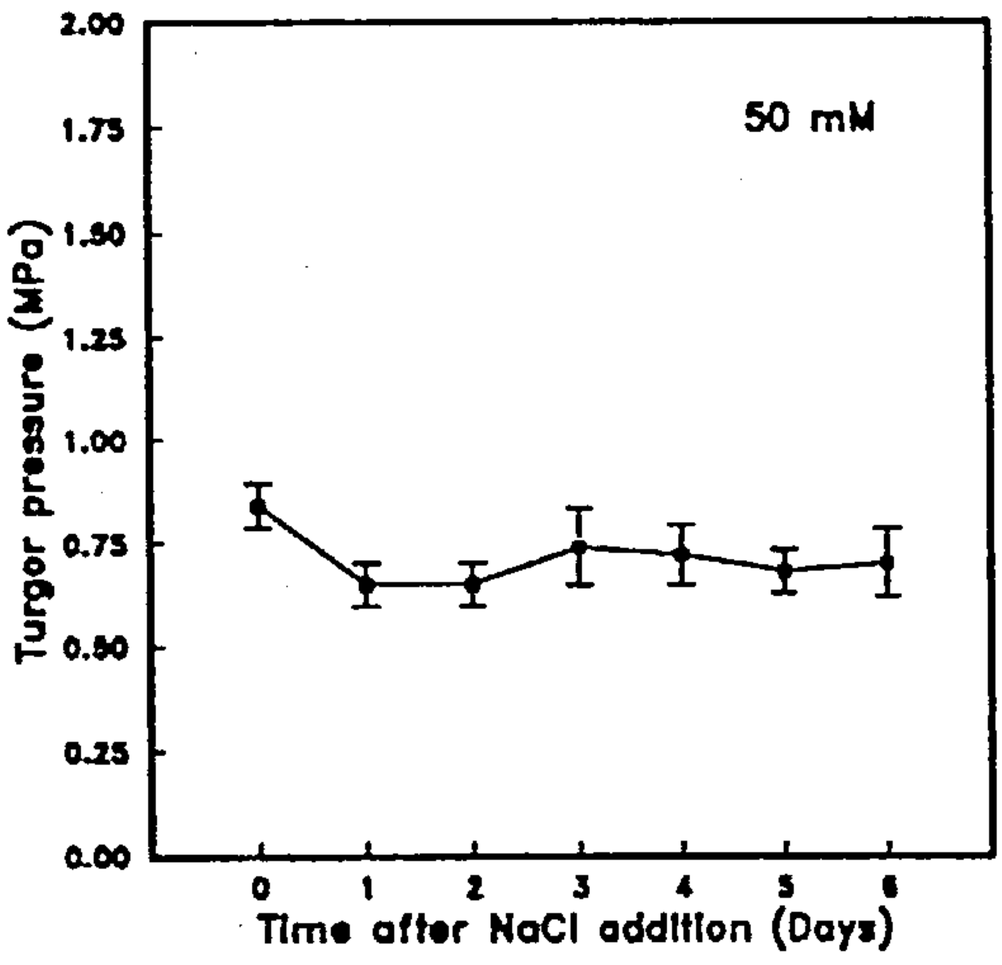
### 6.2.4 Cell wall solutes

The cell wall osmotic pressure ($\pi_w$) was calculated for LU26S (see section 5.1.7). For the control conditions, the $\pi_w$ did not change for all the experimental time and was almost uniformally equivalent to 0.55 ± 0.03 MPa (Fig. 6.13). Whilst, it rose steadily with the time for the plants stressed with different concentrations of NaCl. At the 6 d significant amounts of the osmotically active solutes were present in the cell walls of the growing zone. The magnitude of it was even higher than the amount of the solutes present in the external media for 50 and 150 mol $m^{-3}$ NaCl, and was, however, corresponding qualitatively to the external levels of salinity.

**Fig. 6.1** The response of turgor pressure to the NaCl stress studied in LU26S, a salt tolerant wheat variety, in a long term experiment i.e. 6 days. Turgor pressure was measured using pressure probe in the mature cells of leaf. NaCl treatments as in Fig. 3.3. Each point is the mean of 5-10 replicates.


Control
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time (Days)


25 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Turgor pressure (MPa)
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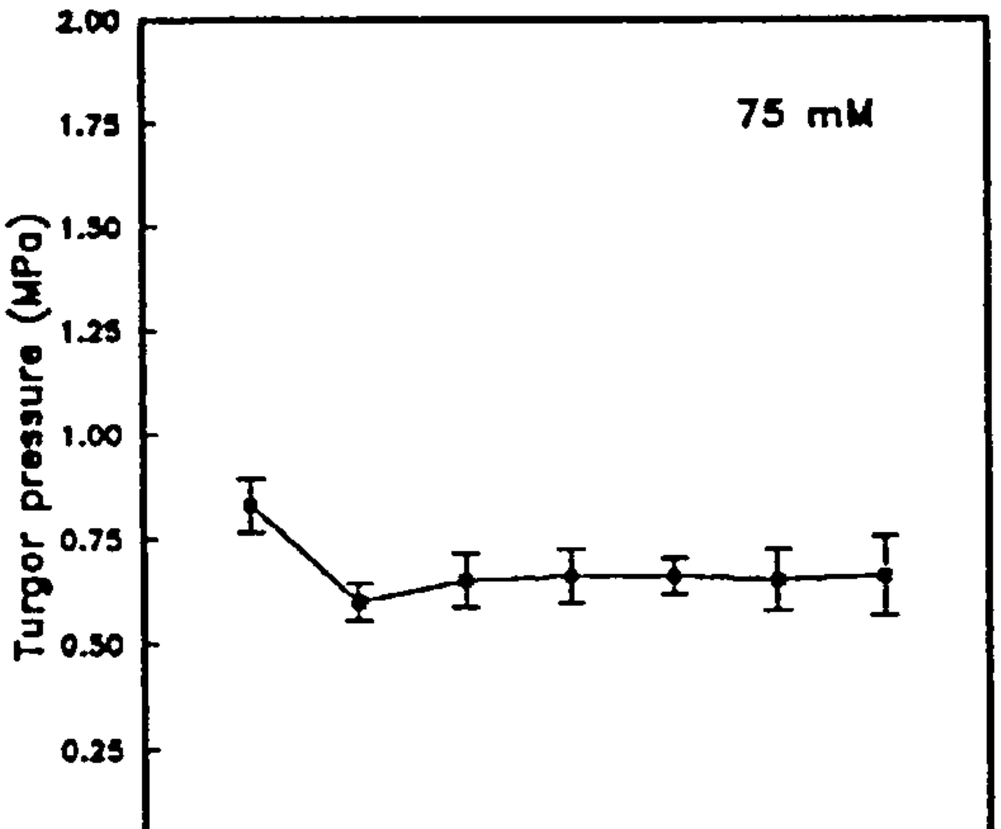
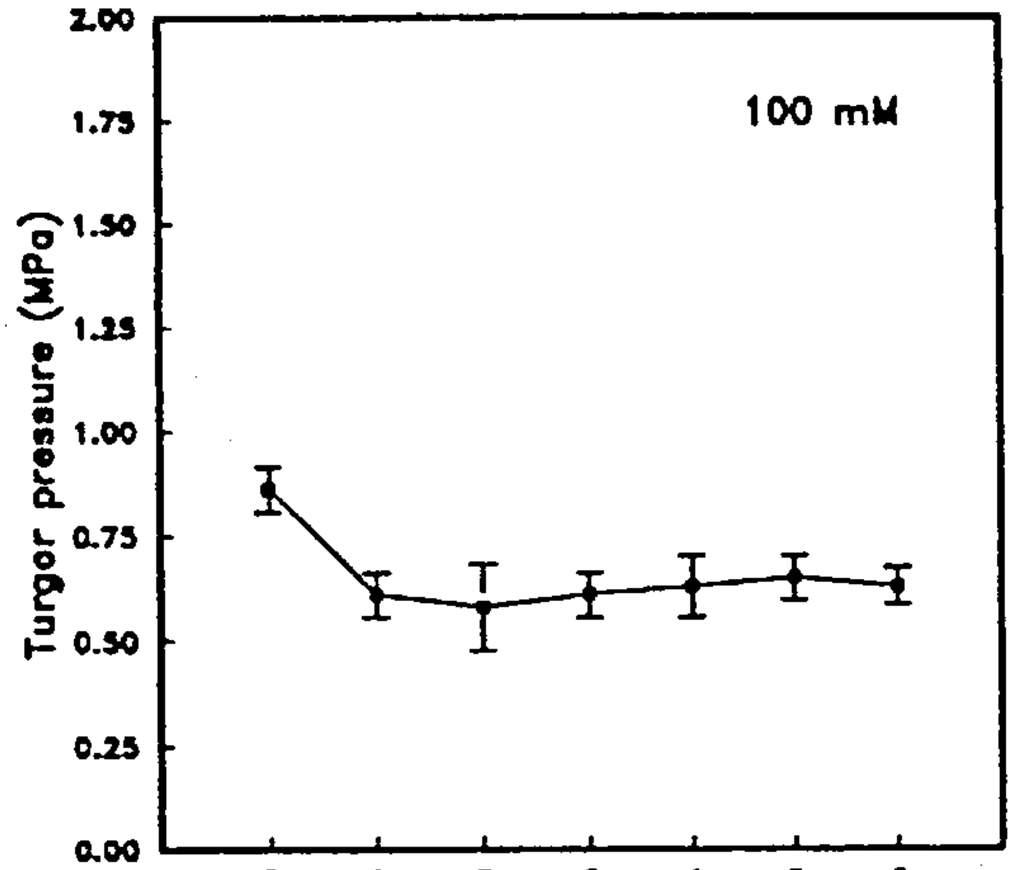
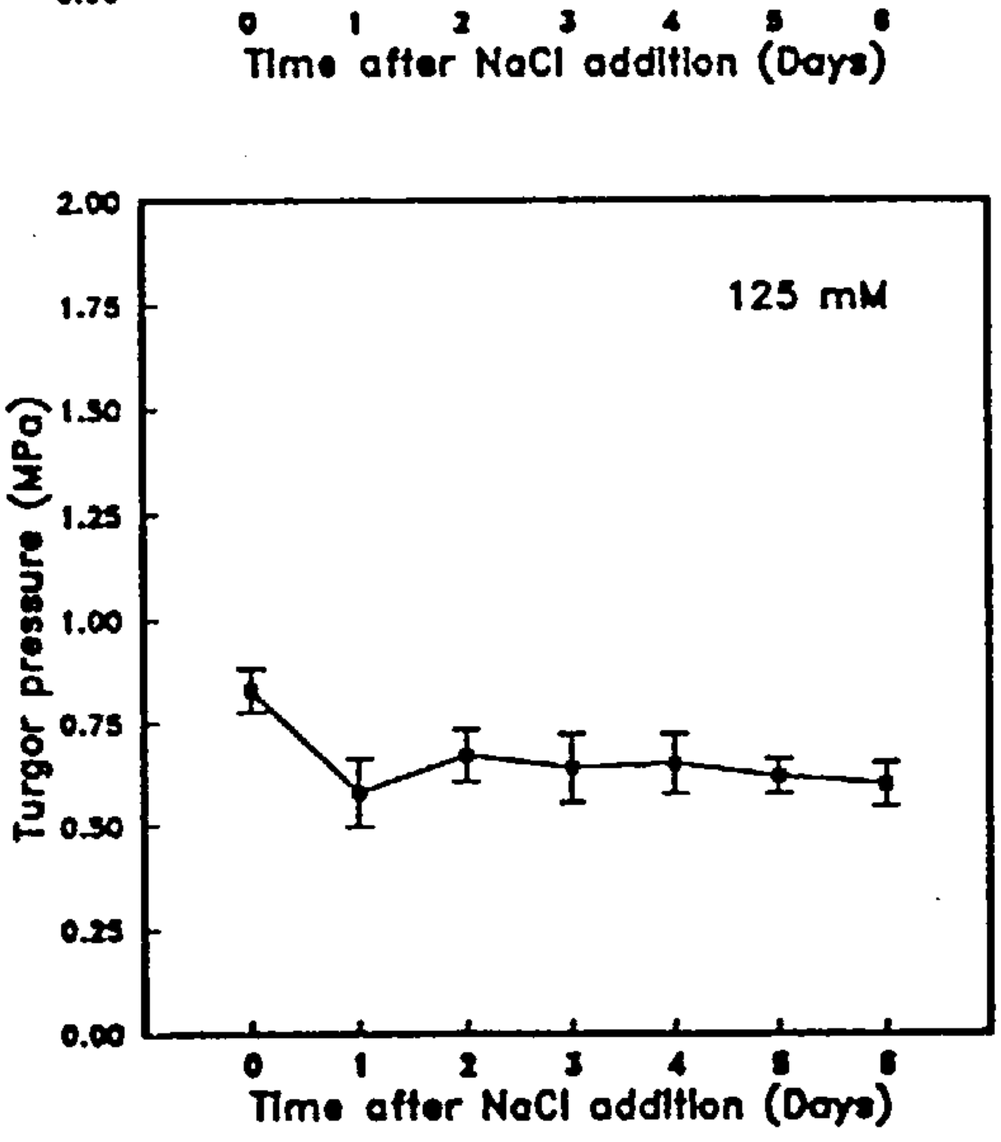
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Turgor pressure (MPa)
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1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

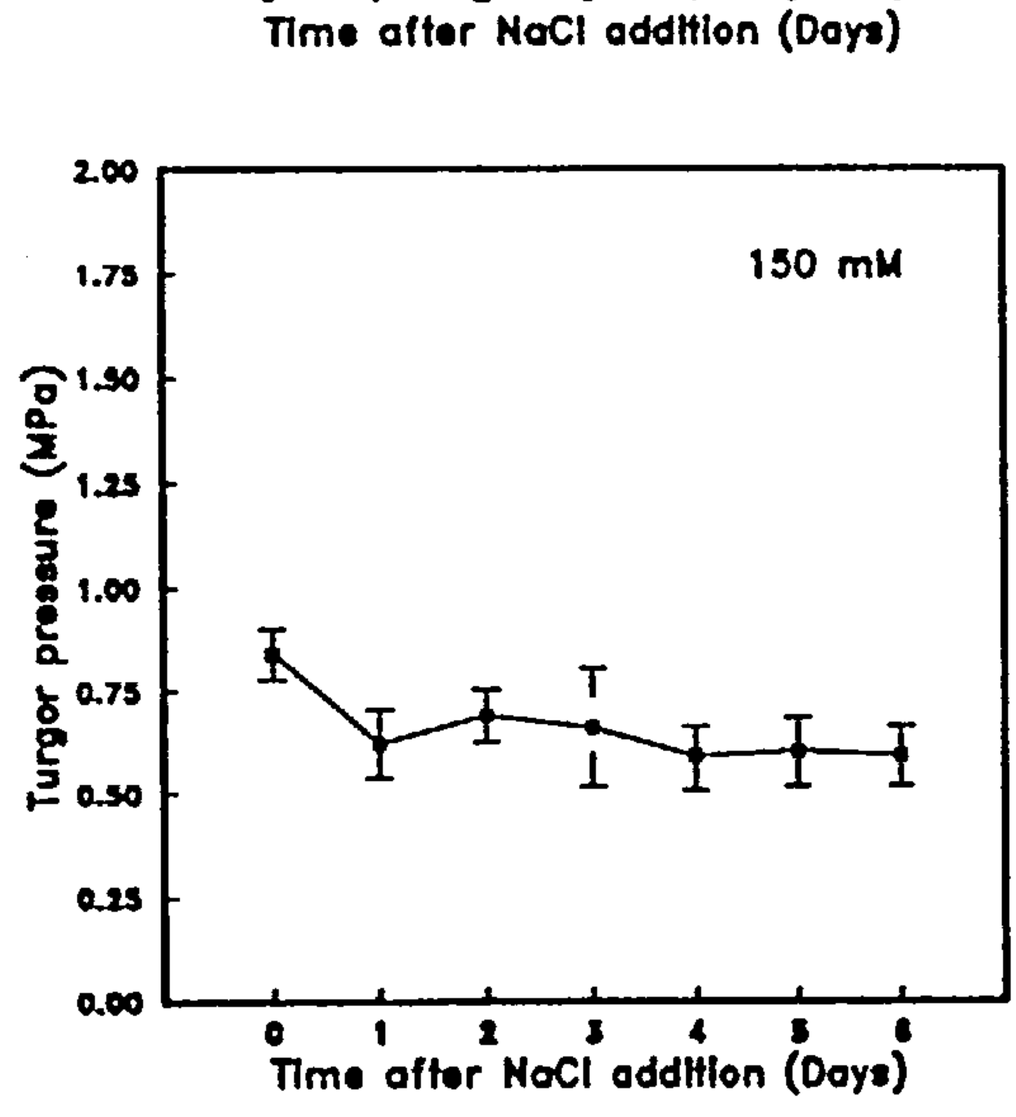
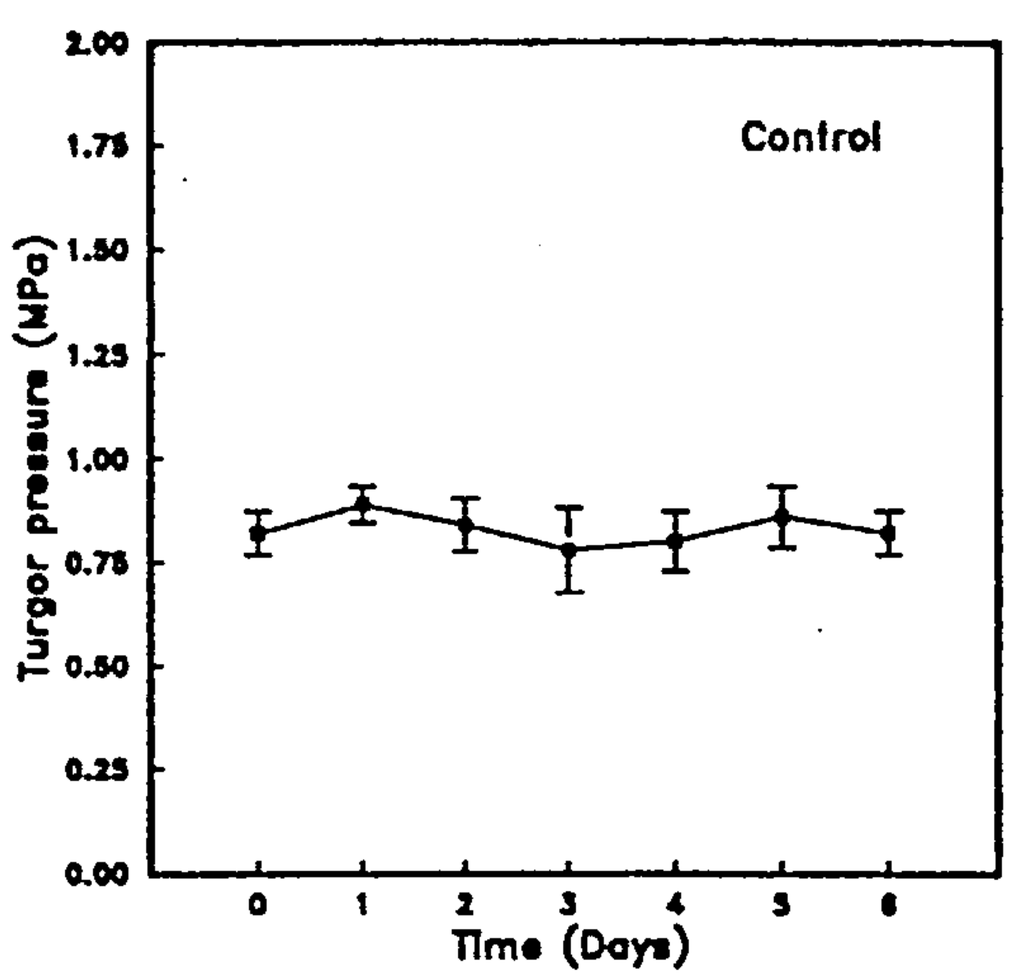
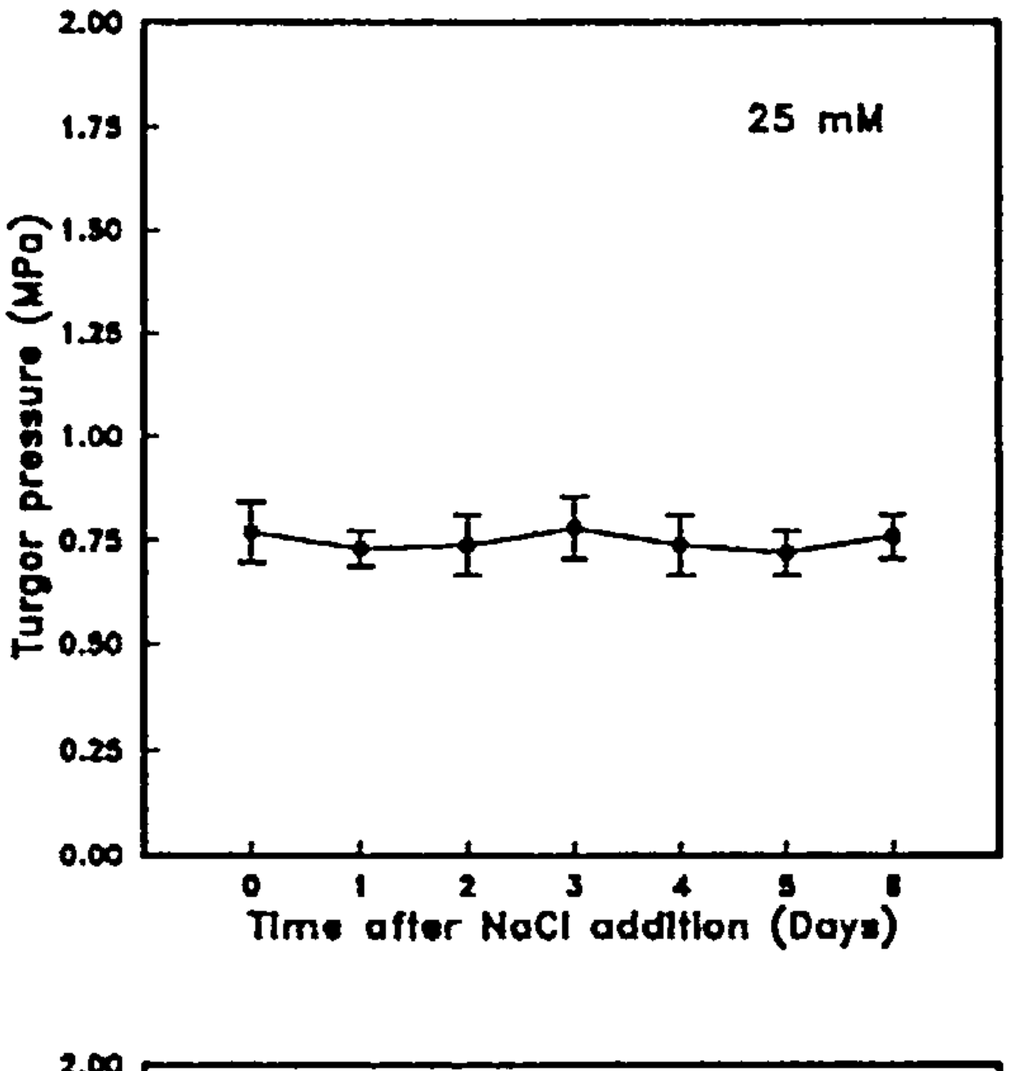
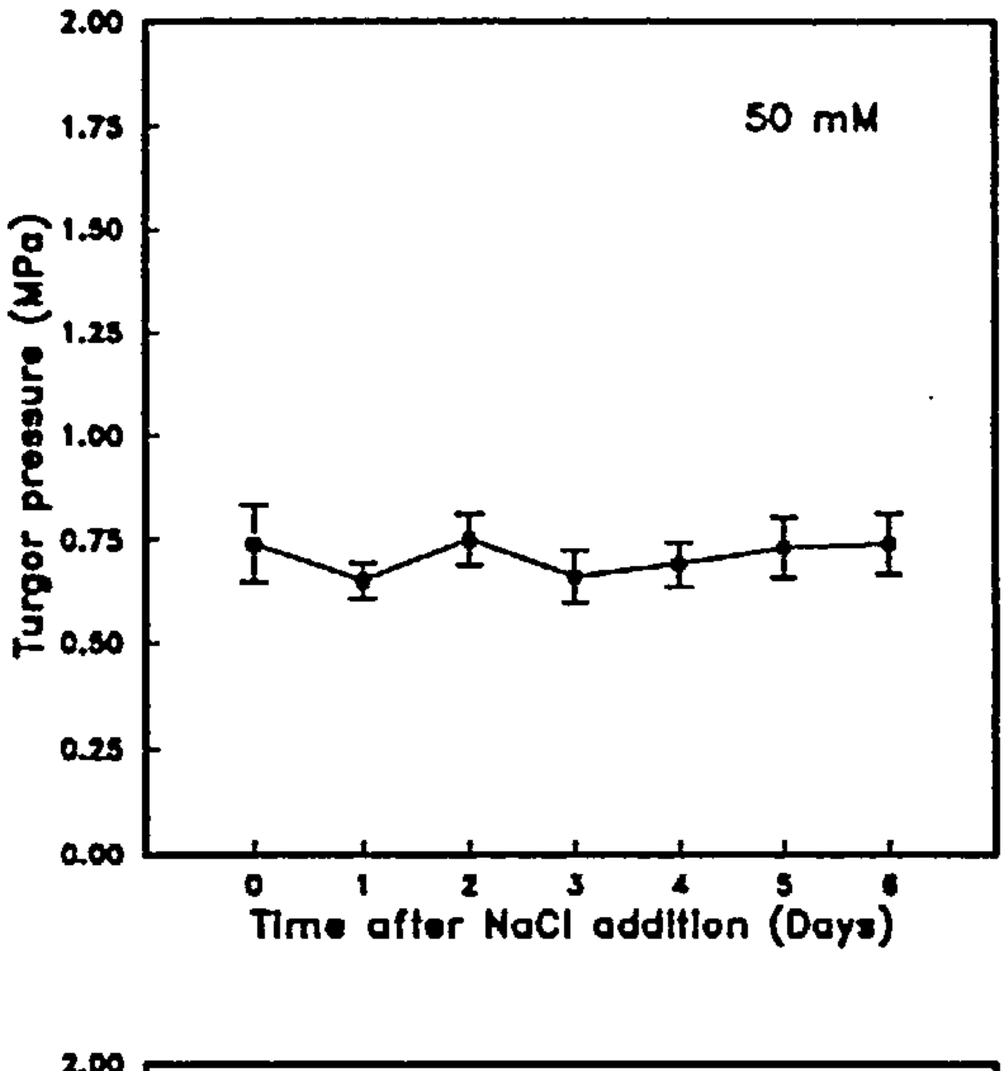
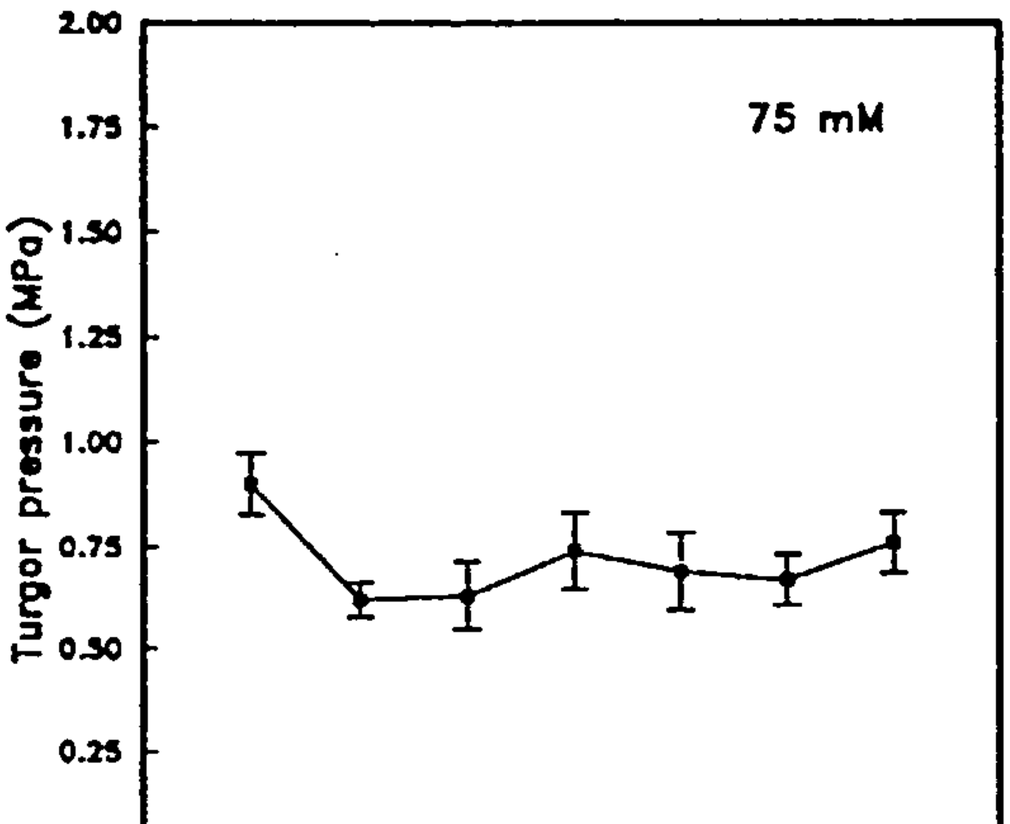

150 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

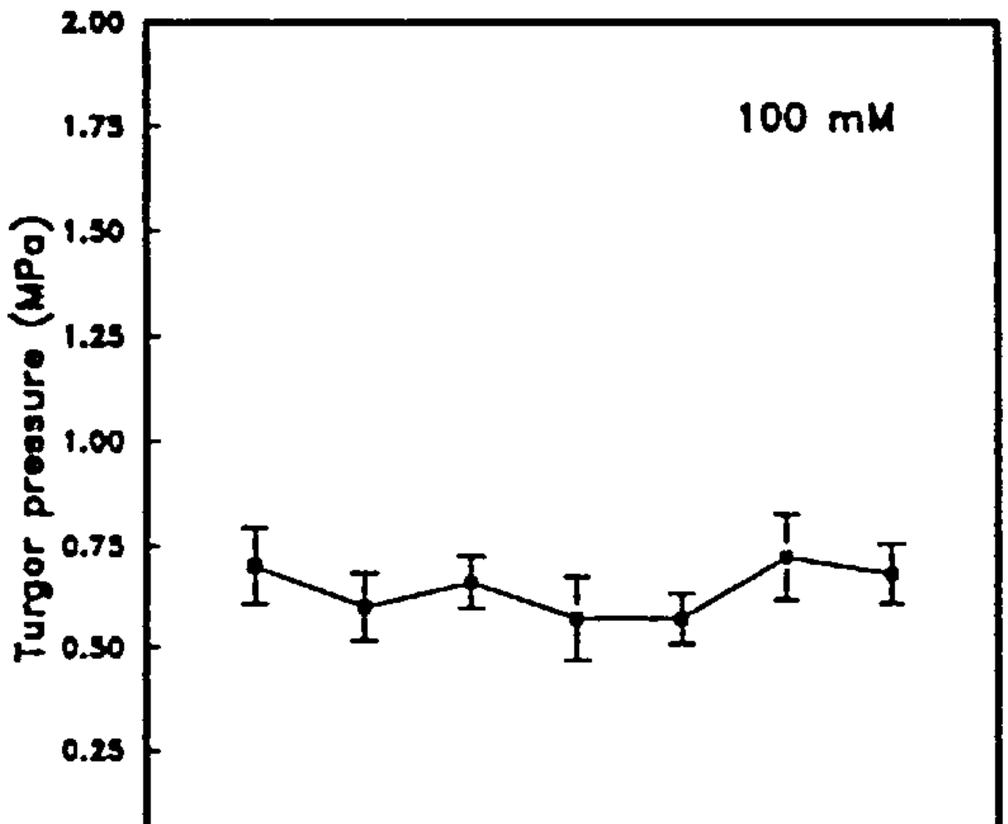
**Fig. 6.2** The response of turgor pressure to the NaCl stress studied in LYP-73, a moderately salt tolerant wheat variety, in a long term experiment i.e. 6 days. Parallel experiment to Fig. 6.1.

