STUDIES ON ROLES OF CALCIUM IONS AND ABSCISIC ACID IN MODULATING DETOXIFICATION PATHWAYS DURING OSMOTIC STRESS IN SEEDLINGS OF MAIZE (*Zea mays* L.)

By

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DECLARATION

I, Lalliansanga, hereby declare that the subject matter of this thesis entitled "Studies on roles of Calcium ions and Abscisic acid in modulating detoxification pathways during osmotic stress in seedlings of maize (*Zea mays* L.)" is the record of work done by me. The contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University for the award of the degree of Doctor of Philosophy in Botany.

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ABBREVIATIONS

ABA	: Abscisic Acid
ABRE	: ABA-responsive element
ADP	: Adenine Dinucleotide Phosphate
APX	: Ascorbate Peroxidase
ASA	: Ascorbate
BCIP/ NBT	: 5-bromo-4-chloro-3-indolyl phosphate/ Nitro blue Tetrazolium
BSA	: Bovine Serum Albumin
CaM	: Calmodulins
CAT	: Catalases
CBLs	: Calcineurin B-like proteins
CDPK	: Calcium dependent protein kinase
Chl	: Chlorophyll
CRT	: Cold Responsive Sensitive transcription factors
$cytCa^{2+}$	: Cytosolic Calcium
DAG	: Diacyl Glycerol
DHAR	: Dehydroascorbate Reductase
DNA	: Deoxyribonucleic acid
DRE	: Dehydration Responsive Elements
DTNB	: 5,5'-dithiobis-(2-nitrobenzoic acid)
EDTA	: Ethylenediaminetetraacetic Acid
EGTA	: Ethylene glycol-bis(-amino ethyl ether)-N,N,N',N'-tetra acetic acid
EL	: Electrolyte leakage
GM	: Gujarat Makki

GPX : Glutathione Peroxidase

GR : Glutathione Reductase

GSH : Redused Glutathione

GSSG : Oxidized glutathione

GST : Glutathione-S Transferase

H_2O_2 : Hydrogen Peroxide

HOG : High Osmolarity Glycerol

IP_3 : Inositol Triphosphate

kD : Kilo Dalton

KI : Potassium Iodide

LEA : Late Embryogenis Abundant

MAPK : Mitogen Activated Protein Kinase

MDA : Malondialdehyde

MDHAR : Monodehydro Ascorbate Reductase

$NADP^+$: Nicotinic Acid Adenine Dinucleotide Phosphate

PA : Phosphatidic acid

PBS : Phosphate Buffered Saline

PCD : Programmed Cell Death

PEG : *Polyethylene Glycol*

PIP_2 : Phosphatidyl- inositol 4,5 bisphosphate

PI-PLC : Phosphoinositide specific Phospholipase C

PLC : Phospholipase C

PLD : Phospholipase D

PMSF : Phenyl Methyl Sulfonyl Fluoride

POX : Peroxidases

PVP	: Polyvinylpyrrolidone
R_f	: Electrophoretic mobility
RNS	: Reactive Nitrogen Species
ROS	: Reactive Oxygen Species
RWC	: Relative Water Content
S1P	: Sphingosine-1-phosphate
SDS-PAGE	: Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis
SEM	: Standard Error Mean
SIPK	: Salicylic Acid-Induced Protein Kinase
SLNI	: Synthetic lethal of N-end rule 1
SOD	: Superoxide dismutase
SOS	: Salt Overly Sensitive

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Chapter: 1

INTRODUCTION

Water is a fundamentally important component of the metabolism of all living organisms. As a universal solvent, it facilitates many vital biological reactions in the living system (Bohnert *et al.,* 1995). One of the major consequences of reduced availability is the loss of protoplasmic water leading to increase in the concentration of ions such as Cl^- and NO_3^-. At high concentrations, these ions effectively inhibit metabolic functions (Hartung *et al.,* 1998). Increased concentration of protoplasmic constituents and loss of water from the cells leads to the formation of "glassy state" in which the cytoplasmic water attains very high viscosity, thereby increasing the chances of molecular interactions that can cause protein denaturation and membrane fusion (Hartung *et al.,* 1998; Hoekstra *et al.,* 2001).

Salinity is one of the major abiotic stresses affecting plant growth and productivity globally. Modern-day plants are products of eons of evolution from primal living organisms in response to abiotic and biotic environmental changes.

Water is one of the major abiotic factors that has shaped evolution of living organisms. Water stress, in its broadest sense, encompasses both drought and salt stress. Drought, salt stress and low temperature are the major problems for agriculture because these adverse environmental factors prevent plants from realizing their full genetic potential. Salt stress afflicts plant agriculture in many parts of the world (Epstein *et al.,* 1980). Compared to salt stress, the problem of drought is even more pervasive and economically damaging (Boyer, 1982). Nevertheless, most studies on water stress signaling have focused on salt stress primarily because plant responses to salt and drought are closely related and the mechanisms overlap.

Since the response of plants to drought is complex and diverse (Alpert and Oliver, 2002; Levitt, 1980; Walters *et al.,* 2002), the need for an understanding of the physiological response of plants under water-limited conditions is of critical importance. Plants can perceive abiotic stresses at the molecular and cellular levels as well as at the physiological and biochemical levels, and elicit appropriate responses with altered metabolism, growth and development. Salt stress is known to induced the expression of a wide array of genes (Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 2000; Thomashow, 1999). The products of these genes function not only in stress tolerance but also in the regulation of gene expression and signal transduction in stress responses (Bartels and Sunkar, 2005; Shinozaki and Yamaguchi-Shinozaki, 2000; Xiong *et al.*, 2002). This cascade of events acts through second messengers leads to physiological responses that are exhibited by plants under stress (Trewavas *et al.*, 1997). The signal transduction pathways can operate independent of each other or may also share components and second messengers (Knight and Knight, 2001). A common aspect of most adverse environmental conditions is the increased production of reactive oxygen species (ROS) within several subcellular compartments of the plant cell (Van Breusegem *et al.*, 2001). While ROS levels can change during cellular metabolic events such as photosynthesis, their formation is usually exacerbated during stressful environmental conditions.

Salt and drought stresses affect virtually every aspect of plant physiology and metabolism. Plants responses to salt and drought stress can be grouped into three categories: (a) homeostasis that includes ion homeostasis, which is mainly relevant to salt stress, and osmotic homeostasis or osmotic adjustment; (b) stress damage control and repair, or detoxification; and (c) growth control (Zhu, 2001). Accordingly, salt and drought stress signaling can be divided into three functional categories: (a) ionic and osmotic stress signaling for the re-establishment of cellular homeostasis under stress conditions, (b) detoxification signaling to control and repair stress damages, and (c) signaling to coordinate cell division and expansion to levels suitable for the particular stress conditions. Current evidence supports the concept that ROS represent a significant point of convergence between pathways that respond to biotic and abiotic stresses. To date, the biological significance of crosstalk between signaling pathways that operate under stress conditions and the mechanisms that underlie this crosstalk remain obscure. Nevertheless, our current understanding of ROS participation in crosstalk between these pathways is very limited. Thus, dissection of the networks that influence ROS levels in response to abiotic stress merits extensive future study. The present work was undertaken to investigate the role of calcium ions and abscisic acid in modulating detoxification systems during early stages of salt stress in maize.

Chapter: 2

REVEW OF LITERATURE

Water is a fundamentally important component of the metabolism of all living organisms. As a universal solvent it facilitates many vital biological reactions in the living system. Salinization is the accumulation of water-soluble salts in the soil solum (the upper part of a soil profile) or regolith (the layer or mantle of fragmental and unconsolidated rock material) to a level that would affect agricultural production. Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations affect seed germination, water deficit, cause ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress (Khan *et al.*, 2002; Khan and Panda, 2008). Plants undergo continuous exposure to various biotic and abiotic stresses in their natural environment. To survive under such conditions, plants have evolved intricate mechanisms to perceive external signals, allowing optimal response to environmental conditions. Phytohormones such as salicylic acid (SA), jasmonic acid (JA), ethylene

(ET), and abscisic acid (ABA) are endogenous, low-molecular-weight molecules that primarily regulate the protective responses of plants against both biotic and abiotic stresses via synergistic and antagonistic actions,

The most important process that is affected in plants, growing under saline conditions, is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO_2 concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns, 2002). The complexity of the plant responses to salt stress can be partially explained by the fact that salinity imposes both hyperionic and hyperosmotic stress on the plants (Glenn *et al.*, 1999) and inhibits plant growth by inhibiting cell division and expansion, imbalance of the cellular ions, change of cell volume or turgor pressure and activity and stability of macromolecules. The degree of growth inhibition due to osmotic stress depends on the severity of the treatment, time scale of the response and tissue and species in question. Whereas mild osmotic stress is known to cause inhibition of growth of leaves and stems, it does not affect the growth of roots (Westgate and Boyer, 1985; Sharp *et al.*, 1988; Spollen *et al.*, 1993). Osorio *et al.* *(*1998) have suggested that the inhibition of shoot growth during water deficit could contribute to solute accumulation and thus eventually to osmotic adjustment. On the other hand, continuation of root growth under drought stress has been suggested to be an adaptive mechanism that facilitates water uptake from deeper soil layers (Munns *et* *al.*, 2000). It has been suggested that continued root growth may provide additional surfaces for sequestration of toxic ions, leading to lower salt concentration.

An initial rapid and transient drop in growth rate followed by a gradual recovery to a new reduced rate of growth upon exposure to salinity in plant leaves has been recorded in maize (Cramer and Bowman, 1991; Neumann, 1993), rice (Yeo *et al.*, 1991), wheat (Passioura and Munns, 2000) and barley (Fricke *et al.*, 2006). Yeo *et al.* (1991) have ascribed the initial responses of plants to salt stress to change in cell water relations imposed by external osmotic pressure. Plants sense salt stress through both ionic (Na^+) and osmotic stress signals. Zhu (2003) has suggested that plants sense excess Na^+ levels either on the surface of the plasma membrane protein or within the cell by membrane proteins including Na^+ sensitive enzymes. It has been suggested that in addition to its role as an antiporter, the plasma membrane Na^+/H^+ antiporter SOS1, having 10 to 12 transmembrane domains and a long cytoplasmic trail, may also act as a Na^+ sensor (Zhu, 2003). Sanders *et al.* (1999) have observed that entry of Na^+ through nonspecific ion channels under salinity caused membrane depolarization that activates Ca^{2+} channels generating Ca^{2+} oscillations. They have suggested that the Ca^{2+} oscillations could act as stress signals for the plant during salt stress. Addition of external Ca^{2+}, however, alleviated the salt-induced reduction of growth in the root of maize and sorghum (Cramer and Läuchli, 1986; Cramer *et al.*, 1988; Colmer *et al.*, 1996).

Given the multiplicity of stress signals, many different sensors are expected, although none have been confirmed for cold, drought, or salinity. In plants many of the different types of stresses *viz.* cold, drought, and salt stimulate the accumulation of compatible osmolytes and antioxidants (Hasegawa *et al.*, 2000). Knight and Knight (2001) have suggested that the multiple signal pathways associated with different types of stress can operate independent of each other and may even modulate other pathways. Each of these pathways is expected to have a component of input signal and a component of output response. For the ionic aspect of salt stress, the input of the SOS pathway has been suggested to be the likely excess intracellular or extracellular Na^+ (Zhu, 2000). The output responses are expression and activity changes of transporters for ions such as Na^+, K^+, and H^+. Zhu (2001) has suggested that the input for osmotic stress signaling pathway could be change in turgor with a corresponding output response being activation of osmolyte biosynthesis enzymes as well as osmolyte transport systems. Xiong *et al.* (2002) divided the signal transduction pathways in plants under environmental stresses into three major types: (i) Ca^{+2}-dependent salt overly sensitive (SOS) signaling that results in ion homeostasis (ii) Ca^{+2}-dependent signaling that leads to activation of LEA-type genes such as DRE/CRT class of genes and (iii) osmotic/oxidative stress signaling that makes use of MAPK modules.

Plants respond to salt stress induced disturbances in ionic homeostasis by restricting salt intake, increased electrolyte leakage and maintenance of a favorable K^+/Na^+ ratio in the cytosol (Niu *et al.*, 1995; Serrano *et al.*, 1999). Salt stress is known to increase the levels of free cytosolic Ca^{+2} either through influx from the apoplastic space or as a consequence of release from the Calcium sequestration compartments (Knight, 2000; Sanders *et al.*, 1999). Schroeder *et al.* (2001) have indicated that inositol polyphosphates, cyclic ADP ribose and NADP could act as second messengers in the salt stress signaling cascade and induce release of Ca^{+2} from sequestered compartments in cells. Elevated levels of free cytosolic calcium have been shown to reduce binding of Na^+ ions to cell walls and plasma membranes, relieve membrane leakiness and prevent salt-induced decline in cell elongation (Stassart *et al.*, 1981; Cramer *et al.*,1985; Kurth *et al.*, 1986; Lynch *et al.*,1987; Zidan *et al.*, *1990*; Picchioni *et al.*, *1991*). However, Bliss *et al.* *(1986)* have suggested that supplemental Ca^{2+} alleviated deleterious effects of salt by mitigating the toxic effects of Na^+ ions rather than the osmotic effects associated with salt stress. Ca^{2+} has been reported to improve germination and plumule emergence (Bliss *et al.*, 1986), root elongation (Cramer *et al.*, 1986; Nakamura *et al.*, 1990; Zidan *et al.*, 1990), shoot growth (Cramer *et al.*, 1989; Grieve and Fujiyama, 1987; Grieve and Maas, 1988; Maas and Grieve, 1987; Subbarrao *et al.*, 1990; Yeo *et al.*, 1991), prevent nuclear deformation and degradation (Katsuhara and Kawasaki, 1996), increase uptake and transport of K^+ (Cramer *et al.*, 1985, 1987; Grieve and Fujiyama, 1987; Nakamura *et al.*, 1990; Subbarrao *et al.*, 1990) and reduce Na^+ accumulation (Cramer *et al.*, 1987, 1989; Ehret *et al.*, 1990; Grieve and Fujiyama, 1987; Grieve and Maas, 1988; Maas and Grieve, 1987; Subbarrao *et al.*, 1990; Zidan *et al.*, 1991) in plants during salt stress.

While Liu and Zhu (1998) and Chinnusamy *et al.* (2005) have attributed the effect of Ca^{2+} on Na^+ and K^+ transport SOS signaling pathway, Shi *et al.* (2002) have shown that the sensor protein for this salt-induced calcium signature is the Ca^{+2}-binding protein SOS1 and SOS3. Loss of function of these proteins due to mutation

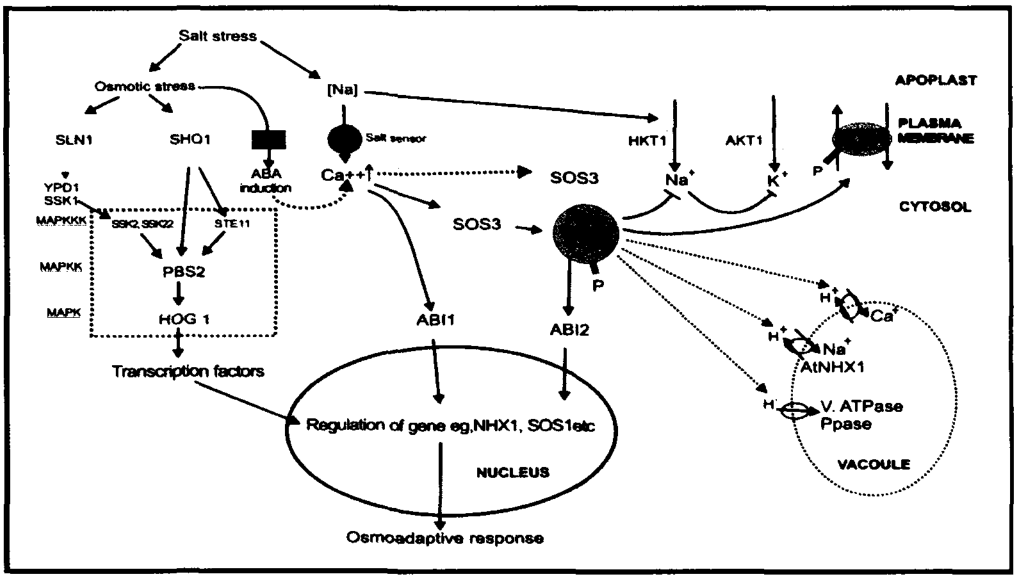


Fig. 2.1: Schematic representation of SOS pathway in cells.

has been shown to render the plants hypersensitive to salt stress (Wu *et al.*, 1996; Shi *et al.*, 2000; Shi *et al.*, 2002). Halfter *et al.* (2000) have suggested that extracellular Ca^{2+} could directly alter Na^+ influx into cells leading to activation of the SOS pathway via SOS3. Using *Arabidopsis* as a model system Liu *et al.* (2000) have proposed that salt stress signaling is perceived by the calcineurin- β -like Ca^{+2} sensor SOS3. However, unlike the calcineurin-β in yeast that acts through activation of a protein phosphatase, SOS3 has been reported to interact with and activate protein kinase SOS2 (Halfter *et al.*, 2000). Thus, SOS3 resembles an adapter or scaffold protein that mediates the interaction of SOS2 with other proteins such as ion transporters. While the increased levels of cytosolic calcium are known to be perceived by various calcium-binding proteins *viz.* CDPKs and the SOS3 family of Ca^{+2} sensors, Knight *et al.* (1997) have suggested that CDPK were the prime candidates that linked calcium signal to downstream responses of osmotic stress. Osmotic stress-induced CDPKs have been reported from several plants including rice (Saijo *et al.*, 2000; Kawasaki *et al.*, 2001; Oztur *et al.*, 2002), *Arabidopsis* (Sheen, 1996), alfalfa (Munnik *et al.*, 1999; Kiegerl *et al.*, 2000) and tobacco (Elizabeth and Zhang, 2000; Yang *et al.*, 2001; Zhang and Klessig, 2001). Besides CDPKs MAPKs are also known to be components of intracellular signal modules that mediate signal transduction from the cell surface to the nucleus. There is accumulating evidence indicating that plants rapidly activate MAPK when exposed to multiple abiotic stress stimuli (Ligterink and Hirt, 2001; Kiegerl *et al.*, 2000). MAPKs are known to be activated in response to drought and other environmental stresses (Zhu, 2002; Agrawal *et al.*, 2003). Xiong and Yang (2003) have shown that the activated MAPKs are transported into the nucleus, where they phosphorylate and activate specific downstream signaling components, such as transcription factors to induce altered gene expression. Osmotic stress signaling MAPK modules which have been identified from different systems include SIMK and SIMKK–SIMK55 from alfalfa and Nt MEKZ–SIPK/ WIPK and SIPK from *Nicotiana tobacum,* NPK1 from *Arabidopsis thaliana* (Munnik *et al.*,1999, Kiegerl *et al.*, 2000; Kovtun *et al.*, 2000; Mikolajczyk *et al.*, 2000; Liu *et al.*, 2000; Yang *et al.*, 2001; Zhang and Klessig, 2001).

In addition to serving important role as components of plasma membranes membrane phospholipids constitute a dynamic system that generate a multitude of signal responses during stress (Munnik and Meijer, 2001). Like ROS, low levels of phospholipid messengers such as IP_3, DAG, PA are known to activate downstream adaptive responses (Sang *et al.*, 2001). IP_3 has been shown to induce release of Ca^{2+} from isolated vacuoles or tonoplast vesicles thereby increasing the levels of free cytosolic calcium in cells (Schumaker and Sze, 1987; Munnik *et al.*, 1998 and DeWald *et al.*, 2001). IP_3 has also been shown to induce increase in the levels of free cytosolic calcium in guard cells and consequent stomatal closure (Sanders *et al.*, 1999). Wu *et al.* (1997) have suggested that increased level of cytosolic calcium cause by IP_3 could be one of the components in the signaling cascade which caused expression of osmotic stress-responsive genes. Like IP_3, other inositol phosphates such as IP_6 and $I(1,3,4)P_3$ have also been reported to be involved in release of Ca^{2+} from internal stores (Lee *et al.*, 1996; Lemtiri-Chlieh *et al.*, 2000).

Several studies have reported that inositol 4,5-bisphosphate is the primary and immediate catabolite of ^{3}H-labeled IP_3 in plants (Joseph *et al.*, 1989; Drøbak *et al.*, 1991; Brearley *et al.*, 1997), suggesting that in these plants, IP_3 was first hydrolyzed through a 1-phosphatase pathway. However, the Ins1Pase responsible for this early termination of the IP_3 signal in plants has not been identified. While the activity of FRY1/SAL1 in the hydrolysis of Ins(1,4)P_2 and inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3] had been demonstrated earlier in *Arabdiopsis thaliana* (Quintero *et al.*, 1996), its ability to hydrolyze IP_3 was not known. Using IP_3 as a substrate, FRY1 recombinant protein was found to have a measurable albeit limited activity [approx. 13% relative to its ability to hydrolyze Ins(1,4)P_2 or Ins(1,3,4)P_3] (Xiong *et al.*, 2001c). The in vivo activity of FRY1 on IP_3 and its significance in overall IP_3 metabolism have yet to be determined. Measurement of IP_3 levels in *fry1* and wild-type plants treated with ABA indicated that, whereas ABA induced a transient increase in IP_3 levels in wild-type plants, the IP_3 levels in *fry1*mutant plants were higher and more sustained (Xiong *et al.*, 2001c). Sustained IP_3 levels likely contributed to the enhanced expression of stress-responsive genes in *fry1*mutant plants.

Accumulating evidence suggests that phospholipase D (PLD) is also involved in the transduction of stress signals. PLD hydrolyzes phospholipids to generate phosphatidic acid (PA), another second messenger in animal cells that can activate PI-PLC and protein kinase C (English, 1996). Wang (1999) has suggested that PA may also serve as a messenger in plants. Jacob *et al.* (1999) have demonstrated PLD activity mediated ABA-induced stomatal closure in guard cell protoplasts. Drought and hyperosmolarity have been shown to activate PLD and lead to transient increases in PA levels in plants (Frank *et al.*, 2000; Munnik *et al.*, 2000; Katagiri *et al.*, 2001). It has been suggested that PLD activation might be taking place through a G-protein(Frank *et al.*, 2000) and the activation may be independent of ABA (Frank *et al.,* 2000; Katagiri *et al.*, 2001).

Xiong *et al.* (2001) and Burnette *et al.* (2001) have demonstrated the role of ABA in increasing IP3 levels in *Vicia faba* guard cell protoplasts and *Arabidopsis* seedlings indicating thereby the involvement of ABA as a component in the stress response signaling cascade. Sanchez and Chua (2001) and Burnette *et al.* (2001) have demonstrated that inositol-5-phosphatase could regulate ABA signal transduction pathway through changes in levels of endogenous IP_3. Takahashi *et al.* (2001) have shown that suppression of increase in levels of IP_3 because of inhibition of PI-PLC activity inhibited the osmotic stress induction of the stress-responsive genes *RD29A* and *COR47.*

Xiong *et al.* (2001) have shown that mutations in the *FRY1* gene encoding an inositol polyphosphate-1-phosphatase resulted in enhanced ABA induced gene transcription in *Arabidopsis thaliana.* Despite increased IP_3 levels and enhanced expression of *fry1* gene, the mutants were more susceptible to damage by salt, drought, or freezing stress. Xiong *et al.* (2001) have postulated that the enhanced expression was likely a compensatory mechanism to limit or repair stress injury. In this regard, FRY1 gene product might represent an interesting point of crosstalk between the stress homeostasis and detoxification pathways. Hyperosmotic stress has also been reported to stimulate PLA_2 activity thereby generating lyso-phospholipids and free fatty acids in algae (Einspahr *et al.,* 1988; Meijer *et al.,* 1999). Munnik and

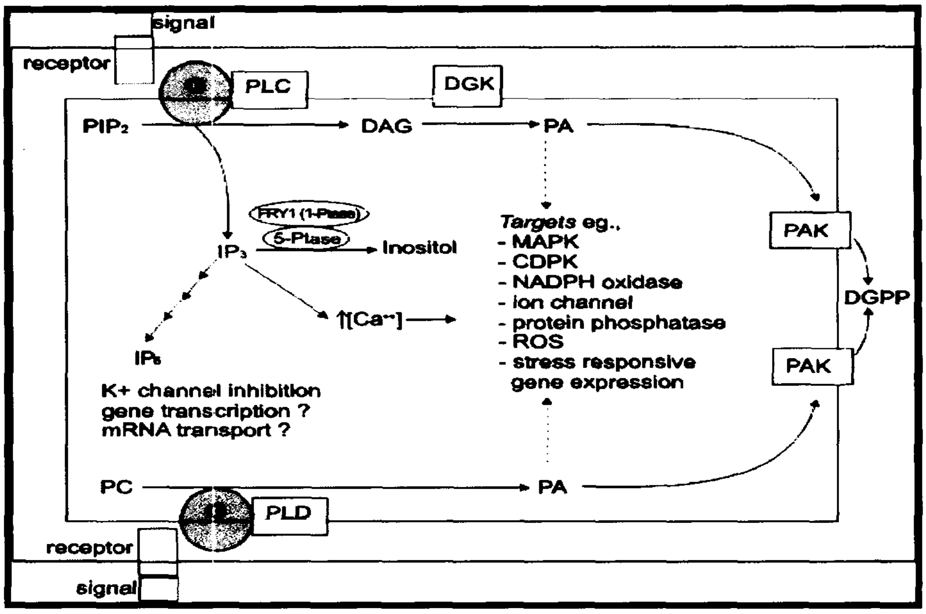


Fig. 2.2: Schematic representation of phospholipid signaling pathway in cells during salt stress.

Meijer (2001) have suggested that this novel lipid messenger may have a role in osmoregulation by stimulating tonoplast H^+-ATPase activity in the cells under stress.

ABA plays a crucial role in higher plants in their response to various environmental stresses and serves as a regulatory link between stress factors and plant responses (Seemann and Sharkey, 1987; Chandler and Robertson, 1994). Physiological studies and molecular analysis have demonstrated that ABA may regulate the adaptation of plants to environmental stresses (Stewart and Voetberg, 1985; Skriver and Mundy, 1990). The increase in endogenous ABA levels under water stress or application of exogenous ABA is often accompanied by enhanced synthesis of dehydration-related proteins and other compatible solutes such as proline, glycine-betaine, cyclitols and enhanced expression of a number of specific genes in plant tissues (Hanson *et al.*, 1985; Stewart and Voetberg, 1985; Gomez *et al.*, 1988; Mundy and Chua, 1988; Bray, 1988; Blackman *et al.*, 1991, 1992; Parcy *et al.*, 1994; Seo *et al.*, 1995; Beardmore and Charest, 1995; Liotenberg *et al.*, 1999; Sun *et al.*, 1999; Xiong *et al.*, 2001). ABA is also known to play an important role in acquisition of desiccation tolerance in seeds during embryo maturation (Quatrano, 1987; Blackman *et al.*, 1991, 1992; Meurs *et al.*, 1992). Exogenously applied ABA has been shown to be able to induce desiccation tolerance in immature zygotic embryos (Wakui *et al.*, 1994; Blackman *et al.*, 1992) as well as somatic embryos (Park *et al.*, 1988; Bochicchio *et al.*, 1991; Etienne *et al.*, 1993). Even though many of the osmotic stress responsive genes are known to be induced by ABA (Liotenberg *et al.*, 1999), *AAO3* (Seo *et al.*, 1995), and *ABA3* (Xiong *et al.*, 2001). Shinozaki and

Yamaguchi-Shninozaki (1997) have showed that induction of many other osmotic stress responsive genes could be independent ABA accumulation in the cells. There is evidence that although ABA does not activate the DRE in the *RD29A* promoter, it may be required for full activation of DRE by osmotic stress (Yamaguchi-Shninozaki and Shinozaki, 1994). Xiong *et al.* 2001 have proposed that activation of DRE by DREB2A and related transcription factors may require ABA-dependent factor(s). An analysis of double mutants between *fry1* and *aba1* or *abi1* indicated that the cold or osmotic stress hypersensitivity in the mutant is not dependent on ABA (Zhu *et al.*, 2002). Sharing of ABA-dependent and ABA-independent pathways may also occur downstream of the first stress recognition and signaling events, and/or a gene may contain both DRE and ABRE elements in its promoter. Several genes that are upregulated under drought conditions are known to contain a conserved ABRE in their promoter region (Qin and Zeevart, 1999; Uno *et al.*, 2000).

A secondary effect of salt stress is the increase in levels of reactive oxygen species (ROS) (Smirnoff, 1998; Bartels, 2001; Apel and Hirt, 2004). ROS's are partially reduced forms of atmospheric oxygen, which are produced in vital processes such as photorespiration, photosynthesis and respiration (Mittler, 2002; Uchida *et al.*, 2002). To produce water in these processes, four electrons are required for perfect reduction of oxygen. But ROS typically results from the transference of one, two and three electrons, respectively, to O_2 to form superoxide ($O^{2\cdot -}$), peroxide hydrogen (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) (Mittler, 2002). These species of oxygen are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc, causing lipid peroxidation, protein denaturing and DNA mutation, respectively (Breusegem *et al.*, 2001; Scandalios, 1993; Quiles and Lopez, 2004). Increased levels of ROS cause oxidative stress in the tissues and consequent damage to proteins, lipids and nucleic acids; the magnitude of damage depending upon the balance between the formation of ROS and their removal by the antioxidative scavenging systems (Hernandez and Almansa, 2002). Even though ROS have been implicated in the regulation of several physiological processes such as cell proliferation (Shibanuma *et al.*, 1990), differentiation (Allen and Balin, 1989), senescence (de Haan *et al.*, 1996), and apoptosis (Mignotte and Vayssiere, 1998), activation of defense responses (Dat *et al.*, 2000; Mittler, 2002) at low concentrations, they are highly cytotoxic at higher concentrations and induce DNA damage, lipid peroxidation, and protein degradation (Sun, 1990; Scandalios, 1993; Breusegem *et al.*, 2001; Quiles and Lopez, 2004). The capacity to tolerate abiotic stress is also know to be associated with a more efficient antioxidative system (Foyer *et al.*, 1994; Gosset *et al.*, 1996; Hernandez *et al.*, 1993, 1995, 1999, 2002; Bor *et al.*, 2003; Vaidyanathan *et al.*, 2003). The enzymatic and nonenzymatic systems involved in maintenance of ROS balance in plants include enzymes like ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) as well as low molecular mass antioxidants *viz.* glutathione and ascorbate (Foyer *et al.*, 1994). Other enzymes that are important in the ROS scavenging system and function in the ascorbate-glutathione cycle are glutathione reductase (GR), monodehydro ascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) (Candan and Tarhan, 2003; Yoshimura *et al.*, 2000). Aziz and Larher (1998) have observed a sharp increase in the activities of superoxide dismutase and ascorbate peroxidase in osmotically stressed leaf discs of rape plants. Increases in the activities of ascorbate peroxidase, superoxide dismutase and glutathione reductase have also been observed in the drought tolerant wheat cultivar 'Massai' when the leaves were subjected to osmotic stress *in vitro* (Lascano *et al.*, 2001). Alscher *et al.* (2002) have suggested that since the antioxidant systems play an important role in scavenging ROS, their improvement could increase the ability of plants to tolerate adverse environmental conditions. Lai *et al.* (2007) have demonstrated a marked increase in the activities of SOD, CAT, APX, GPX and DHAR in leaf discs treated with polyethylene glycol (PEG). The increase in activities of the enzymes in leaf discs of plants transformed with the clone PSAG12-*IPT* was much higher than that in the untransformed controls particularly at 40% PEG treatment. Higher activities of SOD, CAT, APX, GPX and DHAR in leaf discs of plants transformed with the clone PSAG12-*IPT* treated with PEG coincided with decrease in the TBARS concentration, suggesting that oxidative damage induced by osmotic stress could be alleviated by transformation by the chimeric gene PSAG12-*IPT*. This result was consistent with the observation of Dertinger *et al.* (2003) who have reported that the activities of antioxidative enzymes (APX and GR) in old leaves of PSAG12-*IPT* modified tobacco plants were higher than that in the wild-type leaves.

Amongst the reactive oxygen species which mediate responses to various stimuli, H_2O_2 seems best suited to play the role of signaling molecule due to its higher stability and longer half-life. Yu *et al.* (2002, 2003) suggest that H_2O_2 is a signal mediator for programmed cell death of plants as a response to pathogens, elicitors, and hormones (Desikan *et al.*, 1998; Mittler *et al.*, 1999; Solomon *et al.*,

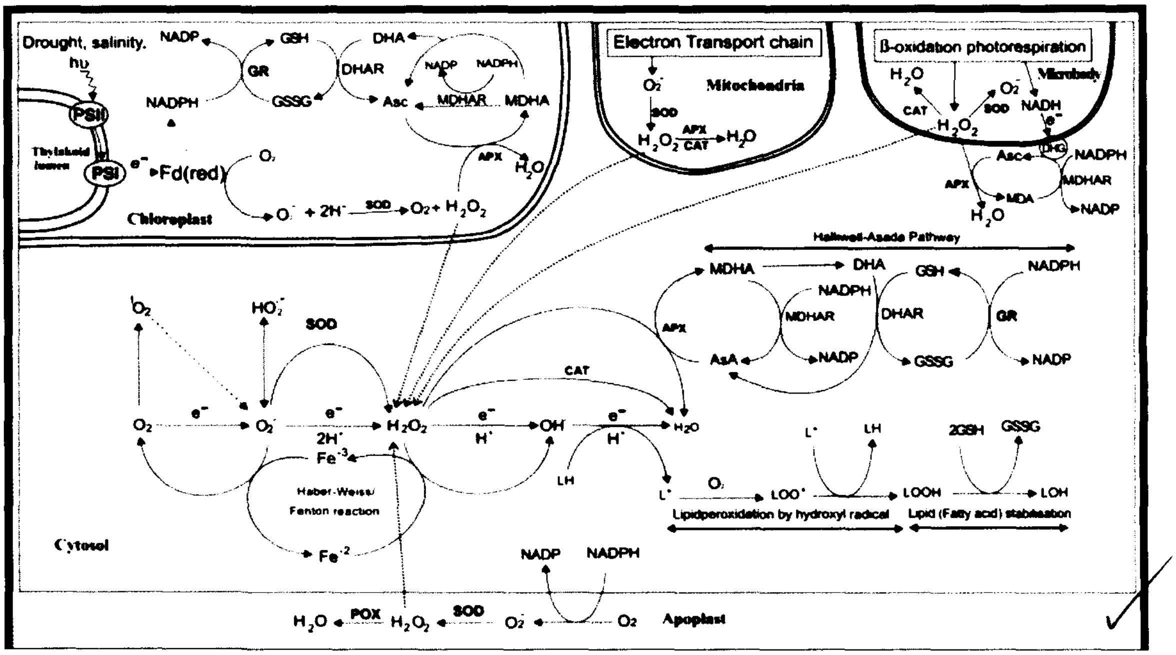


Fig. 2.3: Schematic representation of antioxidative pathways in cells during salt stress.

1999; Bethke and Jones, 2001). Furthermore, a number of studies indicate that H_2O_2 is synthesized in response to application of exogenous ABA and that H_2O_2 mediates ABA induced stomatal closure in leaves (Guan *et al.*, 2000; Pei *et al.*, 2000; Desikan *et al.*, 2001a). H_2O_2 has also been reported to be involved in regulating differential expression of genes during stress including genes encoding potential transcription factors. These results suggest that transcription factors mediate further downstream H_2O_2 responses and finally induce physiological changes to promote adaptation to stresses. Yang and Poovaiah (2002) have demonstrated that treatment with H_2O_2 activated Ca^{2+} channels leading to elevation of cytCa^{2+} level. Increased levels of cytosolic calcium would activate the calcium sensor 'calmodulin' which would through a downstream signaling cascade induce the expression of gene coding for catalase for scavenging the H_2O_2. Evidence suggests that membranes are the primary sites of salinity injury to cells and organelles (Candan and Tarhan, 2003). ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasmalemma or intracellular organelles (Karabal *et al.*, 2003; Stewart and Bewley, 1980).

Current evidence supports the concept that ROS represent a significant point of convergence between pathways that respond to both biotic as well as abiotic stresses. Nevertheless, our current understanding of ROS participation in crosstalk between these pathways is very limited. Moreover, the view that the ABA mediated abiotic stress signaling potentially takes precedence over biotic stress signaling (Ghassemian *et al.*, 2000) also supports the notion that water stress more significantly threatens plant survival than pathogen infection. To date, the biological significance of crosstalk between signaling pathways that operate under stress conditions and the mechanisms that underlie this crosstalk remain obscure. Thus, dissecting the genetic network that regulates ROS signaling in response to such stressful conditions merits extensive future study. When combined, the results of large-scale transcriptome, proteome, and metabolome analyses in plants could enable the elucidation of the ROS network components that govern multiple stress signaling pathways.

Chapter: 3

MATERIALS AND METHODS

MATERIALS:

Plant materials and growth conditions: Seeds of maize (*Zea mays* L.) [variety RCM 1-1 and Gujarat Makki] were procured from the ICAR complex for North East Region, Umroi, Shillong. Healthy seeds were washed with distilled water and germinated in a seed germinator on germinating paper at 25 ± 2°C, 14hours photoperiod and 75 ± 10% air relative humidity. Plants were well-watered and maintained as described above till the second leaf was fully expanded.

TREATMENTS:

Experiment I: The fully grown seedlings at 2nd leaf stage were transferred to culture tubes containing Hoagland's nutrient solution containing 100mM, 150mM, 200mM and 250mM NaCl with and without 10 mM $CaCl_2$. Salt strength of each solution was monitored and adjusted periodically over the entire period. A set of plants maintained over Hoagland's Nutrient solution without NaCl served as the control. The seedlings were maintained for 7 days in a Plant Growth chamber at 25 ± 2°C, under 14 h light 75 ± 10 % R.H after which the seedlings were harvested. The harvested seedlings were washed with distilled water and wiped dry with tissue paper. The wiped seedlings were used for measurement of length, fresh weight and dry weight of shoots and roots, relative water content (RWC), electrolytic leakage (EL), content of cytosolic sodium, total chlorophyll, soluble protein and activities of ascorbate peroxidase (APX) and glutathione reductase (GR).

Experiment II: In order to study the early responses of the seedlings to the presence of 200 mM sodium chloride in the nutrient solution alone or in combination with 10mM $CaCl_2$ and/or 100µM ABA, fully grown seedlings at 2nd leaf stage were transferred to culture tubes containing Hoagland's nutrient solution containing 200 mM NaCl, 10mM $CaCl_2$, 100 µM ABA, 200 mM NaCl with 10mM $CaCl_2$, 200 mM NaCl with 100 µM ABA, 200 mM NaCl with10mM $CaCl_2$ and 100 µM ABA and 10mM $CaCl_2$ and 100 µM ABA and maintained for 144 hours in a growth chamber at 25 ± 2°C, under 14 h light 75 ± 10 % R.H. Salt strength of each solution was monitored and adjusted periodically over the entire period. A set of seedlings was pretreated for 4 hour separately with 10mM EGTA or 1mM Verapamil before transfer to Hoagland's nutrient solution containing 200 mM NaCl with or withjout 10 mM $CaCl_2$ or 100µM ABA. The treatments comprised:

1. Hoagland's nutrient solution containing 200 mM NaCl
2. Hoagland's nutrient solution containing 10mM $CaCl_2$
3. Hoagland's nutrient solution containing 100 µM ABA
4. Hoagland's nutrient solution containing 200 mM NaCl with 10mM $CaCl_2$

5. Hoagland's nutrient solution containing 200 mM NaCl with 100 µM ABA
6. Hoagland's nutrient solution containing 200 mM NaCl with10mM $CaCl_2$ and 100 µM ABA
7. Hoagland's nutrient solution containing 10mM $CaCl_2$ and 100 µM ABA
8. A set of seedlings was maintained in Hoagland's nutrient solution under the same conditions as decscribed above to serve as a control.

The seedlings were maintained for 144 hours in a growth chamber at 25 ± 2°C, under 14 h light 75 ± 10 % R.H. The concentration of NaCl of each solution was monitored and adjusted periodically over the entire period. The seedlings were harvested after 24, 48, 96 and 144 hours of treatment. The harvested seedlings were washed with distilled water and wiped dry with tissue paper. The wiped seedlings were used for measurement of length, fresh weight and dry weight of shoots and roots, relative water content (RWC), electrolytic leakage (EL), content of cytosolic sodium and potassium, total chlorophyll, MDA content, protein carbonyl content, H_2O_2 content, GSH content, AsA content, soluble protein and activities of ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and glutathione reductase (GR).

The experiments were set up in a completely randomized design with three replicates for each set and 10 seedlings for each replicate. The data have expressed as the means ± SEM.

Reagents:

Chemicals used in the present investigation were of analytical/ molecular Biologygrade and were procured from Sigma chemical co., St. Louis (USA), Bangalore Genei, Bangalore, HiMedia, Sisco Research Laboratory (SRL), Bombay (India) and CDH, Bombay. The SDS-PAGE molecular weight markers were purchased from Bangalore Genei, India and Roche Molecular Biological Ltd.

METHODS:

Relative water content (RWC): The relative water content of the plants was determined according to the method of Barrs and Weatherley (1962). The harvested plants were washed with distilled water and wiped clean with filter paper. The leaves were separated from the seedlings and their fresh weight was measured over Metler Toledo digital balance. The weighed leaves were soaked in distilled water for 4hours at room temperature. After 4 hours of incubation the leaves were wiped dry with tissue paper and weighed again to determine the turgid weight. Dry weight of the soaked leaves was determined after drying the leaves at 85°C for 72 hours. RWC of the leaf samples was calculated according to the formula:

$$\mathrm{RWC} = \frac{(\mathrm{FW}-\mathrm{DW})}{(\mathrm{SW}-\mathrm{DW})} \mathrm{x} 100$$

where, FW = weight of fresh leaves, SW = the turgid weight of leaves after soaking in water for 4 hours at room temperature; DW = dry weight of leaves after drying at 85°C for 72 hours.

Percent moisture and dry weight: The harvested seedlings were rinsed with water and wiped with a clean tissue paper. The fresh weight of seedlings was determined with Metler Toledo digital balance. Dry weight of the seedlings was determined after drying the tissues at 85°C for 72 hours. The percent moisture content and dry weight of the seedlings was calculated according to the formulae;

$$\% \text{ moisture } = \frac{(\text{FW}-\text{DW})}{\text{FW}} \text{x} 100; \quad \% \text{ dry weight } = 100 - \% \text{ moisture}$$

where, FW = leaf fresh weight and DW= dry weight of leaves after drying at 85°C.

Total chlorophyll: The content of total chlorophyll in the leaf tissues of the seedlings has been determined according to the method of Arnon (1949). The harvested plants were washed with distilled water and wiped dry with tissue paper. A suitable mass of the leaf tissues was homogenized in 80% acetone in a pre chilled pestle and mortar to extract the pigment molecules. The slurry was filtered through double layer of muslin cloth and the filtrate was centrifuged at 4000g for 5 min at 4°C to pellet the cell debris. The supernatant filtered through Whatman No. 1 filter paper and the absorbance of the filtrate was determined at 645 nm and 663 nm. The content of chlorophyll a and chlorophyll b in the sample was calculated according to the formula:

$$\text{Chl 'a' (mg/g leaf)} = \frac{\{(12.7 \text{ x Abs663}) - (2.6\text{x Abs645})\} \text{ x V}}{\text{leaf tissue (gm)}}$$

$$\text{Chl 'b' (mg/g leaf)} = \frac{\{(22.9 \text{ x Abs645}) - (4.68\text{x Abs663})\} \text{ x V}}{\text{leaf tissue (gm)}}$$

Where V= volume of the extract.

The total Chlorophyll content has been calculated as the summation of values for the content of chlorophyll a and chlorophyll b.

Electrolyte leakage: The electrolyte leakage (EL), expressed as percent of total conductivity, was measured according to the method described by Dionisio Sese and Tobita (1998). The harvested plants were rinsed with distilled water and wiped dry with a clean tissue paper. A suitable mass of the leaf tissues was excised and cut into 5-mm segments. The cut segments were rinsed thrice with deionized water and transferred to test tubes containing 10ml of deionized water. The test tubes were incubated at 32°C for 2 hours in water bath. The initial electrical conductivity of the solution (EC_1) was measured with a conductivity meter (DiST 3, Hanna Instruments). The leaf samples were subsequently autoclaved at 121°C for 20min to release the electrolytes and the electrical conductivity (EC_2) of the solution was measured after tubes cooled down to room temperature. Electrolyte leakage (EL) of the sample was calculated according to the formula : $EL = (EC_1/EC_2) \times 100$.

