Salt Tolerance and Modification of Wheat Salt Resistance by Plant Hormones

By

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Submitted in partial fulfillment of the requirements for the degree of Ph.D in Plant Eco-physiology in the Department of Biodiversity and Conservation Biology, University of the Western Cape, South Africa

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Key Words

Salt tolerance, Salt resistance, Salinity, Salt stress, NaCl, Na, Cl, K, Na/K, Ca, Mg, Wheat, *Triticum*, Gibberellic acid, GA_3, Benzyladenine, Compatible solutes, Osmotic effects, Proline, Seed germination, Citric acid, Malic acid, pH effects.

DECLARATION

I declare that **Salt Tolerance and Modification of Wheat Salt Resistance by Plant Hormones.**

Is my own work, that it has not been submitted for any degree or examination in any other university, and that all the source I have used or quoted have been indicated and acknowledged by complete references.

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ABSTRACT

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The history of salt tolerance and the factors effecting salt resistance of plants were literature surveyed, and it was concluded that ion concentration, salt accumulation, compatible solutes and the genetic traits play a major role in the salt tolerance of plants. Differences in salt resistance of wheat cultivars were investigated at the germination and early seedling stages. Considerable intervarietal differences in salt resistance between wheat cultivars were reported. The interaction between salinity and plant hormones was studied and showed that N^6-benzyladinin treatments caused some changes in some parameters that were studies, GA_3 treatments showed more effects on these parameters of salt stressed plants. This study showed that the treatment with some organic acids, citric acid and malic acid, did not cause significant changes in the parameters measured of the wheat plants. No effects on seed germination were due to the decreases in the pH value due to the GA_3 treatment were found. The study concludes that treatment of salt stressed wheat cultivars with GA_3 could alleviate some of harmful effects of high salt levels, and that it could be useful to treat plants grown in brackish soil or saline environment.

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Muftah A. Adam

I Dedicate This Work

To:

My Parents,

My Wife

And My All Family Members

Muftah

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CHAPTER 1

Plants and Salt Tolerance

1.Salt tolerance history:

Salt stress is certainly one of the most serious environmental factors limiting the productivity of crop plants (Ashraf, 1999). Plants differ in salt tolerance and differences in salt tolerance between genotypes within species and the varietal differences in salt tolerance have been known since the 1930's (Epstein, 1977).

Intraspecific selection for salt tolerance was, by the 1980's, shown to be possible with rice (Akbar & Yabuno, 1977) and barley (Epstein *et al.*, 1980). The topic of the effects of salinity and saline water on plant growth has continued to draw the attention of many research groups across the world and the interest has increased significantly during the past five decades or so (Sharma & Goyal, 2003). More than 40 years of research on salinity have produced an uncountable number of papers and unpublished results that reflect the importance of salinity problems in agriculture (Borsani, *et al.*, 2003). Sharma & Goyal (2003) showed that Flowers & Yeo (1995) searched the "BIOSIS" literature database for papers dealing with salinity and/or salt resistance and vascular plants and reported that in the fifteen years (between 1978 and 1993) 4 231 papers were published, and Sharma & Goyal (2003) found 1 890 papers published between 1993-2001.

Salt tolerance in plants is a complex phenomenon involving morphological, physiological, and biochemical processes (Jacoby, 1999).

Hester *et al.* (1996) showed that the death of 50% of aboveground tissue of plants is an indicator for lethal salinity levels of a specific plant genotype. The U.S. Salinity Laboratory characterizes the salt tolerance of a plant on the basis of which electrical conductivity would cause a 50% yield reduction when compared with plants growing under similar conditions on non-saline soils. For field crops, barley was the most tolerant (EC_e X 10^3 =16) and field beans the most sensitive (an EC_e X 10^3 = 4 gave a 50% yield reduction) (U.S. Salinity Lab. Staff, 1954). Spikelet number per panicle was determined to be more important to grain yield than fertility and kernel weight. And the spikelets per panicle was the most salt-sensitive yield component; fertility and kernel weight were less sensitive to salinity. Because spikelets per panicle can be visually estimated, it should be a desirable and rapid selection criterion for screening a large number of plants for salt tolerance (Zeng & Shannon, 2000). Storey & Wyn Jones (1979) suggested that the capacity to maintain high shoot K/Na is an important element of salt tolerance, especially in species, which lack foliar salt-excretion mechanisms.

Pearce (2003) showed that salt tolerance depends upon physiological [morphological compartmentation, compatible solute production, regulation of transpiration, control of ion movement, membrane characteristics, toleration of high Na/K ratios in the cytoplasm and salt glands] and genetic traits; that salt tolerance is determined by a number of genes. Research on the physiology of salt tolerance suggests that the overall performance is determined by a number of sub-traits any of which might, in turn, be determined by any number of genes. These sub-traits generally include an ability to minimize the net accumulation of sodium and/or chloride ions and

to select potassium from a background of high sodium concentration (Pearce, 2003).

Results from crop salt tolerance tests conducted worldwide between 1950 and 1975 were reviewed by Maas & Hoffman (1977). These reviewers rated irrigated wheat crops as moderately salt tolerant. Salt tolerance appeared to increase under conditions of limited irrigation (Shani & Dudley, 2001). The heritability and inheritance of salt tolerance in a species determines the selection intensity and the number of cycles required, and the lack of genetic variation and a poor understanding of physiology and genetics are generally the major barriers in the improving of salt tolerance of plants (Shannon & Noble, 1990). Our understanding of the physiological/biochemical processes contributing to salt resistance is far from complete (Sharma & Goyal, 2003). Table 1 below has classified various field and forage crops according to their salt tolerance (Lamond, 1992).

Table1. Salt tolerance ratings for various field and forage crops.

Salt tolerance level	Sensitive	Moderately tolerant	Tolerant	Highly tolerant
Soil conductivity	0-4 $S.cm^{-1}$	4-6 $S.cm^{-1}$	6-8 $S.cm^{-1}$	8-12 $S.cm^{-1}$
Crop	Field Bean Red Clover Alsike Clover	Corn Grain sorghum Soybean Bromegrass Sudangrass Sorghum- Sudans	Wheat Oats Triticale Sunflower Alfalfa Tall Fescue Sweet Clovers	Barley Rye Bermudagrass Crested- wheatgrass

1.1 Osmotic effects:

High salinity causes hyperosmotic stress and ion disequilibrium that produce secondary effects or pathologies (Hasegawa *et al.*, 2000b; Zhu, 2000). The earlier belief that it was the actual lack of water that limited growth with a saline root medium has generally been rejected because plants have been shown to adjust osmotically (Maas & Nieman, 1978). The general effect of soil salinity on plants is called an osmotic effect. This means that salts increase the energy with which water is held in the soil (Blaylock, 1994). Most plants are harmed by salinities of 3000 $mg.L^{-1}$ (51 mM NaCl) and cannot survive more than 5000 $mg.L^{-1}$ (86 mM NaCl) (Toenniessen, 1984). Marcum & Murdoch (1992) suggested that 200 to 300 mM of organic osmotica in the cytoplasm is sufficient for osmotic adjustment at higher salinities.

Plants cope by either avoiding or tolerating salt stress. That is plants are either dormant during the salt episode or there must be cellular adjustment to tolerate the saline environment. Tolerance mechanisms can be classified as those that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by these stresses. The chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease, which if severe enough can cause growth reduction (Bohnert *et al.*, 1995) and the ion uptake by nongrowing plant cells becomes saturated when ion concentrations rise above 100-200 mM (Oertli, 1968). Growth cessation occurs when turgor is reduced below the yield threshold of the cell wall. Cellular dehydration begins when the water potential difference is greater than can be compensated for by turgor loss (Yokoi, *et al.*, 2002). The cellular response to turgor reduction is osmotic adjustment. A decrease in water availability under soil salinity causes osmotic stress, which leads to decreased

turgor (Chinnusamy & Zhu, 2003). The cytosolic and organellar machinery of glycophytes and halophytes is equivalently Na^+ and Cl^- sensitive, so osmotic adjustment is achieved in these compartments by accumulation of compatible osmolytes and osmoprotectants (Bohnert, *et al.*, 1995; Bohnert & Jensen, 1996). However, Na^+ and Cl^- are energetically efficient osmolytes for osmotic adjustment and are compartmentalized into the vacuole to minimize cytotoxicity (Blumwald *et al.*, 2000; Niu *et al.*, 1995). Since plant cell growth occurs primarily because of directional expansion mediated by an increase in vacuolar volume, compartmentalization of Na^+ and Cl^- facilitates osmotic adjustment that is essential for cellular development. Osmotic stress may induce ion (Na^+ & K^+) uptake and compartmentalization into the vacuole, and synthesis of organic compatible solutes such as proline, betaine, polyols, and soluble sugars (Chinnusamy & Zhu, 2003). Use of ions for osmotic adjustment may be energetically more favorable than organic osmolyte biosynthesis under stress, as ion uptake and sequestration into the vacuole may cost only 3-4 moles of ATP compared with the 30-50 moles of ATP needed for synthesis of one mole of organic osmolytes (Raven, 1985).

1.2 Ionic effects:

Under salinity conditions, mutual effects of ions on their absorption are of particular interest. Ions in high concentration in the external solution, like Na^+ or Cl^-, are taken up at high rates, which may lead to excessive accumulation in the tissue. These ions may inhibit the uptake of other ions into the roots such as K^+ and Ca^{2+}, and their transport to the shoots through the xylem, eventually leading to a deficiency in the plant tissue (Cramer *et al.*, 1991). No toxic substance restricts plant growth more than does salt on a world scale (Xiong & Zhu, 2002). It is estimated that salinity affects at least 20% of world's arable land and more than 40% of irrigated land to various degrees (Rhoades

& Loveday, 1990). The effects of high ambient NaCl concentrations on the uptake and internal concentrations of macronutrient elements have been extensively studied (Greenway & Munns, 1980; Cramer *et al.*, 1991; Al-Raway *et al.*, 1992; Ashraf & Khanum, 1997; Drihem & Pilbeam, 2002; Poustini & Siosemardeh, 2004). In terms of the interaction between Na^+ and compatible solutes in plant cell sap, cytoplasmic ion homeostasis by exclusion of excess Na^+ from the cytoplasm may necessitate the plant to synthesize compatible osmolytes to reduce the osmotic potential, which is required for water uptake under salt stress. Thus, K^+ uptake is pivotal for cell turgor and maintenance of biochemical processes under salinity and the role of potassium has been investigated because it is involved in many physiological processes such as turgor potential regulation, cell elongation, growth of shoot and roots, stomatal movement, transpiration, and under drought conditions, potassium application has shown effects on growth, water use efficiency, dry matter production and yield (Paauw, 1958; Andersen *et al.*, 1991).

In plants, Na^+ competes with K^+ for uptake under saline conditions. (Chinnusamy & Zhu, 2003). Salt stress induced decrease in the K^+/Na^+ ratio is inimical to cellular biochemical processes (Asch, *et al.* 2000; Wei, *et al.* 2003). In addition to this, K^+ and Na^+ provide the necessary osmotic potential for water uptake by plant cells (Keller & Volkenburgh, 1996; Claussen *et al.* 1997). The specific symptoms of sodium toxicity include high tissue sodium concentrations and low K/Na ratios; inhibition of root elongation and calcium deficiency (Maas & Grieve, 1987). In sugarcane, ion-toxicity was the main determinant of salt tolerance at the grand growth stage, while the osmotic component of NaCl mainly appeared to affect the transport of sucrose to stalks, followed by stimulated sucrolytic activity in the internodes, resulting in reduced final cane yield (Abdul Wahid, 2004). Mentz (2001) saw in his thesis that sodium toxicity is not as widespread as that of Cl, but unfavorable

ratios of Na/Ca and Na/K may disturb inorganic nutrition. High Na can furthermore disturb the Ca-homeostasis of root and leaf cells and therefore the uptake of essential nutrients (Rengel, 1992). It can also indirectly affect growth by its influence on soil structure and fertility, and the formation of a dense natric B-horizon that can obstruct downward water percolation and root growth. A high percentage of exchangeable Mg may also affect soil structure in a similar way to a high exchangeable sodium percentage (ESP) (Driessen & Dudal, 1991).

Other effects of salts on plants are ion toxicities of specific salts and nutritional imbalances. Some elements, such as sodium, chlorine, and boron, have specific toxic effects on plants. Plants sensitive to these elements may be affected at relatively low salt levels if the soil contains enough of the toxic element. Because many salts are also plant nutrients, high salt levels in the soil can upset the nutrient balance in the plant or interfere with the uptake of some nutrients. Ungar (1978) reported that inorganic ions were not more inhibitory than mannitol and polyethylene glycol (PEG) in several halophytes, indicating that seeds are mainly affected by osmotic stress rather than specific ion toxicities. Movement of ions into the vacuole might occur directly from the apoplast into the vacuole through membrane vesiculation or a cytological process that juxtaposes the plasma membrane to the tonoplast (Hasegawa *et al.* 2000b). The cytotoxic ions in saline environments, typically Na^+ and Cl^-, are compartmentalized into the vacuole and used as osmotic solutes (Blumwald *et al.*, 2000; Niu *et al.*, 1995). It follows then that many of the molecular entities that mediate ion homeostasis and salt stress signaling are similar in all plants (Hasegawa *et al.*, 2000b). The bulk of Na^+ and Cl^- movement from the apoplast to the vacuole is mediated through ion transport systems located in the plasma membrane and tonoplast. Presumably, tight coordinate regulation of these ion transport systems is required in order to

control net influx across the plasma membrane and vacuolar compartmentalization. The Salt-Overly-Sensitive (SOS) signal pathway is a pivotal regulator of, at least some, key transport systems required for ion homeostasis (Hasegawa *et al.*, 2000a; Sanders, 2000; Zhu, 2000). Research of more than 30 years (Yokoi, *et al.*, 2002) established that intracellular Na^+ homeostasis and salt tolerance are modulated by Ca^{2+} and high external Na^+ negatively affects K^+ acquisition (Rains & Epstein, 1967). Na^+ competes with K^+ for uptake through common transport systems and does this effectively since the external Na^+ concentration in saline environments is usually considerably greater than that of K^+. Ca^{2+} enhances K^+/Na^+ selective intracellular accumulation (Maathuis *et al.*, 1996; Rains & Epstein, 1967). Responses to ion toxicity, osmotic stress and oxidative stress may be integrated by signaling pathways including MAPK and its negative regulator MAPK phosphatase.

2.Soil salinity and physiological strategies:

Although salinity affects plants physiologically in many different ways, injury is not readily seen morphologically, except at extreme salt concentrations. The most general effect is a reduction in growth and growth rate. Plants that are salt-sensitive or moderately tolerant show a progressive decline in growth and yield as salinity levels increase (Bernstein, 1964, 1974). Plant parts are not all equally affected: shoot growth is usually influenced more than root growth with a concomitant decrease in the shoot to root ratio. The leaf to stem ratio is also often affected, which could be important when crops are used for forage (Maas & Hoffman, 1977).

2.1.Salt accumulation and salt exclusion:

Excessive salt accumulation in soils, in fact, has been recognized as a limiting factor for crop production of one-third of the world's limited arable land (Epstein *et al.*, 1980). Salt accumulates in soil because of different factors: 1- Along the coastline and barrier islands where seawater may wash over, and where salt from spray may collect in the soil. 2- Along brackish tidal rivers and estuaries. Flooding during storms and high tides can deposit salt in lowlying areas. Wooded wetlands are frequently found in these locations. 3- Along sidewalks and roads where salt is used to remove ice and snow, where treated ice and snow are piled when pavements are cleared, or where vehicles cause salt spray. As the snow melts, runoff carries the salt to low-lying areas. Salt accumulation usually occurs within 30-50 feet of roads. 4- In cultivated areas when fertilizers are over applied, when high salt index fertilizers are used, or when fresh animal wastes (manures) are spread on fields. 5- In areas where crops or landscape plants are irrigated with water containing dissolved salts. Repeated light watering without leaching or adequate drainage can result in salt accumulation in the soil. 6- In areas with high groundwater tables (Appleton, 2002). 7-Deforestation (Hatton 2002); removing the trees that were using a high quantity of underground water has led to a rise in the groundwater table, moving salts up to the soil surface as a consequence of deforestation.

Chinnusamy & Zhu, (2003) reported that the emergence of this problem (soil salt accumulation) could be due to one or more of these factors; 1- the use of poor quality water for irrigation, 2- improper drainage in canal-irrigated wetland agro-ecosystems, 3- entry of seawater during cyclones in coastal areas, and 4- salt accumulation in the root zone in arid and semi-arid regions due to high evaporative demand and insufficient leaching of ions as the rainfall is inadequate.

Table.2 shows how salt accumulation in soil has changed the soil characteristics (Lamond, 1992).

Table.2. Classification of salt-affected soil.

Soil Classification	Electrical conductivity (mmhos/cm	Soil pH	Exchangeable sodium percentage	Soil physiological condition
Saline	>4.0	<8.5	<15	Normal
Sodic (alkali)	<4.0	>8.5	>15	Poor
Saline-sodic	>4.0	<8.5	>15	Normal

Ions at high concentrations in the external solution (e.g. Na^+ or Cl^-) are taken up at high rates, which may lead to excessive accumulation in plant tissues (Mer, *et al.* 2000). There are contributory features that function to maintain low rates of salt accumulation in leaves. Sodium translocation from the leaves (Lauchi, 1984) and lower leaf accumulation of Na (Ashraf & O'Leary, 1996), could result in the maintenance of a higher K/Na ratio, which would be suitable for the metabolic processes occurring within the plants and it is proposed that salinity tolerance might be related to some extent, to an ability to restrict or control ion accumulation in shoot tissue (Poustini & Siosemardeh, 2004). It has been reported that the salt tolerant barley variety maintained a cytosolic sodium concentration ten times lower than the more sensitive variety (Carden *et al.* 2003). High shoot/root ratios and high intrinsic growth rates (Pitman, 1984), and absence of an apoplastic pathway in roots (Garcia *et al.*, 1997) will all serve to reduce the rate at which salt enters the transpiration stream and accumulates in the shoot. Neumann (1997) reported that higher rates of salt accumulation in more sensitive varieties then lead to accelerated leaf senescence. This further inhibits new growth, as compared with more resistant varieties, and he suggested that breeders aiming to

increase crop growth under salinity should focus efforts on manipulating genes, which can decrease rates of salt accumulation. Munns (1993) hypothesized that plant growth is initially inhibited (phase 1) by cellular responses to the osmotic effects of external salt, i.e. by responses to the decreased availability of soil water. In a later, second response (phase 2), growth is further inhibited by the toxic effects of excessive salt accumulation within the plant.

2.2.Compatible solutes:

Compatible solutes are synthesised in response to osmotic stress and can occur at high intracellular concentrations without hindering normal cellular metabolism (Ramanjulu & Bartels, 2002). The organic solute in the cytoplasm can have following roles: 1- a contribution to the osmotic balance when electrolytes are lower in the cytoplasm than in the vacuole (Stewart & Lee, 1974), and 2- a protective effect on enzymes in the presence of high electrolytes in the cytoplasm (Pollard & Wyn Jones, 1979). Under osmotic stress, an important consideration is to accumulate osmotically active compounds, called osmolytes, in order to lower the osmotic potential.

A majority of plants divert normal metabolic pathways and increasingly synthesize the compatible solutes to mitigate the adverse effects of salinity (Hare *et al.*, 1998). These are referred to as compatible metabolites because they apparently do not interfere with the normal cellular metabolism. Molecules like glycerol and sucrose were discovered by empirical methods to protect biological macromolecules against the damaging effects of salinity (Sairam & Tyagi, 2004). Later, a systematic examination of the molecules, which accumulate in halophytes and halo-tolerant organisms, led to the identification of a variety of molecules also able to provide protection (Arabawa & Timasheff, 1985; Wiggins, 1990). Characteristically, these

molecules are not highly charged, but are polar, highly soluble and have a large hydration shell. Such molecules will be preferentially solubilized in the bulk water of the cell where they could interact directly with the macromolecules. Osmolytes for which some progress has been made are indicated in Table.3 (Sairam & Tyagi, 2004).

Table.3. Important osmolytes that accumulate in plants during drought and salinity.

Carbohydrate	Nitrogenous compound	Organic acid
Sucrose	Proteins	Oxalate
Sorbitol	Betaine	Malate
Mannitol	Glutamate	
Glycerol	Aspartate	
Arabinitol	Glycine	
Pinitol	Choline	
Other polyols	Putrescine	

Mannitol: many organisms, including some plants synthesize and accumulate mannitol. Abebe *et al.* (2003) found that transgenic plants has demonstrated that cellular accumulation of mannitol can alleviate abiotic stress, and ectopic expression of the *mtlD* gene for the biosynthesis of mannitol in wheat improves tolerance to water stress and salinity, but they reported that the function of mannitol in stress tolerance has not been evaluated in plants of agronomic importance. Transgenic tobacco plants synthesize mannitol-1-phosphate from fructose-6-phosphate. In the absence of salt-stress, wild and transformed plants had similar height and fresh weight gains, but in the presence of 250 mol m^{-3} salt, the *mtlD* gene-transformed plants had a growth

advantage over the wild type in terms of better height grain, less fresh-weight loss and more new leaf and root production (Sairam & Tyagi, 2004). Binzel *et al.* (1988) found that tobacco cells adapted to 428 mM NaCl could maintain cytosolic Na^+ and Cl^- level at less than 100 mM. Though mannitol only partially decreased the amount of inorganic ion accumulation in the cytosol, its protective effect as a compatible solute may have been sufficient to give the marginal growth advantage observed in transformed plants. Su *et al.* (1999) demonstrated that biosynthesis and accumulation of mannitol in plants was correlated with the salt-stress tolerance of plants. These solutes are widely believed to function as a protector or stabilizer of enzymes or membrane structures that are sensitive to dehydrations or ionically induced damage.

Pinitol: The cyclic sugar alcohols, pinnitol and ononitol, are stored in a variety of species which are consistently exposed to saline conditions or accumulate in tolerant species when exposed to saline environments (Paul & Cockburn, 1989).

Sorbitol: This sugar alcohol of glucose is found in a variety of plant species, usually as a constituent of seeds. Sorbitol accumulation has been reported in seeds of many crop plants (Kuo *et al.* 1990). Increasing salinity from 0 to 400 mM resulted in an eightfold increase of sorbitol concentration in shoot tissues and a 100-fold increase in root tissues (Sairam & Tyagi, 2004).

Proline: In organisms ranging from bacteria to higher plants, there is a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effects of high environmental salinity (Flowers *et al.*, 1977; Gorham *et al.*, 1981; Yancey *et al.*, 1982; Singh *et al.* 1972). The intermediates of proline biosynthesis and catabolism, such as glutamine and d-1-pyrroline-5-carboxylic acid could increase the expression

of several osmotically regulated genes in rice (Iyer & Caplan, 1998). There is also evidence that degradation of proline in the mitochondria is directly coupled to the respiratory electron transport system and ATP production (Sairam & Tyagi, 2004).

A relationship between proline accumulation and salt tolerance was found in many previous studies (Rabe, 1993; Viegas *et al.* 1999; Sakhabutdinova *et al.*, 2003; Murphy *et al.* 2003). Proline is a protector of enzyme activity; it minimizes the inhibition of the activity of rubisco by NaCl (Solomon *et al.*, 1994).

Glycine-betaine: It was also found that it correlated with salt tolerance, in *Poaceae* species where the highly tolerant species accumulated the highest levels, moderately tolerant species accumulated intermediate levels and sensitive species accumulated low levels or no glycine-betaine (Rhodes *et al.* 1989). Huang, *et al* (2000) reported that the levels of glycine betaine were 18.6, 12.8 and 13.0 mmol g^{-1} dry weight in *Arabidopsis thaliana*, *Brassica napus* and *Nicotiana tobbacum* respectively, 10-20 fold lower than the levels found in natural betaine producers.

Polyamines: A number of stress factors such as potassium deficiency, osmotic stress, low pH, nutrient deficiency or light have been shown to stimulate the accumulation of polyamines, and particularly putrescine in plants. Putrescine accumulation during environmental stress correlated with increased argenine decarboxylase (ADC) activity in oats. Recent studies with transgenic carrot cells over-expressing ornithine decarboxylase (ODC) cDNA showed that these cells were significantly more tolerant to both salt stress as well as water stress (Bohnert *et al.* 1995). Polyamines have recently gained importance due to their role in the escape of seedlings from the adverse effects of salinity.