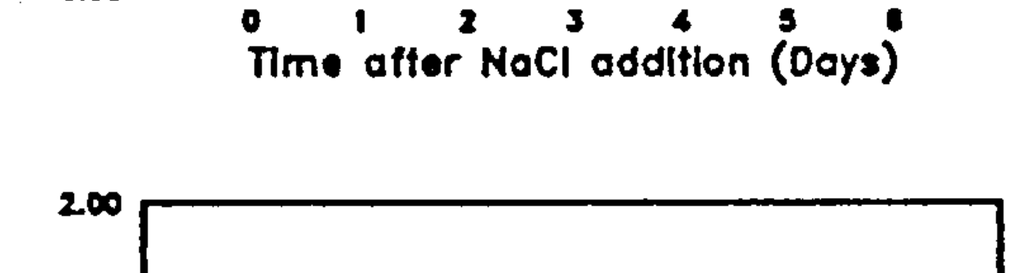

Control
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time (Days)


25 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Turgor pressure (MPa)
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1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

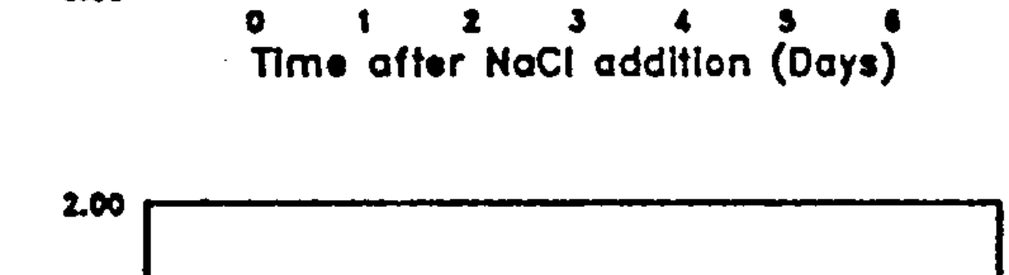
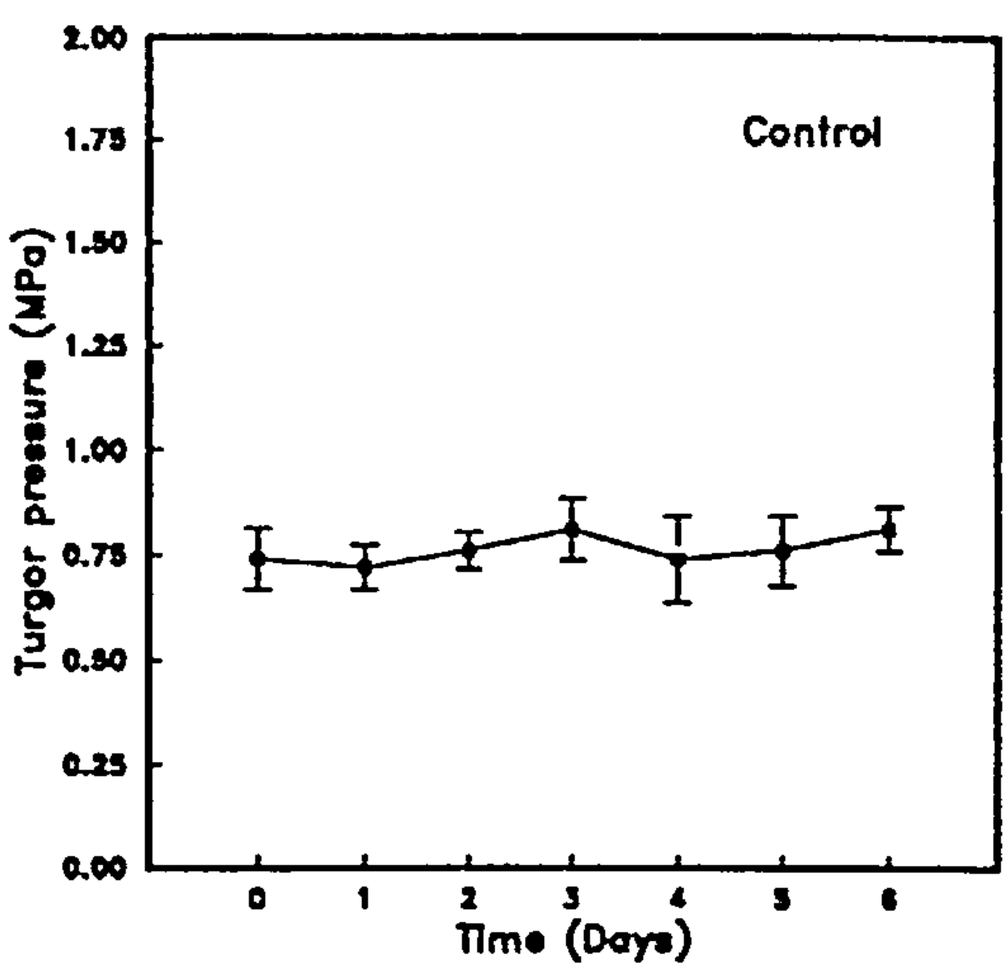
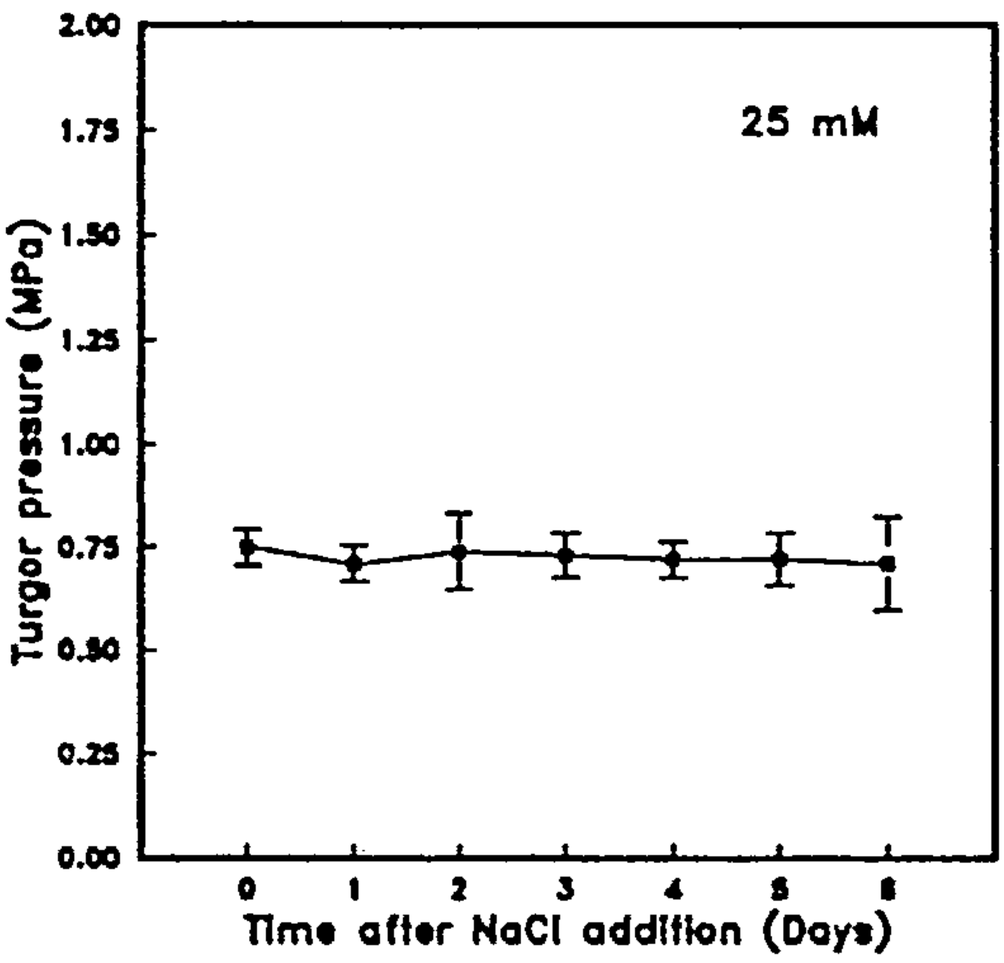
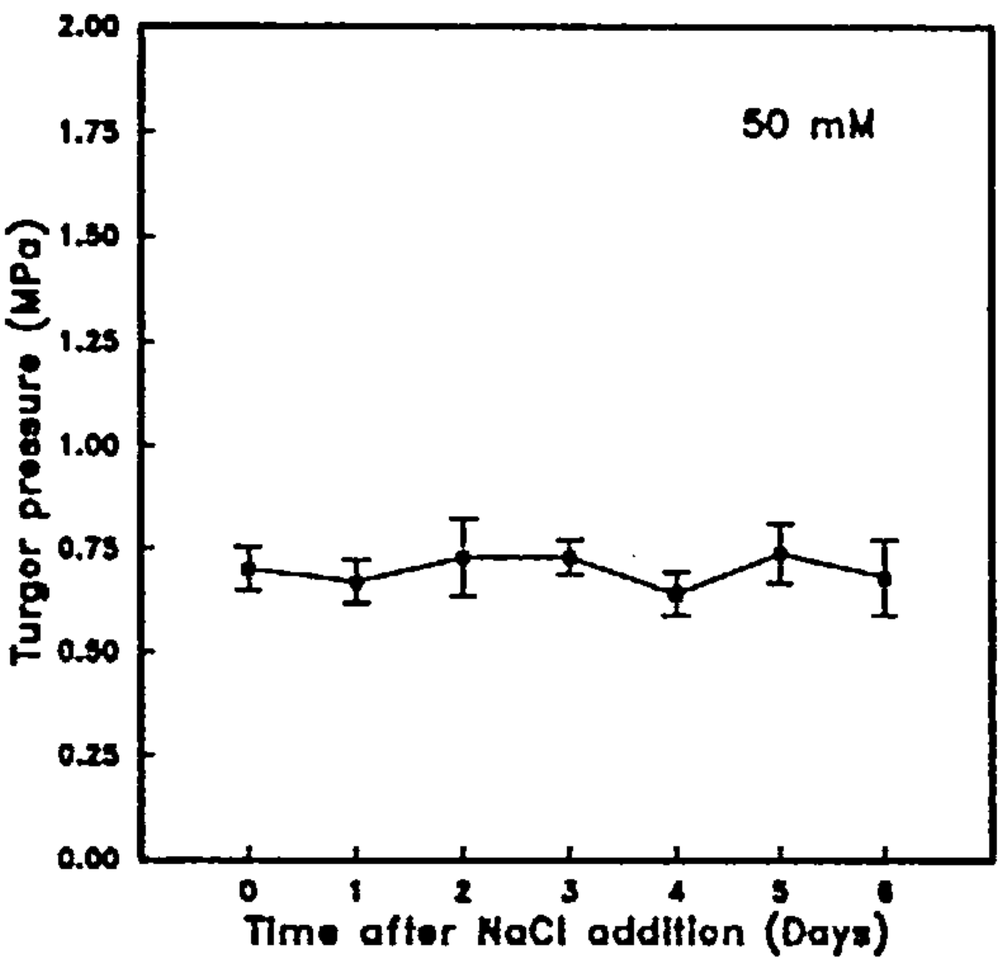
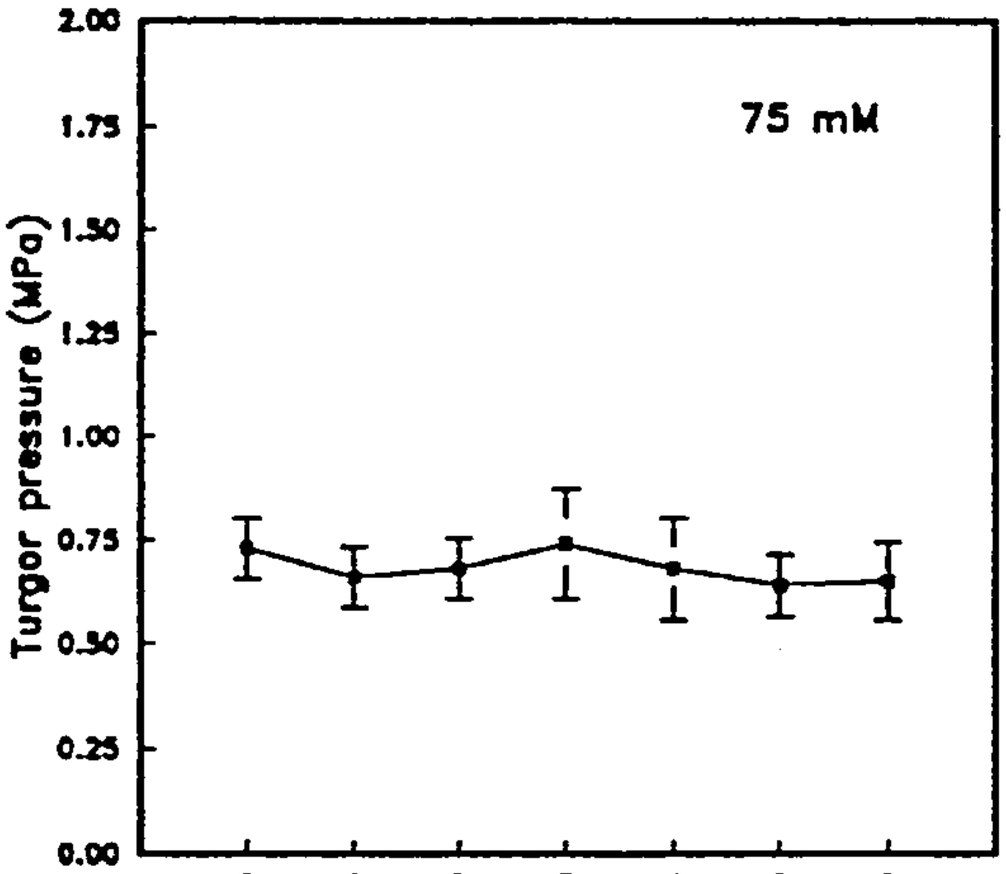

150 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

**Fig. 6.3** The response of turgor pressure to the NaCl stress studied in Pb-81, a moderately salt sensitive wheat variety, in a long term experiment i.e. 6 days. Parallel experiment to Fig. 6.1.


Control
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time (Days)


25 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)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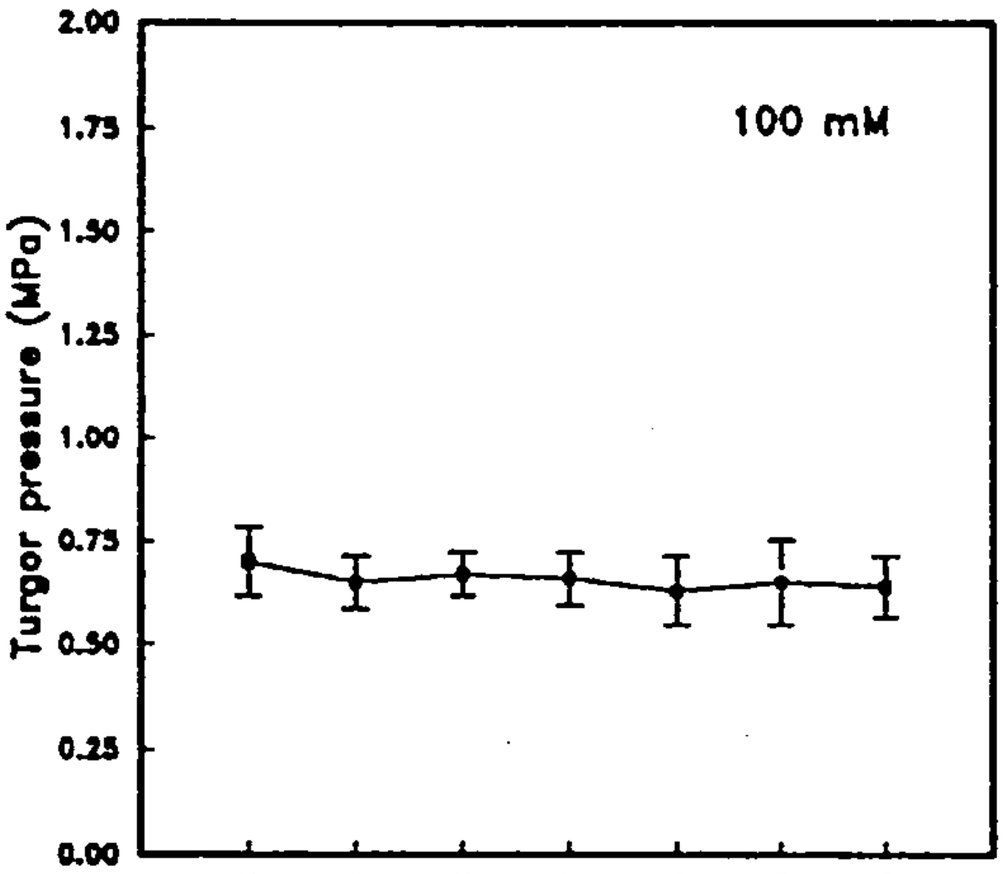
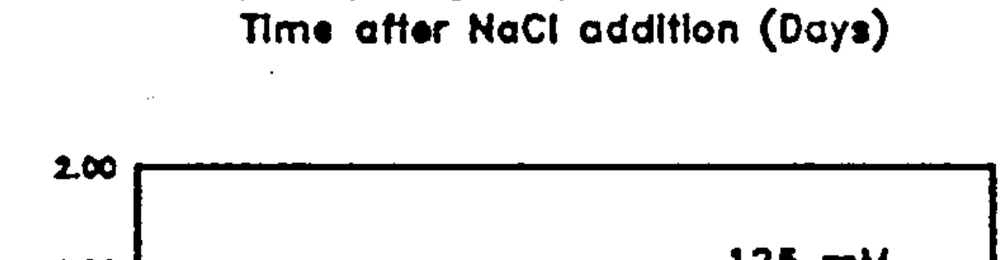
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

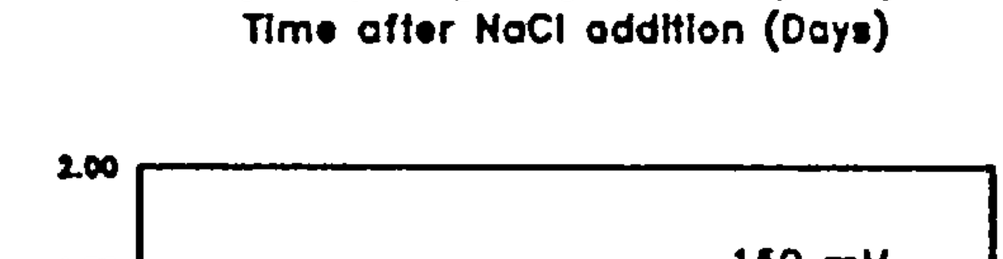
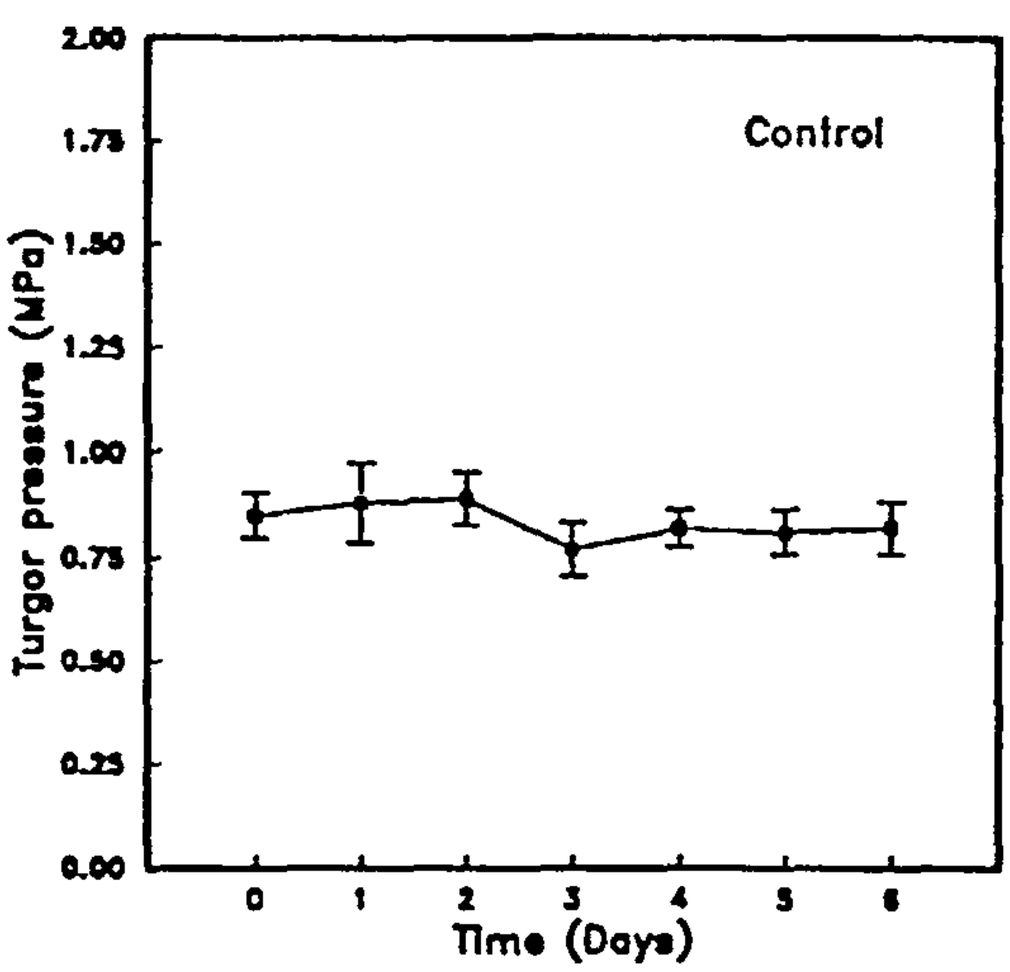

150 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

**Fig. 6.4** The response of turgor pressure to the NaCl stress studied in Indus-79, a salt sensitive wheat variety, in a long term experiment i.e. 6 days. Parallel experiment to Fig. 6.1.


Control
Turgor pressure (MPa)
2.00
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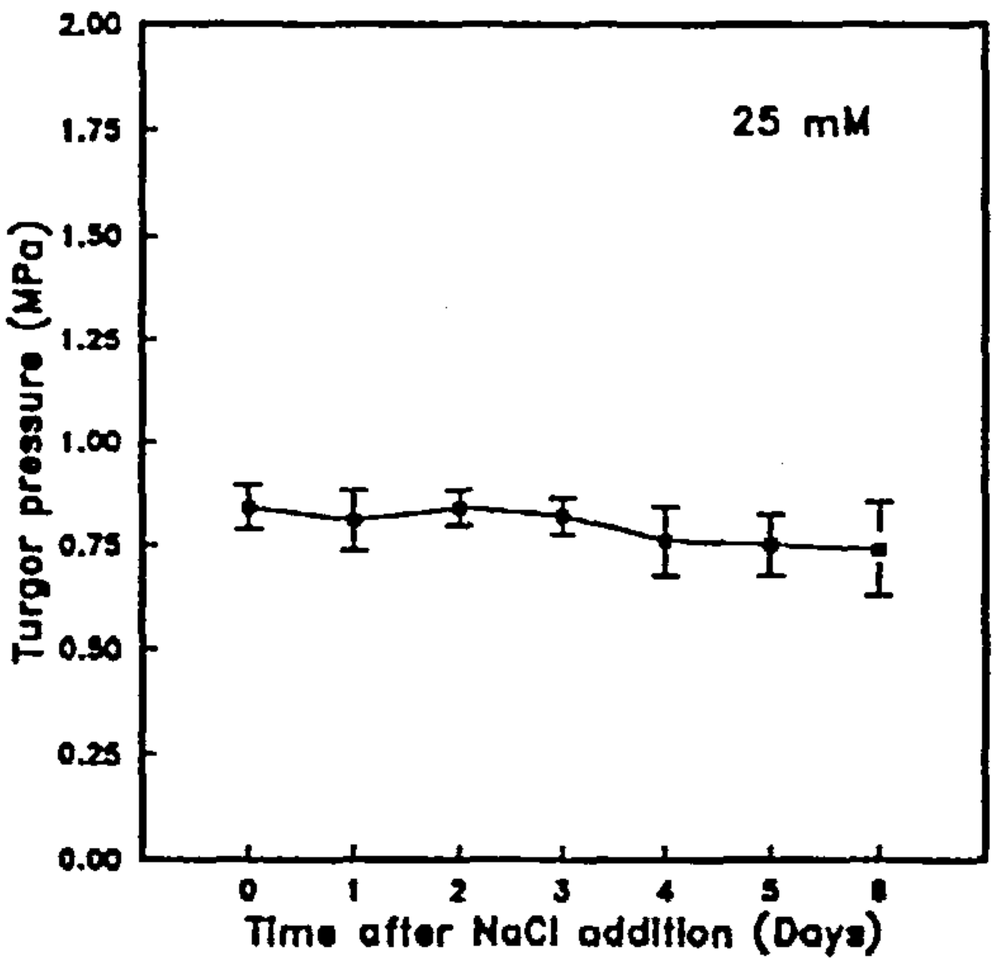
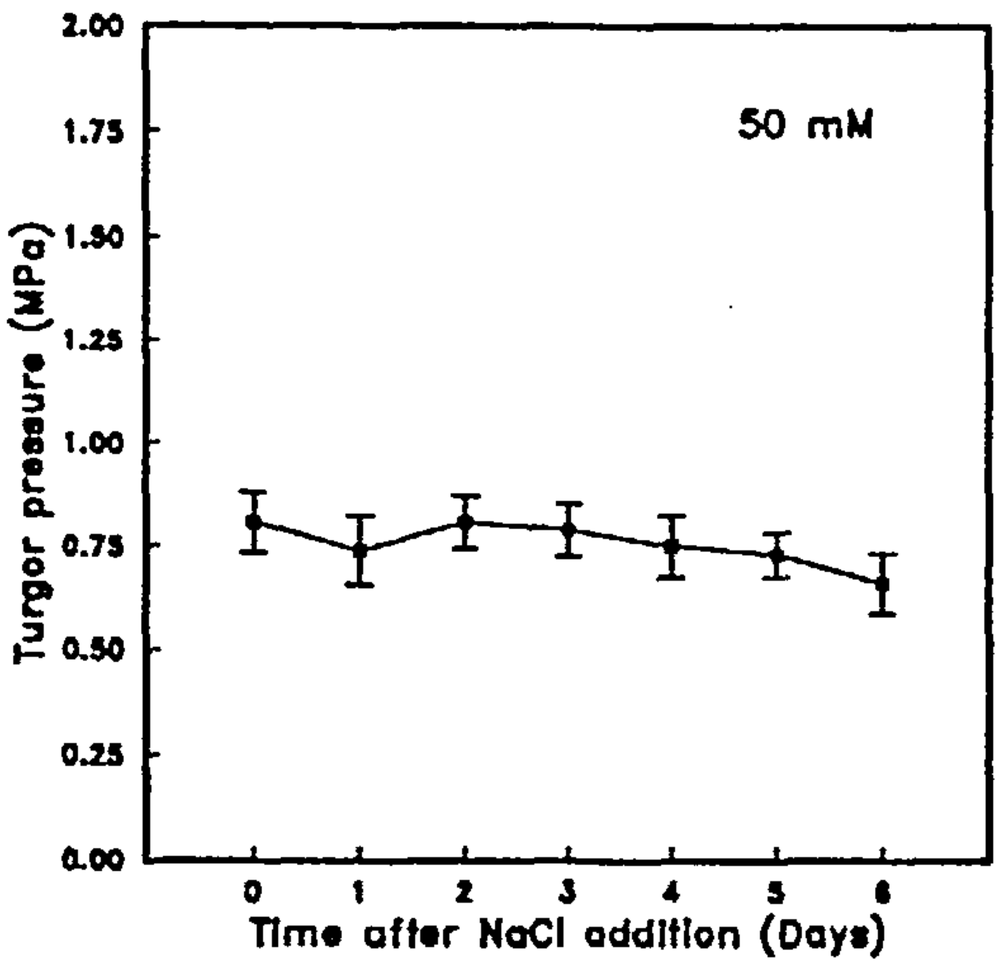
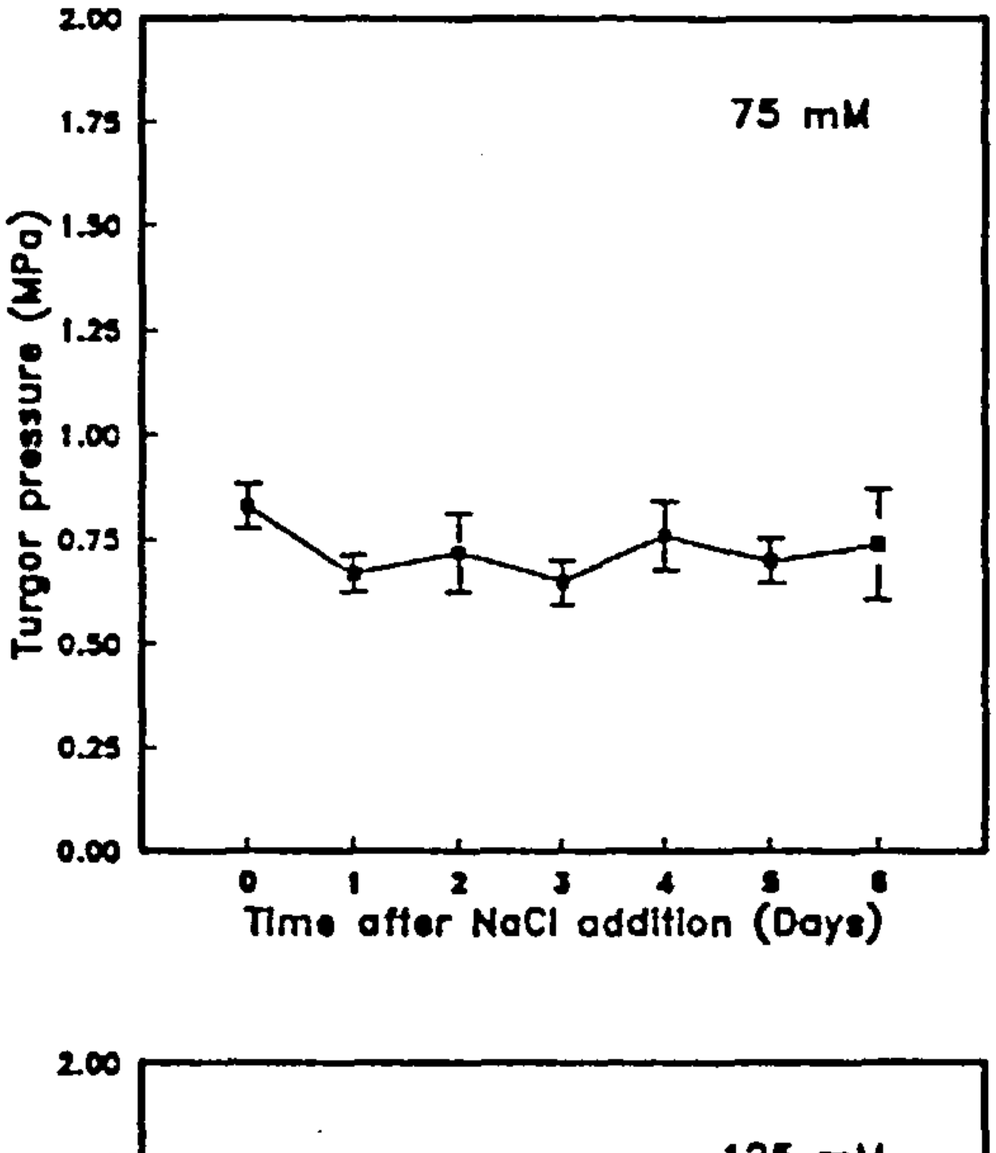
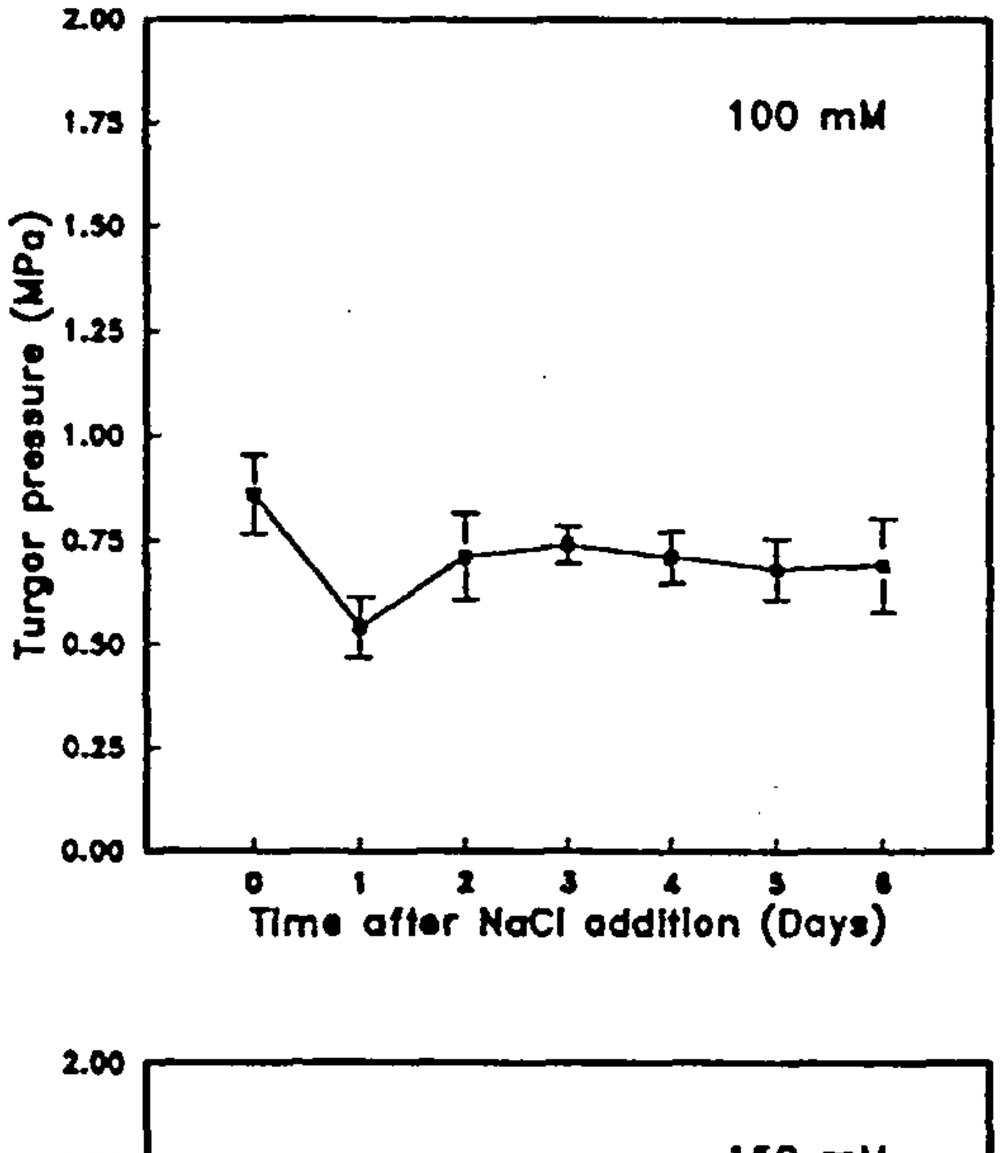
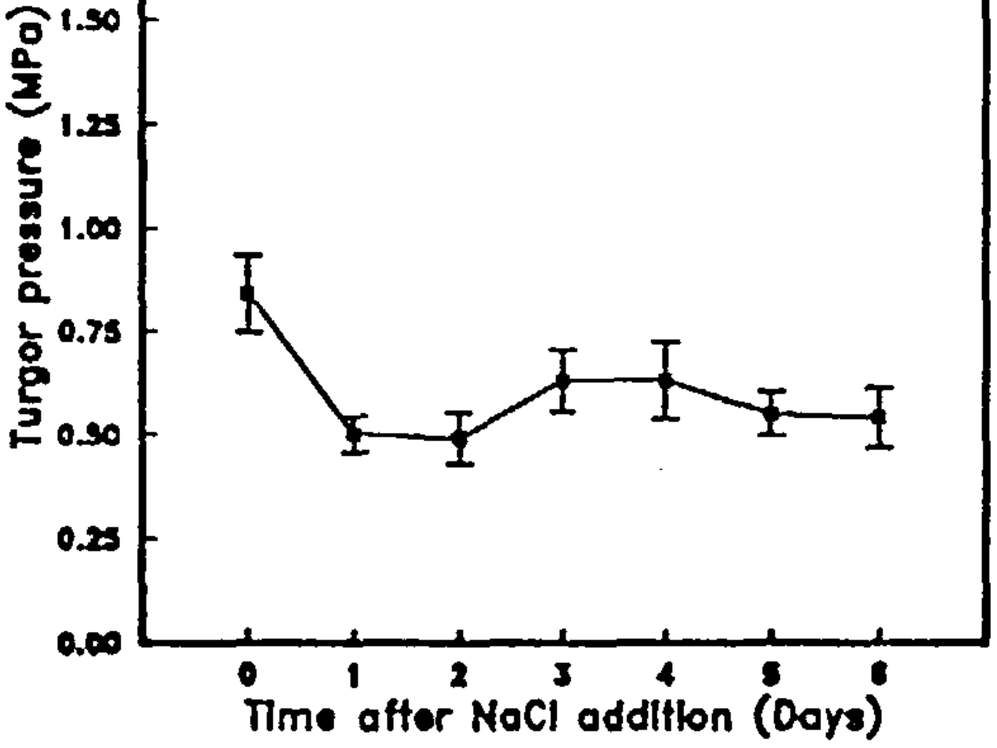
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time (Days)