Na^+ and K^+ content: The content of Na^+ and K^+ in the leaf tissues of the harvested seedlings was determined by flame photometry according to the method of Dionisio - Sese and Tobita (1998). A suitable fresh weight of the leaf tissues was dried in a hot air at 70°C for 48 hours. The dried powder was weighed and digested in an autoclave at 121°C for 20min. The samples were cooled and centrifuged at 5000 rpm for 10 minutes to sediment the debris. The content of cytosolic Na^+ and potassium K^+ in the extract was measured under a flame photometer (Mediflame 127) by reference to known standards

Carbonyl content: The carbonylation of proteins was measured as the protein carbonyl content according to the method by Mercier *et al.*, (1998). A suitable mass of the leaf tissues of harvested seedlings was washed with distilled water, rinsed with filter paper and ground to a fine powder with liquid Nitrogen in a pre chilled mortar and pestle and homogenized in 0.1M phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000rpm for 10 min at 4°C to sediment the cell debris. A suitable aliquot of the supernatant was made to 500µl with 0.1M phosphate buffer (pH 7.0) and 3 ml of 10% TCA was added to it to precipitate the proteins. The solution was centrifuged at 10,000rpm for 10 min at 4°C to sediment the protein pellet. The sedimented pellet was washed thrice ethanol: ethyl acetate (1:1 v/v). The washed pellet was finally dissolved by vortexing for 10 minutes in 3ml of 6M guanidine-HCl prepared in 20mM phosphate buffer (pH 6.5). An additional centrifugation for 5 minutes at 10,000 rpm was carried out at this stage to sediment any suspended particulate matter. The solution was scanned for absorbance between 350 and 390nm in a Perkin Elmer Lamda 650 UV-Vis spectrophotometer. The absorbance value at 372nm was used to determine the content of protein carbonylation with the extinction coefficient of Dinitrophenyl Hydrazine = 22,000/10^6 nmole/ml. Since 5-10% of proteins were lost in various washing steps, the protein in the final 6M guanidine hydrochloride solution was quantified by measurement of absorption of the solution at 280nm. The amount of protein was calculated from a standard curve prepared with BSA dissolved in 6M guanidine hydrochloride as the standard. The values are expressed as nmole carbonyl/mg protein.

Lipid peroxidation: Lipid peroxidation was estimated as the amount of malondialdehyde (MDA) in the harvested leaf tissues according to the method of Heath and Packer (1968). The harvested plants were rinsed with distilled water and wiped dry with a clean tissue paper. A suitable mass of the leaf tissues was ground to fine powder with liquid nitrogen in a pre chilled pestle and mortar. The powdered sample was homogenized in 1.5 ml of a 20% TBA and 0.5% TCA mixture. The homogenate, so obtained, was incubated at 95°C for 45 min followed by centrifugation at 4,000g for 35 min at 4°C. The supernatant was collected diluted with water in the ratio if 1:1 (v:v). Absorbance of the solution was determined at 532 and 600 nm in a Perkin Elmer Lamda 650 spectrophotometer. The specific absorbance of the coloured supernatant was measured at 532nm and value of non-specific absorbance at 600nm was corrected by subtracting it from 532nm. MDA concentration in the leaf tissues of seedlings was calculated by using its molar extinction coefficient of 155 mM^{-1} cm^{-1}at 532 nm.

Hydrogen peroxide (H_2O_2): The concentration of H_2O_2 in leaf tissues was measured according to the method as described by Harinasut *et al.,* (2003). H_2O_2 was extracted from leaf tissues of the harvested plants by homogenizing a suitable mass of the freshly harvested leaf tissues with 0.1% TCA (w/v) in a pre chilled pestle and mortar. The extract was centrifuged at 12,000rpm for 15 minutes at 4°C to pellet the cell debris. For estimation of H_2O_2 , 0.5ml of the supernatant was mixed with 0.5ml of 10mM Potassium Phosphate buffer (pH 7.0) and 1ml of 1M KI. The absorbance of the solution was determined at 390 nm in a Perkin Elmer Lamda 650 spectrophotometer. The concentration of H_2O_2 in the sample was determined by reference to the standard curve prepared using the same method.

Glutathione (GSH): Glutathione content in the seedlings was estimated according to the method described by Sedlak and Lindsay (1968). The harvested plants were rinsed with distilled water and wiped dry with a clean tissue paper. A suitable mass of the leaf tissues from the harvested seedlings was ground to fine powder with liquid nitrogen in a pre-chilled pestle and mortar. The powder was homogenized with 0.2M Tris EDTA buffer (pH 8.2) containing 0.02M EDTA in the ratio of 1:2 (w/v) in a pre-chilled pestle and mortar. The homogenate was incubated at room temperature for 30 minutes and then centrifuged at 3000g for 15 minutes at 4°C. A suitable aliquot of the sample was made to 1ml with the homogenization buffer and mixed with 20µl Ellman's reagent (10mmol/L DTNB in methanol). Absorbance of the supernatant was determined at 412 nm in a Perkin Elmer Lamda 650 spectrophotometer. The amount of GSH in the sample was calculated by reference to the standard curve prepared using GSH as the standard.

Ascorbate (AsA): AsA content in the seedlings was determined according to modified method of Wise and Naylor (1987). The harvested seedlings were rinsed with distilled water and wiped dry with tissue paper. A suitable mass of leaf tissue of the harvested seedlings was powdered in liquid nitrogen and then homogenized in 6% (v/v) $HClO_4$. The slurry was centrifuged at 10,000rpm for 10 min at 4°C. The supernatant was transferred to another tube and 2 volumes of 1.25M Na_2CO_3 was added to it. The solution was then centrifuged again at 10,000rpm for 10 min at 4°C.

A known aliquot of the supernatant was diluted in the ratio of 1:10 with 100mM potassium phosphate buffer (pH 5.6) and transferred to a cuvette for measurement of absorbance at 265 nm in a Perkin Elmer Lamda 650 spectrophotometer. The concentration of AsA was determined from the absorbance values by using the extinction coefficient of 14 $mM^{-1}cm^{-1}$.

Determination of soluble protein: The content of soluble Protein in the leaf tissues of the harvested seedlings was determined according to the method of Lowry (1951).

A suitable mass of the leaf tissues of harvested seedlings was ground to a fine powder in a pestle and mortar using liquid nitrogen. The powder was homogenized to a slurry with chilled 50mM Borate buffer (pH 7.0) containing 1% β-mercaptoethanol and 1mM PMSF. The homogenate was allowed to stand for 45 min over ice after which it was centrifuged at 10,000rpm for 15min at 4°C. A suitable aliquot of the supernatant was made to 200 µl with distilled water. 1ml of Lowry Reagent C (Reagent A [2% Na_2CO_3 solution in NaOH 0.1 N] and Reagent B [0.5% $CuSO_4$ 5 H_2O solution in either 1% Sodium tartrate or 1% trisodium citrate] mixed in the ratio of 20:0.4) was added and the solution mixed thoroughly by gentle vortexing. The solution was kept at room temperature for 10 min after which 100 µl of 1N Folin- Ciocalteu's reagent was added to it. The solution was kept at room temperature for 30 min. after which it was transferred to a cuvette for measurement of absorbance at 660 nm in a Perkin Elmer Lamda 650 spectrophotometer. The concentration of soluble protein in the sample was calculated from the absorbance values by reference to a standard curve prepared using BSA.

Enzyme assay:

Extraction: All the enzyme assays were carried out in crude tissue extracts. For preparation of crude tissue extracts the freshly harvested seedlings were washed thoroughly with water followed by rinsing with distilled water. The rinsed seedlings were wiped dry with a clean tissue paper. A suitable mass of leaf tissues from the seedlings was ground to a fine powder with liquid nitrogen in a pre-chilled pestle and mortar. The tissues were homogenized in 0.1 M sodium phosphate (pH 7.8), containing 1.0 mM EDTA, 1.0 mM PMSF and 2% of PVP. The homogenization buffer for assay of ascorbate peroxidase (APX) activity also contained 5 mM ascorbate besides 1.0 mM EDTA, 1.0 mM PMSF and 2% of PVP. The homogenate was centrifuged at 12,000 g for 15 min at 4°C and the supernatant used for assay of enzyme activity immediately thereafter. The activity units have been described as the amount of substrate being transformed into products per unit soluble protein present in the extract per minute.

Ascorbate peroxidase (APX): The activity of ascorbate peroxidase (EC 1.11.1.1) was assayed according to the method of Nakano and Asada (1981). The assay mixture consisted of 50 mM phosphate buffer (pH 6.0), 0.1 μM EDTA, 0.5 mM ascorbate, and 1.0 mM H_2O_2 and 50 μl of tissue extract in a total volume of 1.5 ml. The reaction was started by addition of H_2O_2 to the assay mixture. Ascorbate oxidation was monitored as the change in absorbance at 290 nm per unit time in a perkin Elmer Lamda 650 spectrophotometer with ascorbate molar extinction coefficient value of 2.8 $mM^{-1}cm^{-1}$ (McKersie and Leshem, 1994).

Superoxide dismutase (SOD): Superoxide dismutase (EC 1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), according to the method of Gionnopolitis and Ries (1977). The assay mixture for measurement of superoxide dismutase activity consisted of 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 50 mM Na_2CO_3, 2μM riboflavin, 13mM methionine, 75 μM NBT, appropriate aliquot of the tissue extract and 2 μM riboflavin in a final volume of 3 ml. The assay mixture was illuminated for 15 min at light intensity of 350 μmol m^{-2} s^{-1}. A set of tubes with all the components of the assay mixture maintained in dark served as the blank. Absorbance values were recorded at 560 nm in a Perkin Elmer Lamda 650 spectrophotometer. One unit of SOD activity (U) has been defined as the amount of enzyme required to cause 50 % inhibition in the rate of reduction of NBT.

Catalase (CAT): The activity of catalase (EC 1.11.1.6) was measured according to the method of Beers and Sizer (1952). The assay mixture consisted of, 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, 20 mM H_2O_2 and 50 μl of the tissue extract in a total volume of 1.5 ml. The reaction was started by addition of H_2O_2 to the assay mixture. Activity of the enzyme was monitored as the decrease in H_2O_2 recorded as change in absorbance at 240 nm per unit time with H_2O_2 molar extinction coefficient of 36 $M^{-1}cm^{-1}$.

Peroxidase (POX): The activity of peroxidase (EC 1.11.1.7) was determined using the method of Srinivas *et al.* (1999). The assay mixture (final volume 1 ml) consisted of 20 mM phosphate buffer (pH 6.0), 5mM 2-methoxy phenol (guaiacol), 1 mM hydrogen peroxide and an appropriate aliquot of the extract. The reaction was started with the addition of the tissue extract and allowed to proceed for 3 minutes. The changes in absorbance per unit time was measured at 470 nm in a Perkin Elmer Lamda 650 spectrophotometer. The amount of tetraguaiacol formed per unit time was calculated using the extinction coefficient value of 26.6 $mM^{-1}cm^{-1}$. One unit of peroxidase activity represents the amount of enzyme catalyzing the oxidation of 1 μmol of guaiacol per unit time per unit protein.

Glutathione reductase (GR): The activity of glutathione reductase (EC 1.6.4.2) was assayed according to the method described by Foyer and Halliwell (1976). The assay mixture for measurement of glutathione reductase consisted of 100 mM phosphate buffer (pH 7.8), 0.1 μM EDTA, 0.05 mM NADPH, 3.0 mM GSSG and 50 μl of the tissue extract in a total volume of 1.0 ml. The reaction was started with the addition of NADPH to the assay mixture. The rate of oxidation of NADPH was monitored by measuring the change in absorbance values with time at 340 nm. The difference in absorbance (ΔA_{340}) was divided by the molar extinction coefficient of NADPH (6.22 $mM^{-1}.cm^{-1}$) and the enzyme activity expressed as mmole of NADPH oxidized min^{-1} mg $protein^{-1}$.

Gel electrophoresis:

SDS-PAGE of the total soluble proteins was carried out on 12% polyacrylamide gel following the method of Laemmli (1970). Suitable aliquots of the tissue extracts, representing 50μg protein from each sample, were mixed separately with 2X laemmli buffer in the ratio 1:1. The mixture was heated for 5minutes in a boiling water bath followed by a brief centrifugation to sediment the debris. The supernatant was loaded into the well of a 1.5mm thick 12% acrylamide gel. An aliquot of the mixture of standard molecular weight markers (Bangalore Genei/Roche) was also denatured similarly and loaded on the same gel to serve as reference for determination of molecular mass of the resolved bands. Electrophoresis was carried out at a constant voltage of 100V for 5 hours. After electrophoresis the gel was removed and washed briefly with distilled water. Proteins in the gel were fixed by immersing the gel for 30 minutes in methanol: Glacial acetic acid: water (4:1:5). The gel was subsequently stained for 3 hours in 0.25% (w/v) Coomassie Brilliant Blue R-250 prepared in methanol: Glacial acetic acid: water (4:1:5). Destaining was carried out in a destaining solution I composed of methanol: water: Glacial acetic acid (4:5:1). The gels were further destained in destaining solution II composed of methanol: glacial acetic acid: water (4:5.3:0.7) till the background was cleared. Protein bands were visualized on KODAK Gel Logic 200 gel documentation system under white light. R_f of the bands was calculated as the ratio of the distance travelled by band to the distance travelled by the dye front. For deriving a relationship between the molecular mass of the protein and its mobility on the polyacrylamide gel, the R_f value of the standard molecular weight markers was plotted against log molecular weight of the protein to obtain a standard curve. The molecular mass of the resolved proteins was determined by comparing their electrophoretic mobility of the resolved proteins with that of the standard molecular weight markers and by reference to the standard curve.

Isozyme analysis: A suitable mass of the leaf tissues of harvested seedlings was ground to fine powder with liquid Nitrogen in a pre-chilled mortar and pestle followed by homogenization in 10 mM potassium phosphate buffer (pH 7.8) containing 0.5% triton X-100 and 1.0% PVP. The samples were centrifuged at 10,000rpm for 15 min at 4°C and the clear supernatant was used for determination of isoenzyme profiles of ascorbate peroxidase, superoxide dismutase, catalase and peroxidase .

Ascorbate peroxidase (APX): The isoenzyme profiles of ascorbate peroxidase were determined following the method of Lee and Lee (2000). A known volume of the tissue extract representing 50µg of protein was loaded in the wells of polyacrylamide slab comprising 5% stacking gel and 10% separating gel. Electrophoresis was carried out in a cold room at 30 mA for 1.5 h in a tank buffer comprising 25 mM Tris-Cl (pH 8.3), 2 mM ascorbic acid and 192 mM glycine. After the electrophoresis was over the gel was equilibrated for 30 min with 50 mM phosphate buffer (pH 7.0) containing 2 mM ascorbate and then incubated for 20 min. in a solution containing 50 mM phosphate (pH 7.0), 4 mM ascorbic acid and 2 mM H_2O_2. After incubation the gel was washed with distilled and then submerged for 20 min with gentle agitation in 50 mM sodium phosphate buffer (pH 7.8) containing 28 mM TEMED and 2.45 mM NBT when the isozymes of ascorbate peroxidase became visible as clear areas against a purple background. The gels were stored in 10% acetic acid at 4°C.

Superoxide dismutase: The isoenzyme profiles of superoxide dismutase were determined following the method of Mc Kersie *et al.*, (2000). A known volume of the tissue extract representing 50µg of protein was loaded in the wells of polyacrylamide slab comprising 5% stacking gel and 12% separating gel. Electrophoresis was carried out in a cold room at 30 mA for 1.5 h in a tank buffer comprising 25 mM Tris-Cl (pH 8.3) and 192 mM glycine. After the electrophoresis was over the gel was stained for 30 minutes in dark in 50 mM phosphate buffer (pH 7.8) containing 0.03 mM riboflavin, 0.325% (w/v) TEMED, and 1.25 mM NBT. The gel was then incubated in the solution for 20 minutes under white light till the isozymes appeared as clear areas bands against a dark brown background.

Catalase: The isoenzyme profiles of catalase have been determined following the method of Manchenko (1994). A known volume of the tissue extract representing 50µg of protein was loaded in the wells of polyacrylamide slab comprising 5% stacking gel and 10% separating gel. Electrophoresis was carried out at in a cold room at 30 mA for 1.5 h in a tank buffer comprising 25 mM Tris-Cl (pH 8.3) and 192 mM glycine. After the electrophoresis was over the gel was incubated for 15 minutes in 0.03% H_2O_2 after which the gel was rinsed with water and then incubated for 5 minutes in a 1:1 mixture of 2% potassium ferricyanide and 2% ferric chloride. The isozymes of catalase appeared as light yellow bands against dark blue background. The gel was washed thoroughly with water and photographed immediately.

Peroxidase (POX): The isoenzyme profiles of peroxidase were determined following the method of Manchenko (1994). A known volume of the tissue extract representing 50µg of protein was loaded in the wells of polyacrylamide slab comprising 5% stacking gel and 10% separating gel. Electrophoresis was carried out at in a cold room at 30 mA for 1.5 h in a tank buffer comprising 25 mM Tris-Cl (pH 8.3) and 192 mM glycine. After the electrophoresis was over the gel was incubated at room temperature in dark in a staining solution comprising of 0.5% benzidine, 25% acetic acid and 0.03% H_2O_2. Incubation was carried till the peroxidase isozymes appeared as blue bands against a clear background. The gel was washed with distilled water and stored under 50% glycerol.

Chapter: 4

RESULTS

Experimental:

Seeds of maize (*Zea mays* variety RCM 1-1 and Gujarat Makki) were germinated in a seed germinator at 25 ± 2°C under 14 H photoperiod and 75 ± 10 % R.H. Plants were watered at regular intervals and maintained as described above till the second leaf was fully expanded. The fully grown seedlings at 2nd leaf stage were transferred to culture tubes containing Hoagland's nutrient solution supplemented with 100mM, 150mM, 200mM and 250mM NaCl. A set of plants maintained over Hoagland's Nutrient solution without NaCl served as the controls. The seedlings were maintained for 7 days in a Plant Growth chamber at 25 ± 2°C under 14 h light and 75 ± 10 % R.H. After 7 days the seedlings were harvested, washed with distilled water and wiped dry with tissue paper. The wiped seedlings were used for measurement of length, fresh weight and dry weight of shoots and roots, relative water content (RWC), electrolytic leakage (EL), content of cytosolic sodium, total chlorophyll, soluble protein and activities of ascorbate peroxidase (APX) and glutathione reductase (GR).

Results:

When compared with the untreated controls, seedlings of both varieties of maize *viz.* RCM1-1 and Gujarat Makki, which were maintained over Hoagland's nutrient solution containing different concentrations of NaCl, showed markedly reduced shoot length. The magnitude difference in the shoot length increased with increasing concentration of NaCl in the nutrient solution. Compared to the controls, seedlings cultured in nutrient solution supplemented with 250 mM NaCl showed an almost 2 fold reduction in shoot length. There was no marked difference in the effect of the treatment on shoot length between the two varieties studied in the present investigation (Table 4.1; Fig. 4.1a). There was no significant difference in the root length between the untreated controls and those treated with different concentrations of NaCl (Table 4.1; Fig. 4.1b).

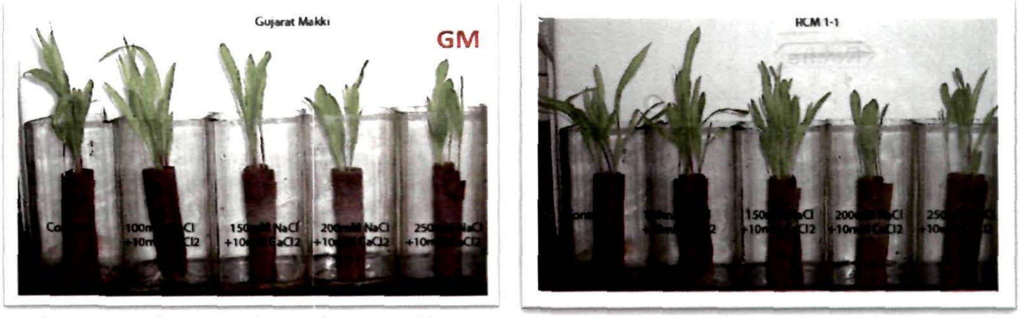
Compared to the untreated controls seedlings of both the varieties of maize *viz.*, RCM1-1 and Gujarat Makki cultured in Hoagland's nutrient solution supplemented different concentrations of NaCl showed significantly reduced moisture content as well as relative water content (RWC); the magnitude of difference in the moisture content as well as RWC between the untreated controls and the salt stressed seedlings increased with increasing concentration of sodium

Control 100 mM NaCl 150mM NaCl 200mM NaCl 250mM NaCl

(a)

Control 100mM NaCl 150mM NaCl 200mM NaCl 250mM NaCl

(b)



Control 100 mM NaCl + 10mM $CaCl_2$ 150mM NaCl+ 10mM $CaCl_2$ 200mM NaCl+ 10mM $CaCl_2$ 250mM NaCl+ 10mM $CaCl_2$

(c)

Control 100mM NaCl + 10mM $CaCl_2$ 150mM NaCl + 10mM $CaCl_2$ 200mM NaCl + 10mM $CaCl_2$ 250mM NaCl+ 10mM $CaCl_2$

(d)

a,b: Effect of different concentrations of NaCl on the seedlings of maize (*Zea mays* L.) var. Gujarat Makki (GM) and RCM 1-1 after 7days of treatment.

c,d. Effect of different concentrations of NaCl and 10mM $CaCl_2$ on the seedlings of maize (*Zea mays* L.) var. Gujarat Makki (GM) and RCM 1-1 after 7days of treatment.

PLATE 1

Table 4.1: Effect of different concentrations of NaCl on the shoot and root length of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment.

Treatments	Variety	Shoot length (cm)	Root length (cm)
control	GM	41.0±0.88	20±0.58
	RCM 1-1	35.5±1.49	15±0.44
100mM NaCl	GM	34.0±0.88	20±0.67
	RCM 1-1	27.0±1.83	17±0.73
150mM NaCl	GM	32.0±1.15	22±1.16
	RCM 1-1	25.5±2.75	21±0.33
200mM NaCl	GM	24.5±0.87	18±1.20
	RCM 1-1	23.0±1.78	21±0.73
250mM NaCl	GM	22.0±0.58	16±0.87
	RCM 1-1	22.5±2.37	20±0.73

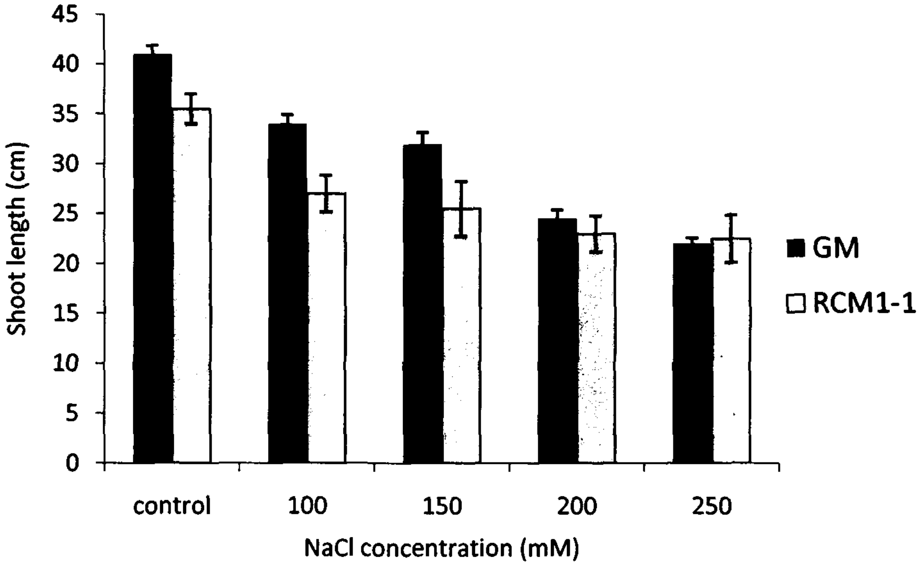
±SEM

Table 4.2: Effect of different concentrations of NaCl on the relative water content (RWC), % dry weight and % moisture content of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment.

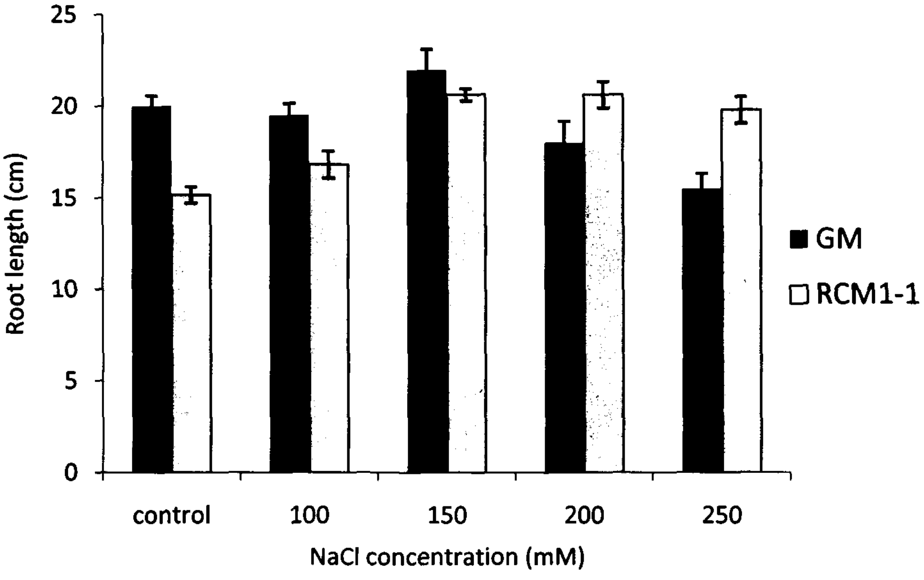
Treatments	Variety	% moisture	RWC	% dry weight
control	GM	91.24±0.561	98.87±2.17	8.76±0.56
	RCM 1-1	90.50±0.343	97.76±2.61	9.50±0.34
100mM NaCl	GM	88.29±0.773	97.95±1.38	11.71±0.77
	RCM 1-1	88.59±0.663	75.81±2.00	11.41±0.66
150mM NaCl	GM	88.56±0.736	93.19±1.29	11.44±0.74
	RCM 1-1	88.64±0.737	70.68±3.24	11.36±0.74
200mM NaCl	GM	86.65±0.373	87.95±1.59	13.35±0.37
	RCM 1-1	88.04±0.613	65.39±2.70	11.96±0.61
250mM NaCl	GM	84.38±0.497	69.85±2.25	15.62±0.50
	RCM 1-1	87.68±0.383	59.66±3.06	12.32±0.38

±SEM

Figure 4.1: Effect of different concentrations of NaCl on the length of shoot (a) and root (b) in seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment. Vertical lines on each bar represent SEM.

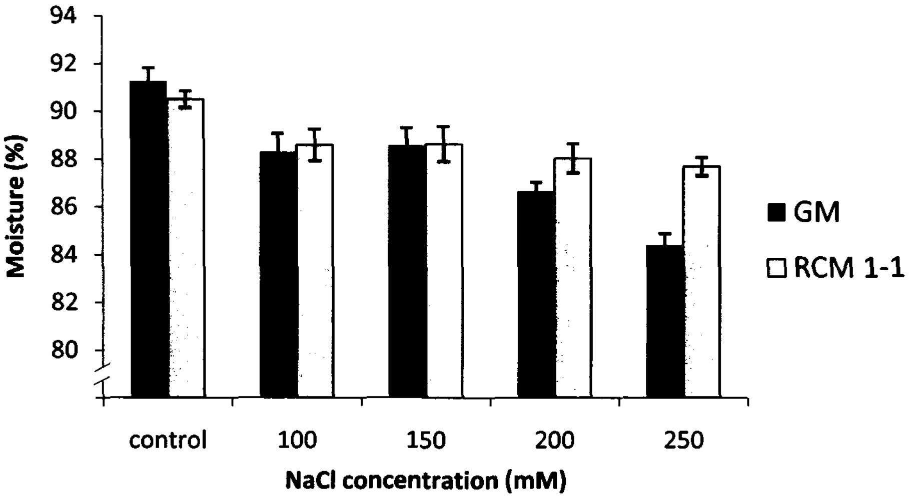

45
40
35
30
25
20
15
10
5
0
Shoot length (cm)
control
100
150
200
250
NaCl concentration (mM)
■ GM
□ RCM1-1

(a)

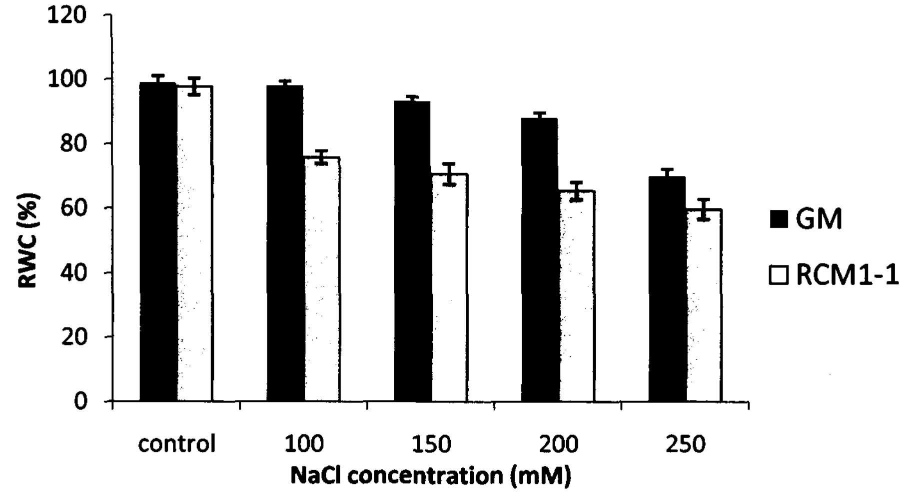

25
20
15
10
5
0
Root length (cm)
control
100
150
200
250
NaCl concentration (mM)
■ GM
□ RCM1-1

(b)

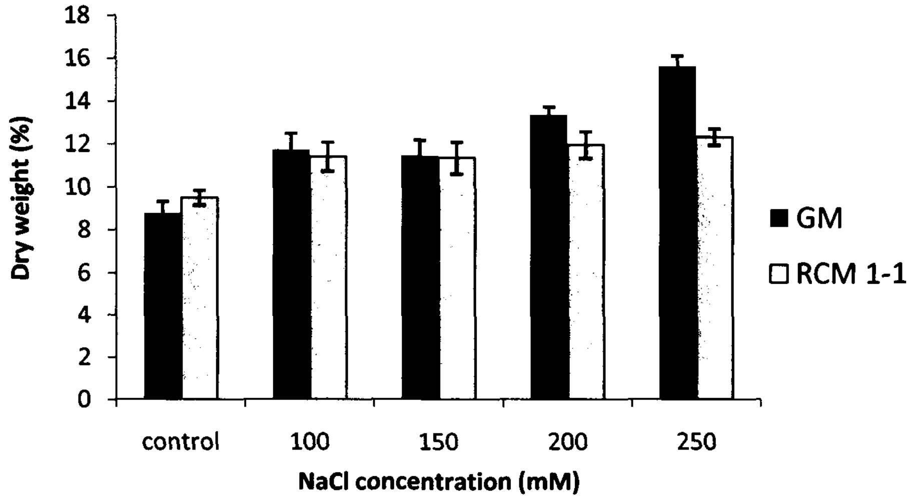
Figure 4.2: Effect of different concentrations of NaCl on (a)% moisture content, (b) relative water content (RWC) and (c) % dry weight of shoot tissues of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment. Vertical lines on each bar represent SEM.


94
92
90
88
86
84
82
80
Moisture (%)
control
100
150
200
250
NaCl concentration (mM)
■ GM
□ RCM 1-1

(a)


120
100
80
60
40
20
0
RWC (%)
control
100
150
200
250
NaCl concentration (mM)
■ GM
□ RCM1-1

(b)


18
16
14
12
10
8
6
4
2
0
Dry weight (%)
control
100
150
200
250
NaCl concentration (mM)
■ GM
□ RCM 1-1

(c)

chloride in the nutrient solution (Table 4.2; Fig. 4.2). The decrease in moisture content in the variety Gujarat Makki showed a linear relationship with increasing concentration of sodium chloride in the nutrient solution. The reduction in moisture content in the variety RCM1-1 was, however, much less marked in the variety RCM1-1 than that in the variety Gujarat Makki . Seedlings of the variety RCM1-1, *cultured in nutrient solution supplemented with varying concentrations of* NaCl, showed a greater level of reduction in RWC than the variety Gujarat Makki (Table 4.2; Fig. 4.2b). There was a marked increase in percent dry weight of the seedlings with increase in the concentration of NaCl in the nutrient solution. The magnitude of increase in percent dry matter was, however, more marked in the variety Gujarat Makki. Thus, seedlings of the variety Gujarat Makki, cultured in nutrient solution supplemented with 250 mM NaCl had two fold higher dry matter content than the untreated controls. On the other hand, seedlings of the variety RCM1-1 showed only a marginal increase in dry weight with increase in the concentration of NaCl in the nutrient solution (Table 4.2; Fig. 4.2c).

When expressed as percent of fresh weight, the content of total chlorophyll in shoot tissues of both varieties of maize showed a marginal increase at 100 and 150 mM sodium chloride in the nutrient solution. However, the content of total chlorophyll was markedly less in seedlings cultured in nutrient medium supplemented with 250 mM sodium chloride than those cultured in nutrient medium supplemented with 150 and 200 mM NaCl (Table 4.3; Fig. 4.3a). When expressed as percent of dry weight, the content of total chlorophyll in shoot tissues of the variety Gujarat Makki showed a marked decrease with increasing concentration of NaCl in the nutrient solution. Thus, seedlings of variety Gujarat Makki cultured in nutrient solution supplemented with 250 mM NaCl showed a nearly 40% decrease in the content of total chlorophyll than the untreated controls. On the other hand seedlings of the variety RCM1-1 showed only marginal differences in the content of total chlorophyll between the untreated controls and the treated ones (Table 4.3; Fig. 4.3b).

Expressed either as percent of fresh weight or as percent of dry weight, seedlings of both varieties of maize growing in Hoagland's nutrient solution supplemented with varying concentrations of sodium chloride showed markedly higher levels of cytosolic sodium than the untreated controls. Irrespective of the reference base, the concentration of cytosolic sodium showed a marked increase with increase in the concentration of NaCl in the ambient nutrient solution. When expressed as percent of fresh weight, seedlings of the variety Gujarat Makki, cultured in nutrient solution supplemented with 250 mM NaCl, had 8 fold higher levels of cytosolic sodium than the untreated controls. Similarly seedlings of the variety RCM1-1, cultured in nutrient solution supplemented with 250 mM NaCl, had six fold higher levels of cytosolic sodium than the untreated controls (Table 4.4; Fig. 4.3c,d). When the tissue concentration of total chlorophyll was expressed as percent of dry weight, seedlings of both the varieties, cultured in nutrient solution supplemented with 250 mM NaCl, showed >4 fold higher levels of cytosol sodium than the untreated controls (Table 4.4; Fig. 4.3c,d).

There was a marked increase in the electrolyte leakage, expressed as percent of total conductivity, from cells of both the varieties of maize studied in the present

Table 4.3: Effect of different concentrations of NaCl on the total chlorophyll content and cytosolic sodium content in shoots of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 after 7days of treatment.

Treatments	Variety	Total chlorophyll (mg/100mg fresh weight)	Total chlorophyll (mg/100mg dry weight)
control	GM	0.070±0.00083	0.7962±0.01083
	RCM 1-1	0.043±0.00030	0.4569±0.01030
100mM NaCl	GM	0.076±0.00149	0.6454±0.01488
	RCM 1-1	0.064±0.00099	0.5572±0.01990
150mM NaCl	GM	0.086±0.00142	0.7535±0.01142
	RCM 1-1	0.070±0.00098	0.6133±0.01098
200mM NaCl	GM	0.087±0.00133	0.6536±0.01233
	RCM 1-1	0.062±0.00225	0.5200±0.01250
250mM NaCl	GM	0.075±0.00137	0.4790±0.01137
	RCM 1-1	0.052±0.00127	0.4237±0.01127

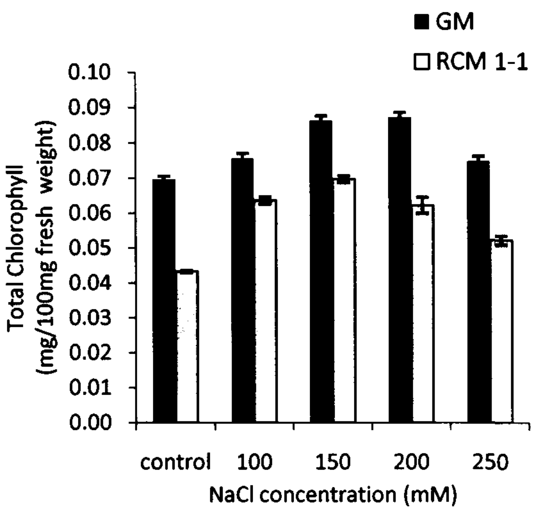
±SEM

Table 4.4: Effect of different concentrations of NaCl on the electrolyte leakage (EL), concentration of soluble protein, activities of glutathione reductase (GR) and ascorbate peroxidase (APX) in shoots of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment.

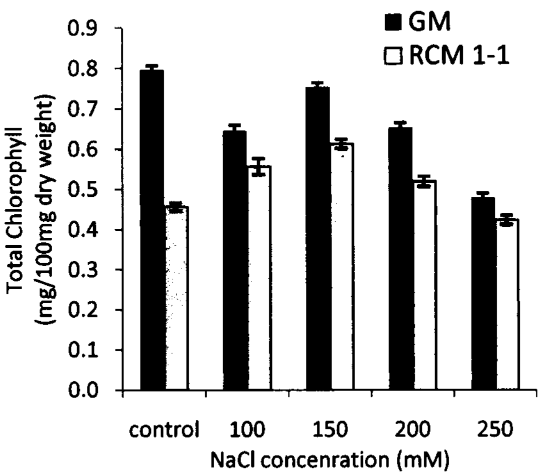
Treatments	Variety	Cytosolic Na$^+$ (nmol/100mg fresh weight)	Cytosolic Na$^+$ (nmol/100mg dry weight)	Electrolyte leakage
Control	GM	0.0034±0.0003	0.039±0.0006	9.16±0.032
	RCM 1-1	0.0021±0.0001	0.022±0.0020	10.63±0.032
100mM NaCl	GM	0.0079±0.0003	0.068±0.0023	28.99±0.313
	RCM 1-1	0.0093±0.0002	0.081±0.0024	33.23±0.071
150mM NaCl	GM	0.0138±0.0002	0.121±0.0021	41.75±0.740
	RCM 1-1	0.0104±0.0004	0.091±0.0035	43.34±0.051
200mM NaCl	GM	0.0176±0.0008	0.132±0.0021	60.66±1.281
	RCM 1-1	0.0114±0.0003	0.095±0.0032	48.24±0.061
250mM NaCl	GM	0.0239±0.0004	0.153±0.0034	70.73±1.199
	RCM 1-1	0.0136±0.0003	0.110±0.0020	60.84±0.046

±SEM

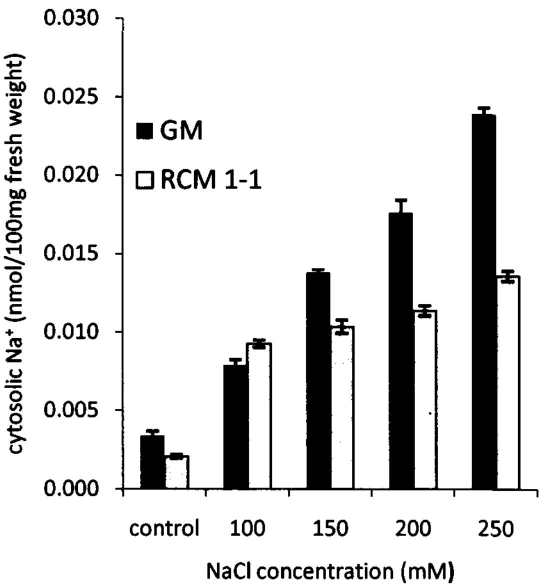
Figure 4.3: Effect of different concentrations of NaCl on the content of total chlorophyll (a,b), cytosolic sodium content (c,d) and electrolyte leakage (e) in shoots of seedlings of two varieties of maize *viz.* Gujarat Makki (GM) and RCM 1-1 after 7days treatment. Vertical lines on each bar represent SEM.


■ GM
□ RCM 1-1
Total Chlorophyll (mg/100mg fresh weight)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
control
100
150
200
250
NaCl concentration (mM)

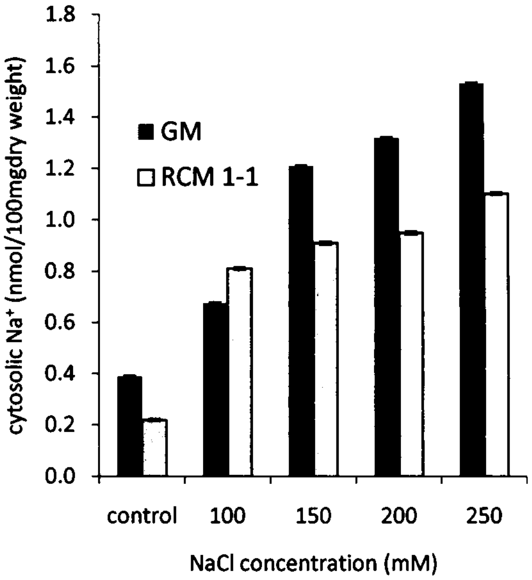
(a)


■ GM
□ RCM 1-1
Total Chlorophyll (mg/100mg dry weight)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
control
100
150
200
250
NaCl concenration (mM)

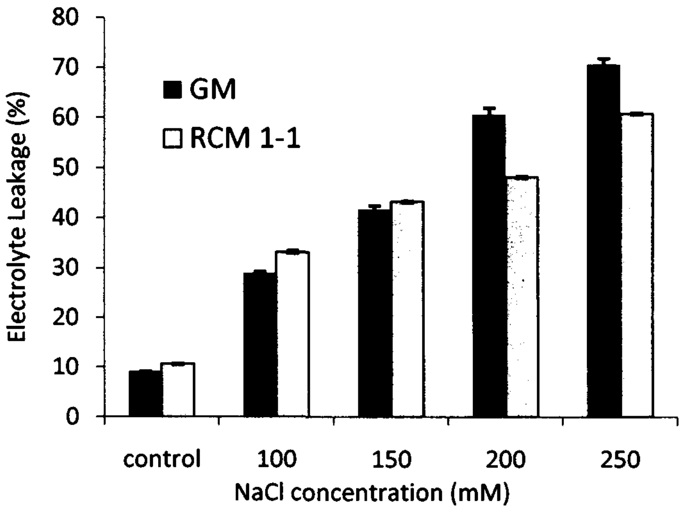
(b)


■ GM
□ RCM 1-1
cytosolic Na⁺ (nmol/100mg fresh weight)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
control
100
150
200
250
NaCl concentration (mM)

(c)


■ GM
□ RCM 1-1
cytosolic Na⁺ (nmol/100mgdry weight)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
control
100
150
200
250
NaCl concentration (mM)

(d)


■ GM
□ RCM 1-1
Electrolyte Leakage (%)
80
70
60
50
40
30
20
10
0
control
100
150
200
250
NaCl concentration (mM)

(e)

investigation with increase in the concentration of NaCl in the ambient nutrient solution. The electrolyte leakage was, however, relatively more marked in the variety Gujarat Makki than the variety RCM1-1(Table 4.4; Fig. 4.3e). Compared to the untreated controls seedlings of the variety Gujarat Makki showed 7 fold higher electrolyte leakage at ambient NaCl concentration of 250 mM. Similarly, at 250 mM ambient NaCl concentration seedlings of the variety RCM1-1 showed 6 fold higher leakage of electrolytes (Table 4.4; Fig. 4.3e).

Expressed as percent of fresh weight, the tissue content of soluble protein in seedlings of both varieties of maize *viz.* RCM1-1 and Gujarat Makki grown in nutrient solution containing different concentrations of sodium chloride was marginally higher than that in the untreated controls. The content of soluble protein showed a marginal increase with increase in the concentration of NaCl in the ambient nutrient solution. However, when the tissue level of soluble protein was expressed as percent of dry weight the concentration of soluble protein in the seedlings of variety RCM1-1 showed a marginal increase with increase in the concentration of NaCl in the ambient nutrient solution. However, in case of the variety Gujarat Makki the concentration of soluble protein showed a decreasing trend with increase in the concentration of NaCl in the nutrient solution. Thus, seedlings of variety Gujarat Makki growing in nutrient solution supplemented with 150, 200 and 250 mM NaCl showed significantly lower levels of soluble protein than the variety RCM1-1 cultured under same concentrations of NaCl in the nutrient solution (Table 4.5; Fig. 4.4a,b).

Seedlings of both the varieties of maize grown on Hoagland's nutrient medium supplemented with 100, 200 and 250 mM NaCl showed marginally higher activity of glutathione reductase than the untreated controls. Thus, compared to the untreated controls, seedlings cultured in nutrient solution supplemented with 200 mM NaCl showed almost 1.5 fold higher activity of glutathione reductase than that of the untreated controls (Table 4.5; Fig. 4.4c). The activity of the enzyme showed a marginal decline at 250 mM ambient NaCl concentration. However even at 250 mM ambient NaCl concentration, the activity of GR in the seedlings was higher than that in the untreated controls (Table 4.5; Fig. 4.4c). There were no significant differences in the activity of the enzyme between the two varieties studied in the present investigation.