Suppression of polyamine biosynthesis by cyclohexylamine has been reported to result in increased ethylene synthesis as well as seed germination (Gallardo *et al.* 1995). Lin & Kao (1995) reported an increase in the level of spermidine under salinity, but a low level of putrescine in the shoot and roots of rice seedlings. Under salinity and drought conditions, polyamines, as well as their corresponding enzyme activities, are substantially enhanced (Lefevre & Lutts, 2000). Transgenic rice for argenine decarboxylase (ADC) cDNA showed an increase in biomass under salinity stress conditions compared to the control (Roy & Wu, 2000). Lefevre *et al.*, (2001) suggested that the ionic component by itself might trigger short-term polyamine accumulation.

Compatible solutes have also been shown to function as free radical scavengers, protecting DNA from the degradative effects of reactive oxygen species (Akashi *et al.*, 2001). Roles for galactinol and raffinose as compatible solutes under osmotic stress was found by Mundree *et al.* (2002). Other organic solutes such as certain polyols and cyclitols, can be accumulated under salinity conditions to osmotically adjust the cytoplasm without inhibiting enzymes (Gorham, 1996). Ashraf & Harris (2004) found that myo-inositol could serve not only as a substrate for the production of compatible solutes but also as a leaf-to-root signal that promotes Na^+ uptake.

The successful engineering of metabolic pathways for a number of compatible solutes such as glycine betaine, sorbitol, mannitol, trehalose and proline have led to reported results of transgenic plants which display increased resistance to drought stress, high salinity and cold stress (Chen & Murata, 2002). Murphy, *et al.* (2003) suggested the use of the ^1H nuclear magnetic resonance (NMR) method (Ratcliffe, 1994) to identify and quantify compatible solutes in leaf tissue, as the NMR is a non-invasive technique that can be used to determine solute contents and concentrations within cells.

2.3.Role of hormones:

Several reports indicated changes in the levels of endogenous growth regulators under stress conditions. Ramana (1968) observed a large reduction in gibberellin activity under saline conditions in groundnut. Cytokinin and gibberellin were reduced under salinity stress (Boucard & Unger, 1976; Itai & Vaadia, 1965). Application of abiotic stresses during germination and the early cycle of plant species, results in altered levels of plant hormones and decreased plant growth (Morgan, 1990). Ethylene increases rapidly as a response to water stress (Zhang, 1997). Lipid peroxidation, due to activated oxygen under water stress may be related to the increase of the ethylene level (Hildebrand & Grayburn, 1991; Smirnoff, 1993). It was reported that water stress enhances respiration, senescence and ripening which are all related to increased ethylene levels (Morgan, 1990). One of the major signals operating during water stress is the plant hormone abscisic acid, ABA. Studies on ABA have shown that this hormone mediates various developmental and physiological processes that affect the agronomic performance of crop plants such as embryo maturation and germination, as well as the response of vegetative tissues to osmotic stress (Ramagopal, 1987; Zeevaart & Creelman, 1988; Mizrahi *et al.*, 1970; Itai & Vaadia, 1965). ABA increases as a result of water stress and has important roles in the tolerance of plants to drought, high salinity and cold (Mundree *et al.*, 2002).

Most of the earlier studies indicated that changes in hormonal balance during saline conditions depend more on the total concentration of soluble salts than on specific ions (Ramana, *et al.*, 1984). The direct effect of salt on hormone levels remained unclear till the late 70s as reviewed in the study of Jennings, (1976). A reduction in hormone levels can be compensated for by exogenous hormone treatments, which can ameliorate the deleterious effects of salinity

as demonstrated in several studies (Odegbaro & Smith, 1969; Singh & Dara 1971; Darra *et al.*, 1973; Adam 1996; Shonjani, 2002). Kahn *et al.* (1957) reported that an osmotic inhibition of the germination of lettuce seed by mannitol might be overcome by treatment of the seeds with gibberellin. Chaudhuri & Wiebe (1968) found that GA and kinetin increased salt resistance in wheat by increasing the percentage of germination. In more recent studies, the application of exogenous hormone has been found to ameliorate the deleterious effects of salinity. Kazama & Katsumi (1983) reported that treatment with GA_3 reduced the osmotic potential and increased the starch content in cucumber plants. Setia & Narang (1983) found that the application of growth substance (GA_3, IAA and kinetin) could enhance the germination percentage of salt stressed pea plants. Gadallah (1999) found that application of kinetin to wheat plants reduced Na^+, Ca^{2+} and Cl^- accumulation and improved K^+ uptake under salinity stress, and kinetin also reduced membrane injury.

Several previous studies reported that the application of GA for improving salt tolerance and tolerance to other stresses, was more effective than the application of other hormones (Setia & Narang 1983; Wang *et al.*, 1996; Kaur *et al.*, 1998).

The role of plant hormones and their interaction with salinity in wheat plants will be further examined and reviewed in Chapters 3 and 5, which present our study.

3.Genetics and breeding for salt tolerance:

The extensive genetic diversity for salt tolerance that exists in plant taxa is distributed over numerous genera (Flowers *et al.*, 1986; Greenway & Munns, 1980). One of the earliest report of variability and inheritance of salt tolerance of tomato was by Lyon (1941) who reported that *Lycopersicum pimpinellifolium* was less sensitive than L. *esculentum* to salinity. Strogonov (1954) suggested that by crossing selections for salt tolerance taken from saline fields with vigorous plants taken from non-saline fields, and then screening the progeny of the subsequent generation in saline fields could obtain increasing tolerance. However, it was only about the seventies and the eighties that workers realized the importance of finding salt-tolerant varieties and cultivars by screening a wider range of germplasm. Subsequently such studies in different crops provided evidence that promising genotypes do exist and there is a hope that they can be exploited for improving salt tolerance (Flowers & Yeo, 1995). Cassells & Doyle (2003) have seen that genetic engineering is often presented as a one-step, rapid solution to the improvement of stress tolerance in plants. While it may benefit from but not necessitate the requirement for backcrossing for gene introgression, it does not remove the requirement for field trials.

A major requirement in traditional breeding for salt tolerance is that genetic variation exists for that specific character in the gene pool. Such variation may be between individuals, varieties, or species along with some degree of sexual compatibility, so that genes may be transferred from one individual to another (Sharma & Goyal, 2003).

Research of recent decades has established that most halophytes and glycophytes tolerate salinity by rather similar strategies often using analogous tactical processes (Hasegawa *et al.*, 2000b).

Research on the plant genetic model *Arabidopsis* has greatly increased our understanding of how cellular salt tolerance mechanisms are integrated and coordinated in an organismal context, and are linked to essential phenological adaptations (Yokoi *et al*, 2002). Since *Arabidopsis* is a glycophyte, a salt tolerant genetic model will be required to delineate if salt tolerance is affected most by form or function of genes or more by differences in the expression of common genes due either to transcriptional or post-transcriptional control (Zhu, 2001).

The genetic study of salt tolerance has been linked with two other major abiotic stresses, drought and cold, and many genes that are regulated by salt stress are also responsive to drought or cold stress (Zhu *et al*., 1997).

Some attempts in this line have been successful, a transformed tomato with yeast HAL1 (salt tolerance) is reported to improve its level of salt tolerance (Gisbert *et al*., 2000), while Zhang *et al*. (1999) were able to demonstrate that allelic variation in one copy of a small family of H1 ATPase genes was correlated with a quantitative trait locus (QTL) for salt tolerance in rice. The identification of QTL has, therefore, practical importance to attempts to enhance stress tolerance (Koyama *et al*., 2001). They also reported that identifying and mapping the major QTL associated with the salinity tolerance traits of low sodium uptake and regulation of Na/K ratio, has been accomplished. Markers closely associated with major QTL for salt tolerance might then be used for breeding programs in rice.

However, most of the processes found, empirically, to be important in plant resistance or tolerance of salinity, exhibit quantitative inheritance; that is they show continuous variation and a high degree of environmental sensitivity (Koyama *et al*., 2001; Oerke *et al*., 1999). Several authors have argued for

engineering of specific stress pathways for constitutive higher expression, but this would imply a significant yield penalty (Cassells &Doyle, 2003).

So, the genetics attempts are still facing many problems that make its goals elusive. An important reason for the limited success of the genetic approach has been the lack of standardized and uniform conditions in the experimental conditions conducted in different laboratories, and many studies were carried out under artificial laboratory conditions with little or no similarity to the prevailing natural saline conditions. Many potential criteria or traits have been proposed for screening, often unrelated to each other, and giving different estimates of salt tolerance. Moreover, the salt tolerance differs at different growth stages (Sharma & Goyal, 2003).

4.The study Hypotheses:

It clear that the salinity problem is a major agriculture problem in the entire world, thus attempts to solve that problem are needed.

This study hypothesizes two different ways to attempt reduce salinity effects on wheat plants as one of the most important annual crops. Hypothesis 1: There is sufficient genetic variation with respect to salinity in wheat cultivars to enable the selection of resistant cultivars. Hypothesis 2: hormonal treatments will be able to enhance wheat performance under saline conditions.

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CHAPTER 2

Differential Salt Resistance Of Selected Wheat Cultivars

2.1. Summary:

Thirty eight wheat cultivars were investigated in terms of salinity resistance at the germination and early seedling stages under 0, 100, 200, 300, and 400 mM NaCl salt levels. Palmiet, SST 65, Adam Tas, Chokka, Nantes, Dias, SST 16, SST 38, SST 66, SST 55, SST 825, SST 57, Kariega, SST 75, Daeraad, Sterling, Flameks, Bona, Impala, Sokkies, K20, Rooi Indies, Keniagover, Van Dyk, Unie 52, Palala, Eksteen, Klein Trou, Knoppies, Du Toit, Liesbeeck, Drommedaris, Rooiwol, Rooigys, Yecoro Royo, Charchia, Losper, and Losper 52 were used. The paper towel (paper roll) method was used to germinate the seeds. After seven days the percentage seed germination, coleoptile length, root length, and root mass were determined as indicators of cultivar performance. Results were subjected to variance analysis and the cultivars were arranged in a table in order of decreasing salinity resistance. Results showed considerable intervarietal differences (between the thirty eight wheat cultivars) in the above parameters at different salt levels.

2.2. Introduction:

Excess soil salinity is a major agricultural concern in arid and semiarid regions. Salt can be found in the soil naturally, as in salt marshes and the areas around salt marshes, and the areas close to the sea, or due to the use of irrigation water with a high salt concentration. Areas like these, which suffer from a decrease in fresh water availability, occur in North Africa and the Middle East. High concentrations of salts have detrimental effects on plant growth (Bernstein, 1961; Kramer 1983; Pandey & Thakarar, 1997) and excessive concentrations kill growing plants (Donahue *et al.* 1983). Many investigators have reported inhibition or retardation of germination and growth of seedlings at high salinity, as did Bernstein (1962).

Crop yields are usually higher under irrigation and less dependent on the effects of the weather. While only 15% of the world's cultivated land is irrigated, it accounts for 35-40% of the global food harvest. Projected population growth rates for the next 30 years will require an increase in food production equal to 20% in developed countries and 60% in developing countries to maintain present levels of food consumption (U.S. Salinity Laboratory, Riverside, California). To solve the problem, either the salt concentration of the root growth zone in the soil needs to be decreased, or plant salt tolerance must be improved. Selection of salt tolerant cultivars, and the development of new plant varieties that can grow under high salinity levels, may help. An alternative is to stimulate the plant to resist the excessive salinity levels, which could be achieved via treatment of the plant with some chemicals that improve osmotic adjustment. Adult plants grow in substrate salinities of 50-425 mM NaCl (Blits & Gallagher, 1990). However, plant species differ in their sensitivity or tolerance to salts (Troech & Thompson 1993). Varietal differences in salt tolerance have been known since the 1930's

(Epstein, 1977). One of the most efficient methods of improving turfgrass growth in salt-stressed situations is to use salt tolerant species and/or cultivars (Qian *et. al,* 2001).

The success achieved in producing salt-tolerant crops has, however, been very limited. Over the years that records have been kept, only about 25 cultivars of just 12 species have been released for their salt tolerance (Flowers & Yeo, 1995; Shannon & Noble, 1990).

In 1898, the first results of studying the tolerance of plants to salinity in the germination period were reported (Zeynalabedin & Mohammed, 2002). While Pearce (2003) believes that the first attempt to evaluate the inheritance of salt tolerance was made by Lyon in 1941.

Salinity resistance in plants is a complex trait involving several interacting physiological properties, the expression of which is strongly influenced by environmental factors (Yeo 1983). Salt tolerance in plants is a complex phenomenon involving morphological, physiological, and biochemical processes (Jacoby, 1999).

Many plants in saline or dry habitats are known to accumulate organic solutes such as glycine-betaine or proline (Poljakoff-Mayber *et al.*, 1987). It is assumed that under stress condition these substances serve as compatible cytoplasmic solutes that compensate osmotically for external osmolarity or for ions sequestered in the vacuole (Singh *et al.*, 1972; Storey & Wyn Jones 1975; Ahmed *et al.*, 1979).

The wheat plant is one of the most important economic crop plants. It is one of the top three crops in the world (Miller 2000). Previous studies on wheat cultivars have shown that there are intravarietal differences in salt tolerance (Qureshi *et al.* 1980; Kingsbury & Epstein 1984; Rashid 1986; Troech & Thompson 1993). Different physiological traits such as potassium (K^+) selectivity, exclusion and/or compartmentation of sodium (Na^+) and chloride (Cl^-) ions, balance of nitrate and chloride (NO_3^- /Cl^-), osmotic adjustment, and the accumulation of organic (compatible) solutes have all been related to the salt tolerance of cultivars of different species (Abel & MacKenzie, 1964; Storey & Wyn Jones, 1978; Yeo & Flowers, 1982; Kingsbury *et al.*, 1984; Kingsbury & Epstein, 1986; Weimberg, 1987. Yeo *et al.*, 1990). Young seedlings of wheat exhibited a gross ability to adjust osmotically in response to high salinity stress with the decrease in external osmotic potential being compensated for by the decrease in mean shoot sap osmotic potential (Rashid *et al.*, 1999). Wheat plants possess the ability to exclude Na^+ and Cl^- ions from the expanding leaf as salinity levels increase concentrating these ions in the older leaves.

No consistent relationship exists between growth, stomatal conductance, the assimilation rate and the degree of salt tolerance, (Ashraf & O'Leary, 1996). According to the FAO report of 1985, irrigation water can be classified into three groups as listed in Table 2.1.

Table 2.1. FAO Classification of irrigation water, (1985).

	Group 1 (Slight salinity)	**Group 2** (Moderate salinity)	**Group 3** (Excess salinity)
EC	<0.7 $mS.cm^{-1}$	0.7-3 $mS.cm^{-1}$	3-7.5 $mS.cm^{-1}$
Na	<3 mM/L	3-9 mM/L	>9 mM/L
Cl	<4 mM/L	4-10 mM/L	>10 mM/L
SAR	<3 mM/L	3-9 mM/L	>9 mM/L
B	<0.7 ppm	0.7-3 ppm	>3 ppm
$HCO3^-$	<1.50 mM/L	1.50-8.5 mM/L	>8.5 mM/L
N-NO3	<5 ppm	5-30 ppm	>30 ppm

EC= Electric Conductivity. SAR= Sodium Adsorption Ratio.

Soil salinity can reduce plant growth by perturbing matter allocation, ion relations, water relations, and other biochemical and physiological processes. Variability in osmotic adjustment capacity was observed between many barley genotypes under low water potential in the study by Arnau *et al.* (1997).

2.3. Materials and Methods:

2.3.1.Plant material: Thirty eight wheat cultivars were investigated in order to select cultivars suitable to the further aims of this study. Thirty four cultivars were obtained from the Agronomy Department of the University of Stellenbosch, and four salt resistant cultivars were supplied by the Small Grain Institute in Bethlehem, South Africa.

The cultivars Palmiet, SST 65, Adam Tas, Chokka, Nantes, Dias, SST 16, SST 38, SST 66, SST 55, SST 825, SST 57, Kariega, and SST 75 are known as modern cultivars, because they have been selected during the last ten years in South Africa. Cultivars Daeraad, Sterling, Flameks, Bona, Impala, Sokkies, K20, Rooi Indies, Keniagover, Van Dyk, Unie 52, Palala, Eksteen, Klein Trou, Knoppies, Du Toit, Liesbeeck, Drommedaris, Rooiwol, and Rooigys are older cultivars, up to fifty years of age. Cultivars Yecoro Royo, Charchia, Losper, and Losper 52 are salt tolerant cultivars, which were supplied by the Small Grain Institute in Bethlehem, South Africa. Table 2.2. Lists these cultivars with the number assigned to each for convenience.

Table 2.2. The wheat cultivars that were studied.

Assigned #	Modern Cultivars	Assigned #	Traditional Cultivars	Assigned #	Salt Resistant Cultivars
1-	Palmiet	15-	Daeraad	35-	Yecoro Royo
2-	SST 65	16-	Sterling	36-	Charchia
3-	Adam Tas	17-	Flameks	37-	Losper
4-	Chokka	18-	Bona	38-	Losper 52
5-	Nantes	19-	Impala		
6-	Dias	20-	Sokkies		
7-	SST 16	21-	K 20		
8-	SST 38	22-	Rooi Indies		
9-	SST 66	23-	Kenia Gover		
10-	SST 55	24-	Van Dyk		
11-	SST 825	25-	Unie 52		
12-	SST 57	26-	Palala		
13-	Kariega	27-	Eksteen		
14-	SST 75	28-	Klein Trou		
		29-	Knoppies		
		30-	Du Toit		
		31-	Liesbeek		
		32-	Drommedaris		
		33-	Rooiwol		
		34-	Rooigys		

2.3.2. Equipment and Supplies: The method of the International Seed Testing Association (TSTA) (Hampton & Tekrony, 1995) was used to study germination and initial growth of wheat cultivars (Fig 2.1). Commercially produced germination paper towels of the same weigh, thickness, and size (65x30 cm) were used. Twenty seeds were placed on a paper towel (in a

Laminar Flow Cabinet), then the mean of the twenty were taken as a one replicate. Five plastic containers (30x22x12 cm) were used to soak the papers in the various salt solutions. Plastic bags 42x33 cm were used to keep the towels rolls moist. Plastic pots were used to keep rolled towels in an upright position. A Fisons Growth Chamber Model: L.T.G.C. was maintained under a 10/20°C regime (night/day respectively), and twelve hours day/night setting for seven days. Four NaCl solutions (100, 200, 300, and 400 mM) were used as germination media, and for a control, distilled water was used.

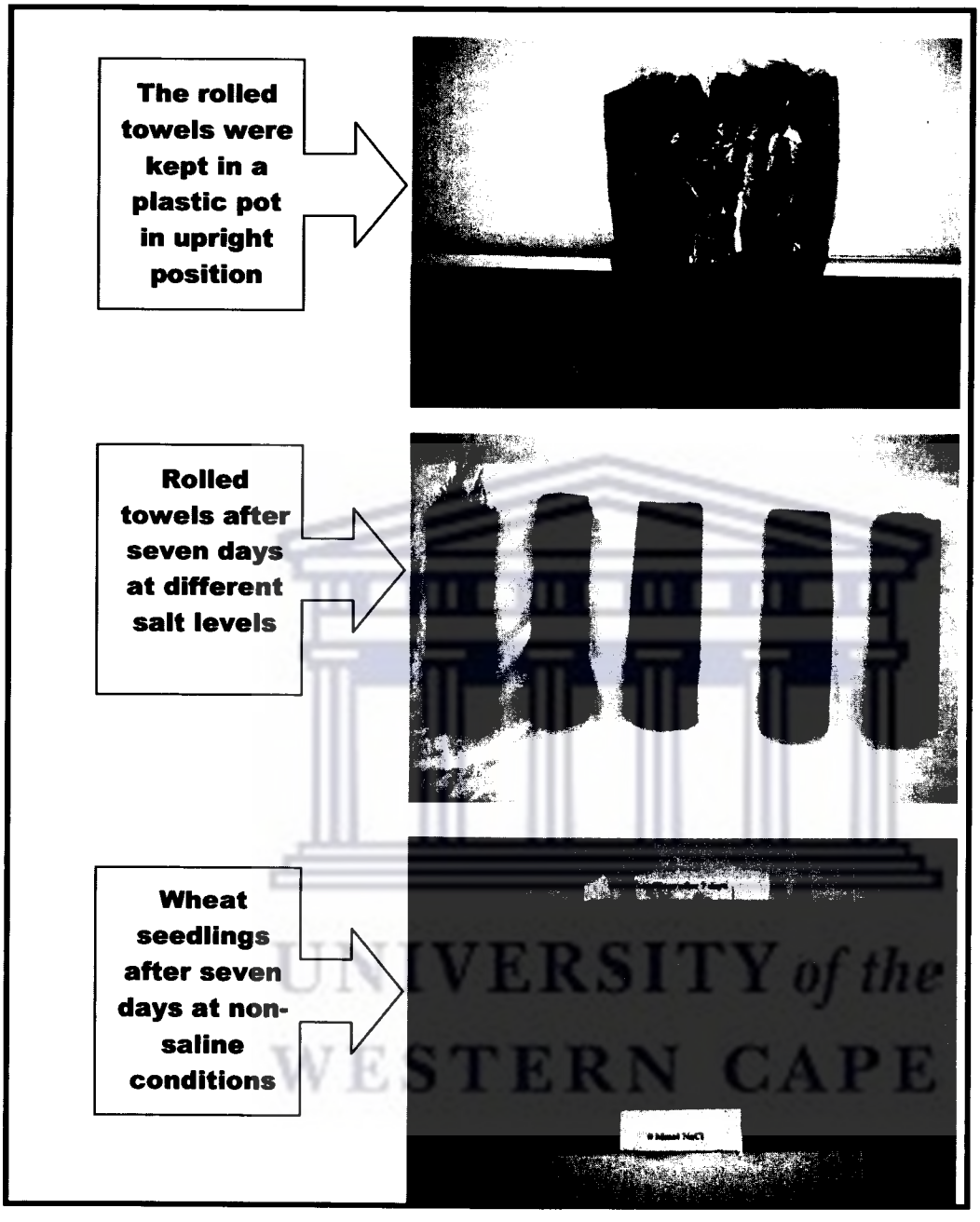
2.3.3. Experimental design: A chain block method was used involving the 38 wheat cultivars, five salt concentrations and two replicates. The thirty eight wheat cultivars were distributed in six successive experiments. Five wheat cultivars plus one as a control were used in each experiment, except in experiment one where twelve cultivars were used. Cultivar Adams Tas was used as a standard control in each experiment.