25 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

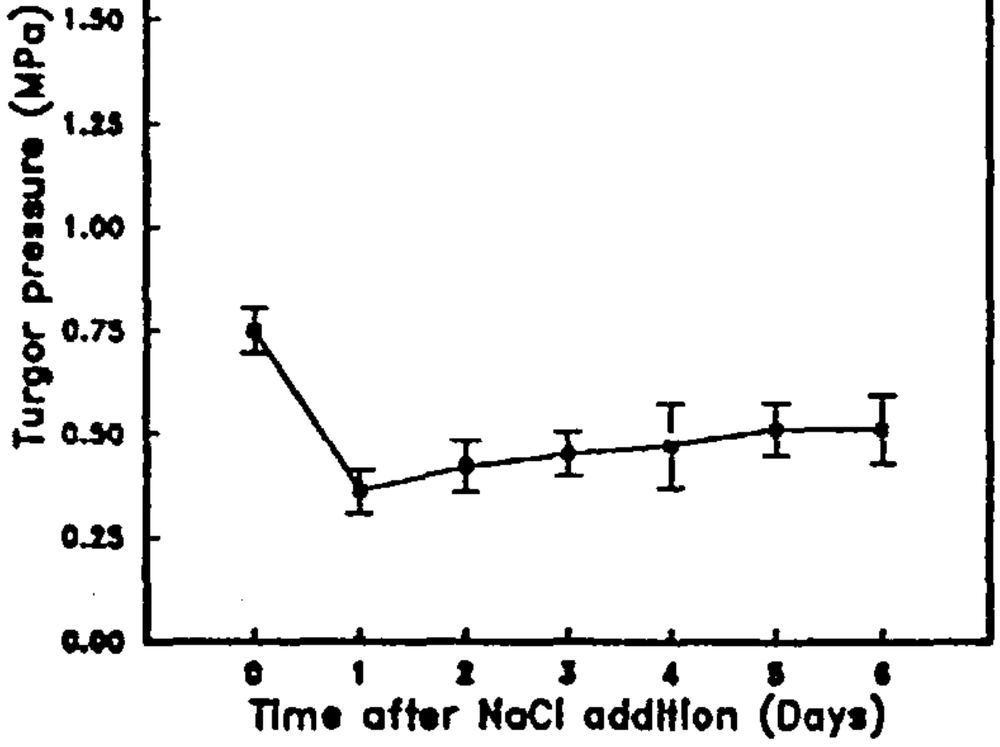
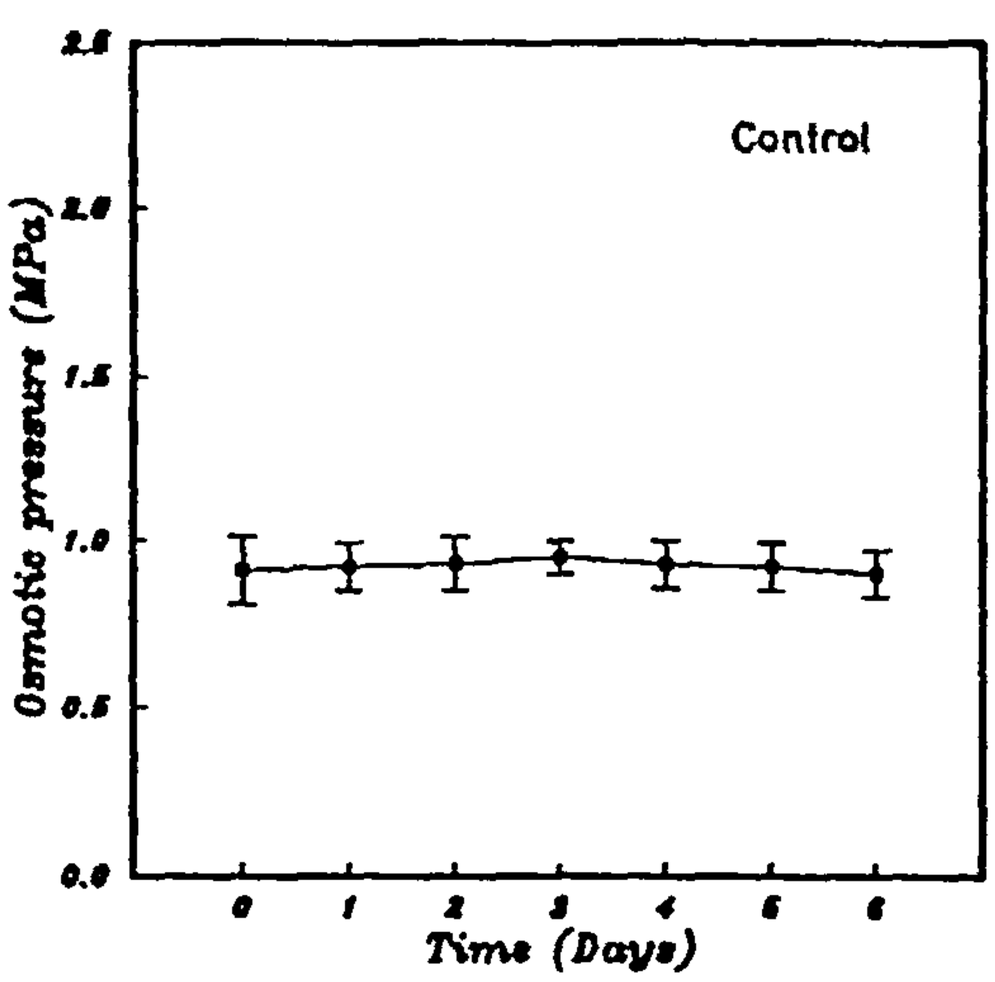
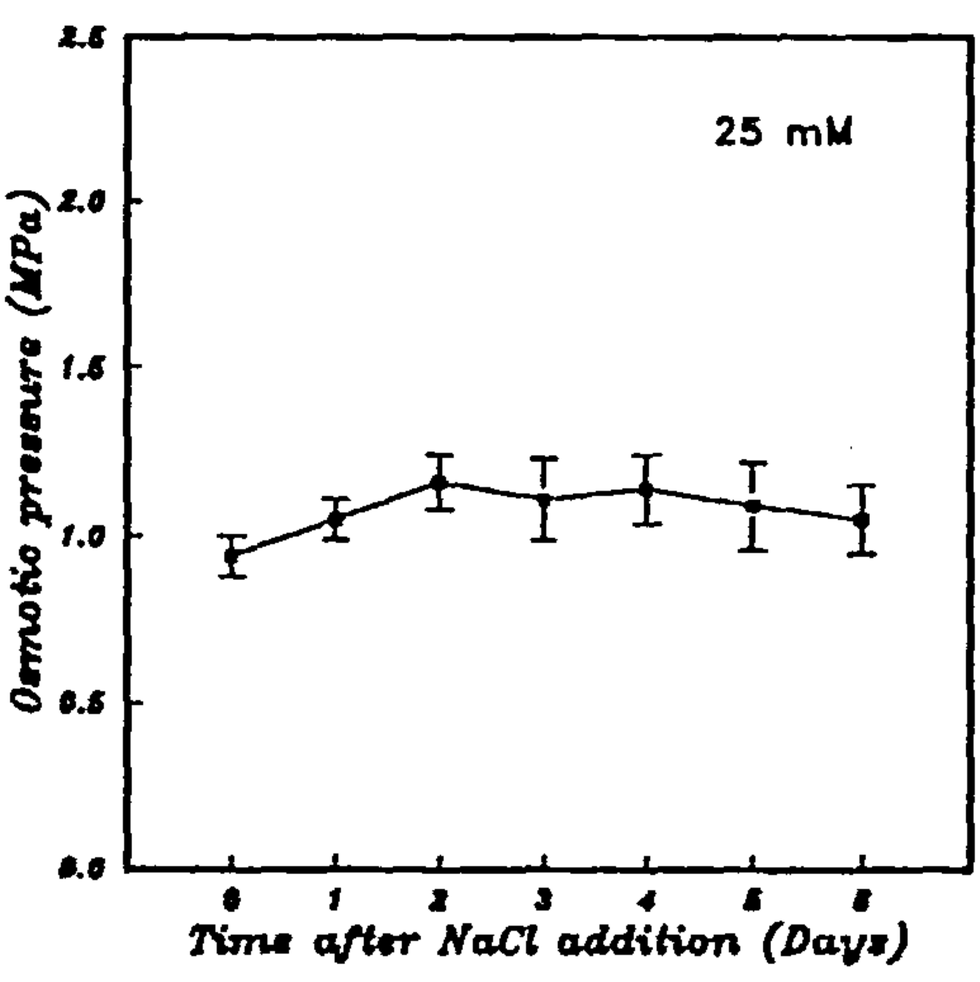
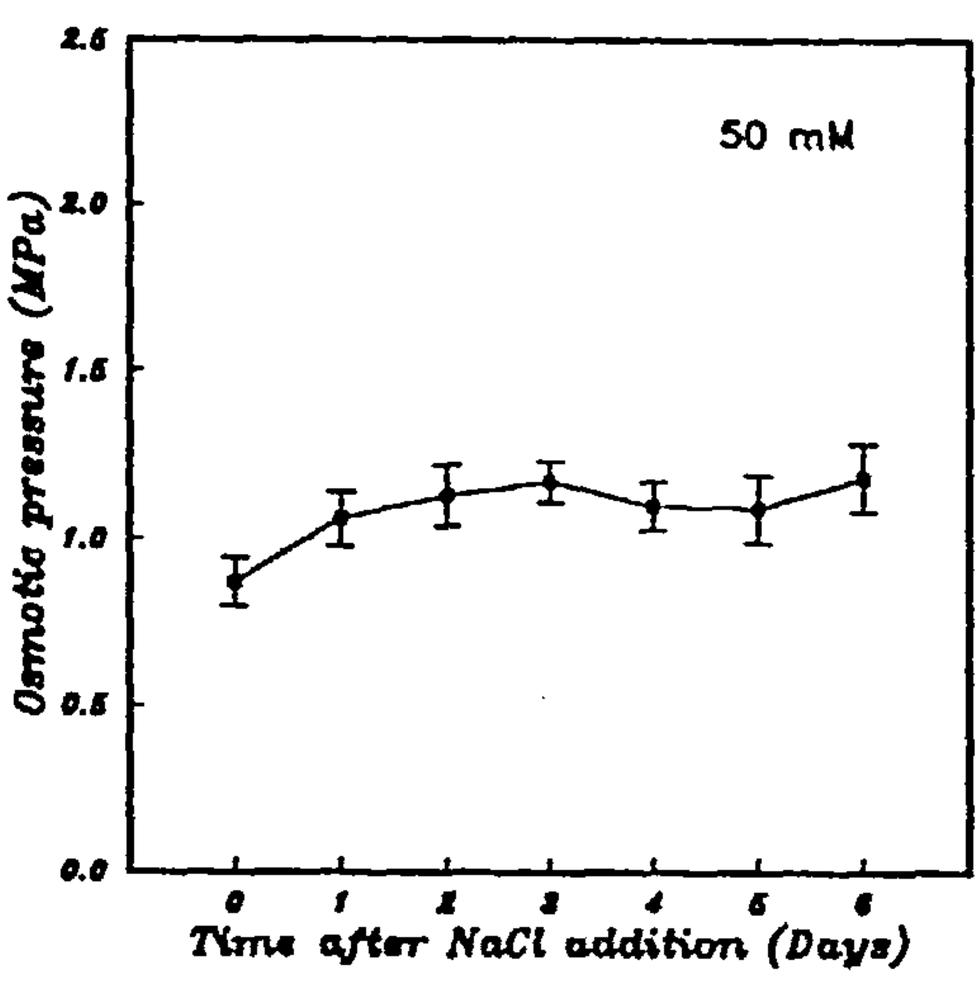
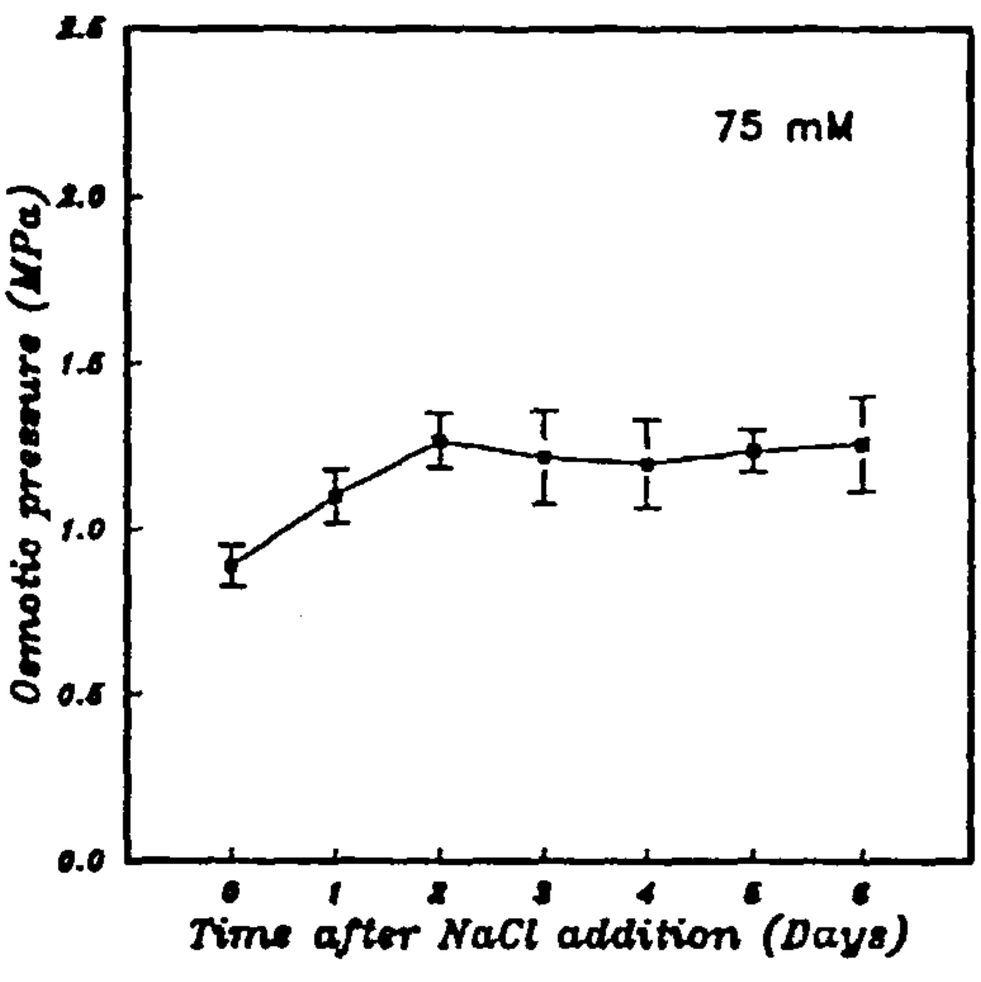

150 mM
Turgor pressure (MPa)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6
Time after NaCl addition (Days)

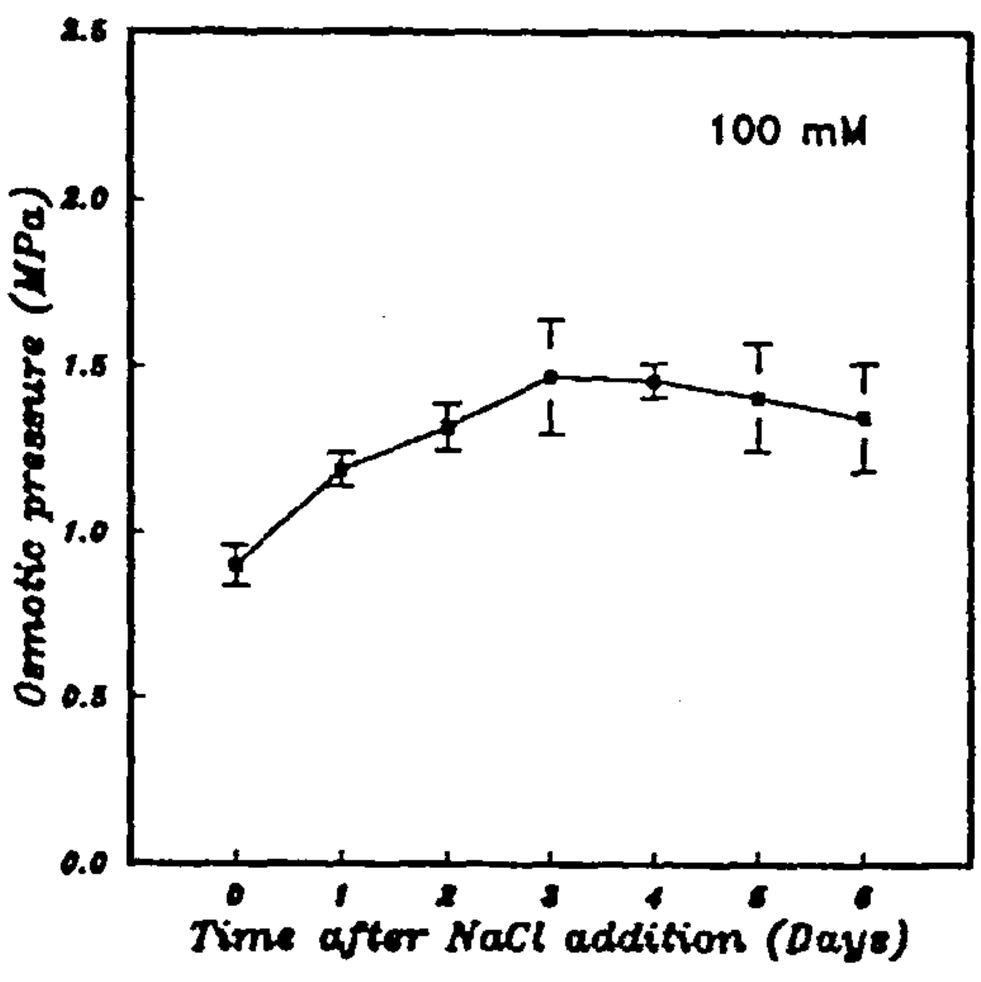
**Fig. 6.5** The response of osmotic pressure to the NaCl stress studied in LU26S, a salt tolerant wheat variety, in a long term experiment i.e. 6 days. Osmotic pressure was measured in expressed cell sap from the leaf mature zone using vapour pressure osmometry on the whole tissue basis. Measurements correspond to Fig. 6.1 (for turgor pressure). Each point is the mean of 3-5 replicates.

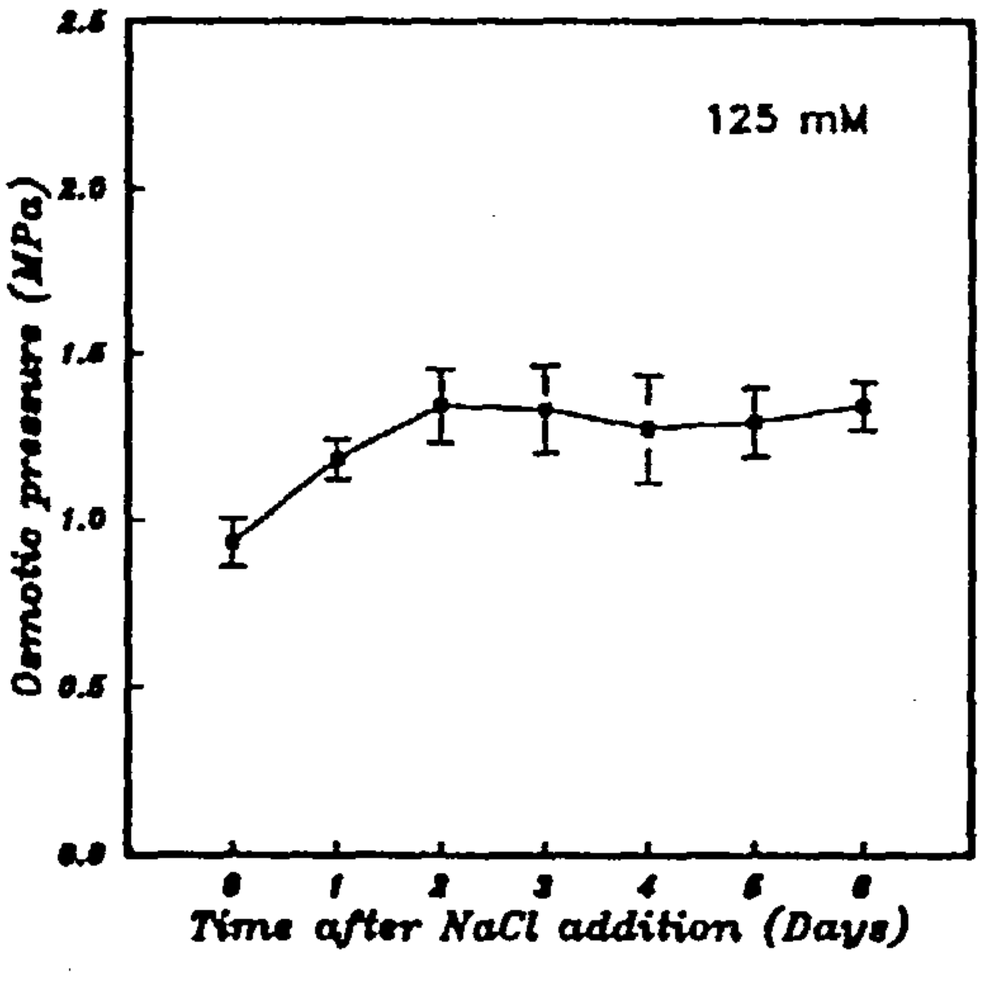

Control
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time (Days)