There were no marked differences in the activity of ascorbate peroxidase in leaf tissues of control seedlings and those which were grown in nutrient solution supplemented with either 100 or 150 mM NaCl. While the activity of the enzyme was significantly higher in shoot tissues of seedlings of the variety RCM1-1 grown in nutrient solution supplemented with either 200 or 250 mM NaCl, the activity of APX in leaf tissues of seedlings of the variety Gujarat Makki showed a marked decrease with increase in the concentration of sodium chloride in the nutrient solution (Table 4.5; Fig. 4.4d).

In order to study the role of calcium ions in modulating metabolic pathways during salt stress in seedlings of maize, seeds of maize (*Zea mays* L.) variety RCM 1-1 and Gujarat Makki) were germinated in a seed germinator at 25 ± 2°C under 14

Table 4.5: Effect of different concentrations of NaCl on the electrolyte leakage (EL), concentration of soluble protein, activities of glutathione reductase (GR) and ascorbate peroxidase (APX) in seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment.

Treatments	Variety	Soluble protein (µg/mg fresh weight)	Soluble protein (µg/mg dry eight)	GR activity (µmol mg protein^{-1} min^{-1})	APX activity (mmol mg protein^{-1} min^{-1})
Control	GM	1.297±0.010	14.812±0.20	2.47±0.021	0.247±0.026
	RCM 1-1	1.250±0.014	13.160±0.22	2.16±0.017	0.233±0.014
100mM NaCl	GM	1.401±0.008	11.961±0.17	2.96±0.025	0.233±0.012
	RCM 1-1	1.345±0.013	11.784±0.13	2.97±0.012	0.214±0.019
150mM NaCl	GM	1.427±0.010	12.473±0.11	2.89±0.029	0.268±0.004
	RCM 1-1	1.780±0.021	15.663±0.15	3.61±0.020	0.321±0.017
200mM NaCl	GM	1.540±0.009	11.532±0.28	3.48±0.012	0.190±0.006
	RCM 1-1	1.814±0.014	15.164±0.20	3.66±0.041	0.568±0.017
250mM NaCl	GM	1.555±0.009	9.957±0.10	3.16±0.038	0.106±0.003
	RCM 1-1	1.830±0.016	14.853±0.17	2.77±0.015	0.393±0.009

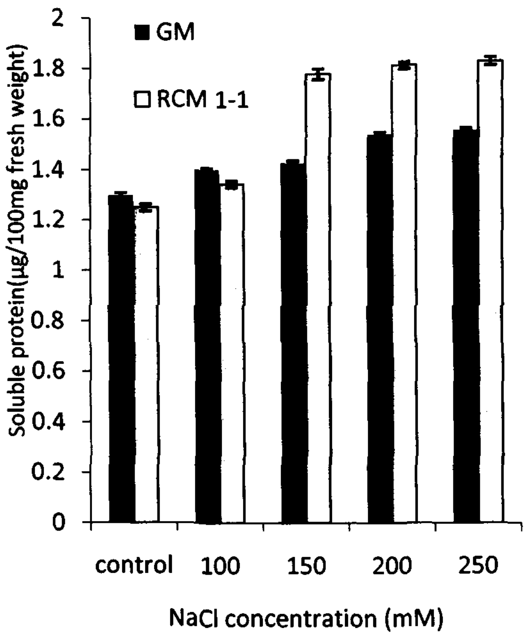
±SEM

Table 4.6: Effect of different concentrations of NaCl with or without 10 mM $CaCl_2$ on the length of shoot and root in seedlings of two varieties of maize *viz.* Gujarat Makki (GM) and RCM 1-1 after 7 days of treatment.

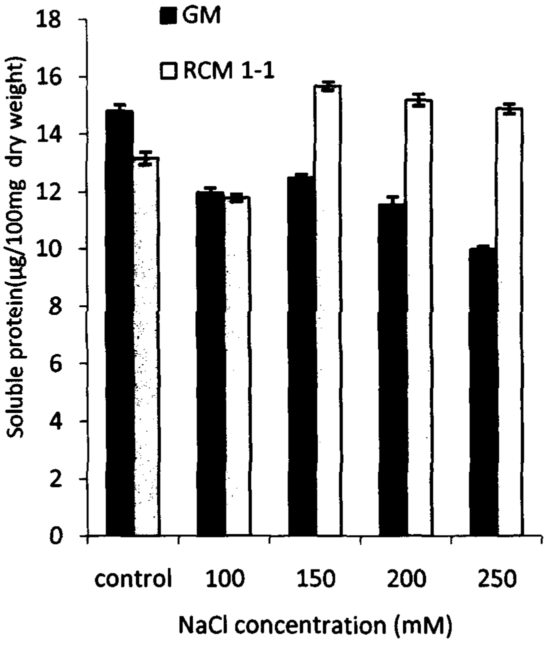
Treatments	Variety	Shoot length (cm)	Root length (cm)
control	GM	41.0±0.88	20±0.577
	RCM 1-1	35.5±1.49	15±0.441
100mM NaCl	GM	34.0±0.88	20±0.667
	RCM 1-1	27.0±1.83	17±0.726
100mM NaCl +10mM $CaCl_2$	GM	39.0±0.58	23±0.333
	RCM 1-1	29.5±0.30	21±0.289
150mM NaCl	GM	32.0±1.15	22±1.155
	RCM 1-1	25.5±2.75	21±0.333
150mM NaCl +10mM $CaCl_2$	GM	31.5±0.44	23±0.577
	RCM 1-1	29.0±1.33	20±0.726
200mM NaCl	GM	24.5±0.87	18±1.202
	RCM 1-1	23.0±1.78	21±0.726
200mM NaCl +10mM $CaCl_2$	GM	31.0±2.19	24±0.667
	RCM 1-1	24.5±1.76	21±0.764
250mM NaCl	GM	22.0±0.58	16±0.866
	RCM 1-1	22.5±2.37	20±0.726
250mM NaCl +10mM $CaCl_2$	GM	29.0±0.58	18±0.289
	RCM 1-1	23.5±2.46	20±0.601

±SEM

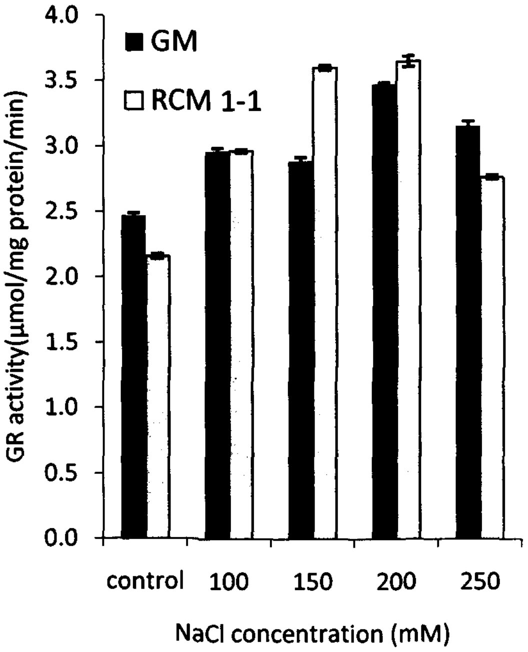
Figure 4.4: Effect of different concentrations of NaCl on the concentration of total soluble protein (a,b), activities of glutathione reductase (GR) (c) and ascorbate peroxidase (APX) (d) in shoots of seedlings of two varieties of maize *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment. Vertical lines on each bar represent SEM.


■ GM
□ RCM 1-1
Soluble protein(µg/100mg fresh weight)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
control
100
150
200
250
NaCl concentration (mM)

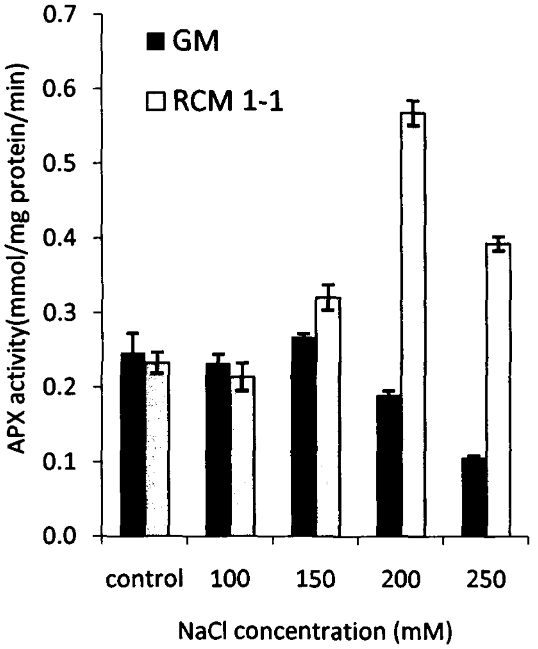
(a)


■ GM
□ RCM 1-1
Soluble protein(µg/100mg dry weight)
0
2
4
6
8
10
12
14
16
18
control
100
150
200
250
NaCl concentration (mM)

(b)

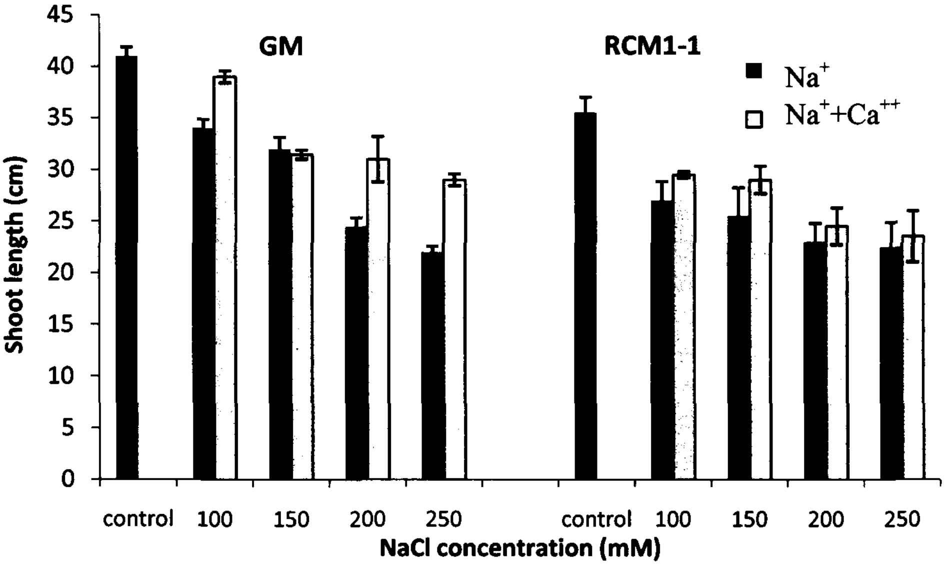

■ GM
□ RCM 1-1
GR activity(µmol/mg protein/min)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
control
100
150
200
250
NaCl concentration (mM)

(c)

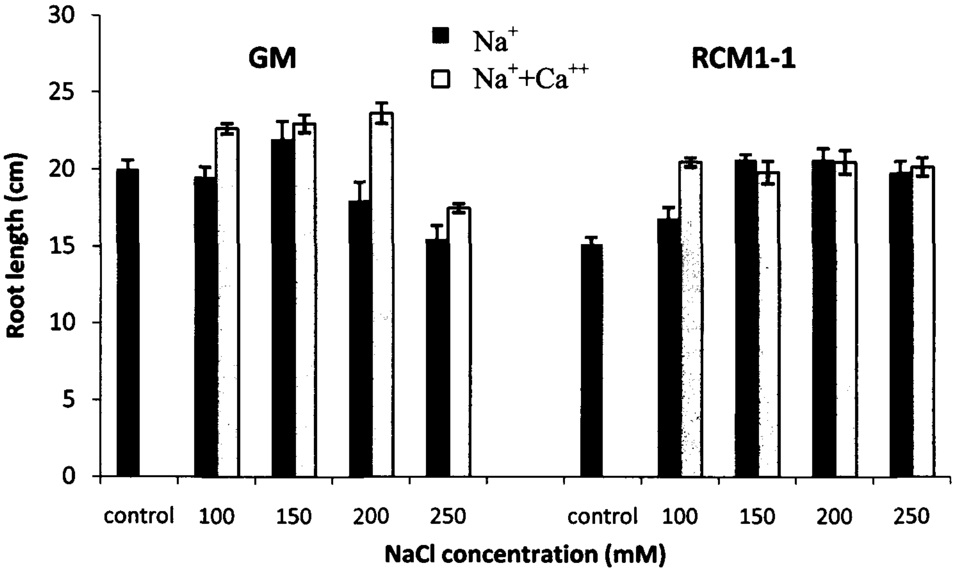

■ GM
□ RCM 1-1
APX activity(mmol/mg protein/min)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
control
100
150
200
250
NaCl concentration (mM)

(d)

Figure 4.5: Effect of different concentrations of NaCl with (▨) or without (■) 10 mM $CaCl_2$ on the shoot length (a) and root length (b) of seedlings of two varieties of maize *viz.* Gujarat Makki (GM) and RCM 1-1 after 7days of treatment. Vertical lines on each bar represent SEM.


GM
RCM1-1
■ Na⁺
☐ Na⁺+Ca⁺⁺
Shoot length (cm)
45
40
35
30
25
20
15
10
5
0
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(a)


GM
■ Na⁺
☐ Na⁺+Ca⁺⁺
RCM1-1
Root length (cm)
30
25
20
15
10
5
0
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(b)

h photoperiod and 75 ± 10 % R.H. Plants were watered at regular intervals and maintained, as described above, till the second leaf was fully expanded. The fully grown seedlings at 2nd leaf stage were transferred to culture tubes containing Hoagland's nutrient solution supplemented with 100mM, 150mM, 200mM and 250mM NaCl with and without 10 mM $CaCl_2$. A set of plants maintained over Hoagland's nutrient solution without NaCl or any other additional supplements served as controls for the corresponding NaCl or NaCl+$CaCl_2$ treated sets.

Seedlings of both maize varieties *viz.* RCM1-1 and Gujarat Makki, cultured in Hoagland's nutrient solutions supplemented with different concentrations of NaCl showed marked decrease in the shoot length than the untreated controls; the length of the shoots decreased progressively with increase in the concentration of NaCl in the nutrient solution (Table 4.6; Fig. 4.5a). However, the magnitude of decrease in shoot length was less marked in the variety RCM1-1 than the variety Gujarat Makki. Seedlings cultured in nutrient solutions supplemented with different concentrations of NaCl and 10 mM $CaCl_2$, however, showed greater shoot length than the corresponding control seedlings in which the nutrient solutions were not supplemented with $CaCl_2$ (Table 4.6; Fig. 4.5a). While the seedlings grown in nutrient solution containing 250mM NaCl recorded >40% suppression in shoot length than that of the untreated control, seedlings which were cultured in nutrient solution containing 250 mM NaCl along with 10 mM $CaCl_2$ showed only 30% inhibition in shoot length. Incorporation of 10mM calcium chloride in the nutrient solutions containing different concentrations of sodium chloride did not have any marked effect on the roots length of the seedlings in either of the two varieties (Table 4.6; Fig. 4.5b).

Seedlings of both maize varieties *viz.* RCM1-1 and Gujarat Makki, cultured in Hoagland's nutrient solutions supplemented with different concentrations of NaCl showed marked decrease in the percent moisture content as well as relative water content than the untreated controls; the percent moisture content as well as relative water content showed progressive decrease with increase in the concentration of NaCl in the nutrient solution (Table 4.7; Fig. 4.6a,b). There was no significant effect of calcium chloride on the RWC in either of the two varieties. Seedlings of variety Gujarat Makki cultured in Hoagland's nutrient solutions containing different concentrations of NaCl supplemented with 10 mM $CaCl_2$ showed higher tissue moisture content than those in which the nutrient solution was not supplemented with $CaCl_2$. However, there was no marked effect of $CaCl_2$ on the moisture levels in seedlings of the variety RCM1-1 cultured in nutrient solutions supplemented with different concentrations of sodium chloride (Table 4.7; Fig. 4.6a). Seedlings of the variety Gujarat Makki, cultured in Hoagland's nutrient solutions supplemented with different concentrations of sodium chloride and 10 mM $CaCl_2$ showed a marginally lower % dry matter than the corresponding control seedlings in which the nutrition solution was not supplemented $CaCl_2$. However, there was no marked effect of $CaCl_2$ on the dry matter content of seedlings of variety RCM1-1 (Table 4.7; Fig. 4.6c).

Expressed either as percent of fresh weight or as percent of dry weight seedlings of both the varieties of maize, cultured in Hoagland's nutrient solutions supplemented with different concentrations of NaCl and 10 mM $CaCl_2$, showed

Table 4.7: Effect of different concentrations of NaCl with or without 10 mM $CaCl_2$ on the % moisture content, relative water content (RWC) and % dry weight content in shoots of seedlings of two varieties of maize *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment.

Treatments	Variety	% moisture	RWC	% dry weight
control	GM	91.24±0.561	98.87±2.17	8.76±0.560
	RCM 1-1	90.50±0.343	97.76±2.61	9.50±0.340
100mM NaCl	GM	88.29±0.773	97.95±1.38	11.71±0.770
	RCM 1-1	88.59±0.663	75.81±2.00	11.41±0.660
100mM NaCl +10mM $CaCl_2$	GM	90.87±0.643	98.26±4.31	9.13±0.643
	RCM 1-1	89.53±0.405	89.32±2.35	10.94±0.405
150mM NaCl	GM	88.56±0.736	93.19±1.29	11.44±0.740
	RCM 1-1	88.64±0.737	70.68±3.24	11.36±0.740
150mM NaCl +10mM $CaCl_2$	GM	89.82±0.465	95.62±3.33	10.18±0.465
	RCM 1-1	88.17±0.652	82.66±2.41	11.83±0.652
200mM NaCl	GM	86.65±0.373	87.95±1.59	13.35±0.370
	RCM 1-1	88.04±0.613	65.39±2.70	11.96±0.610
200mM NaCl +10mM $CaCl_2$	GM	89.72±0.262	88.54±3.14	10.28±0.262
	RCM 1-1	89.42±0.850	74.61±3.20	10.58±0.850
250mM NaCl	GM	84.38±0.497	69.85±2.25	15.62±0.500
	RCM 1-1	87.68±0.383	59.66±3.06	12.32±0.380
250mM NaCl +10mM $CaCl_2$	GM	87.55±0.336	78.13±3.65	12.45±0.336
	RCM 1-1	86.84±0.118	63.69±2.34	13.16±0.118

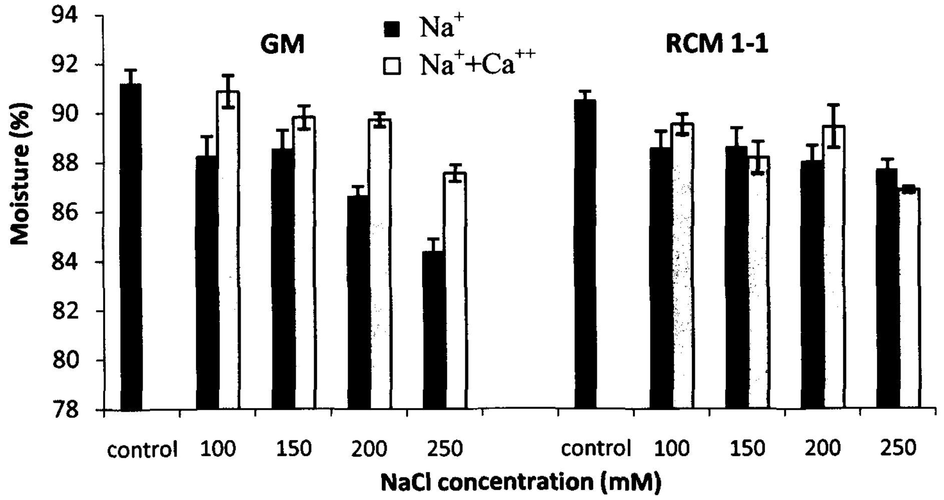
±SEM

Table 4.8: Effect of different concentrations of NaCl with or without 10mM $CaCl_2$ on the concentration of total chlorophyll in shoots seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment.

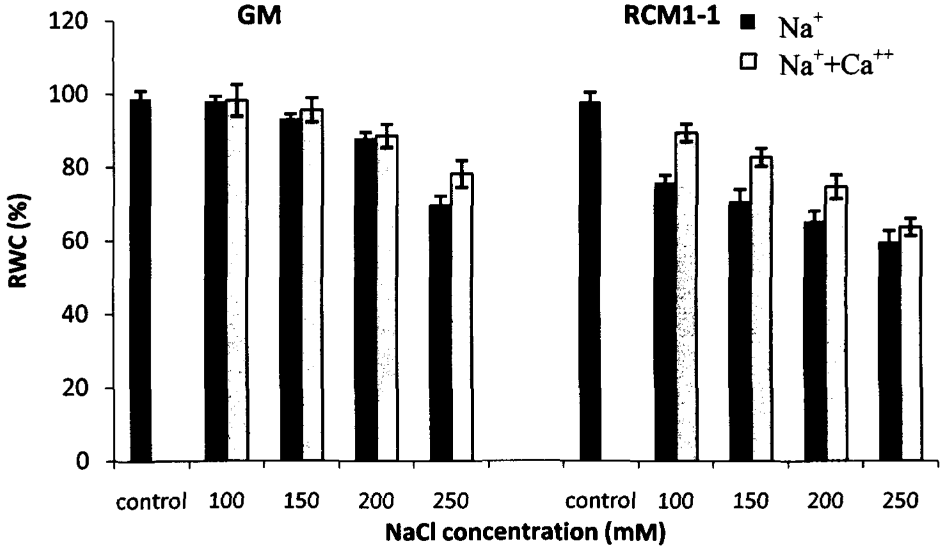
Treatments	Variety	Total chlorophyll (mg/100mg fresh weight)	Total chlorophyll (mg/100mg dry weight)
control	GM	0.070±0.0008	0.7962±0.011
	RCM 1-1	0.043±0.0003	0.4569±0.010
100mM NaCl	GM	0.076±0.0015	0.6454±0.015
	RCM 1-1	0.064±0.0010	0.5572±0.020
100mM NaCl +10mM $CaCl_2$	GM	0.078±0.0012	0.8585±0.022
	RCM 1-1	0.066±0.0015	0.6071±0.025
150mM NaCl	GM	0.086±0.0014	0.7535±0.011
	RCM 1-1	0.070±0.0010	0.6133±0.011
150mM NaCl +10mM $CaCl_2$	GM	0.089±0.0008	0.8710±0.019
	RCM 1-1	0.081±0.0008	0.6848±0.018
200mM NaCl	GM	0.087±0.0013	0.6536±0.012
	RCM 1-1	0.062±0.0023	0.5200±0.013
200mM NaCl +10mM $CaCl_2$	GM	0.094±0.0007	0.9192±0.017
	RCM 1-1	0.095±0.0020	0.8994±0.030
250mM NaCl	GM	0.075±0.0014	0.4790±0.012
	RCM 1-1	0.052±0.0013	0.4237±0.011
250mM NaCl +10mM $CaCl_2$	GM	0.092±0.0006	0.7358±0.016
	RCM 1-1	0.093±0.0020	0.7057±0.030

±SEM

Figure 4.6: Effect of different concentrations of NaCl with (▨) or without (■)10mM $CaCl_2$ on the % moisture content (a), relative water content (b) and % dry weight (c) in shoots of seedlings of two varieties of maize *viz.* Gujarat Makki (GM) and RCM 1-1 after 7days of treatment. Vertical lines on each bar represent SEM.


GM
RCM 1-1
■ Na^+
□ Na^++Ca^{++}
Moisture (%)
94
92
90
88
86
84
82
80
78
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(a)


GM
RCM1-1
■ Na^+
□ Na^++Ca^{++}
RWC (%)
120
100
80
60
40
20
0
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(b)

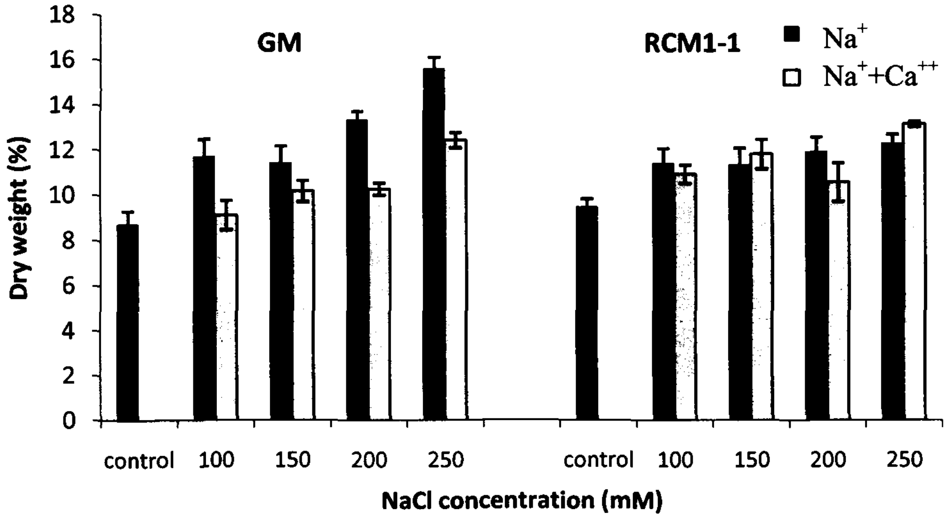
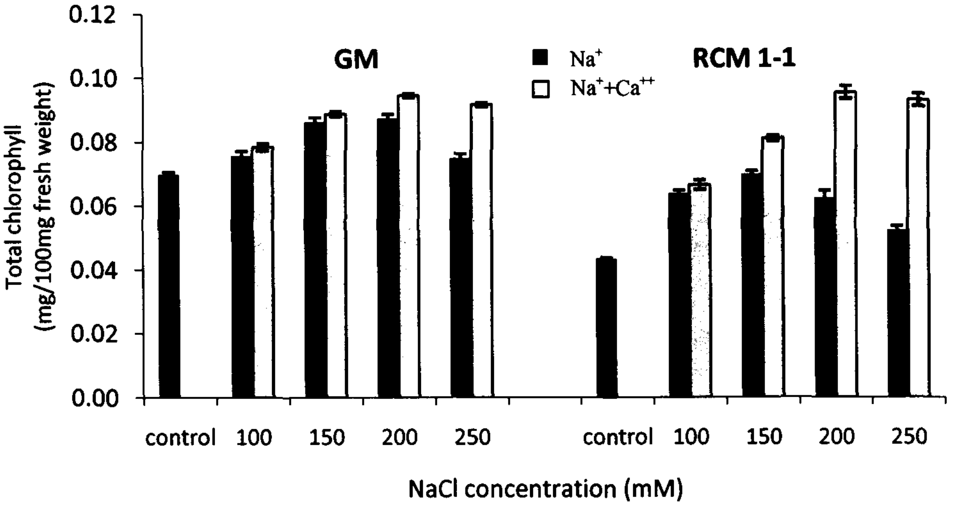
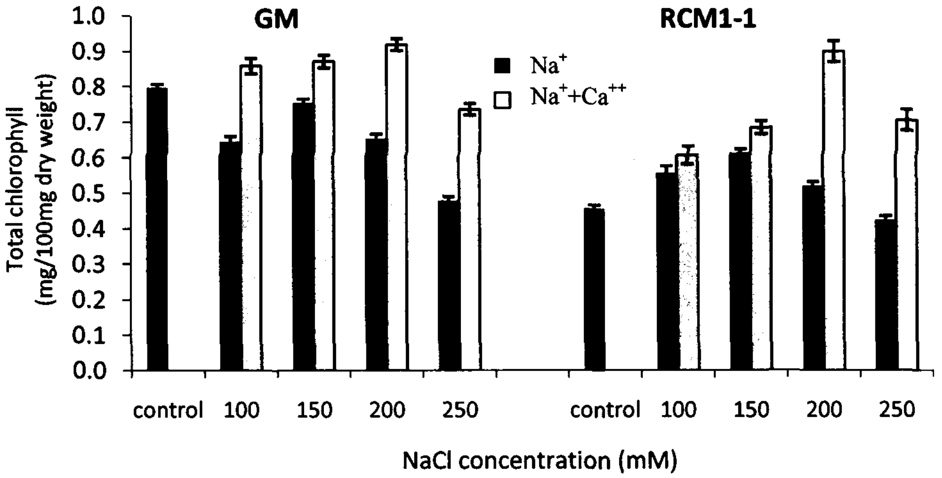

GM
RCM1-1
■ Na^+
□ Na^++Ca^{++}
Dry weight (%)
18
16
14
12
10
8
6
4
2
0
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

Figure 4.7: Effect of different concentrations of NaCl with (▨) or without (■) 10mM $CaCl_2$ on the content of total chlorophyll in leaves of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 after 7days of treatment. Vertical lines on each bar represent SEM.


0.12
0.10
0.08
0.06
0.04
0.02
0.00
Total chlorophyll
(mg/100mg fresh weight)
GM
RCM 1-1
Na$^+$
Na$^+$+Ca^{++}
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(a)


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Total chlorophyll
(mg/100mg dry weight)
GM
RCM1-1
Na$^+$
Na$^+$+Ca^{++}
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(b)

higher content of total chlorophyll than those in which the nutrient solution was not supplemented with $CaCl_2$. (Table 4.8; Fig. 4.7). Expressed as percent of fresh weight the tissue content of total chlorophyll in seedlings of the variety Gujarat Makki did not show any marked change in with increase in the concentration of NaCl in the nutrient solution. On the other hand the tissue content of total chlorophyll in the variety RCM1-1, cultured in nutrient solution containing only varying dozes of NaCl, registered a marginal increase with increase in the concentration of NaCl in the nutrient solution from 100 to 150 mM. The concentration of chlorophyll showed a progressive decrease with further increase in the concentration of NaCl in the nutrient solution upto 250 mM. The total chlorophyll content of the seedlings of this variety, cultured in nutrient solutions supplemented with different concentrations of NaCl and 10 mM $CaCl_2$ showed a progressive increase with increase in the concentration of NaCl in the nutrient solution (Table 4.8; Fig. 4.7a). Expressed as percent of dry weight the tissue level total chlorophyll in the variety Gujarat Makki, cultured in nutrient solutions supplemented only with different concentrations of NaCl, showed a marked decrease with increase in the concentration of NaCl in the nutrient solution. However, the tissue level total chlorophyll in the variety Gujarat Makki, cultured in nutrient solutions supplemented with different concentrations of NaCl and 10 mM $CaCl_2$, did not show any such decrease with increase in the concentration of NaCl in the nutrient solution (Table 4.8; Fig. 4.7b). On the other hand the tissue content of total chlorophyll in RCM1-1, cultured in nutrient solutions supplemented with only NaCl, registered a slight increase with increase in the concentration of NaCl in the nutrient solution from 100 to 150 mM. The concentration of chlorophyll showed a progressive decrease with further increase in the concentration of NaCl in the nutrient solution. The total chlorophyll content of the seedlings of this variety, cultured in nutrient solutions supplemented with different concentrations of NaCl and 10 mM $CaCl_2$ showed a progressive increase with increase in the concentration of NaCl upto 200 mM. At the ambient NaCl concentration of 250 mM, the tissue concentration of total chlorophyll in this variety showed a marginal decrease compared to the concentration of chlorophyll in seedlings cultured at ambient NaCl concentration of 200 mM (Table 4.8; Fig. 4.7b).

There as a marked increase in the tissue concentration of free cytosolic sodium in both the varieties of maize studied in the present investigation with increase in the concentration of NaCl in the nutrient solution. However, when the tissue content of free cytosolic sodium in seedlings cultured in nutrient solutions containing different concentrations of NaCl and 10 mM $CaCl_2$ was compared with those which were cultured in nutrient solution supplemented with NaCl only, seedlings of both the varieties of maize *viz.* RCM1-1 and Gujarat Makki cultured in Hoagland's nutrient solutions containing different concentrations of NaCl supplemented with 10mM $CaCl_2$ showed significantly lower levels of cytosolic sodium (Table 4.9; Fig. 4.8a,b). While seedlings grown in nutrient solution containing 250 mM sodium chloride had 4 fold higher levels of free cytosolic sodium than the untreated controls it was only two fold higher in seedlings cultured in Hoagland's nutrient solutions containing 250 mM NaCl and 10 mM $CaCl_2$.

Expressed as percent of total conductivity, seedlings of both varieties of maize growing in Hoagland's nutrient solution containing different concentrations of sodium chloride showed markedly higher electrolyte leakage than the untreated controls. The percentage of total conductivity showed a significant increase with increasing concentration of sodium chloride in the nutrient solution. Thus, compared with the untreated controls, seedlings grown in nutrient solution containing 250 mM NaCl showed more than six fold increase in electrolyte leakage. However, seedlings of both the varieties of maize cultured in Hoagland's nutrient solutions supplemented with different concentrations of NaCl and 10 mM $CaCl_2$ showed significantly lower electrolyte leakage than those in which 10 mM calcium chloride was not incorporated in the nutrient solution (Table 4.9; Fig. 4.8c).

Expressed either as percent of fresh weight or as percent of dry weight, there was no marked difference in the tissue content of soluble protein in the shoot tissues of seedlings of both varieties of maize *viz.* RCM1-1 and Gujarat Makki between the untreated controls and those cultured in nutrient solutions containing different concentrations of sodium chloride. Incorporation of 10 mM calcium chloride in the nutrient solution had no significant effect on the tissue concentration of soluble protein in the seedlings (Table 4.10; Fig. 4.9a,b).

When compared with the untreated controls, the leaf tissues of seedlings grown on Hoagland's nutrient medium containing either 100, 200 or 250 mM NaCl showed marginally higher activity of glutathione reductase. The activity of the enzyme did not show any significant increase with increase in the concentration of sodium chloride in the nutrient solution (Table 4.10; Fig. 4.9c). Incorporation of

Table 4.9: Effect of different concentrations of NaCl with or without 10mM $CaCl_2$ on the cytosolic sodium content and electrolyte leakage (EL) in shoots of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM1-1 harvested after 7days of treatment.

Treatments	Variety	Na^+ (nmol/100mg fresh weight)	Na^+ (nmol/100mg dry weight)	Electrolyte leakage
control	GM	0.0034±0.0003	0.039±0.0006	9.16±0.032
	RCM 1-1	0.0021±0.0001	0.022±0.0020	10.63±0.032
100mM NaCl	GM	0.0079±0.0003	0.068±0.0023	28.99±0.313
	RCM 1-1	0.0093±0.0002	0.081±0.0024	33.23±0.071
100mM NaCl +10mM $CaCl_2$	GM	0.0045±0.0004	0.049±0.0044	18.88±0.236
	RCM 1-1	0.0022±0.0001	0.020±0.0028	20.00±0.064
150mM NaCl	GM	0.0138±0.0002	0.121±0.0021	41.75±0.740
	RCM 1-1	0.0104±0.0004	0.091±0.0035	43.34±0.051
150mM NaCl +10mM $CaCl_2$	GM	0.0051±0.0006	0.050±0.0059	38.74±0.150
	RCM 1-1	0.0035±0.0002	0.029±0.0022	26.07±0.058
200mM NaCl	GM	0.0176±0.0008	0.132±0.0021	60.66±1.281
	RCM 1-1	0.0114±0.0003	0.095±0.0032	48.24±0.061
200mM NaCl +10mM $CaCl_2$	GM	0.0067±0.0003	0.065±0.0035	46.59±0.138
	RCM 1-1	0.0030±0.0001	0.028±0.0018	28.89±0.162
250mM NaCl	GM	0.0239±0.0004	0.153±0.0034	70.73±1.199
	RCM 1-1	0.0136±0.0003	0.110±0.0020	60.84±0.046
250mM NaCl +10mM $CaCl_2$	GM	0.0090±0.0005	0.072±0.0054	48.33±0.170
	RCM 1-1	0.0052±0.0002	0.040±0.0041	45.39±0.071

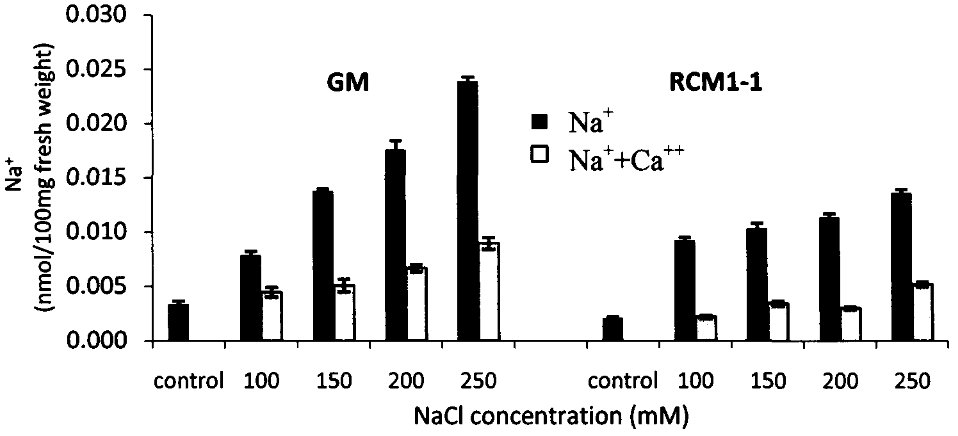
±SEM

Table 4.10 : Effect of different concentrations of NaCl with or without 10mM $CaCl_2$ on the concentration of soluble protein, activities of glutathione reductase and ascorbate peroxidase in shoot tissues of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM1-1 after 7days of treatment.

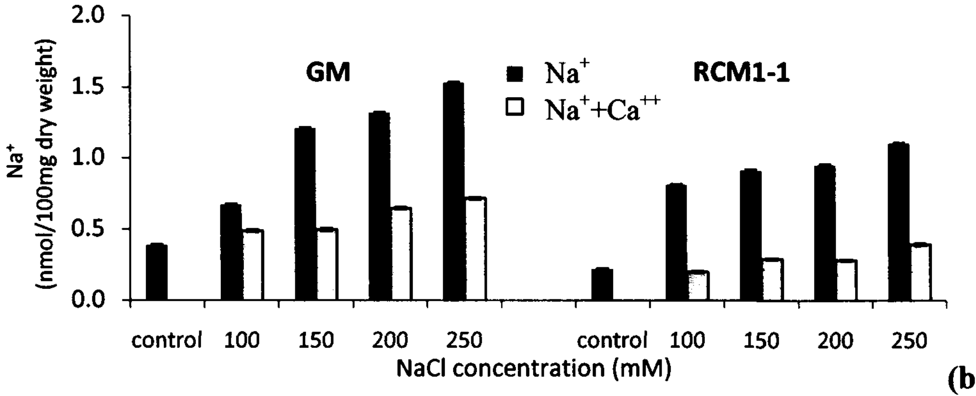
Treatments	Variety	Soluble protein (µg/mg fresh weight)	Soluble protein (µg/mg dry weight)	GR activity (µmol mg protein^{-1} min^{-1})	APX activity (mmol mg protein^{-1} min^{-1})
control	GM	1.297±0.010	14.81±0.20	2.47±0.021	0.247±0.026
	RCM 1-1	1.250±0.014	13.16±0.22	2.16±0.017	0.233±0.014
100mM NaCl	GM	1.401±0.008	11. 96±0.17	2.96±0.025	0.233±0.012
	RCM 1-1	1.345±0.013	11.78±0.12	2.97±0.012	0.214±0.019
100mM NaCl +10mM $CaCl_2$	GM	1.390±0.017	15.22±0.20	2.80±0.008	0.245±0.003
	RCM 1-1	1.333±0.006	12.19±0.21	3.93±0.020	0.250±0.006
150mM NaCl	GM	1.427±0.010	12.47±0.10	2.89±0.029	0.268±0.004
	RCM 1-1	1.780±0.021	15.66±0.15	3.61±0.020	0.321±0.017
150mM NaCl +10mM $CaCl_2$	GM	1.511±0.015	14.84±0.17	3.15±0.017	0.217±0.004
	RCM 1-1	1.801±0.009	15.23±0.12	3.85±0.024	0.339±0.003
200mM NaCl	GM	1.540±0.009	11.53±0.27	3.48±0.012	0.190±0.006
	RCM 1-1	1.814±0.014	15.16±0.20	3.66±0.041	0.568±0.017
200mM NaCl +10mM $CaCl_2$	GM	1.650±0.021	16.05±0.17	3.64±0.005	0.352±0.003
	RCM 1-1	1.877±0.010	17.73±0.15	4.09±0.035	0.750±0.001
250mM NaCl	GM	1.555±0.009	9.96±0.10	3.16±0.038	0.106±0.003
	RCM 1-1	1.830±0.016	14.85±0.17	2.77±0.015	0.393±0.009
250mM NaCl +10mM $CaCl_2$	GM	1.690±0.020	13.58±0.28	3.48±0.004	0.405±0.006
	RCM 1-1	1.921±0.018	14.59±0.21	3.77±0.032	0.535±0.019

±SEM

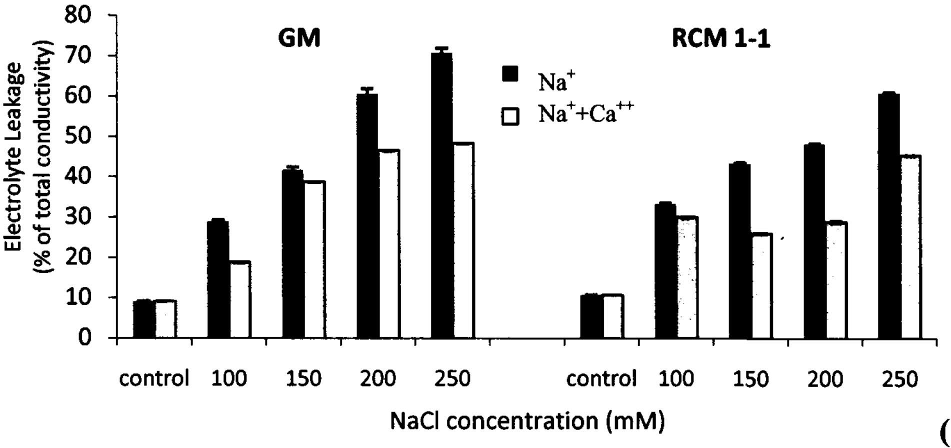
Figure 4.8 : Effect of different concentrations of NaCl with (▨) or without (■) 10mM $CaCl_2$ on the cytosolic sodium content (a,b) and electrolyte leakage (c) in shoots of seedlings of two varieties of maize (*Zea mays* L.) *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment. Vertical lines on each bar represent SEM .


0.030
0.025
0.020
0.015
0.010
0.005
0.000
Na+
(nmol/100mg fresh weight)
GM
RCM1-1
■ Na+
□ Na++Ca++
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(a)

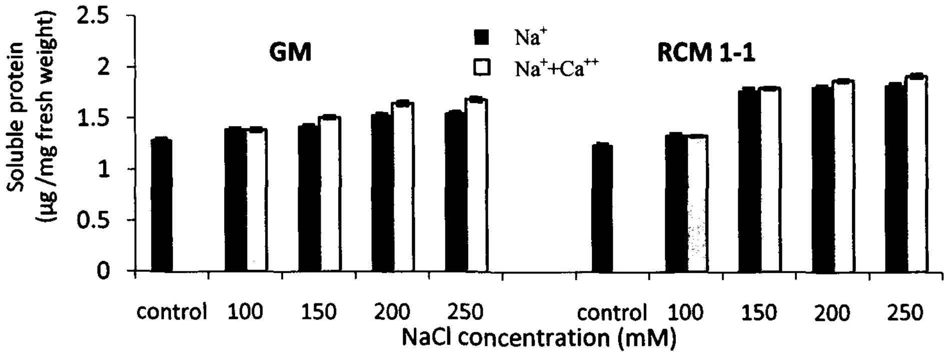

2.0
1.5
1.0
0.5
0.0
Na+
(nmol/100mg dry weight)
GM
RCM1-1
■ Na+
□ Na++Ca++
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(b)

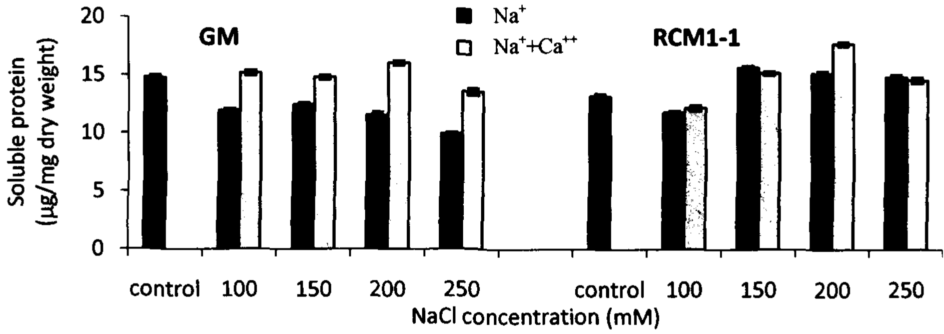

80
70
60
50
40
30
20
10
0
Electrolyte Leakage
(% of total conductivity)
GM
RCM 1-1
■ Na+
□ Na++Ca++
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(c)

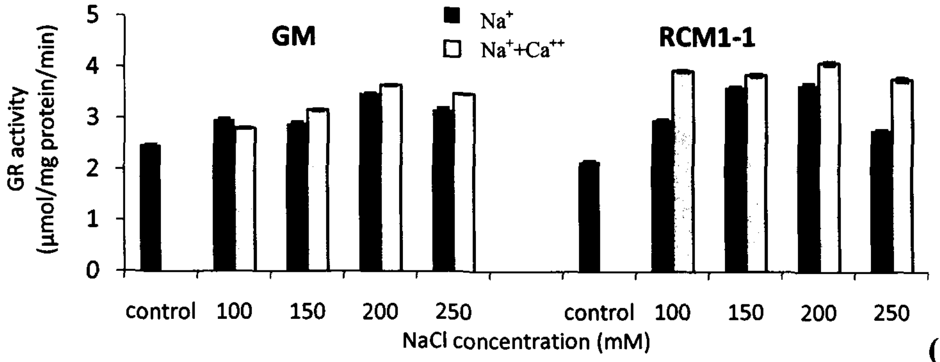
Figure 4.9: Effect of different concentrations of NaCl with (□) or without (■) 10mM $CaCl_2$ on the concentration of soluble protein (a,b), activities of glutathione reductase (GR) (c) and ascorbate peroxidase (APX) (d) in the shoots of seedlings of two varieties of maize *viz.* Gujarat Makki (GM) and RCM 1-1 harvested 7days after treatment. Vertical lines on each bar represent SEM.