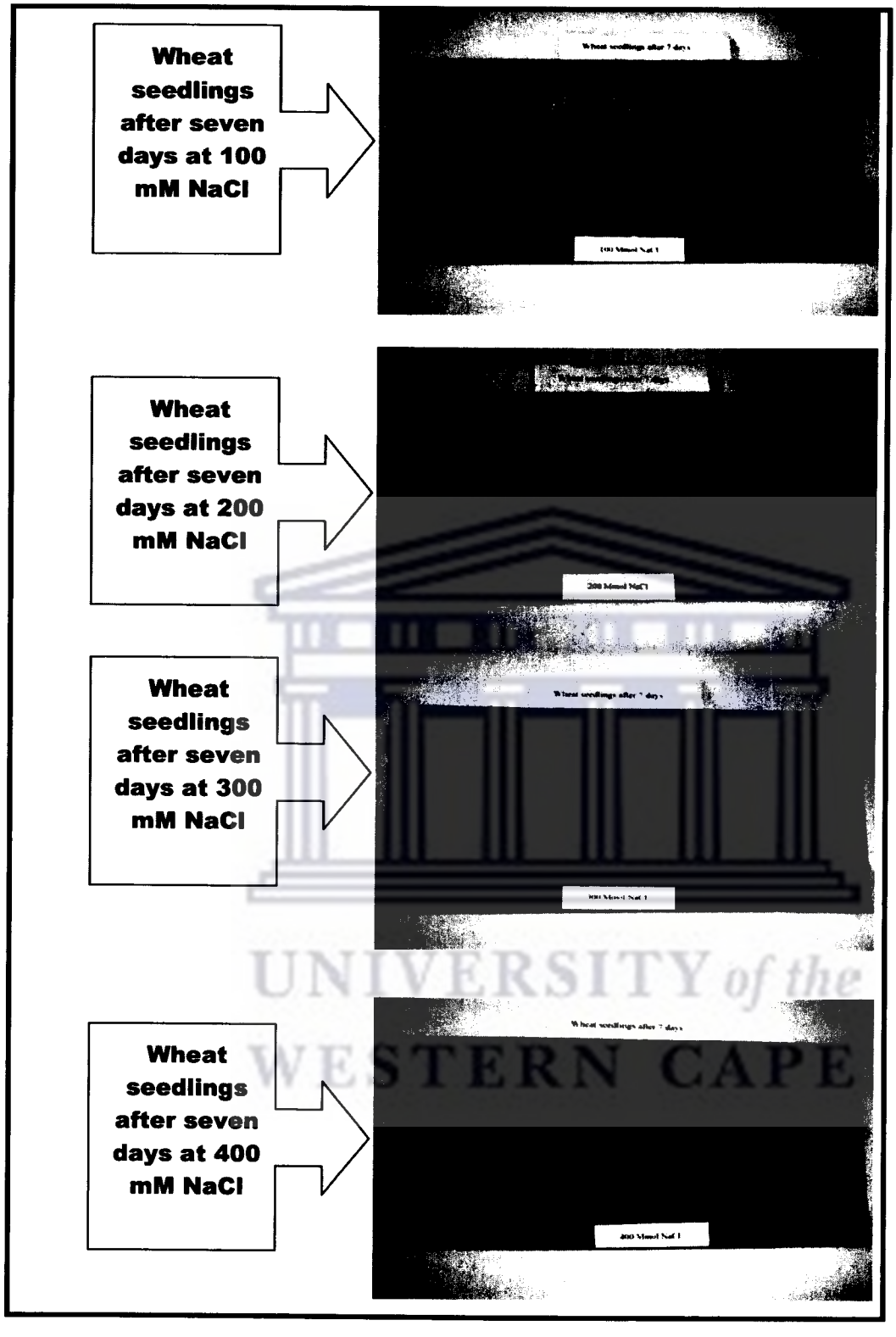
2.3.4. Seed sterilization: Directly before placing seeds on the paper towels the seeds were surface sterilized in 3.5% sodium hypochlorite solution (NaOCl) for 3 minutes and then immediately gently washed with sterilized, distilled water.

2.3.5 Parameters Measured: After seven days of germination the percentage seed germination, coleoptile length, root length, and fresh root mass were determined as indicators of cultivar performance.

2.3.6. Statistical analysis: The results were subjected to variance analysis.

Fig 2.1. The steps of the Paper Towel method, to test one wheat cultivar in five salt concentrations.





2.4. Results:

All experimental variables (Cultivars, salinity, & their interaction) proved to be significant ($P<0.01$ – Table 2.3). The same table shows error not to have been significant.

Table 2.3: ANOVA Table of the effects of five salt treatments on thirty eight wheat cultivars:

ANOVA		Root Length		Col. Length		%Germination		Root Mass	
Source	*d.f*	*MS*	*P*	*MS*	*P*	*MS*	*P*	*MS*	*P*
Trial	5	33.99	0.0001	6.884	0.0001	758.5	0.0001	0.0007	0.0001
Error (a)	6	0.33	0.8269	0.16	0.3785	93.41	0.0982	0.0000	0.977
Cultivar	39	2.53	0.0001	1.20	0.0001	384.2	0.0001	0.0001	0.0001
Salinity	4	2722	0.0001	685.1	0.0001	27094	0.0001	0.0436	0.0001
Cul*Sal	156	2.47	0.0001	0.806	0.0001	132	0.0001	0.0001	0.0001
Error(b)	239	0.70		0.149		51.66		0.00002	
Cor total	449								

Tables 2.4, 2.5, 2.6 and 2.7 give the results of root length, coleoptile length, germination, and root mass respectively.

Table 2.4: The effect of five salt treatments on the root length (cm) of thirty eight wheat cultivars: (Means with the same letter are not significantly different).

	Salt treatments (mM)						
Cultivar	0	100	200	300	400	Mean	T GROUPING
33	17.96	14.90	8.91	1.51	0.46	8.75	A
34	16.37	15.27	7.31	1.65	0.43	8.21	A B
25	16.59	12.98	6.20	2.87	0.68	7.86	B C
29	15.76	12.89	6.76	1.58	0.56	7.51	B C D
31	15.50	11.94	7.25	1.96	0.59	7.45	E C D
13	14.08	12.06	7.11	2.80	0.67	7.34	E C D
17	11.47	11.92	8.88	3.33	0.95	7.31	E C D F
21	15.32	10.67	6.20	2.43	0.63	7.05	E G D F
15	14.56	11.03	6.59	2.20	0.75	7.03	E G D F
30	15.05	11.45	5.84	1.61	0.78	6.95	E G D F H
28	12.66	11.63	6.61	2.63	1.02	6.91	E G D F H I
4	13.46	11.44	6.39	2.44	0.73	6.89	E G D F H I
3	13.65	11.32	6.38	2.39	0.71	6.89	E G D F H I
22	13.84	11.46	5.82	2.31	0.70	6.83	E G D F H I
19	13.05	11.92	6.23	1.97	0.65	6.76	E G J F H I
26	13.64	11.42	5.02	2.88	0.78	6.75	E G J F H I
20	13.46	11.74	6.06	1.87	0.52	6.73	E G J F H I
14	13.59	11.72	5.12	1.83	0.68	6.59	K G J F H I
11	13.62	10.02	6.06	2.20	0.99	6.58	K G J F H I
32	14.94	10.96	4.81	1.45	0.65	6.56	K G J H I
1	14.01	9.65	6.40	1.80	0.85	6.54	K G J H I
5	12.54	10.70	6.35	1.89	0.71	6.44	K G J L H I
6	12.81	9.39	6.83	2.24	0.59	6.37	K G J L H I
37	10.71	11.69	5.50	2.83	1.09	6.36	K G J L H I
7	12.33	10.01	6.70	1.83	0.84	6.34	K G J L H I
36	10.34	10.78	6.65	2.89	1.03	6.34	K G J L H I
24	10.84	10.49	6.80	2.10	0.97	6.24	K J L H I
9	12.28	10.75	5.02	2.00	0.98	6.21	K J L I
16	12.16	8.95	6.77	1.75	0.80	6.09	K N J L M
12	11.70	10.58	5.10	2.11	0.93	6.08	K N J L M
10	11.13	10.42	5.63	2.30	0.80	6.06	K N J L M
2	12.90	9.38	5.50	1.78	0.41	5.99	K N L M
27	12.47	9.39	4.97	2.01	0.81	5.93	K N L M
38	10.60	11.10	4.81	2.38	0.68	5.91	K N L M
18	12.29	9.55	4.90	1.49	0.53	5.75	N L M
8	10.68	9.90	5.56	1.68	0.92	5.75	N L M
23	11.17	9.15	4.60	1.93	0.54	5.48	N M
35	9.54	8.19	5.77	2.43	0.88	5.36	N
LSD 5%						0.73	

Table 2.5: The effect of five salt treatments on the coleoptile length (cm) of thirty eight wheat cultivars (Means with the same letter are not significantly different).

	Salt treatments (mM)						
Cultivar	0	100	200	300	400	Mean	T GROUPING
33	9.26	6.73	2.40	0.12	0.06	3.71	A
34	8.56	7.44	1.94	0.10	0.05	3.62	A B
29	8.24	6.46	1.76	0.14	0.10	3.34	B
26	7.42	4.95	1.15	0.45	0.16	2.83	C
20	8.26	4.57	0.86	0.21	0.09	2.80	C D
21	7.55	4.19	1.65	0.39	0.11	2.78	C D
22	8.29	4.30	0.99	0.18	0.12	2.78	C D
30	7.42	5.26	0.80	0.14	0.10	2.74	C D E
17	5.71	5.19	2.12	0.37	0.19	2.72	C D E
24	7.27	4.34	1.25	0.26	0.14	2.65	C D E F
3	6.30	4.46	1.69	0.26	0.13	2.57	C D E F G
19	6.68	4.48	1.19	0.25	0.18	2.56	C D E F G H
15	6.31	4.45	1.23	0.28	0.15	2.48	C D E F G H I
4	6.06	4.32	1.43	0.31	0.21	2.47	D E F G H I
10	6.17	4.29	1.22	0.29	0.21	2.44	E F G H I
27	6.80	3.77	1.04	0.36	0.19	2.43	E F G H I
8	5.56	4.49	1.24	0.27	0.17	2.35	J F G H I
36	6.01	3.90	1.27	0.33	0.13	2.33	J F G H I
1	5.90	3.44	1.69	0.36	0.23	2.32	J F G H I
5	5.37	4.27	1.49	0.25	0.18	2.31	J K G H I
14	6.03	4.37	0.87	0.16	0.12	2.31	J K G H I
32	7.10	3.62	0.51	0.13	0.10	2.29	J K G H I
37	6.24	3.79	0.92	0.33	0.10	2.28	J K G H I
7	5.34	4.01	1.64	0.24	0.13	2.27	J K G H I
2	5.41	4.27	1.07	0.32	0.21	2.26	J K G H I
28	5.91	3.80	1.13	0.30	0.13	2.25	J K G H I
38	5.73	4.00	1.04	0.36	0.11	2.25	J K G H I
25	6.60	3.41	0.85	0.23	0.11	2.24	J K G H I
9	5.43	4.19	1.09	0.27	0.17	2.23	J K G H I
13	5.45	3.94	1.36	0.25	0.12	2.22	J K H I
6	5.34	3.56	1.60	0.38	0.18	2.21	J K I
18	6.08	3.51	0.93	0.29	0.14	2.19	J K I
16	5.92	3.15	1.29	0.26	0.17	2.16	J K I
12	5.88	3.13	0.88	0.31	0.14	2.07	J K L
11	5.13	3.28	1.40	0.30	0.17	2.06	J K L
31	5.40	3.45	0.85	0.14	0.10	1.99	K L
35	4.63	2.46	1.12	0.27	0.14	1.72	L M
23	4.79	2.43	0.55	0.19	0.19	1.63	M
LSD 5%						0.34	

Table 2.6: The effect of five salt treatments on the percentage germination thirty eight wheat cultivars (Means with the same letter are not significantly different).

	Salt treatments (mM)						
Cultivar	0	100	200	300	400	Mean	T GROUPING
4	100.00	97.50	92.50	95.00	87.50	94.50	A
7	100.00	95.00	97.50	87.50	67.50	89.50	A B
17	97.50	100.00	95.00	82.50	65.00	88.00	B C
26	100.00	95.00	92.50	82.50	70.00	88.00	B C
34	97.50	100.00	95.00	87.50	55.00	87.00	E B C D
37	97.50	95.00	80.00	95.00	57.50	85.00	E B C D F
6	100.00	87.50	95.00	77.50	57.50	83.50	E B C D F G
21	95.00	95.00	87.50	72.50	67.50	83.50	E B C D F G
33	100.00	92.50	90.00	67.50	67.50	83.50	E B C D F G
2	97.50	92.50	85.00	75.00	65.00	83.00	E H C D F G
18	95.00	95.00	80.00	85.00	55.00	82.00	E H C D F G
22	95.00	92.50	87.50	85.00	50.00	82.00	E H C D F G
30	95.00	95.00	90.00	67.50	62.50	82.00	E H C D F G
3	94.50	93.00	89.00	76.00	55.00	81.50	E H I D F G
38	90.00	90.00	90.00	82.50	52.50	81.00	E H I J F G
20	100.00	95.00	80.00	80.00	47.50	80.50	H I J F G
25	97.50	100.00	85.00	65.00	55.00	80.50	H I J F G
31	97.50	97.50	90.00	65.00	52.50	80.50	H I J F G
5	100.00	97.50	85.00	65.00	52.50	80.00	H I J F G
1	97.50	92.50	67.50	77.50	60.00	79.00	H I J F G
8	95.00	82.50	90.00	72.50	55.00	79.00	H I J F G
11	95.00	97.50	85.00	62.50	55.00	79.00	H I J F G
24	87.50	90.00	95.00	60.00	62.50	79.00	H I J F G
29	92.50	90.00	80.00	70.00	57.50	78.00	H I J K G
9	97.50	97.50	77.50	77.50	35.00	77.00	H I J K
12	95.00	95.00	92.50	67.50	35.00	77.00	H I J K
15	95.00	95.00	70.00	72.50	52.50	77.00	H I J K
16	100.00	82.50	90.00	67.50	45.00	77.00	H I J K
10	95.00	95.00	82.50	57.50	47.50	75.50	I J K
19	95.00	97.50	72.50	70.00	42.50	75.50	I J K L
35	85.00	87.50	75.00	62.50	65.00	75.00	M J K L
36	92.50	85.00	77.50	60.00	60.00	75.00	M J K L
28	77.50	87.50	85.00	55.00	57.50	72.50	M N K L
14	92.50	92.50	77.50	55.00	32.50	70.00	M N O L
13	92.50	95.00	70.00	50.00	37.50	69.00	M N O
27	95.00	90.00	67.50	50.00	35.00	67.50	N P O
23	95.00	85.00	75.00	45.00	20.00	64.00	P O
32	100.00	82.50	70.00	37.50	17.50	61.50	P
LSD 5%						6.27	

Table 2.7: The effect of five salt treatments on the root mass (g) of thirty eight wheat cultivars (Means with the same letter are not significantly different).

	Salt treatments (mM)						
Cultivar	0	100	200	300	400	Mean	T GROUPING
25	0.086	0.062	0.0305	0.013	0.0025	0.0388	A
36	0.061	0.0615	0.036	0.019	0.0065	0.0368	A B
38	0.062	0.058	0.027	0.0165	0.0045	0.0336	B C
35	0.064	0.0425	0.034	0.018	0.0055	0.0328	B C D
37	0.058	0.051	0.032	0.017	0.0055	0.0327	E C D
31	0.062	0.0515	0.0345	0.0095	0.003	0.0321	E F C D G
33	0.0645	0.057	0.034	0.001	0.003	0.0319	E F C D G
11	0.0655	0.0415	0.029	0.012	0.0045	0.0305	E F C D G H
30	0.062	0.0515	0.027	0.009	0.003	0.0305	E F C D G H
34	0.0605	0.056	0.0315	0.001	0.0035	0.0305	E F C D G H
28	0.0625	0.04	0.028	0.013	0.005	0.0297	E F C D G H I
13	0.0555	0.046	0.0295	0.013	0.004	0.0296	E F C D G H I
27	0.064	0.0395	0.024	0.0125	0.0045	0.0289	E F J D G H I
32	0.0635	0.048	0.022	0.0065	0.0035	0.0287	E F J G H I
14	0.0585	0.047	0.025	0.0095	0.003	0.0286	F J G H I
3	0.0576	0.045	0.027	0.0102	0.0032	0.0286	F J G H I
17	0.0355	0.0505	0.035	0.015	0.005	0.0282	K J G H I
15	0.0495	0.045	0.03	0.0115	0.0045	0.0281	K J G H I
29	0.0505	0.0505	0.0305	0.0055	0.002	0.0278	K J L H I
21	0.0605	0.0395	0.0265	0.0095	0.0025	0.0277	K J L H I
4	0.0505	0.0465	0.0245	0.011	0.004	0.0273	K J L H I
7	0.0545	0.039	0.028	0.009	0.004	0.0269	K J L M H I
1	0.0485	0.042	0.027	0.011	0.005	0.0267	K J L M H I
20	0.053	0.043	0.024	0.0105	0.002	0.0265	K J L M H I N
9	0.055	0.041	0.0205	0.0095	0.0045	0.0261	K J L M O I N
19	0.0485	0.041	0.027	0.01	0.0035	0.0260	K J L M O I N
22	0.0505	0.042	0.025	0.0095	0.003	0.0260	K J L M O I N
10	0.045	0.042	0.025	0.011	0.004	0.0254	K J L M O N
24	0.0485	0.038	0.0255	0.01	0.0025	0.0249	K J L M O N
26	0.049	0.038	0.02	0.0115	0.0035	0.0244	K L M O N
6	0.042	0.036	0.0295	0.011	0.003	0.0243	K L M O N
12	0.0465	0.045	0.0175	0.007	0.0035	0.0239	L M O N
5	0.039	0.0415	0.026	0.0095	0.0035	0.0239	L M O N
23	0.048	0.0345	0.0205	0.0095	0.0035	0.0232	M O N
16	0.037	0.038	0.028	0.0075	0.004	0.0229	M O N
8	0.043	0.036	0.0205	0.009	0.004	0.0225	O N
2	0.041	0.039	0.0215	0.0075	0.002	0.0222	O
18	0.0415	0.0365	0.0225	0.0035	0.002	0.0212	
LSD 5%						0.004	

The analysis of variance (ANOVA) data in the Tables (2.4, 2.5, 2.6, and 2.7), are summarized in Table 2.8, and this table shows the intervarietal differences between the thirty eight wheat cultivars at different salt levels. Significant differences were found in different parameters that were used to investigate the germination and initial growth at the five NaCl levels (0, 100, 200, 300, and 400 mM). The least salt sensitive cultivars are at the top of the table, and the most salt sensitive cultivars are at the bottom. The four cultivars that appear at the top of the table (Table 2.8) are in order of decreasing resistance as follow: Yecoro Royo, Charchia, Flameks, and Losper. The three cultivars that appear at bottom of Table 2.8 are in order of decreasing sensitivity: Rooiwol, Rooigys, and Knoppies. The other cultivars were on a continuum with these seven, but intermediate in their responses.

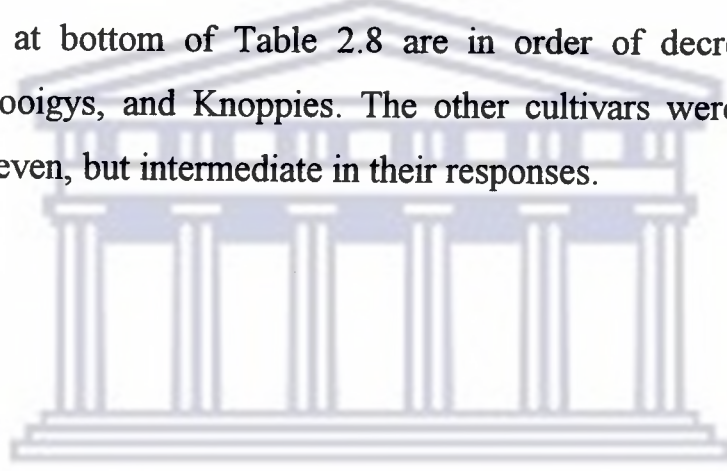


Table 2.8: Thirty eight wheat cultivars numbered as in Table 2.2 are arranged in order of decreasing salinity resistance. Data from table 2.3-2.7 and from log transformation of the same tables.

Col	L.Col	Grm	L.Grm	RLeng	L.RLeng	RMass	L.RMass
35	1	4	35	35	36	16	17
23	6	35	28	36	35	17	36
11	2	26	4	8	37	6	35
6	23	7	38	37	17	5	37
31	35	28	24	24	24	8	1
7	11	21	36	23	28	18	16
12	4	37	21	38	8	2	8
13	17	24	29	10	12	10	15
2	10	2	8	17	9	23	28
5	27	38	18	16	10	26	10
16	16	17	22	12	11	1	5
1	5	18	37	27	27	24	27
9	9	1	3	7	38	19	6
38	8	8	30	9	7	15	9
8	12	29	2	6	16	12	4
28	18	33	1	18	26	22	26
18	26	36	7	28	1	4	38
36	36	30	26	5	4	7	23
4	28	6	15	2	5	9	13
37	19	3	17	11	22	20	11
17	7	22	11	1	3	13	19
10	15	34	6	26	15	37	7
14	38	15	33	4	6	35	12
25	13	11	34	19	13	29	22
15	37	20	14	3	23	28	18
3	24	25	27	22	19	3	14
27	3	31	13	14	21	27	3
19	25	16	10	20	14	21	2
32	21	5	12	13	30	14	24
24	31	19	31	15	25	36	31
21	14	10	19	21	18	11	21
26	22	9	16	32	20	38	32
30	32	12	20	30	32	31	20
22	20	13	25	31	31	30	30
20	30	14	9	29	2	32	25
29	29	27	5	25	29	34	29
34	33	23	23	34	34	33	34
33	34	32	32	33	33	25	33

Col= coleoptile length

Grm= percentage of germination

RLeng= root length

RMass= root mass

L= log

1-Palmiet	9-SST 66	17-Flameks	25-Unie 52	33-Rooiwol
2-SST 65	10-SST 55	18-Bona	26-Palala	34-Rooigys
3-Adam Tas	11-SST 825	19-Impala	27-Eksteen	35-Yecoro Royo
4-Chokka	12-SST 57	20-Sokkies	28-Klein Trou	36-Charchia
5-Nantes	13-Kariega	21-K20	29-Knoppies	37-Losper
6-Dias	14-SST 75	22-Rooi Indies	30-Du Toit	38-Losper 52
7-SST 16	15-Daeraad	23-Kenia Gover	31-Liesbeeck	
8-SST 38	16-Sterling	24-Van Dyk	32-Drommedaris	

All the parameters used decreased with increasing salt concentration, but growth stimulation was observed in some cases at the lowest salt concentration (100 mM), with the percentage germination increasing in the following cultivars: Impala, Adam Tas, Yecoro Royo, Vandyk, Unie 52, Rooigys, Kariega, Knoppies, and Du Toit. The root mass of some wheat cultivars was promoted at low salt concentrations (100mM), namely Nantes, Flameks, and SST 75. The same observation was made for the root length of Charchia, and Losper.

2.5. Discussion:

It is well known that the germination stage is a very sensitive period of the plant life cycle. Obviously plants that do not establish them selves do not survive. However, evaluating tolerance is made more complex by variation in sensitivity to salt during the life cycle (Pearce, 2003). Flowers (2003), reported that salt tolerance is genetically and physiologically complex. Plants can have a similar tolerance at germination and during vegetative growth (Foolad & Chen, 1999). So the tolerance at germination stage can be an indicator of salt tolerance in the wheat plant although some other plants may differ. In some species yield may be the best indicator of salt resistance (Khatun & Flowers, 1995).

Decreasing the external osmotic potential, generally led to a decrease in percentage germination and a delay in seed germination (Ashraf & Abu-Shakra, 1978; Lafond & Baker, 1986; Hampson & Simpson, 1990; Poljakoff-Mayber, *et al.*, 1993 & 1994. Bell, *et al.*, 1993. Baalbaki, *et al.*, 1998). Salinity has three potential effects on plants: lowering of the water potential, direct toxicity of any Na and Cl absorbed, and interference with the uptake of essential nutrients (Munns, 1993).

This study shows that there are obvious differences in salt responses between the thirty-eight wheat cultivars that were assayed. These differences were found in all parameters that were measured. Different responses from the wheat cultivars are due to individual characteristics, either morphological or physiological, due to differences in genetics. One of the most obvious physiological factors to be adjusted during salinity resistance is the osmotic potential of the cell sap (Rashid, *et al.* 1999), and large variations in Na^+ and Cl^- ion concentrations have been found in individual plants of some cultivars (Baalabaki, *et al.*, 1999).

The most salt sensitive cultivars showed lower root lengths, coleoptile lengths, and germination percentages. Ryan, *et al.* (1975), and Zeynalabedin, & Mohammed, (2002), also reported this. The root mass showed a quite different result; the salt tolerant cultivars appeared below the middle of Table 2.8 which means they had less root mass response to salt effects (Table 2.4). In term of salt damage mechanisms, cell membranes of seeds in a weaker physiological condition, progressively lose their biochemical functions (Laudman, *et al.*, 1979), and leaching of cell contents occurs.

Table 2.8 shows the most salt tolerant wheat cultivars at the top, and the most salt sensitive cultivars at the bottom. The data in Table 2.8 was based on t test and data stability of the salt sensitivity behavior, i.e. how consistent the position of the cultivar in Table 2.8. For instance Table 2.4 shows that the mean of longest roots was in cultivar Rooiwol, but Table 2.8 showed that cultivar Yecoro Royo had the longest root under salinity effects, which considers the salt responses of the interaction between other parameters that were used, and avoids the individual cultivar growth under non saline conditions (control plants).