25 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

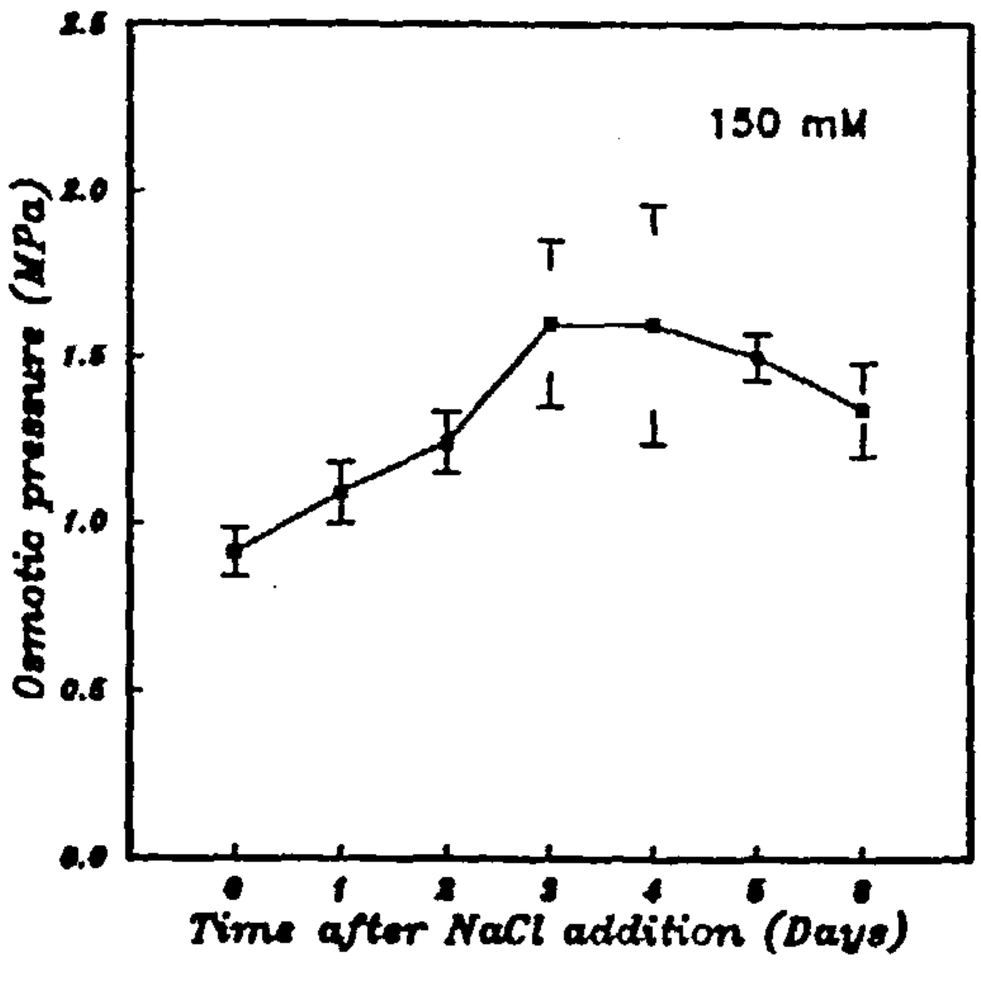
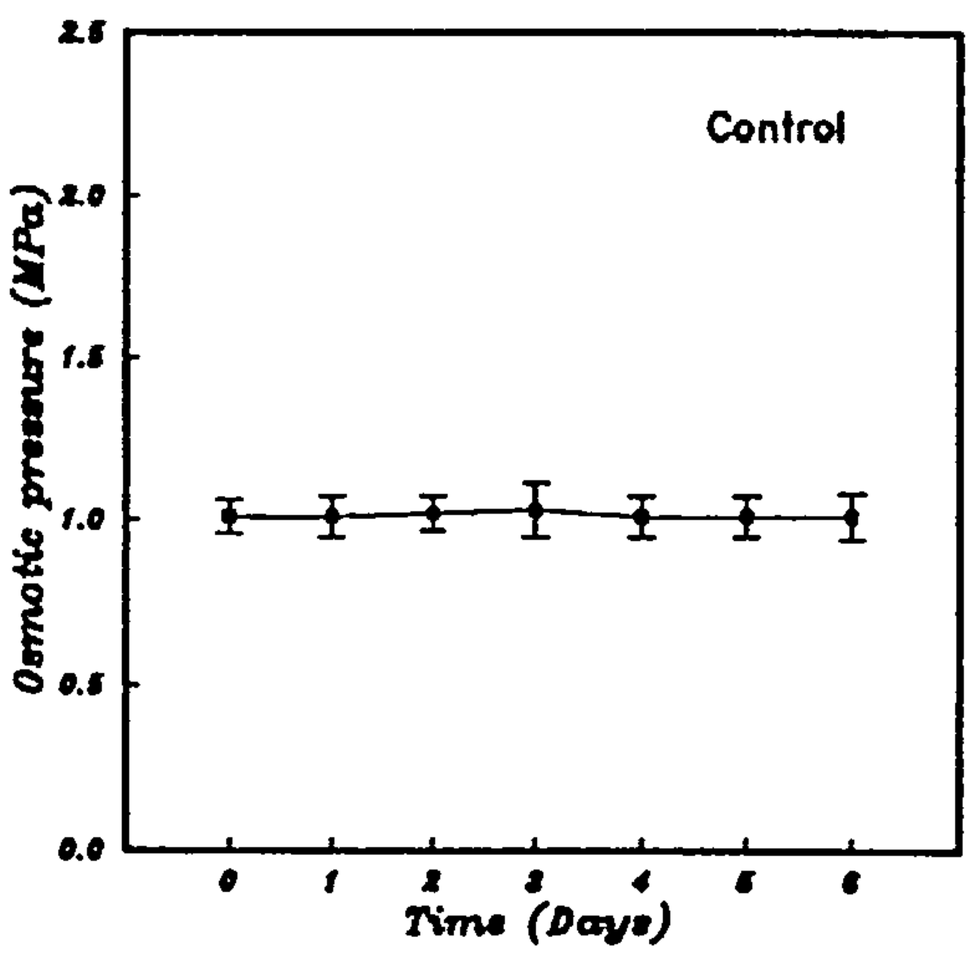
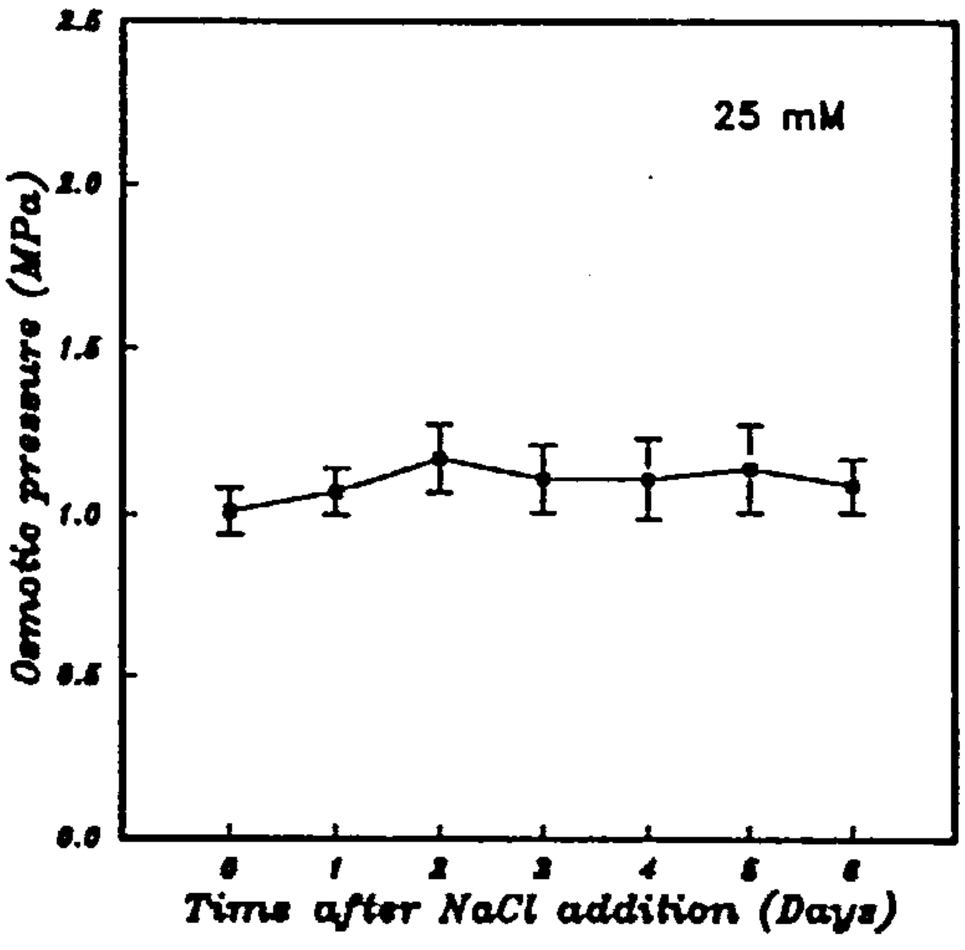
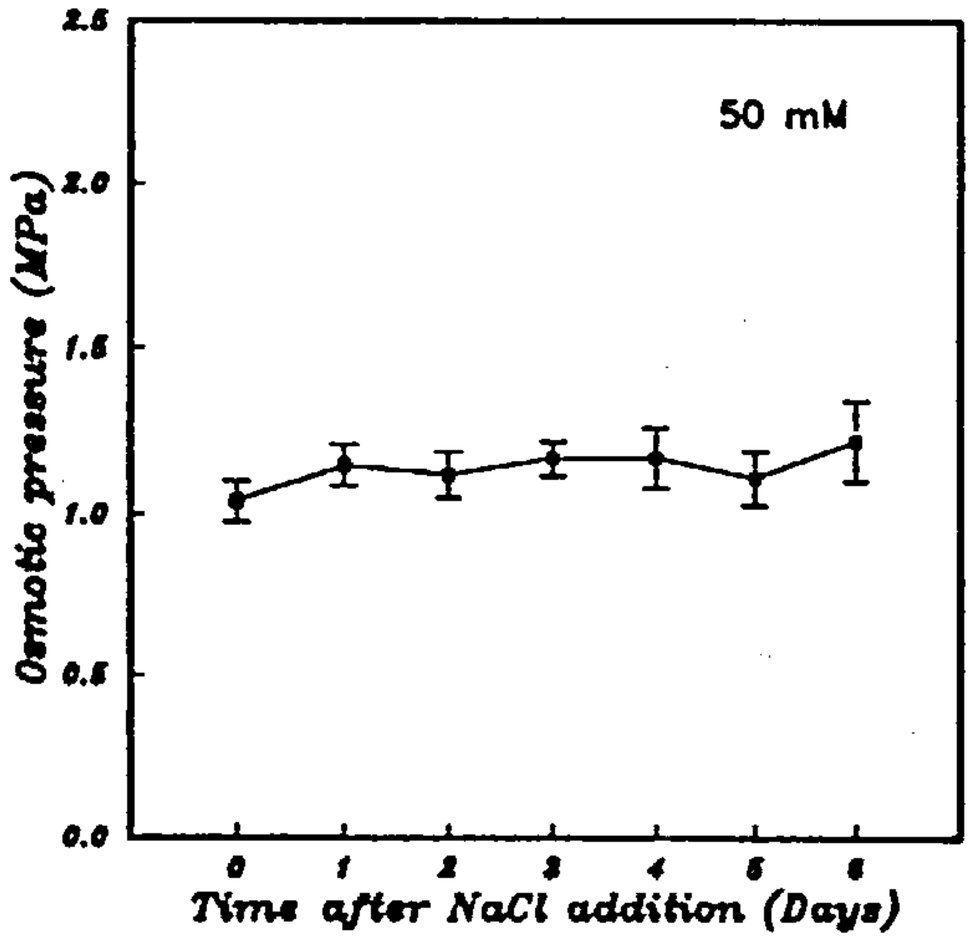
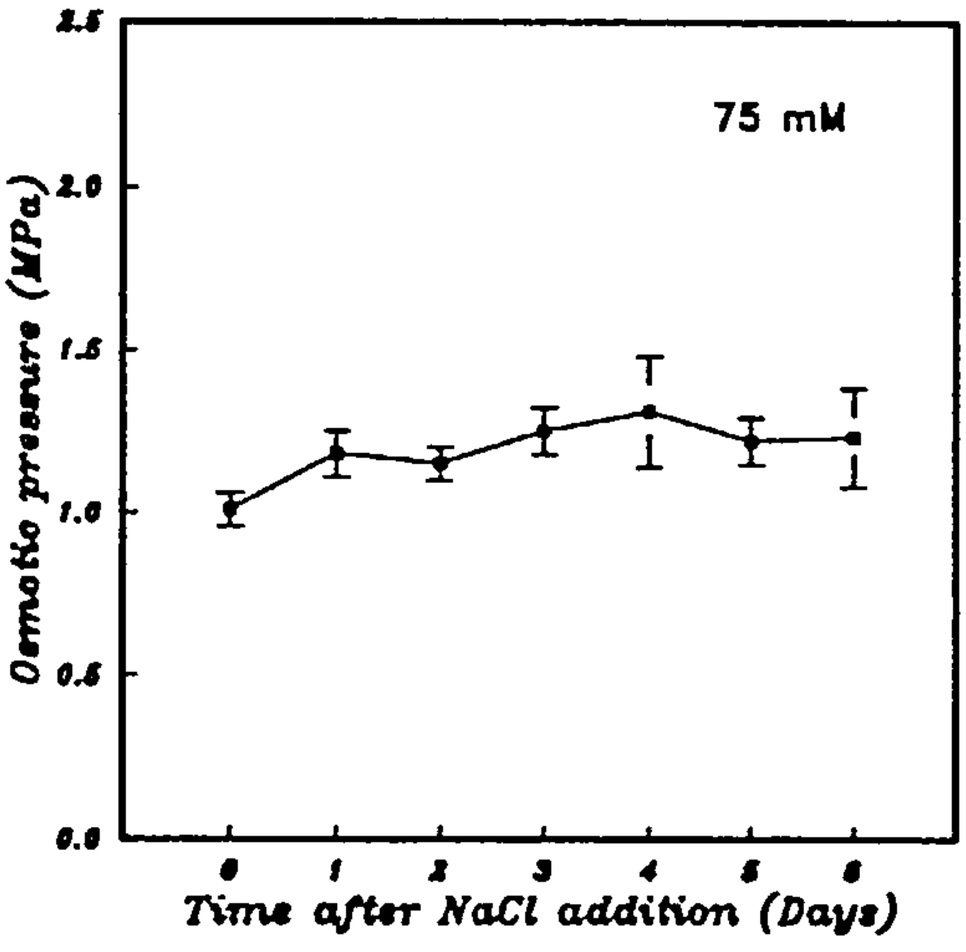

150 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

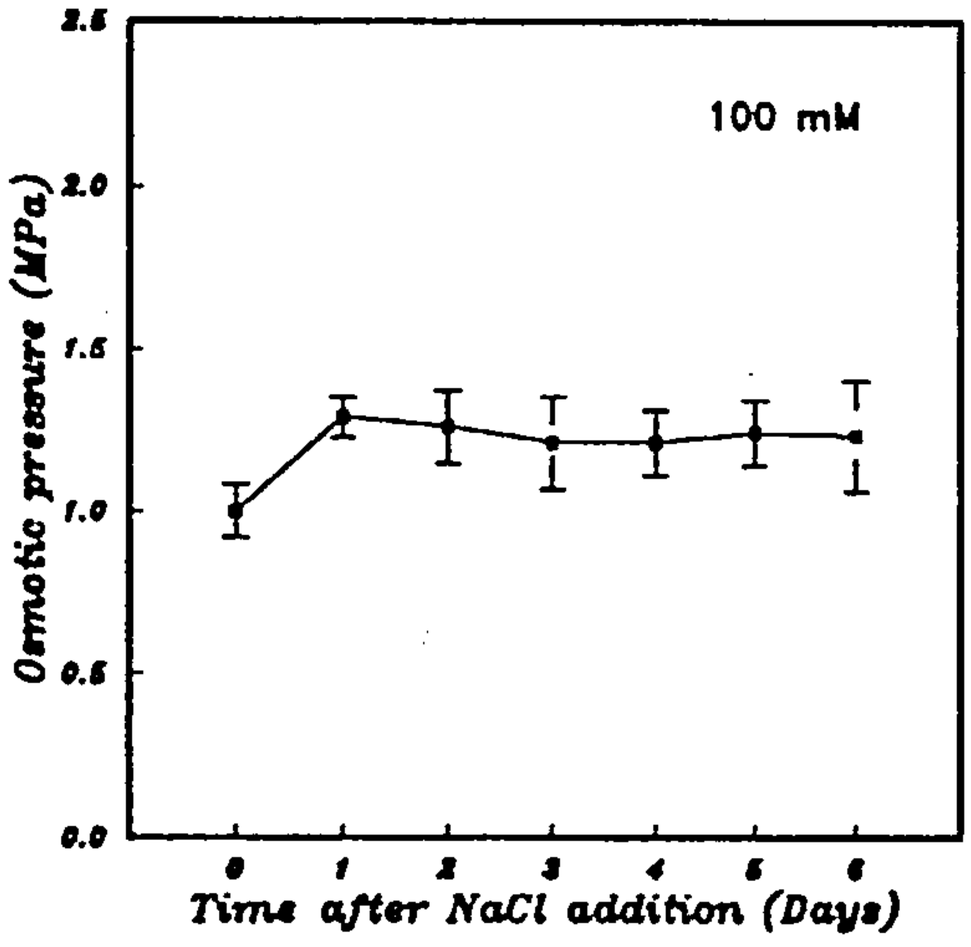
**Fig. 6.6** The response of osmotic pressure to the NaCl stress studied in LYP-73, a moderately salt tolerant wheat variety, in a long term experiment i.e. 6 days. Measurements correspond to Fig. 6.2 (for turgor pressure). Parallel experiment to Fig. 6.5.

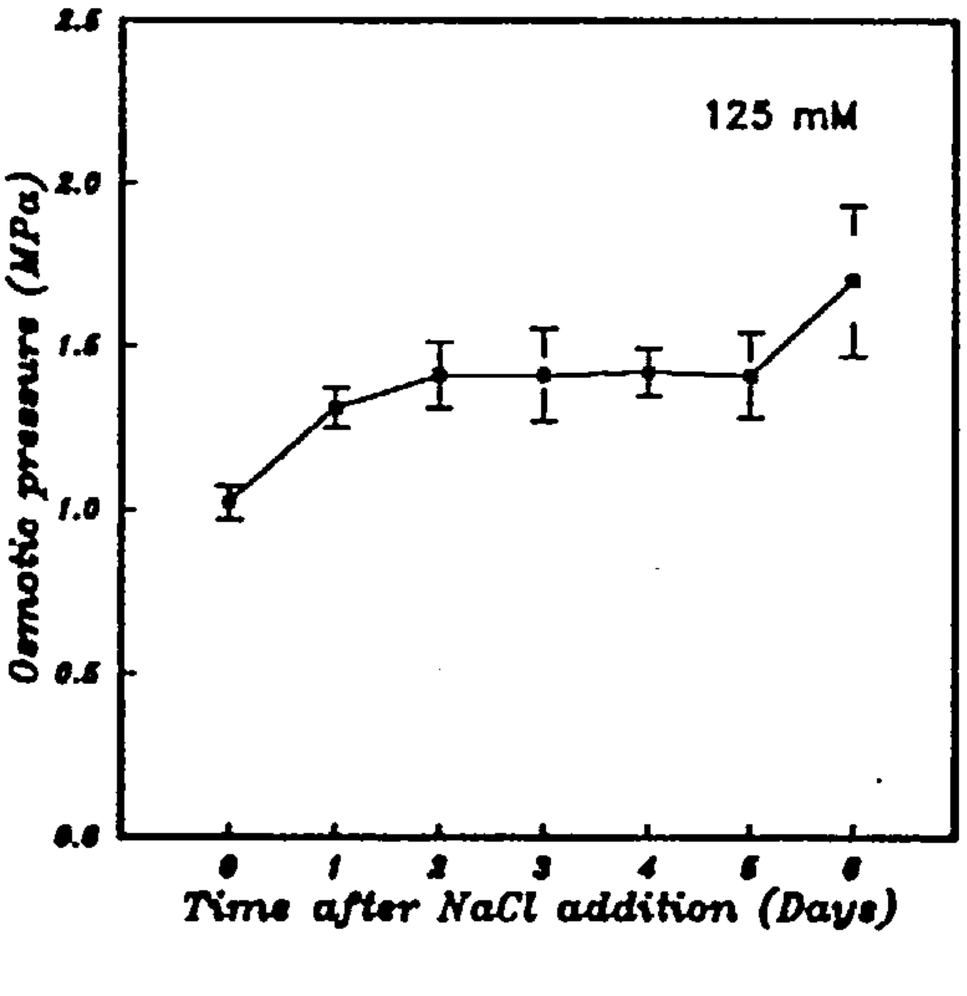

Control
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time (Days)


25 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

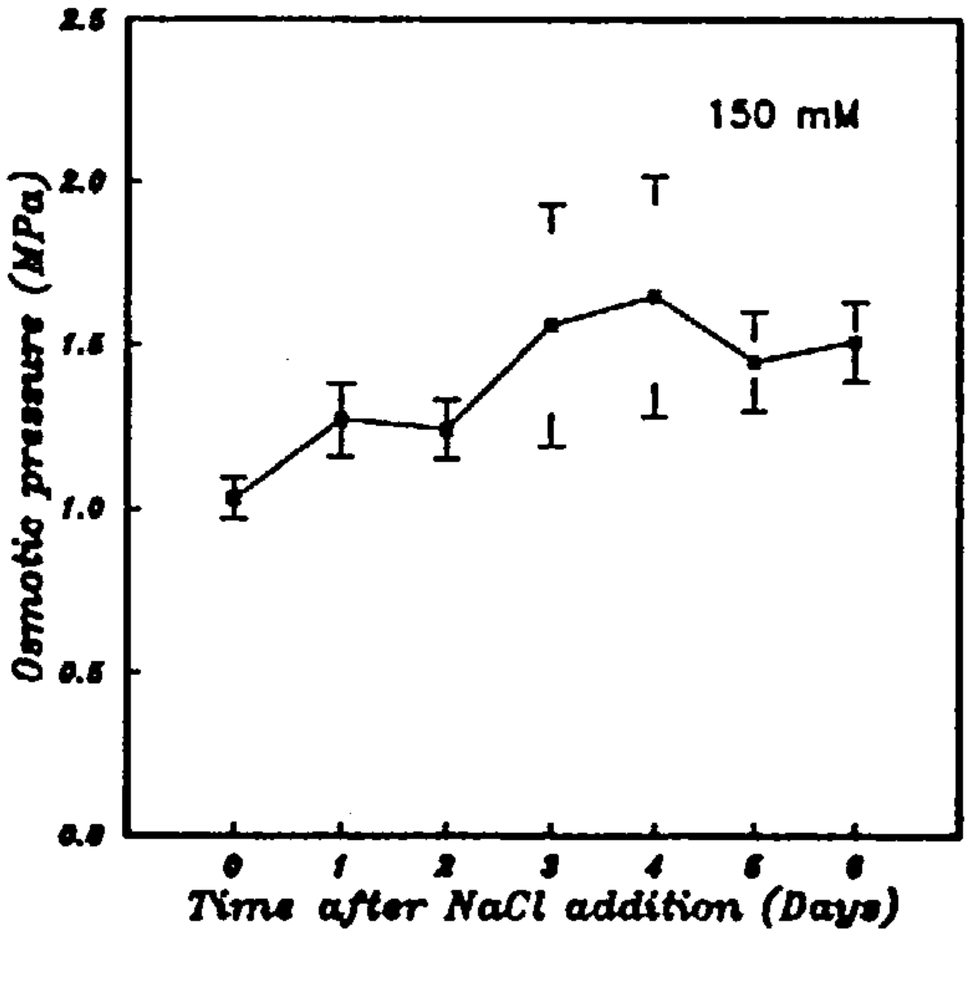
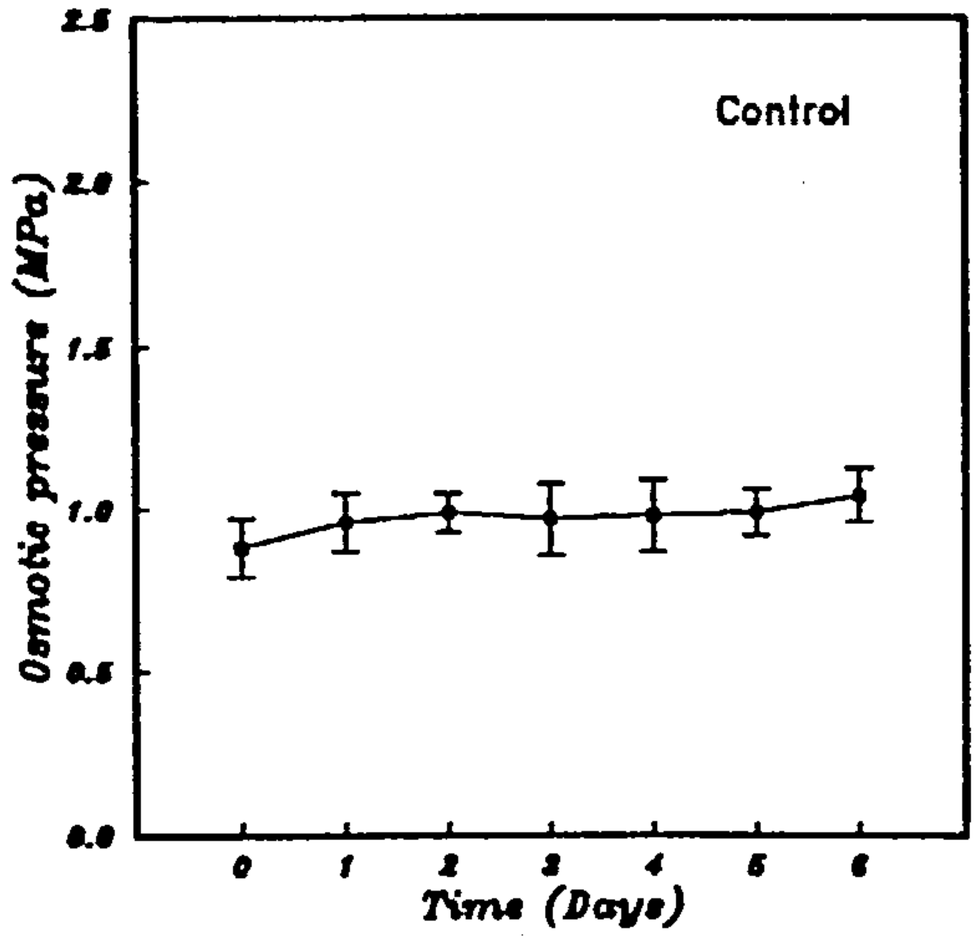
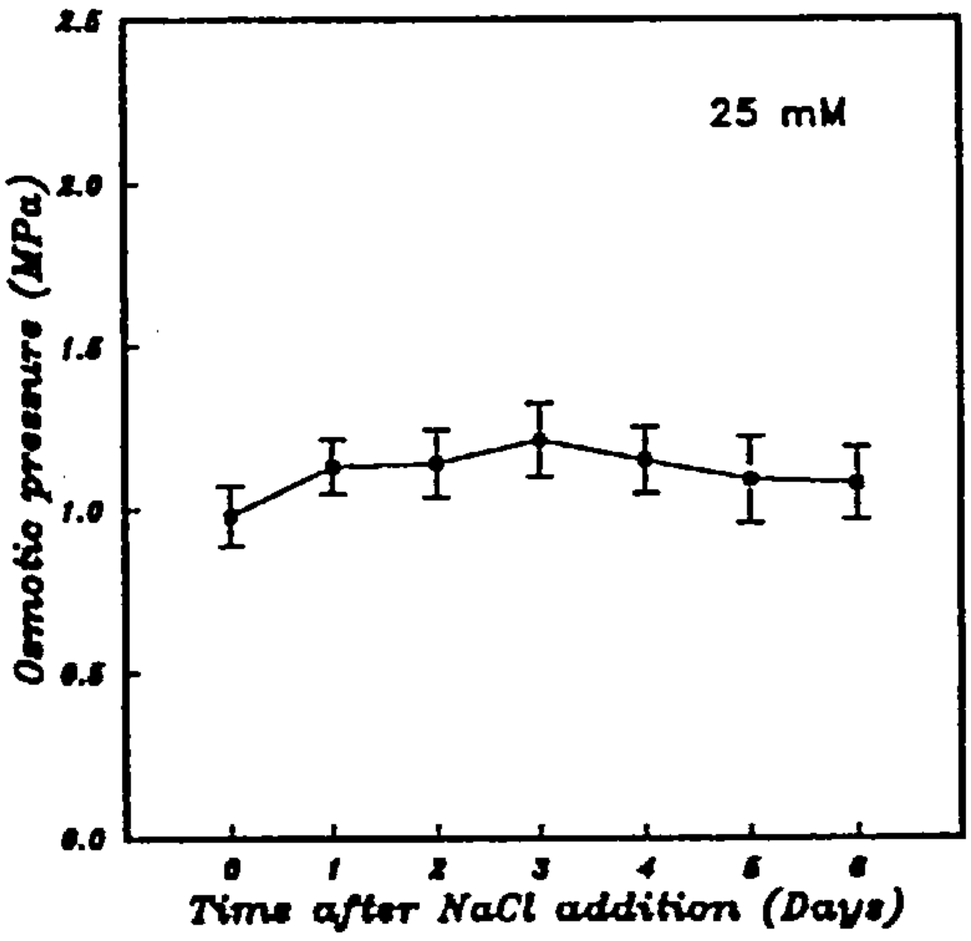
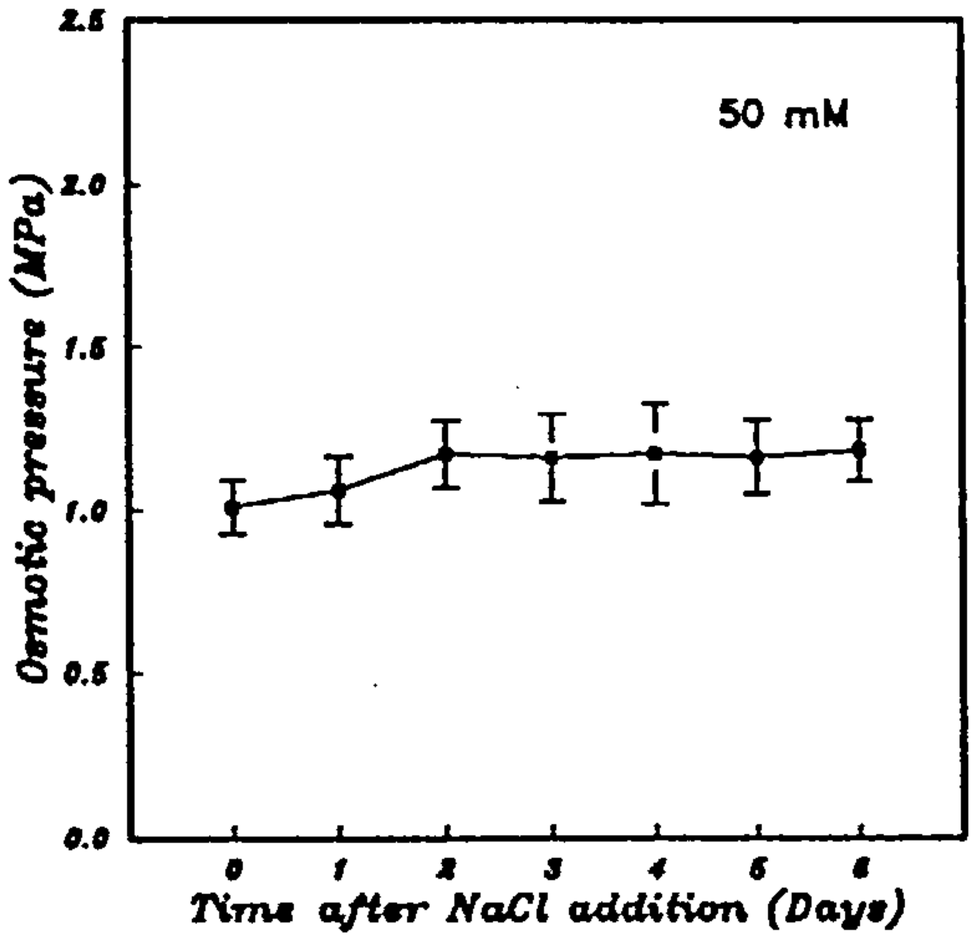
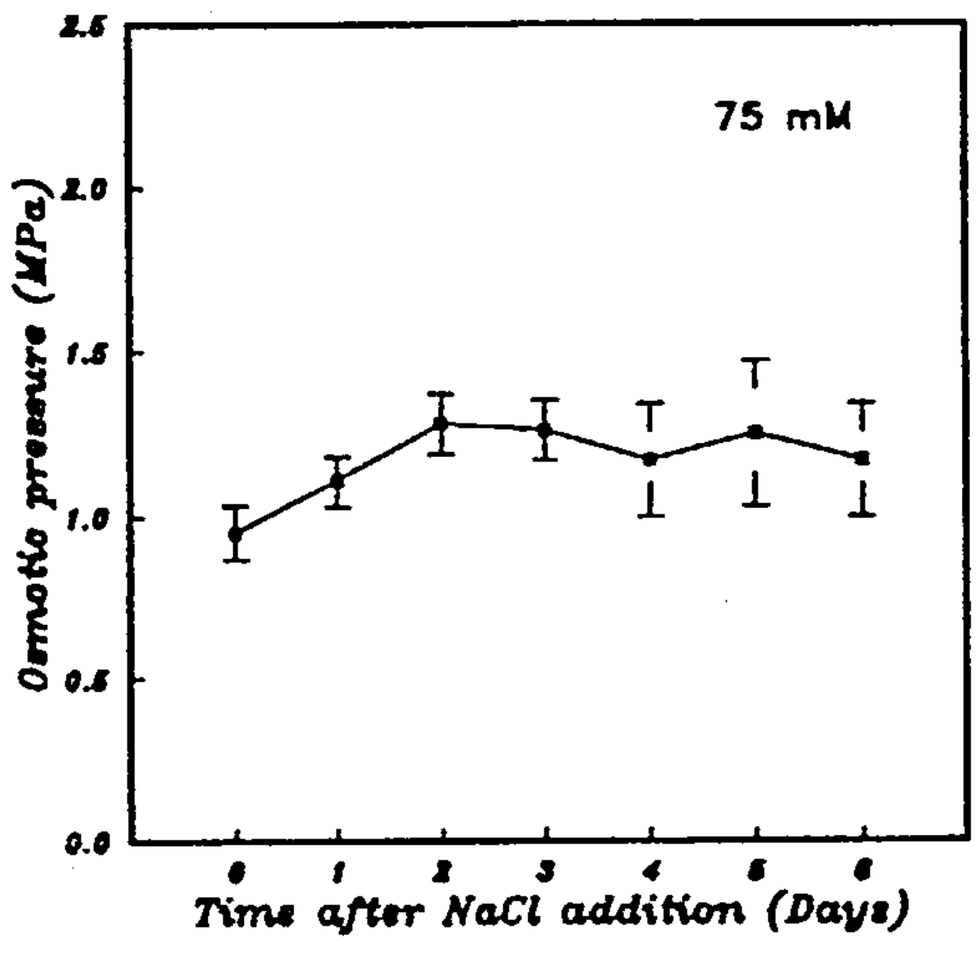