Soluble protein
(µg/mg fresh weight)
2.5
2
1.5
1
0.5
0
GM
Na+
Na++Ca++
RCM 1-1
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

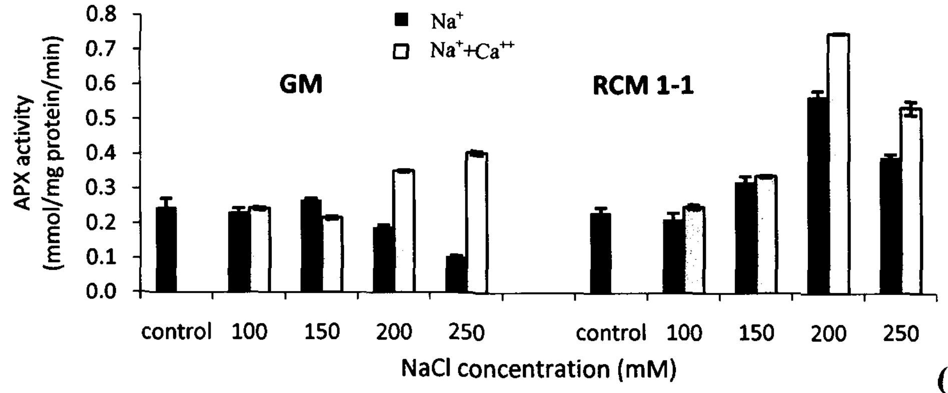
(a)


Soluble protein
(µg/mg dry weight)
20
15
10
5
0
GM
Na+
Na++Ca++
RCM1-1
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(b)


GR activity
(µmol/mg protein/min)
5
4
3
2
1
0
GM
Na+
Na++Ca++
RCM1-1
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

(c)


APX activity
(mmol/mg protein/min)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Na+
Na++Ca++
GM
RCM 1-1
control
100
150
200
250
control
100
150
200
250
NaCl concentration (mM)

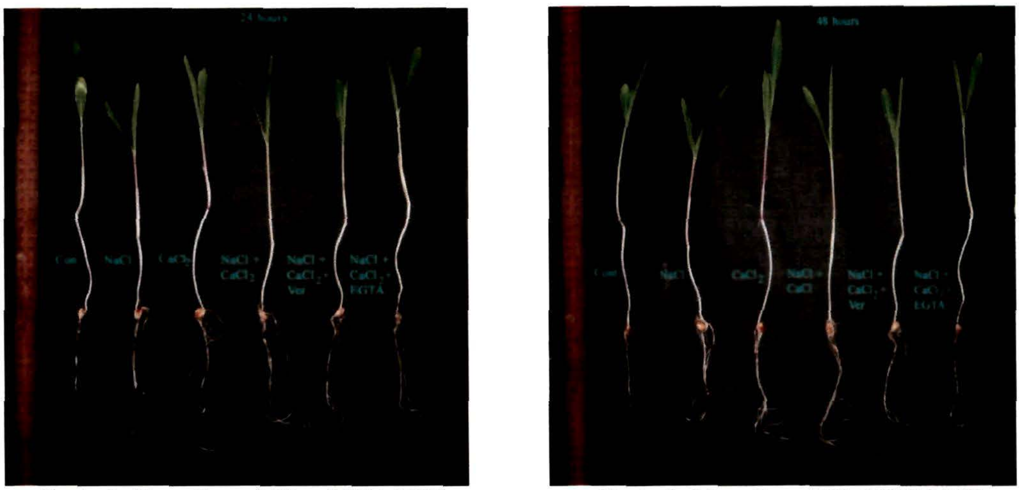
(d)

10 mM calcium chloride in the nutrient solution had no significant effect on the activity of glutathione reductase in the seedlings (Table 4.10; Fig. 4.9c).

There were no marked differences in the activity of ascorbate peroxidase in leaf tissues of seedlings of the variety Gujarat Makki with increase in the concentration of NaCl in the nutrient solution upto 150 mM. The activity of the enzyme showed marked decrease with increase in the concentration of NaCl in the nutrient solution from 150 to 250 mM. On the other hand the activity of ascorbate peroxidase in leaf tissues of the variety RCM1-1 showed a progressive increase with increase in the concentration of NaCl in the nutrient solution upto 200 mM; the activity of the enzyme showed a marginal decrease with further increase in the concentration of NaCl in the nutrient solution upto 250 mM (Table 4.10; Fig. 4.9d). Seedlings of both the varieties cultured in Hoagland's nutrient solution containing different concentrations of NaCl supplemented with 10 mM $CaCl_2$ showed significantly higher activity of ascorbate peroxidase than those which were grown in nutrient solution was supplemented with only sodium chloride (Table 4.10; Fig. 4.9d). The activity of ascorbate peroxidase in leaf tissues of seedlings cultured in nutrient solutions supplemented with different concentrations of NaCl and 10 mM $CaCl_2$ showed a progressive increase with increase in the concentration of NaCl in the nutrient solution upto 200 mM; the activity of the enzyme showed a marginal decrease with further increase in the concentration of NaCl in the nutrient solution upto 250 mM.

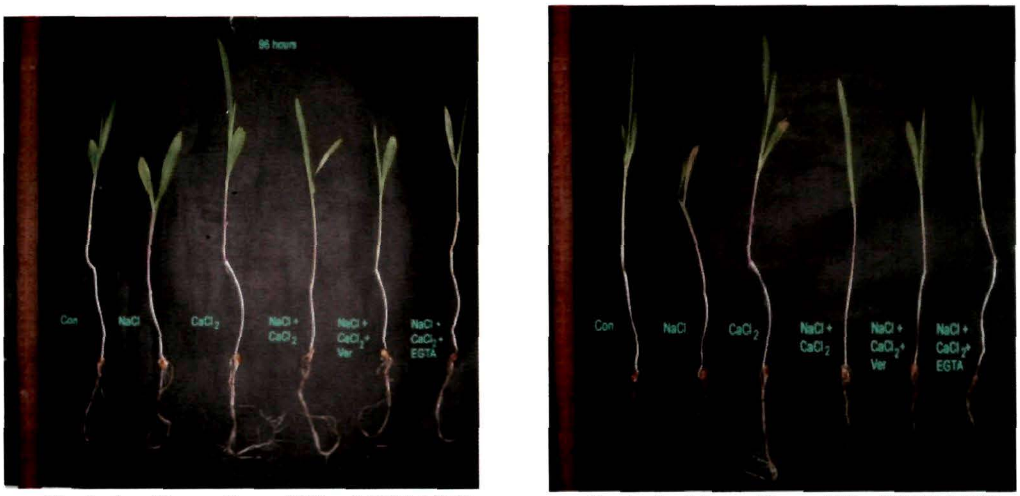
In order to study the effects of calcium ions and abscisic acid in modulating salt stress with time during the early stage of germination in seedlings of maize, seeds of maize (*Zea mays* L.) var. RCM 1-1 were germinated in a seed germinator at 25 ± 2°C under 14 h photoperiod and 75 ± 10 % R.H. Plants were watered at regular intervals and maintained as described above till the second leaf was fully expanded. A set of seedlings was rinsed with distilled water and pretreated with either 1 mM verapamil or 10 mM EGTA for 4 hours before transfer to Hoagland's nutrient solution containing 200mM NaCl with or without 10mM $CaCl_2$ and 100μM ABA. The seedlings were harvested after 0, 24, 48, 96 and 144 hours of treatment. The harvested seedlings were rinsed with water, wiped dry and used for determination of shoot length, root length, RWC, % moisture and dry weight, total chlorophyll, electrolyte leakage, H_2O_2 content, cytosolic sodium and potassium, tissue content of soluble protein, carbonyl and MDA concentration, GSH and AsA content and activities of APX,GR, SOD, POX and CAT.

Irrespective of the treatment the seedlings showed a consistent increase in the length of shoots with progressing time till 144 hours. Seedlings maintained in native Hoagland's nutrient solution or those maintained in nutrient solution supplemented with either 10 mM $CaCl_2$ or 100 μM ABA showed more than 2 fold increase in shoot length during the 144 hours of incubation (Table 4.11; Fig. 4.10). On the other hand seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride showed a marked suppression of growth. These seedlings registered only 1.2 fold increase in shoot length during the 144 hours of incubation (Table 4.11; Fig. 4.10).



Control N C NC NCV NCE
t_{24hour}

Control N C NC NCV NCE
t_{48hour}

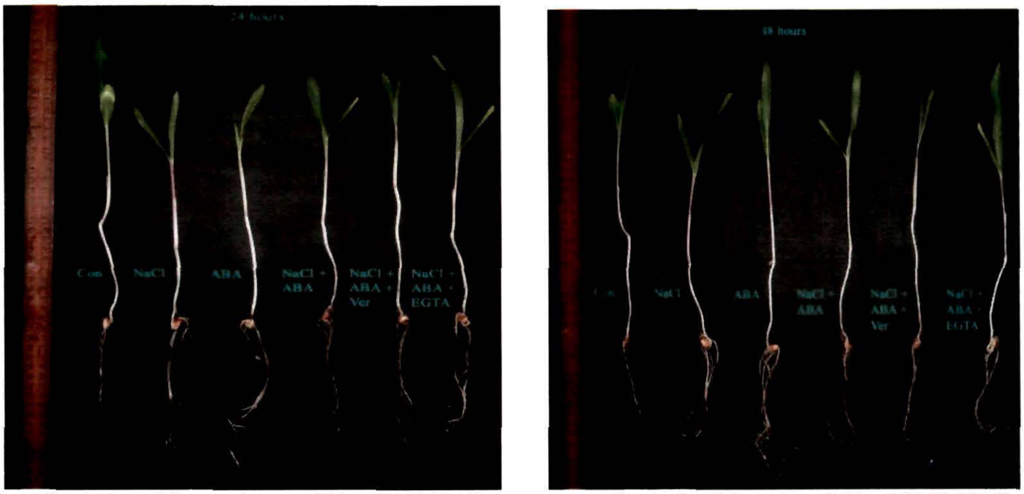


Control N C NC NCV NCE
t_{96hour}

Control N C NC NCV NCE
t144hour

Seedlings of maize (*Zea mays* L.) var. RCM 1-1 cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (N); seedlings cultured in Hoagland's nutrient solution containing 10 mM $CaCl_2$ (C); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ (NC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCE).The seedlings were harvested after 24, 48, 96 and 144 hours of treatment.

PLATE 2



Control N A NA NAV NAE Control N A NA NAV NAE

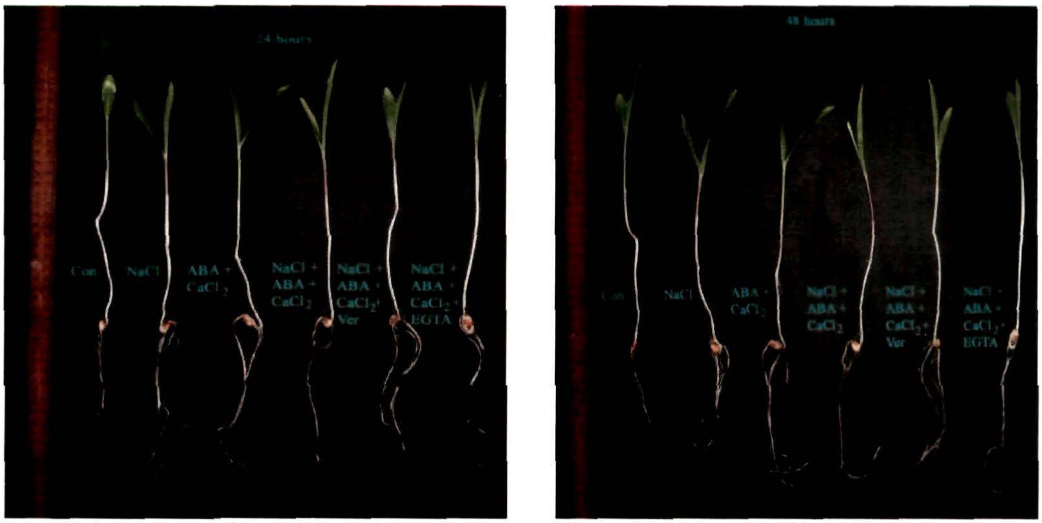
t_{24hour} t_{48hour}

Control N A NA NAV NAE Control N A NA NAV NAE

t_{96hour} $t_{144hour}$

Seedlings of maize (*Zea mays* L.) var. RCM 1-1 cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (N); seedlings cultured in Hoagland's nutrient solution containing 100 μM ABA (A); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 100 μM ABA (NA); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100 μM ABA (NAV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100 μM ABA (NAE).The seedlings were harvested after 24, 48, 96 and 144 hours of treatment.

PLATE 3

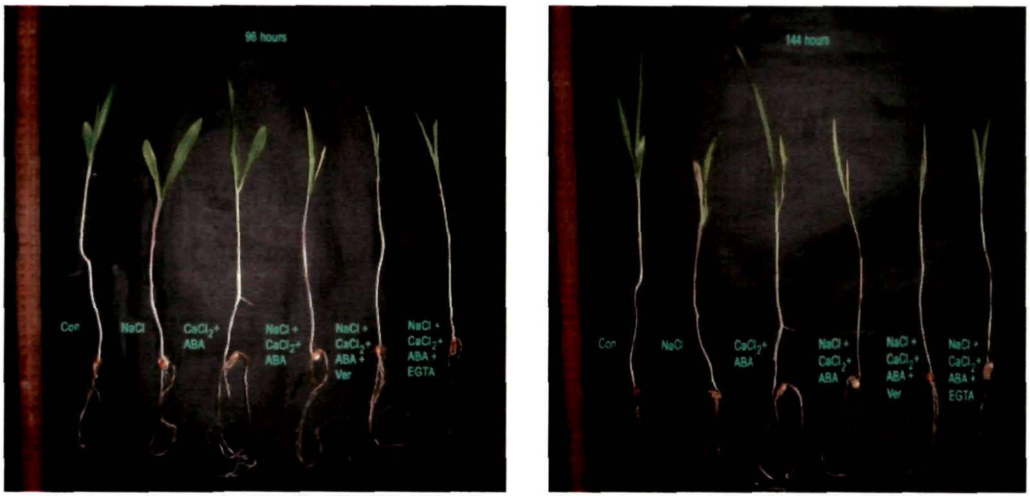


Control N AC NAC NACV NACE

t_{24hour}

Control N AC NAC NACV NACE

t_{48hour}



Control N AC NAC NACV NACE

t_{96hour}

Control N AC NAC NACV NACE

$t_{144hour}$

Seedlings of maize (*Zea mays* L.) var. RCM 1-1 cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (N); seedlings cultured in Hoagland's nutrient solution containing 100 μM ABA and 10mM $CaCl_2$ (AC); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl, 10mM $CaCl_2$ and 100 μM ABA (NAC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100 μM ABA and 10mM $CaCl_2$ (NACV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100 μM ABA and 10mM $CaCl_2$ (NACE).The seedlings were harvested after 24, 48, 96 and 144 hours of treatment.

PLATE 4

Table 4.11: Effect of 200mM NaCl (Na$^+$), 100μM ABA and 10mM CaCl$_2$ (Ca^{++}) and combinations of the above on the shoot length (cm) of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Shoot length (cm)				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	12.0±0.29	18.0±0.58	22.0±1.53	27.0±0.87	30.0±1.04
Na$^+$	-	13.0±0.89	13.0±1.32	14.0±0.87	14.5±1.02
Ca^{++}	-	17.5±0.29	21.0±1.20	25.5±0.48	28.5±0.29
Na$^+$+Ca^{++}	-	16.0±1.10	17.0±0.58	18.0±0.50	22.7±0.67
Na$^+$+Ca^{++}+Ver	-	15.0±0.29	15.5±0.29	16.0±0.58	18.0±1.26
Na$^+$+Ca^{++}+EGTA	-	15.0±0.76	15.0±0.29	15.5±0.76	17.5±0.76
ABA	-	13.0±1.04	13.0±0.76	14.0±0.76	14.5±2.02
Na$^+$+ABA	-	16.2±0.61	20.0±1.00	20.0±0.29	20.0±0.50
Na$^+$+ABA+Ver	-	14.0±1.04	14.0±1.04	16.0±0.76	16.0±0.29
Na$^+$+ABA+EGTA	-	14.0±0.76	14.5±0.50	17.0±0.76	18.0±0.58
ABA+Ca^{++}	-	17.0±1.04	21.0±1.32	28.5±0.87	29.0±0.87
Na$^+$+ABA+Ca^{++}	-	14.6±0.31	15.0±0.29	17.0±0.76	17.5±1.04
Na$^+$+ABA+Ca^{++}+Ver	-	14.0±1.15	14.5±0.29	16.5±0.87	16.5±0.58
Na$^+$+ABA+Ca^{++}+EGTA	-	15.0±0.58	15.5±0.58	17.0±0.29	17.5±0.87

± SEM

Table 4.12. Effect of 200mM NaCl (Na$^+$), 100μM ABA and 10mM CaCl$_2$ (Ca^{++}) and combinations of the above on the root length (cm) of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Root length (cm)				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	16.0±0.58	20.0±1.15	21.0±2.52	24.0±1.15	30.0±1.00
Na$^+$	-	19.0±1.61	19.5±1.04	20.0±2.06	21.0±1.65
Ca^{++}	-	21.0±2.00	21.5±1.61	23.5±0.87	28.0±1.31
Na$^+$+Ca^{++}	-	19.0±2.06	19.0±0.58	19.5±2.08	19.5±1.08
Na$^+$+Ca^{++}+Ver	-	20.0±2.08	20.0±1.00	20.0±0.58	20.0±1.08
Na$^+$+Ca^{++}+EGTA	-	20.0±1.53	19.5±1.32	20.5±0.58	20.5±1.08
ABA	-	20.0±1.08	20.5±1.76	23.5±0.87	28.5±1.75
Na$^+$+ABA	-	19.0±1.00	19.0±1.53	19.0±0.76	19.0±1.08
Na$^+$+ABA+Ver	-	17.0±1.15	17.0±0.58	19.5±1.55	20.5±1.04
Na$^+$+ABA+EGTA	-	17.5±3.12	18.5±1.61	19.0±1.51	19.0±0.58
ABA+Ca^{++}	-	19.5±1.89	20.0±2.52	20.0±2.52	20.0±1.53
Na$^+$+ABA+Ca^{++}	-	19.0±1.73	20.5±1.80	21.0±1.00	23.0±1.00
Na$^+$+ABA+Ca^{++}+Ver	-	20.0±1.00	20.0±1.52	20.5±1.01	21.0±0.58
Na$^+$+ABA+Ca^{++}+EGTA	-	19.0±1.04	20.0±1.53	20.5±1.47	22.5±1.02

± SEM

Figure 4.10: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the shoot length (cm) of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM .

Figure 4.11: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the root length (cm) of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM .

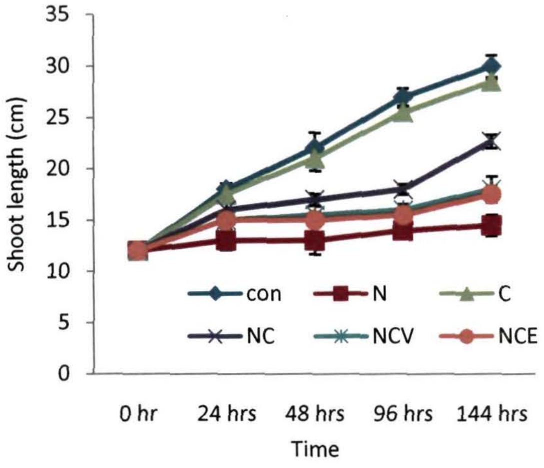


Fig. 4.10.a

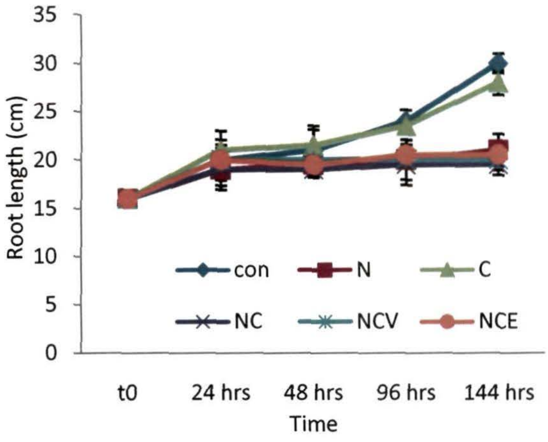


Fig. 4.11.a

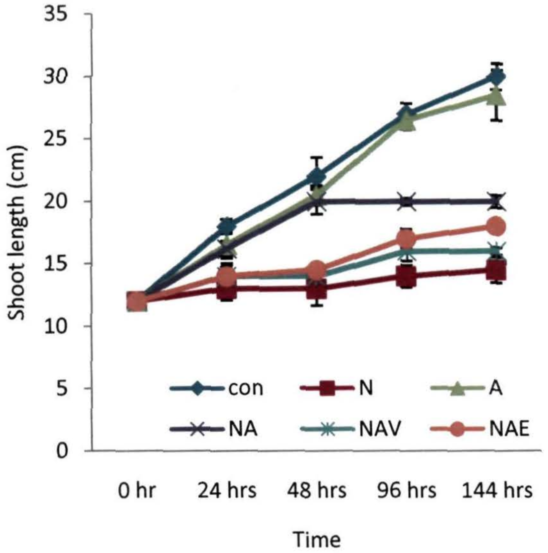


Fig. 4.10.b

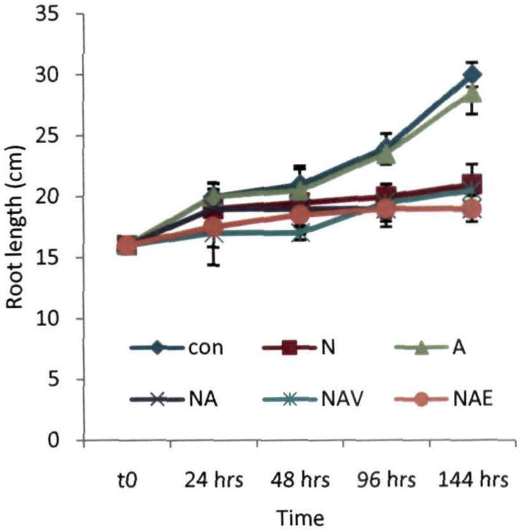


Fig. 4.11.b

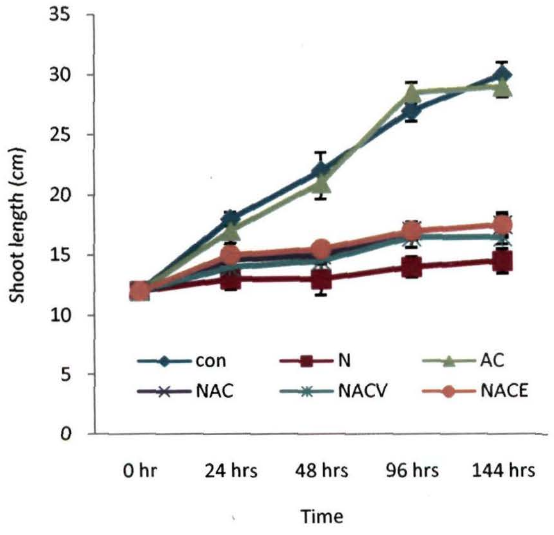


Fig. 4.10.c

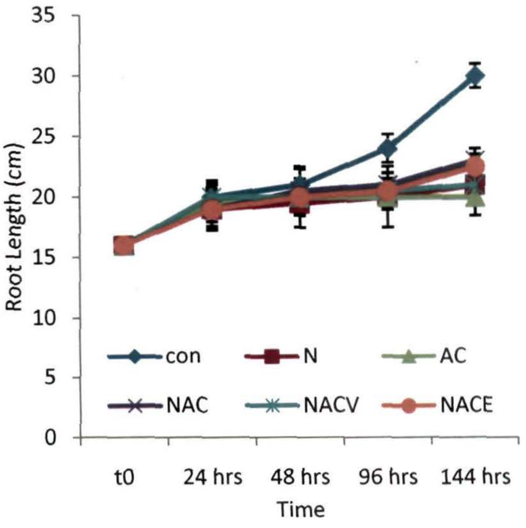


Fig. 4.11.c

Seedlings cultured in nutrient solution containing 10 mM $CaCl_2$ with 200 mM NaCl or 100 µM ABA with 200 mM NaCl registered 1.8 and 1.6 fold increase in shoot length during the 144 hours of incubation (Table 4.11; Fig. 4.10). Seedlings pretreated with 1 mM verapamil or 10 mM EGTA before their transfer to nutrient solution containing 200 mM NaCl along with either 10 mM $CaCl_2$ or 100 µM ABA, however, showed a marked suppression in shoot length. The length of shoots in these seedlings was only marginally higher than the seedlings which were incubated in nutrient solution containing only 200 mM NaCl (Table 4.11; Fig. 4.10). Similarly seedlings maintained in native Hoagland's nutrient solution or in nutrient solution supplemented with either 10 mM $CaCl_2$ /100 µM ABA showed nearly 2 fold increase in length of the primary roots during the 144 hours of incubation. On the other hand seedlings incubated in nutrient solution containing 200 mM sodium chloride showed a marked suppression of growth. These seedlings registered only 1.3 fold increase in length of the primary roots during the 144 hours of incubation (Table 4.12; Fig. 4.11). Seedlings cultured in nutrient solution containing 10 mM $CaCl_2$ with 200 mM NaCl or 100 µM ABA with 200 mM NaCl did not show any marked increase in root length during the 144 hours of incubation. These seedlings registered only 1.2 fold increase in shoot length during the 144 hours of incubation (Table 4.12; Fig. 4.11). Seedlings pretreated with 1 mM verapamil or 10 mM EGTA before their transfer to nutrient solution containing 200 mM NaCl along with either 10 mM $CaCl_2$ or 100 µM ABA also showed a marked suppression in root growth during the 144 hour incubation period (Table 4.12; Fig. 4.11).

There was no marked change in the moisture content of seedlings cultured in native Hoagland's nutrient solution during the 144 hours of incubation. However, seedlings incubated in nutrient solution supplemented with 200 mM NaCl showed a marked decrease in moisture content with progressing time. Seedlings incubated in nutrient solution supplemented with either 100 μM ABA or 10mM $CaCl_2$ also showed a decrease in moisture content with progressing time (Table 4.13; Fig. 4.12). While the seedlings cultured in nutrient solution supplemented with 200 mM NaCl and either 10 mM $CaCl_2$ or 100 μM ABA also showed a decrease in moisture content with time, the magnitude of decrease was marginally lower than that observed in seedlings which were incubated in nutrient solution containing only 200 mM NaCl (Table 4.13; Fig. 4.12). The change in % moisture content in seedlings pretreated with either 1 mM verapamil or 10 mM EGTA followed the same pattern as that observed in seedlings cultured in nutrient solution containing 200 mM NaCl (Table 4.13; Fig. 4.12).

There was no marked change in the % dry matter content of seedlings cultured in native Hoagland's nutrient solution during the 144hours of incubation. Seedlings cultured in Hoagland's nutrient solution supplemented with 100 μM ABA, however, showed marginally higher % dry matter than the untreated controls. On the other hand, seedlings incubated in nutrient solution supplemented with 200 mM NaCl showed a marked increase in % dry matter with progressing time. Thus, at 144 hours of incubation, seedlings cultured in nutrient solution supplemented with 200 mM NaCl showed 1.6 fold higher % dry matter than the untreated controls (Table 4.14; Fig. 4.13). Similar pattern of change in % dry matter content was recorded for

Table 4.13. Effect of 200mM NaCl (Na^{+}), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the % moisture content in shoot tissues of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	% moisture content				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	91.89±0.96	91.87±0.97	92.09±1.06	91.50±0.80	91.69±0.83
Na^{+}	-	91.00±0.87	90.81±0.45	89.67±0.85	86.45±0.44
Ca^{++}	-	91.58±0.80	91.55±0.49	91.45±0.51	89.66±0.82
Na^{+}+Ca^{++}	-	91.68±0.64	91.60±0.50	91.35±0.23	88.67±0.55
Na^{+}+Ca^{++}+Ver	-	90.85±0.88	87.95±0.56	87.35±0.46	87.05±1.03
Na^{+}+Ca^{++}+EGTA	-	91.05±1.04	91.21±1.16	90.15±1.12	88.05±0.85
ABA	-	91.55±0.77	91.46±0.54	90.28±1.36	89.67±0.74
Na^{+}+ABA	-	90.00±1.05	90.43±0.63	90.79±0.95	88.73±0.61
Na^{+}+ABA+Ver	-	90.51±0.79	90.27±1.38	90.28±1.39	89.08±0.80
Na^{+}+ABA+EGTA	-	90.44±0.39	89.70±0.63	89.77±0.87	87.84±0.97
ABA+Ca^{++}	-	90.85±0.92	91.77±0.94	88.30±0.26	87.60±0.65
Na^{+}+ABA+Ca^{++}	-	90.88±0.95	91.00±1.10	88.16±1.01	88.44±0.58
Na^{+}+ABA+Ca^{++}+Ver	-	90.12±0.99	90.85±0.86	88.06±0.94	88.18±1.24
Na^{+}+ABA+Ca^{++}+EGTA	-	91.02±1.02	91.58±0.52	88.56±0.68	89.05±1.00

± SEM

Table 4.14. Effect of 200mM NaCl (Na^{+}), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the % dry weight content in shoot tissues of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	% dry weight				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	8.11±0.51	8.13±0.97	7.91±1.06	8.50±0.80	8.31±0.83
Na^{+}	-	9.00±0.87	9.19±0.45	10.33±0.85	13.55±0.44
Ca^{++}	-	8.42±0.80	8.45±0.49	8.55±0.51	10.34±0.82
Na^{+}+Ca^{++}	-	8.32±0.64	8.40±0.50	8.65±0.23	11.33±0.55
Na^{+}+Ca^{++}+Ver	-	9.15±0.88	12.05±0.56	12.65±0.46	12.95±1.03
Na^{+}+Ca^{++}+EGTA	-	8.95±1.04	8.79±1.16	9.85±1.12	11.95±0.85
ABA	-	8.45±0.77	8.54±0.54	9.72±1.36	10.33±0.74
Na^{+}+ABA	-	10.00±1.05	9.57±0.63	9.21±0.95	11.27±0.61
Na^{+}+ABA+Ver	-	9.49±0.79	9.73±1.38	9.72±1.39	10.92±0.80
Na^{+}+ABA+EGTA	-	9.56±0.39	10.30±0.63	10.23±0.87	12.16±0.97
ABA+Ca^{++}	-	9.15±0.92	8.23±0.94	11.70±0.26	12.40±0.65
Na^{+}+ABA+Ca^{++}	-	9.12±0.95	9.00±1.10	11.84±1.01	11.56±0.58
Na^{+}+ABA+Ca^{++}+Ver	-	9.88±0.99	9.15±0.86	11.94±0.94	11.82±1.24
Na^{+}+ABA+Ca^{++}+EGTA	-	8.98±1.02	8.42±0.52	11.44±0.68	10.95±1.00

± SEM

Figure 4.12. Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the % moisture in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM

Figure 4.13. Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the % dry weight in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM

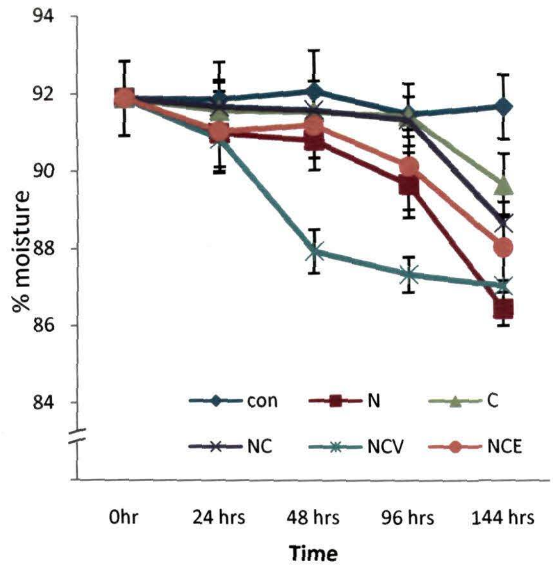


Fig. 4.12.a

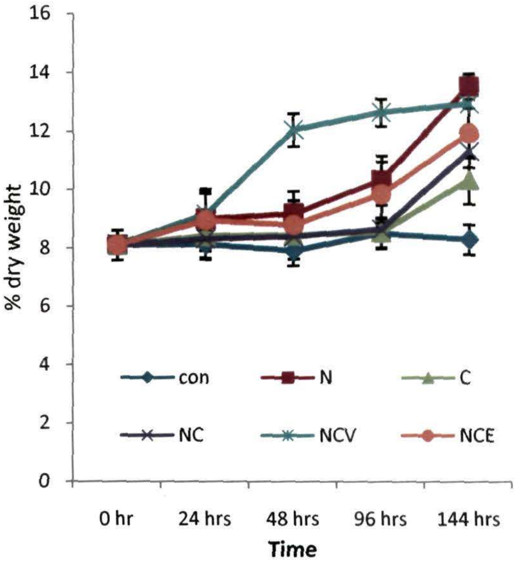


Fig. 4.13.a

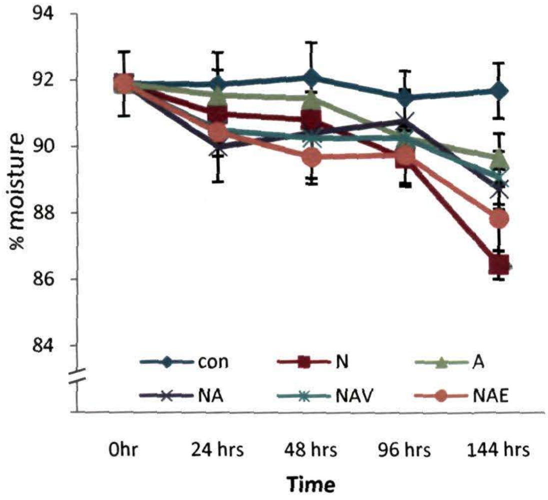


Fig. 4.12.b

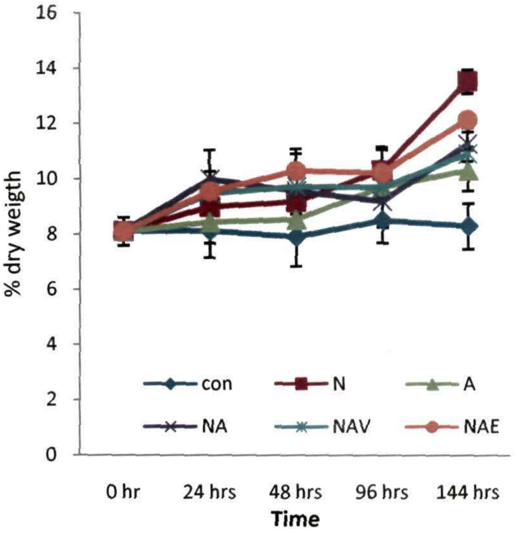


Fig. 4.13.b

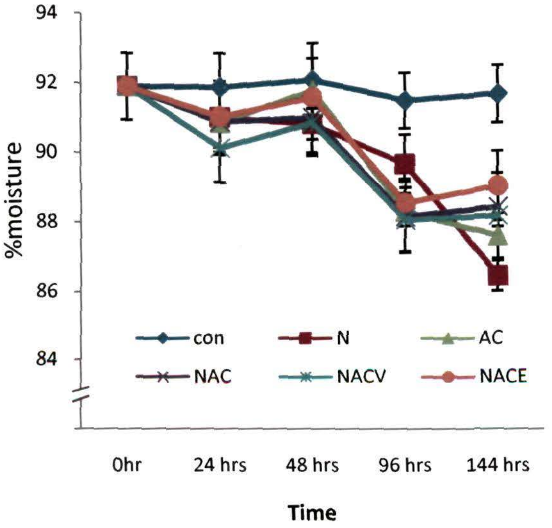


Fig. 4.12.c

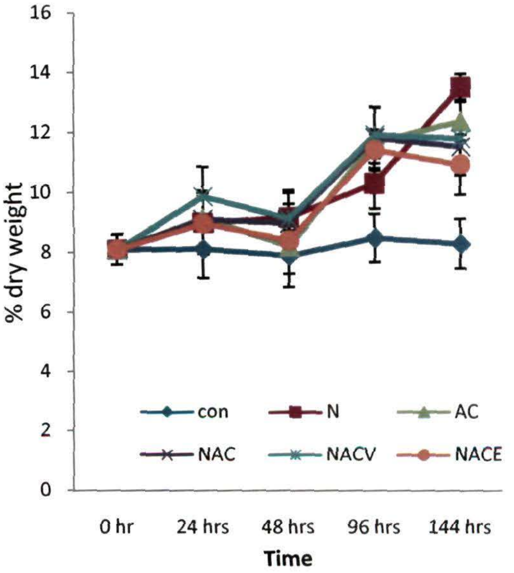


Fig. 4.13.c

seedlings which were pretreated with either 1 mM verapamil or 10 mM EGTA and then incubated in Hoagland nutrient solution supplemented with 200 mM NaCl with either 10 mM $CaCl_2$ or 100 µM ABA or both together. However, seedlings receiving NaCl with $CaCl_2$ or ABA showed only 1.3 fold increase in % dry matter during the same period (Table 4.14; Fig. 4.13).

Compared to the untreated controls, seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride showed a marked decrease in RWC with progressing time, registering a >2 fold decrease in RWC during 144 hours of incubation (Table 4.15; Fig.4.14). However, seedlings cultured in nutrient solution supplemented with 10 mM $CaCl_2$ did not show any marked change in RWC during the 144 hours of incubation. On the other hand, seedlings which were pretreated with 1 mM verapamil and then incubated in Hoagland nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ registered the same pattern and magnitude of change in RWC with time as those which were incubated in nutrient solution supplemented with 200 mM sodium chloride. These seedlings also showed a 2 fold decrease in RWC during 144 hours of incubation (Table 4.15; Fig. 4.14).

Expressed as percent of total conductivity, the electrolyte leakage in shoot tissues in seedlings cultured on Hoagland's nutrient solution lacking any additional supplements showed a marginal increase during the 144 hours of incubation. However, seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride showed a marked increase in electrolyte leakage during the 144 hour incubation period. These seedlings registered a >4 fold increase in electrolyte leakage during the 144 hours of incubation (Table 4.16 ; Fig. 4.15). The increase in electrolyte leakage was, however, more prominent during the initial 24 hours of incubation. While the seedlings cultured in nutrient solution supplemented with either 10 mM $CaCl_2$ or 100 μM ABA registered a 2 fold increase of electrolyte leakage in the shoot tissue during the 144 hours of incubation, those cultured in nutrient solution supplemented with 10 mM $CaCl_2$ and 100 μM ABA showed nearly the same pattern and magnitude of change in electrolyte leakage as the seedlings cultured in nutrient solution lacking any additional supplements (Table 4.16; Fig. 4.15).Seedlings cultured in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed nearly the same pattern of change in electrolyte leakage as those cultured in nutrient solution supplemented with 200 mM NaCl. The magnitude of increase in electrolyte leakage in these seedlings was, however, much less than the seedlings cultured in nutrient solution supplemented with 200 mM NaCl (Table 4.16 ; Fig. 4.15). Seedlings pretreated with 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with either 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed a markedly higher electrolyte leakage than the controls. These seedlings registered the same magnitude in electrolyte leakage as those cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (Table 4.16; Fig. 4.15).