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CHAPTER 3

The Interaction Between Salinity And Gibberellic Acid And N^6-Benzyladenine On Some Wheat Cultivars

3.1. Summary:

Three salt sensitive wheat cultivars (Knoppies, Rooiwol, and Rooigys) and three salt tolerant wheat cultivars (Yecoro Royo, Charchia, and Losper) were examined under five different NaCl concentrations, 0, 100, 200, 300, and 400 mM. Wheat seeds were pretreated with five hormone concentrations, (0, 12.5, 40, and 125 μM), of Gibberellic acid (GA_3) or N^6-Benzyladenine. Root length, shoot length, root mass, shoot mass and percentage seed germination were measured as indicators of hormone effects at different salt levels. Results showed that treatment with GA_3 led to a significant increases in most parameters measured, particularly the percentage seed germination. The results of N^6-Benzyl adenine pretreatment were erratic for both cultivars and salt concentrations.

The results of this study indicate that the use of GA_3 as wheat pretreatment could possibly alleviate the damaging effects of high salt levels on seedlings, particularly in brackish soil.

3.2. Introduction:

The effect of plant hormones on plant growth under salinity stress, has been studied in the hope of improving salt tolerance. The attempt to produce salt tolerant crops was evident in ancient times (Jacobsen & Adams, 1958). Flowers & Yeo (1995) suggested five possible ways, which were appropriate at that time, to develop salt tolerant crops: (1) develop halophytes as alternative crops; (2) use interspecific hybridisation to raise the tolerance of current crops; (3) use the variation already present in existing crops; (4) generate variation within existing crops by using recurrent selection, mutagenesis or tissue culture; and (5) breed for yield rather than tolerance. In addition genetic studies involving gene transfers have had great support from the scientific community.

However, other technical applications, such as treatment with plant hormones, have led to some exciting results in the improvement of salt tolerance. Gibberellins (GAs) play an essential role in many aspects of plant growth and development, such as seed germination (Jones & Stoddard, 1977; Haba *et al.*, 1985; Khafagi *et al.*, 1986; Kumar & Neelakandan, 1992; David *et. al.*, 1993; Maske *et al.*, 1997), stem elongation and flower development (Yamaguchi & Kamiya, 2000). It has been known that plant hormones can regulate plant responses to salt effects, (Camacho *et al*, 1974; Itai *et al.* 1978; Walker & Dumbroff, 1981), increasing wheat and bean seed germination with indolacetic acid (IAA) or gibberellic acid (GA_3) treatment under salt stress (Salama & Ahmed, 1987). Kabar (1990) found the same results in barley and wheat seeds treated with GA_3 and exposed to saline conditions. An increase in the level of transpiration, under salt treatment, was reported in a range of plants that were treated with kinetin (Livne & Vaadia, 1965; Meidner 1967; Cooper *et al.*, 1972; Biddington & Thomas 1978; Salama & Awadalla 1986;

Malibari 1993; Yonis *et al.*, 1994). Gadallah (1999) reported that the association between the internal mineral element concentrations in wheat plants was largely affected by kinetin treatment. Kinetin application ameliorated the deleterious effects of salinity, and oxygen deficiency. It reduced Na^+, Ca^{2+} and Cl^- accumulation and improved K^+ uptake under salinity and waterlogging stresses. An increased K^+/Na^+ ratio helped the plants to avoid Na^+ toxicity and enhanced shoot growth and grain yield. Kinetin also reduced membrane injury by dehydration and heat stress and improved the water status of plants under both aerobic and anaerobic conditions.

Significant growth stimulation under salinity was found in some plants (wheat, barley, rice, broad bean, *Suaeda sp.*, etc.) that were treated with IAA or GA_3 (Boucaud & Ungar, 1976a; Parasher & Varma 1988; Kapchina & Foudouli 1991; Ivanova *et al.*, 1991; Adam, 1996). A significant improvement in plant height, leaf area, grain size, net CO_2 and photosynthetic capacity of the tested wheat plants was caused by treatment with GA_3 under salt stress, as reported by Ashraf *et al.* (2002). Prakash & Prathapasenan (1990) reported a significant increase in rice production in plants that were treated with GA_3 and grown under NaCl treatment. The same researchers reported that GA_3 could enhance the ionic balance in plant cells and reduce the inhibition of growth under saline conditions.

A significant increase in plant pigments was reported in several studies involving treatment with hormones under saline conditions (Varshyney & Baijal 1979; Shaddad & Heikal 1982; Radi *et al.*, 1989; Prakash & Prathapasenan 1990). Treatments with plant hormones such as GA_3 led to increases in carbohydrate content in plants that were grown in saline conditions (Khafagi *et al.*, 1986; Radi *et al.*, 1989; Ivanova

et al., 1991). Salama & Abdel-Basset (1987) reported that treatment of plants with IAA or GA_3 led to significant increases in protein content and decreases in amino acids content in plants that were grown under saline conditions.

Wheat seeds treated with 2,4-D showed a significant improvement in the number of productive tillers, yield of straw and grain, and grain protein content when grown in saline soil (Gulnaz *et. al.*, 1999). Satvir *et. al.* (1998) reported that gibberellic acid (GA_3) and kinetin induced the best germination and seedling growth of chickpea seeds (*Cicer arietinum* L.cv. PBG-1) grown under salt stress. Application of 6-benzyladenine (BA) to bean plants improved recovery of plants during rehydration after water stress, and increased abaxial stomatal conductance, adaxial stomatal conductance, and net photosynthetic rate, *i.e.*, parameters which were markedly decreased by mild water stress (Rulcova & Pospisilova., 2001). Gibberellin (GA) and/or cytokinin supplied to the root medium modified the rate of growth of the shoot and the adventitious and seminal roots in young seedlings of *Sorghum bicolor (L.)* (Amzallag 1999). Variations in hormone metabolism, and especially in cytokinin, have been observed after plant exposure to salinity and water stress (Vaadia 1976). Decreased cytokinin and GA_3 levels were observed in salt stressed plants (Boucaud & Unger, 1976b; Itia *et. al.*, 1968; Mizrahi *et. al.*, 1971).

Other applications have been used to avoid salt injury, such as salicylic acid, which reduced the damaging action of salinity and water deficit on wheat seedling growth; accelerated a restoration of growth processes; and essentially diminished the alteration of phyto-hormone levels in wheat seedlings under salinity and water deficit (Sakhabutdinova, *et. al.*, 2003).

Seed priming has been extensively used to improve germination of many species. Various types of seed treatments, geared towards improving germination under adverse conditions, have been reported. The two most common types of priming treatments are osmotic and solid matrix. These priming treatments rely on the osmotic and matric property of the priming solution or media, respectively (Bino *et al.*, 1998). Seed priming is a controlled hydration process that involves exposing seeds to low water potentials that restrict germination but permits pregerminative physiological and biochemical changes to occur (Bradford, 1986, Khan, 1992, Heydecker & Coolbear, 1977). Osmotic priming involves the imbibition of dry seed with a solution that has a high osmotic potential. This process allows water to enter the seed while still maintaining a low osmotic potential, thereby initiating metabolic activities leading to germination, but preventing or delaying emergence of the radicle (Arteca, 1996). Infusion procedures utilize organic solvents for the incorporation of chemicals such as growth regulators, fungicides, insecticides, antibiotics, and herbicidal antidotes into the seed (Khan 1978). According to the previous studies described above, and considering to the role of some plant hormones in salt tolerance, three salt tolerant and three salt sensitive wheat cultivars will be subjected to gibberellic acid or benzyladenine under salt treatment, to attempt to modify their salt tolerance.

3.3. Materials and Methods:

3.3.1.Plant material: Wheat cultivars (*Triticum aestivum*) Knoppies, Rooiwol, and Rooigys, which were established as salt sensitive cultivars in the previous study, and cultivars Yecoro Royo, Charchia, and Losper, which were found to be salt tolerant in the previous study - chapter 2, were chosen to be examined with GA_3 or cytokinin (CK) in this study.

3.3.2. Equipment and Supplies: The paper towel (paper doll) method of the International Seed Testing Association (TSTA) (Hampton & Tekrony, 1995) (discussed in chapter 2) was used to study germination and initial growth of the treated wheat cultivars. Four NaCl solutions (100, 200, 300, and 400 mM) were used as germination media, and for a control, distilled water was used. Gibberellic acid (GA_3) and 6-benzyladinine were obtained from the Sigma Company.

Benzyladenine was dissolved in 13ml of absolute alcohol at 50°C and left for 15 min. on a magnetic stirrer. Gibberellic acid was dissolved in distilled water and mixed for 15 min. on a magnetic stirrer. Concentrations of 12.5, 40, 125, and 400 μM were prepared from each hormone just before use, and kept in dark bottles. Seeds were surface sterilized in 3.5% sodium hypochlorite solution (NaOCl) as explained in chapter 2. Seeds were washed with hormone solution then soaked in the hormone solutions for 6 hours and then air dried for 24 hours. Control seeds were soaked in distilled water. To keep the seeds uncontaminated, they were manipulated in a laminar flow cabinet, and rolled in plastic bags then kept up right and incubated at 25°C on paper towels. GA_3 and BA were chosen to be used in this study, on the basis of previous studies that were mentioned in the introduction to this chapter.

Other steps (Experimental design, Parameter measurement, and Results analysis) were the same as in chapter 2.

3.4. Results:

3.4.1.The effect of Gibberellic Acid (GA_3) and salinity treatments:

The effect of increasing gibberellin concentrations on the seedling root length of six wheat cultivars (Table 3.1) was in general not consistent for the different cultivars investigated. However, at the highest salt concentration, no differences were found for any of the cultivars with gibberellin treatments. At 300 mM NaCl, intermediate gibberellin levels promoted root growth in four cultivars, three of which were salt sensitive, and the 40 μM GA_3 concentration was most effective. At 200 mM salt, three cultivars showed no effects, two showed negative effects and only one (#36-Charchia) gave an increase in root length. With the lowest (100 mM) salt concentrations, increased root growth was found in three cases, and decreased growth in two. When there was no salt, all cultivars, except Yecoro Royo (#35), showed reduced root length.

The applied gibberellin treatments had no effect on shoot length (Table 3.2) at the two highest salt concentrations (300 & 400 mM). At the lower concentrations, results varied with the cultivar, as was the case with root length. At 200 mM NaCl, gibberellin had no effect on two cultivars, a negative effect on three and in one case a positive effect. At 100 mM salt, GA_3 application resulted in increased shoot growth in four cultivars, and was negative for two, and positive at low concentration and negative at high concentrations in the case of one cultivar (#36-Charchia). There were similar mixed effects in the absence of salt: with no effect in one case, a negative effect on one cultivar, a positive effect on two, and a mixed effect on two.

Table 3.1. Different effects of five gibberellic acid treatments (μM) on root length (cm) of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Knoppies	0	16.3	12.6	9.7	0.9	0.0
	12.5	14.9 n	16.4 +*	9.1 n	3.1 +*	0.3 n
	40	13.4 -*	14.4 +*	9.4 n	3.7 +*	0.9 n
	125	13.8 -*	13.1 n	9.8 n	3.1 +*	0.7 n
	400	12.5 -*	13.6 n	8.4 n	1.6 n	0.7 n
Rooiwol	0	19.7	16.0	8.5	1.7	0.8
	12.5	12.6 -*	16.1 n	8.5 n	2.9 n	0.8 n
	40	15.9 -*	16.5 n	7.4 n	4.2 +*	0.9 n
	125	16.2 -*	16.7 n	9.3 n	3.3 n	1.3 n
	400	17.3 -*	17.4 n	7.5 n	1.5 n	0.6 n
Rooigys	0	20.5	17.0	9.7	1.7	0.4
	12.5	19.0 n	13.8 -*	7.9 -*	3.2 n	0.5 n
	40	15.5 -*	14.4 -*	7.1 -*	3.7 +*	0.9 n
	125	13.3 -*	16.1 n	8.8 n	2.8 n	1.0 n
	400	18.2 -*	14.1 -*	9.8 n	3.0 n	1.6 n
Yecoro Royo	0	18.1	16.4	10.4	2.7	0.7
	12.5	17.5 n	17.9 n	7.8 -*	4.2 n	1.1 n
	40	20.0 +*	15.9 n	9.6 n	4.3 n	1.3 n
	125	20.4 +*	18.2 +*	9.4 n	3.8 n	1.1 n
	400	21.4 +*	16.7 n	9.5 n	3.2 n	0.9 n
Gharchak	0	18.9	15.8	8.9	3.5	0.9
	12.5	17.2 n	16.0 n	11.5 +*	4.1 n	1.6 n
	40	14.1 -*	13.9 -*	11.1 +*	5.8 +*	1.0 n
	125	12.5 -*	17.5 n	11.9 +*	4.2 n	1.2 n
	400	14.3 -*	12.0 -*	10.3 n	3.1 n	0.8 n
Tesper	0	17.9	13.2	10.7	4.6	0.7
	12.5	19.4 n	15.3 +*	10.5 n	4.3 n	1.6 n
	40	13.4 -*	13.3 n	10.2 n	4.3 n	1.8 n
	125	15.4 -*	15.6 +*	10.4 n	5.1 n	1.2 n
	400	16.0 -*	15.0 +*	9.0 n	4.9 n	1.3 n

LSD= 1.77 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

Table 3.2. Different effects of five gibberellic acid treatments (μM) on shoot length (cm) of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Knoppies	**0**	10.3	8.3	4.2	0.1	0.0
	12.5	10.8 n	10.1 +*	1.9 -*	0.4 n	0.1 n
	40	10.8 n	9.2 +*	2.6 -*	0.4 n	0.3 n
	125	11.2 +*	8.4 n	2.2 -*	0.7 n	0.4 n
	400	10.6 n	9.3 +*	2.1 -*	0.5 n	0.4 n
Rooiwol	**0**	10.9	9.2	2.6	0.3	0.1
	12.5	9.6 -*	9.4 n	2.8 n	0.6 n	0.3 n
	40	9.6 -*	11.4 +*	2.5 n	0.7 n	0.5 n
	125	11.2 n	11.7 +*	2.9 n	0.8 n	0.4 n
	400	11.9 +*	10.2 +*	2.4 n	0.9 n	0.4 n
Rooigys	**0**	10.6	9.1	2.1	0.2	0.1
	12.5	10.7 n	7.4 -*	1.6 n	0.5 n	0.2 n
	40	9.0 -*	8.5 n	2.0 n	0.5 n	0.3 n
	125	10.0 n	9.1 n	2.7 n	0.5 n	0.2 n
	400	10.1 n	9.3 n	2.0 n	0.5 n	0.3 n
Yecoro Rojo	**0**	5.9	5.9	2.2	0.3	0.1
	12.5	5.9 n	5.1 n	1.2 -*	0.6 n	0.5 n
	40	6.0 n	3.6 -*	1.6 n	0.5 n	0.4 n
	125	7.2 +*	6.3 n	2.2 n	0.5 n	0.5 n
	400	6.5 n	4.9 -*	1.5 n	0.6 n	0.3 n
Gtaedtia	**0**	9.1	7.0	2.7	0.4	0.2
	12.5	6.7 -*	8.9 +*	3.4 n	0.8 n	0.4 n
	40	8.3 -*	7.4 n	3.4 n	0.8 n	0.2 n
	125	6.9 -*	7.8 +*	3.5 +*	1.1 n	0.6 n
	400	9.8 +*	5.5 -*	2.6 n	0.5 n	0.2 n
Lesoer	**0**	10.1	4.3	4.3	0.6	0.2
	12.5	10.3 n	7.6 +*	2.6 -*	0.5 n	0.4 n
	40	10.6 n	8.7 +*	4.1 n	0.6 n	0.5 n
	125	10.6 n	6.6 +*	3.6 n	0.9 n	0.5 n
	400	10.7 n	7.9 +*	1.9 -*	0.7 n	0.4 n

LSD= 0.73 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

The effect of GA_3 and salinity on the percentage germination of the six wheat cultivars tested, is presented in Table 3.3. The percentage germination increased significantly in the salt sensitive and salt tolerant seedlings treated with the GA_3 concentrations under the highest salt conditions, except for one salt tolerant cultivar (#36-Charchia). Under 300 mM NaCl, GA_3 had the same enhancing effect on Knoppies, Rooiwol and Charchia treated with the lowest and highest GA_3 concentrations. Negative effects of GA_3 on germination were found in Rooigys and Yecoro Royo in plants that were grown at the same salt levels (300 mM). At mild, low and no salt, the highest GA_3 concentrations (400 μM) led to decreases in germination in all tested cultivars, except Charchia, where it increased germination in the absence of salt. An increase in GA_3 concentration had no effect on the percentage germination of the seeds of cultivar Rooigys (#34) at the zero and moderate salt treatments. Charchia showed the most positive effects under non-saline conditions, while Yecoro Royo and Knoppies showed the opposite effect.

The results show that the GA_3 seed priming treatments could improve the seed germination of all the tested salt sensitive cultivars and two salt tolerant cultivars (Yecoro Royo and Losper) under salt stress conditions.

The effects of increasing GA_3 and salt treatments on the root mass of the six investigated wheat cultivars, are presented in Table 3.4. As expected, an increase in salinity at the zero GA_3 treatments caused a decrease in root mass, especially at the two highest salt treatments (300 and 400 mM). An increase in GA_3 concentration at the zero NaCl treatment had some negative effects on the root mass with most cultivars, while it had no effect at the highest salt levels, and a positive effect with 300 mM NaCl especially at 40 and 125μM GA_3. In 200 mM NaCl GA3 caused an increase in root mass in Charchia plants and decrease in Rooiwol and Yecoro Royo.

Table 3.3. Different effects of five gibberellic acid treatments (μM) on percentage Germination of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	GA_3 \ NaCl	0	100	200	300	400
Knoppies	0	100	90	90	50	0
	12.5	90 -*	100 +*	100 +*	100 +*	40 +*
	40	100 n	100 +*	90 n	100 +*	80 +*
	125	70 -*	100 +*	90 n	100 +*	100 +*
	400	80 -*	80 -*	80 -*	80 +*	70 +*
Roolwol	0	90	100	70	60	10
	12.5	90 n	90 -*	90 +*	60 n	30 +*
	40	100 +*	100 n	80 +*	80 +*	40 +*
	125	100 +*	100 n	90 +*	80 +*	50 +*
	400	80 -*	80 -*	60 -*	80 +*	60 +*
Roolgys	0	100	80	100	90	10
	12.5	100 n	80 n	100 n	70 -*	60 +*
	40	100 n	80 n	90 -*	90 n	80 +*
	125	100 n	100 +*	90 -*	90 n	60 +*
	400	80 -*	80 n	80 -*	80 -*	80 +*
Yecoro Royo	0	90	90	90	80	30
	12.5	70 -*	70 -*	70 -*	50 -*	60 +*
	40	70 -*	90 n	50 -*	60 -*	80 +*
	125	50 -*	90 n	90 n	60 -*	80 +*
	400	60 -*	80 -*	70 -*	40 -*	50 +*
Charlie	0	60	90	90	70	60
	12.5	70 +*	80 -*	60 -*	80 +*	50 -*
	40	70 +*	80 -*	90 n	70 n	60 n
	125	80 +*	80 -*	70 -*	60 -*	50 -*
	400	80 +*	80 -*	50 -*	80 +*	40 -*
Loerpar	0	90	80	100	80	50
	12.5	100 +*	80 n	90 -*	80 n	80 +*
	40	100 +*	100 +*	100 n	100 +*	50 n
	125	90 n	80 n	90 -*	80 n	80 +*
	400	80 -*	80 n	80 -*	80 n	80 +*

LSD=10 *P*= 0.05

n = Non significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

Table 3.4. Different effects of five gibberellic acid treatments (μM) on root mass (gram) of six wheat Cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Knoppies	**0**	0.041	0.034	0.034	0.003	0.000
	12.5	0.040 n	0.042 +*	0.027 n	0.009 n	0.001 n
	40	0.031 -*	0.034 n	0.030 n	0.012 +*	0.004 n
	125	0.039 n	0.035 n	0.026 -*	0.012 +*	0.003 n
	400	0.040 n	0.046 +*	0.040 n	0.007 n	0.004 n
Rooiwol	**0**	0.041	0.043	0.037	0.007	0.004
	12.5	0.036 n	0.047 n	0.029 -*	0.014 n	0.004 n
	40	0.044 n	0.034 -*	0.024 -*	0.019 +*	0.006 n
	125	0.040 n	0.037 n	0.028 -*	0.013 n	0.008 n
	400	0.049 n	0.052 +*	0.029 -*	0.009 n	0.005 n
Roolgys	**0**	0.067	0.060	0.037	0.008	0.002
	12.5	0.060 n	0.047 -*	0.029 -*	0.011 n	0.003 n
	40	0.049 -*	0.053 n	0.031 n	0.014 n	0.003 n
	125	0.040 -*	0.039 -*	0.032 n	0.016 +*	0.005 n
	400	0.056 -*	0.054 n	0.049 +*	0.013 n	0.008 n
Vecora Roqo	**0**	0.096	0.068	0.063	0.017	0.003
	12.5	0.080 -*	0.081 +*	0.040 -*	0.029 +*	0.007 n
	40	0.092 n	0.072 n	0.048 -*	0.034 +*	0.009 n
	125	0.083 -*	0.076 +*	0.053 -*	0.026 +*	0.007 n
	400	0.090 n	0.072 n	0.060 n	0.019 n	0.005 n
Gtarchia	**0**	0.078	0.056	0.044	0.020	0.006
	12.5	0.071 n	0.056 n	0.053 +*	0.027 n	0.002 n
	40	0.057 -*	0.055 n	0.052 +*	0.027 n	0.006 n
	125	0.044 -*	0.057 n	0.053 +*	0.033 +*	0.010 n
	400	0.061 -*	0.049 n	0.051 n	0.019 n	0.010 n
Tosstar	**0**	0.064	0.052	0.051	0.022	0.004
	12.5	0.060 n	0.064 +*	0.051 n	0.021 n	0.007 n
	40	0.053 -*	0.037 -*	0.048 n	0.023 n	0.009 n
	125	0.059 n	0.054 n	0.048 n	0.027 n	0.007 n
	400	0.057 n	0.065 +*	0.057 n	0.025 n	0.007 n

LSD=0.0081 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

The effects of increasing salinity and GA_3 treatments on shoot mass of the six investigated wheat cultivars are presented in Table 3.5. The shoot mass of seedlings of salt sensitive cultivars germinated under non-saline and slightly saline conditions, tended to increase with an increase in GA_3 concentration at some treatments, while the shoot masses of the salt resistant cultivars under similar conditions, showed more erratic responses. No effect on shoot mass was found for GA_3 treatments in all plants germinated under highest salt levels (300 & 400 mM), except with 12.5 and 125 μM GA_3 treatments where two cultivars had positive effect. A negative effect was caused in shoot mass by GA_3 treatment in three cultivars that were grown in 200 mM NaCl, and one cultivar had a positive effect (#36-Charchia). The effect of GA_3 treatments under 100 mM NaCl tended to have a positive effect on shoot mass in most cultivars, except Rooigys and Yecoro Royo, which showed a negative effect.