150 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

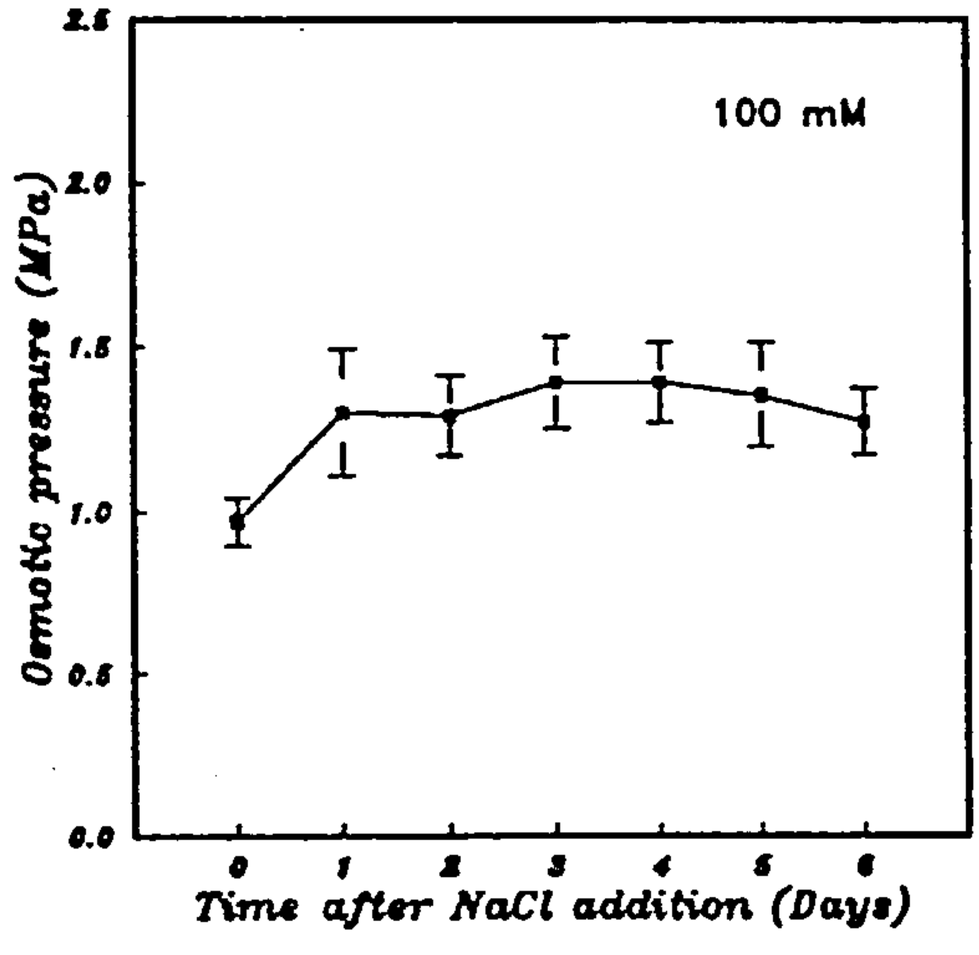
Fig. 6.7 The response of osmotic pressure to the NaCl stress studied in Pb-81, a moderately salt sensitive wheat variety, in a long term experiment i.e. 6 days. Measurements correspond to Fig. 6.3 (for turgor pressure). Parallel experiment to Fig. 6.5.

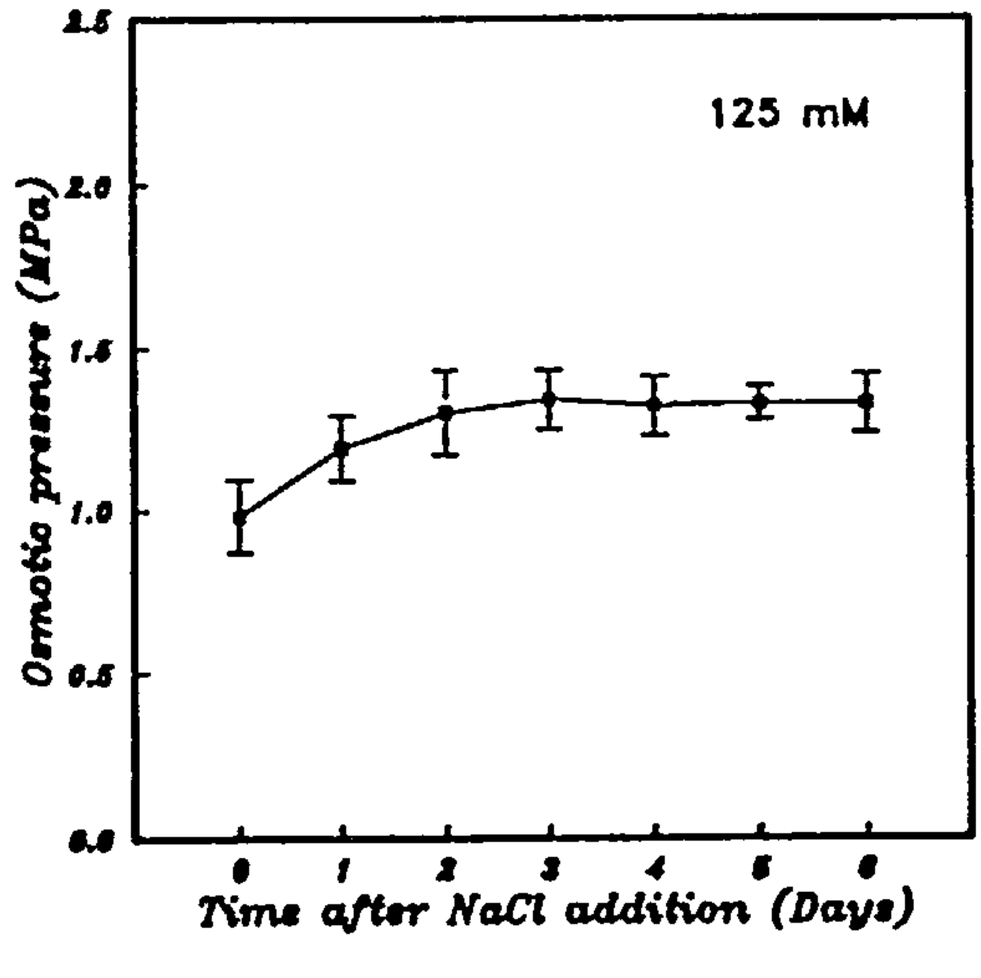

Control
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time (Days)


25 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

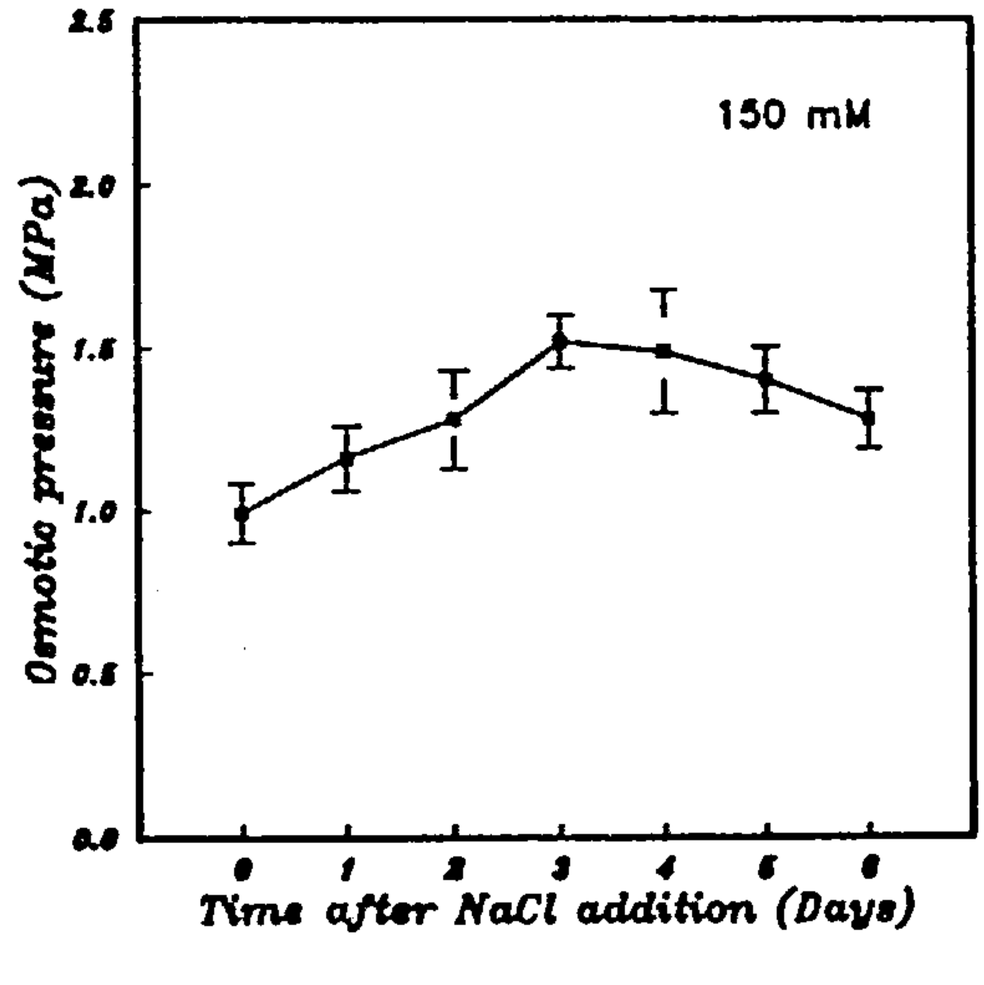
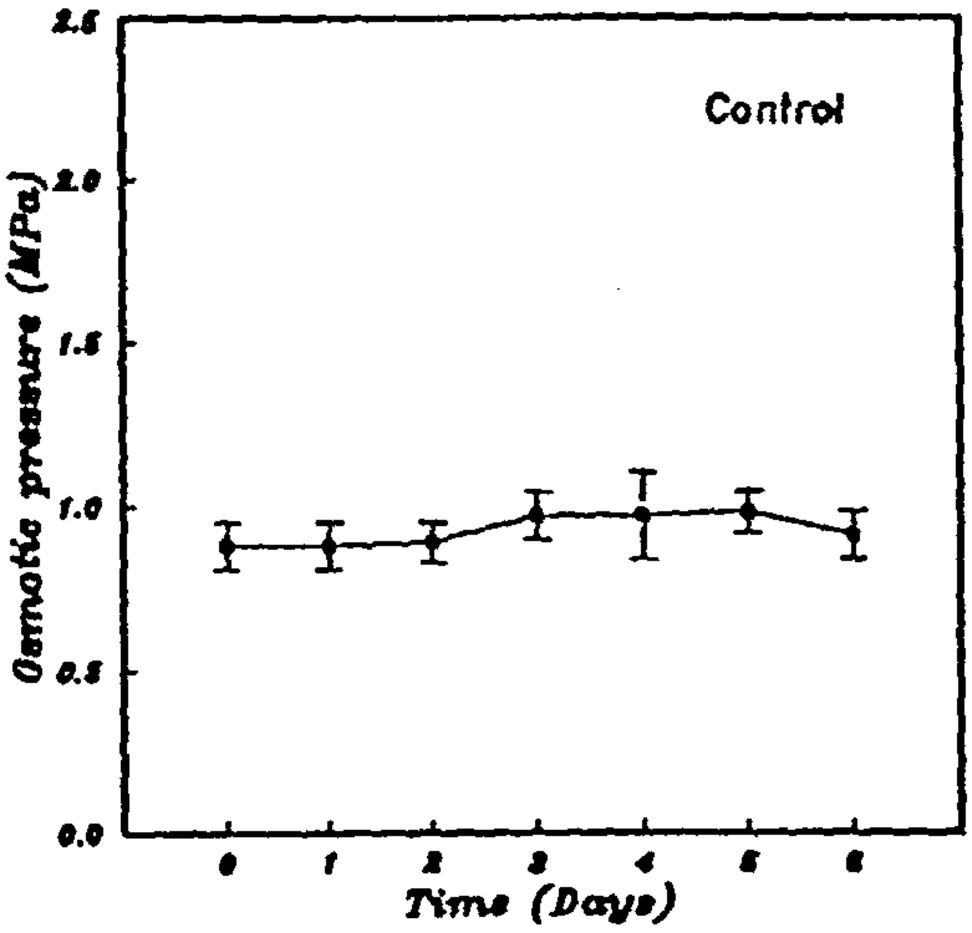
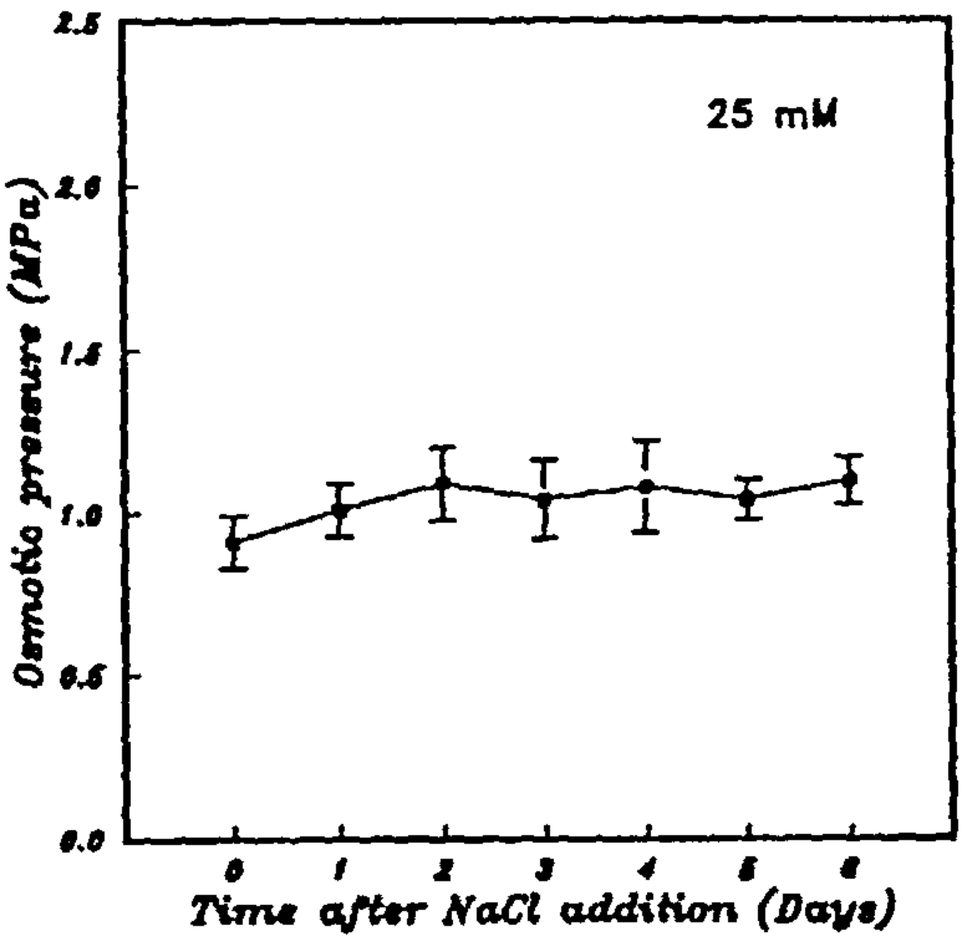
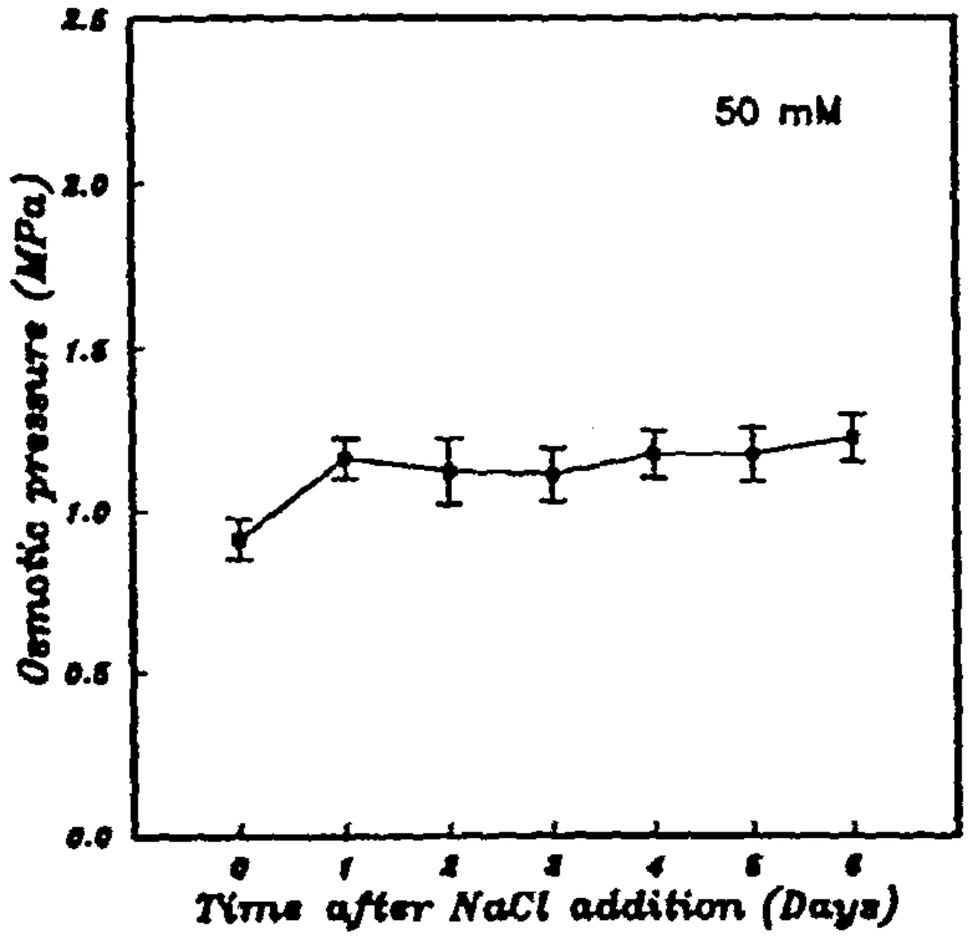
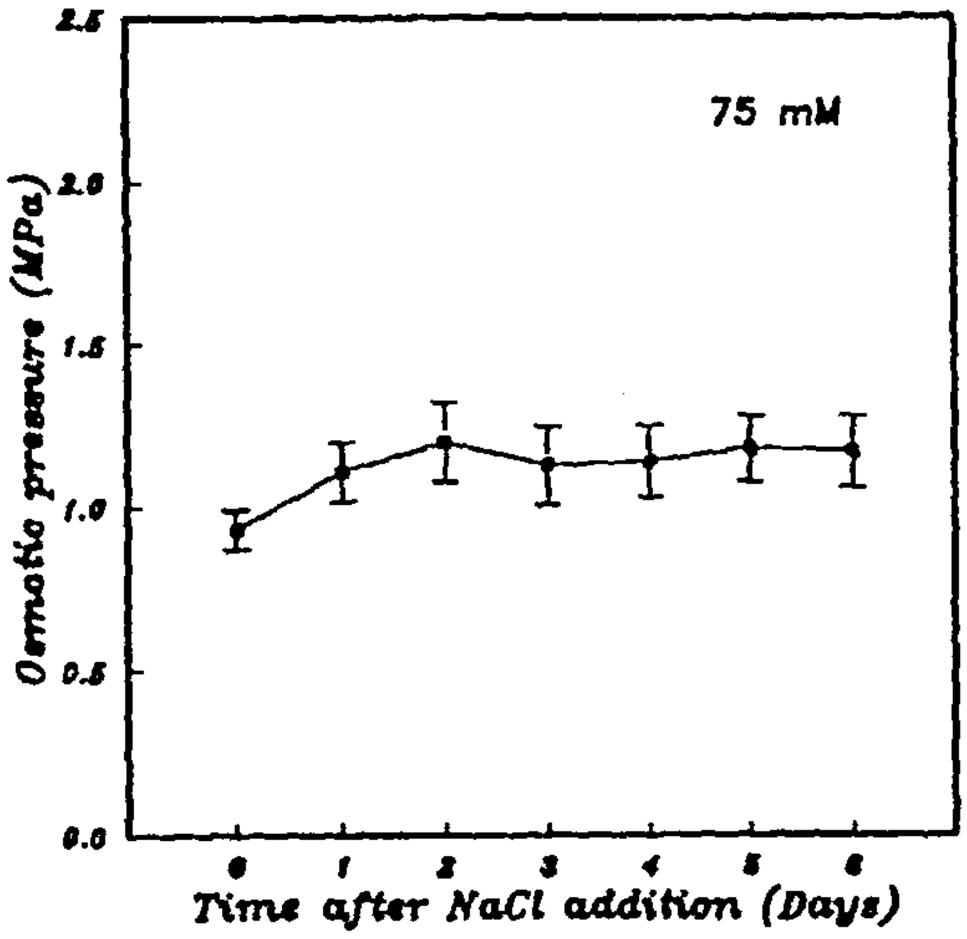

150 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

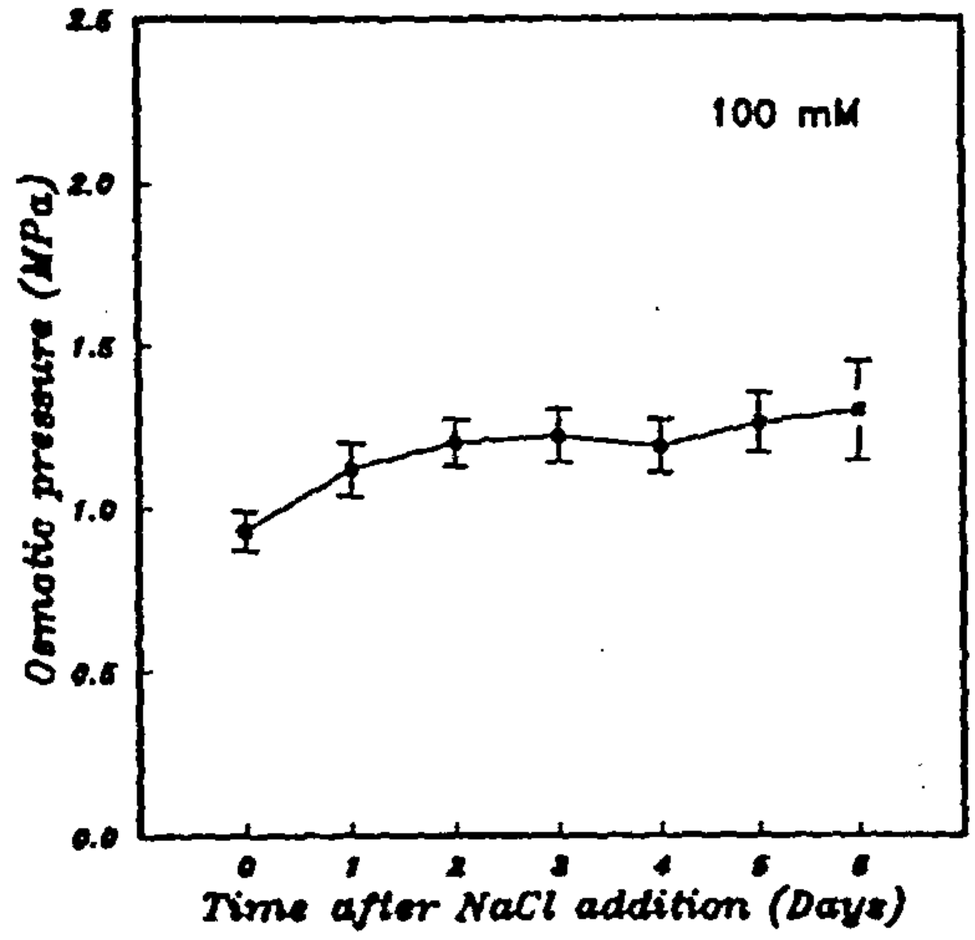
**Fig. 6.8** The response of osmotic pressure to the NaCl stress studied in Indus-79, a salt tolerant wheat variety, in a long term experiment i.e. 6 days. Measurements correspond to Fig. 6.4 (for turgor pressure). Parallel experiment to Fig. 6.5.

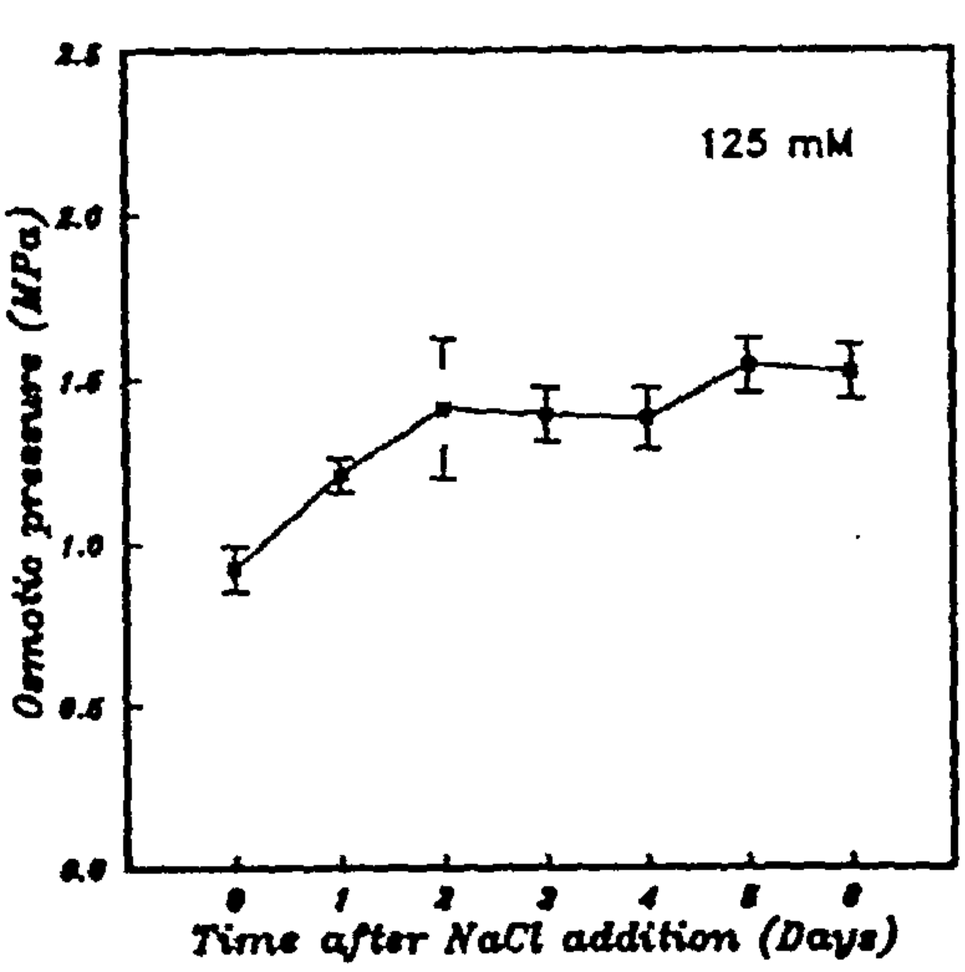

Control
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time (Days)