Expressed as percent of fresh weight the content of total chlorophyll in the shoot tissues of untreated seedlings showed a marginal decrease during the initial 24 hours of incubation. After 24 hours the content of total chlorophyll in these seedlings showed a consistent increase with progressing time till 144 hours of incubation. A

Table 4.15. Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the Relative water content (%) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Relative water content (%)				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	92.60±0.73	87.23±0.72	83.63±0.42	79.92±1.02	84.77±0.42
Na^+	-	84.07±0.75	78.99±0.62	74.43±0.72	52.25±0.64
Ca^{++}	-	86.59±0.70	82.51±0.36	77.58±0.36	83.61±0.57
Na^++Ca^{++}	-	89.81±0.46	85.93±0.89	83.35±0.73	71.91±0.83
Na^++Ca^{++}+Ver	-	79.25±0.64	88.25±0.87	65.25±0.72	60.15±0.92
Na^++Ca^{++}+EGTA	-	85.62±0.37	89.58±0.40	82.56±0.38	70.59±0.31
ABA	-	85.63±0.62	80.54±0.34	77.87±0.48	64.51±0.33
Na^++ABA	-	88.12±0.62	87.90±0.79	79.03±0.71	67.43±0.76
Na^++ABA+Ver	-	80.57±0.32	83.64±0.36	72.66±0.38	80.84±0.44
Na^++ABA+EGTA	-	80.20±0.62	73.92±0.91	75.87±0.48	75.29±0.65
ABA+Ca^{++}	-	90.30±0.70	90.02±0.85	66.04±0.86	59.44±0.77
Na^++ABA+Ca^{++}	-	85.44±0.72	84.25±0.84	68.92±1.03	67.77±0.46
Na^++ABA+Ca^{++}+Ver	-	83.86±0.46	82.98±0.85	66.85±0.47	66.85±0.44
Na^++ABA+Ca^{++}+EGTA	-	87.85±0.47	86.95±0.89	70.56±0.37	68.65±0.32

± SEM

Table 4.16: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the electrolyte leakage in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Electrolyte leakage				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	8.84±0.31	14.22±0.43	14.58±0.70	15.39±1.45	15.36±0.91
Na^+	-	30.44±1.60	37.89±1.71	40.13±1.72	42.43±1.70
Ca^{++}	-	19.21±0.45	20.23±0.59	25.58±0.95	28.71±0.97
Na^++Ca^{++}	-	22.06±0.45	25.49±1.92	27.38±0.85	28.94±0.98
Na^++Ca^{++}+Ver	-	27.25±0.95	30.54±1.78	32.69±1.40	36.87±2.04
Na^++Ca^{++}+EGTA	-	24.51±0.65	26.58±1.45	30.54±0.86	31.85±1.10
ABA	-	20.12±0.58	21.85±0.56	26.25±1.03	30.12±0.75
Na^++ABA	-	28.00±1.81	32.41±0.30	34.43±1.12	31.73±0.85
Na^++ABA+Ver	-	26.62±2.03	26.69±1.18	32.65±0.83	30.96±1.56
Na^++ABA+EGTA	-	27.36±0.23	30.65±0.48	38.80±1.01	40.98±2.95
ABA+Ca^{++}	-	17.53±0.80	19.62±0.80	22.22±1.20	20.17±0.35
Na^++ABA+Ca^{++}	-	28.68±0.85	30.87±0.70	35.01±1.83	36.42±0.41
Na^++ABA+Ca^{++}+Ver	-	27.51±0.80	32.15±0.81	35.89±1.37	36.25±0.65
Na^++ABA+Ca^{++}+EGTA	-	29.54±1.33	36.58±1.80	41.25±0.63	43.26±0.60

± SEM

Figure 4.14. Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the relative water content (RWC) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.15: Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the electrolyte leakage (EL) (% of total conductivity) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

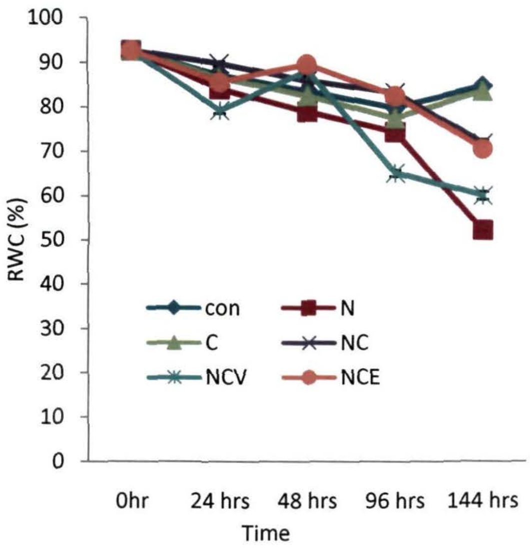


Fig. 4.14.a

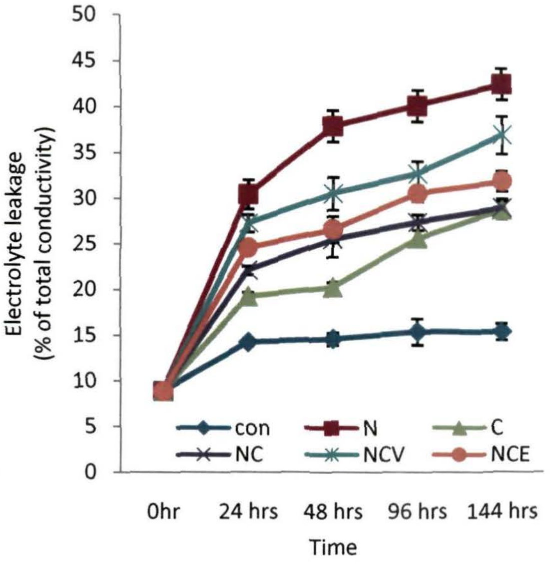


Fig. 4.15.a

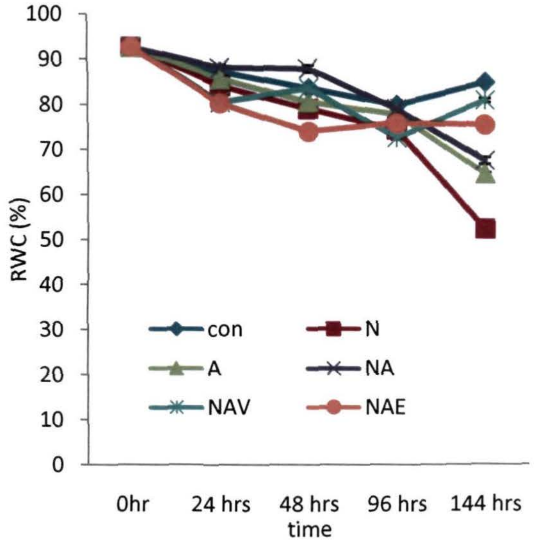


Fig. 4.14.b

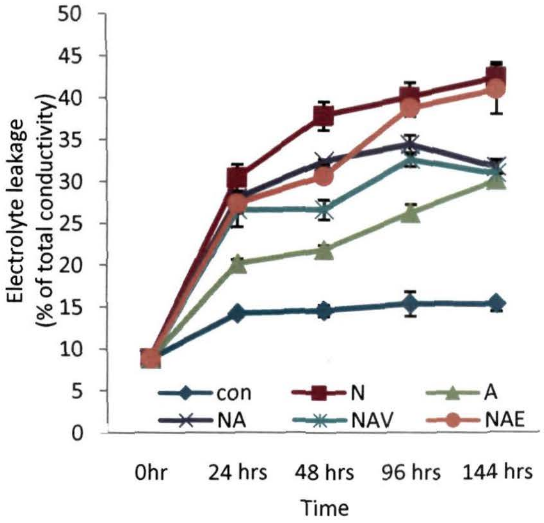


Fig. 4.15.b

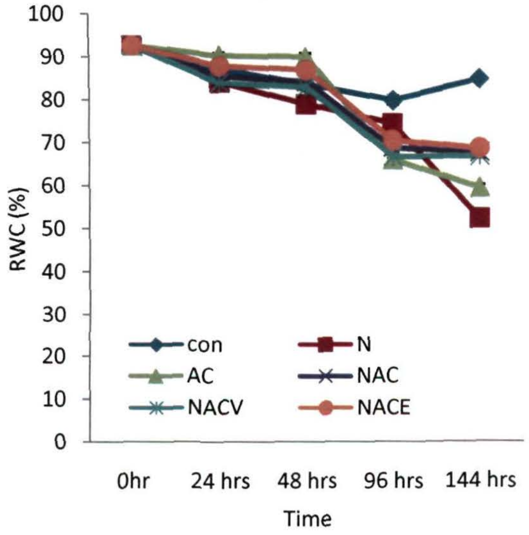


Fig. 4.14.c

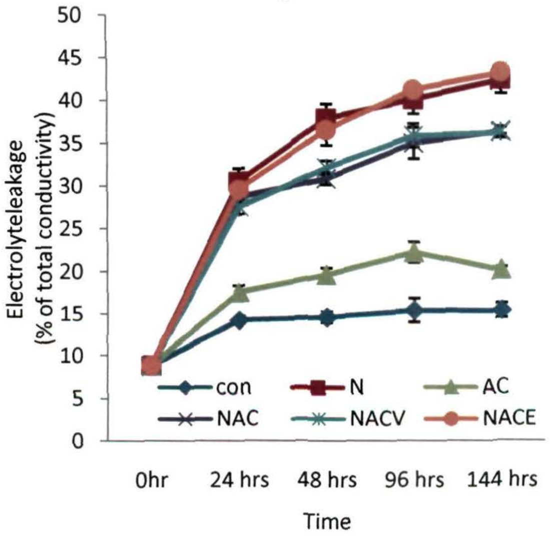


Fig. 4.15.c

similar pattern and magnitude of change in the content of total chlorophyll was recorded for seedlings incubated in nutrient solution supplemented with either 10mM $CaCl_2$ or 100 µM ABA. On the other hand seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride showed a significant decrease in the tissue content of total chlorophyll during the 144 hour incubation period (Table 4.17; Fig. 4.16). Seedlings incubated in the nutrient solution supplemented with 200 mM NaCl and either 10 mM $CaCl_2$ or 100 µM ABA also showed marked decrease in tissue total chlorophyll content during the same period. The magnitude of decrease was, however, less than that in seedlings cultured in nutrient solution supplemented with only 200 mM NaCl. (Table 4.18; Fig.4.17). When expressed as percent of dry weight, there was marginal increase in the total chlorophyll content in the shoot tissues of seedlings incubated in Hoagland's nutrient lacking any additional supplements during the 144 hours of incubation. While the seedlings incubated in nutrient solution supplemented with 10 mM CaCl2 showed the same pattern and magnitude of change in the chlorophyll content as the untreated controls those cultured in nutrient solution supplemented with 100 µM ABA did not registered any significant change in total chlorophyll content in shoot tissues during the 144 hours of incubation. However, seedlings incubated in nutrient solution containing 200 mM sodium chloride showed marked decrease in content of total chlorophyll in the shoot tissues. These seedlings registered almost 2 fold decrease in the total chlorophyll content of leaf tissues during the 144 hours of incubation (Table 4.18; Fig. 4.17). Seedlings incubated in the nutrient solution supplemented with 200 mM NaCl and either 10 mM $CaCl_2$ or 100 µM ABA also showed marked decrease in tissue total

Table 4.17: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the total chlorophyll (mg 100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Total chlorophyll (mg 100mg fresh weight^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	0.0879±0.003	0.0847±0.002	0.0876±0.002	0.0943±0.002	0.0942±0.002
Na^+	-	0.0768±0.002	0.0758±0.003	0.0717±0.002	0.0676±0.002
Ca^{++}	-	0.0844±0.003	0.0865±0.001	0.0951±0.002	0.0950±0.003
Na^++Ca^{++}	-	0.0796±0.003	0.0765±0.003	0.0755±0.004	0.0826±0.001
Na^++Ca^{++}+Ver	-	0.0821±0.002	0.0785±0.005	0.0795±0.003	0.0835±0.003
Na^++Ca^{++}+EGTA	-	0.0825±0.005	0.0805±0.005	0.0820±0.001	0.0825±0.005
ABA	-	0.0840±0.002	0.0866±0.002	0.0921±0.003	0.0935±0.003
Na^++ABA	-	0.0785±0.002	0.0750±0.002	0.0745±0.005	0.0735±0.002
Na^++ABA+Ver	-	0.0825±0.002	0.0815±0.003	0.0820±0.002	0.0830±0.003
Na^++ABA+EGTA	-	0.0825±0.002	0.0820±0.007	0.0815±0.001	0.0830±0.001
ABA+Ca^{++}	-	0.0835±0.001	0.0855±0.001	0.0915±0.002	0.0920±0.002
Na^++ABA+Ca^{++}	-	0.0780±0.002	0.0765±0.006	0.0750±0.003	0.0745±0.003
Na^++ABA+Ca^{++}+Ver	-	0.0830±0.003	0.0825±0.003	0.0825±0.005	0.0825±0.004
Na^++ABA+Ca^{++}+EGTA	-	0.0815±0.002	0.0815±0.004	0.0820±0.003	0.0825±0.002

± SEM

Table 4.18: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the content of total chlorophyll (mg 100mg dry weight^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Total chlorophyll (mg 100mg dry weight^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	0.096±0.0043	0.093±0.0080	0.111±0.0080	0.111±0.0089	0.113±0.0064
Na^+	-	0.085±0.0049	0.083±0.0049	0.069±0.0038	0.050±0.0069
Ca^{++}	-	0.100±0.0038	0.102±0.0089	0.111±0.0094	0.092±0.0024
Na^++Ca^{++}	-	0.096±0.0094	0.091±0.0058	0.087±0.0039	0.067±0.0063
Na^++Ca^{++}+Ver	-	0.090±0.0080	0.065±0.0089	0.063±0.0086	0.064±0.0079
Na^++Ca^{++}+EGTA	-	0.092±0.0044	0.092±0.0080	0.083±0.0054	0.069±0.0046
ABA	-	0.099±0.0094	0.101±0.0040	0.095±0.0069	0.091±0.0026
Na^++ABA	-	0.079±0.0005	0.078±0.0086	0.081±0.0037	0.065±0.0053
Na^++ABA+Ver	-	0.087±0.0032	0.084±0.0099	0.084±0.0028	0.076±0.0088
Na^++ABA+EGTA	-	0.086±0.0094	0.080±0.0099	0.080±0.0068	0.068±0.0057
ABA+Ca^{++}	-	0.091±0.0032	0.104±0.0050	0.072±0.0099	0.069±0.0012
Na^++ABA+Ca^{++}	-	0.086±0.0038	0.085±0.0040	0.063±0.0038	0.064±0.0054
Na^++ABA+Ca^{++}+Ver	-	0.084±0.0086	0.090±0.0020	0.069±0.0054	0.070±0.0088
Na^++ABA+Ca^{++}+EGTA	-	0.091±0.0038	0.097±0.0094	0.072±0.0020	0.075±0.0038

± SEM

Figure 4.16. Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the content of total chlorophyll (mg $100mg^{-1}$ fresh weight) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.17. Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the content of total chlorophyll (mg $100mg^{-1}$ dry weight) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

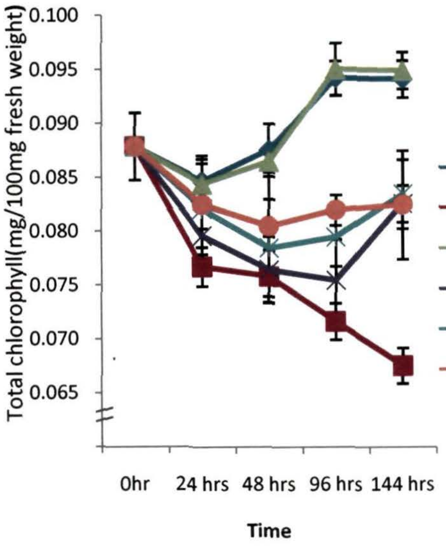


Fig. 4.16.a

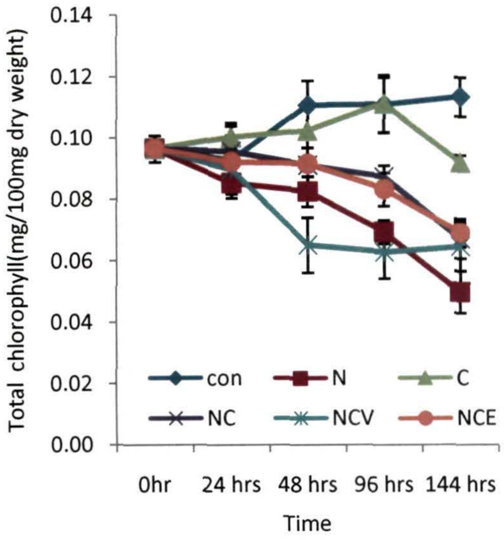


Fig. 4.17.a

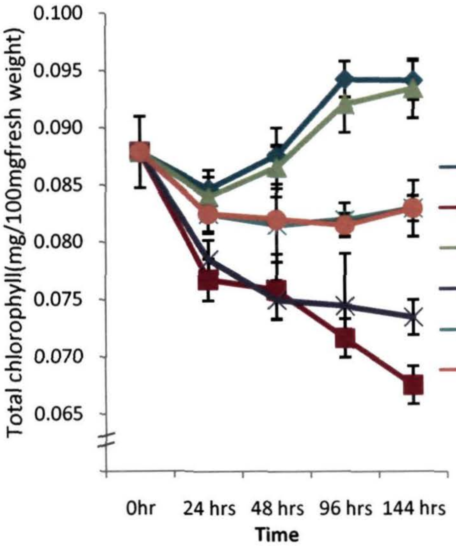


Fig. 4.16.b

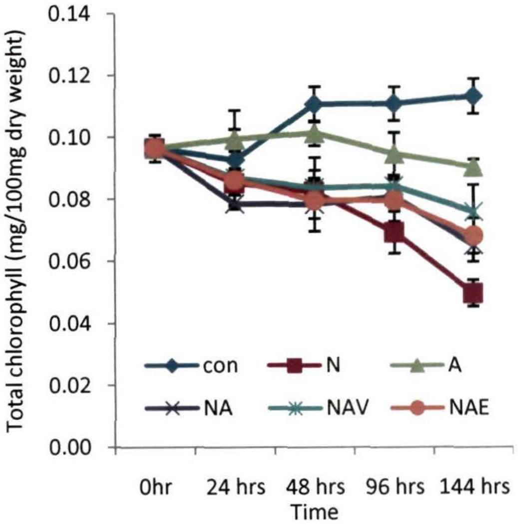


Fig. 4.16.b

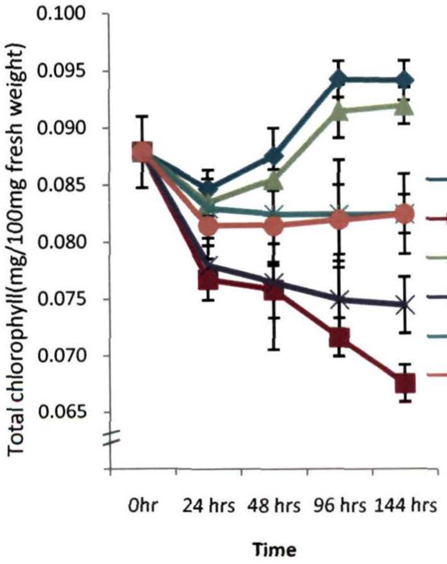


Fig. 4.16.c

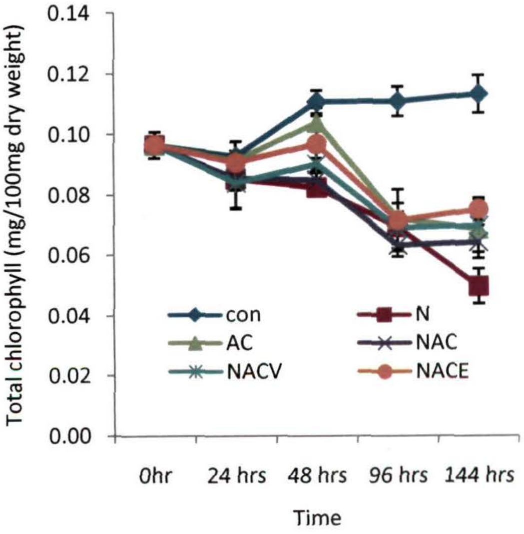


Fig. 4.17.c

chlorophyll content during the same period. The magnitude of decrease was, however, less than that in seedlings cultured in nutrient solution supplemented with only 200 mM NaCl (Table 4.18; Fig. 4.17).

Seedlings incubated in Hoagland's nutrient solution lacking any additional supplements did not show any significant change in tissue level of free cytosolic Na^+ during the 144 hours of incubation (Table 4.19,20; Fig. 4.18,19). Similarly seedlings incubated in Hoagland's nutrient solution supplemented with either 10 mM $CaCl_2$ or 100 µM ABA did not show any significant change in tissue level of free cytosolic Na^+ during the 144 hours of incubation. The change in tissue level of free cytosolic Na^+ in seedlings maintained over Hoagland's nutrient solution containing either 10 mM $CaCl_2$ or 100 µM ABA followed the same pattern as that observed in seedlings cultured in nutrient solution lacking any additional supplements. However, seedlings incubated in nutrient solution supplemented with 200 mM NaCl showed a marked increase in cytosolic Na^+ levels during the 144 hours of incubation. These seedlings registered more than 4 fold increase in cytosolic Na^+ content of the shoot tissues during the 144 hours of incubation (Table 4.19,20; Fig. 4.18,19). The increase in Na^+ levels showed a linear relationship with progressing time during the 144 hours of incubation. Incorporation of calcium chloride in the nutrient solution containing 200 mM NaCl significantly inhibited the uptake of sodium by the seedlings. Thus, seedlings incubated in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ showed less than two fold increase in tissue level of free cytosolic Na^+ during the 144 hour incubation period compared to the >4 fold increase observed in seedlings cultured in nutrient solution supplemented with 200 mM NaCl (Table

4.19,20; Fig. 4.18,19). On the other hand, seedlings incubated in nutrient solution supplemented with 200 mM NaCl and 100 µM ABA showed a threefold increase in the level of cytosolic Na^+ during the same period. However, seedlings pretreated with 1 mM verapamil and then incubated in nutrient solution containing 200 mM NaCl, and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed a significantly higher level of tissue Na^+ than the corresponding controls which were not pretreated with verapamil. These seedlings registered nearly six fold increase in the level of cytosolic Na^+ during the 144 hours of incubation (Table 4.19, 20; Fig. 4.18, 19). Seedlings pretreated with EGTA and then incubated in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed the same pattern and magnitude of changes in cytosolic Na^+ as those which were not subjected to any pretreatment (Table 4.19,20; Fig. 4.18,19).

Seedlings incubated in Hoagland's nutrient solution lacking any additional supplements did not show any significant change in tissue level of free cytosolic K^+ during the 144 hours of incubation. Similarly seedlings maintained over Hoagland's nutrient solution containing either 10 mM $CaCl_2$ or 100 µM ABA also did not show any significant change in tissue level of free cytosolic K^+ during the 144 hours of incubation. On the other hand seedlings incubated in nutrient solution supplemented with 200 mM NaCl showed a marked decrease in the cytosolic K^+ levels during the 144 hours incubation period. After 144 hours of incubation, seedlings growing in nutrient solution containing 200 mM NaCl had 40% lower levels of cytosolic K^+ than the corresponding untreated controls (Table 4.21,22; Fig. 4.20,21). Pretreatment of

Table 4.19. Effect of 200mM NaCl (Na^+), 100µM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the level of cytosolic sodium (nmol100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Cytosolic Na^+ (nmol 100mg fresh weight^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	0.0039±0.00013	0.0024±0.00008	0.0024±0.00008	0.0032±0.00011	0.0041±0.00014
Na^+		0.0063±0.00022	0.0132±0.00045	0.0182±0.00062	0.0282±0.00097
Ca^{++}		0.0030±0.00010	0.0026±0.00009	0.0030±0.00010	0.0042±0.00015
Na^++Ca^{++}		0.0055±0.00019	0.0055±0.00019	0.0073±0.00025	0.0102±0.00035
Na^++Ca^{++}+Ver		0.0096±0.00033	0.0188±0.00064	0.0278±0.00095	0.0372±0.00127
Na^++Ca^{++}+EGTA		0.0085±0.00029	0.0160±0.00055	0.0185±0.00064	0.0269±0.00092
ABA		0.0035±0.00012	0.0039±0.00013	0.0051±0.00017	0.0063±0.00022
Na^++ABA		0.0065±0.00022	0.0102±0.00035	0.0118±0.00041	0.0145±0.00050
Na^++ABA+Ver		0.0090±0.00031	0.0161±0.00055	0.0192±0.00066	0.0263±0.00090
Na^++ABA+EGTA		0.0094±0.00032	0.0194±0.00066	0.0203±0.00070	0.0269±0.00092
ABA+Ca^{++}		0.0044±0.00015	0.0035±0.00012	0.0042±0.00014	0.0041±0.00014
Na^++ABA+Ca^{++}		0.0095±0.00033	0.0092±0.00031	0.0140±0.00048	0.0161±0.00055
Na^++ABA+Ca^{++} +Ver		0.0114±0.00039	0.0184±0.00063	0.0274±0.00094	0.0290±0.00099
Na^++ABA+Ca^{++} +EGTA		0.0085±0.00029	0.0145±0.00050	0.0247±0.00085	0.0234±0.00080

± SEM

Table 4.20. Effect of 200mM NaCl (Na^+), 100µM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the level of cytosolic sodium (nmol100mg dry weight^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Cytosolic Na^+ (nmol 100mg dry weight^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	0.048±0.004	0.030±0.003	0.030±0.006	0.038±0.004	0.049±0.004
Na^+	-	0.070±0.003	0.144±0.002	0.176±0.005	0.208±0.002
Ca^{++}	-	0.036±0.006	0.031±0.001	0.035±0.005	0.041±0.004
Na^++Ca^{++}	-	0.066±0.005	0.065±0.007	0.084±0.004	0.090±0.007
Na^++Ca^{++}+Ver	-	0.105±0.012	0.156±0.005	0.220±0.007	0.287±0.004
Na^++Ca^{++}+EGTA	-	0.095±0.012	0.182±0.003	0.188±0.004	0.225±0.008
ABA	-	0.042±0.002	0.046±0.004	0.052±0.006	0.061±0.003
Na^++ABA	-	0.065±0.005	0.107±0.004	0.128±0.004	0.129±0.007
Na^++ABA+Ver	-	0.095±0.019	0.166±0.006	0.197±0.004	0.241±0.006
Na^++ABA+EGTA	-	0.098±0.009	0.188±0.002	0.198±0.010	0.221±0.007
ABA+Ca^{++}	-	0.048±0.005	0.042±0.006	0.036±0.003	0.033±0.005
Na^++ABA+Ca^{++}	-	0.104±0.005	0.102±0.002	0.118±0.002	0.139±0.006
Na^++ABA+Ca^{++}+Ver	-	0.115±0.005	0.201±0.001	0.229±0.001	0.245±0.005
Na^++ABA+Ca^{++}+EGTA	-	0.095±0.004	0.172±0.007	0.216±0.004	0.214±0.005

± SEM

Figure 4.18. Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the level of cytosolic sodium (nmol100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.19. Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the level of cytosolic sodium (nmol100mg dry weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

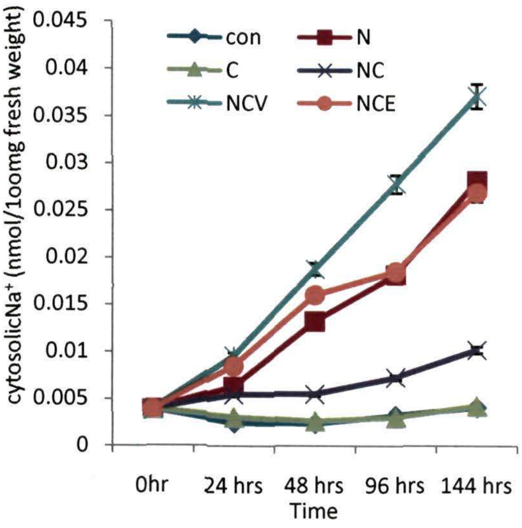


Fig. 4.18.a

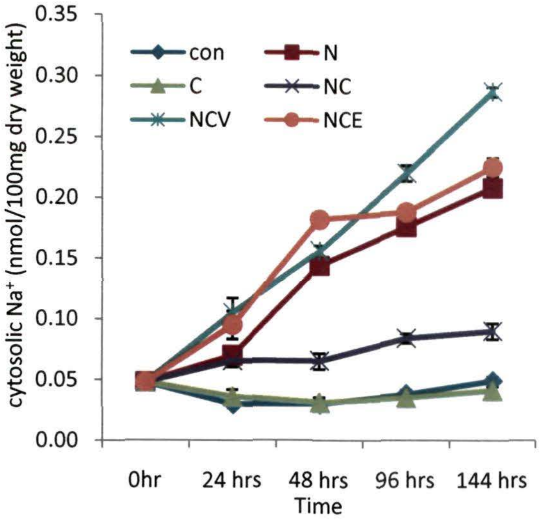


Fig. 4.19.a

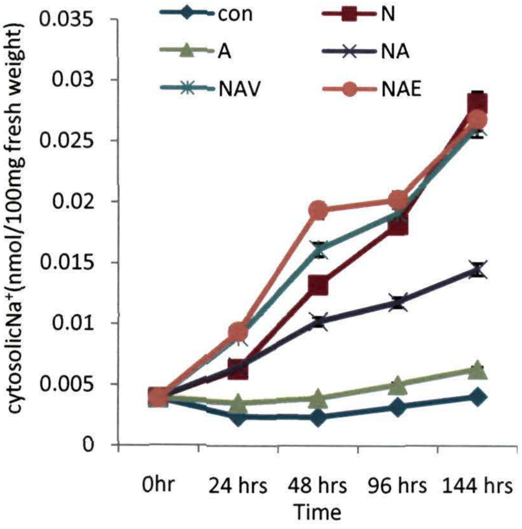


Fig. 4.18.b

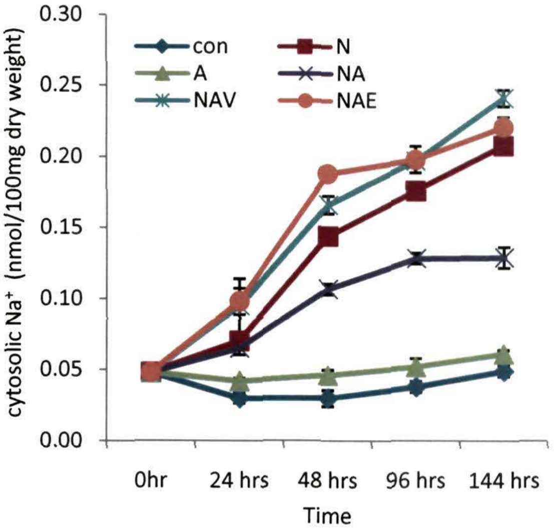


Fig. 4.19.b

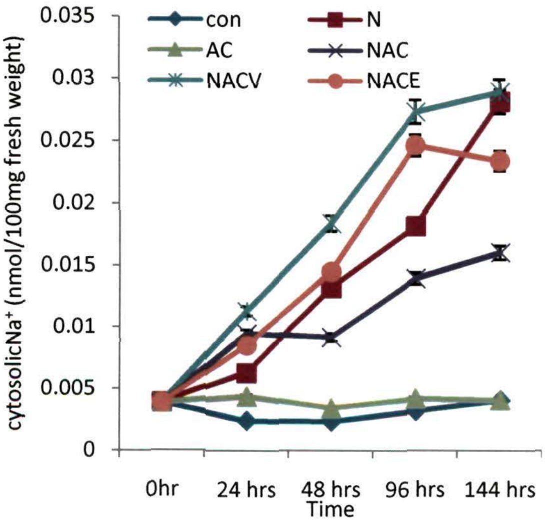


Fig. 4.18.c

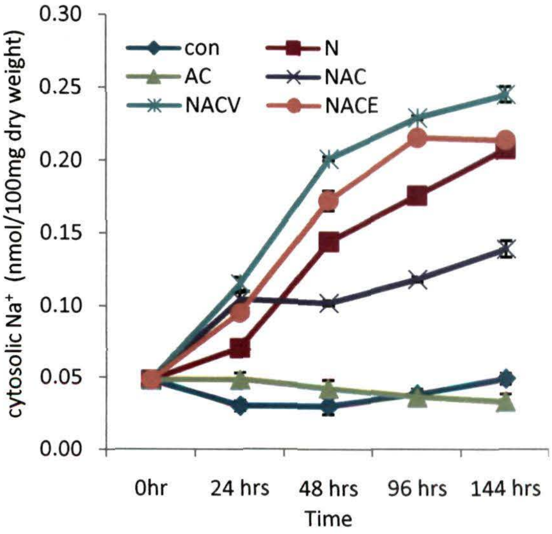


Fig. 4.19.c

Table 4.21. Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the level of cytosolic potassium (nmol/100mg fresh weight) in shoots in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Cytosolic K^+ (nmol/100mg fresh weight)				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	0.037±0.0010	0.034±0.0010	0.035±0.0010	0.043±0.0012	0.043±0.0012
Na^+		0.034±0.0010	0.036±0.0010	0.033±0.0009	0.041±0.0012
Ca^{++}		0.035±0.0010	0.036±0.0010	0.034±0.0010	0.043±0.0012
Na^++Ca^{++}		0.033±0.0009	0.035±0.0010	0.036±0.0010	0.048±0.0014
Na^++Ca^{++}+Ver		0.037±0.0011	0.051±0.0014	0.056±0.0016	0.058±0.0017
Na^++Ca^{++}+EGTA		0.034±0.0010	0.034±0.0010	0.037±0.0011	0.042±0.0012
ABA		0.033±0.0009	0.035±0.0010	0.034±0.0010	0.040±0.0011
Na^++ABA		0.037±0.0010	0.037±0.0010	0.031±0.0009	0.041±0.0012
Na^++ABA+Ver		0.036±0.0010	0.039±0.0011	0.033±0.0009	0.039±0.0011
Na^++ABA+EGTA		0.036±0.0010	0.040±0.0011	0.037±0.0010	0.047±0.0013
ABA+Ca^{++}		0.043±0.0012	0.026±0.0007	0.038±0.0011	0.041±0.0012
Na^++ABA+Ca^{++}		0.041±0.0012	0.053±0.0015	0.071±0.0020	0.070±0.0020
Na^++ABA+Ca^{++}+Ver		0.045±0.0013	0.055±0.0016	0.073±0.0021	0.073±0.0021
Na^++ABA+Ca^{++}+EGTA		0.039±0.0011	0.048±0.0014	0.066±0.0019	0.064±0.0018

± SEM

Table 4.22. Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the level of cytosolic potassium (nmol/100mg dry weight) in shoots in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Cytosolic K^+ (nmol/100mg dry weight)				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	0.455±0.034	0.424±0.035	0.440±0.073	0.510±0.033	0.520±0.048
Na^+	-	0.380±0.046	0.390±0.019	0.320±0.028	0.300±0.023
Ca^{++}	-	0.414±0.034	0.430±0.055	0.392±0.045	0.412±0.021
Na^++Ca^{++}	-	0.395±0.025	0.415±0.020	0.421±0.023	0.420±0.026
Na^++Ca^{++}+Ver	-	0.405±0.025	0.422±0.048	0.444±0.032	0.451±0.009
Na^++Ca^{++}+EGTA	-	0.375±0.049	0.389±0.032	0.375±0.035	0.351±0.037
ABA	-	0.395±0.026	0.405±0.025	0.352±0.053	0.385±0.038
Na^++ABA	-	0.365±0.045	0.384±0.032	0.341±0.039	0.362±0.049
Na^++ABA+Ver	-	0.380±0.025	0.400±0.022	0.340±0.019	0.360±0.053
Na^++ABA+EGTA	-	0.380±0.036	0.390±0.009	0.360±0.052	0.390±0.034
ABA+Ca^{++}	-	0.465±0.034	0.315±0.020	0.322±0.013	0.332±0.044
Na^++ABA+Ca^{++}	-	0.450±0.003	0.590±0.024	0.600±0.037	0.610±0.042
Na^++ABA+Ca^{++}+Ver	-	0.455±0.026	0.605±0.021	0.615±0.017	0.620±0.031
Na^++ABA+Ca^{++}+EGTA	-	0.432±0.058	0.566±0.039	0.575±0.040	0.582±0.016

± SEM

Figure 4.20: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the level of cytosolic potassium (nmol100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.21: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the level of cytosolic potassium (nmol100mg dry weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

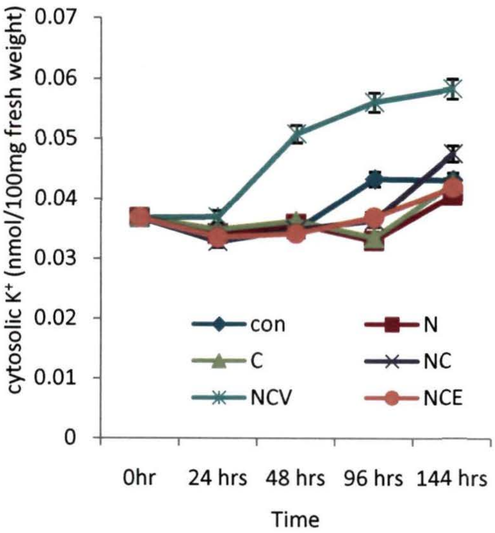
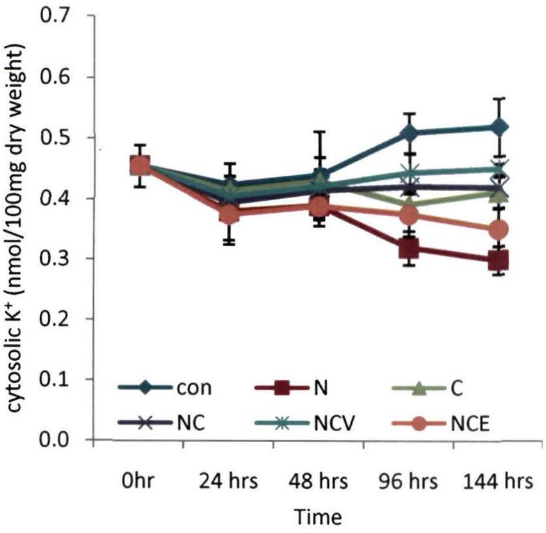


Fig. 4.20.a



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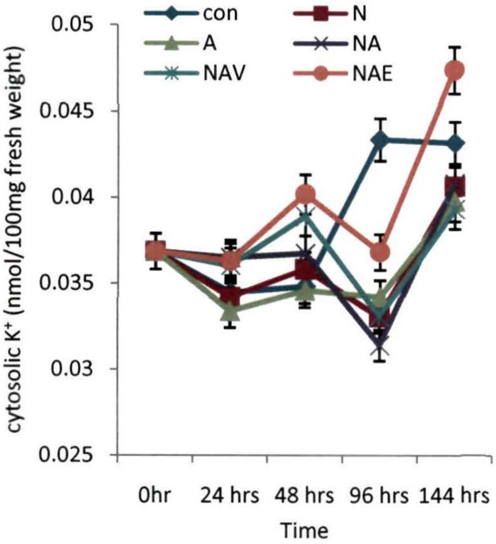
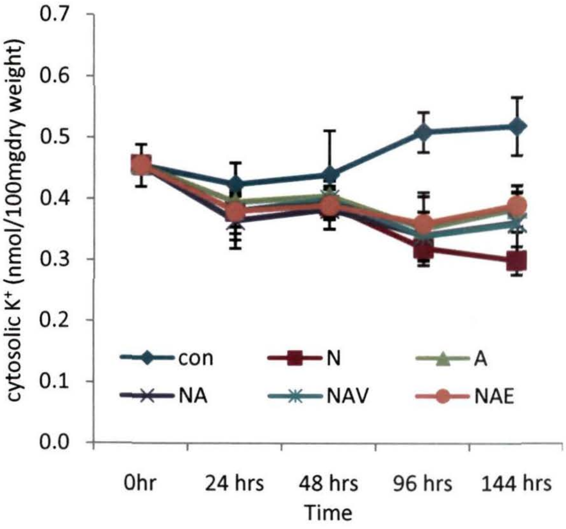


Fig. 4.20.b



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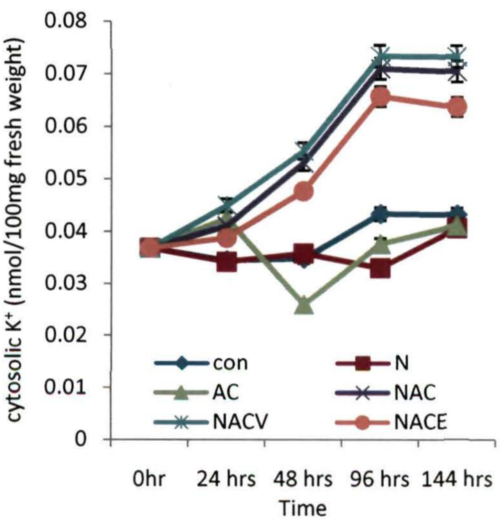


Fig. 4.20.c

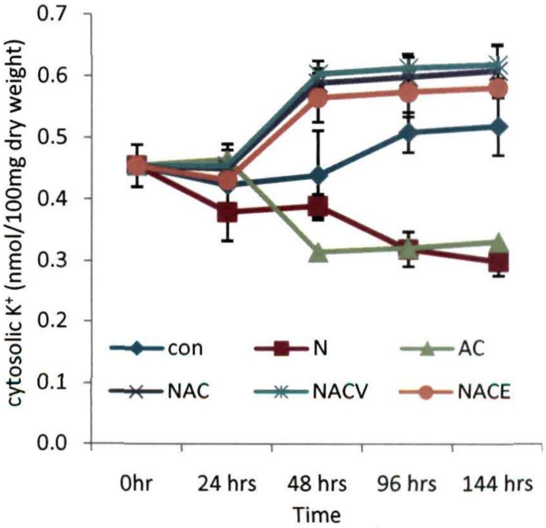


Fig. 4.21.c

the seedlings with either 1 mM verapamil or 10 mM EGTA did not have any significant effect on the levels of cytosolic K^+ in the seedlings.

There was no marked change in the Na^+/K^+ ratio in the shoot tissues of seedlings cultured in native Hoagland nutrient solution during the 144 hours of incubation. Seedlings maintained over Hoagland's nutrient solution supplemented with either 10 mM $CaCl_2$ or 100 μM ABA also did not show any marked change in the cellular Na^+/K^+ ratio during the 144 hour incubation period. However, seedlings incubated in nutrient solution containing 200 mM sodium chloride showed a consistent increase in Na^+/K^+ ratio of the shoot tissues with progressive time. These seedlings registered a 6 fold increase in Na^+/K^+ ratio of the shoot tissues during the 144 hours of incubation (Table 4.23; Fig. 4.22).On the other hand seedlings incubated in nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed a markedly lower ratio of Na^+ to K^+ than the corresponding control seedlings which were incubated in nutrient solution supplemented with only 200 mM NaCl. While the seedlings incubated in nutrient solution supplemented with 200 mM NaCl registered a six fold increase in Na^+/K^+ ratio those incubated in nutrient solution supplemented with 200 mM NaCl and 10 mM registered only 2 fold increase in Na^+/K^+ ratio during the 144 hours of incubation. However, seedlings incubated in nutrient solution supplemented with 200 mM NaCl and 100 μM ABA registered 3.5 fold increase in the ratio of Na^+ to K^+ during the same period. Seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed a significantly higher ratio of Na^+

Table 4.23: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the ratio of Na^+/K^+ in shoots in shoots in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Na^+/K^+				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	0.105±0.005	0.070±0.003	0.068±0.003	0.074±0.003	0.094±0.004
Na^+	-	0.184±0.008	0.369±0.017	0.550±0.025	0.693±0.031
Ca^{++}	-	0.086±0.004	0.072±0.003	0.089±0.004	0.099±0.004
Na^++Ca^{++}	-	0.166±0.008	0.157±0.007	0.199±0.009	0.214±0.010
Na^++Ca^{++}+Ver	-	0.259±0.012	0.369±0.017	0.495±0.022	0.636±0.029
Na^++Ca^{++}+EGTA	-	0.253±0.011	0.467±0.021	0.501±0.023	0.641±0.029
ABA	-	0.106±0.005	0.113±0.005	0.147±0.007	0.158±0.007
Na^++ABA	-	0.178±0.008	0.277±0.013	0.376±0.017	0.356±0.016
Na^++ABA+Ver	-	0.250±0.011	0.415±0.019	0.579±0.026	0.669±0.030
Na^++ABA+EGTA	-	0.257±0.012	0.482±0.022	0.550±0.025	0.566±0.026
ABA+Ca^{++}	-	0.103±0.005	0.133±0.006	0.112±0.005	0.099±0.004
Na^++ABA+Ca^{++}	-	0.231±0.010	0.172±0.008	0.196±0.009	0.227±0.010
Na^++ABA+Ca^{++}+Ver	-	0.252±0.011	0.332±0.015	0.372±0.017	0.395±0.018
Na^++ABA+Ca^{++}+EGTA	-	0.219±0.010	0.304±0.014	0.374±0.017	0.367±0.017

± SEM

Table 4.24: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the content of hydrogen peroxide (H_2O_2) (ng 100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	H_2O_2 (ng 100mg fresh weight^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	10.2±1.53	12.5±1.53	12.1±2.08	13.0±4.16	13.6±3.61
Na^+	-	25.4±3.51	29.1±3.79	30.6±4.16	32.0±4.16
Ca^{++}	-	15.4±4.00	15.1±4.73	16.3±2.52	17.2±2.52
Na^++Ca^{++}	-	23.4±3.51	22.1±2.08	20.5±3.61	21.9±3.61
Na^++Ca^{++}+Ver	-	25.0±4.58	30.2±2.65	30.5±3.61	31.0±2.00
Na^++Ca^{++}+EGTA	-	24.1±3.79	30.8±4.16	31.9±4.73	24.7±2.00
ABA	-	14.5±2.08	14.0±2.65	15.6±5.00	16.3±2.52
Na^++ABA	-	22.3±2.08	21.9±4.73	21.0±3.06	20.8±3.06
Na^++ABA+Ver	-	24.5±2.08	36.5±2.52	34.6±4.16	38.6±4.16
Na^++ABA+EGTA	-	24.6±3.21	35.0±3.21	32.1±3.61	25.1±6.43
ABA+Ca^{++}	-	15.9±4.51	18.2±2.00	19.5±2.52	17.9±3.61
Na^++ABA+Ca^{++}	-	21.5±3.21	25.5±2.65	25.3±5.86	26.6±4.16
Na^++ABA+Ca^{++}+Ver	-	26.5±2.65	30.1±2.08	34.5±3.61	36.8±5.29
Na^++ABA+Ca^{++}+EGTA	-	25.1±6.08	28.9±4.73	28.4±2.52	34.9±5.86

± SEM

Figure 4.22: Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the Na^+/K^+ in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.23: Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the content of H_2O_2 (ng 100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

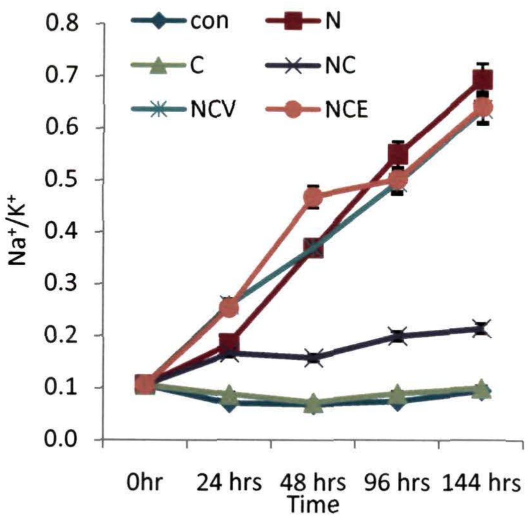


Fig. 4.22.a

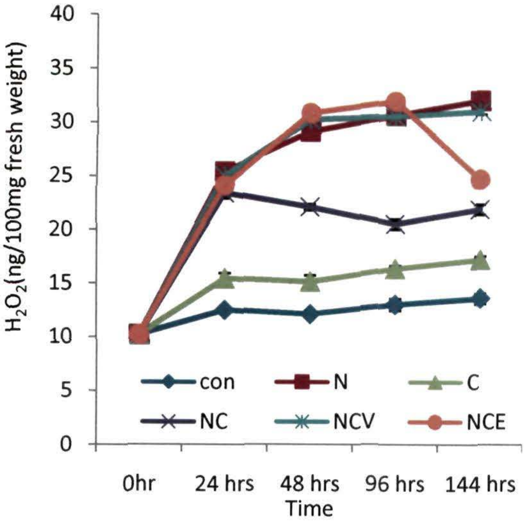


Fig. 4.23.a

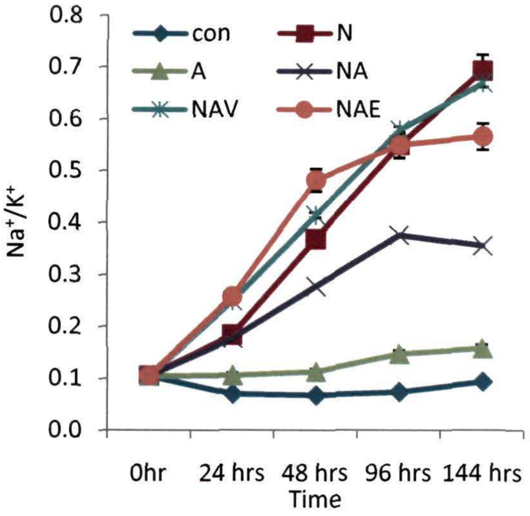


Fig. 4.22.b

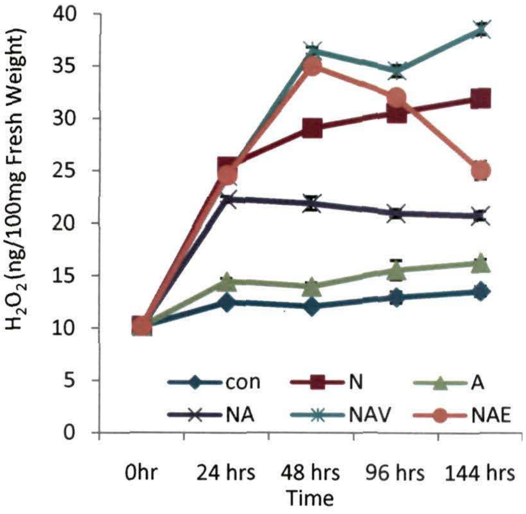


Fig. 4.23.b

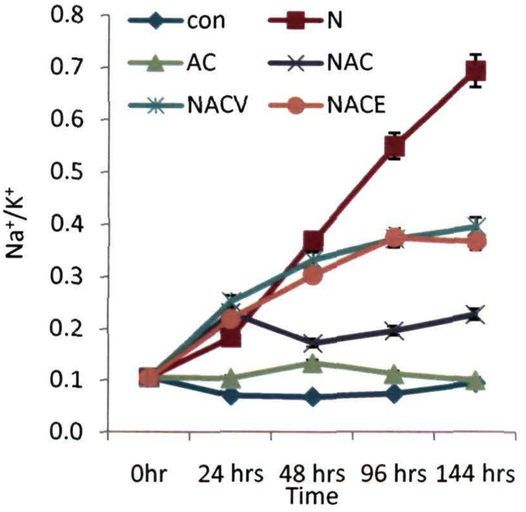


Fig. 4.22.c

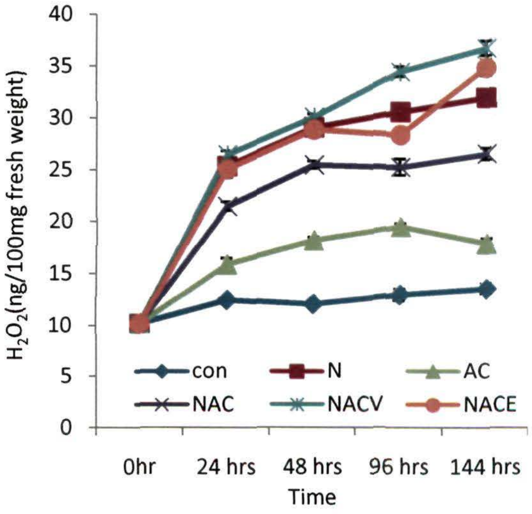


Fig. 4.23.c

to K^+ than the corresponding controls which were not pretreated with either verapamil or EGTA. These seedlings registered nearly six fold increase in the level of ratio of Na^+ to K^+ during the 144 hours of incubation (Table 4.23; Fig. 4.22).