Table 3.5. Different effects of five gibberellic acid treatments (μM) on shoot mass (gram) of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Knoppies	**0**	0.107	0.078	0.039	0.000	0.000
	12.5	0.113 n	0.097 +*	0.018 -*	0.003 n	0.000 n
	40	0.102 n	0.078 n	0.024 -*	0.005 n	0.001 n
	125	0.122 +*	0.084 n	0.019 -*	0.006 n	0.003 n
	400	0.119 +*	0.113 +*	0.025 -*	0.005 n	0.003 n
Rooiwol	**0**	0.089	0.082	0.026	0.006	0.000
	12.5	0.085 n	0.094 +*	0.026 n	0.007 n	0.000 n
	40	0.088 n	0.077 n	0.023 n	0.007 n	0.000 n
	125	0.096 n	0.100 +*	0.027 n	0.008 n	0.000 n
	400	0.143 +*	0.125 +*	0.022 n	0.011 n	0.005 n
Rooigys	**0**	0.122	0.106	0.020	0.003	0.000
	12.5	0.125 n	0.074 -*	0.015 n	0.032 +*	0.000 n
	40	0.099 -*	0.088 -*	0.020 n	0.006 n	0.000 n
	125	0.102 -*	0.089 -*	0.027 n	0.006 n	0.000 n
	400	0.134 +*	0.113 n	0.026 n	0.005 n	0.003 n
Kraaibos Rooi	**0**	0.076	0.071	0.026	0.003	0.000
	12.5	0.070 n	0.057 -*	0.015 -*	0.008 n	0.003 n
	40	0.074 n	0.050 -*	0.022 n	0.005 n	0.000 n
	125	0.095 +*	0.077 n	0.027 n	0.006 n	0.005 n
	400	0.075 n	0.065 n	0.019 -*	0.005 n	0.004 n
Gladiola	**0**	0.113	0.093	0.032	0.004	0.000
	12.5	0.107 n	0.113 +*	0.000 -*	0.009 n	0.009 +*
	40	0.103 -*	0.093 n	0.044 +*	0.010 n	0.000 n
	125	0.089 -*	0.093 n	0.042 +*	0.014 +*	0.007 n
	400	0.132 +*	0.073 -*	0.034 n	0.005 n	0.003 n
Tasper	**0**	0.139	0.075	0.052	0.005	0.000
	12.5	0.121 -*	0.112 +*	0.037 -*	0.006 n	0.003 n
	40	0.124 -*	0.096 +*	0.050 n	0.008 n	0.000 n
	125	0.148 +*	0.078 n	0.045 -*	0.010 n	0.007 n
	400	0.149 +*	0.117 +*	0.032 -*	0.011 n	0.006 n

LSD= 0.00693 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

3.4.2. The effect of N6-Benzyladenine and salinity treatments:

The effect of increasing benzyladenine and sodium chloride concentrations on the root length of germinated seeds of six wheat cultivars is presented in Table 3.6. All wheat cultivars that were treated with benzyladenine showed a general tendency towards a decrease in root length with increasing benzyladenine concentration, although only one significant decrease was recorded at the 300 & 400 mM salt levels. A decreased root length with increased benzyladenine concentrations was found at 0, 100 and 200mM salinity levels. In some cases low benzyladenine concentrations resulted in increased root length without salt, or at 100mM salinity. This was also true at 200mM in the case of the salt resistant cultivars.

The effects of five different benzyladenine treatments and salt concentrations on the shoot lengths of germinating seeds of six wheat cultivars are presented in Table 3.7. The shoot length of the salt sensitive cultivars and Losper at the zero salt treatment and Yecoro Royo at 100 mM salt level tended to decrease with increasing benzyladenine concentration. At the 200 mM NaCl, and above, no significant differences were found in salt sensitive cultivars except one positive case of Rooigys (200mM salt +400µM BA). A positive effect of benzyladenine occurred at the zero salt level in salt tolerant cultivars (Yecoro Royo and Charchia), and the same is true at the 100 and 200 mM salt in Charchia and Losper plants, while at the 300 mM NaCl and above no significant differences were found in salt tolerant cultivars except one positive case of Charchia (300mM salt +400µM BA).

The results of the effects of increased benzyladenine and salt concentrations on germination (Table 3.8), show that benzyladenine in general seems to have a negative effect on germination in salt sensitive cultivars and erratic effects on the salt tolerant cultivars. Benzyladenine had some positive effects on seed germination, especially with the highest salt levels in the salt resistant cultivars.

Table 3.6. Different effects of five N6-benzyladenine treatments (μM) on root length (cm) of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Kraaidjy	**0**	15.6	11.0	7.4	1.2	0.0
	12.5	13.0 -*	12.5 +*	6.9 n	1.6 n	0.0 n
	40	13.9 -*	11.1 n	5.3 -*	1.5 n	0.0 n
	125	8.2 -*	12.6 +*	4.4 -*	1.1 n	0.3 n
	400	5.2 -*	7.0 -*	3.9 -*	1.0 n	0.0 n
Rooimol	**0**	16.2	14.1	5.2	2.3	0.5
	12.5	14.4 -*	12.9 n	5.1 n	2.3 n	0.5 n
	40	15.3 n	12.7 -*	4.6 n	2.1 n	0.3 n
	125	14.1 -*	10.0 -*	3.0 -*	2.5 n	0.0 n
	400	8.7 -*	12.7 -*	2.9 -*	0.6 -*	0.2 n
Roelgys	**0**	14.7	12.8	6.0	2.1	1.1
	12.5	14.5 n	14.3 +*	4.8 n	1.8 n	0.0 n
	40	11.1 -*	14.1 n	5.2 n	2.0 n	0.3 n
	125	9.1 -*	11.2 -*	4.2 -*	1.5 n	0.9 n
	400	7.8 -*	8.5 -*	4.2 -*	1.9 n	0.6 n
Veldcoo Rooi	**0**	12.9	16.6	5.1	2.5	0.5
	12.5	15.1 +*	13.3 -*	5.0 n	1.8 n	0.5 n
	40	14.0 n	9.4 -*	6.5 +*	2.3 n	1.0 n
	125	10.1 -*	12.0 -*	5.4 n	2.8 n	0.9 n
	400	9.0 -*	8.1 -*	4.7 n	2.7 n	0.9 n
Gariep	**0**	10.5	10.8	6.0	3.2	0.5
	12.5	13.7 +*	14.7 +*	8.0 +*	3.6 n	1.1 n
	40	12.8 +*	8.1 -*	4.6 -*	3.0 n	1.2 n
	125	10.7 n	9.4 -*	6.8 n	2.8 n	1.2 n
	400	8.2 -*	5.8 -*	4.8 n	3.4 n	1.2 n
Tugela	**0**	16.8	11.8	6.0	3.1	1.0
	12.5	15.7 n	12.1 n	7.8 +*	3.0 n	1.3 n
	40	13.6 -*	13.2 +*	5.8 n	3.5 n	0.8 n
	125	7.9 -*	11.7 n	6.2 n	2.4 n	0.6 n
	400	7.1 -*	7.1 -*	3.8 -*	2.0 n	1.0 n

LSD= 1.32 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

Table 3.7. Different effects of five N_6-benzyladenine treatments (μM) on shoot length (cm) of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Knoppies	0	11.0	6.8	1.2	0.1	0.0
	12.5	9.9 -*	8.8 +*	1.1 n	0.2 n	0.0 n
	40	10.1 -*	7.2 n	0.9 n	0.1 n	0.0 n
	125	9.6 -*	9.0 +*	1.1 n	0.1 n	0.1 n
	400	8.3 -*	7.4 +*	1.7 n	0.3 n	0.0 n
Rooiwol	0	9.7	8.8	0.9	0.2	0.1
	12.5	8.4 -*	8.1 -*	0.7 n	0.4 n	0.1 n
	40	10.4 +*	8.0 -*	0.7 n	0.1 n	0.1 n
	125	9.2 n	6.6 -*	0.5 n	0.2 n	0.0 n
	400	7.4 -*	7.1 -*	1.2 n	0.3 n	0.1 n
Roolgys	0	9.2	6.2	0.6	0.1	0.1
	12.5	7.9 -*	7.5 +*	0.5 n	0.1 n	0.0 n
	40	9.1 n	6.4 n	0.7 n	0.1 n	0.1 n
	125	7.3 -*	6.4 n	0.5 n	0.1 n	0.1 n
	400	7.5 -*	6.2 n	1.3 +*	0.2 n	0.1 n
Yecoro Royo	0	4.9	4.9	0.9	0.2	0.1
	12.5	4.9 n	4.5 n	0.6 n	0.2 n	0.1 n
	40	5.7 +*	3.1 -*	1.1 n	0.2 n	0.2 n
	125	5.0 n	3.7 -*	0.9 n	0.2 n	0.1 n
	400	5.6 +*	4.5 n	1.5 n	0.4 n	0.2 n
Ciarchia	0	6.1	4.5	0.9	0.3	0.1
	12.5	7.2 +*	7.4 +*	1.0 n	0.2 n	0.2 n
	40	4.8 -*	2.9 -*	0.8 n	0.4 n	0.3 n
	125	7.3 +*	4.7 n	2.1 +*	0.5 n	0.2 n
	400	7.0 +*	6.2 +*	1.6 +*	0.9 +*	0.3 n
Lesopr	0	10.1	4.9	1.0	0.3	0.2
	12.5	8.4 -*	6.3 +*	1.7 +*	0.1 n	0.3 n
	40	7.6 -*	6.6 +*	1.5 n	0.3 n	0.1 n
	125	5.9 -*	4.4 n	1.2 n	0.4 n	0.1 n
	400	7.2 -*	5.8 +*	1.8 +*	0.5 n	0.3 n

LSD= 0.62 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

The effects of increasing benzyladenine and salt concentrations on root mass (Table 3.9) shows that the root mass of salt sensitive cultivars decreased with increasing benzyladenine concentration at the zero salt treatment for all salt sensitive cultivars and 400 mM salt with cultivar Rooiwol, and at any other salt level where significant differences were found. The salt resistant cultivars also showed a decrease in root mass at zero salt and higher concentrations. A few positive significant differences were found with salt resistant cultivars where higher salt concentrations were used, and a negative result was obtained with Rooiwol, resulting in reduced root mass.

The results for shoot mass at the different salt and benzyladenine concentrations are summarized in Table 3.10. The effect of salinity on shoot mass was manifested by a decrease in shoot mass with an increase in salinity. The shoot mass of salt sensitive and salt resistant cultivars, germinated under non-saline and slightly saline conditions, decreased with low and sometimes moderate benzyladenine treatments where significant differences occurred, except in Charchia plants, where it had a positive effect. No other significant differences were found in plants at the two highest salt levels due to benzyladenine treatments.

Table 3.8. Different effects of five N6-benzyladenine treatments (μM) on percentage germination of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Knoppies	0	90.0	80.0	100.0	70.0	0.0
	12.5	80.0 n	90.0 n	80.0 -*	70.0 n	0.0 n
	40	100.0 n	70.0 n	70.0 -*	40.0 -*	0.0 n
	125	100.0 n	100.0 +*	80.0 -*	40.0 -*	10.0 n
	400	80.0 n	80.0 n	70.0 -*	70.0 n	0.0 n
Roolwol	0	90.0	90.0	80.0	70.0	20.0
	12.5	100.0 n	100.0 n	70.0 n	60.0 n	20.0 n
	40	100.0 n	80.0 n	90.0 n	40.0 -*	20.0 n
	125	100.0 n	100.0 n	90.0 n	60.0 n	0.0 -*
	400	80.0 n	80.0 n	80.0 n	50.0 -*	30.0 n
Rooigys	0	90.0	100.0	90.0	80.0	40.0
	12.5	100.0 n	100.0 n	100.0 n	70.0 n	0.0 -*
	40	100.0 n	90.0 n	90.0 n	60.0 -*	40.0 n
	125	100.0 n	100.0 n	90.0 n	80.0 n	40.0 n
	400	80.0 n	80.0 -*	70.0 -*	70.0 n	50.0 n
Yecoro Royo	0	90.0	80.0	60.0	70.0	40.0
	12.5	100.0 n	60.0 -*	60.0 n	50.0 -*	20.0 -*
	40	90.0 n	90.0 n	70.0 n	80.0 n	60.0 +*
	125	90.0 n	90.0 n	60.0 n	80.0 n	60.0 +*
	400	70.0 -*	50.0 -*	80.0 +*	60.0 n	40.0 n
Chacrita	0	80.0	70.0	80.0	50.0	40.0
	12.5	80.0 n	100.0 +*	70.0 n	70.0 +*	60.0 +*
	40	80.0 n	70.0 n	70.0 n	50.0 n	50.0 n
	125	90.0 n	90.0 +*	50.0 -*	60.0 n	70.0 +*
	400	80.0 n	60.0 n	70.0 n	50.0 n	30.0 n
Losgar	0	100.0	80.0	70.0	70.0	80.0
	12.5	80.0 -*	90.0 n	100.0 +*	80.0 n	50.0 -*
	40	100.0 n	100.0 +*	70.0 n	80.0 n	30.0 -*
	125	100.0 n	90.0 n	80.0 n	90.0 +*	70.0 n
	400	80.0 -*	80.0 n	80.0 n	70.0 n	70.0 n

LSD= 18.83 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

Table 3.9. Different effects of five N6-benzyladenine treatments (μM) on root mass (gram) of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Koppies	0	0.047	0.036	0.025	0.004	0.000
	12.5	0.045 n	0.033 n	0.026 n	0.006 n	0.000 n
	40	0.033 -*	0.035 n	0.020 n	0.004 n	0.000 n
	125	0.023 -*	0.033 n	0.019 n	0.005 n	0.002 n
	400	0.020 -*	0.024 -*	0.019 n	0.007 n	0.000 n
Rooiwol	0	0.057	0.036	0.019	0.009	0.015
	12.5	0.046 -*	0.035 n	0.021 n	0.010 n	0.003 -*
	40	0.040 -*	0.040 n	0.020 n	0.012 n	0.002 -*
	125	0.032 -*	0.038 n	0.016 n	0.012 n	0.000 -*
	400	0.033 -*	0.035 n	0.019 n	0.004 n	0.002 -*
Roolgys	0	0.060	0.044	0.024	0.008	0.006
	12.5	0.052 n	0.047 n	0.017 n	0.009 n	0.000 n
	40	0.043 -*	0.049 n	0.024 n	0.011 n	0.002 n
	125	0.028 -*	0.038 n	0.019 n	0.009 n	0.005 n
	400	0.034 -*	0.040 n	0.017 n	0.013 n	0.004 n
Yecoro Rojo	0	0.068	0.082	0.031	0.015	0.003
	12.5	0.081 +*	0.061 -*	0.024 n	0.014 n	0.003 n
	40	0.063 n	0.057 -*	0.032 n	0.015 n	0.009 n
	125	0.042 -*	0.060 -*	0.036 n	0.017 n	0.009 n
	400	0.048 -*	0.052 -*	0.027 n	0.020 n	0.011 n
Chiarchia	0	0.053	0.043	0.031	0.018	0.003
	12.5	0.061 +*	0.056 +*	0.042 +*	0.010 n	0.008 n
	40	0.046 n	0.029 -*	0.029 n	0.021 n	0.009 n
	125	0.043 -*	0.047 n	0.029 n	0.005 -*	0.010 n
	400	0.035 -*	0.036 n	0.029 n	0.029 +*	0.013 +*
Lesper	0	0.074	0.046	0.032	0.017	0.007
	12.5	0.077 n	0.043 n	0.040 +*	0.012 n	0.008 n
	40	0.050 -*	0.052 n	0.029 n	0.022 n	0.006 n
	125	0.032 -*	0.052 n	0.034 n	0.016 n	0.005 n
	400	0.040 -*	0.039 n	0.024 n	0.018 n	0.006 n

LSD= 0.0081 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

Table 3.10. Different effects of five N6-benzyladenine treatments (μM) on shoot mass (gram) of six wheat cultivars under five NaCl (mM) treatments.

Cultivar	NaCl / GA_3	0	100	200	300	400
Knopies	0	0.113	0.073	0.010	0.003	0.000
	12.5	0.103 -*	0.084 +*	0.009 n	0.000 n	0.000 n
	40	0.111 n	0.072 n	0.009 n	0.000 n	0.000 n
	125	0.102 -*	0.097 +*	0.009 n	0.000 n	0.000 n
	400	0.106 n	0.086 +*	0.019 +*	0.003 n	0.000 n
Roiwol	0	0.093	0.081	0.007	0.001	0.000
	12.5	0.084 -*	0.068 -*	0.005 n	0.000 n	0.000 n
	40	0.105 +*	0.072 -*	0.005 n	0.000 n	0.000 n
	125	0.088 n	0.066 -*	0.005 n	0.000 n	0.000 n
	400	0.093 n	0.064 -*	0.010 n	0.004 n	0.000 n
Roigys	0	0.105	0.065	0.005	0.001	0.000
	12.5	0.087 -*	0.082 +*	0.004 n	0.000 n	0.000 n
	40	0.104 n	0.077 +*	0.007 n	0.000 n	0.000 n
	125	0.081 -*	0.077 +*	0.005 n	0.000 n	0.000 n
	400	0.111 n	0.086 +*	0.013 +*	0.002 n	0.000 n
Yesora Roye	0	0.057	0.070	0.009	0.002	0.000
	12.5	0.061 n	0.057 -*	0.007 n	0.000 n	0.000 n
	40	0.069 +*	0.041 -*	0.012 n	0.000 n	0.000 n
	125	0.059 n	0.052 -*	0.010 n	0.003 n	0.000 n
	400	0.086 +*	0.050 -*	0.018 +*	0.003 n	0.000 n
Charlie	0	0.079	0.063	0.011	0.002	0.000
	12.5	0.099 +*	0.090 +*	0.010 n	0.000 n	0.000 n
	40	0.061 -*	0.034 -*	0.009 n	0.000 n	0.000 n
	125	0.100 +*	0.065 n	0.020 +*	0.025 +*	0.000 n
	400	0.089 +*	0.084 +*	0.019 +*	0.010 +*	0.000 n
Laspar	0	0.123	0.074	0.010	0.002	0.000
	12.5	0.115 -*	0.072 n	0.018 +*	0.000 n	0.000 n
	40	0.106 -*	0.088 +*	0.016 n	0.000 n	0.000 n
	125	0.086 -*	0.067 n	0.014 n	0.004 n	0.000 n
	400	0.116 -*	0.093 +*	0.024 +*	0.006 n	0.000 n

LSD= 0.0072 *P*= 0.05

n = Non Significant

+*= Positive Effects

-* = Negative Effects

Salt sensitive cultivars

Salt tolerant cultivars

3.5 Discussion:

This study showed that treatment of salt sensitive and salt tolerant wheat cultivars with NaCl led to significant decreases in all parameters of growth that have been studied (Table 3.1-3.10). The effects of increasingly saline media on wheat growth have been described in chapter 2. GA_3 decreased root length of all tested wheat cultivars under non-saline conditions except the cultivar Yecoro Royo where the root length significantly increased (Table 3.1). The length-shortening effect of GA_3 on roots might have occurred because of an increase in the production of ethylene, caused by an increase in the synthesis of ACC (1-Aminocyclopropane-1-carboxilic acid) (Kaneta *et al.*, 1997). This result was confirmed by Leite *et al.* (2003). GA_3 caused a significant increase in root length of Knoppies seedlings that were grown under 100 and 300 mM NaCl and treated with 12.5, 40, and 125 µM GA_3, the same results were found with Rooiwol and Rooigys plants that were grown in 300 mM NaCl and were treated with 40 µM GA_3. An increase in root length was reported for salt tolerant cultivars, Yecoro Royo (100 mM salt +125 µM GA_3), Charchia (200 mM+12.5, 40, and 125 µM GA_3 and 300 mM NaCl+40 µM GA_3). Losper plants showed increases in root length only with 0 and 100 mM salt levels when treated with 12.5, 125, and 400 µM GA_3.

The stimulation of growth via GA_3 could be due to an inherent attribute of GA_3 in increasing cell division and cell elongation (Tanimoto, 1990; Scott, 1984; El Fouly *et al.*, 1988). The improving of salt tolerance via application of GA_3 could also be due to a decrease in osmotic potential of cell sap (Pallas & Box, 1970; Tal & Imber, 1971; Salama & Awadalla, 1989). GA_3 appears to be essential for seed germination and almost universally stimulates germination being frequently associated with mobilization of endosperm reserves and growth of embryonic tissues (Jones & Stoddard, 1977), particularly in grasses. Addition of exogenous GA_3 could cause an increase in

germination and seedling growth probably by enhancing the availability of endogenous GA_3. The reduced seedling growth under salt stress conditions correlated with the decreased amylase activity and high starch content in seeds of stressed seedlings. Increases in shoot and root length with GA_3 treatments could be due to an inherent attribute of the hormone to increase cell division and cell elongation (Scott, 1984). GA_3 is an important factor in enhancing the α-amylase activities in germinating seeds (Palmiano & Juliano, 1972). It is conceivable that the mechanism by which NaCl-induced inhibition of α-amylase activities is counteracted by GA_3, is related to a deficiency of GA_3 in NaCl stressed endosperm and the reduction on NaCl inhibition of shoot growth by GA_3 resulted from an enhancement of the hydrolysis of starch in the endosperm (Lin & Kao, 1995).

Table 3.11 summarizes these results; GA_3 was more positive in its effects (99x) than benzyladenine (57x) on salt sensitive and salt tolerant cultivars. GA_3 could improve root length (15x) and root mass (14x) more successfully than benzyladenine 8x, and 5x. On other hand, GA_3 caused a less negative effect on root length (8x), while benzyladenine caused 24 negative effects. In contrast GA_3 caused less positive effects on shoot length (13x, and 15x for benzyladenine) and shoot mass (14x, and 20x for benzyladenine) (Table 3.11). So from the above table, GA_3 treatments were more effective than cytokinin treatments, and a similar situation was reported by Leite *et al.*, (2003) and Kaur, *et al.*, (1998). Increases of root against shoot growth have been considered to be a salt tolerance factor, similar results were found by Adam (1996) and El-Sharkawi & Salama (1984).

Seed germination was the most affected parameter in this experiment. Salt sensitive cultivars were clearly enhanced by treating them with GA_3 and gave

significantly positive results at mild and extreme salt levels (Table 3.3) and this result is more obvious when presented graphically (Fig 3.1).

It is well known that GA_3 can activate the transcription and translation of α-amylase and other hydrolases (Fincher, 1989; Jones & Jacobsen, 1991). The decreased amylase activity in the stressed seeds will result in a reduced formation of glucose from starch, thereby leading to a reduce synthesis of sucrose, resulting in its restricted supply to the embryonic axis, resulting in reduced seedling growth under salt stress conditions, and so GA_3 could avoid salt damage via increased amylase activity in treated seeds under salt stress (Kaur, *et al.*, 1998). Reduced synthesis of sucrose leads to an increase in osmotic potential, which in turn prevents the stressed seeds from absorbing more water, which then affects other physiological processes.

Another possibility is that gibberellin effects ion transport. Sodium ions may pass through membranes (Hasegawa, *et al.*, 2000) via ion channels as the HKT family of transporters (Very & Sentenac, 2003) and low selectivity channels (Demidchik *et al*, 2002) Na^+/H^+ antiporters (Maeshima, 2001; Zhu, 2002) are also means for sodium to cross membranes. It is possible that the remarkable effect of gibberellin on the germination of salt sensitive cultivars, may be due to changes in ion channel activity similar to those found by Bethke & Jones (1994) in barley aleurone cells.

Table 3.11. The number of times significant differences were found in the parameters measured when salt treatments were presented along with GA_3 or BA treatments.

GA_3		Root L.	Shoot L.	G %	Root M.	Shoot M.	Total
Salt sensitive cultivars	+	7	6	27	8	6	**54**
	-	5	5	10	10	7	**37**
Salt tolerant cultivars	+	8	7	11	11	8	**45**
	-	3	3	23	4	9	**42**
All cultivars	+	**15**	**13**	**38**	**19**	**14**	**99**
	-	**8**	**8**	**33**	**14**	**16**	**79**
BA							
Salt sensitive cultivars	+	3	5	1	0	9	**18**
	-	14	4	12	5	4	**39**
Salt tolerant cultivars	+	5	10	8	5	11	**39**
	-	10	3	7	6	5	**31**
All cultivars	+	**8**	**15**	**9**	**5**	**20**	**57**
	-	**24**	**7**	**19**	**11**	**9**	**70**

Benzyladenine had a less positive effects on salt sensitive cultivars (18x) than on salt tolerant cultivars (39x), and more negative effects on salt sensitive cultivars (39x) than on salt tolerant cultivars (31x) Table 3.11. Cytokinin may be involved in the development of leaves and branches in plants under adverse conditions, such as low luminosity, as demonstrated by Sharma & Walia (1996), or high aluminum concentration, as shown by Pan *et al.* (1988; 1989). Cytokinins (CK) antagonize many physiological processes induced by water stress, mainly those mediated by abscisic acid (Rulcova & Pospisilova, 2001). CK may partially ameliorate negative effects of water stress, by stimulation of osmotic adjustment (Yadav *et al.*, 1997; Agarwal & Gupta, 1995), and Cowan *et al.* (1999) proposed a model illustrating metabolic antagonism between CK and ABA.