25 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


50 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


75 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


125 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

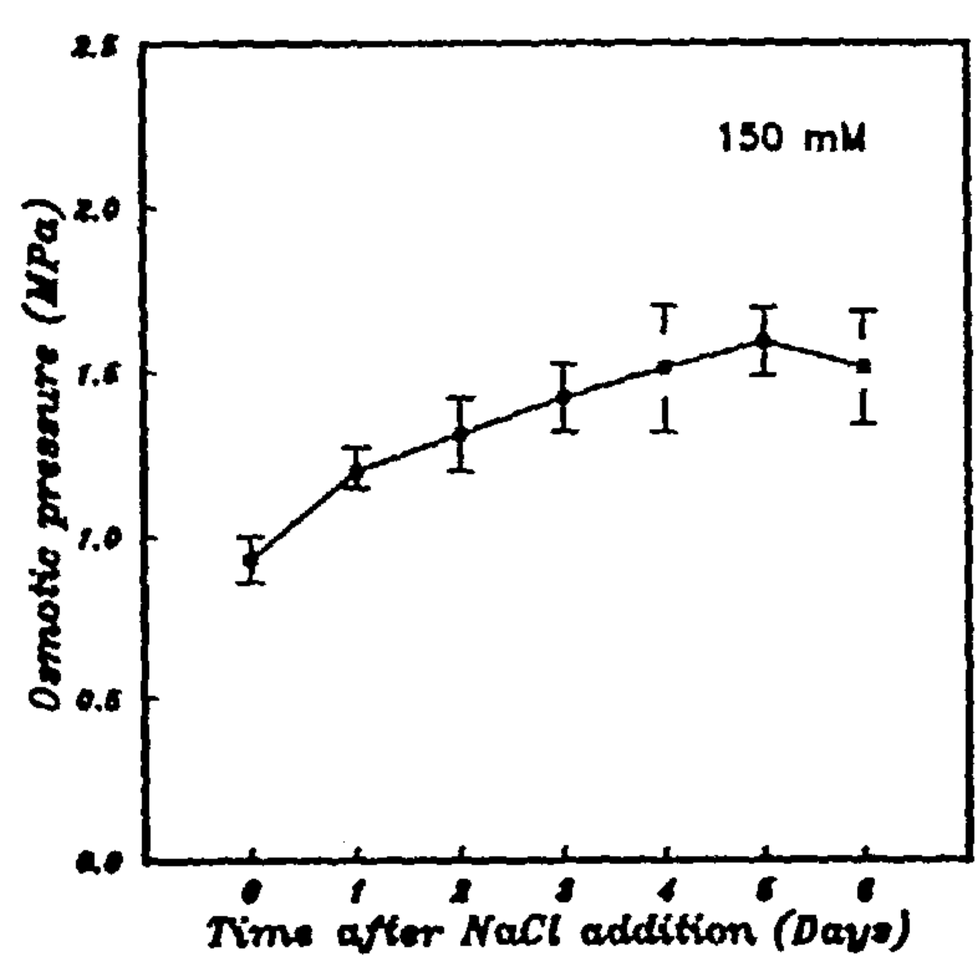
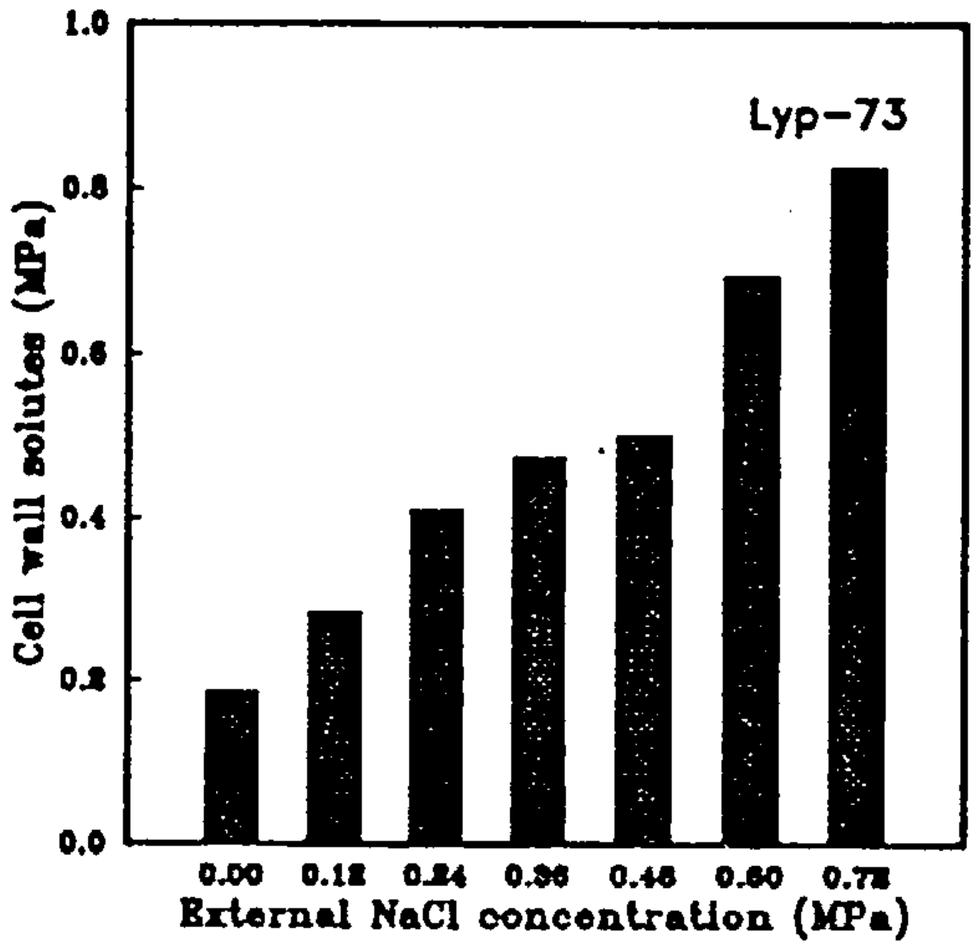
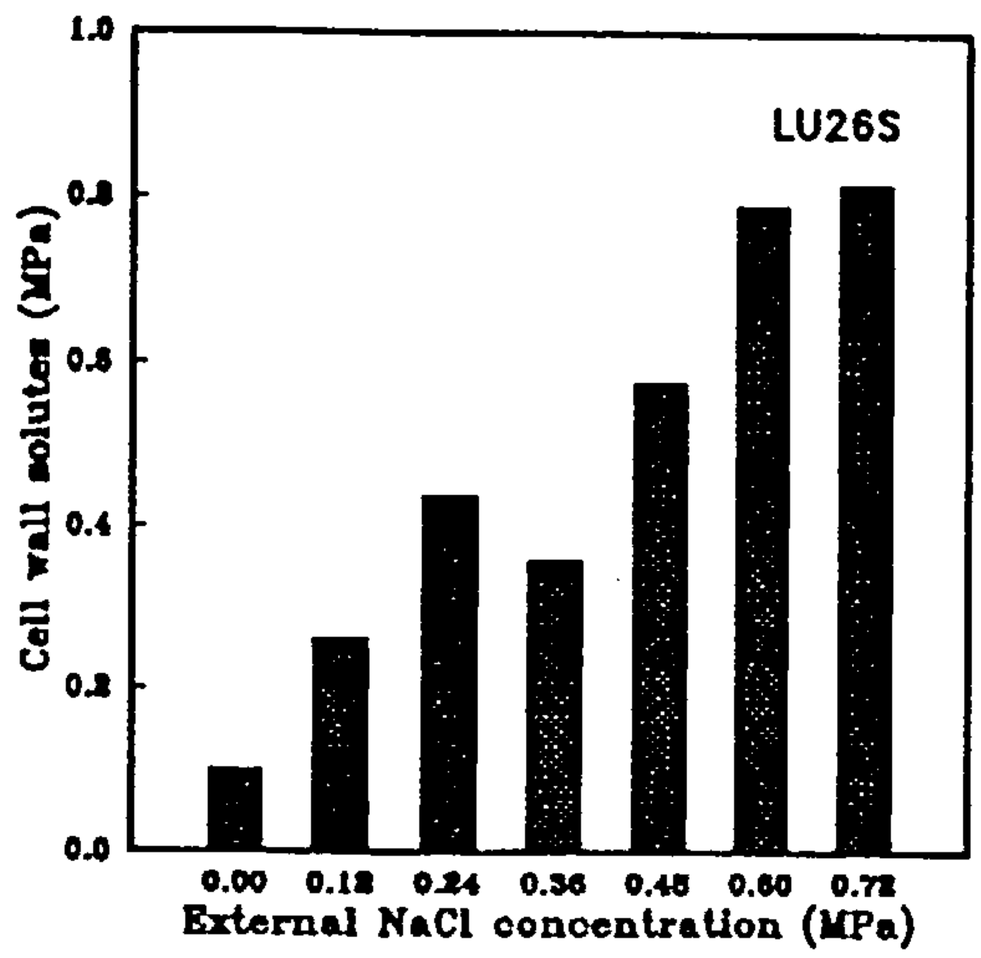
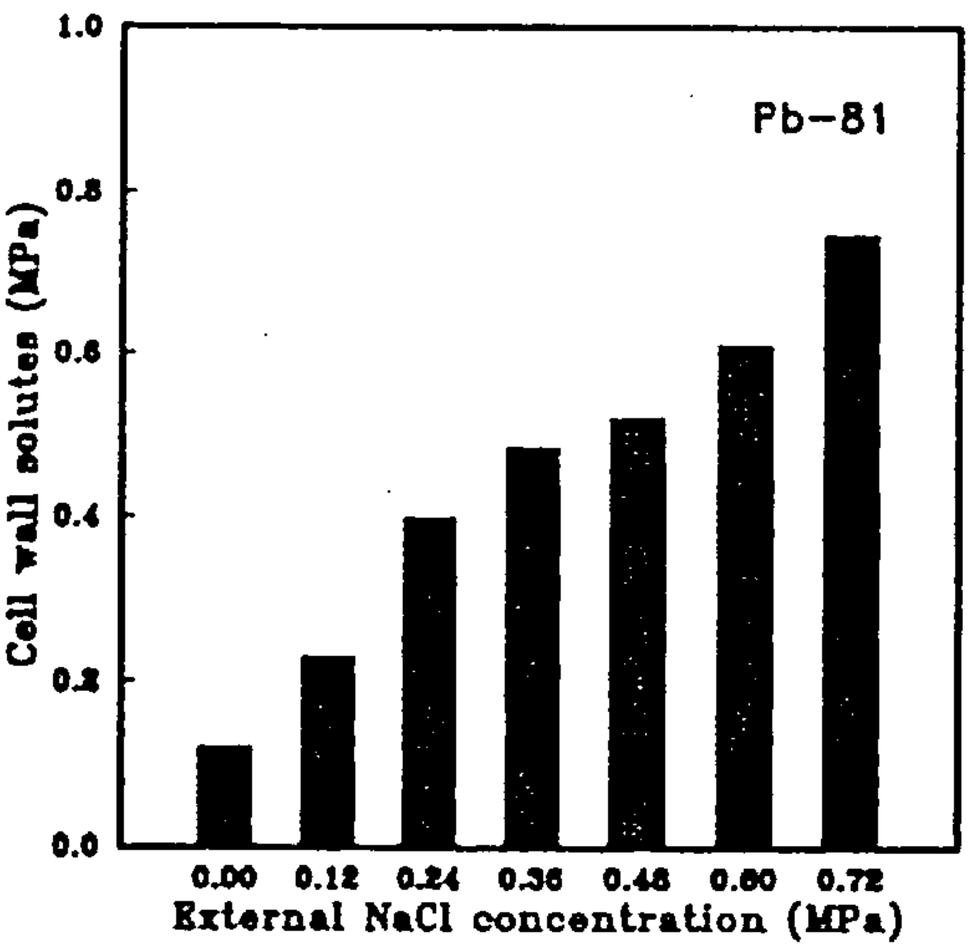

150 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

**Fig. 6.9** An estimation of the concentration of osmotically active solutes present in the cell wall of leaf mature zone of all the varieties studied, measured after 6 days of the stress application. The cell wall solutes were estimated by the difference of cell turgor pressure, tissue osmotic pressure and the cell wall transpiration tension.


Lyp-73
Cell wall solutes (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0.00 0.12 0.24 0.36 0.48 0.60 0.72
External NaCl concentration (MPa)


LU26S
Cell wall solutes (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0.00 0.12 0.24 0.36 0.48 0.60 0.72
External NaCl concentration (MPa)


Pb-81
Cell wall solutes (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0.00 0.12 0.24 0.36 0.48 0.60 0.72
External NaCl concentration (MPa)

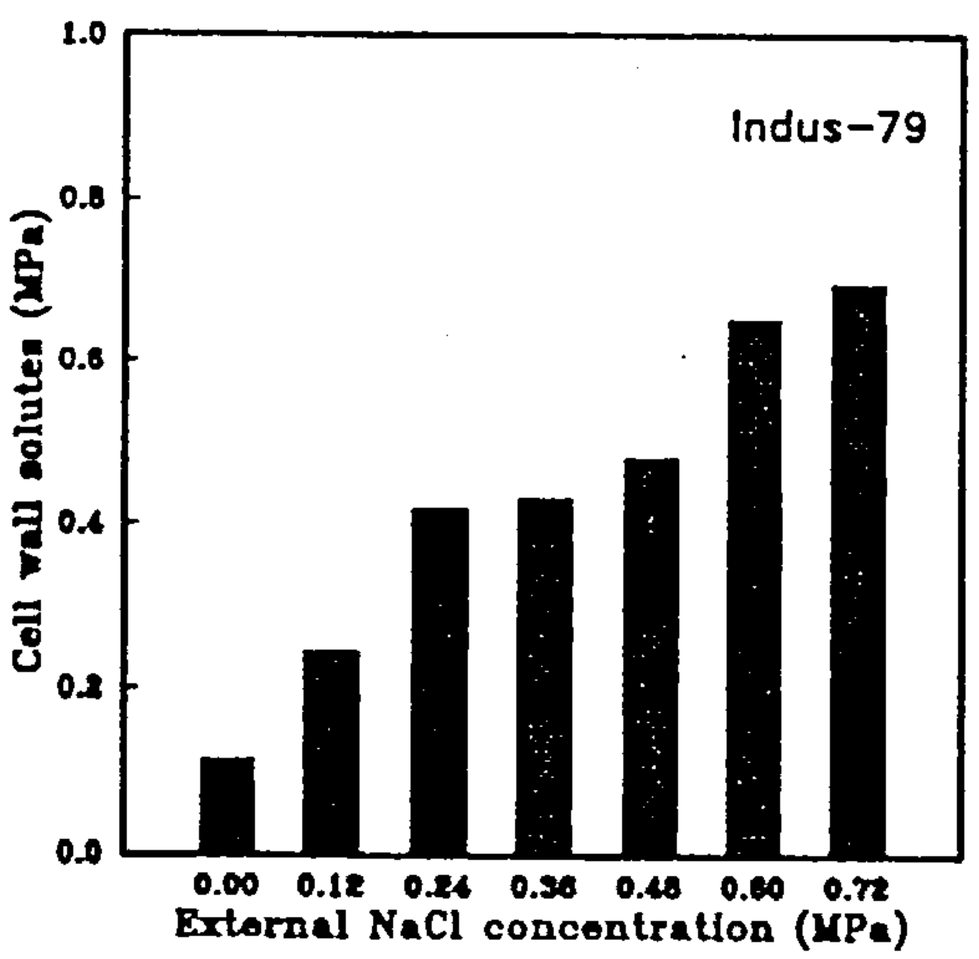
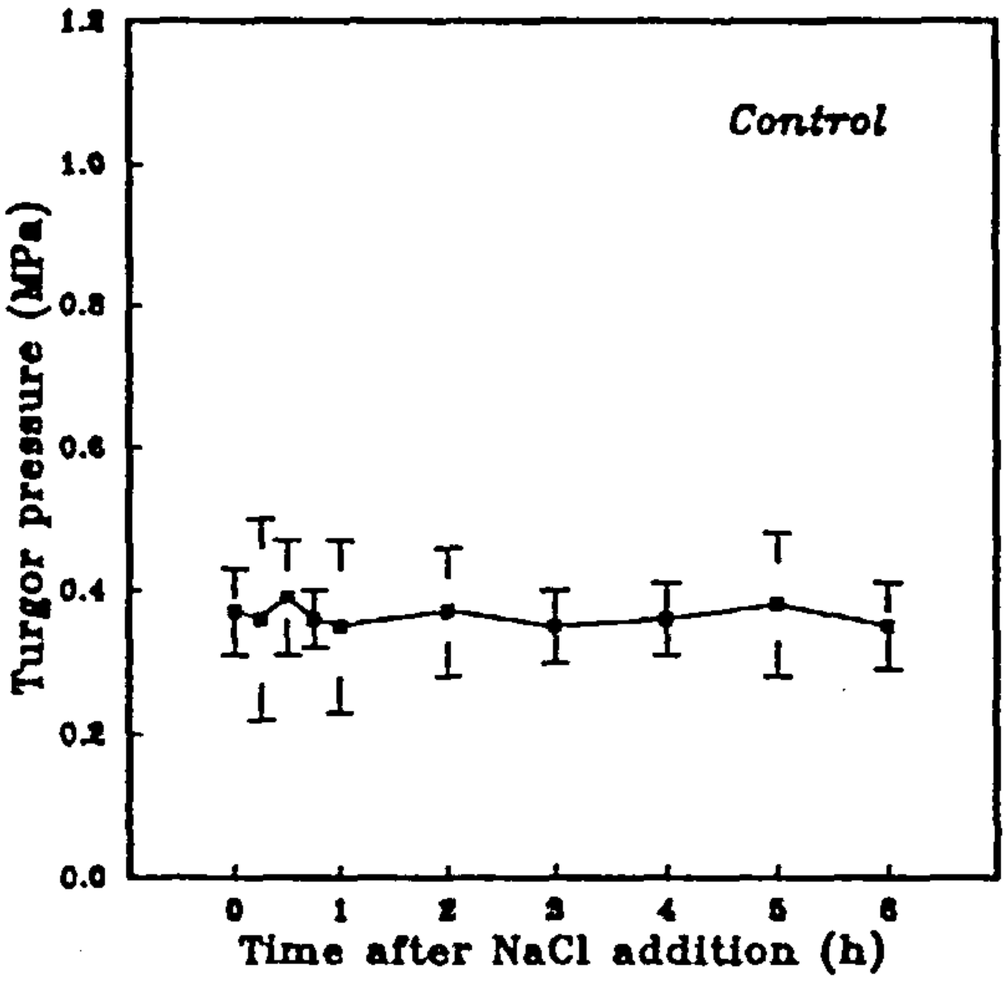
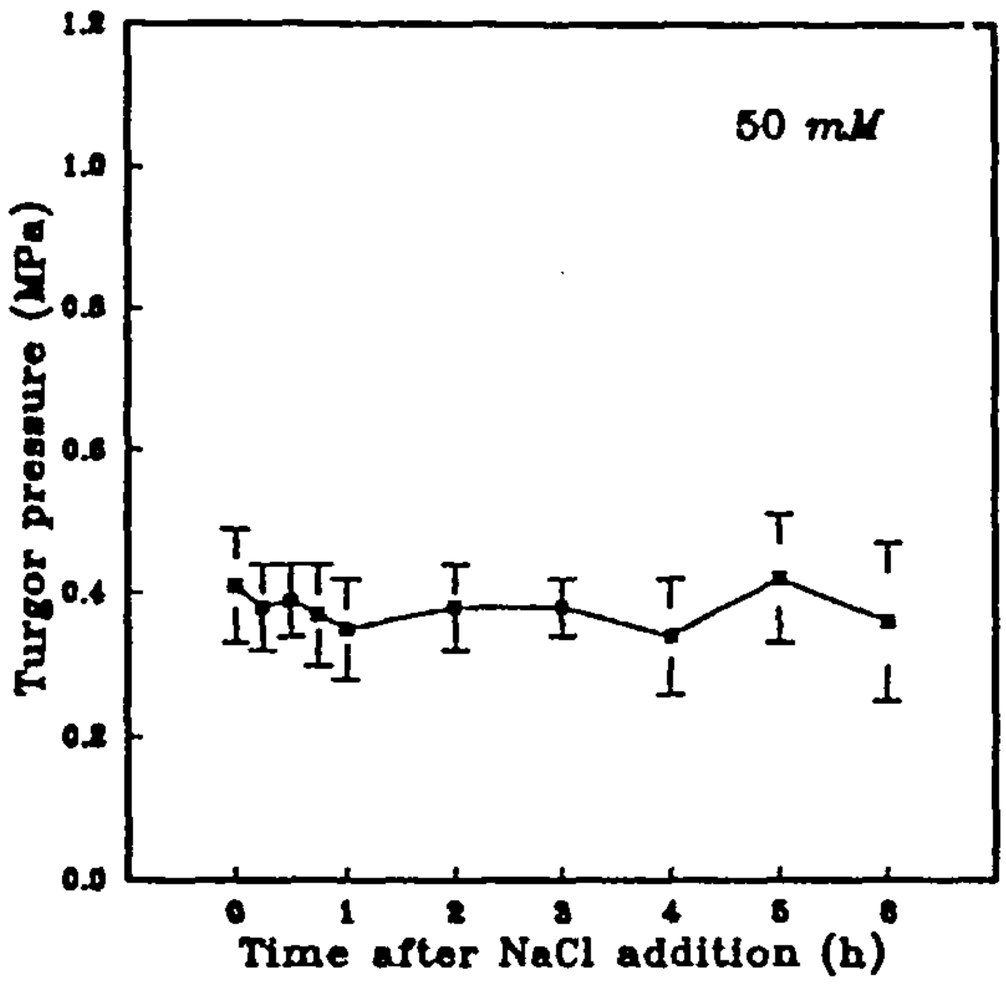
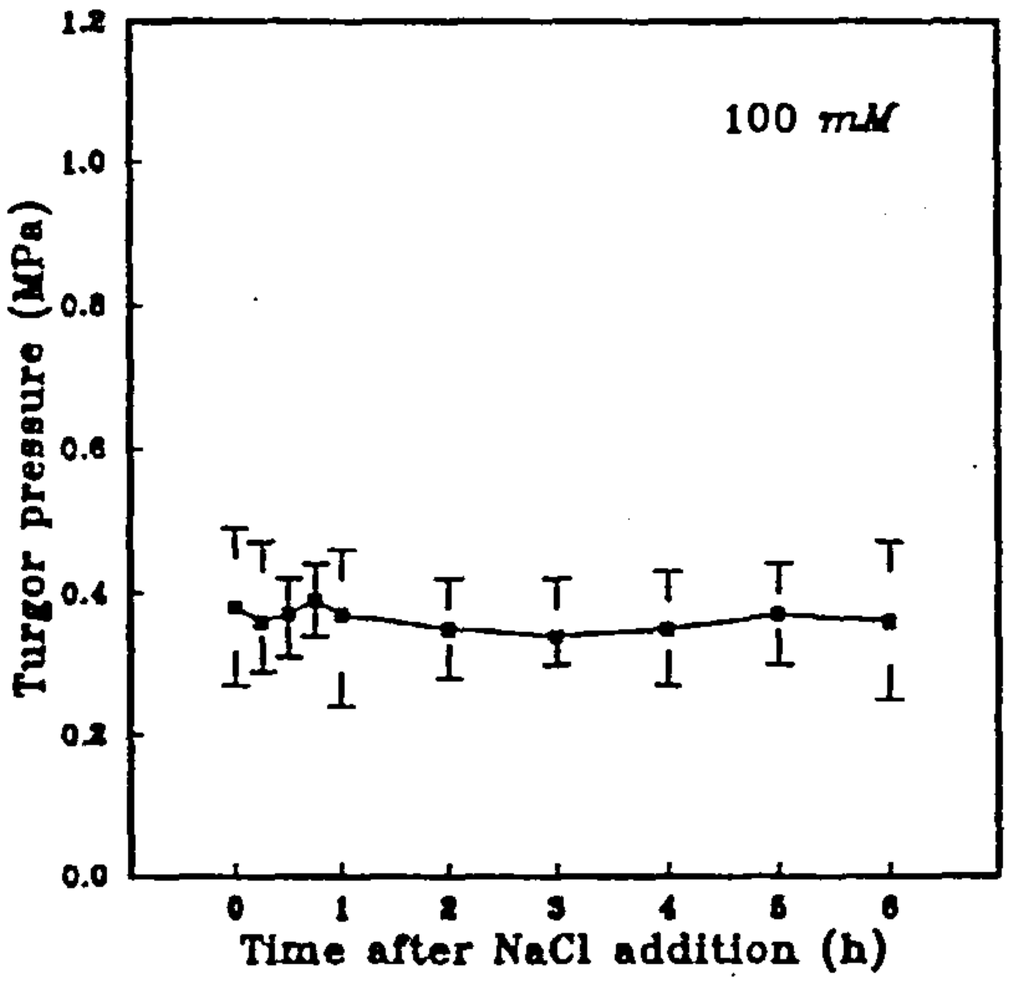

Indus-79
Cell wall solutes (MPa)
1.0
0.8
0.6
0.4
0.2
0.0
0.00 0.12 0.24 0.36 0.48 0.60 0.72
External NaCl concentration (MPa)

**Fig. 6.10** Time course of turgor pressure response to the NaCl stress measured in the expanding cells of LU26S, a salt tolerant variety, studied in a short term experiment i.e. over 6 h. Salt treatments as in Fig. 3.3. Each point is the mean of 3-5 replicates.


Control
Turgor pressure (MPa)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)


50 mM
Turgor pressure (MPa)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)


100 mM
Turgor pressure (MPa)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

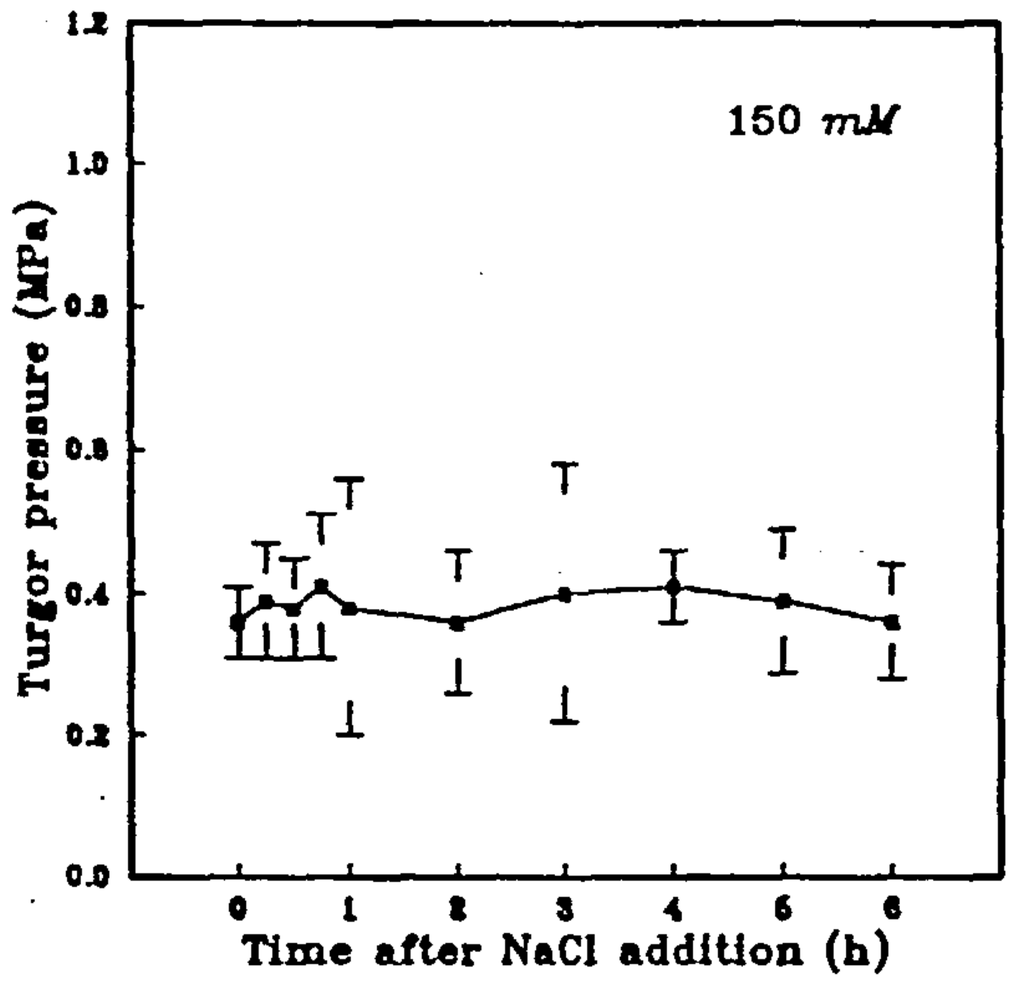
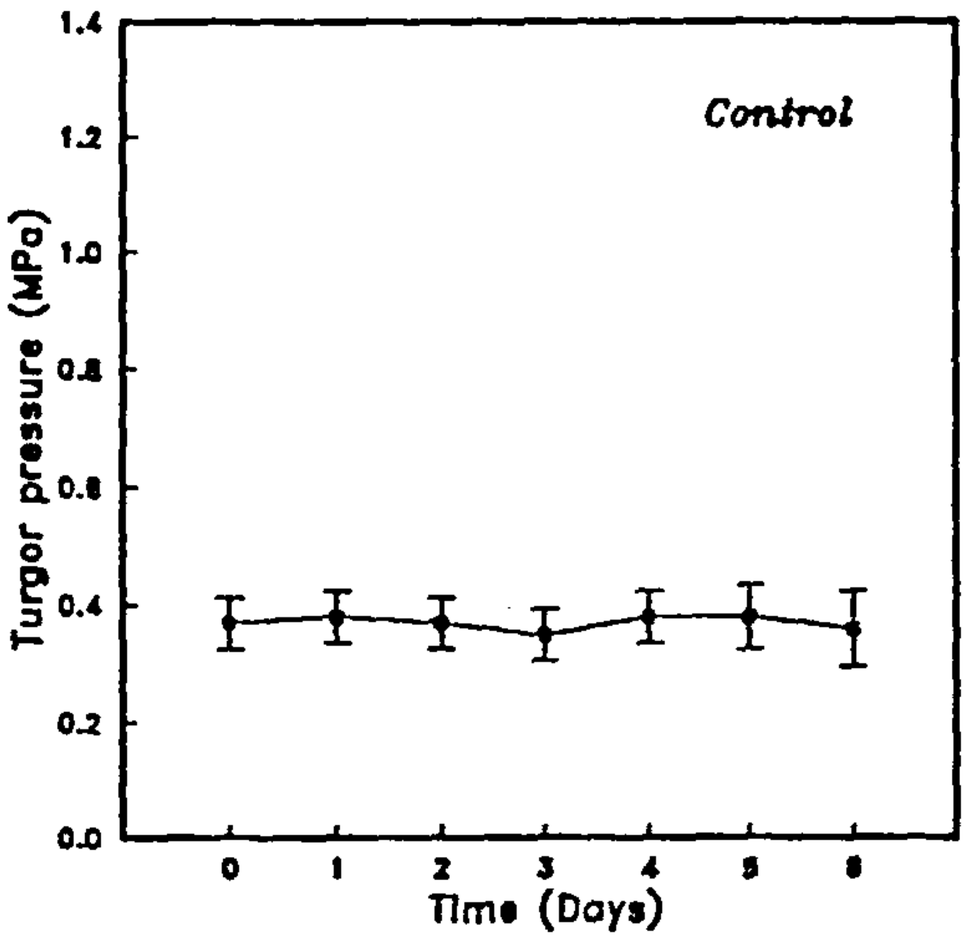
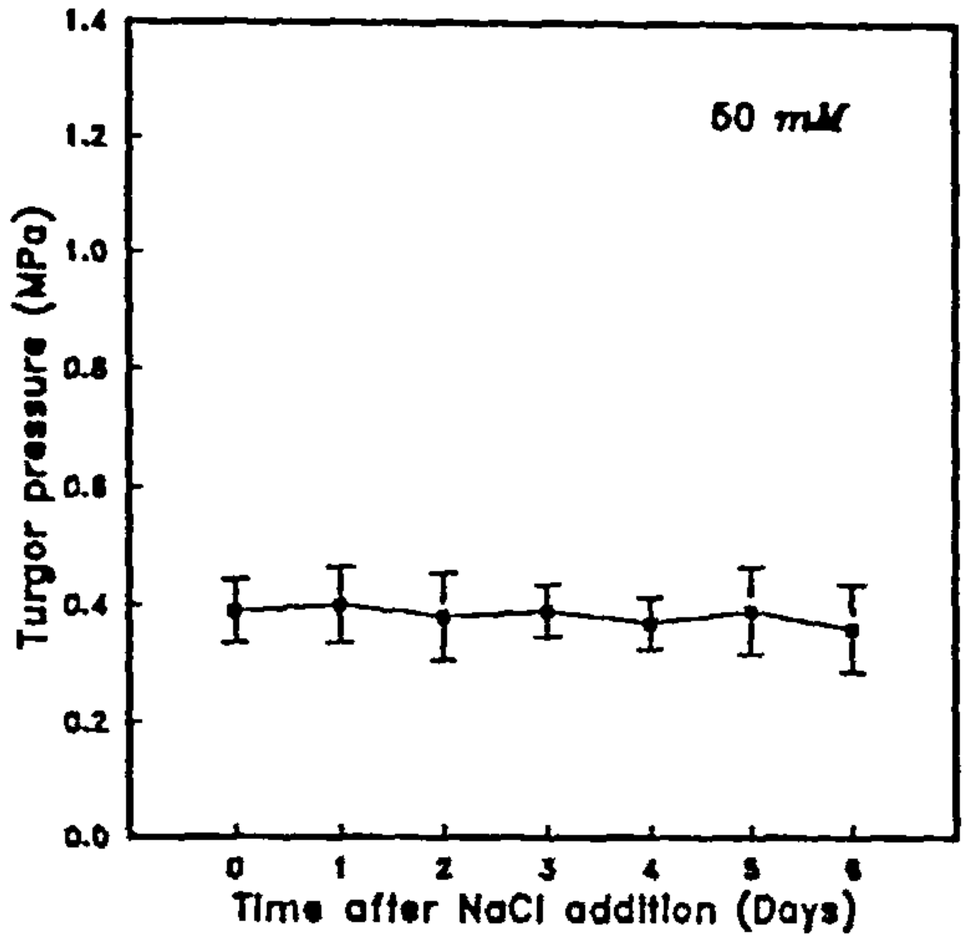
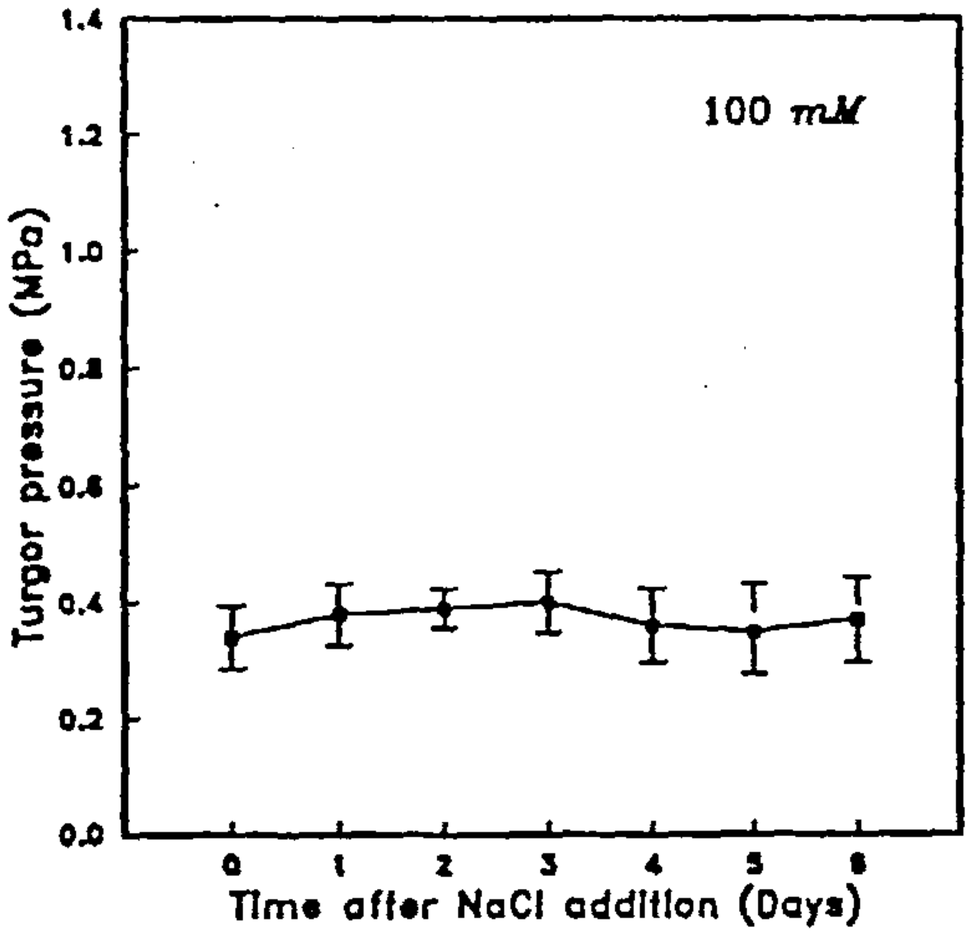