Compared to the untreated controls seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride showed a marked increase in the tissue level of hydrogen peroxide (H_2O_2) during the 144 hours of incubation. The increase in tissue level of hydrogen peroxide was, however, more prominent during the initial 24 hours of incubation. While the increase in level H_2O_2 was only marginal in the untreated controls, it was more than 3 fold in seedlings cultured in nutrient solution supplemented with 200 mM NaCl (Table 4.24; Fig. 4.23). Compared to the seedlings cultured in nutrient solution supplemented with 200 mM NaCl, seedlings incubated in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed markedly lower tissue levels of hydrogen peroxide. These seedlings registered a 2 fold increase in H_2O_2 levels during the 144 hours of incubation (Table 4.24; Fig. 4.23). The increase in tissue levels of H_2O_2 during the 144 hours of incubation was significantly higher in seedlings pretreated with either verapamil or EGTA.

Expressed as percent fresh weight there was a marked decrease in the tissue content of soluble protein in seedlings during 144 hours of incubation in Hoagland's nutrient lacking any additional supplements. The decrease in the content of soluble protein was, however, much less marked in seedlings incubated in nutrient solution supplemented with 200 mM NaCl (Table 4.25; Fig. 4.24). Seedlings cultured in nutrient solution containing 200 mM NaCl showed marginally higher soluble protein

Table 4.25: Effect of 200mM NaCl (Na$^+$), 100μM ABA and 10mM CaCl$_2$ (Ca^{++}) and combinations of the above on the concentration of soluble protein (mg 100mg^{-1} fresh weight) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Soluble protein (mg 100mg fresh weight^{-1})				
	0hr	24hr	48hr	96 hr	144 hr
control	0.56±0.010	0.54±0.012	0.48±0.017	0.45±0.015	0.45±0.003
Na$^+$	-	0.61±0.019	0.58±0.009	0.56±0.003	0.56±0.004
Ca^{++}	-	0.54±0.017	0.48±0.017	0.45±0.015	0.46±0.012
Na$^+$+Ca^{++}	-	0.58±0.015	0.54±0.009	0.53±0.015	0.55±0.008
Na$^+$+Ca^{++}+Ver	-	0.52±0.015	0.49±0.015	0.46±0.012	0.45±0.002
Na$^+$+Ca^{++}+EGTA	-	0.53±0.021	0.47±0.007	0.46±0.013	0.44±0.003
ABA	-	0.58±0.041	0.51±0.008	0.47±0.011	0.46±0.013
Na$^+$+ABA	-	0.56±0.013	0.50±0.018	0.46±0.026	0.45±0.014
Na$^+$+ABA+Ver	-	0.53±0.026	0.49±0.017	0.44±0.009	0.45±0.014
Na$^+$+ABA+EGTA	-	0.53±0.022	0.47±0.008	0.46±0.011	0.43±0.017
ABA+Ca^{++}	-	0.53±0.026	0.50±0.020	0.46±0.031	0.45±0.016
Na$^+$+ABA+Ca^{++}	-	0.52±0.018	0.49±0.018	0.45±0.016	0.44±0.015
Na$^+$+ABA+Ca^{++}+Ver	-	0.51±0.015	0.48±0.017	0.45±0.018	0.44±0.015
Na$^+$+ABA+Ca^{++}+EGTA	-	0.52±0.019	0.50±0.009	0.46±0.009	0.45±0.011

± SEM

Table 4.26: Effect of 200mM NaCl (Na$^+$), 100μM ABA and 10mM CaCl$_2$ (Ca^{++}) and combinations of the above on the concentration of soluble protein (mg 100mg^{-1} dry weight) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Soluble protein (mg 100mg dry weight^{-1})				
	0hr	24hr	48hr	96 hr	144 hr
control	0.691±0.029	0.666±0.028	0.614±0.026	0.524±0.022	0.536±0.023
Na$^+$	-	0.674±0.029	0.636±0.027	0.541±0.023	0.414±0.018
Ca^{++}	-	0.639±0.027	0.579±0.025	0.532±0.023	0.442±0.019
wNa$^+$+Ca^{++}	-	0.703±0.030	0.646±0.027	0.609±0.026	0.485±0.021
Na$^+$+Ca^{++}+Ver	-	0.577±0.025	0.406±0.017	0.364±0.015	0.344±0.015
Na$^+$+Ca^{++}+EGTA	-	0.595±0.025	0.537±0.023	0.469±0.020	0.369±0.016
ABA	-	0.692±0.029	0.594±0.025	0.488±0.021	0.446±0.019
Na$^+$+ABA	-	0.564±0.024	0.518±0.022	0.495±0.021	0.404±0.017
Na$^+$+ABA+Ver	-	0.564±0.024	0.506±0.022	0.456±0.019	0.410±0.017
Na$^+$+ABA+EGTA	-	0.561±0.024	0.454±0.019	0.449±0.019	0.355±0.015
ABA+Ca^{++}	-	0.586±0.025	0.603±0.026	0.390±0.017	0.369±0.016
Na$^+$+ABA+Ca^{++}	-	0.563±0.024	0.542±0.023	0.382±0.016	0.389±0.017
Na$^+$+ABA+Ca^{++}+Ver	-	0.517±0.022	0.528±0.022	0.377±0.016	0.379±0.016
Na$^+$+ABA+Ca^{++}+EGTA	-	0.580±0.025	0.597±0.025	0.405±0.017	0.417±0.018

± SEM

Figure 4.24. Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the concentration of soluble protein (mg $100mg^{-1}$ fresh weight) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.25. Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the concentration of soluble protein (mg $100mg^{-1}$ dry weight) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM

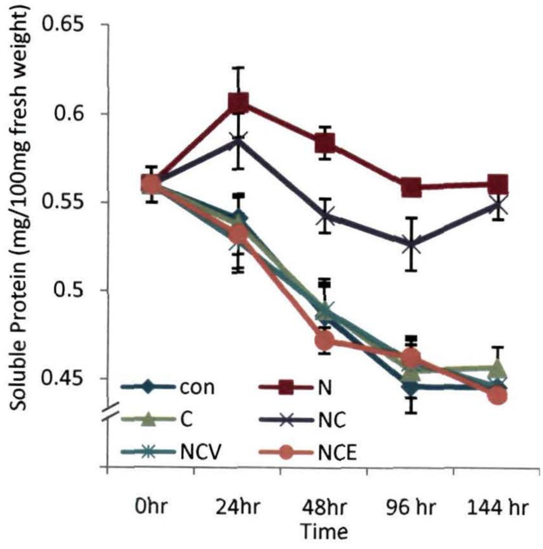
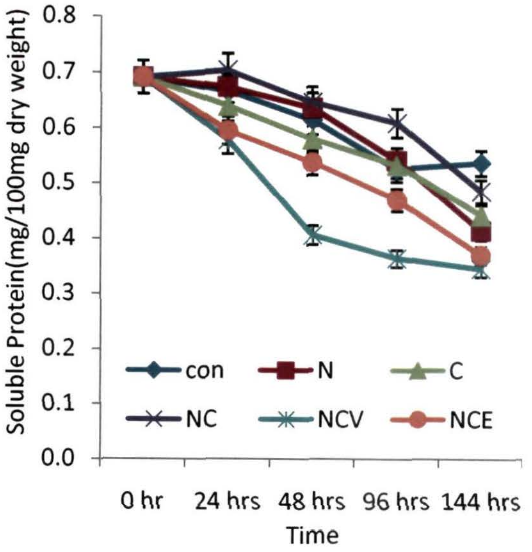


Fig. 4.24.a



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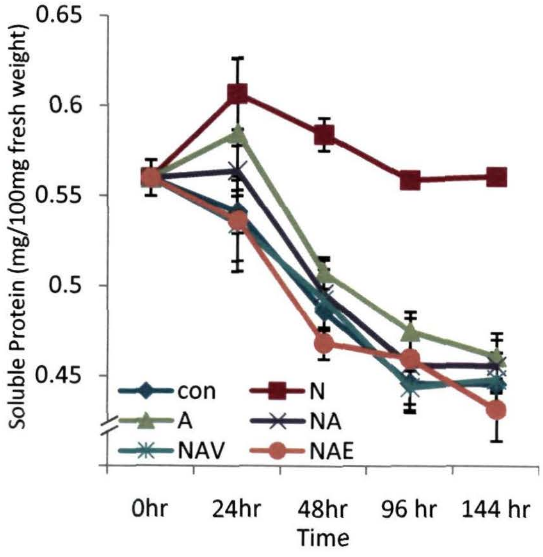

Fig. 4.24.b

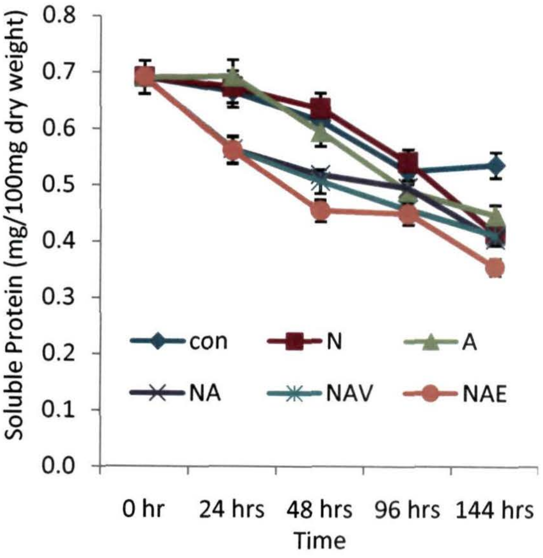

Fig. 4.25.b

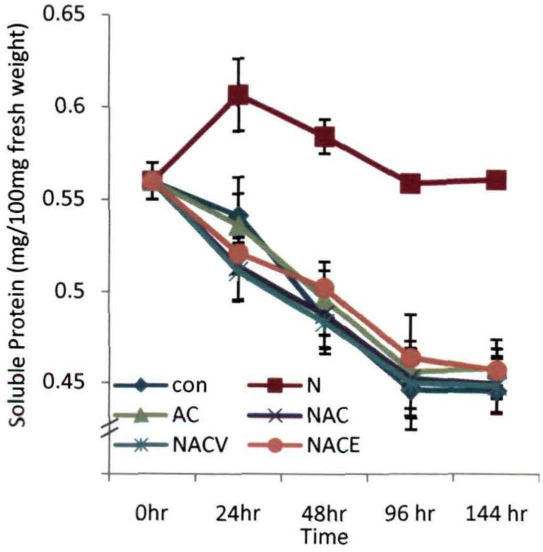

Fig. 4.24.c

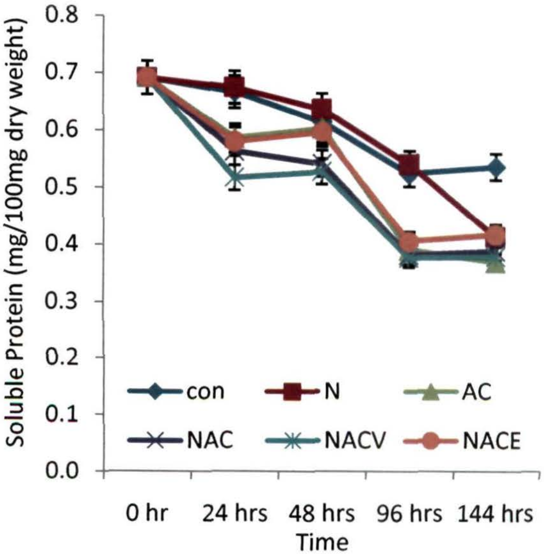

Fig. 4.25.c

content than those incubated in nutrient solution without any supplements. Compared to the seedlings cultured in nutrient solution containing 200 mM NaCl, seedlings incubated in nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed marginally lesser soluble protein content in the shoot tissue during the 144 hours of incubation (Table 4.25; Fig. 4.24). However, when the content of soluble protein was expressed as percent of tissue dry weight, there was a marked decrease in the tissue content of soluble protein in seedlings during 144 hours of incubation in Hoagland's nutrient lacking any additional supplements. Seedlings incubated in nutrient solution supplemented with 200 mM NaCl showed two fold decrease in the tissue content of soluble protein during the 144 hours of incubation (Table 4.24; Fig. 4.23). There were no significant differences in the soluble protein content of the leaf tissues between seedlings cultured in nutrient solution containing 200 mM NaCl and those cultured in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA.

Expressed either as percent of fresh weight or as percent of total soluble protein, the level of protein carbonylation showed a marginal increase with progressing time in the shoot tissues of seedlings incubated in Hoagland's nutrient lacking any additional supplements (Table 4.27; Fig. 4.26). The change in protein carbonyl level of seedlings maintained over Hoagland's nutrient solution containing either 10 mM $CaCl_2$ or 100 μM ABA showed the same pattern and magnitude of change as in seedlings incubated in Hoagland's nutrient lacking any additional supplements. However, seedlings cultured in nutrient solution containing 200mM

Table 4.27: Effect of 200mM NaCl (Na^{+}), 100μM ABA and 10mM CaCl$_2$ (Ca^{++}) and combinations of the above on the content of protein carbonyl (nmol 100mg^{-1} fresh weight) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Protein carbonyl (nmol 100mg fresh weight^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	6.52±0.78	6.80±0.51	7.50±0.10	7.50±0.49	7.80±0.40
Na^{+}	-	8.20±0.10	9.10±0.20	10.20±0.49	11.00±0.06
Ca^{++}	-	7.00±0.50	7.70±0.36	7.60±0.15	7.90±0.45
Na^{+}+Ca^{++}	-	8.00±0.43	7.80±0.50	8.10±0.70	8.90±0.67
Na^{+}+Ca^{++}+Ver	-	7.80±0.26	9.70±0.30	11.50±0.31	11.50±0.81
Na^{+}+Ca^{++}+EGTA	-	9.20±0.40	8.50±0.25	10.10±0.68	12.50±0.67
ABA	-	7.50±0.41	7.70±0.11	7.80±0.36	7.90±0.42
Na^{+}+ABA	-	7.90±0.66	7.80±0.35	7.90±0.26	8.10±0.36
Na^{+}+ABA+Ver	-	6.20±0.15	9.10±0.55	10.50±0.47	12.30±0.23
Na^{+}+ABA+EGTA	-	7.60±0.25	9.90±1.12	10.40±0.55	10.70±1.12
ABA+Ca^{++}	-	6.65±0.39	7.45±0.85	7.60±0.25	8.05±0.33
Na^{+}+ABA+Ca^{++}	-	8.30±0.20	8.10±0.43	9.50±0.26	9.40±0.40
Na^{+}+ABA+Ca^{++}+Ver	-	8.55±0.17	8.40±0.26	9.65±0.3	9.60±0.38
Na^{+}+ABA+Ca^{++}+EGTA	-	8.05±0.27	7.95±0.57	9.45±0.56	9.10±0.78

± SEM

Table 4.28: Effect of 200mM NaCl (Na^{+}), 100μM ABA and 10mM CaCl$_2$ (Ca^{++}) and combinations of the above on the content of protein carbonyl (nmol mg protein^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Protein carbonyl (nmol mg protein^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	11.64±0.08	12.56±0.08	15.43±0.10	16.82±0.11	17.51±0.11
Na^{+}	-	13.52±0.09	15.57±0.10	18.25±0.12	19.61±0.13
Ca^{++}	-	13.01±0.08	15.73±0.10	16.71±0.11	17.29±0.11
Na^{+}+Ca^{++}	-	13.68±0.09	14.36±0.09	15.38±0.10	16.20±0.10
Na^{+}+Ca^{++}+Ver	-	14.76±0.10	19.83±0.13	25.00±0.16	25.78±0.17
Na^{+}+Ca^{++}+EGTA	-	17.28±0.11	17.99±0.12	19.68±0.13	23.80±0.15
ABA	-	12.82±0.08	15.17±0.10	16.43±0.11	17.14±0.11
Na^{+}+ABA	-	14.01±0.09	15.73±0.10	17.32±0.11	17.78±0.11
Na^{+}+ABA+Ver	-	11.60±0.07	18.47±0.12	23.66±0.15	27.44±0.18
Na^{+}+ABA+EGTA	-	14.17±0.09	21.14±0.14	22.63±0.15	24.81±0.16
ABA+Ca^{++}	-	12.41±0.08	15.01±0.10	16.67±0.11	17.59±0.11
Na^{+}+ABA+Ca^{++}	-	16.15±0.10	16.61±0.11	19.89±0.13	20.92±0.13
Na^{+}+ABA+Ca^{++}+Ver	-	16.73±0.11	17.38±0.11	21.44±0.14	21.41±0.14
Na^{+}+ABA+Ca^{++}+EGTA	-	15.46±0.10	15.82±0.10	20.38±0.13	19.92±0.13

± SEM

Figure 4.26: Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the content of carbonyl (nmol 100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM

Figure 4.27: Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the content of carbonyl (nmol mg protein^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM

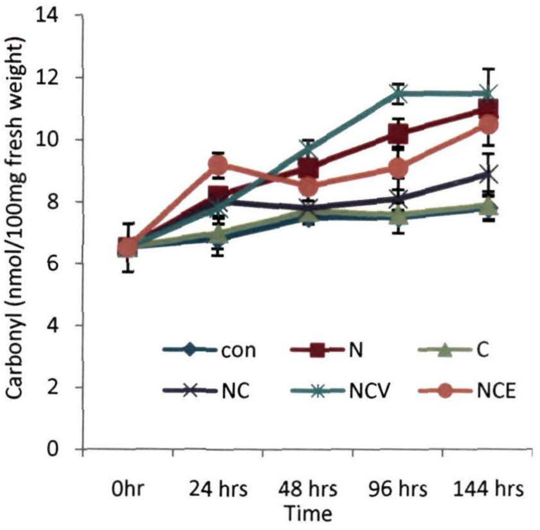

Fig. 4.26.a

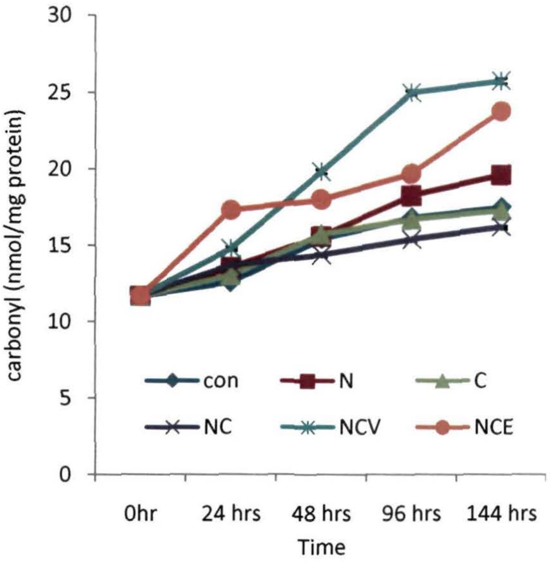

Fig. 4.27.a

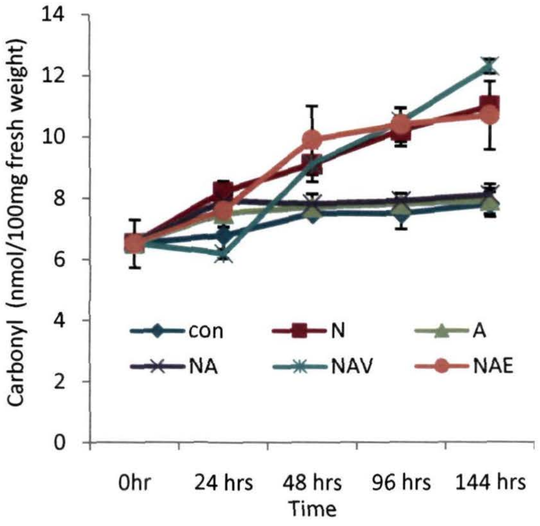

Fig. 4.26.b

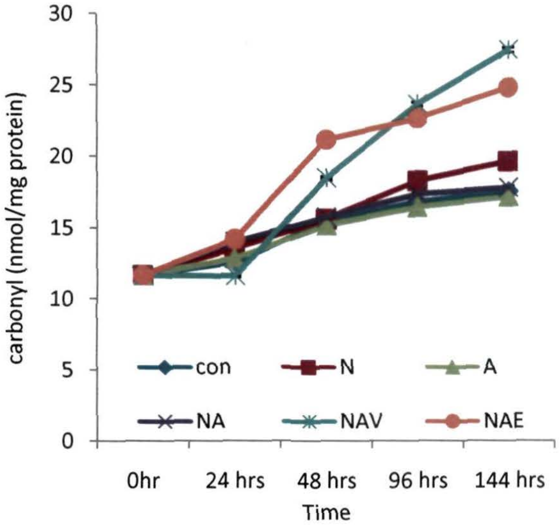

Fig. 4.27.b

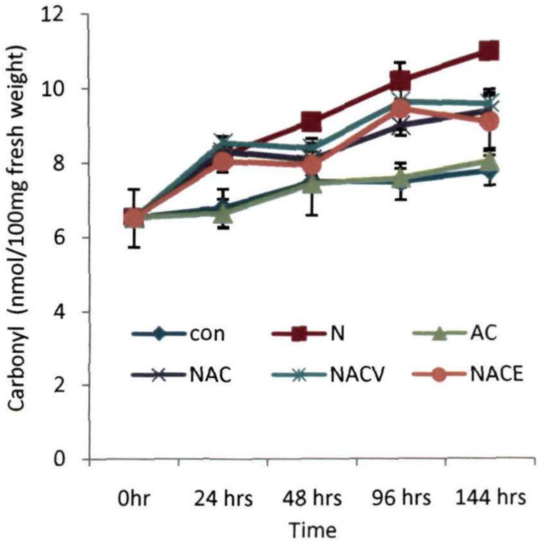

Fig. 4.26.c

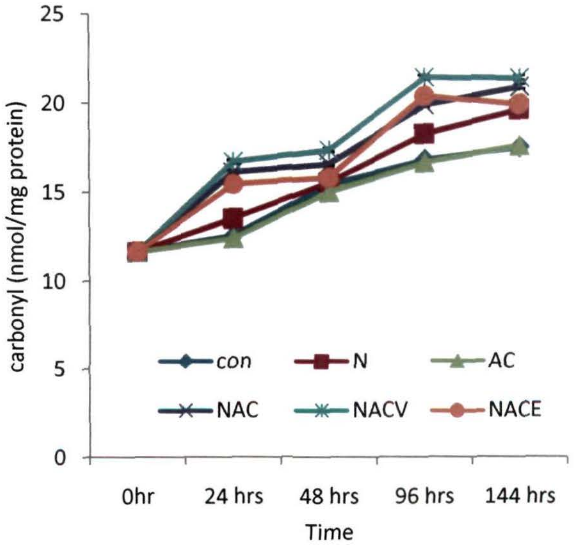

Fig. 4.27.c

NaCl showed markedly higher level of protein carbonylation than the untreated controls. These seedlings registered almost 2 fold higher protein carbonylation than the corresponding controls cultured in nutrient solution without any additional supplements (Table 4.27; Fig. 4.26). On the other hand, seedlings incubated in nutrient solution supplemented with 200 mM NaCl and either 10 mM $CaCl_2$ or 100 μM ABA showed only marginal increase in the level of protein carbonylation during the 144 hours of incubation. However seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed a significantly higher carbonyl than those which were not pretreated with either verapamil or EGTA. These seedlings registered more than 60% increase in the level of protein carbonylation during the 144 hours of incubation (Table 4.28; Fig. 4.27).

Expressed as percent of fresh weight, there was an almost two fold increase in the malondialdehyde (MDA) content in shoot tissues of seedlings during 144 hours of incubation in Hoagland's nutrient lacking any additional supplements (Table 4.29; Fig. 4.28). Seedlings incubated in Hoagland's nutrient solution supplemented with either 10 mM $CaCl_2$ or 100 μM ABA also registered nearly the same magnitude of change in MDA levels during the 144 hours of incubation (Table 4.29; Fig. 4.28). However, seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride showed significantly higher levels of malondialdehyde (MDA) in the shoot tissues. These seedlings registered a more than 3 fold increase in the tissue level of malondialdehyde during the 144 hours of incubation (Table 4.29; Fig.4.28). On the other hand seedlings incubated in the solution supplemented with 200 mM

Table 4.29: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the lipid peroxidation products i.e., level of MDA (nmol 100mg^{-1} fresh weight) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	MDA (nmol 100mg fresh weight^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	1.956±0.66	2.039±0.65	2.212±0.43	2.954±0.65	3.671±0.95
Na^+	-	2.836±0.64	4.686±0.62	5.865±0.66	6.939±0.80
Ca^{++}	-	2.155±0.65	2.382±0.65	3.074±0.65	3.824±0.41
Na^++Ca^{++}	-	2.967±0.66	3.212±0.71	4.129±0.64	5.000±0.72
Na^++Ca^{++}+Ver	-	3.050±0.75	3.265±0.45	5.025±0.40	5.556±0.31
Na^++Ca^{++}+EGTA	-	3.285±0.65	2.945±0.56	4.213±0.45	4.758±0.29
ABA	-	2.235±0.34	2.416±0.44	3.498±0.78	4.521±0.42
Na^++ABA	-	2.751±0.37	3.529±0.39	5.040±0.34	6.178±0.90
Na^++ABA+Ver	-	3.319±0.61	3.208±0.73	5.486±0.90	7.136±0.36
Na^++ABA+EGTA	-	3.477±0.65	3.082±0.87	4.586±0.07	5.054±0.33
ABA+Ca^{++}	-	3.161±0.28	3.097±0.81	2.387±0.57	4.452±0.31
Na^++ABA+Ca^{++}	-	3.091±0.76	2.876±0.65	5.129±0.58	6.055±0.46
Na^++ABA+Ca^{++}+Ver	-	3.156±0.65	2.925±0.65	6.521±0.47	7.089±0.56
Na^++ABA+Ca^{++}+EGTA	-	2.925±0.67	2.658±0.65	5.452±0.30	5.994±0.92

± SEM

Table 4.30: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the content of glutathione (GSH) (nmol/100mg fresh weight) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Glutathione (GSH) (nmol 100mg fresh weight^{-1})				
	0hr	24hrs	48hrs	96hrs	144hrs
control	74.09±0.85	78.64±0.54	72.27±0.57	70.45±0.80	69.55±1.49
Na^+	-	69.64±1.06	68.55±1.27	67.73±0.91	66.82±1.04
Ca^{++}	-	79.13±1.14	73.16±0.57	71.71±0.80	70.10±1.93
Na^++Ca^{++}	-	77.73±0.93	73.18±0.88	72.09±0.66	71.73±0.87
Na^++Ca^{++}+Ver	-	72.50±1.77	70.00±1.27	69.03±1.05	67.86±0.76
Na^++Ca^{++}+EGTA	-	72.51±1.30	70.24±0.60	69.13±1.03	69.05±2.18
ABA	-	79.26±0.58	73.63±0.60	69.93±0.46	69.59±1.52
Na^++ABA	-	75.91±2.20	75.00±1.35	75.00±1.86	74.55±1.50
Na^++ABA+Ver	-	68.04±0.84	66.55±1.01	65.73±1.48	64.82±1.04
Na^++ABA+EGTA	-	73.10±1.11	72.08±0.96	70.66±1.44	71.19±4.28
ABA+Ca^{++}	-	79.55±1.06	67.73±0.79	70.46±1.02	67.73±1.44
Na^++ABA+Ca^{++}	-	72.29±2.63	69.58±0.75	69.36±1.92	68.09±1.20
Na^++ABA+Ca^{++}+Ver	-	63.23±0.88	62.56±0.38	60.67±0.63	59.17±0.23
Na^++ABA+Ca^{++}+EGTA	-	64.53±0.64	64.22±0.91	62.57±0.97	62.64±1.85

± SEM

Figure 4.28. Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the lipid peroxidation i.e., MDA content (nmol 100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.29: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the content of glutathione (GSH) (nmol 100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

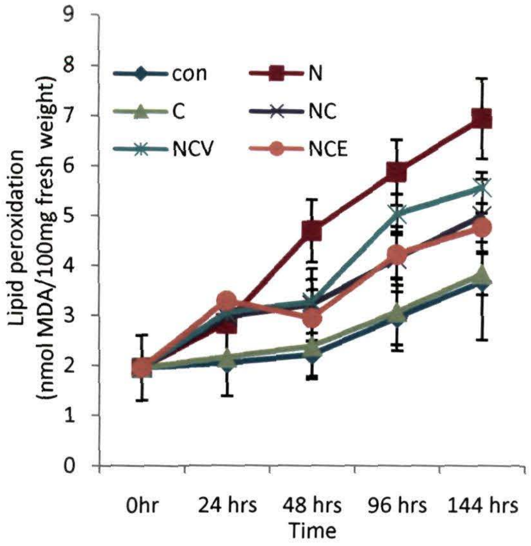

Fig. 4.28.a

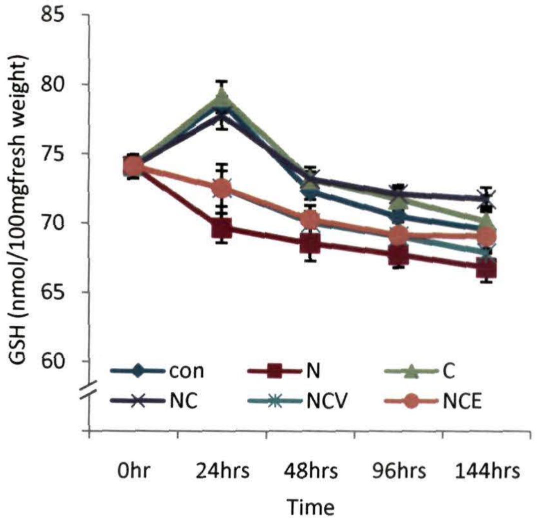

Fig. 4.28.a

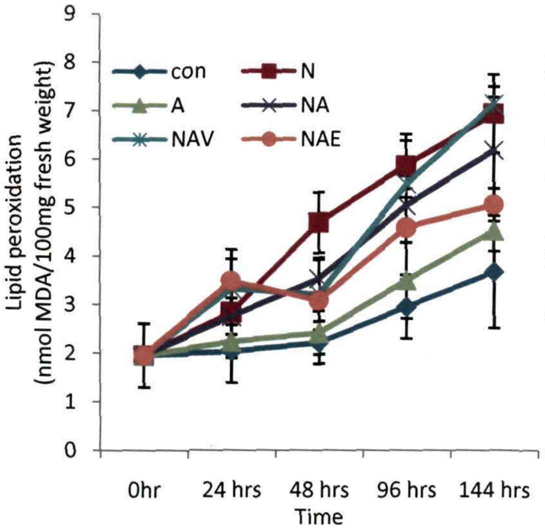

Fig. 4.28.b

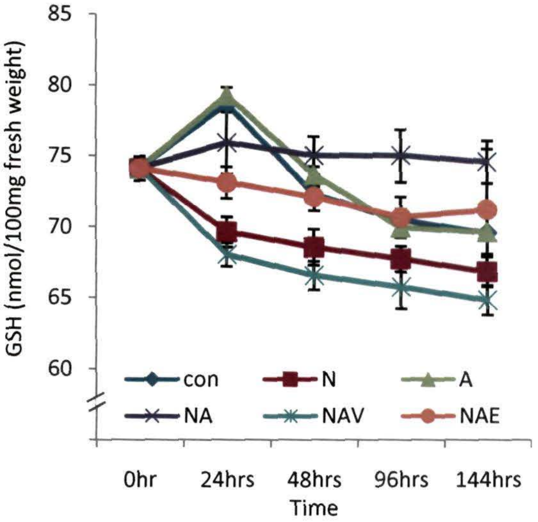

Fig. 4.29.b

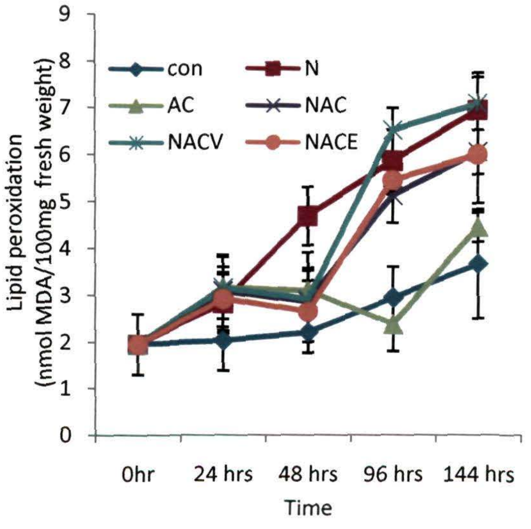

Fig. 4.28.c

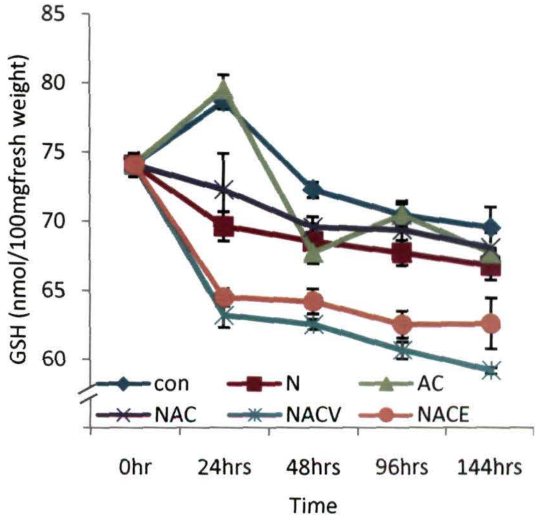

Fig. 4.29.c

NaCl and either 10 mM $CaCl_2$ or 100 μM ABA showed only two fold increase in tissue level of malondialdehyde during the 144 hours of incubation. However, seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl, and 10 mM $CaCl_2$ or 200 mM NaCl, and 100 μM ABA showed a significantly higher malondialdehyde levels than the corresponding controls which were not pretreated with either verapamil or EGTA. These seedlings registered nearly 3 fold increase in the level of tissue level of malondialdehyde during the 144 hours of incubation (Table 4.29; Fig. 4.28).

Expressed as percent of fresh weight, there was a marginal decrease in the shoot tissue GSH content of the seedlings with progressing time during the 144 hours of incubation in Hoagland's nutrient lacking any additional supplements. The decrease in GSH content was, however, significantly more pronounced in seedlings incubated in Hoagland's nutrient solution supplemented with 200 mM NaCl (Table 4.30; Fig. 4.29). On the other hand, seedlings incubated in the nutrient solution supplemented with 200 mM NaCl and either 10 mM $CaCl_2$ or 100 μM ABA showed marginally higher tissue level of GSH than those incubated in nutrient solution supplemented with only 200 mM NaCl (Table 4.30; Fig. 4.29). However, seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed a marginally lower GSH content than the corresponding controls which were not pretreated with either verapamil or EGTA (Table 4.30; Fig. 4.29).

Expressed as percent of fresh weight, the AsA content in shoot tissues of seedlings cultured in Hoagland's nutrient solution lacking any additional supplements showed a marginal increase with progressing time during the 144 hours of incubation. The increase in AsA content was, however, significantly more pronounced in seedlings which were incubated in Hoagland's nutrient solution supplemented with 200 mM NaCl than the untreated controls (Table 4.31; Fig. 4.30). On the other hand, seedlings cultured in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed marked higher AsA content than those cultured in nutrient solution supplemented with only 200 mM NaCl (Table 4.31; Fig. 4.30). However, seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed a marginally higher AsA content than the corresponding controls which were not pretreated with either verapamil or EGTA (Table 4.31; Fig.4.30).

There was no marked change in the activity of glutathione reductase in the leaf tissues of seedlings cultured in Hoagland nutrient solution lacking any additional supplements during the 144 hours of incubation (Table 4.32; Fig.4.31). The activity of GR in shoot tissues of seedlings cultured in Hoagland's nutrient solution supplemented with either 10 mM $CaCl_2$ or 100 µM ABA showed the same pattern as that observed in seedlings cultured in nutrient solution without any supplements. However, seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride showed a marked increase of shoot GR activity during the initial 48 hours after transfer to nutrient solution. After 48 hours the activity of the enzyme

Table 4.31: Effect of 200mM NaCl (Na$^+$), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the content of ascorbate (AsA) (μmol 100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	Ascorbate (AsA) (μmol 100mg fresh weight^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	0.203±0.004	0.204±0.006	0.205±0.004	0.206±0.008	0.205±0.008
Na$^+$	-	0.206±0.003	0.208±0.005	0.209±0.005	0.206±0.004
Ca^{++}	-	0.209±0.007	0.212±0.014	0.212±0.024	0.213±0.015
Na$^+$+Ca^{++}	-	0.209±0.003	0.209±0.013	0.211±0.014	0.212±0.010
Na$^+$+Ca^{++}+Ver	-	0.206±0.003	0.207±0.005	0.208±0.003	0.209±0.014
Na$^+$+Ca^{++}+EGTA	-	0.208±0.005	0.210±0.015	0.213±0.004	0.214±0.004
ABA	-	0.209±0.004	0.211±0.016	0.212±0.003	0.213±0.004
Na$^+$+ABA	-	0.207±0.010	0.208±0.005	0.210±0.008	0.211±0.008
Na$^+$+ABA+Ver	-	0.207±0.008	0.207±0.012	0.208±0.007	0.209±0.006
Na$^+$+ABA+EGTA	-	0.209±0.007	0.211±0.014	0.211±0.015	0.212±0.006
ABA+Ca^{++}	-	0.209±0.003	0.210±0.015	0.212±0.007	0.212±0.010
Na$^+$+ABA+Ca^{++}	-	0.203±0.003	0.204±0.008	0.205±0.005	0.205±0.006
Na$^+$+ABA+Ca^{++}+Ver	-	0.201±0.005	0.202±0.004	0.204±0.006	0.204±0.017
Na$^+$+ABA+Ca^{++}+EGTA	-	0.202±0.003	0.202±0.006	0.205±0.003	0.205±0.003

Table 4.32: Effect of 200mM NaCl (Na$^+$), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the glutathione reductase (GR) activity (μmol mg protein^{-1} min^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	GR activity (μmol mg protein^{-1} min^{-1})				
	0hr	24 hr	48 hr	96 hr	144 hr
control	2.38±0.02	2.36±0.05	1.83±0.08	1.87±0.35	2.01±0.35
Na$^+$	-	4.04±0.06	4.34±0.23	3.31±0.09	2.48±0.42
Ca^{++}	-	2.20±0.10	1.98±0.40	2.02±0.51	2.26±0.19
Na$^+$+Ca^{++}	-	3.38±0.22	2.68±0.48	2.65±0.29	3.36±0.32
Na$^+$+Ca^{++}+Ver	-	2.10±0.28	1.98±0.43	2.15±0.38	2.35±0.35
Na$^+$+Ca^{++}+EGTA	-	3.87±0.15	3.35±0.34	2.51±0.35	1.68±0.40
ABA	-	2.56±0.13	2.01±0.41	2.11±0.16	2.23±0.29
Na$^+$+ABA	-	3.00±0.40	3.38±0.44	2.90±0.33	3.05±0.28
Na$^+$+ABA+Ver	-	1.61±0.26	1.21±0.27	1.22±0.18	1.25±0.36
Na$^+$+ABA+EGTA	-	3.97±0.29	3.20±0.33	2.31±0.18	1.52±0.35
ABA+Ca^{++}	-	2.33±0.43	2.40±0.42	2.07±0.36	2.38±0.44
Na$^+$+ABA+Ca^{++}	-	2.64±0.17	2.74±0.28	2.90±0.27	2.97±0.37
Na$^+$+ABA+Ca^{++}+Ver	-	2.61±0.20	2.48±0.45	2.69±0.49	2.66±0.23
Na$^+$+ABA+Ca^{++}+EGTA	-	2.66±0.26	2.60±0.18	2.75±0.26	2.69±0.30

± SEM

Figure 4.30: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the content of ascorbate (AsA) (µmol 100mg fresh weight^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.31: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the glutathione reductase (GR) activity (µmol mg protein^{-1} min^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

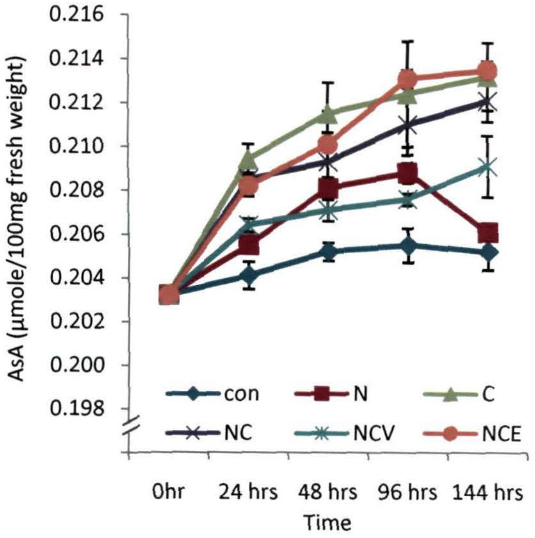

Fig. 4.30.a

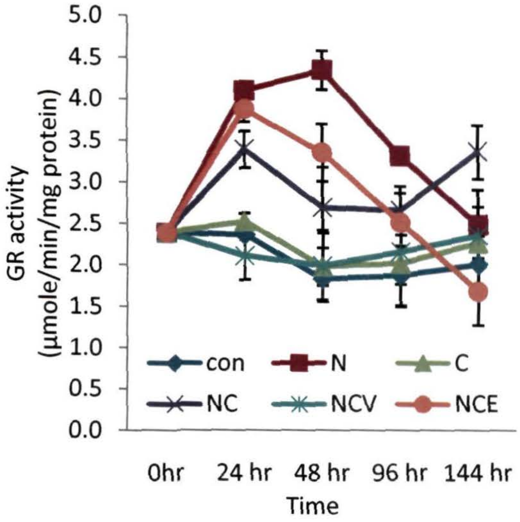

Fig. 4.31.a

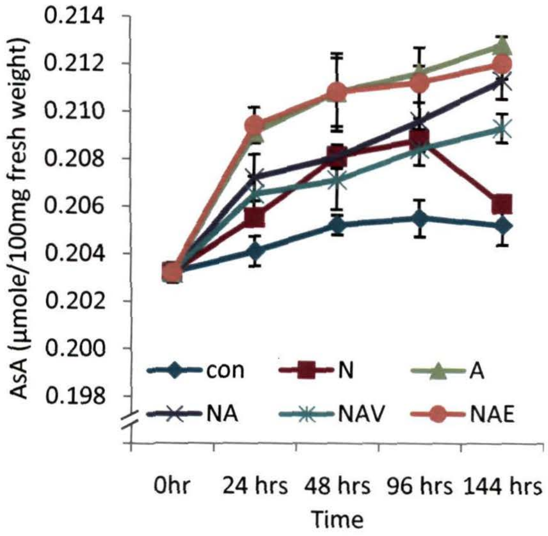

Fig. 4.30.b

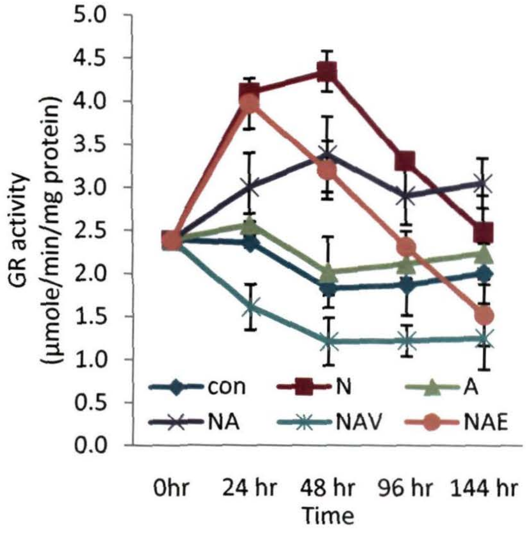

Fig. 4.31.b

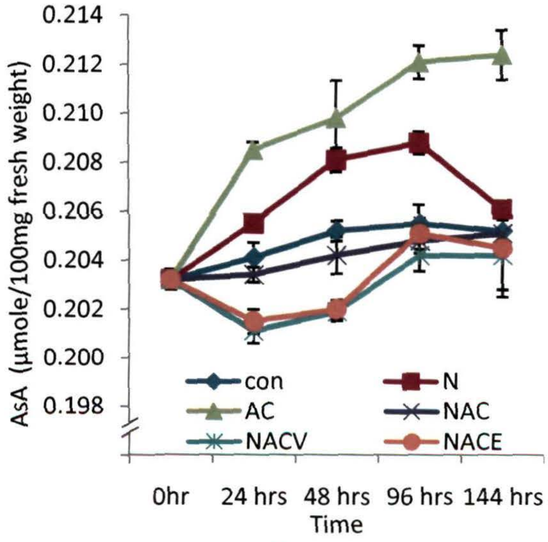

Fig. 4.30.c

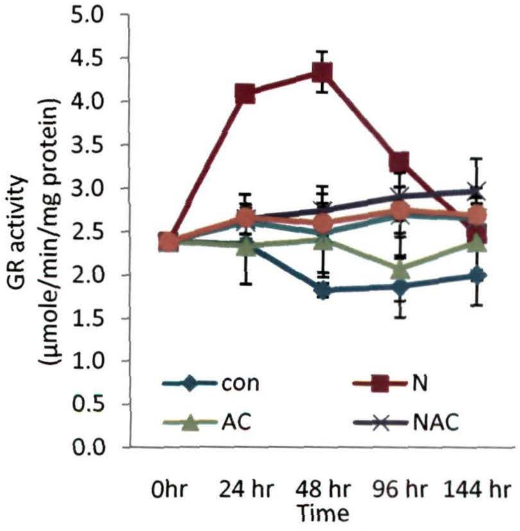

Fig. 4.31.c

showed a marked decrease with progressing time till 144 hours of incubation (Table 4.32; Fig.4.31). On the other hand, seedlings cultured in nutrient solution supplemented with 200 mM NaCl and 10mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed a marginal increase in GR activity with progressing time till 144 hours of incubation . At 144 hours of incubation there was no significant difference in the activity of GR between the seedlings which were cultured in nutrient solution supplemented with NaCl alone and those cultured in nutrient solution supplemented with 200 mM NaCl and 10mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA (Table 4.32; Fig.4.31). Seedlings pretreated with 1 mM verapamil before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ showed the same pattern of change in GR activity as the untreated controls. On the other hand seedlings pretreated with 1 mM verapamil before incubation in nutrient solution supplemented with 200 mM NaCl and 100 µM ABA showed a marginal decrease in the activity of GR with progressing time during the 144 hours of incubation. Seedlings pretreated with EGTA before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed a rapid increase in the activity of GR during the initial 24 hours of incubation. After 24 hours, the activity of the enzyme showed a rapid decrease with progressing time till 144 hours of incubation (Table 4.32; Fig.4.31).