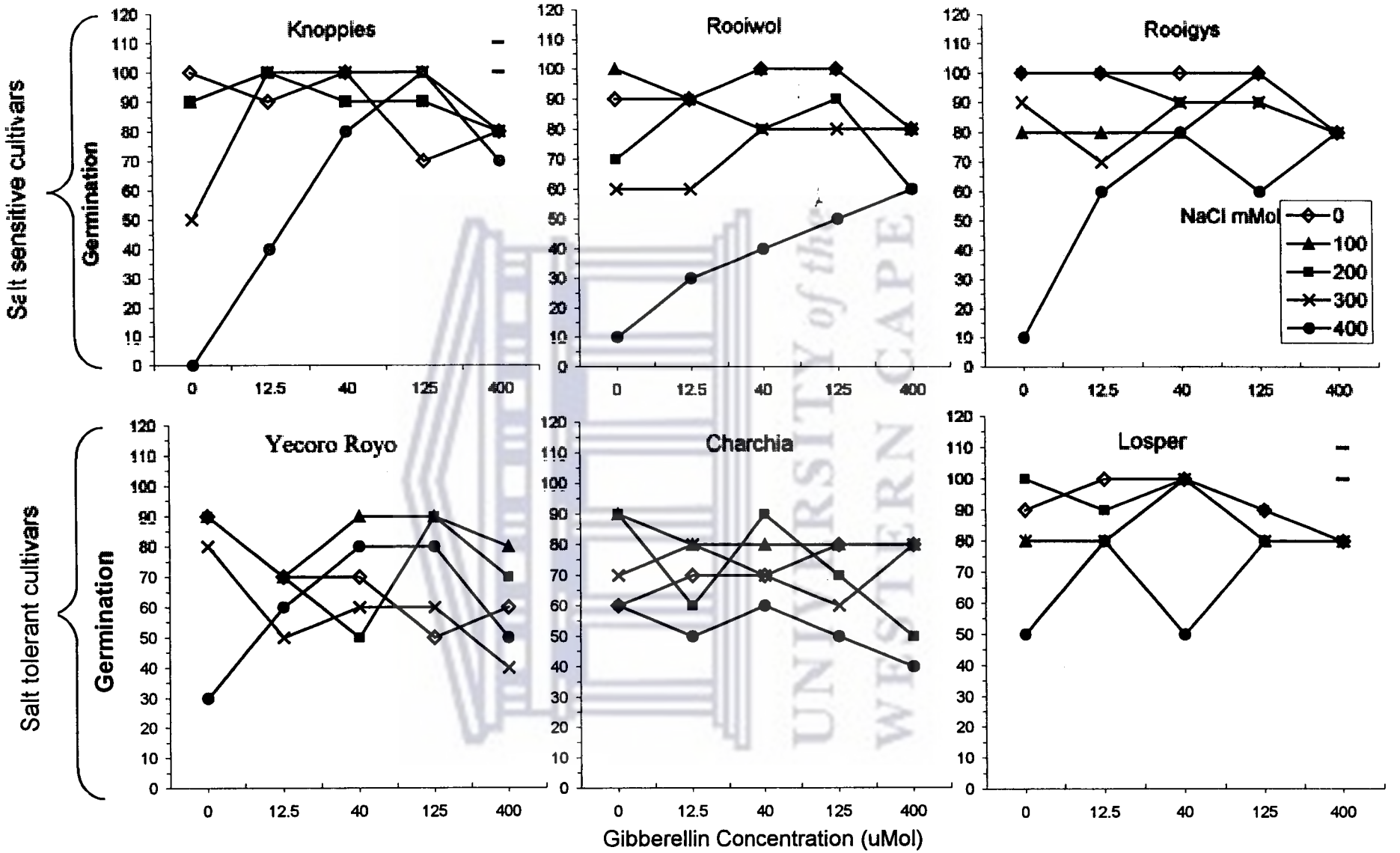

Fig 3.1: Effects of gibberellin and salinity (mM) on the percentage germination (%) of six wheat cultivars.

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CHAPTER 4

The Interaction Between Salinity, Citric Acid and Malic Acid on Two Wheat Cultivars (Knoppies & Losper)

4.1. Summary:

Two wheat cultivars, salt sensitive (Knoppies) and salt tolerant (Losper) were treated with two organic acids (Citric acid, at 13.33 µM, or 133.33 µM, or Malic acid at 20 µM, or 200 µM). Seeds were soaked in one of the organic acid solutions, and then germinated under different NaCl solutions (200 or 400 mM). Root length, root mass, shoot length, shoot mass and percentage germination were determined. Results showed some negative effects of citric acid on root mass and germination of Knoppies seedlings and negative effects of malic acid on shoot mass in Losper plants. No positive contribution of organic acids on salt resistance was found in this study. This study showed that the positive effects of gibberellic acid (GA_3) on the salt sensitive cultivars under saline conditions (chapter 3) was due to hormonal effects and not by the reduction of the pH of the germination media.

4.2. Introduction:

It is well known that seed germination is affected by several factors such as temperature, light, and moisture. The pH levels or the degree of the acidity is one these factors affecting seed germination. The acidity of irrigation water is expressed as pH (< 7.0 acidic; > 7.0 basic). The normal pH range for irrigation water is from 6.5 to 8.4. (Bauder, *et al.* 2004).

Reductions in soil pH can cause an inhibition of seed germination and seedling growth (Gow & Pidwirny, 1996; Marsh, 1993; MacDonald *et al.* 1986). A pH of 2.0 in the germination medium seemed to be a threshold level for inhibition of seed germination and seedling growth (Fan & Wang, 2000). The typical effect of low pH level in nature is that of acid soil and acid rain. Acid rain is affecting plant regeneration by impacting seedbed properties, seed germination, seedling nutrient relations, and seedling growth. Effects of acid rain on seed germination vary with species and method of treatment, producing inhibition of germination in some species, but stimulation of germination in others (MacDonald *et al.* 1986). The seedlings of *Pisum sativum* L. tolerated simulated acid rain exposure down to pH 2.2. Below this, the seedling growth was reduced and the seeds succumbed at pH levels of 1.2 and pH 0.5. A reduction of about 48.7% in root length and 67.3% in shoot length was observed between pH 6.8 (control) and pH 2.2. The shoot dry weight showed a reduction of 48.5% while root dry weight decreased by about 56.4% (Deepika & Khan, 2002). Other studies that have a connection with acid effects on plants include the role of cyclic hydroxamic acids and benz-oxazolinones on the germination and growth of plants (Nair *et al.*1990; Chase *et al.*1991; Perez & Ormeno-Nunez 1991, 1993; Petho 1993). The role of cyclic hydroxamic acids in allelopathy, presents in higher concentration in the form of glucosides, than that of the aglucones. In higher concentrations

they have an inhibitory effect on the growth of cucumber. The level of the inhibition increases with the increase of the pH of the nutrient solution, and the length of time of administration of the cyclic hydroxamic acids (Petho, 1993). Organic acids from root exudates in some plants can solubilize unavailable soil Ca, Fe and Al phosphates, reduce the rhizosphere pH and decrease the availability of some mineral nutrients as well as the effective functioning of some soil bacteria, such as the rhizobial bacteria themselves. Some plants such as Rooibos tea (*Aspalathus linearis* L.) actively modify their rhizosphere pH by extruding OH^- and HCO_3^- to facilitate growth in low pH soils (pH 3-5) (Dakora & Phillips, 2002).

The objective of the study in this chapter was to investigate the effect of some acid solutions on seed germination of a salt sensitive and a salt resistant wheat cultivar under salt stress. This was to test whether or not the GA, being an acid, could have had effects (chapter 3) by reducing the pH levels.

4.3. Materials and methods:

The salt sensitive wheat cultivar (Knoppies) and salt tolerant cultivar (Losper) were chosen to be tested for possible acid alleviation of salt stress. Citric acid (tri-protic acid), and malic acid (di-protic acid) were used to treat seeds as was done in the hormone experiment. Gibberellic acid is a mono-protic acid, so accordingly, the concentrations that were used were as follow:

GA_3	40 µM	400 µ M
	*pH= 4.4	*pH= 3.3
Malic acid	20 µM	200 µM
	*pH= 4.2	*pH= 3.3
Citric acid	13.33 µM	133.33 µM
	*pH= 4.5	*pH= 3.4

*(pH value as measured)

Seeds were surface sterilized in 3.5% sodium hypochlorite (NaOCl) solution as explained in chapters 2 and 3. Seeds were washed with organic acid solution, then soaked in the organic acid solutions for 6 hours, and air dried for 24 hours. Control seeds were soaked in distilled water. To keep the seeds uncontaminated they were manipulated in a laminar flow cabinet, and rolled in plastic bags then kept up right and incubated at 25°C on toweling paper in an incubator. Other steps (Experiment design, Parameter measurement, and Results analysis [Table 4.1]) were the same as in chapters 2 and 3. Two NaCl solutions (200 and 400 mM) were used as germination media, and for a control, distilled water was used.

4.4. Results:

Treatment of the salt sensitive wheat cultivar (Knoppies) and the salt resistant cultivar (Losper) with salinity led to decreases in all parameters that were measured, except that the germination rate of Losper was not affected. (Fig 4.1-4.4). The interaction between salinity and citric acid led to a decrease in root mass and germination percentage of Knoppies seedlings, particularly at the highest salt level (400 mM) that was treated with 133.3 μM citric acid, and had no significant effect on other parameters, and had no effect on the salt tolerant cultivar (Losper) (Fig 4.1), and had no effect on Losper seedlings (Fig. 4.2). Treating wheat seed with malic acid caused an increase in root mass and shoot mass of Losper plants without salt (Fig 4.4), while it had no effect on Knoppies seedlings (Fig 4.3). This was also established in an ANOVA Table (Table 4.1), which shows the effect of each factor (organic acid, salinity and the interaction between organic acid and salinity). So the effects of organic acids were either negative, or they had no effect on the growth of tested plants when applied with salt. This established that the effect of GA_3 in the promotion of germination under high salinity, particularly in salt sensitive cultivars, (given in the previous chapter) was due to the hormonal effects and not the lowering of the pH that might be caused by GA_3.

Table 4.1. ANOVA-table (organic acid, salinity and the interaction) for the various parameters measured on Losper and Knoppies seedlings.

		Knoppies						Losper					
		Citric Acid			Malic Acid			Citric Acid			Malic Acid		
Source of Variation		*F*	*P-value*	*F crit*	*F*	*P-value*	*F crit*	*F*	*P-value*	*F crit*	*F*	*P-value*	*F crit*
Root Length	Org. Acid	11.91 *	0.002962	4.26	4.45 *	0.045367	4.26	0.22	0.80571	4.26	1.24	0.334178	4.26
	Salinity	367.17 *	2.36E-09	4.26	86.93 *	1.3E-06	4.26	190.11 *	4.35E-08	4.26	98.53 *	7.61E-07	4.26
	Interaction	3.45	0.056735	3.63	1.42	0.302077	3.63	2.51	0.115889	3.63	2.19	0.151626	3.63
			LSD=	1.212		LSD=	2.381		LSD=	1.456		LSD=	2.36
Root Mass	Org. Acid	12.95 *	0.002247	4.26	1.29	0.320879	4.26	0.57	0.585823	4.26	3.32	0.083118	4.26
	Salinity	323.57 *	4.15E-09	4.26	124.40 *	2.78E-07	4.26	91.54 *	1.04E-06	4.26	74.71 *	2.48E-06	4.26
	Interaction	3.74 *	0.04658	3.63	1.44	0.297726	3.63	3.04	0.076638	3.63	5.32 *	0.017678	3.63
			LSD=	0.0057		LSD=	0.01016		LSD=	0.0115		LSD=	0.0175
Shoot Length	Org. Acid	1.53	0.268171	4.26	1.26	0.329423	4.26	0.19	0.82667	4.26	2.73	0.11838	4.26
	Salinity	213.05 *	2.63E-08	4.26	147.46 *	1.32E-07	4.26	110.88 *	4.57E-07	4.26	370.41 *	2.27E-09	4.26
	Interaction	1.36	0.320869	3.63	0.87	0.517172	3.63	1.06	0.429816	3.63	3.43	0.057448	3.63
			LSD=	0.789		LSD=	0.958		LSD=	0.928		LSD=	0.499
Shoot Mass	Org. Acid	5.35 *	0.029425	4.26	0.81	0.473956	4.26	0.15	0.860406	4.26	5.17 *	0.032053	4.26
	Salinity	672.75 *	1.59E-10	4.26	188.35 *	4.53E-08	4.26	102.68 *	6.37E-07	4.26	202.13 *	3.32E-08	4.26
	Interaction	2.23	0.145592	3.63	0.47	0.759007	3.63	0.47	0.760154	3.63	6.15 *	0.011457	3.63
			LSD=	0.0045		LSD=	0.0089		LSD=	0.0103		LSD=	0.009
Germination	Org. Acid	67.44 *	3.83E-06	4.26	15.90 *	0.001112	4.26	9.20 *	0.006678	4.26	9.25 *	0.006563	4.26
	Salinity	26.78 *	0.000163	4.26	2.73	0.118728	4.26	0.74	0.502993	4.26	0.25	0.784034	4.26
	Interaction	6.11 *	0.011668	3.63	0.28	0.880925	3.63	0.22	0.920763	3.63	0.39	0.808956	3.63
			LSD=	0.16		LSD=	0.3804		LSD=	0.43		LSD=	0.4882

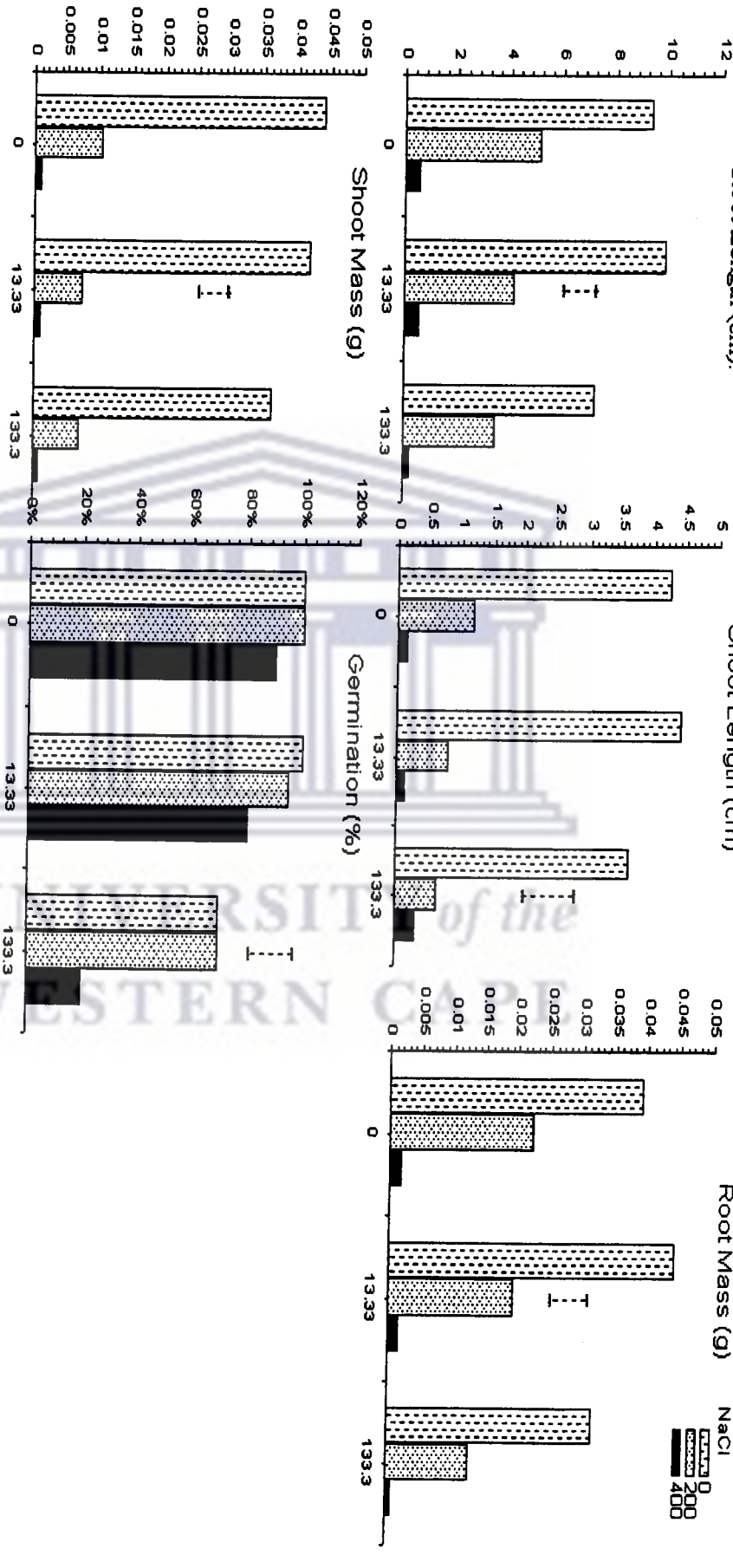

Fig.4.1. Effects of citric acid (µM) and salinity (mM) on the growth of the seedlings of the salt sensitive cultivar (Knoppies).

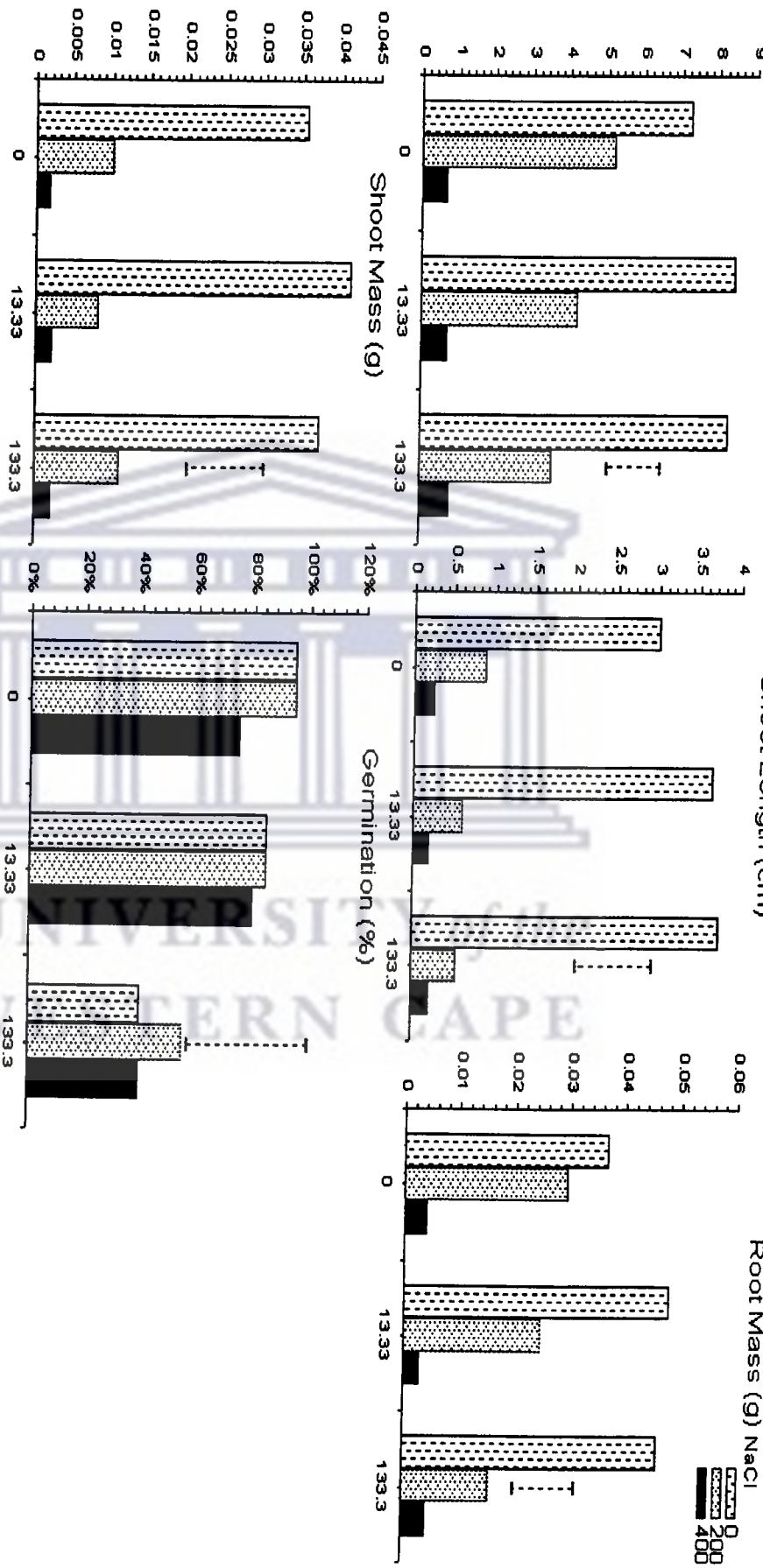

Fig.4.2. Effects of citric acid (µM) and salinity (mM) on the growth of the seedlings of the salt resistant cultivar (Losper).

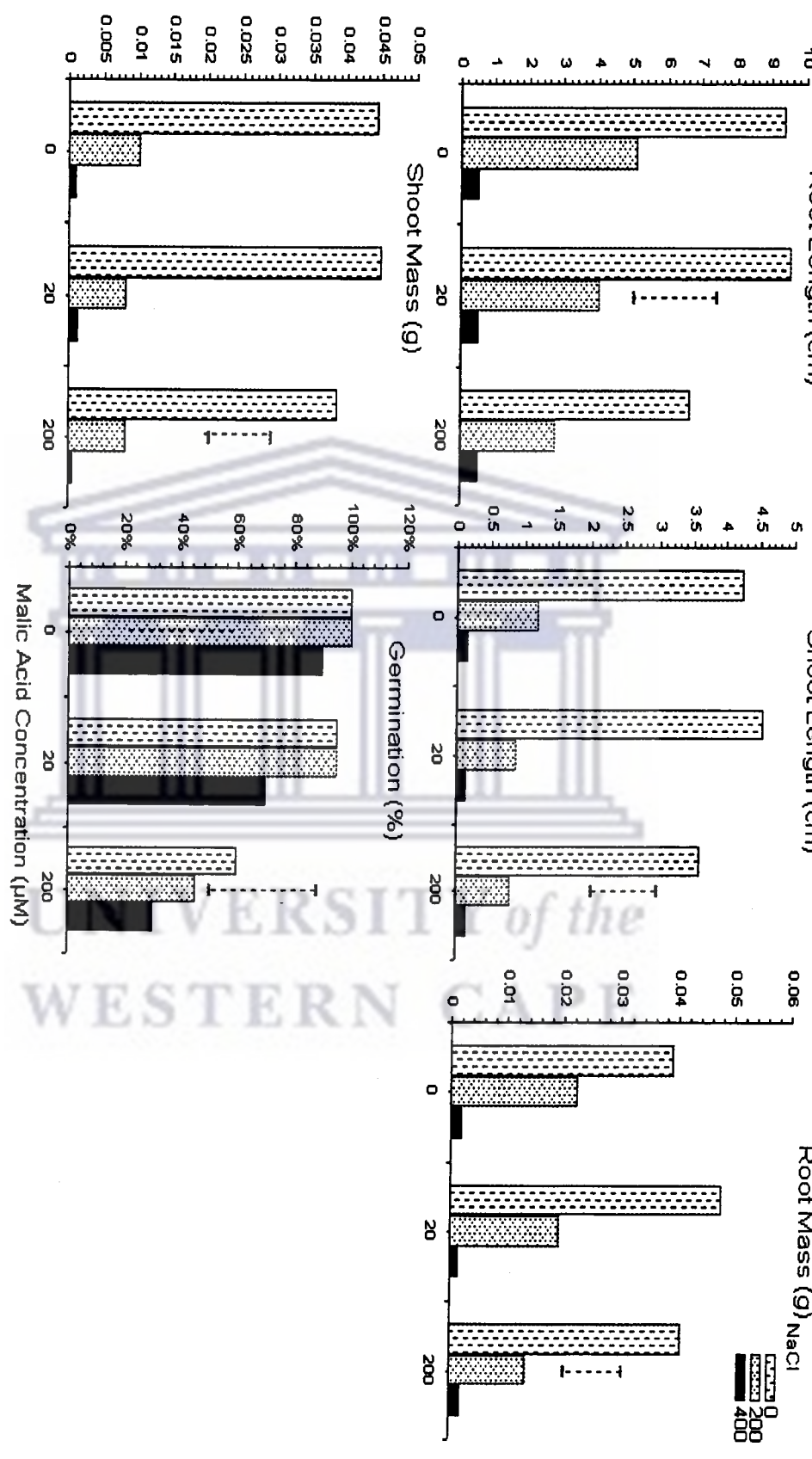

Fig.4.3. Effects of malic acid (µM) and salinity (mM) on the growth of the seedlings of the salt sensitive cultivar (Knoppies).