150 mM
Turgor pressure (MPa)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Time after NaCl addition (h)

**Fig. 6.11** Time course of turgor pressure response to the NaCl stress measured in the expanding cells of LU26S, a salt tolerant variety, studied in a longer term experiment i.e. over 6 days. Salt treatments as in Fig. 3.3. Each point is the mean of 3-5 replicates.


Control
Turgor pressure (MPa)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6
Time (Days)


50 mM
Turgor pressure (MPa)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)


100 mM
Turgor pressure (MPa)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

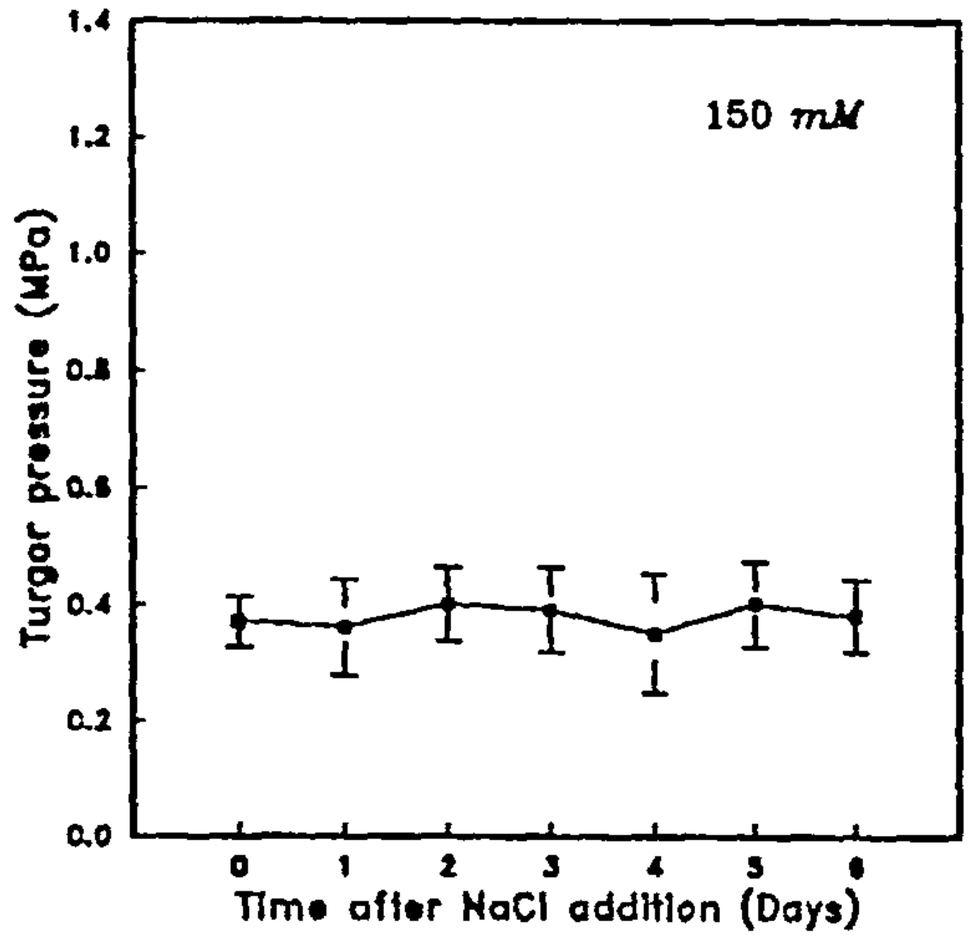
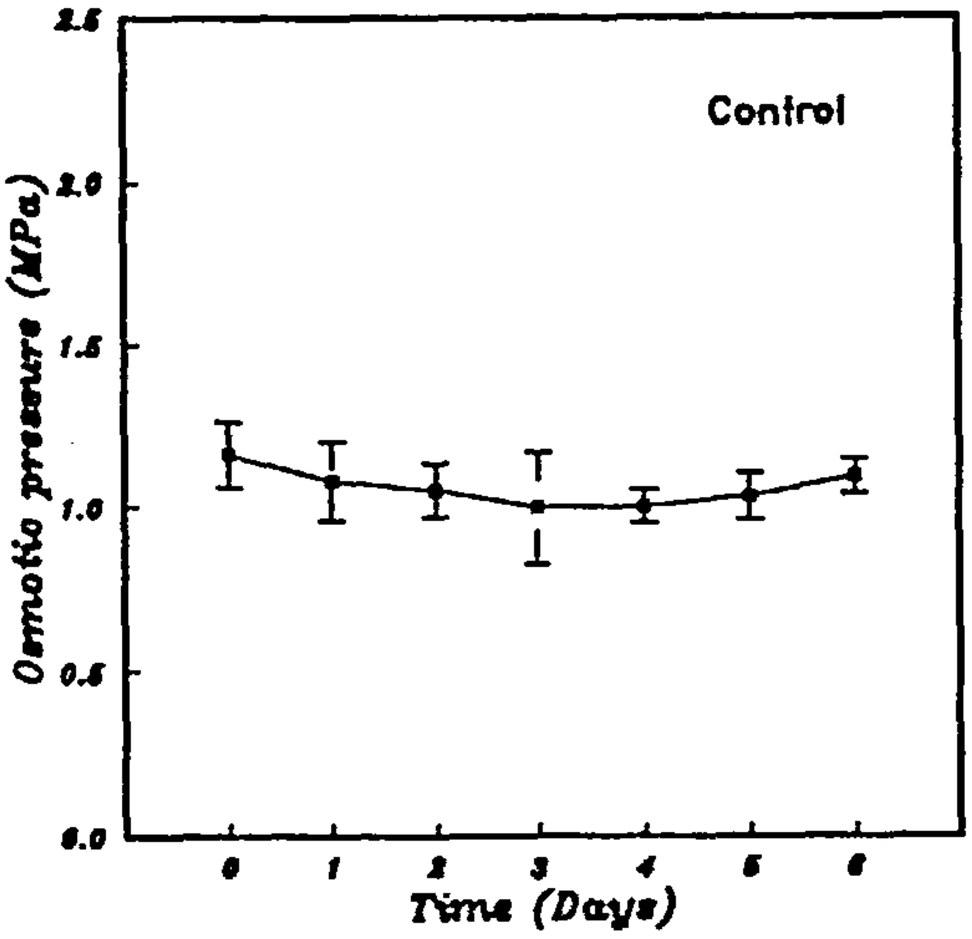
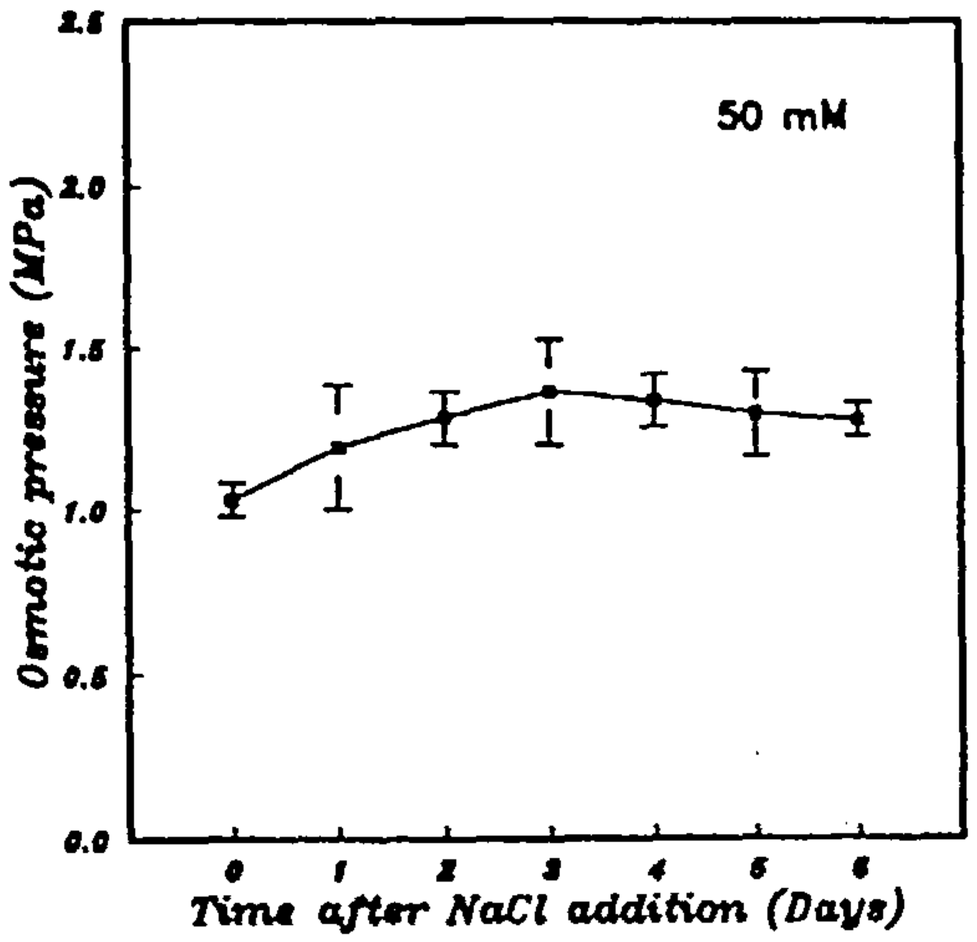
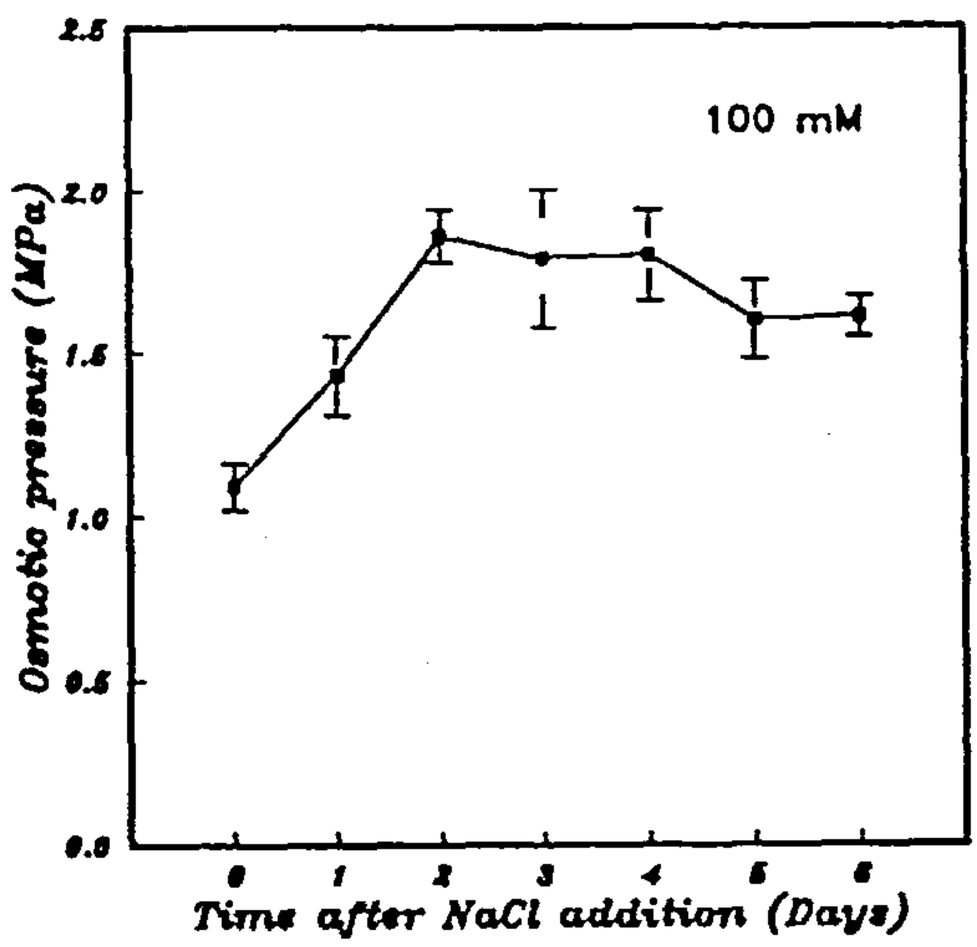

150 mM
Turgor pressure (MPa)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6
Time after NaCl addition (Days)

**Fig. 6.12** The osmotic pressure response to the NaCl stress studied in the leaf growing zone of LU26S, a salt tolerant wheat variety, in a long term experiment i.e. 6 days. Osmotic pressure was measured using vapour pressure osmometer in expressed cell sap on the whole tissue basis. Parallel experiment to Fig. 6.11. Each point is the mean of 5-8 replicates.


Control
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time (Days)


50 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)


100 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)

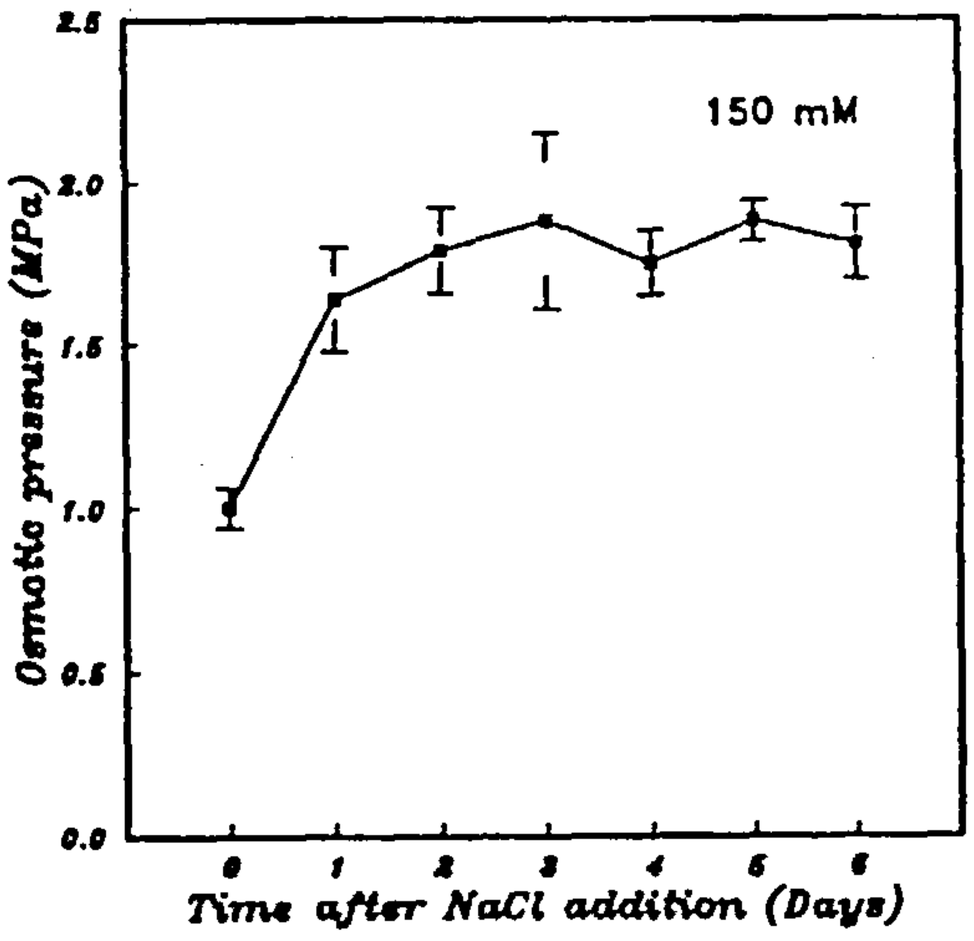
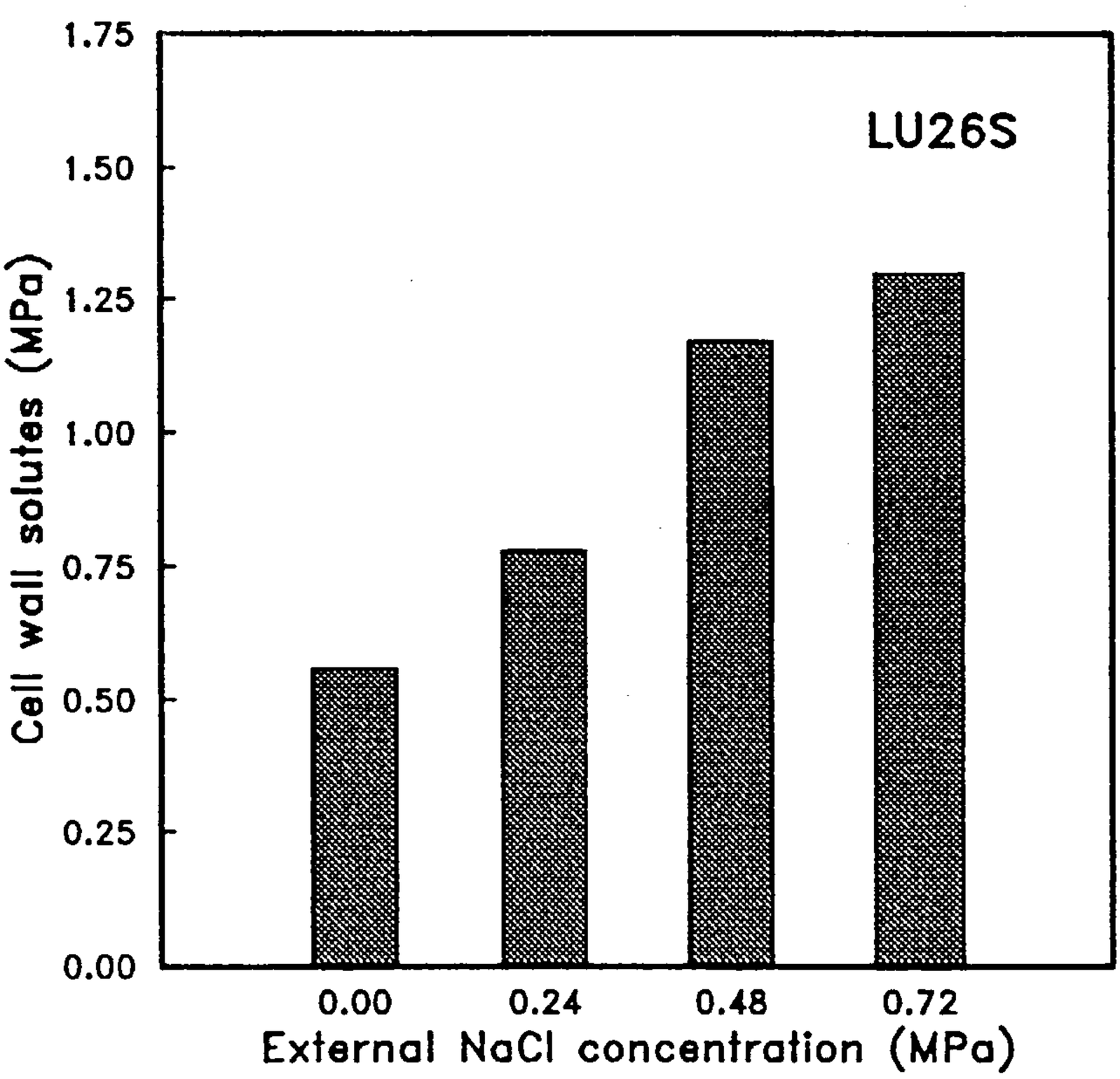

150 mM
Osmotic pressure (MPa)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
Time after NaCl addition (Days)

**Fig. 6.13** The concentration of osmotically active solutes present in the cell wall of leaf growing zone of LU26S, a salt tolerant wheat variety, measured after 6 days of the stress application. The cell wall solutes were estimated by the difference of cell turgor pressure, tissue osmotic pressure and the cell wall transpiration tension.


LU26S
Cell wall solutes (MPa)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.00
0.24
0.48
0.72
External NaCl concentration (MPa)

# CHAPTER 7

# *DISCUSSION*

## 7.1 Summary of Results

The effect of NaCl stress was studied on leaf growth rate and on cellular water relations in growing and in mature zone of the first emerged leaf of wheat seedlings. Varying degrees of NaCl stress (0, 25, 50, 75, 100, 125 and 150 mol $m^{-3}$) were applied to salinize the medium.

In case of leaf growth study a two phase response was observed;

a) a rapid decrease and later,

b) a recovery in the elongation rate.

Leaf elongation rate decreased within 1-2 minutes of the stress onset and it started recovering after 1-2 h of the salt application. The time taken for the recovery was proportional to the levels of external salinity. The elongation rate was almost fully recovered for all the NaCl levels after 24 h. A more or less similar response was observed when equi-osmolal concentrations (with NaCl) of mannitol were added to the media.

Turgor pressure in the elongating zone was about 0.45 MPa in non-stressed plants and tissue osmotic pressure was equal to 1.1 MPa showing that the cell had a low water potential (-0.6 MPa). The transpiration tension was equivalent to about 0.1 MPa. Turgor pressure in the growing cells did not change after addition of up to 150 mol $m^{-3}$ NaCl. However, the tissue osmotic pressure continuously increased with time. In contrast turgor pressure dropped when 200 and 250 mol $m^{-3}$ NaCl were applied to the medium. The mature cells exhibited a different turgor pressure response. In non-stressed plants turgor pressure was about 1.0 MPa, almost

twice that in the growing cells, while tissue osmotic pressure was similar to that found in the growing cells i.e. 1.1 MPa. The turgor pressure dropped in response to application of all the concentrations of NaCl. Turgor pressure recovery started due to osmotic adjustment after about 10-12 h of the stress onset. The complete turgor recovery was achieved after 24 h for NaCl concentrations up to 100 mol $m^{-3}$. While, tissue osmotic pressure increased continuously with time. A large increase in the osmotic pressure of cell wall solutes was observed during the whole experimental period. After 6 d of the stress application the increase corresponded to the magnitude of external stress.

The concentrations of various ionic and osmotic solutes were determined to observe their contribution to the biochemical osmotic adjustment. In non-stressed plants $Na^+$, $Cl^-$, $PO_4^{3-}$, $SO_4^{2-}$, glucose, fructose and sucrose were present in small amounts, however, $K^+$ and $NO_3^-$ were the major osmotica as their concentrations were about 200 mol $m^{-3}$. A large increase in the concentrations of $Na^+$ and $Cl^-$ was observed after the stress application, while sucrose level increased to a small extent. However, other osmotica remained uniform for whole of the experimental time.

Efforts were made to use turgor pressure recovery in the mature cells for designing a tool for the assessment of the salt tolerance of various Pakistani wheat varieties which were already rated according to their performance in absolute grain yield in response to NaCl stress. Turgor pressure response was similar as expected from their previous rating in case of three varieties.

However, LU26S responded differently where turgor pressure could not be recovered for the final two concentrations of NaCl. In case of their tissue osmotic pressure and cell wall solutes all the varieties responded similarly to that of the Flanders, assumed to be a model.

## 7.2 Salt Effects on Leaf Growth

In the study the leaf growth declined within 1 to 2 minutes, after the onset of NaCl stress (Fig. 3.1). These results were in accordance with the responses observed firstly by Acevedo et al. (1971). Similar rapid responses have also been studied in barley leaves in response to the salt stress (Matsuda and Riazi, 1981; Thiel et al. 1988). The growth inhibition in earlier studies was not dependent on either $Na^+$ or on $Cl^-$ (Thiel et al. 1988). They showed that salinity reduced leaf elongation in short term by osmotic effects on water availability in the expanding leaf tissue. In our study at the time of growth cessation cell turgor pressure of the growing zone remained unchanged or at least changed below the level measurable (Fig. 4.1). From equation 1.12 this indicates either that growth rate is influenced by change in wall properties or that the yield threshold, Y, is so close to the cell turgor pressure, P, that it can not be distinguished by the pressure results presented. In this current study pressure closer than 0.05 MPa would not have been distinguished. We shall discuss this further in the next section 7.3.

It, therefore, seems likely that the rapid recovery of growth rate on the removal of NaCl was not due to change in growing cell

Conversly, it might be due to the stored growth as metabolic processes for cell expansion (Acevedo et al. 1971), and cell division, might have continued unchecked during the stress period. When plants were kept in the saline media for 24 h a gradual recovery in the elongation rate was observed and after about 24 h the initial rates were attained for all the NaCl concentrations. The time taken for the process was irrespective of the salt concentration. On the whole we studied a two phased response (inhibition and subsequent recovery) in the leaf elongation rate when exposed to the salt stress. This contrasts the observation of Thiel et al. (1988) who studied the decreased but more stable elongation rate after about 1 h of the stress initiation. Their observation would probably be due to the shorter experimental period otherwise they would have observed the complete recovery of leaf elongation rate.

In our experiments the response of leaf elongation rate to the equi-osmolal concentrations of mannitol was similar (Fig. 3.2). This may seem to contrast the findings of Thiel et al. (1988) who proposed high ionic concentration to be the controlling factor for NaCl effect through its influence on ionic strength of the medium and on the ionic transport to the leaf expanding zone. Further, it contrasts the 'mannitol effect' i.e. mannitol caused an immediate reduction in leaf elongation rate when plants were transferred from NaCl to isosmotic solution of mannitol, as observed by Termaat and Munns (1986). Hence the leaf elongation rate may be affected by osmotic solutes irrespective of their types or nature, as our data showed.

## 7.3 Water Relations of the Growing Zone

### 7.3.1 Turgor pressure

The data indicate that turgor pressure in the leaf growing zone did not change and kept uniform while growth was markedly inhibited in response to the varying degree of the NaCl stress. The turgor constancy over the entire range of salt concentration indicated that growth inhibition was not mediated via a change in turgor pressure. Therefore, the observed decline in growth can be attributed to other biophysical parameters such as the cell wall extensibility and the yield threshold. In this context a number of research works have been done. For example Thomas et al. (1989) suggest the changes in cell wall rheology to be the cause of growth reduction at low temperature. Because of the absence of turgor pressure change in this case, they argue that the growth rate is controlled by wall rheological properties and does not involve changes in tissue hydraulic conductivity. From equation 1.12 we see that wall rheology can occur in two ways - by altering yield threshold or by altering wall extensibility. Cosgrove and Sovonick-Dunford (1989), studying the influence of gibberellin on pea stem elongation, proposed that the growth retardation is effected via a reduction in the cell wall extensibility and an increase in the yield threshold. However, under saline conditions, the work of Margritz and Cosgrove (1987) has suggested the cell wall extensibility rather than the yield threshold to be the controlling factor since the latter was unaffected by salt treatment. Cell wall extensibility was reduced by 50 percent. However, according to Thiel et al. (1988) it is

not entirely appropriate to estimate the yield threshold from comparison of the the individual cell turgor pressure and total leaf extension, especially when different plants are used for the measurements. However, Thomas et al. (1989) have recently observed the similar elongation rates for the whole leaf and for the single cells in the growing region of Lolium temulentum. Therefore, the argument put forward by Thiel et al. (1988) needs at least some experimental evidence. Nevertheless, the suggestions of Cosgrove and Sovonick-Dunford (1988) and Thomas et al. (1989) are consistent with the findings of several studies on wheat roots (Jones et al. 1987; Pritchard et al. 1987; 1988; 1989), phototropic mustard seedlings (Rich and Tomos, 1988), maize leaves (Michelena and Boyer, 1982) and soybean leaves (Randall and Sinclair, 1987). In all these systems turgor pressure remained unaltered after the stress onset implying change in wall properties. One contradicting observation has been put forward by Thiel et al. (1988) who found a drop in growing zone turgor pressure in response to salinity using pressure probe. They argue that the drop is restricted to the autotrophic leaves of the plants (third leaf in their case) not to the first leaf that utilize seed reserves for its growth. On the other hand Thomas et al. (1989) could not detect such specificity even in the fourth leaf, exposed to low temperature stress.