There was no marked change in the activity of ascorbate peroxidase in seedlings cultured in Hoagland's nutrient solution lacking any additional supplements during the 144 hours of incubation. However, seedlings cultured in Hoagland's nutrient solution containing either 10 mM $CaCl_2$ or 100 µM ABA showed a marginal increase in the activity of the enzyme during the 144 hours of incubation. On the other hand, seedlings incubated in nutrient solution containing 200 mM sodium chloride showed a marked increase in the activity of the enzyme during the 144 hours of treatment. The seedlings registered more than 2 fold increase in APX activity during the 144 hours of incubation (Table 4.33; Fig. 4.32). Compared to that in the seedlings cultured in nutrient solution supplemented with 200 mM NaCl the activity of the enzyme in seedlings incubated in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ showed a 130% increase during the 144 hours of incubation (Table 4.33; Fig.4.32). Seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed markedly lower activity of ascorbate peroxidase than those which were not pretreated with either verapamil or EGTA (Table 4.33; Fig. 4.32).

In relation to progressing time, the activity of catalase, in seedlings cultured in Hoagland's nutrient solution which did not contain any additional supplements or those cultured in nutrient solution supplemented with either 10 mM $CaCl_2$, showed a marginal increase till 48 hours of incubation after which it decreased progressively with progressing time till 144 hours (Table 4.34; Fig.4.33). On the other hand seedlings incubated in nutrient solution containing 200 mM sodium chloride or those incubated in nutrient solution supplemented with 100 µM ABA showed a 2 fold increase of CAT activity by 48 hours of treatment. After 48 hours the activity of the enzyme showed a significant decrease with progressing time till 144 hours (Table 4.34; Fig.4.33). However, seedlings incubated in the nutrient solution supplemented

Table 4.33: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the ascorbate peroxidase (APX) activity (mmol mg $protein^{-1}$ min^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	APX activity (mmol mg $protein^{-1}$ min^{-1})				
	0hr	24hr	48hr	96hr	144hr
control	0.243±0.028	0.195±0.014	0.212±0.040	0.186±0.032	0.259±0.050
Na^+	-	0.427±0.013	0.442±0.057	0.524±0.030	0.564±0.032
Ca^{++}	-	0.214±0.061	0.254±0.030	0.236±0.031	0.246±0.030
Na^++Ca^{++}	-	0.371±0.064	0.564±0.029	0.547±0.035	0.586±0.029
Na^++Ca^{++}+Ver	-	0.275±0.057	0.350±0.057	0.335±0.026	0.340±0.028
Na^++Ca^{++}+EGTA	-	0.320±0.070	0.350±0.081	0.312±0.041	0.285±0.028
ABA	-	0.311±0.085	0.324±0.030	0.319±0.049	0.361±0.078
Na^++ABA	-	0.461±0.037	0.438±0.038	0.336±0.030	0.321±0.078
Na^++ABA+Ver	-	0.421±0.066	0.356±0.030	0.298±0.111	0.281±0.045
Na^++ABA+EGTA	-	0.315±0.045	0.345±0.028	0.301±0.055	0.254±0.030
ABA+Ca^{++}	-	0.267±0.035	0.331±0.020	0.468±0.041	0.497±0.045
Na^++ABA+Ca^{++}	-	0.272±0.036	0.289±0.021	0.328±0.040	0.345±0.058
Na^++ABA+Ca^{++}+Ver	-	0.242±0.041	0.245±0.028	0.295±0.028	0.252±0.052
Na^++ABA+Ca^{++}+EGTA	-	0.252±0.041	0.259±0.049	0.315±0.028	0.284±0.040

± SEM

Table 4.34: Effect of 200mM NaCl (Na^+), 100μM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the catalase (CAT) activity (μmol mg $protein^{-1}$ min^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	CAT activity (μmol mg $protein^{-1}$ min^{-1})				
	0hr	24 hr	48 hr	96 hr	144 hr
control	0.639±0.05	0.975±0.03	1.037±0.04	0.873±0.04	0.812±0.06
Na^+	-	0.871±0.05	1.195±0.06	0.879±0.06	0.396±0.03
Ca^{++}	-	0.812±0.04	0.825±0.03	0.845±0.10	0.816±0.03
Na^++Ca^{++}	-	0.665±0.03	0.636±0.03	0.645±0.05	0.632±0.07
Na^++Ca^{++}+Ver	-	0.850±0.07	0.835±0.02	0.845±0.03	0.855±0.07
Na^++Ca^{++}+EGTA	-	0.915±0.03	0.810±0.05	0.821±0.06	0.819±0.06
ABA	-	0.885±0.03	1.120±0.06	0.875±0.05	0.556±0.04
Na^++ABA	-	0.642±0.04	0.641±0.05	0.642±0.06	0.643±0.04
Na^++ABA+Ver	-	0.716±0.03	0.722±0.04	0.727±0.04	0.518±0.04
Na^++ABA+EGTA	-	0.911±0.05	0.816±0.03	0.822±0.05	0.827±0.04
ABA+Ca^{++}	-	0.659±0.06	0.718±0.04	1.135±0.05	1.017±0.04
Na^++ABA+Ca^{++}	-	1.040±0.04	0.932±0.04	0.841±0.06	0.759±0.05
Na^++ABA+Ca^{++}+Ver	-	1.031±0.04	0.721±0.07	0.519±0.05	0.641±0.10
Na^++ABA+Ca^{++}+EGTA	-	1.115±0.03	0.851±0.11	0.573±0.04	0.686±0.03

± SEM

Figure 4.32. Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the ascorbate peroxidase (APX) activity (mmol mg protein^{-1} min^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.33. Effect of 200mM NaCl (N), 100μM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the catalase (CAT) activity (μmol mg protein^{-1} min^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

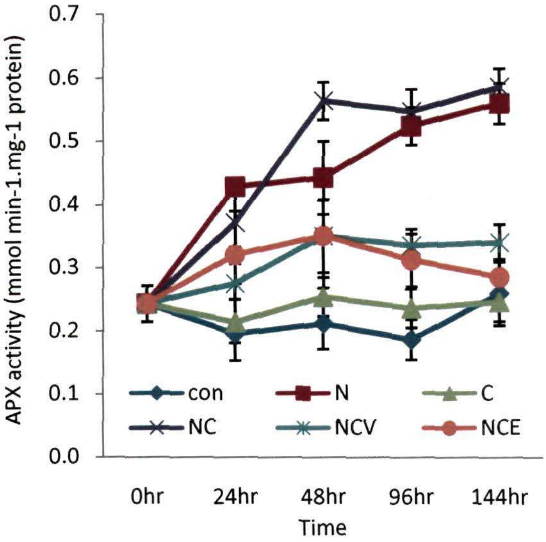

Fig. 4.32.a

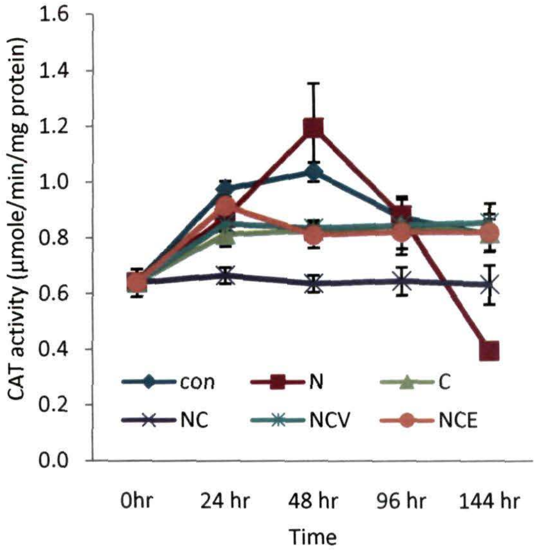

Fig. 4.33.a

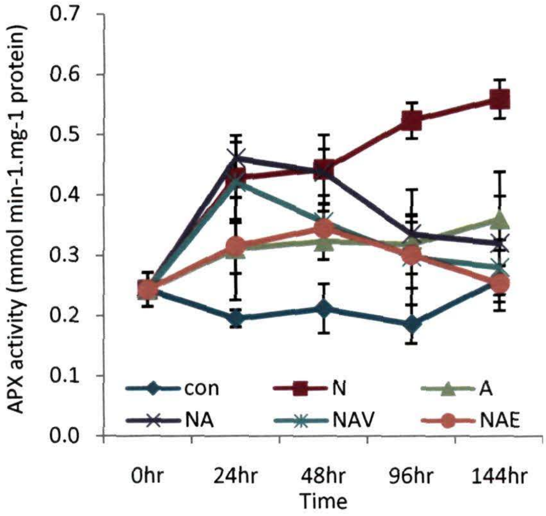

Fig. 4.32.b

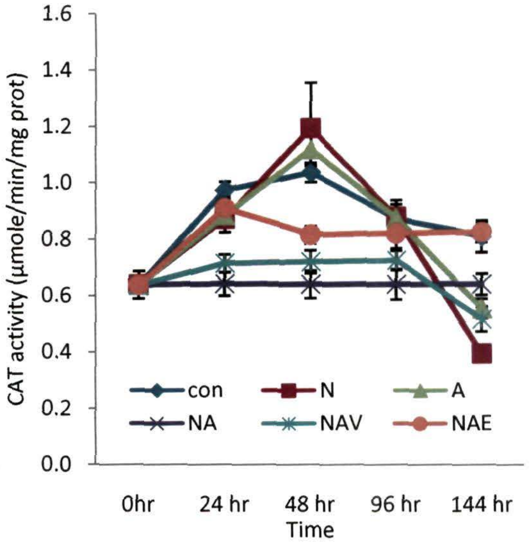

Fig. 4.33.b

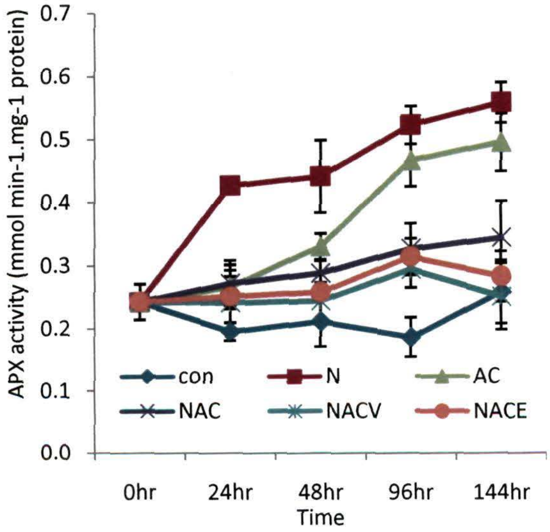

Fig. 4.32.c

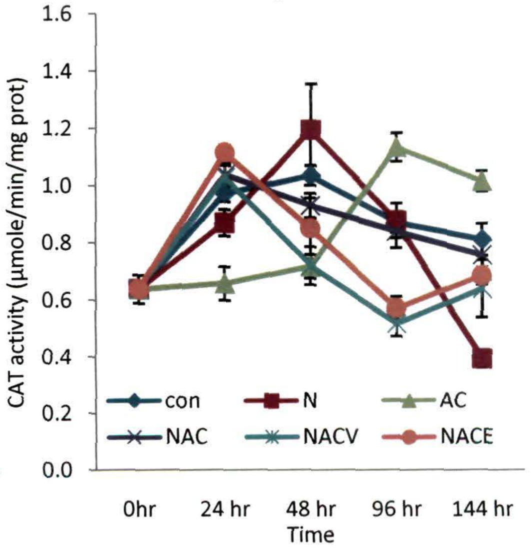

Fig. 4.33.c

with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed no marked change in the activity of the enzyme during the 144 hours of incubation. Seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl, and 10 mM $CaCl_2$ or 200 mM NaCl, and 100 µM ABA registered a marginally higher CAT activity than those which were not pretreated with either verapamil or EGTA (Table 4.34; Fig.4.33).

In relation to progressing time, the activity of superoxide dismutase, in seedlings cultured in Hoagland's nutrient solution which did not contain any additional supplements, showed an almost 2 fold decrease during the 144 hours of incubation in the nutrient solution (Table 4.35; Fig. 4.34). Seedlings cultured in nutrient solution supplemented with either 10 mM $CaCl_2$, showed a marginal increase till 48 hours of incubation after which it decreased progressively with progressing time till 144 hours. On the other hand, seedlings cultured in nutrient solution supplemented with either 10 mM $CaCl_2$ or 100 µM ABA showed a marginal increase in SOD activity of the shoot tissue during 144 hours of incubation. Seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride also showed a marginal increase of SOD activity during 144 hours of incubation. After showing a marginal decrease in the activity of SOD during initial 24 hours of treatment, seedlings incubated in the nutrient solution supplemented with 200 mM NaCl and either 10 mM $CaCl_2$ or 100 µM ABA registered almost 2 fold increase in SOD activity of the shoot tissues between 24 and 144 hours of incubation (Table 4.35; Fig. 4.34). Compared to these seedlings, those incubated in nutrient solution supplemented with 200 mM NaCl showed markedly lower activity of SOD. While the seedlings pretreated with 1 mM verapamil before incubation in nutrient solution supplemented with 200 mM NaCl, and 10 mM $CaCl_2$ or 200 mM NaCl, and 100 μM ABA registered a marginally higher SOD activity than those which were not receiving any pretreatment, those seedlings pretreated with EGTA before incubation in nutrient solution supplemented with 200 mM NaCl, and 10 mM $CaCl_2$ or 200 mM NaCl, and 100 μM ABA registered a marginally lower SOD activity than those which were not receiving any pretreatment (Table 4.33; Fig. 4.32).

In relation to progressing time there was a consistent increase in the activity of peroxidase in shoot tissues of seedlings cultured in Hoagland's nutrient solution which lacked any additional supplements or those cultured in Hoagland's nutrient solution supplemented with either 10 mM $CaCl_2$ or 100 μM ABA (Table 4.36; Fig. 4.35). However, seedlings incubated in nutrient solution supplemented with 200 mM sodium chloride also showed a marked increase of shoot POX activity with increase in time during 144 hours of treatment; the magnitude of increase in the activity of the enzyme in these seedlings was significantly more pronounced than the untreated controls. Seedlings cultured in nutrient solution containing NaCl along with $CaCl_2$ or ABA showed a marginal decrease in the activity of the enzyme during the initial 48 hours of treatment after which it showed a sharp increase with progressing time till 144 hours (Table 4.36; Fig. 4.35). However, while the seedlings pretreated with 1 mM verapamil before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed a marginally lower activity of peroxidase than those which were not receiving any pretreatment.

Table 4.35: Effect of 200mM NaCl (Na^{+}), 100µM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the superoxide dismutase (SOD) activity (U mg protein^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	SOD activity (U mg protein^{-1})				
	0hr	24hr	48hr	96hr	144hr
control	210.23±19.07	206.75±34.27	190.31±26.73	190.16±07.42	123.77±18.85
Na^{+}	-	148.72±26.67	241.04±03.82	245.12±19.04	252.54±07.49
Ca^{++}	-	159.65±26.56	251.45±19.09	252.36±01.21	256.74±15.14
Na^{+}+Ca^{++}	-	154.12±41.94	174.41±26.73	363.20±03.86	357.18±11.22
Na^{+}+Ca^{++}+Ver	-	211.12±15.21	245.14±26.73	239.84±11.32	256.68±15.00
Na^{+}+Ca^{++}+EGTA	-	162.23±15.17	181.58±11.46	245.29±22.60	251.71±11.42
ABA	-	154.25±26.56	241.85±15.28	248.21±15.24	250.12±15.24
Na^{+}+ABA	-	151.25±15.10	162.85±11.46	301.84±07.50	306.94±15.18
Na^{+}+ABA+Ver	-	189.72±19.03	221.04±15.28	255.12±19.04	282.54±07.49
Na^{+}+ABA+EGTA	-	202.12±19.02	233.13±15.28	188.57±18.85	135.67±18.86
ABA+Ca^{++}	-	211.13±15.17	232.23±11.46	220.33±07.53	258.62±22.88
Na^{+}+ABA+Ca^{++}	-	199.12±26.65	210.13±15.28	258.57±18.85	266.67±15.07
Na^{+}+ABA+Ca^{++}+Ver	-	201.25±22.74	226.12±19.09	182.21±11.42	119.14±07.64
Na^{+}+ABA+Ca^{++}+EGTA	-	189.65±26.56	204.56±26.73	142.51±22.88	78.36±19.10

± SEM

Table 4.36: Effect of 200mM NaCl (Na^{+}), 100µM ABA and 10mM $CaCl_2$ (Ca^{++}) and combinations of the above on the peroxidase (POX) activity (U mg protein^{-1}) in shoots of seedlings of maize var. RCM1-1 without any pretreatment or after pretreatment with verapamil (+ver) /EGTA (+EGTA). The seedlings were harvested after 24, 48, 96 and 144 hours of culture in the nutrient solution.

Treatments	POX activity (U mg protein^{-1})				
	0hr	24 hrs	48 hrs	96 hrs	144 hrs
control	7.31±0.377	7.24±0.528	7.54±0.609	7.62±0.393	7.82±0.318
Na^{+}	-	8.40±0.637	8.58±0.432	8.65±0.461	8.84±0.225
Ca^{++}	-	7.54±0.252	8.05±0.422	8.00±0.315	8.16±0.415
Na^{+}+Ca^{++}	-	7.85±0.327	8.26±0.574	8.45±0.417	8.74±0.312
Na^{+}+Ca^{++}+Ver	-	7.12±0.273	7.05±0.503	7.80±0.304	8.80±0.491
Na^{+}+Ca^{++}+EGTA	-	8.50±0.423	8.71±0.396	8.95±0.378	9.42±0.192
ABA	-	7.52±0.375	7.68±0.511	7.89±0.244	7.91±0.294
Na^{+}+ABA	-	7.69±0.293	7.89±0.387	7.92±0.325	8.59±0.256
Na^{+}+ABA+Ver	-	8.58±0.384	8.88±0.293	9.15±0.349	9.24±0.470
Na^{+}+ABA+EGTA	-	8.60±0.520	8.52±0.467	8.54±0.351	8.35±0.588
ABA+Ca^{++}	-	8.50±0.532	8.55±0.433	8.60±0.415	8.62±0.449
Na^{+}+ABA+Ca^{++}	-	8.48±0.394	8.65±0.459	8.86±0.288	8.98±0.352
Na^{+}+ABA+Ca^{++}+Ver	-	8.35±0.572	8.60±0.474	8.65±0.442	8.75±0.309
Na^{+}+ABA+Ca^{++}+EGTA	-	8.55±0.506	8.70±0.422	8.90±0.322	8.90±0.324

± SEM

Figure 4.34: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the superoxide dismutase (SOD) activity (U mg protein^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

Figure 4.35: Effect of 200mM NaCl (N), 100µM ABA (A) and 10mM $CaCl_2$ (C) and combinations of above on the peroxidase (POX) activity (U mg protein^{-1}) in shoots of seedlings of maize var. RCM1-1 after 24, 48, 96 and 144 hours of treatment. con: untreated control V: seedlings pretreated with 1 mM verapamil before transfer to culture medium; E: seedlings pretreated with 10 mM EGTA before transfer to culture medium. Vertical bars represent SEM.

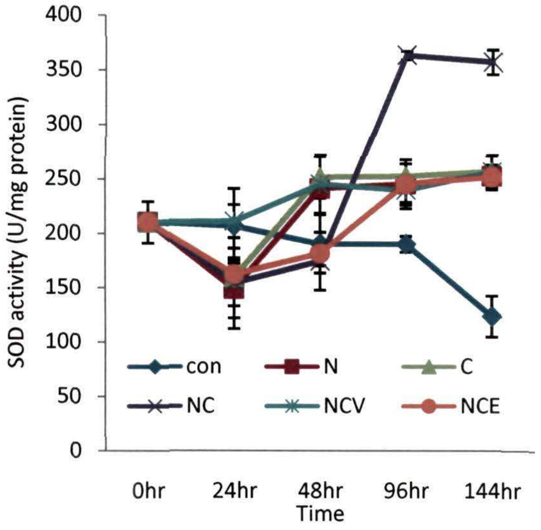

Fig. 4.34.a

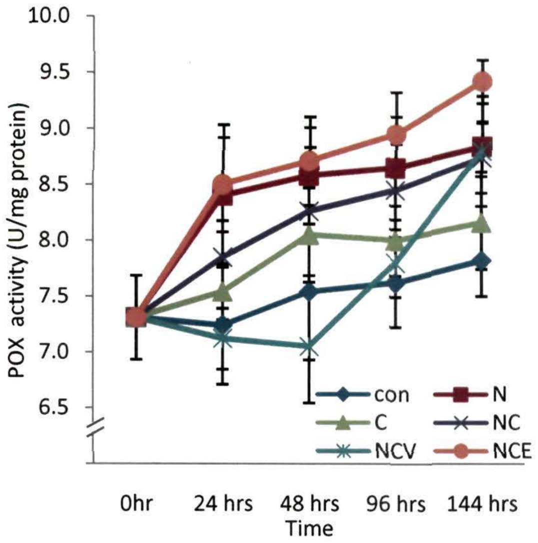

Fig. 4.35.a

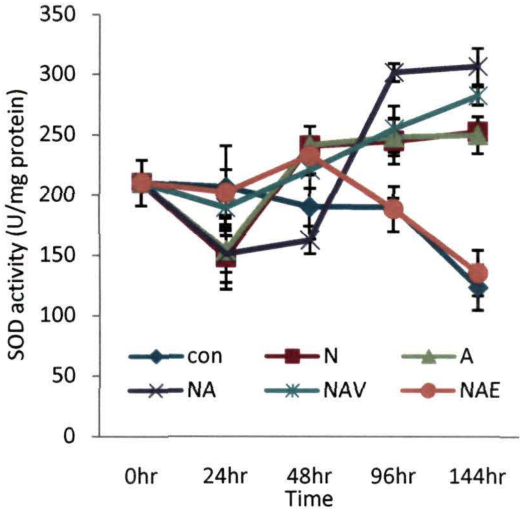

Fig. 4.34.b

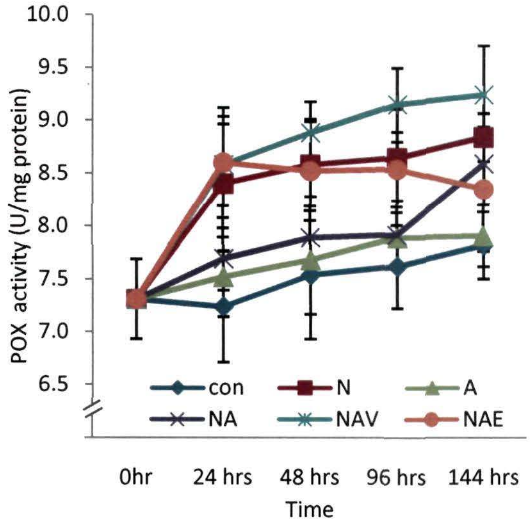

Fig. 4.35.b

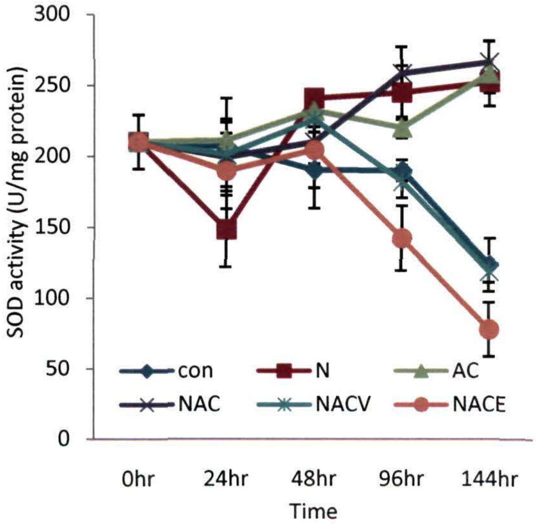

Fig. 4.34.c

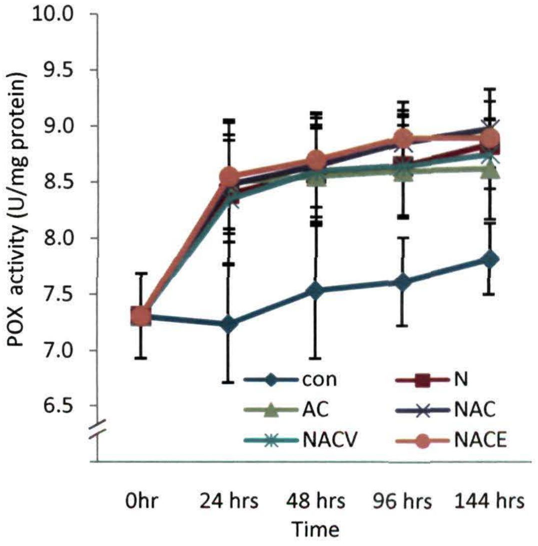

Fig. 4.35.c

On the other hand, seedlings pretreated with EGTA before incubation in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 μM ABA showed markedly higher activity of the enzyme than those which were not receiving any pretreatment (Table 4.36; Fig. 4.35).

The SDS-PAGE profile of total soluble proteins of maize seedlings (var. RCM 1-1) receiving various treatments are presented in (Plate 5). SDS-PAGE profiles of both untreated controls as well as the treated seedlings showed similar pattern with regard to the number of bands as well as their size. However, differences were observed in the intensities of the protein bands between different treatments. The intensity of the two major bands with high molecular weights of 50kD and 90kD was observed to be more intense in seedlings receiving NaCl than the untreated controls. Correspondingly, the intensity of the bands representing molecular mass of 50 and 90 kDa was lower in the seedlings cultured in nutrient solution supplemented with 200 mM NaCl and 100 μM ABA or NaCl and 10 mM $CaCl_2$.

Isozyme profiles of APX from leaf tissues of maize seedling revealed the presence of 3 activity zones (Plate 6). The intensity of activity zones was higher in seedlings cultured in nutrient solution supplemented with 200 mM NaCl but lower in seedlings cultured in nutrient solution supplemented with NaCl and 10 mM $CaCl_2$ or NaCl and 100 μM ABA. Isozyme profiles of CAT revealed the presence of one isoenzyme of CAT in the leaf tissues of maize seedlings (Plate 7). Compared to the untreated controls, the extracts from seedlings cultured in nutrient solution supplemented with 200 mM NaCl showed higher intensity of CAT isozyme. Isozyme profiles of superoxide dismutase from leaf tissues of maize seedling revealed the presence of 5 isoenzymes of SOD in the leaf tissues of maize seedlings (Plate 8). These isozymes were identified as MnSOD, FeSOD (I and II) and Cu/ZnSOD (I and II) by using KCN to inhibit Cu/ZnSOD and H_2O_2 to inhibit both Cu/ZnSOD and FeSOD. Isozyme profiles of POX from leaf tissues of the maize seedlings revealed the presence of 4 activity zones corresponding to 4 isozymes of the enzyme (Plate 9). The intensity of the bands was greater in extracts from leaf tissues of seedlings cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl.

SDS-PAGE profiles of soluble protein from leaves of maize (*Zea mays* L.) seedlings cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (N); seedlings cultured in Hoagland's nutrient solution containing 10 mM $CaCl_2$ (C); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ (NC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCE); seedlings cultured in Hoagland's nutrient solution containing 100µM ABA (A); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 100µM ABA (NA); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA (NAV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA (NAE); seedlings cultured in Hoagland's nutrient solution containing 100µM ABA, 10 mM $CaCl_2$ (AC); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl,100µM ABA and 10 mM $CaCl_2$ (NAC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA and10 mM $CaCl_2$ (NACV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA and 10 mM $CaCl_2$ (NACE). The seedlings were harvested at 24 hours (a), 48 hours (b), 96 hours (c) and 144 hours (d) of treatment. Con: untreated control.

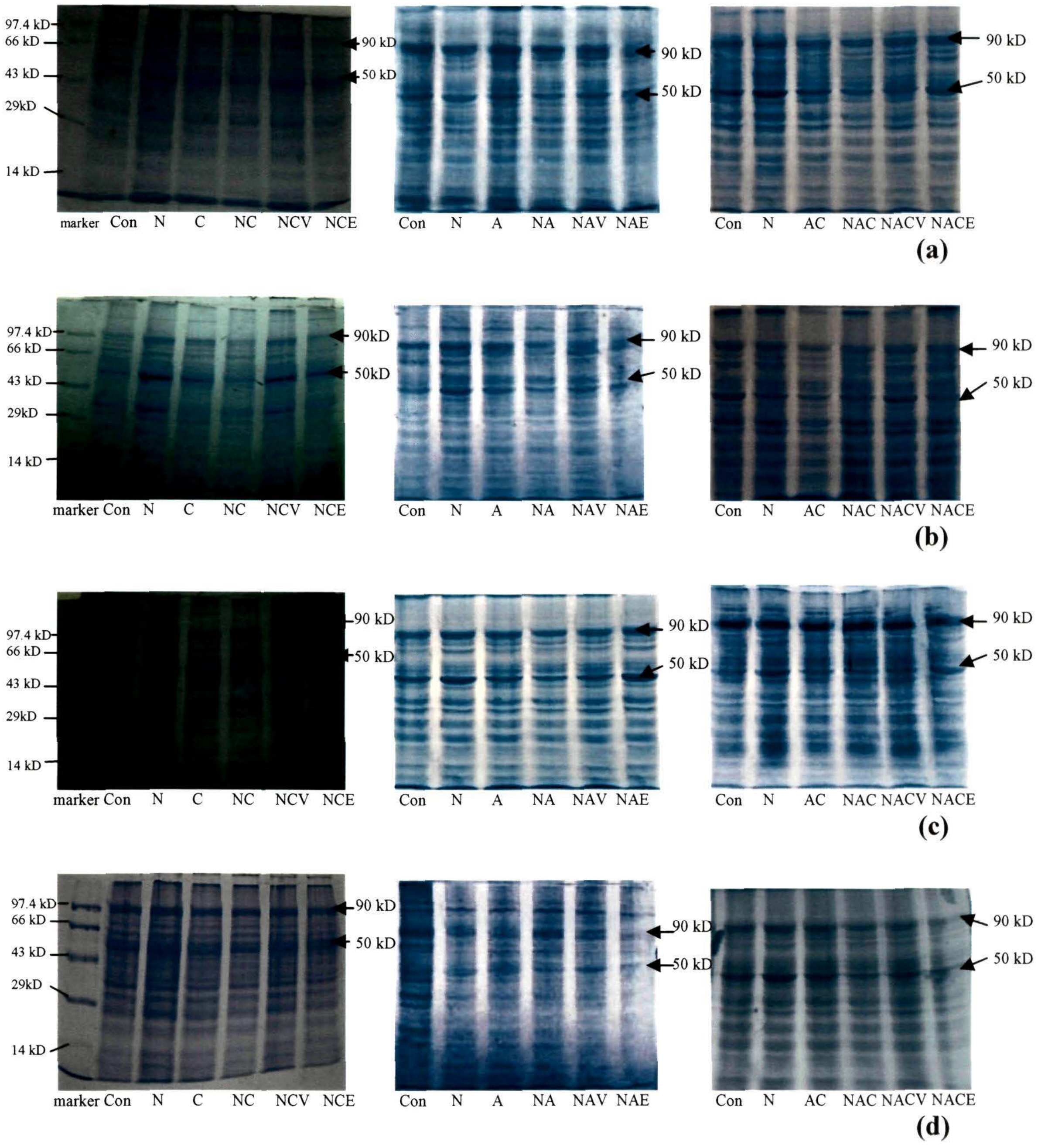
97.4 kD
66 kD
43 kD
29kD
14 kD
90 kD
50 kD
marker
Con
N
C
NC
NCV
NCE
90 kD
50 kD
Con
N
A
NA
NAV
NAE
90 kD
50 kD
Con
N
AC
NAC
NACV
NACE
(a)
97.4 kD
66 kD
43 kD
29kD
14 kD
90kD
50kD
marker
Con
N
C
NC
NCV
NCE
90 kD
50 kD
Con
N
A
NA
NAV
NAE
90 kD
50 kD
Con
N
AC
NAC
NACV
NACE
(b)
97.4 kD
66 kD
43 kD
29kD
14 kD
90 kD
50 kD
marker
Con
N
C
NC
NCV
NCE
90 kD
50 kD
Con
N
A
NA
NAV
NAE
90 kD
50 kD
Con
N
AC
NAC
NACV
NACE
(c)
97.4 kD
66 kD
43 kD
29kD
14 kD
90 kD
50 kD
marker
Con
N
C
NC
NCV
NCE
90 kD
50 kD
Con
N
A
NA
NAV
NAE
90 kD
50 kD
Con
N
AC
NAC
NACV
NACE
(d)

PLATE 5

APX isozymes from leaves of maize (*Zea mays* L.) seedlings cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (N); seedlings cultured in Hoagland's nutrient solution containing 10 mM $CaCl_2$ (C); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ (NC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCE); seedlings cultured in Hoagland's nutrient solution containing 100μM ABA (A); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 100μM ABA (NA); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100μM ABA (NAV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100μM ABA (NAE); seedlings cultured in Hoagland's nutrient solution containing 100μM ABA, 10 mM $CaCl_2$ (AC); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl,100μM ABA and 10 mM $CaCl_2$ (NAC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100μM ABA and10 mM $CaCl_2$ (NACV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100μM ABA and 10 mM $CaCl_2$ (NACE). The seedlings were harvested at 24 hours (a), 48 hours (b), 96 hours (c) and 144 hours (d) of treatment. Con: untreated control.

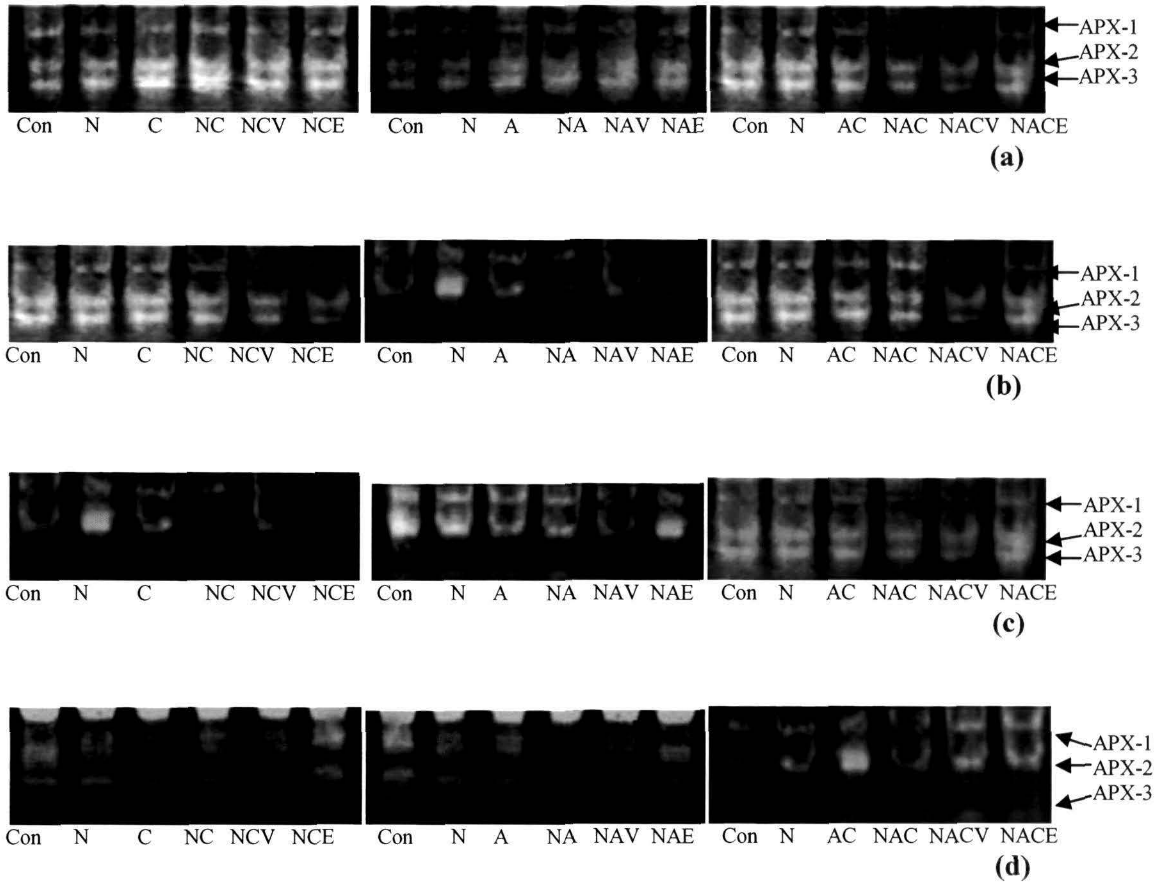
APX-1
APX-2
APX-3
Con N C NC NCV NCE
Con N A NA NAV NAE
Con N AC NAC NACV NACE
(a)
APX-1
APX-2
APX-3
Con N C NC NCV NCE
Con N A NA NAV NAE
Con N AC NAC NACV NACE
(b)
APX-1
APX-2
APX-3
Con N C NC NCV NCE
Con N A NA NAV NAE
Con N AC NAC NACV NACE
(c)
APX-1
APX-2
APX-3
Con N C NC NCV NCE
Con N A NA NAV NAE
Con N AC NAC NACV NACE
(d)

PLATE 6

CAT isozymes from leaves of maize (*Zea mays* L.) seedlings cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (N); seedlings cultured in Hoagland's nutrient solution containing 10 mM $CaCl_2$ (C); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ (NC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCE); seedlings cultured in Hoagland's nutrient solution containing 100μM ABA (A); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 100μM ABA (NA); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100μM ABA (NAV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100μM ABA (NAE); seedlings cultured in Hoagland's nutrient solution containing 100μM ABA, 10 mM $CaCl_2$ (AC); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl,100μM ABA and 10 mM $CaCl_2$ (NAC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100μM ABA and10 mM $CaCl_2$ (NACV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100μM ABA and 10 mM $CaCl_2$ (NACE). The seedlings were harvested at 24 hours (a), 48 hours (b), 96 hours (c) and 144 hours (d) of treatment. Con: untreated control.


Con N C NC NCV NCE
Con N A NA NAV NAE
Con N AC NAC NACV NACE
(a)
Con N C NC NCV NCE
Con N A NA NAV NAE
Con N AC NAC NACV NACE
(b)
Con N C NC NCV NCE
Con N A NA NAV NAE
Con N AC NAC NACV NACE
(c)
Con N C NC NCV NCE
Con N A NA NAV NAE
Con N AC NAC NACV NACE
(d)

PLATE 7

SOD isozymes from leaves of maize (*Zea mays* L.) seedlings cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (N); seedlings cultured in Hoagland's nutrient solution containing 10 mM $CaCl_2$ (C); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ (NC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCE); seedlings cultured in Hoagland's nutrient solution containing 100µM ABA (A); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 100µM ABA (NA); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA (NAV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA (NAE); seedlings cultured in Hoagland's nutrient solution containing 100µM ABA, 10 mM $CaCl_2$ (AC); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl,100µM ABA and 10 mM $CaCl_2$ (NAC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA and10 mM $CaCl_2$ (NACV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA and 10 mM $CaCl_2$ (NACE). The seedlings were harvested at 24 hours (a), 48 hours (b), 96 hours (c) and 144 hours (d) of treatment. Con: untreated control.