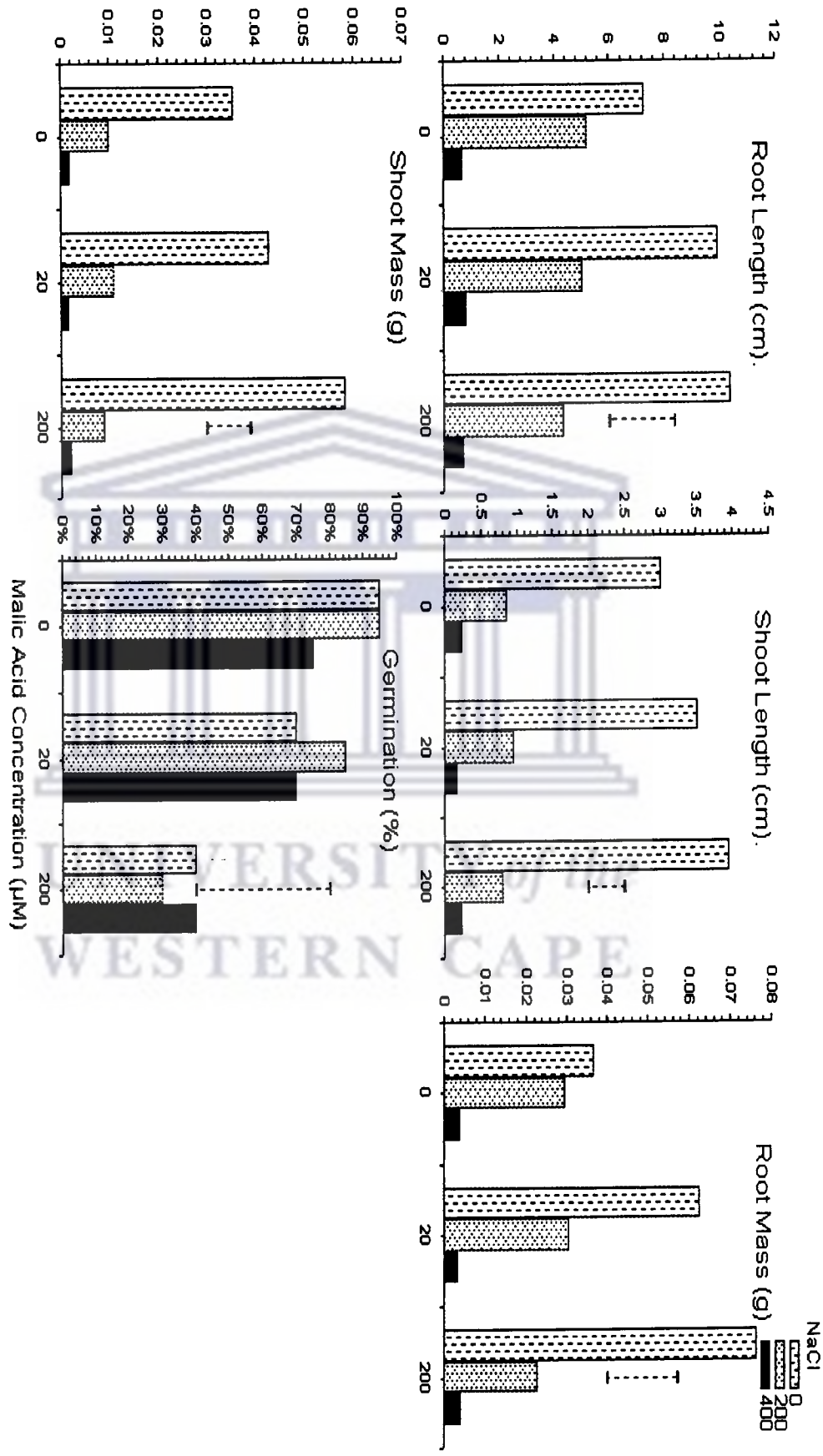

Fig.4.4. Effects of malic acid (µM) and salinity (mM) on the growth of the seedlings of the salt resistant cultivar (Losper).

4.5. Discussion:

It is clear that treatments with the organic acids caused decreases in pH (Malic acid, 20 µM pH=4.2, 200 µM pH=3.3, and Citric acid 13.33 µM pH= 4.5, 133.3 µM pH= 3.4). The significant effects that were caused by treatments with malic acid and citric acid in this study were decreased growth wherever accompanied by salt. Several studies showed that decreasing pH, due to acid rain or acid soil, caused various injuries to the exposed plants; injury to foliage (Leith *et al.*, 1989; Back & Huttunen, 1992), interference with normal metabolism (Pell, 1988; Magel *et al.* 1990), accelerated leaching of nutrients from plant foliage and soil (Turner & Tingey, 1990; Reddy *et al.* 1991), effects of increasing Al^{3+} in soil solution on the fine roots (Schaedle *et al.* 1989), influences on seedling emergence and growth (Lee & Weber, 1979; McColl & Johnson, 1983; Haines & Carson, 1989), alterations of symbiotic associations and host-parasite interactions (Shriner, 1976; Moor & Gillette, 1989; Walker & McLaughlin, 1991; Marsh, 1993), and increased susceptibility to some environmental stress factors (Tomlinson, 1983; Johnson & Siccama, 1983).

Marsh (1993) found promotion of seed germination of some plants by acid treatments (pH 2.0).

In our study, no positive effects of the tested organic acids were found in treated plants under salt stress, this agrees with some previous studies; no role for citric and malic acid treatments were found in term of aluminum stress resistance in rice plants (Macedo *et al.*, 2001). In a study done by Elhaak (1999) on some desert species, an accumulation of malic acid was found in plants that were exposed to water stress and CO_2 deficiency. This accumulation can be a benefit in osmotic adjustment under water stress.

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CHAPTER 5

The Interaction Between GA_3 and Salinity in Vegetative Growth of Two Wheat Cultivars (Flameks and Drommedaris)

5.1. Summary:

A salt tolerant wheat cultivar (Flameks) and a salt sensitive wheat cultivar (Drommedaris) were examined under three NaCl concentrations, 0, 200 and 400 mM. Wheat plants were watered with three GA_3 concentrations, 0, 40 and 125 µM throughout the experiment period. Plants were harvested after two weeks under the full dose of the salt treatments. Chlorophyll content (a and b), shoot fresh mass, dry mass, water content, root dry mass, Mg^{2+}, Ca^{2+}, Na^+, K^+, Na/K ratio Cl^- concentrations and proline concentrations were measured as indicators of hormone effects at different salt levels.

Results showed that the treatment with GA_3 and salinity caused significant increases in the shoot fresh mass in the salt tolerant cultivar and a reduction in the salt sensitive one.

Significant reductions in the proline content was recorded in the shoots of Flameks plants and the roots of the both cultivars treated with GA_3 at the highest salt concentrations. The treatment with GA_3 led to significant decreases in shoot Mg^{2+} and Na^+ content of the salt tolerant cultivar and increases in the shoot Ca^{2+} content of both cultivars and this was more

pronounced in the salt sensitive cultivar (Drommedaris). The root Na^+ content was also decreased by GA_3 treatment under the highest salt levels. The K^+ content decreased in the shoots and increased in the roots of Flameks plants. GA_3 treatments led to decreases in the shoot Na/K ratios in the salt tolerant plants and in some cases also in the salt sensitive plants. The Cl^- content decreased in shoots and roots of the both wheat cultivars with GA_3 treatments. The treatments with GA_3 did not cause significant changes in the chlorophyll content, shoot dry mass and shoot water content in the wheat cultivars.

The results of this study indicate that treatment of salt stressed wheat cultivars with GA_3 could alleviate some of the harmful effects of high salt levels on the pre-reproductive growth and development of wheat plants, in other words wheat plants can be grown in brackish soil if the seeds are pre-treated wit GA_3.

This study was a follow up of the previous study reported in the Chapter 3 and helps to explain the role of GA_3 in alleviating salt damage in several spheres; decreases in Na^+, Cl^-, Mg^{2+} content and Na/K ratio and increases in fresh mass and Ca^{2+} contents. The reduction in proline content may be an indicator of the reduction in the stress caused by high salt treatments.

5.2. Introduction:

The presence of high concentrations of salt (NaCl) in the soil solution induces a wide range of physiological and biochemical perturbations at the whole plant level. It has been demonstrated in several glycophyte species such as rice (Lutts *et al.*, 1996a,b), potato (Sabbah & Tal, 1990) and alfalfa (Winicov & Krishman, 1996) but also in halophyte species like *Mesembryanthemum crystallinum* (Vera-Estrella *et al.*, 1999), that the responses exhibited by salt stressed plants are, at least partly, determined by cellular properties. Salinity resistance is a complex trait resulting from the interaction of several morphological and physiological traits. Salinity induces both an ionic and an osmotic strain in plant tissues (Drihem & Pilbeam, 2002; Almansouri *et al.* 2000; Borsani *et al.*, 2001; Lazof & Bernstein, 1999; Greenway & Munns, 1980). While electrical conductivity (EC) is an assessment of all soluble salts in a soil sample, sodium hazard is defined separately because of its specific detrimental effects on soil physical properties. The sodium hazard is typically expressed as the sodium adsorption ratio (SAR). This index quantifies the proportion of sodium (Na^{+}) to calcium (Ca^{++}) and magnesium (Mg^{++}) ions in a sample. General classifications of irrigation water based upon SAR values are presented in Table 5.1 below.

Table 5.1. The sodium hazard of water based on SAR values (Bauder *et al*. 2004).

SAR values	**Sodium hazard of water**	**Comments**
1-9	Low	Use on sodium sensitive crops must be cautioned.
10-17	Medium	Amendments (such as gypsum) and leaching needed.
18-25	High	Generally unsuitable for continuous use.
≥ 26	Very high	Generally unsuitable for use.

The high concentrations of sodium in irrigation water can cause toxicity problems for some crops, especially when sprinkler applied. Crops vary in their susceptibility to this type of damage (Bauder *et al*., 2004; Rashid *et al*., 1999) as show in the table below (5.2).

Table 5.2. Susceptibility ranges for selected crops to foliar injury from saline sprinkler water (Maas 1990).

	Na or Cl concentration ($mg.L^{-1}$) causing foliar injury			
Na concentration	<46	46-230	231-460	>460
Cl concentration	<175	175-350	351-700	>700
Crop	Apricot	Pepper	Alfalfa	Sugarbeet
	Plum	Potato	Barley	Sunflower
	Tomato	Corn	Sorghum	

Salinity tolerance of cereals may be related to the accumulation of Na in old leaves and the continued transport of K to young leaves (Greenway *et al.*, 1965; Yeo *et al.*, 1985; Yeo & Flowers, 1986; Wolf *et al.*, 1991). Sodium accumulation has often been implicated as one of the mechanisms of salt tolerance in non-halophytes, although this conclusion cannot be generalized (Cramer *et al.*, 1994). Limited accumulation of Na^+ and Cl^- in the shoots of salt-tolerant non-halophytes diminishes the role of these solutes in osmotic adjustment (Greenway & Munns, 1980).On the other hand, Munns & James, (2003) found that Na^+ exclusion is a robust trait that should help to confer salinity tolerance in the field, and they found no additional advantage in measuring K^+/Na^+, when the Na^+ concentrations were already being determined.

An increased Ca/ Na ratio in the soil solution enhances the capacity of roots to restrict Na^+ influx (Marschner 1995). An exogenous supply of calcium may significantly alleviate the detrimental effects of Na^+ on the physiological performance of hydroponically grown plants (La Haye & Epstein, 1969; Cramer *et al.* 1985 1989; Kurth *et al.*, 1986; Lauchli 1990; Rengel 1992; Colmer *et al.*, 1996; Reid & Smith 2000; Shabala 2000; Elphick *et al.*, 2001; Shabala *et al.*, 2003).

Chloride is a common ion in irrigation waters. Although chloride is essential to plants in low amounts, it can cause toxicity to sensitive crops at high concentrations (Table 5.3.). Like sodium, high chloride concentrations cause more problems when applied with sprinkler irrigation (Table 5.2 and 5.3). Leaf burn, under sprinkler irrigation, from both sodium and chloride, can be reduced by night time irrigation or application on cool, cloudy days (Bauder *et al.* 2004).

Table 5.3. Chloride classification of irrigation water.

Chloride ($mg.L^{-1}$)	Effect on Crops
Below 70	Generally safe for all plants.
70-140	Sensitive plants show injury
141-350	Moderately tolerant plants show injury.
Above 350	Can cause severe problems.

Drihem & Pilbeam (2002) found that potassium concentrations in wheat plants decreased with high salinity. Feigin *et al.*, (1987); Silberbush (2001); Botella *et al.* (1997) and Wei *et al.* (2003), found the same in other species.

The toxic influences and nutritional imbalances are recognized, and some authors maintain that it is mainly the total salt concentration of the soil solution that causes growth reduction (Bernstein, 1964, 1974; Maas & Hoffman, 1977; Maas & Nieman, 1978). Evidence connected to the direct toxic influence of some ions or the accumulation of toxic amounts of salts in the leaf tissues, leads others to attach more importance to growth inhibition through ion toxicity or accumulation (Maas, 1990; Munns, 1993; Termaat & Munns, 1986). It is generally recognized that these adverse effects could simultaneously be responsible for growth reduction, but the relative contribution of the three major constraints to growth inhibition at high substrate salinity, is difficult to assess (Marschner, 1995; Jacoby, 1994). However, the opinion that growth reduction is primarily due to the osmotic potential is being reviewed as many nutritional and also membrane related studies indicate other possibilities (Reinhold, *et al.*, 1989; Lauchli & Epstein, 1990; Grattan & Grieve, 1992; Rengel, 1992). Lowered osmotic potential

may also influence cell wall hardening and eventually growth (Neumann, 1995).

The chlorophyll content (chlorophyll a, b and total) usually decreases in plants that are grown under salt stress and this will affect the photosynthetic rate. This result has been found in several studies (Ashraf *et al.*, 2002; Gadalla, 1999; Khavari-Nejad & Chaparzadeh, 1998 and Lutts *et al.*, 1996c). A significant change in chlorophyll content will cause a change in the photosynthetic process in plants.

About 7% of the world's total land area is affected by salt, as is a similar percentage of its arable land (Ghassemi *et al.*, 1995; Szabolcs, 1994). The area is increasing as a result of irrigation and land clearing (Munns *et al.*, 2002). So the enhancement of crop salt tolerance is necessary.

Improving salt tolerance of crops has been studied for many years and since times of old, it has been attempted and the possible ways to increase tolerance have been extensively researched (Jacobsen & Adams, 1958). Salt tolerance depends upon: morphology, compartmentation and compatible solutes, regulation of transpiration, control of ion movement, membrane characteristics, tolerace of high Na concentrations in the cytoplasm and salt glands, that is in both structure and function (Pearce, 2003).

Adaptation to salt stresses is associated with metabolic adjustments that lead to the accumulation of organic solutes such as sugars, polyols, betaines and proline (Flowers *et al.*, 1977; Gorham *et al.*, 1981; Yancey *et al.*, 1982). Singh *et al.* (1972) were probably the first to assign a correlation between proline accumulation and water deficiency resistance in barley. They showed that resistant cultivars accumulated many fold higher free proline than the

susceptible cultivars and proline can act as a non-toxic osmolyte, and they also pointed out that proline was about 300 times more soluble in water than other amino acids. Many other studies have since showed a correlation between water stress and proline accumulation (*e.g.* Waldren & Tear, 1974; Hanson *et al.*, 1977; 1979; Singh *et al.*, 1985; Rai, 2002). Proline accumulation has been studied in many plants, and its accumulation has also been described in salt-tolerant mutants of *Nicotiana plumbaginifolia* obtained from protoplast cultures (Sumarayati *et al.* 1992) as well as in NaCl-resistant lines of *Brassica juncea* obtained *in vitro* (Kirti *et al.* 1991). Proline markedly increased under low water potential in *Zea mays* L (Verslues & Sharp, 1999). In *Arabidopsis thaliana rss* mutants exposed to NaCl, proline levels were lower than those of the wild-type controls (Werner & Finkelstein 1995) showing variation within a species.

Methods and the attempts to improve crop salt tolerance have been widely discussed in chapter 3. One of those important methods is that of plant hormone application to alleviate salt effects. Use of GA_3 for that purpose has been demonstrated in chapter 3. The experiment in chapter 3 was done at the germination stage. In this chapter we report on the pre-reproductive stage of the vegetative growth of two wheat cultivars (salt sensitive [Drommedaris], and a salt tolerant cultivar [Flameks]), under GA_3 application.

5.3. Materials and Methods:

5.3.1. Plant material:

Two wheat cultivars (Flameks and Drommedaris) were chosen to be tested in this experiment. From the Chapter 2, Flameks was classified as a salt tolerant cultivar and the Drommedaris as a salt sensitive cultivar at the germination stage. And they were supplied by the same source (Agronomy Department of the University of Stellenbosch, South Africa).

5.3.2. Equipment and supplies:

Plastic pots of 15 cm diameter and height were used. Silica sand was used for the germination medium. The field capacity was determined and it was approximately 35%. Twenty five seeds were placed in each pot and after one-week seedlings were reduced to 15 seedlings per pot. Concentrations of 0, 200 and 400 mM NaCl were used for salt treatments, and 0, 40 and 125 μM GA_3 for the hormone treatments. The experiment was carried out in two growth chambers (Fisons Model: L.T.G.C.) and they were maintained under a 10/20 °C regime (night/day respectively), and 12 hours day/night setting. One half strength Chemicult nutrient solution was used to provide tested plants with the necessary inorganic elements. A Shimadzu spectrophotometer (Model UV-160A) was used for the chlorophyll and proline determinations. A Unicam SOLAR Atomic Absorption Spectrophotometer (Model GF95) was used for the analysis of inorganic elements (Na^+, K^+, Ca^{2+}, Mg^{2+}).

5.3.3. Experimental design:

A random block method was used involving two wheat cultivars, three salt concentrations and three hormone concentrations. Two replicates for each

treatment were used. The pots were completely randomized and distributed in two blocks, and each block was placed in a separate growth chamber.

5.3.4. Hormone and Salt treatments:

The plants were watered with a half strength Chemicult solution and a full dose of GA_3 throughout the experimental period. To reduce salt stress, the salt treatments were increased gradually. After two weeks the salt treatment started with 100 ml of 100 mM NaCl given every day to each pot except the control, to reach to the 700 ml after a week which was the field capacity of the sand used, then 200 mM NaCl started for the next salt concentration for a week as well, then 400 mM NaCl was started and given to the plants due to receive the highest salt level. Plants were left to grow for two more weeks at their respective NaCl-concentrations and every two days watered with 700 ml of the full dose of NaCl to avoid salt accumulation on the soil surface.

5.3.5. Plant harvesting:

The wheat plants were harvested after two weeks at the highest salt treatments. On the same day, and just before harvesting, 0.25g was cut from the leaves of each pot and placed in a small bottle and frozen for later chlorophyll determinations. The roots were cut and washed with normal water and blotted by tissue. The fresh weight of shoots was measured, and then the shoots and roots of each pot were placed in paper bags and dried in an oven at 80°C for 72 hours. The dry samples were weighed, ground and stored in plastic containers in a fridge until further use.

5.3.6.Chlorophyll determination:

The Todd & Basler method (1965) was used to determine the chlorophyll concentration in plant samples as follows:

- A 0.25 g portion of frozen fresh leaf was ground in a mortar for 5 min in 50 ml 85% acetone.
- The supernatant was transferred to a centrifuge tube and centrifuged at 4000 rpm for 30 min in a Beckman centrifuge.
- The solution was then transferred to a 100 ml volumetric flask and filled to the mark with 85% Acetone.
- The absorption was determined at 663 nm for chlorophyll a, and 645 nm for chlorophyll b.
- The concentration of the chlorophyll in plant tissue was calculated using the Vishniac (1957) equation:

$$\text{Chlorophyll a} = 12.7\ D_{663} - 2.69\ D_{645}$$

$$\text{Chlorophyll b} = 22.9\ D_{645} - 4.68\ D_{663}$$

D = the absorption value at the specific wave length.

5.3.7.Plant extraction:

The El-Sharkawi & Michel (1977) method was used to prepare a plant extract as follows:

- A 0.5 g sample of dry plant material was placed in a test tube with 10 ml distilled water, and then placed in a water bath on a shaker at 90°C for an hour.
- The material was centrifuged at 3000 *g* for 15 minutes. then transferred to a 25 ml volumetric flask with 10 ml distilled water.
- The same process was repeated for the precipitate.
- Finally the solution was made up to the mark with distilled water.

5.3.8.Proline analysis:

The Bates *et. al.*, (1973) method was used to measure the proline concentration in plant samples as follows:

Reagents:

- 1.25 g Ninhydrin was dissolved in 30 ml glacial acetic acid with 20 ml phosphoric acid (6M) with heating until completely dissolved, and then kept in the fridge.
- 3.0 g Sulfo salicylic acid dissolved in 100 ml distilled water (3%).
- Toluene solvent.

The method:

- A 0.1 g dry powder was ground in a mortar with 10 ml of 3% sulfo salycilic acid, and then the solution was centrifuged at 3000 *g* for 15 min.
- 2 ml was taken from the solution and 2 ml acid ninhydrin added with 2 ml glacial acetic acid, then left for an hour in a boiling water bath.
- The samples were quickly cooled in ice.
- 4 ml Toluene solvent were added to each sample and mixed for 2 min.
- The solution was left for 10 min before the spectrophotometer readings were taken at 520 nm,
- Toluene solvent was used as a blank.
- A calibration curve was set up using different proline concentrations (0 to 5mg/L).

5.3.9.Digestion of plant samples:

The sulphuric acid-hydrogen peroxide method was used to digest plant material for inorganic element determinations according to (Allen, 1982) as follow:

Reagents:

- Sulphuric acid, concentrated.
- Hydrogen peroxide, 100 volume.

- Selenium, powder.
- Lithium sulphate, monohydrate.
- Mixed digestion reagent, 350 ml H_2O_2 added to 0.42 g Se and 14 g $LiSO_4$ in a litre boiling flask. 420 ml H_2SO_4 added slowly whilst mixing and cooling.

Procedure:

- 0.5 g of the ground samples was weighed into 50 ml digestion flask.
- 4.4 ml from the mixed digestion reagent was added.
- Heated gently to 600 °C in a Buchi digestion block and continued heating until the digest became clear.
- The digested samples were diluted and quantitatively transferred through filter paper into a 50 ml volumetric flask and then diluted to volume with deionized water, and mixed.

5.3.10.Elemental analysis:

A SOLAAR atomic absorption spectrophotometer was used to evaluate the sodium, potassium, magnesium and calcium concentrations in plant samples, according to the methods of Allen (1982).

5.3.11.Chloride analysis:

For chloride analysis a potentiometeric method involving a silver sensitive electrode (radiometer) and automatic titrator was used.

5.4. Results:

The results of effects of the GA_3 treatments (0, 40, and 125 μM) on some minerals (Mg, Ca, Na, K, and Na/K) in the shoots and roots of salt tolerant wheat cultivar (Flameks) and the salt sensitive wheat cultivar (Drommedaris), grown at three different salt levels (0, 200, and 400 mM), are presented in Tables 5.4. and 5.6. Treatment of salt tolerant (Flameks) and salt sensitive (Drommedaris) cultivars with salinity led to significant decreases in magnesium and calcium content (at 400 mM NaCl in Flameks plants and 200 mM in Drommedaris plants) and potassium contents in shoots. A significant increase was found in the sodium and chloride content and Na/K ratio of shoots of plants that were grown under saline conditions and without hormone treatments.