Our explanation based on changes in wall properties is in contrast to the work of Boyer and colleagues (Boyer, 1985) who claim that changes in the water conductivity of tissues is involved in controlling growth rate. Recently, for example,

Nonami and Boyer (1989) have argued that growth in soybean stem in response to water stress was inhibited by a collapse of the growth-induced water potential gradients necessary for water to enter the elongating tissue, not by turgor loss in the bulk of the tissue. Using the pressure probe they showed a drop in turgor pressure in a few cells next to the xylem only. These cells recovered turgor within 30 min after the stress. We found no evidence of turgor pressure gradient, either standing or changing, during our work and feel that the implication of such work as that of Nonami and Boyer (1989) can not as yet be assessed for wheat leaves.

### 7.3.2 Cell wall extensibility

The cell wall extension has been termed as a viscoelastic phenomenon resulting from biochemical modification of the wall (Cleland, 1987; Ray, 1987). Therefore, Cosgrove and Sovonick-Dunford (1989) argue that the wall yield threshold (Y) and extensibility (ø) should not correlate with mechanical measures of the wall when growth is not under the control of viscoelastic process (mechanical or physical extension). Further, when biochemical processes control the growth, it should be influenced by a single chemorheological process (biochemical reaction-dependent extension) with no correlation with the mechanical properties of the wall.

In our experiments the extensibility of excised cell wall was also measured (in vitro). Results obtained using the pressure probe implies wall rheological changes. Although, as discussed

above, the Instron tensiometer (Cleland, 1984) does not measure the parameters described in the Lockhart equation. We need the technique to see if there are any changes in the wall that could be detected by tensiometry. Despite the change in growth rate in vitro extensibility was found to be unchanged in the salt treated plants even after 5 h of the stress onset (Table 3.4). After 144 h both, elastic and plastic, components of the in vitro wall extensibility decreased for both stressed and control plants. This decline can be attributed to the tissue hardening with age and not to the salinity. At the same time a potential error in the technique should not be ruled out since the whole whorl of leaves in the stem was taken to be representing the measurements on the first leaf extensibility. Our results are similar to the findings of Cosgrove and Sovonick-Dunford (1989) who suggest that for pea stems Instron-extensibility is not a reliable indicator of the wall properties that governs growth as the two do not correlate.

### 7.3.3 Cell wall solutes : The role in turgor maintenance

There remains the question of how turgor pressure is kept constant in the expanding cells despite the osmotic stress. The turgor pressure in the expanding cells (of control plants) is very much lower than the osmotic pressure (even after transpiration tension is abolished). This either suggests a growth-induced water potential gradient from xylem (Nonami and Moyer, 1989) to cell, a wall hydrostatic tension that is independent of transpiration or the presence of osmotically active solutes in the cell wall. This last possibility has been

argued for the other cases by several colleagues e.g. (non-growing tissues) Leigh and Tomos (1983), (growing tissue) Cosgrove and Cleland (1983 a and b), and Clipson et al. (1985). Aspects of this have been reviewed by Tomos (1988). It makes a great deal of sense to have high solute concentrations in the cell wall for the cells with very low water potentials such as salt adjusted halophytes. If the wall water potentials were maintained very low by its hydrostatic component i.e. transpiration tension, the high atmospheric humidity would stop transpiration and allow cells to reach full turgor resulting in the cell bursting. A similar situation was observed when salt treated Suaeda maritima leaves were infiltrated with distilled water (Tomos, 1988). In further support to this argument Cosgrove and Cleland (1983 a and b) propose that hydraulic conductance is large enough and only small water potential gradients are necessary to sustain water influx for growth. If large growth-induced water potential gradients are necessary for growth (as proposed by Nonami and Boyer, 1989) then a reduced growth rate should be associated with a high water potential and turgor pressure (provided L and $\pi$ remained constant) (Cosgrove, 1986). However, this was not the case when growth rate was decreased by various treatments such as cyanide, auxin application and low temperature the turgor pressure in growing cells remained unchanged (Cosgrove and Cleland, 1983b; Thomas et al. 1989).

The amount of osmotically active solutes present in the cell wall in the growing zone of leaf of control plants was estimated (Fig. 4.4) and was found to be about 0.6 to 0.7 MPa. This represents an

osmolality equivalent to 145 to 185 mOsmol $kg^{-1}$ of water. This much osmolality can be equivalent to 122 to 142 mol $m^{-3}$ NaCl. Therefore, one possible mechanism responsible for turgor maintenance in growing cells in response to up to 150 mol $m^{-3}$ NaCl is proposed from this observation.

It is proposed that growing cells maintain their turgor pressure by regulating the cell wall solutes which are high even under non-stressed conditions. The cells take up the solutes from the surrounding cell walls immediately after receiving the specific signal (Termaat and Munns, 1985) from the roots about the salt stress onset. Therefore, they managed to maintain their turgor pressure despite in the presence of large change in water potential. This mechanism would have a significant impact on the cell wall osmotic pressure with little effect on that of the cell. This can be illustrated if we assume that wall free space water and cell sap occupy about 5 and 90 % of the tissue volume, respectively. The uptake of all the osmotically active wall solutes i.e. equivalent to 0.6 to 0.7 MPa, into the cell would result in an increase of only 0.033 to 0.038 MPa in the tissue sap osmotic pressure (which will be dominated by solutes from the protoplast). The measurements imply that the walls contain 0.6-0.7 MPa of solutes that affect the osmotic balance of cells (i.e. they do not include solutes held in Donnan equilibrium of the Donnan Free Space that will have their effect cancelled out by the Donnan Hydrostatic Pressure). This sets a "upper limit" on the stress that can be accommodated. Further evidence for this proposal, therefore, comes from the observation that on application of more than this amount of salt (200 and 250 mol $m^{-3}$

i.e. 0.49 and 0.61 MPa) a drop in turgor pressure was observed. The uptake of all the cell wall solutes could not help in the turgor maintenance. Our results are in agreement with the findings of Schmalstig and Cosgrove (1988), who studied a similar phenomenon in pea epicotyls in response to stoppage of the solute import into the growing region by removal of the cotyledons. In their study stem elongation was inhibited by 60 % within 5 h of excision. Dry weight accumulation into the growing region stopped and osmotic pressure of the cell sap declined by 0.14 MPa over 5 h. Despite the drop in cell osmotic pressure, turgor pressure did not decline. Turgor maintenance was postulated to occur via uptake of solutes from the free space, thereby maintaining the osmotic pressure difference across the plasma membrane. However, in their system about 37 % of the solutes were taken into the cells which probably depended upon the extent of the stress applied.

### 7.3.4 Footnote

In concluding this section this must be noted that our explanation of wall properties regulating growth is based on the apparent absence of turgor pressure change in expanding cells upon salt stress. In conclusion, it must be stated that it is impossible to exclude regulation by turgor pressure change below our limit of resolution. The data presented here represent the current state of the art in studying growth at single cell resolution. Hopefully future developments in techniques will throw even more light on the issue.

## 7.4 Water Relations of the Mature Zone

### 7.4.1 Turgor pressure

Turgor pressure determined in the mature zone was about 1.02 ± 0.08 MPa (possibly the highest turgor values measured in any tissue of higher plants). The turgor values were twice as high as those measured in the growing zone of the same leaf (Fig. 5.1). This observation is in agreement with the previously directly (Thiel et al. 1988) and indirectly (Matsuda and Riazi, 1981) measured turgor values of barley leaves. However, a contrasting trend exists in Suaeda maritima leaves in which turgor pressure is higher in expanding cells than that in the mature cells, measured with pressure probe (Clipson et al. 1985). Turgor maintenance in response to different types of stresses has also been studied in maize leaves (Michelena and Boyer, 1982; Westgate and Boyer, 1984) and in wheat roots (Pritchard et al. 1987). Our turgor pressure values in non-stressed plants were quite uniform, however, such uniformity was absent in the results of Thiel et al. (1988) who found a big variation in mature zone turgor pressure values for barley i.e. 0.57 to 0.82 MPa.

The observed decline in turgor pressure on addition of salt to the medium is presumably due to the osmotic effects of the external media that were transmitted from roots to the leaves. A similar turgor pressure response has been reported by Thiel et al. (1988), and by Nonami and Boyer (1989) in barley and soybean after NaCl and water stress, respectively. The drop in turgor pressure corresponded quantitatively to the lower NaCl concentrations (25, 50 and 75 mol $m^{-3}$), however, it was not in

accordance with the higher concentrations (100, 125 and 150 mol $m^{-3}$). We have no explanation for this, other than the possibly that root reflection coefficient ($\sigma$) may be altered by salt concentration. Indeed a recent report on root cells of Mesembryanthemum crystallinum indicate that not only is $\sigma$ affected by high salt but that it can assume negative values (Rygol and Zimmermann, Personal communication). Our results are similar to the observation of Thiel et al. (1988) who found an inconsistent turgor drop of about 56 % of the osmotic stress i.e. 80 mol $m^{-3}$ NaCl, assuming an osmotic coefficient of 0.9. They could not manage to observe any change in turgor pressure after treating the plants with 40 mol $m^{-3}$ NaCl. This response could be due to the excessive amount of salts present in the root medium i.e. producing higher osmotic pressure than the root cell osmotic pressure (which is about 200 to 250 mOsmol $kg^{-1}$, Pritchard, 1988). Under such conditions water transport in the roots changes its pathway from transcellular to symplasmic (J. Frensch, Personal communication) increasing certain type of resistance to the water flow (Nonami and Boyer, 1989). Thiel et al. (1988) have argued that in short term leaf responses are partially buffered from media salinization by some unknown mechanism, perhaps water storage or redistribution in the plant or rapid osmotic adjustment. Steudle and Tyerman (1983) have reported root reflection coefficient to be of about 0.6 for NaCl, however, our results indicate much higher reflection coefficient values for low NaCl concentrations i.e. 25, 50 and 75 mol $m^{-3}$.

### 7.4.2 Volumetric elastic modulus

Another, if unlikely, explanation for the lack of correlation between external osmotic pressure and cell turgor pressure would be a large effect of the stress on values of the instantaneous volumetric elastic modulus of the cells. This was measured in the mature region in order to find whether this parameter is responsible for the inconsistent response of turgor pressure. The uniformity of the parameter (Fig. 5.2) over a wide range of NaCl concentrations for 6 d indicated no relation with the extent of the turgor pressure decline. These results are in accordance with the results of Margritz and Cosgrove (1987), who measured the instantaneous volumetric elastic modulus in Pisum sativum seedlings. The ε reported was 4.8 to 4.9 MPa and was unaffected by the salt treatment, which would have caused water efflux from the root cells. However, the findings of Frey et al. (1988) contradict our results which show an increase in ε in single-celled alga Eremosphaera virdis with the increase in ion concentration of the culture medium. They suggest that the presence of sodium has a pronounced influence on the elsticity of the cell wall. This response would probably be specific for the unicellular lower plants only e.g. algae, which are in direct contact with the external media. In the support of this argument are the findings of the Rygol et al. (1989) who studied the water relations of leaf cells of Mesembryanthemum crystallinum plants. They could not measure any dependence of absolute values of the elastic moduli on the salinity because of the large fluctuations in the ε values of bladder cells. Balanõs and Longstreth (1984) measured an increase in bulk elastic modulus in alligator weed

(Alternanthera philoneroids) under salt stress. The observed increase would probably be due to difference in the technique employed. Therefore, a conclusion can be drawn from our findings i.e. the turgor pressure response is irrespective of the ε in context of the salt stress.

### 7.4.3 Root hydraulic conductivity

Finally the effect of salt stress on root $L_{pr}$ was analysed to see if there would be the basis of the lack of correlation between external osmotic pressure and leaf cell turgor pressure. $L_{pr}$ was measured in single excised roots and in whole root systems. The values of $L_{pr}$ for the whole root system showed a relationship with the turgor pressure response in salt stressed plants. Among the single and whole root systems, the latter simulates the situation under which turgor pressure was measured. The $L_{pr}$ values in single roots were higher than in the whole root system, these results are similar to the results of Jones et al. (1988), however, the overall higher values in our case indicate that the time after excision can be crucial in such measurements. Therefore, the lower values of $L_{pr}$ reported by Jones et al. (1988) could have been due to long time elapse (90 minutes) between the excision and the measurements, whereas in our case the measurements were performed after 30 minutes of excision. This could also be due to variation in the root length. Our results suggest that NaCl and mannitol have similar effects on the $L_{pr}$ (Fig. 5.3), as it decreases slightly but the differences were not statistically significant. The overll $L_{pr}$ values remained almost uniform for all the concentration jumps (see

section 5.1.3). This uniformoity could be due to the possible decrease in the root reflection coefficient with the increase in external salt concentrations. A decrease in σ would result an increase in $L_{pr}$ (see Equation 1.14).

During the measurements, water efflux from roots (as indicated by a drop in the exudate level in glass capillary) in response to osmotic stress corresponded to external stress level in the single excised roots, however, in the whole root system exudation rate did not correspond to the external stress level. This discrepancy could be due to high resistance to water flow at root-shoot interface in the whole root system (Jones et al. 1983). This is not clear whether the different $L_{pr}$ values for different type of tissues are due to change in the water flow pathway (Jones et al. 1988). $L_{pr}$ values obtained, however, are found not to support the turgor pressure response to higher NaCl concentrations, where it did not correspond to external stress level. Therefore, the leaf turgor pressure could be assumed to be independent of the $L_{pr}$.

### 7.4.4 Turgor pressure regulation

As shown in results section (5.1.4), the turgor pressure started recovering after 5 to 6 h of stress initiation and with time was fully recovered. However, the time required for complete recovery varied with the varying NaCl concentrations in the root media. The process of turgor recovery was completed after 24 and 48-72 h for the low and high salt concentrations, respectively. Turgor regulation was carried due to conventional osmotic adjustment (in

contrast with the role of wall solutes discussed above) resulting from the uptake and the subsequent transport of the solutes to the leaves. A similar turgor recovery have been reported recently by Nonami and Boyer (1989) in the mature zone of soybean stems that occured after 45 h of their transplant to lower water potential (-0.028 MPa ≈ 57 mol $m^{-3}$ NaCl). They also suggested that turgor recovered due to the osmotic adjustment which was caused by an accumulation of solutes in the tissue.

During our studies we discovered (by accident) that the nutrient status of growing media can have a profound effect on the water relations of plant cells particularly the turgor pressure. The large increase observed in turgor pressure of leaf epidermal cells, when salt stress was accompanied by supply of full nutrient solution to plants previously growing in low nutrient medium i.e. 0.5 mol $m^{-3}$ $CaCl_2$, (Fig. 5.5) might have been due to rapid and large uptake of $K^+$ into the leaf cells when the growing media was changed from 'low nutrient' to 'high nutrient' (see Materials and Methods). This assumption is similar to those derived from the results of Pritchard et al. (1987) who observed turgor pressure response in the wheat root epidermal cells 32 to 40 mm away from the meristem on changing the nutrient status of the growing media. Turgor pressure was found to be 0.3 to 0.35 MPa for low salt plants and in the plants supplied with sodium salts (10 mol $m^{-3}$), while the turgor pressure of 0.55 to 0.74 MPa was recorded when $K^+$ salts were applied in the medium. The crops well fertilized with $K^+$ contain high vacuolar concentration of $K^+$ as a major contributor to the osmotic pressure of cell sap (Hsiao

and Läuchli, 1986). However, in the absence of $K^+$ salts the plants can maintain their turgor pressure by substituting other solutes such as $Na^+$, $Mg^{2+}$, $Ca^{2+}$, organic solutes for vacuolar $K^+$ (Leigh and Wyn Jones, 1984). That turgor pressure is a function of these changes is rather unexpected and suggests that turgor pressure, per se, is not an important parameter. Indeed, this has been recently argued by Munns (1988).

### 7.4.5 Transpiration tension

In the discussion so far we have discussed transpiration tension from the consideration of wall water potential. The cell wall transpiration tension is the hydrostatic component of the wall water potential which plays an important role in the control of water relation of the cell (as above Donnan Hydrostatic Pressure is ignored since it will be cancelled out by the Donnan Osmotic Pressure). As summarised in the results section (see 5.1.7) the salt stress appear to have no profound effects on this parameter. If hydrostatic tension was a major component of the wall water potential, the turgor pressure would have increased much more than measured by seedling immersion in the growing medium. Our observation agrees with the measurements of the wall transpiration tension (0.1 to 0.2 MPa) in Suaeda maritima using the similar technique (Clipson et al. 1985). However, in contrast to our findings, the transpiration rates of wheat and barley decreased by 32 and 44 % respectively, at 100 mol $m^{-3}$ NaCl stress (Termaat et al. 1985). The transpiration tension is a small component of transpiration stream in cell wall and therefore large changes in transpiration rate have small influence on the

transpiration tension. However, transpiration tension have large effects on transpiration stream.

### 7.4.6 Cell wall solutes.

Our results suggest that under control conditions negligible amounts of osmotically active solutes are present in the cell wall of mature cells (Fig. 5.9). The cell turgor pressure corresponds to the tissue-based osmotic pressure (Arif and Tomos, 1988). Malone et al. (1989) have recently confirmed it by measuring the cell osmotic pressure directly using nanoliter osmometer along with the pressure probe for the sap extraction. The results presented here are in contrast with those of the growing tissue discussed (7.3.3) above and of the growing tissue of other systems (Cosgrove and Cleland, 1983a; Nonami and Boyer, 1987). In apparently mature tissue using the pressure probe Leigh and Tomos (1983) indicated that turgor pressure in the cells of red beet tap-root were 0.4 to 0.5 MPa lower than the full turgor expected from the tissue osmotic pressure, under the conditions of negligible transpiration tension in the cell wall.

Our results indicate following application of salt stress that the osmotic pressure of cell wall solutes increases with time and after 6 d of the stress initiation it corresponds to the amount of salts present in external media. This observation agrees with the postulate of Gorham et al. (1985) who assumed that toxic ions might be located in the older leaves which are shed as a characteristic mechanism of the salt tolerance in Gramineae. However, their measurements were carried out on whole tissue

basis instead of measuring the osmotic pressure of the cell and the cell wall separately. This was done in our study for the cell wall solutes. Our results are analogous to those of Clipson et al. (1985) who, also using the pressure probe, found that cell wall osmotic pressure in the leaf mature zone of the Suaeda maritima was about 2 to 3 MPa (depending on external salt concentration), estimated by the difference of the measurements of turgor pressure, osmotic pressure and cell wall transpiration tension. In the same plant species Clipson and Flowers (1987) have suggested xylem sap concentrations equal to 0.6 and 0.3 MPa for night and day conditions, respectively. This value was much lower than that of the leaf apoplast. Therefore, the xylem sap may not be representative of the leaf cell wall solution and the apoplast might not be uniform osmotically (Tomos 1988). Palta et al. (1988) have measured a constant turgor pressure in sugar beet tap root cells under non-illuminated conditions despite the tissue osmotic pressure rising from 1 MPa to 2.7 MPa. They proposed that the increase was due to the accumulation of osmotic solutes in the cell wall. Therefore, a link can be suggested between the wall solutes and the turgor pressure in a feedback system to maintain turgor pressure (Wyse et al. 1985).

Harvey and Thorpe (1986) measured the ion distribution within wheat leaf mesophyll cells from the plants grown in the presence of 100 mol $m^{-3}$ NaCl by x-ray microanalysis. They found that quite high concentrations of $Na^+$, $K^+$ and $Cl^-$ were present in the cell wall i.e. 733, 137 and 105 mol $m^{-3}$, respectively, which were measured per unit of total volume of material present. They also

observed salinity-induced increase and decrease in the volume fraction of the cell wall and vacuole, respectively.

### 7.4.7 Solutes used for osmotic adjustment in mature tissue

Salinity induces a change in concentrations of ionic and osmotic solutes present in plant cells. The results show that a large increase in the leaf $Na^+$ occured after the NaCl stress (Fig. 5.10). However, the leaf concentration of the other major cation, $K^+$ remained almost unchanged for all the experimental duration (Fig. 5.11). Among the four measured anions only $Cl^-$ concentration increased after the stress application (Fig. 5.12). However, the leaf $NO_3^-$ level appeared to be unchanged by the increase in the extent of the stress (Fig. 5.13). The concentrations of the two other anions, $PO_4^{3-}$ and $SO_4^{2-}$ were found to be almost uniform under the similar experimental conditions, however, sulphate showed a tendency of increase at 125 and 150 mol $m^{-3}$ NaCl (Fig. 5.14 and 5.15).

The increase in concentrations of $Na^+$ and $Cl^-$ are consistent with the previous findings in wheat (Gorham et al. 1985; Rashid, 1986), barley (Delane et al. 1982; Munns et al. 1982), rice (Flowers and Yeo, 1981; Yeo and Flowers, 1986), stone fruit crops (Maas and Hoffman, 1977), Suaeda maritima (Yeo 1981; Gorham and Wyn Jones, 1983), avocado (Downton, 1978), soybean (Abel, 1969), castor bean (Jeschke and Wolf, 1988) and perennial Triticeae (Gorham et al. 1984, 1985 and 1986), where these ions were accumulated in plant cells as a consequence of the salt stress.

No change in $K^+$ concentration after the stress onset was seen by

Jeschke and Wolf (1988) in leaf lamina of the castor bean, while a decrease in $K^+$ concentration was observed in the petioles. Therefore, it could be assumed that $K^+$ concentration does not change in response to salt stress in the mature zone of the leaf. Hsiao and Läuchli (1986) argue that $K^+$ is a major contributor to the osmotic pressure of the cell sap. They propose that in crops well fertilized with $K^+$ the vacuolar $K^+$ concentration increases up to about 200 mol $m^{-3}$. It is observed that $Na^+$ and $Cl^-$ concentrations in the plant cells do change in response to salt addition to external media. Their concentrations change immediately after the stress onset. The concentrations and the responses of $PO_4^{3-}$ and $SO_4^{2-}$ are in consistency with the findings of Gorham et al. (1986), Jeschke and Wolf (1988) and Frey et al. (1988). Frey et al. (1988) measured these ions in NaCl-treated alga Eremosphaera viridis. However, very high $NO_3^-$ concentration would possibly be due to high $NO_3^-$ concentration of $NO_3^-$ salts in growing medium (50 % Hoagland solution; Hoagland and Arnon, 1950).

In the case of sugars, no changes were observed in the glucose and fructose contents of the NaCl-treated plants (Figs. 5.16 and 5.17), however, a large increase was observed in the sucrose concentration (Fig. 5.18). The higher content of sucrose than both of the reducing sugars in the mature zone of leaf was also shown by Munns and Weir (1981), while glucose was found to be much higher in the growing zone. This might be associated with the high activities of acid invertase in rapidly growing cells (ap Rees, 1974). Munns and Weir (1981) observed an increase in

the sugar accumulation in wheat leaves after water stress, however, the increase was approximately equivalent to the decreased decomposition of carbohydrates during the reduction in growth in response to the stress. The significance of the role of the carbohydrates is quite controversial. However, they contribute significantly to the osmotic adjustment in many plants depending upon the plants species, type of tissue, plant growth stage and even sampling time (Flowers et al. 1977; Greenway and Munns, 1980; Gorham et al. 1981). But their contribution has been negligible in comparison with total osmotic pressure.

The contribution of these ionic osmotic solutes towards the osmotic adjustment was determined by their summation and then comparison with the measured osmotic pressure (Fig. 5.19). $K^+$ and $NO_3^-$ were the major osmotica and they contributed about 85 % and 80 % towards the calculated and the measured osmotic pressure respectively, under the control conditions. Hence, the calculated osmotic pressure represented about 94 % of the measured one, therefore, only 6 % was contributed by other osmotica such as $Ca^+$, $Mg^+$, amino acids, glycinebetaine and proline etc. However, these osmotica were not measured in this study.

After six days of the stress onset the difference between $\pi_c$ and $\pi_m$ became greater and the contribution of $\pi_c$ towards the $\pi_m$ reduced to approximately 80 %. This must be due to an increase in the concentrations of the osmotica not measured in this study. Rashid (1986) measured an increase in the concentrations of the proline and glycinebetaine in the NaCl-treated wheat leaves. However, no changes in the $Ca^{2+}$ and $Mg^{2+}$ and total amino acid

content could be found in the same plants. Gorham et al. (1986) observed the salinity-induced changes in solute composition in the leaf of Thinopyrum junceiforme, a perennial triticeae. They showed a little change in overall amino acid concentrations (14 amino acids were measured), however, an increase was observed in proline content being balanced by decreases in asparagine and glutamine. The total concentration of $Ca^{2+}$, $Mg^{2+}$, glycinebetaine, proline and other organic osmotica contribute towards the 20 % discrepancy between the measured and the calculated osmotic pressures (see Rashid, 1986).

In conclusion, $K^+$ and $NO_3^-$ are the major osmotica in non-stressed wheat leaves and the concentrations of only $Na^+$ and $Cl^-$ increase in response to the salt stress. The concentration of sucrose increases as well, however, its contribution to the total osmotic pressure is almost negligible. In other studies an increase in the content of proline and glycinebetaine has also been reported in wheat leaves in response to NaCl stress (Rashid, 1986).

## 7.5 Variability in the Varietal Response

Many types of criterion have been used for the assessment of salt tolerance in crops which help in breeding of plants for improved plant growth and high economic yields under saline conditions. These criteria are based on certain physical and metabolic parameters which have been measured in different types of plants. Examples are sodium uptake and chlorophyll concentration in rice (Yeo and Flowers, 1983), $Na^+$ and $Cl^-$ uptake in rice (Flowers and Yeo, 1981) and in wheat (Sharma et al. 1984), germination

percentage, germination rate and economic yields in wheat (Ashraf and McNielly, 1988), ionic discrimination in legume forage species (Ashraf et al. 1986b), dry matter production, tiller number, root/shoot ratio and seedling root growth in grasses (Ashraf et al. 1986a) and $K^+/Na^+$ discrimination ratio in wheat (Shah et al. 1987; Gorham et al. 1988). Anyhow, among all these criteria ion concentration and selectivity have been used most extensively. However, the measures of salt tolerance determined at various growth stages and using different criteria do not provide similar estimates of the extent of salt tolerance (Srivastava and Jana, 1984).

The Pakistani varieties used in this study are rated into four groups i.e. tolerant, moderately tolerant, moderately sensitive and sensitive according to their varying degree of salt tolerance. The selection criteria was based on the absolute grain yields of the individual varieties obtained as cumulative value of the measurements at two salinity levels (10 and 20 $EC_e$ dS $m^{-1}$ i.e. equivalent to salinity produced by 100 and 200 mol $m^{-3}$ NaCl) for two years (see Rashid, 1986).

The results show that turgor pressure in leaf mature zone of these varieties (Figs. 6.1, 6.2, 6.3 and 6.4) was lower than that of the Flanders (Fig. 5.1). The difference in turgor pressure level would probably be due to adaptation of these varieties to the semi-arid climatic conditions. Such environmental conditions tend to decrease the water content in plant leaves resulting in the decreased turgor pressure (Palta et al. 1987; Frensch and Schulze, 1988).

The turgor pressure recovery has been observed in the Flanders (Fig. 5.4), therefore, it was decided to asses this phenomenon/ parameter for designing a tool to assess the salt tolerance at the seedling stage. It must be said that this is the first example of this type of study.

Considering the turgor pressure response of individual varieties the tolerant variety, LU26S, did not show the response that might have been expected. Because the recovery was not observed for the two higher concentrations of NaCl. Perhaps this variety is not being a true isoline for salt tolerance (Qureshi, R.H., personal communication) did not show the precise trend of salt tolerance as our results indicated. However, the genetic deterioration may cause variability in the nature of isolines. If we rely on the turgor pressure response to assess salt tolerance and to rate accordingly this variety could probably be categorized as 'moderately tolerant'. Further, the response of LYP-73 (rated as moderately tolerant) and Pb-81 (moderately sensitive) did not show the expected response. LYP-73 showed turgor pressure recovery for all concentrations of NaCl and Pb-81 showed the similar to that of the LU26S. However, the recovery trend was as expected from the sensitive variety, Indus-79, i.e. turgor recovery took place for the lower NaCl concentrations only. Therefore, it must be concluded that the turgor pressure regulation is not a good screening tool for the salt tolerance of crops.

From the overall results of the study turgor pressure response did not coincide fully with the absolute grain yield criterion.

The possible reason for this different behaviour could be the difference in the stage of plant development under study. Because the turgor pressure measurements were carried out on the first leaf of wheat seedlings. Whilst the absolute grain yield criterion employs the measurements at the plant maturity. Hence, the turgor pressure response of wheat seedlings does not necessarily have to agree with the absolute grain yield since with the ageing plants must have adapted themselves to salt stress. It is believed that plants accumulate the toxic ions in first emerged leaves which are shed during the growth (Gorham et al. 1986) and that may help adapting to saline conditions.

To check if the tissue osmotic pressure and cell wall solutes vary with the turgor pressure response on salt treatment some experiments were carried out to measure osmotic pressure (Figs. 6.5, 6.6, 6.7 and 6.8). With the help of which cell wall solutes were estimated. However, the results obtained are similar to that of the Flanders (Fig. 5.7). This indicates that regardless of the species and varieties these parameters show similar behaviour.

## 7.6 Conclusions

Various conclusions can be drawn from the experiments performed in this project, as listed below.

a) The immediate effect of NaCl stress on leaf growth is that it stops within 1-2 minutes of the stress application. The normal growth rates are recovered after about 20-24 h of the NaCl addition to the media.

b) During this growth inhibition process the turgor pressure in growing cells remains unchanged. The turgor pressure maintenance may be result of uptake of solutes present in the cell wall by the cells. However, tissue osmotic pressure increases with time and the concentration of cell wall solutes increases, as well.

c) In contrast to the growing cells, turgor pressure in mature cells drops within 15-20 min of the stress onset. The process of osmotic adjustment starts after about 10 h by taking in the solutes necessary for it. The process of osmotic adjustment completes between 24 to 48 h depending upon the level of external stress.

d) During osmotic adjustment the concentrations of $Na^+$ and $Cl^-$ increase with time, while other ionic and osmotic solutes almost remain unchanged in their concentrations. However, in control plants $K^+$ and $NO_3^-$ are the major osmotica.

e) Volumetric elastic modulus remains unchanged by the salt stress. However, the root hydraulic conductivity was almost uniform with the increase in stress level. No relationship could be drawn about the contribution of these parameters in the control of growth and in leaf water relations in context of salt stress.

f) Turgor pressure regulation is not a good criterion in screening the crop plants for salt tolerance.

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