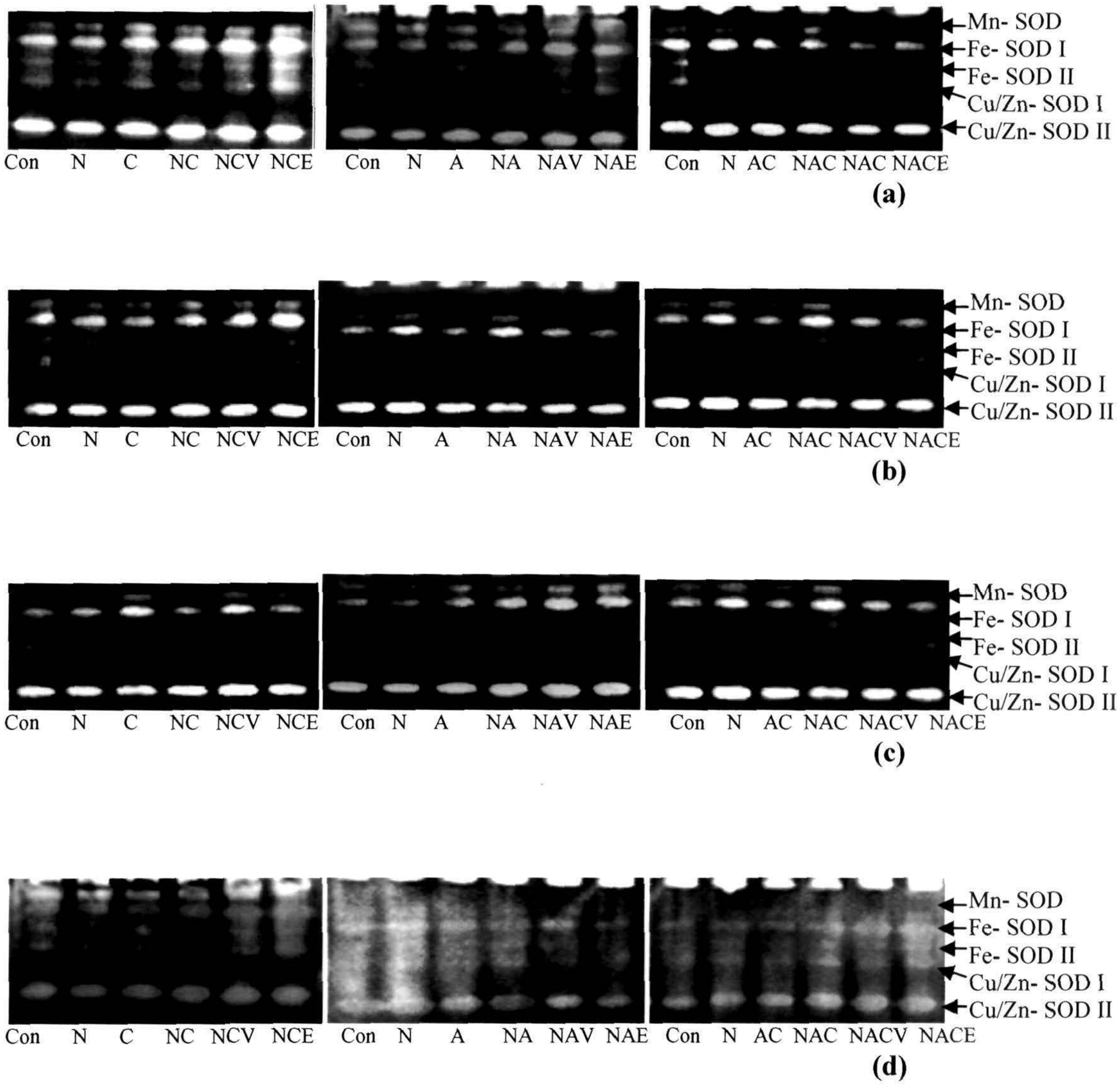
Mn- SOD
Fe- SOD I
Fe- SOD II
Cu/Zn- SOD I
Cu/Zn- SOD II
Con N C NC NCV NCE Con N A NA NAV NAE Con N AC NAC NAC NACE
(a)
Mn- SOD
Fe- SOD I
Fe- SOD II
Cu/Zn- SOD I
Cu/Zn- SOD II
Con N C NC NCV NCE Con N A NA NAV NAE Con N AC NAC NACV NACE
(b)
Mn- SOD
Fe- SOD I
Fe- SOD II
Cu/Zn- SOD I
Cu/Zn- SOD II
Con N C NC NCV NCE Con N A NA NAV NAE Con N AC NAC NACV NACE
(c)
Mn- SOD
Fe- SOD I
Fe- SOD II
Cu/Zn- SOD I
Cu/Zn- SOD II
Con N C NC NCV NCE Con N A NA NAV NAE Con N AC NAC NACV NACE
(d)

PLATE 8

POX isozymes from leaves of maize (*Zea mays* L.) seedlings cultured in Hoagland's nutrient solution supplemented with 200 mM NaCl (N); seedlings cultured in Hoagland's nutrient solution containing 10 mM $CaCl_2$ (C); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ (NC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 10 mM $CaCl_2$ (NCE); seedlings cultured in Hoagland's nutrient solution containing 100µM ABA (A); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl and 100µM ABA (NA); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA (NAV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA (NAE); seedlings cultured in Hoagland's nutrient solution containing 100µM ABA, 10 mM $CaCl_2$ (AC); seedlings cultured Hoagland's nutrient solution containing 200 mM NaCl,100µM ABA and 10 mM $CaCl_2$ (NAC); seedlings pretreated with 1mM Verapamil followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA and10 mM $CaCl_2$ (NACV) and seedlings pretreated with 10 mM EGTA followed by culture on Hoagland's nutrient solution containing 200 mM NaCl, 100µM ABA and 10 mM $CaCl_2$ (NACE). The seedlings were harvested at 24 hours (a), 48 hours (b), 96 hours (c) and 144 hours (d) of treatment. Con: untreated control.

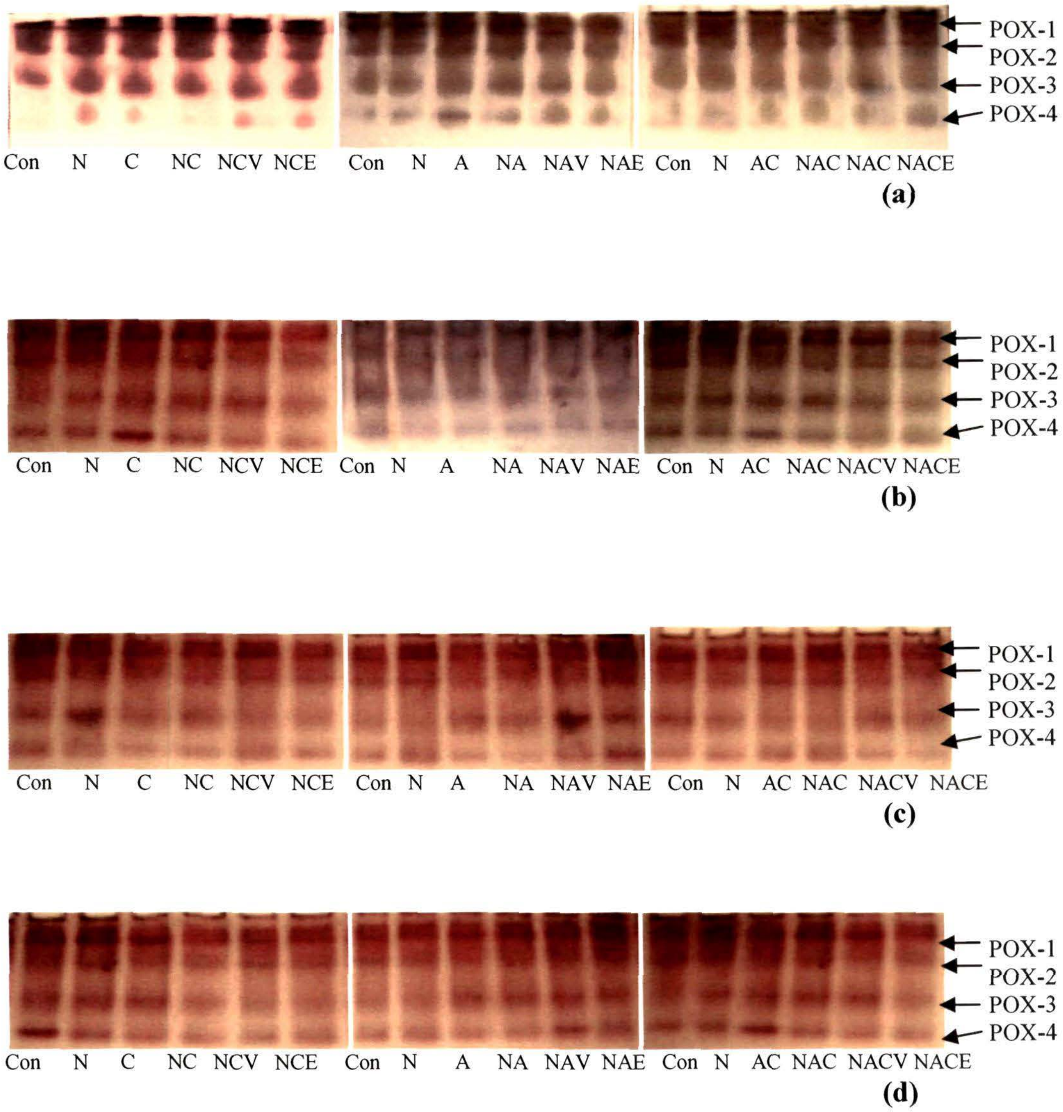
POX-1
POX-2
POX-3
POX-4
Con N C NC NCV NCE Con N A NA NAV NAE Con N AC NAC NAC NACE
(a)
POX-1
POX-2
POX-3
POX-4
Con N C NC NCV NCE Con N A NA NAV NAE Con N AC NAC NACV NACE
(b)
POX-1
POX-2
POX-3
POX-4
Con N C NC NCV NCE Con N A NA NAV NAE Con N AC NAC NACV NACE
(c)
POX-1
POX-2
POX-3
POX-4
Con N C NC NCV NCE Con N A NA NAV NAE Con N AC NAC NACV NACE
(d)

PLATE 9

Chapter: 5

DISCUSSION

Plants are frequently exposed to stress factors, which are defined as external conditions that adversely affect growth, development, or productivity. Response to stress may occur within few seconds (e.g., a change in the phosphorylation status of protein), within few minutes and hours (e.g., a change in gene expression) or several days (e.g., alterations in cellular ultrastructure). Salt stress affects many aspects of plant metabolism and, as a result, growth and yields are reduced (Pasternak *et al.*, 1995). Salinity stress is known to elicit complex effects on plant metabolism resulting from ion toxicity, water deficit, and nutrient imbalances (Pasternak, 1987; Werner and Finkelstein, 1995).

In the present investigation seedlings of *Zea mays* cultured in Hoagland's nutrient solution supplemented with different concentrations of NaCl showed markedly reduced shoot length, reduced RWC, decreased moisture content and a correspondingly higher % dry matter content. Thus, seedlings which were growing in nutrient solution supplemented with 250 mM NaCl showed an almost 2 fold reduction in shoot length compared to the untreated controls. These results are in agreement with similar observations made on lentil (Ashraf and Waheed, 1990), *Hordeum* spp. (Mano and Takeda, 1998), *Zea mays* (Çiçek and Cakirlar, 2003), *Phaseolus* spp. (Bayuelo-Jimenez *et al.*, 2002; Stoeva and Kaymakanova, 2008) who have also demonstrated the inhibitory effects of salt stress on shoot length, moisture content and RWC. Kuhad *et al.* (1987), Greenway and Munns (1980) and Shanon and Nobel (1995) have ascribed the reduction in shoot length to delayed seed germination as a consequence of salt stress in pearl millet and tomato. In the present investigation the seedlings were of same age at the time of treatment. Hence the reduction in shoot length cannot be ascribed to delayed seed germination. The increased % dry matter content of seedlings cultured in nutrient solution supplemented with different concentrations of sodium chloride could be ascribed to the increased loss of moisture from these seedlings as a consequence of more negative water potential of the ambient nutrient solution. Water status of a plant is known to be highly sensitive to salinity and therefore is dominant in determining the plant responses (Stepien and Klobus, 2006). The loss of cellular water in salt stressed tissues, as observed in the present investigation, would lead to a significant decrease in the water potential of the plants and consequently their ability to maintain turgor. Even though both the varieties of maize *viz.* Gujarat Makki and RCM1-1, showed the inhibitory effects of NaCl on growth and moisture retention capacity the reduction in moisture content in the variety RCM1-1 was much less marked compared to that in

Gujarat Makki. Results of the present investigation clearly demonstrate the ameliorative effects of $CaCl_2$ and ABA on the salt stress induced suppression of growth in the seedlings. Similar observations have been made by Akinci and Simsek (2004) in cucumber. ABA treatment has been reported to enhance draught tolerance, as evidence by higher RWC and lower electrolyte leakage, in tall fescue (Jiang and Huang, 2002). Similar observations have also been reported for maize by Jiang and Zhang (2003).

While the seedlings of *Zea mays* var. Gujarat Makki cultured in nutrient solution supplemented with 250 mM NaCl showed almost 40% reduction in the total chlorophyll content, those of the variety RCM1-1 registered only a marginal decrease in the tissue content of total chlorophyll. The differential responses of the two varieties to sodium chloride, as reflected in differences in changes in the tissue content of total chlorophyll, indicate the differences in the tolerance levels of the two varieties to salt stress. Reduction in the tissue content of total chlorophyll in plants under salt stress have been reported in sorghum (Netondo *et al.*, 2004), rice (Baek *et al.*, 2006) and sunflower (Turhan *et. al.*, 2008). Notendo *et al.* (2004) have reported almost 58% decrease in the content of chlorophyll a and 68% decrease in the content of chlorophyll b in seedlings of cultured under 250 mM NaCl. In the present investigation seedlings of *Zea mays* var. Gujarat Makki cultured in nutrient solution containing 250 mM NaCl showed almost 40% reduction in the total chlorophyll content. Reddy and Vora (1986) have ascribed the reduction in chlorophyll to either the inhibitory effects of salinity on chlorophyll synthesis or the acceleration of chlorophyll degradation as a response of plants to salinity. Result of the present investigation showed that Ca^{2+} and ABA prevented the salt stress induced reduction in the total chlorophyll content of maize seedlings. Agarwal *et al.* (2004) have made similar observations about the effect of ABA on chlorophyll content of salt stressed leaves.

Seedlings of both varieties of maize cultured in Hoagland's nutrient solution supplemented with different concentrations of sodium chloride showed markedly higher electrolyte leakage and higher cytosolic Na^+ than the untreated controls. The magnitude of increase in electrolyte leakage and cytosolic Na^+ increased with increasing concentration of NaCl. Similar observations about increased electrolyte leakage as a response to salt stress have been made for fox-tail millet (Sreenivasulu *et al.,* 1999), mulberry (Sudhakar *et al.,* 2001), lentil (Bandeoglu *et al.*, 2004) and rice (Baek *et al.,* 2006). The increased electrolyte leakage could be due to loss of membrane permeability as a consequence of free radical induced membrane lipid peroxidation. Baek *et al.* (2006) have suggested that changes in solute leakage could be used as indicators for determining membrane integrity and permeability during salt stress. Orcutt and Nilsen (2000) have suggested that cell membrane function may be also compromised as a result of Na^+ replacing Ca^{2+}, resulting in increased cell leakiness.

Our results on the effects of increased NaCl concentration in the ambient medium clearly identify enhanced uptake of Na^+ as one of the early responses of the seedlings to salt stress. Increased ion accumulation in plant cells has been reported as an apparent consequence of salt stress (Hayward and Wadleigh, 1949; Grillot, 1954; Gorham *et al.,*1985; Shabala *et al.,* 1998; Schachtman and Munns, 1992; Hasegawa *et al.,* 2000; Neel *et al.,*2002; Tester and Davenport, 2003). Increase in cellular levels of Na^+ as a response to salt stress has also been reported for other genotypes of maize (Alberico and Cramer, 1993; Erdei and Taleisnik, 1993; Azevedo-Neto and Tabosa, 2000). Alberico and Cramer (1993) have suggested that salt tolerance in maize was not related to shoot Na^+ content but to the capacity of the cells to compartmentalize the ions in the vacuole thereby maintaining low Na^+ content in the cytoplasm. In the present investigation, seedlings of both varieties of maize cultured in Hoagland's nutrient solution containing different concentrations of sodium chloride showed markedly higher levels of cytosolic sodium than the untreated controls. However, the level of cytosolic sodium was significantly lower in var. RCM1-1 than the variety Gujarat Makki. RCM1-1 also showed lesser reduction in the content of total chlorophyll. While Azevedo-Neto *et al.* (2004) have indicated that leaf Na^+ content or leaf soluble organic solute content had no relation with salt tolerance in maize, our results indicate that leaf cytosolic Na^+ levels can be used as physiological markers for salt stress in maize.

The content of cytosolic Na^+, H_2O_2 levels and electrolyte leakage in shoots tissue of both varieties of maize *viz.* Gujarat Makki and RCM1-1 showed a marked decline in the presence of 10 mM $CaCl_2$ in the nutrient solution. Although several studies have reported no effect of Ca^{2+} variation in the millimolar range on shoot Na^+ uptake (Aslam *et al.,* 2003; Baba and Fujiyama, 2003; Yeo and Flowers, 1985), a reduction in shoot Na^+ has been observed in others (Song and Fujiyama, 1996; Anil *et al.,* 2005). Our results on the effects of external calcium on the levels of cytosolic sodium in salt stressed plants are in agreement with similar observations on rice (Shah *et al.*,2003), wheat (Hussain *et al.*, 2004), rice (Anil *et al.*, 2005) and cotton (Cramer, 1986, 1997). Shah *et al.* (2003) have attributed the protective effects of Ca^{2+} on growth and Na^+ exclusion in salt stressed rice seedlings. They have concluded that besides its role in overcoming the Na^+ toxicity supplemental Ca^{2+} mitigated the osmotic components of salt stress by enhancing proline accumulation and also helped the salt stressed cells to maintain not ion homeostasis. Hussain *et al.* (2004) have attributed the inhibitory effect of external calcium on Na^+ uptake to a regulation of xylem loading transporters in the plants. Cramer *et al.* (1987) and Liu and Zhu (1998) have suggested that externally supplied Ca^{2+} reduced the toxic effects of NaCl, presumably by facilitating higher K^+/Na^+ selectivity. The tight Na^+/Ca^{2+} interaction has been ascribed to similar crystal ionic radii of the two ions (Allen *et al.*, 1994; Cramer, 2002). While Ca^{2+} has crystal ionic radius of 0.099 nm, sodium ions have a crystal ionic radius of 0.097 nm. Zid and Grignon (1985), Grignon and Senetenac (1991) and Munns (2005) have attributed the Na^+/Ca^{2+} interactions to competition between Na^+ and Ca^{2+} for negatively charged binding sites that have a high specificity for Ca^{2+}.

Seedlings incubated in Hoagland's nutrient solution lacking any additional supplements did not show any significant change in tissue level of free cytosolic K^+ during the 144 hours of incubation. Similarly seedlings maintained over Hoagland's nutrient solution containing either 10 mM $CaCl_2$ or 100 μM ABA also did not show any significant change in tissue level of free cytosolic K^+ during the 144 hours of incubation. On the other hand seedlings incubated in nutrient solution supplemented with 200 mM NaCl showed a marked decrease in the cytosolic K^+ levels during the 144 hours incubation period. The increase in cytosolic K^+ in salt stressed seedlings affected the Na^+/K^+ ratio of the seedlings. There was no marked change in the Na^+/K^+ ratio in the shoot tissues of seedlings cultured in native Hoagland nutrient solution during the 144 hours of incubation. However, seedlings incubated in nutrient solution containing 200 mM sodium chloride showed a consistent increase in Na^+/K^+ ratio of the shoot tissues with progressive time. These seedlings registered a 6 fold increase of Na^+/K^+ ratio of the shoot tissues during the 144 hours of incubation. On the other hand seedlings incubated in nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA showed a markedly lower ratio of Na^+ to K^+ than the corresponding control seedlings which were incubated in nutrient solution supplemented with only 200 mM NaCl. However, seedlings pretreated with either 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl, and 10 mM $CaCl_2$ or 200 mM NaCl, and 100 µM ABA showed a significantly higher ratio of Na^+ to K^+ than the corresponding controls which were not pretreated with either verapamil or EGTA. These seedlings registered nearly six fold increase in the level of ratio of Na^+ to K^+ during the 144 hours of incubation.

The reduction in K^+ content in the shoot tissues of maize seedlings under salt stress has also been reported by Hajibagheri *et al.* (1987), Alberico and Crammer (1993), Azevedo-Neto and Tabosa (2000) and Azevedo-Neto *et al.* (2004). Munns (2002) has suggested that salt induced growth inhibition in salt sensitive genotypes could be mainly due to metabolic changes resulting from ion imbalance or ion toxicity during salt stress. Our results on the effect of pretreatments with calcium channel blocker verapamil or calcium chelator EGTA on Na^+/K^+ ratio in seedlings of maize under salt stress indicate involvement of Ca^{2+} ions in exclusion of Na^+ from the cells during stress.

H_2O_2 levels, MDA concentration and electrolyte leakage are routinely estimated parameters to assess the extent of oxidative stress in plants. Increases in the level of H_2O_2 and MDA upon salt stress has been reported in rice (Dionisio-Sese and Tobita, 1998; Lee *et al.*, 2001), mulberry (Sudhakar *et al.*, 2001), wheat (Sairam and Srivastava, 2002) and lentil (Bandeoglu, 2004). The increase was shown to be related to the magnitude of stress and correlated with membrane lipid damage. The reactive oxygen species produced as a result of stress can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc, causing lipid peroxidation, protein denaturing and DNA mutation (Breusegem *et al.,* 2001; Scandalios, 1993; Quiles and Lopez, 2004). ROS are also known to cause oxidative damage to chlorophyll (Alscher *et al.,* 1997; Pastori and Foyer, 2002).

In the present investigation seedlings cultured in nutrient solution supplemented with 200 mM NaCl registered a more than 3 fold increase in the level of H_2O_2 during the 144 hours of incubation. However seedlings cultured in nutrient solution supplemented with 10 mM $CaCl_2$ and 200 mM NaCl registered only 2 fold increase in the level of H_2O_2 during the 144 hours of incubation. The increase in tissue levels of H_2O_2 during the 144 hours of incubation was, however, significantly higher in seedlings pretreated with the calcium channel blocker verapamil. These results indicate the ameliorative role of calcium ions in preventing oxidative damage to tissues of salt stressed plants. Similar observation on the role of calcium ions in preventing oxidative damage in salt stressed plants have been made by Cachorro *et al.* (1993), Gong *et al.* (1997) and Hawighorst (2007). Neill *et al.* (2002b) and Pastori and Foyer (2002) have explained that H_2O_2 was produced under salt stress either by the dismutation of superoxide and transported from the apoplast to the cytosol or was generated in chloroplasts, mitochondria and peroxisomes from where it would move into cytosol. H_2O_2 has also been shown to be involved in the cellular signaling process as secondary messengers to induce a number of genes and proteins involved in stress defenses including SOD, CAT, APX, GR and POX (Lamb and Dixon, 1997; Karpinski *et al.,* 1999; Morita *et al.,* 1999; Desikan *et al.*, 2001; Neill *et al.,* 2002a; Vranová *et al.,* 2002). Our results clearly show a marked increase in the MDA content of leaf tissues in seedlings cultured in nutrient solution supplemented with 200 mM NaCl. The MDA content, however, significantly lower in seedlings cultured in nutrient solution supplemented with 200 mM and 10 mM $CaCl_2$. On the other hand seedlings pretreated with either verapamil or EGTA, before transfer to nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$, had markedly higher levels of MDA. Analysis of changes observed in the level of protein carbonylation and the MDA content with salt stress, as observed in the present investigation, indicate that membrane lipid peroxidation rather than protein carbonylation was the major cause of enhanced electrolyte leakage during salt stress. MDA content is often used as an indicator of lipid peroxidation resulting from oxidative stress (Smirnoff, 1995), and its accumulation is considered a manifestation of the detriment of ROS in plants.

Increase in the MDA content in seedlings pretreated with calcium channel blocker verapamil or calcium chelator EGTA clearly indicates the protective role of Ca^{2+} in preventing membrane lipid peroxidation during salt stress in maize. Gilroy *et al.* (1991), McAinsh *et al.* (1990, 1992), Chen *et al.* (2001) and Yang and Poovaiah (2002) have suggested that ABA could trigger Ca^{2+} influx thereby enhancing the concentration of cytosolic Ca^{2+} in the cells. The enhanced $cytCa^{2+}$ could through a downstream signaling cascade induce other physiological responses in the salt stressed plants cells. Behl and Jeshke (1981) have suggested that EGTA or verapamil disrupts the deployment of Ca^{2+} as second messenger thereby blocking the action of ABA in preventing salt stress induced damage to plant cells.

Compared to the untreated controls salt stressed seedlings of both varieties of maize showed increased levels of AsA and GSH. While the tissue level of GSH showed a progressive decrease with time that of AsA increased progressively with time till 144 hours of incubation. The tissue concentration of AsA also showed a significant increase with progressing time in seedlings cultured in nutrient solution supplemented with 100 μM ABA. While the seedlings incubated in nutrient solution supplemented with 200 mM NaCl and either 10 mM $CaCl_2$ or 100 μM ABA showed marginally higher tissue level of GSH, those pretreated with verapamil showed markedly lower tissue levels of GSH than the corresponding controls. On the other hand salt stressed seedlings had significantly higher tissue concentration of AsA than the untreated controls. The increased accumulation of AsA in ABA treated seedlings is indicative of the role of ABA depending signaling cascade in regulation of

Ascorbate- glutathione cycle. El-baky *et al.* (2003) have reported significant elevation in the level of GSH in three onion cultivars under salt stress. On the contrary results of the present investigation do not show any significant increase in GSH content during salt stress. Our results clearly show increase in the AsA content in leaf tissues of the seedlings in response to salt stress. Zushi and Matsuzoe (2007) have observed similar changes in AsA content in salt stressed tomato cv. House momotaro. They have also demonstrated the cultivar specific response of the plants in respect of accumulation various components of the antioxidative system. It was shown that while the cultivar House momotaro showed increase in AsA levels in response to salt stress the cultivar mini carol showed decreased levels of AsA under salt stress. El-baky *et al.* (2003) have suggested that the proportional contribution of various components of the antioxidative system in plants under salt stress was always species dependent and hence any change in any of the various components of the antioxidative system would depend on the tolerance level of the species/cultivar. Using a transgenic tobacco system, Lee *et al.* (2007) have demonstrated that simultaneous expression of multiple antioxidant enzymes was more effective than single or double enzyme expression systems for developing transgenic plants with enhanced tolerance to stresses. It was shown that mature leaves of transgenic plants expressing three antioxidant genes viz. Cu/ZnSOD, APX and DHAR had nearly 2 fold higher DHAR activity, higher ratio of reduced ascorbate (AsA) to DHA and oxidized glutathione (GSSG) to reduced Glutathione (GSH). The higher levels of AsA and lower levels of GSH in the variety RCM1-1, as observed in the present indication, is indicative of greater salt tolerance capability in this variety. This also indicates the involvement of Ascorbate-Glutathione pathway in alleviating salt stress induced oxidative damage in the seedlings.

Results from the present study clearly revealed marked increases in the activities of APX, POX and SOD in the shoot tissues of maize seedlings under salt stress. While the salt stressed seedlings maintained higher activities of APX and POX throughout 144 hours of incubation, the activity of GR showed marked increase during the initial 48 hours of stress after which it showed marked decrease with progressing time. Similar pattern of change in activity was recorded for CAT. Simultaneously there was a sharp increase in the tissue content of MDA and protein carbonylation between 48 and 144 hours of stress. The sharp increase in MDA levels and protein carbonyls between 48 to 144 hours of stress indicates that salt stress induced oxidative damage to lipids and proteins might after 48 hours of incubation in nutrient solution supplemented with 200mM NaCl.

The first enzyme of the ascorbate-glutathione cycle, APX, catalyzes the reduction of H_2O_2 to water and has high specificity and affinity for ascorbate as reductant (Asada, 1999). In the present investigation, APX activity in salt stressed seedlings was 54% higher than the untreated controls. Higher activity of APX was also recorded in ABA treated seedlings. Seedlings cultured in nutrient solution supplemented with 200 mM NaCl along with either 10mM $CaCl_2$ or 100μM ABA also showed higher activity of the enzyme. The differential effects of sodium chloride on the APX activity of the two varieties of maize, as observed in the present investigation, indicate the differences in the tolerance levels of the two varieties to salt stress. Guetha-Dahan *et al.* (1997) have suggested that APX was a key enzyme in determining salt tolerance in *Citrus* as its constitutive activity was much higher in salt tolerant cultivar. APX activity has also been shown to be higher in tolerant cultivars of pea (Hernandez *et al.*, 1999), mulberry (Sudhakar *et al.*, 2001), tomato (Rodriguez-Rosales *et al.*, 1999) under salt stress. Compared to the untreated controls, seedlings cultured in nutrient solution supplemented with 200 mM NaCl and 10 mM $CaCl_2$ or 200 mM NaCl and 100 µM ABA also showed higher activities of POX and GR. These results indicate activation of the antioxidant enzyme systems in the seedlings as an immediate response to salt stress. The activation of antioxidant enzyme systems indicates could be a consequence of the seedlings sensing higher levels of ROS in the tissues. These results also indicate that Ca^{2+} or ABA supplementation might have a role to play in preventing generation/accumulation of ROS in the salt stressed plants. Similar observations have been reported in foxtail millet (Sreenivasulu *et al.*, 2000), citrus (Arbona *et al.,* 2003) and maize (Jiang and Zhang, 2003).

SDS-PAGE profiles for total soluble proteins extracted from leaf tissues of the seedlings did not reveal any marked changes in the protein profiles in seedlings under salt stress. While there was no marked variation in the number of bands there were variations in the intensities of some bands particularly the two having molecular mass of 50 kD and 90 kD. Kongngern *et al.* (2005) have also shown increase in the intensity of 90 kDa band in salt stressed rice seedlings. These observations reflect changes in the expression of genes during when the seedlings are subjected to salt stress.

Our results have revealed the presence of 3 activity zones for APX isozymes in shoot tissues of maize seedlings. The intensity of activity zones was higher in seedlings cultured in nutrient solution supplemented with 200 mM NaCl but lower in seedlings cultured in nutrient solution supplemented with NaCl and 10 mM $CaCl_2$ or NaCl and 100 µM ABA. Results of the present investigation have also clearly revealed the presence of 5 isoenzymes of SOD in the leaf tissues of maize seedlings. With the help of appropriate inhibitors these isozymes have been identified as These isozymes were characterized as MnSOD, FeSOD (I and II) and Cu/ZnSOD (I and II).

Which isozyme showed greater changes?

Lee (2001) has reported enhanced intensities of APX isozymes IV, V, VI and VII in salt stressed rice with APX isozymes I, II and II showing no significant change in response to salt stress. Enhanced activities of three isozymes of APX in response to salt stress, as observed in the present investigation, is indicative of its role in alleviating the disruptive effect of H_2O_2 in the shoot tissues during salt stress.

Our results on changes in the profile and intensity of isozymes of SOD in leaf tissues under salt stress are in agreement with earlier findings in a number of plant species including wheat (Sairam and Srivastava, 2002), pea (Hernandez *et al.*, 1999), tomato (Kurepa *et al.*, 1997) and french bean (Kwiatowski and Kaniuga,1984). Lee (2001) have reported enhancement of expression of Cu/Zn-SOD-1, II and Mn-SOD-II isoforms by salt stress in rice. On the other hand the salt sensitive varieties Hitomebore and IR28 of rice have been shown to exhibited decreased SOD activity, increased lipid peroxidation, increased electrolyte leakage and high Na^+ accumulation under high salinization (Dionisio and Tobita, 1998). It has been suggested that low SOD activity could favour accumulation of oxygen radical species which would cause oxidative damage to membranes thereby enhancing electrolyte leakage.

Even though our results showed a marginal decrease in CAT activity in the tissues during salt stress, the activity of the enzyme was enhanced in the presence of $CaCl_2$ or ABA. Salt stressed seedlings of maize showed higher activity of CAT-I and CAT-II isozymes. CAT activity has also been found to increase under salt stress in soybean (Comba *et al.*, 1998), tobacco (Bueno *et al.,* 1998), cucumber (Lechno *et al.*, 1997) and mulberry (Sudhakar *et al.*, 2001). However, the activity of the enzyme did not show any marked change under salt stress in potato (Benavides *et al.*, 2000) and rice (Lin and Kao, 2000). Khan *et al.* (2002) have even reported salt stress induced decrease in the activity of the enzyme in rice..

The last enzyme of ascorbate-glutathione cycle, GR, catalyzes the NADPH-dependent reduction of oxidized glutathione. It is the rate-limiting enzyme and is involved in the maintenance of reduced glutathione required for the regeneration of ascorbate (Sudhakar *et al.,* 2001). GR activates in glutathione-ascorbate cycle and coverts GSSG to reduced glutathione (GSH) (Asada, 2000; Vega *et al.*, 2003). In addition, GR regulates GSH/GSSG ratio and supplies GSH for GPX and DHAR, which convert H_2O_2 to H_2O and reduce oxidized ascorbate, respectively. Although GR acquires the reduction power from NADPH, H^+, it dissipates this power and, in turn, increases $NADP^+$/NADPH, H^+ ratio. Results of the present investigation showed a marked increase in the activity of glutathione reductase within 24 hours of transfer to nutrient solution supplemented with 200 mM NaCl. Seedlings cultured in nutrient solution supplemented with 200 mM NaCl along with either 10mM $CaCl_2$ or 100µM ABA also showed higher activity of the enzyme. Increased activity of GR in response to salt stress has also observed in rice (Lin and Kao, 2000), soybean (Comba *et al.*, 1997), mulberry (Sudhakar *et al.*, 2001), tomato (Guetha-Dahan *et al.*, 1998) and *Chrysanthemum morifolium* (Hossain *et al.*, 2004). On the other hand, Bueno *et al.* (1998) have reported that salt stress increased the activity of all antioxidant enzymes except GR in tobacco plant. Sudhakar *et al.* (2001) mentioned that elevated levels of GR activity could increase the ratio of $NADP^+$/ NADPH thereby ensuring the availability $NADP^+$ to accept electrons from photosynthetic electron transport chain, thus minimizing the reduction of oxygen and formation of superoxide radicals. Salt stress induced elevation of activities of SOD, CAT and APX have been reported in seedlings of *Catharanthus roseus* (Elkahoui *et al.*, 2004), rice (Lee, 2001) and *Bruguiera gymnorrhiza* (Lin, 2001). In the present study, the activity of GR showed a marked decrease after 48 hours of treatment. The decrease in the activity of GR after 48 hours of treatment could lead to limitation of the glutathione-ascorbate cycle and $NADP^+/NADPH,H^+$ ratio leading to accumulation of ROS and a consequent damage to membranes.

Results from the present investigation showed that NaCl stress induced a significant increase in the production H_2O_2 and the activities of SOD, CAT, APX and GR, and the content of AsA. Jiang and Zhang (2001, 2003) have shown a significant increase in H_2O_2 in seedlings of maize as a response to exogenous application of ABA. The increase in H_2O_2 levels was followed by increase activities of SOD, CAT, APX and GR. On the basis of their observations they have suggested the involvement of ABA dependent downstream signaling cascade in modulating the physiological responses of the seedlings to salt stress. Results of the present investigation have clearly revealed that pretreatment of the plants with verapamil or EGTA reduced the ameliorative effect of $CaCl_2$ and ABA in the seedlings during salt stress. Behl and Jeshke (1981) have suggested that EGTA or verapamil disrupt the deployment of Ca^{2+} as second messenger, and then block the action of ABA. Results of the present investigation clearly indicate a link between salt stress and oxidative stress in maize seedlings. The results also indicate a crosstalk between Ca^{2+} and ROS through ABA. This cross talk induces increases in the activities of antioxidant enzymes in the shoot tissues of maize seedlings.

Chapter: 6

GENERAL SUMMARY AND CONCLUSION

Water is a fundamentally important component of the metabolism of all living organisms. As a universal solvent it facilitates many vital biological reactions in the living system. The salinization of soils caused by increasing NaCl concentrations is a major problem for today's agriculture. Salinization is the accumulation of water-soluble salts in the soil solum (the upper part of a soil profile) or regolith (the layer or mantle of fragmental and unconsolidated rock material) to a level that would affect agricultural production. High exogenous salt concentrations are known to affect seed germination, cause water deficit and ion imbalance in the cells resulting in ion toxicity and osmotic stress. There is strong evidence that salt affects tissue concentration of chlorophylls and carotenoids and the activity of photosynthetic enzymes, Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes. The complexity of the plant responses to salt stress can be partially explained by the fact that salinity imposes both hyperionic and hyperosmotic stress on the plants and inhibits plant growth by inhibiting cell division and expansion, imbalance of the cellular ions, change of cell volume or turgor pressure and activity and stability of macromolecules.

Plants respond to salt stress induced disturbances in ionic homeostasis by restricting salt intake, increased electrolyte leakage and maintenance of a favorable K^+/Na^+ ratio in the cytosol. Salt stress is known to increase the levels of free cytosolic Ca^{+2} either through influx from the apoplastic space or as a consequence of release from the calcium sequestration compartments. Elevated levels of free cytosolic calcium have been shown to reduce binding of Na^+ ions to cell walls and plasma membranes, relieve membrane leakiness and prevent salt-induced decline in cell elongation. A secondary effect of salt stress is the increase of ROS which leads to oxidative stress. At higher concentration ROS are highly cytotoxic at higher concentrations and induce DNA damage, lipid peroxidation, and protein degradation. The degree of oxidative damage caused by ROS, however, depends on the balance between the formation of ROS and its removal by the antioxidative scavenging systems. Plant tolerance to adverse biotic and abiotic stress is thus usually correlated with a more efficient antioxidative system. Current evidence supports the concept that ROS represent a significant point of convergence between pathways that respond to biotic and abiotic stresses. To date, the biological significance of crosstalk between signaling pathways that operate under stress conditions and the mechanisms that underlie this crosstalk remain obscure. Nevertheless, our current understanding of ROS participation in crosstalk between these pathways is very limited. Thus, dissection of the networks that influence ROS levels in response to abiotic stress merits extensive future study. The present work was undertaken to investigate the role of calcium ions and abscisic acid in modulating detoxifications systems during early stages of salt stress in maize.

Seedlings of *Zea mays* cv. Gujarat Makki (GM) and RCM1-1 cultured in Hoagland's nutrient solution supplemented with different concentrations of NaCl showed markedly reduced shoot length, reduced RWC, decreased moisture content and a correspondingly higher % dry matter content. When compared with the untreated controls seedlings of the variety Gujarat Makki showed 46% reduction in shoot length at 250 mM ambient NaCl concentration. On the other hand the variety RCM 1-1 showed only 35% reduction in shoot length at 250 mM ambient NaCl concentration. The salt stressed seedlings also showed a marked increase in % dry matter. The increased % dry matter content of seedlings cultured in nutrient solution supplemented with different concentrations of sodium chloride could be ascribed to the increased loss of moisture from these seedlings as a consequence of more negative water potential of the ambient nutrient solution.

While the seedlings of *Zea mays* var. Gujarat Makki cultured in nutrient solution supplemented with 250 mM NaCl showed almost 40% reduction in the total chlorophyll content, those of the variety RCM1-1 registered only a marginal decrease in the tissue content of total chlorophyll. The differential responses of the two varieties to sodium chloride, as reflected in differences in changes in the tissue content of total chlorophyll, indicate the differences in the tolerance levels of the two varieties to salt stress. Exogenous application of Ca^{2+} and ABA prevented the salt stress induced reduction in the total chlorophyll content of maize seedlings. Results of the present investigation clearly demonstrated the ameliorative effects of $CaCl_2$ and ABA on the salt stress induced suppression of growth in the seedlings.

Seedlings of both varieties of maize cultured in Hoagland's nutrient solution supplemented with different concentrations of sodium chloride showed markedly higher electrolyte leakage and higher cytosolic Na^+ than the untreated controls. The magnitude of increase in electrolyte leakage and cytosolic Na^+ increased with increasing concentration of NaCl. The level of cytosolic sodium was, however, significantly lower in var. RCM1-1 than the variety Gujarat Makki. The increased electrolyte leakage could be due to loss of membrane permeability as a consequence of free radical induced membrane lipid peroxidation. Our results clearly reveal the protective effects of Ca^{2+} on growth and Na^+ exclusion in salt stresses seedlings of maize.

There was a marked decrease in the level of free cytosolic K^+ in seedlings incubated in nutrient solution supplemented with 200 mM NaCl. The increase in cytosolic K^+ in salt stressed seedlings had a marked effect on the Na^+/K^+ ratio of the seedlings which showed a 6 fold increase in the Na^+/K^+ ratio. Incorporation of calcium chloride along with NaCl lead to a marked reduction in the ratio of Na^+ to K^+ in the seedlings. However, pretreatment of the seedlings with 1 mM verapamil or EGTA before incubation in nutrient solution supplemented with 200 mM NaCl, and

10 mM $CaCl_2$ or 200 mM NaCl, and 100 µM ABA negated the effect of $CaCl_2$ in restoring the cellular Na^+/K^+ ratio of the salt stressed seedlings.

Seedlings cultured in nutrient solution supplemented with 200 mM NaCl registered more than 3 fold increase in the level of H_2O_2 during 144 hours of incubation. Incorporation of calcium chloride along with NaCl lead to a marked reduction in the H_2O_2 levels of shoot tissues in the salt stressed seedlings. The increase in tissue levels of H_2O_2 was, however, significantly higher in seedlings pretreated with the calcium channel blocker verapamil. These results indicate the ameliorative role of calcium ions in preventing oxidative damage to tissues of salt stressed plants. Results of the present investigation also showed a marked increase in the MDA content of leaf tissues in seedlings cultured in nutrient solution supplemented with 200 mM NaCl. While the MDA content, however, significantly lower in seedlings cultured in nutrient solution supplemented with 200 mM and 10 mM $CaCl_2$, seedlings pretreated with either verapamil or EGTA, before transfer to nutrient solution containing 200 mM NaCl and 10 mM $CaCl_2$, had markedly higher levels of MDA. Analysis of changes observed in the level of protein carbonylation and the MDA content with salt stress, as observed in the present investigation, indicate that membrane lipid peroxidation rather than protein carbonylation was the major cause of enhanced electrolyte leakage during salt stress.

Compared to the untreated controls salt stressed seedlings of both varieties of maize showed increased levels of AsA and GSH. While the tissue level of GSH showed a progressive decrease with time that of AsA increased progressively with time till 144 hours of incubation. The tissue concentration of AsA also showed a significant increase with progressing time in seedlings cultured in nutrient solution supplemented with 100 µM ABA. While the seedlings incubated in nutrient solution supplemented with 200 mM NaCl and either 10 mM $CaCl_2$ or 100 µM ABA showed marginally higher tissue level of GSH, those pretreated with verapamil showed markedly lower tissue levels of GSH than the corresponding controls. The increased accumulation of AsA in ABA treated seedlings is indicative of the role of ABA depending signaling cascade in regulation of Ascorbate-glutathione cycle. The higher levels of AsA and lower levels of GSH in the variety RCM1-1, as observed in the present indication, is indicative of greater salt tolerance capability in this variety. This also indicates the involvement of Ascorbate -Glutathione pathway in alleviating salt stress induced oxidative damage in the seedlings.

Our results clearly reveal marked increases in the activities of APX, POX and SOD in the shoot tissues of maize seedlings under salt stress. While the salt stressed seedlings maintained higher activities of APX and POX throughout 144 hours of incubation, the activity of GR showed marked increase during the initial 48 hours of stress after which it showed marked decrease with progressing time. Simultaneously there was a sharp increase in the tissue content of MDA and protein carbonylation between 48 and 144 hours of stress. The sharp increase in MDA levels and protein carbonyls between 48 to 144 hours of stress indicates that salt stress induced oxidative damage to lipids and proteins occurred after 48 hours stress at 200mM NaCl. Treatment with ABA further enhanced the activities of these antioxidant enzymes and the contents of these antioxidant metabolites in salt stressed leaves. These data strongly suggest that ABA is an essential mediator in triggering salt stress-induced antioxidative defense response against oxidative damage in maize seedlings. Therefore, ABA-enhanced salt stress tolerance is, at least in part, due to the induction of antioxidative defense systems. Our results showed that the key enzymes in modulating the oxidative stress adaptations during salt stress were SOD, APX and GR. The role of SOD and APX enzymes in the antioxidative defense system for detoxification of ROS, played a significant role in the whole network of antioxidative pathway during NaCl stress in maize seedling. Pretreatment of the plants with verapamil or EGTA reduced the ameliorative effect of $CaCl_2$ and ABA in the seedlings during salt stress. It is possible that EGTA and verapamil disrupt the deployment of Ca^{2+} as second messenger and consequently block the action of ABA. Results of the present investigation clearly indicate a link between salt stress and oxidative stress in maize seedlings. The results also indicate a crosstalk between Ca^{2+} and ROS through ABA. This cross talk induces increases in the activities of antioxidant enzymes in the shoot tissues of maize seedlings.

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