Treatment of wheat plants with GA_3 (Table 5.4), caused significant decreases in magnesium content in shoots of both cultivars that were grown under non-saline conditions. Significant decreases in magnesium content were found in the salt tolerant plants (Flameks) that were grown under low and high saline conditions and treated with 125 μM GA_3. No significant effects of GA_3 treatment were found on magnesium content of plants of the salt sensitive cultivar that were grown under saline conditions, except for a decrease in those that were treated with 40 μM GA_3 and were grown under 400mM NaCl. A significant decrease was found in magnesium contents of roots of Flameks plants that were grown under 0 and 200 mM NaCl and treated with 125 μM GA_3. No significant effects were found on the Mg contents of roots of Drommedaris plants except for those plants that were grown at 400 mM NaCl and treated with 40 μM GA_3 where the treatment led to a decrease in root magnesium contents (Table 5.6).

The salt resistant cultivar (Flameks) had no significant response in the calcium content of shoots, except those plants that were treated with 400 mM NaCl and 40 μM GA_3, where there was a significant positive effect. A positive effect for the GA_3 treatments was found in shoots of plants of the salt sensitive cultivar that were grown under 200 mM salt and treated with 40 and 125 μM GA_3, and 400 mM salt combined with 125 μM GA_3 (Table 5.4).

No significant effects were found in the root calcium content of Flameks plants at different salt levels and different hormone treatments. Treatment of Drommedaris plants with GA_3 caused a significant decrease in the calcium content of plants that were grown under non-saline conditions and treated with 40 μM GA_3, and plants that were grown at 400 mM NaCl and were treated with 40, and 125 μM GA_3 (Table 5.6).

Addition of GA_3 to the irrigation water of the Flameks cultivar led to significant decreases in the sodium content of shoots of all plants that were grown under salinity. The shoot sodium content also decreased in the Drommedaris plants, except for those plants that were grown with 200 mM NaCl and treated with 125 μM GA_3 where a significant increase was found (Table 5.4). Significant increases were found in the root sodium content of Flameks plants that were grown at 200 mM NaCl and treated with hormone, while a significant decrease took place in root sodium content in plants that were grown at 400 mM NaCl and treated with 40 and 125 μM GA_3. Treatment of Drommedaris plants with NaCl led to an increase in the root sodium content, GA_3 treatment reduced this detrimental effect at 400 mM NaCl.

Table 5.4. The effects of three GA_3 treatments (μM) and three NaCl treatments (mM) on the Mg^{2+}, Ca^{2+}, Na^+, K^+, and Cl^- content (mM) and Na/K ratio of the shoots of two wheat cultivars.

		Flameks			Drommedaris		
	NaCl / GA_3	0	200	400	0	200	400
	0	0.118	0.071 -$^{\odot}$	0.076 -$^{\odot}$	0.107	0.069 -$^{\odot}$	0.061 -$^{\odot}$
Mg^{2+}	40	0.087 -*	0.080 n	0.068 n	0.081 -*	0.062 n	0.051 -*
LSD=0.009	125	0.086 -*	0.055 -*	0.052 -*	0.083 -*	0.067 n	0.066 n
	0	0.252	0.224 n	0.203 -$^{\odot}$	0.205	0.163 -$^{\odot}$	0.186 n
Ca^{2+}	40	0.223 n	0.232 n	0.242 +*	0.220 n	0.218 +*	0.216 n
LSD=0.035	125	0.226 n	0.234 n	0.207 n	0.235 n	0.231 +*	0.229 +*
	0	0.157	2.860 +$^{\odot}$	3.609 +$^{\odot}$	0.149	1.560 +$^{\odot}$	3.383 +$^{\odot}$
Na^+	40	0.487 n	1.596 -*	2.757 -*	0.257 n	1.296 n	2.558 -*
LSD=0.388	125	0.513 n	1.370 -*	2.390 -*	0.299 n	2.042 +*	3.254 n
	0	1.553	1.085 -$^{\odot}$	1.123 -$^{\odot}$	1.610	1.077 -$^{\odot}$	1.109 -$^{\odot}$
K^+	40	1.422 n	1.224 +*	1.062 n	1.595 n	1.146 n	1.046 n
LSD=0.144	125	1.528 n	0.917 -*	0.921 -*	1.526 n	1.167 n	1.032 n
	0	0.600	1.550 +$^{\odot}$	1.890 +$^{\odot}$	0.050	0.850 +$^{\odot}$	1.800 +$^{\odot}$
Na/K	40	0.200 n	0.750 -*	1.530 -*	0.090 n	0.670 n	1.440 -*
LSD=0.201	125	0.200 n	0.880 -*	1.530 -*	0.120 n	1.030 n	1.850 n
	0	0.007	0.020 +$^{\odot}$	0.019 +$^{\odot}$	0.007	0.015 +$^{\odot}$	0.020 +$^{\odot}$
Cl^-	40	0.008 n	0.013 -*	0.018 n	0.009 n	1.014 n	0.017 n
LSD=0.003	125	0.009 n	0.016 -*	0.016 n	0.011 +*	0.018 n	0.020 n

+$^{\odot}$ = NaCl negative effects

-$^{\odot}$ = NaCl positive effects

+* = GA_3 positive effects

-* = GA_3 negative effects

n = Non significant effects

Treatment of Flameks plants with GA_3 caused decreases in the potassium content of the shoots, except for those that were grown at 200 mM NaCl and 40 μM GA_3, where a significant increase was found. No significant effects were found in the potassium content of shoots of Drommedaris plants with the different hormone treatments (Table 5.4).

The treatment of Flameks plants with GA_3 led to increases in root potassium content in plants that were grown in non-saline solutions and in 200 mM NaCl with 125 μM GA_3. No effects of GA_3 treatment on root potassium contents of Drommedaris plants under different salt levels were found while significant decreases were found in plants that were grown under non-saline conditions (Table 5.6).

Sodium to potassium ratios showed a significant decrease in the shoots of salt tolerant plants under GA_3 treatments. No consistent effects were found in Na/K ratio in shoots of the Drommedaris plants (Table 5.4). No significant effects of GA_3 on sodium : potassium ratios were found in roots of Flameks plants under salt stress conditions while a decrease was found in Drommedaris plants that were grown under extreme salinity and treated with GA_3 (Table 5.6).

Treatments with GA_3 had no effects on chlorine contents in shoots of Flameks plants that were grown under non-saline conditions. A significant increase occurred in the Cl^- contents of shoots of Flameks and Drommedaris plants that were grown with NaCl and without hormone treatment. A significant decrease took place in the Cl^- contents of shoots of Flameks plants that were grown under 200 mM NaCl and treated with GA_3. Treatments of GA_3 had no effects on the chloride contents of shoots of Drommedaris plants that were grown with NaCl and treated with GA_3, except those plants that were grown

without salt and treated with 125 μM GA_3 where the effect was a significant increase (Table 5.4). Decreases in chlorine content took place in roots of Flameks plants that were grown under 200 mM salt and 40 μM GA_3, and the plants that were grown under 400 mM NaCl and GA_3. Chloride contents increased in salt sensitive plants that were grown at 200 mM salt and treated with 40 μM GA_3 while a decrease took place in plants that were grown at 400 mM NaCl and treated with 40 μM GA_3 (Table 5.6).

Effects of GA_3 treatments on the chlorophyll content of shoots of the salt tolerant (Flameks) and salt sensitive (Drommedaris) cultivars that were grown at three different salt levels are presented in Table 5.5. The treatments with NaCl had no significant effect on the chlorophyll content of both cultivars. A significant decreases was caused by 125 μM GA_3 in chlorophyll a, chlorophyll b and chlorophyll a+b at 200 mM NaCl in the salt sensitive cultivar (Drommedaris).

Effects of GA_3 treatments on shoot fresh mass, shoot dry mass, shoot water content, and root dry mass of the salt tolerant cultivar (Flameks) and the salt sensitive (Drommedaris) that were grown at three different salt levels, are presented in Table (5.5). The NaCl treatments applied, led to significant decreases in the shoot fresh mass of both tested cultivars. The interaction between salt and GA_3 treatments caused a significant increases in shoot mass in the Flameks plants that were grown under 200 mM salt and treated with GA_3, while significant decreases took place in plants that grew under non-saline conditions and were treated with hormone. The same was true in the salt sensitive cultivar, and in addition, in the plants that were grown under NaCl and 125 μM GA_3.

The salt treatments led to significant decreases in shoot dry mass of both cultivars. Only a single response was reported in dry mass with GA_3 treatments, where positive effect occurred in Flameks plants that were grown under 200 mM salt and 125 µM GA_3 (Table 5.5).

The situation in roots dry mass is the same as that of shoots with salt treatments. Dry mass of Flameks plants decreased with GA_3 treatments in plants that were grown in non-saline conditions. The same is true in the salt sensitive plants that were treated with 40 µM GA_3. A positive increase in root dry mass is reported for Drommedaris plants that were grown at 400 mM NaCl and treated with 40 µM GA_3 (Table 5.6).

The salt stress caused a significant reduction in shoot water content of both tested cultivars and the applied of GA_3 treatments didn't cause any significant changes in shoot water content of Flameks and Drommedaris plants (Table 5.5).

Application of NaCl treatments led to significant increases in shoot and root proline contents (Table 5.5 and 5.6). GA_3 applied to the salt resistant cultivar caused significant decreases in shoot proline content in all salt stressed plants, while that did not happen in the salt sensitive cultivar, except in plants that were grown at 200 mM salt and 125 µM GA_3. And it is clear that no changes in proline content had occurred in shoots and roots of plants that were grown under non-saline conditions (Tables 5.5 and 5.6). Increases in root proline content were found in Flameks plants that were grown at 200 mM NaCl and 125 µM GA_3, and decreases at 400 mM salt. Significant decreases were also reported in root proline content of the salt sensitive cultivar (Drommedaris) plants that were grown at 200 mM salt and treated with 40 µM GA_3 and the

same in plants that were grown under extreme salt stress and treated with GA_3.

Table 5.5. The effects of three GA_3 treatments (μM) and three NaCl treatments (mM) on the chlorophyll a, b, a+b (mg/g fresh mass), a/b, fresh mass (g), dry mass (g), water content (%) and proline content (mg. g^{-1}), in shoots of two wheat cultivars.

		Flameks			Drommedaris		
	NaCl / GA_3	0	200	400	0	200	400
Chlorophyll A	0	3.79	4.61 n	4.30 n	3.73	5.76 n	4.51 n
	40	3.85 n	4.81 n	3.67 n	4.07 n	5.26 n	3.73 n
LSD=2.62	125	3.89 n	5.01 n	3.10 n	3.45 n	3.05 -*	2.36 n
Chlorophyll B	0	1.20	1.44 n	1.36 n	1.21	1.79 n	1.40 n
	40	1.17 n	1.51 n	1.13 n	1.29 n	1.59 n	1.08 n
LSD=0.82	125	1.17 n	1.57 n	0.93 n	1.02 n	0.85 -*	0.72 n
Chlorophyll a+b	0	4.99	6.04 n	5.66 n	4.94	7.55 n	5.90 n
	40	5.02 n	6.32 n	4.80 n	5.36 n	6.85 n	4.81 n
LSD=3.43	125	5.06 n	6.58 n	4.03 n	4.47 n	3.90 -*	3.08 n
Chlorophyll a/b	0	3.18	3.18 n	3.07 n	3.10	3.22 n	3.23 n
	40	3.30 n	3.19 n	3.24 n	3.15 n	3.31 n	3.59 n
LSD=0.73	125	3.32 n	3.19 n	3.40 n	3.41 n	3.68 n	4.00 +*
Fresh Mass	0	50.30	12.30 -⊙	15.30 -⊙	53.67	21.25 -⊙	15.22 -⊙
	40	45.97 -*	16.21 +*	15.75 n	49.78 -*	19.37 n	13.11 n
LSD=2.87	125	46.39 -*	20.03 +*	15.54 n	50.17 -*	15.02 -*	9.02 -*
Dry Mass	0	6.48	2.08 -⊙	3.40 -⊙	6.64	3.91 -⊙	3.05 -⊙
	40	6.10 n	2.73 n	3.05 n	5.77 n	3.18 n	2.18 n
LSD=1.47	125	5.74 n	3.90 +*	3.33 n	6.90 n	2.77 n	2.35 n
Water content.	0	87.0	78.0 -⊙	78.0 -⊙	88.0	82.0 n	80.0 -⊙
	40	87.0 n	83.0 n	81.0 n	88.0 n	84.0 n	83.0 n
LSD=6.7%	125	88.0 n	81.0 n	78.0 n	86.0 n	82.0 n	74.0 n
Proline	0	0.0096	0.1021 +⊙	0.1140 +⊙	0.0185	0.0601 +⊙	0.1069 +⊙
	40	0.0159 n	0.0493 -*	0.0931 -*	0.0120 n	0.0525 n	0.1015 n
LSD=0.1177	125	0.0161 n	0.0498 -*	0.0926 -*	0.0148 n	0.0783 +*	0.0997 n

+⊙ = NaCl positive effects -* = GA_3 negative effects

-⊙ = NaCl negative effects n = Non significant effects

+* = GA_3 positive effects

Table 5.6. The effects of three GA_3 treatments (μM) and three NaCl treatments (mM) on Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Cl^{-} ($mg.g^{-1}$), Na/K, dry mass (g), and proline content ($mg.g^{-1}$), in roots of two wheat cultivars.

		Flameks			Drommedaris		
	NaCl / GA_3		20	40	0	200	400
	0	1.29	1.21 n	1.28 n	1.24	1.19 n	1.25 n
Mg	40	1.35 n	1.19 n	1.03 n	1.29 n	1.14 n	0.91 -*
LSD=0.3172	125	0.83 -*	0.86 -*	1.17 n	1.40 n	1.40 n	1.44 n
	0	15.50	14.43 n	14.26 n	18.70	14.33 -⊙	19.19 n
Ca	40	17.81 n	16.70 n	14.62 n	16.15 -*	15.87 n	15.98 -*
LSD=2.443	125	14.13 n	15.97 n	16.54 n	19.87 n	16.69 n	15.70 -*
	0	3.52	16.21 +⊙	32.09 +⊙	4.28	22.73 +⊙	29.77 +⊙
Na	40	4.82 n	21.88 +*	22.86 -*	4.60 n	21.23 n	20.14 -*
LSD=2.930	125	4.12 n	20.33 +*	25.19 -*	3.34 n	24.95 n	24.33 -*
	0	11.70	6.78 -⊙	9.98 n	20.04	9.21 -⊙	6.23 -⊙
K	40	15.40 n	9.39 n	5.88 n	13.42 -*	6.20 n	5.27 n
LSD=4.345	125	17.09 +*	11.48 +*	7.30 n	15.51 -*	8.76 n	8.52 n
	0	0.30	2.39 +⊙	3.24 +⊙	0.21	2.50 +⊙	4.80 +⊙
Na/K	40	0.32 n	2.38 n	4.04 n	0.35 n	3.42 +*	3.85 -*
LSD=0.8357	125	0.25 n	1.80 n	3.45 n	0.22 n	2.90 n	2.86 -*
	0	0.61	0.77 +⊙	0.77 +⊙	0.53	0.75 +⊙	0.77 +⊙
Cl-	40	0.81 +*	0.61 -*	0.51 -*	0.50 n	0.82 +*	0.69 -*
LSD=0.0722	125	0.64 n	0.87 +*	0.67 -*	0.52 n	0.74 n	0.80 n
Dry	0	2.04	0.81 -⊙	0.76 -⊙	1.83	0.71 -⊙	0.51 -⊙
Mass	40	1.27 -*	0.47 n	0.65 n	0.89 -*	0.72 n	1.02 +*
LSD=0.3940	125	0.66 -*	0.68 n	0.88 n	1.54 n	0.52 n	0.28 n
	0	0.0059	0.0136 +⊙	0.0468 +⊙	0.0043	0.0209+⊙	0.0401 +⊙
Proline	40	0.0062 n	0.0171 n	0.0465 n	0.0052n	0.0128-*	0.0298 -*
LSD=.0075	125	0.0042 n	0.0231 +*	0.0385 -*	0.0041n	0.0178n	0.0282 -*

+⊙ = NaCl positive effects

-⊙ = NaCl negative effects

+* = GA_3 positive effects

-* = GA_3 negative effects

n = Non significant effects

5.5. Discussion:

It is well known that salinity causes accumulation of some ions such as Na^+ and Cl^- in plant tissues as was found in this study (Tables 5.4 and 5.6). It's also true, that salinity causes a reduction in plant growth (Table 5.5), and that the growth reduction is primarily due to the decreased osmotic potential, however, many nutritional and also membrane related studies indicate other possibilities (Reinhold *et al.* 1989; Lauchli & Epstein, 1990; Grattan & Grieve, 1992; Rengel, 1992). However, lowered osmotic potential may also influence cell wall hardening and eventually growth (Neumann, 1995). The effects of excess salinity on plant growth were presented and discussed in Chapters 1 and 2.

There are very few studies that investigated the interaction between magnesium content and salinity. The magnesium content was decreased in the shoots of both cultivars with increasing NaCl concentration (Tables 5.4 and 5.6); similar results were also found in other studies (Pushpam & Rangasmy, 2000; Ashraf & Sultana, 2000). Magdy *et al.*, (1998) found that NaCl reduced Mg^{+2}ATPase enzyme activity, which in turn inhibited the active transport of magnesium through the cell membrane. The accumulation of Mg^{+2} in plant shoots may be related to the unused magnesium in the chloroplasts, because of the inhibition of photosynthesis caused by salinity (Boyer 1970).

Calcium also decreased with an increase in salt concentrations in the shoots of both cultivars and in the roots of the salt sensitive cultivar (Drommedaris) (Tables 5.4 and 5.6). Similar results were found by Ajmal *et al.* (1999) and Cramer *et al.* (1991), who showed that calcium uptake by barley plants was significantly reduced by salinity, because the Ca^{2+} activity in the external

solution was reduced because the major saline cations (Na^+ and K^+) interfered with its uptake.

The most obvious salinity effects are osmotic stress and Na^+ toxicity (Marschner 1995). In addition, salinity is known to reduce Ca^{2+} activity in aqueous solutions (Reid & Smith 2000).

The enzyme systems of plants are sensitive to high NaCl concentrations and are inhibited at concentrations above 100 to 200 mM (Flowers *et al.*, 1977). In plants and fungi, the major ion pumps in the plasma membrane are P-type H^+-ATPase (proton pumps) (Morsomme & Boutry, 2000; Portillo, 2000) and they play a primary role in providing metabolic energy for ion transport at the plasma membrane of plant cells (Ayala *et al.*, 1997). These pumps function to energize the plasma membrane for nutrient uptake and signal transduction by generating an electrical potential and chemical gradient across the membrane. Many studies have found changes in pump activity in response to a variety of environmental conditions, including salt stress, hormones, light, and pathogens (Assmann, 1993; Mathieu *et al.*, 1994; Niu *et al.*, 1993; Portillo, 2000), and small changes in pump activity are thought to be important for many aspects of plant growth and development (Vitart *et al.*, 2001), Therefore, we can consider that the H^+-ATPase enzyme was one of the important factors responsible for the ion disorder that occurred in the plant tissues under salinity stress. Gong *et al.* (1999) found that salt stress-induced injuries of the plasma membrane, promoted the activities of membrane-protective enzymes, such as superoxide dismutase and peroxidase, and delayed the accumulation of malon-di-aldehyde. Mansour *et al.* (2000) found that NaCl treatment reduced the plasma membrane ATPase activity of the roots of salt resistant and salt sensitive wheat cultivars.

Significant increases in the sodium content of shoots and roots occurred under NaCl treatment, while the potassium content decreased. This was clear in the increases of the Na/K ratio in the shoots and roots of both wheat cultivars (Tables 5.4 and 5.6), these results agreed with other studies (Asch, *et al.* 2000; Vitart, *et al.* 2001; Wei, *et al.* 2003).

Significant reductions in the potassium content under salinity are reported in shoots and roots of the salt resistant and salt sensitive cultivars (Tables 5.4 and 5.6). Potassium is a major osmoticum in plant cells (Marschner 1995), and it may enter root cells by several routes. Both high (HKT1) and low affinity (LCT1) transporters, as well as several types of K^+-permeable channels, are present in the plasma membrane in the root epidermis (Maathuis & Amtmann 1999). Under saline conditions, each of these may be affected (Shabala *et al.*, 2003). Salt stress is known to significantly reduce the intracellular K^+ concentration, especially in the vacuolar pool (Cuin *et al.*, 2003). The K^+ reductions under salt treatment were also found in other studies (e.g. Drihem & Pilbeam 2002; Ayala *et al.*, 1997).

The Cl^- content, which significantly increased in the shoots and roots of the both cultivars with increases in salt concentrations, paralled the sodium content (Tables 5.4 and 5.6), and much of the discussion of the Na^+ behavior can also apply to the Cl^- ions. Leopold & Willing (1984) proposed that salt induced injury in membranes and subsequent leakage of cell contents could be a distinct effect of ion toxicity. That injury can have an effect on the membrane permeability and allow the toxic ions to move through and accumulate in the cell was reported by Lutts *et al.*, (1996c).

The treatment with GA_3 led to decreases in the magnesium content in shoots and roots of both cultivars. This reduction can be of benefit to the plants that are grown under salinity stress because the high concentrations of Mg^{2+}, can be harmful to the plant, not only because they are toxic to the plant tissue, but also because they can greatly reduce the absorption of Ca^{2+} and K^{+} (Hayward & Wardleigh, 1949). This is possible because the treated plants did not show magnesium deficiency and the magnesium that was reduced by GA_3 was probably from luxury consumption. Other evidence is that the chlorophyll content was not affected (Table 5.5).

In some cases the GA_3 treatments increased the calcium content, especially in the salt sensitive cultivar (Drommedaris). Ca^{2+} can play a role in the salinity tolerance mechanism (Ding & Zhu 1997). Elphick *et al.*, (2001) reported that root growth was restored by Ca^{2+} treatment under salt stress, and the beneficial effects of Ca^{2+} on root growth are reported by others (Cramer *et al.* 1989; Rengel 1992; Colmer *et al.* 1996).

Under saline conditions Na^{+} is the principle toxic ion (Fangqing & Zhangcheng, 1999). GA_3 markedly reduced Na concentration in shoots and roots of Flameks and Drommedaris plants, particularly under extreme NaCl effects (Table 5.4 and 5.6).

The Na/K ratio was also significantly reduced by GA_3 treatments in the shoots of both cultivars and in the roots of Drommedaris (Tables 5.4 and 5.6). In wheat, the degree of salt tolerance correlated with low Na/K ratios in the shoots (Wyn Jones *et al.* 1984; Schachtman *et al.* 1989; Gorham 1990) indicating that exclusion of Na^{+} from the shoots was a distinctive salt-tolerant trait (Ayala *et al.* 1997). According to this, GA_3 could enhance salt tolerance in wheat plants. The decreased cytokinin and gibberellic acid and increased

abscisic acid contents observed in salt stressed plants (Boucard & Unger, 1976) has led to the suggestion that salt stress-induced changes in membrane permeability and water relations are related to changes in hormone balance (Shonjani 2002). The subsequent growth reduction could be attributed to altered endogenous hormonal levels, as hormonal regulation is involved in membrane permeability and water relations (Ilan, 1971). Exogenously applied gibberellins probably compensate for a natural or environmentally induced deficiency (Wareing, 1982).

The chlorophyll content didn't show significant changes with NaCl treatment or with GA_3 application (Table 5.5). Lack of effect of GA_3 treatment under salt stress was also found by Boucaud & Ungar (1976). Salinity causes decreases in chlorophyll content in some plants (Ashraf *et al.*, 2002; Munjal & Goswami 1995). On the other hand chlorophyll can be increased in some plants by salt stress (Radi *et al.*, 1989; Adam, 1996) or there may be no effect (Ouerghi, *et al.* 2000). So the behavior of chlorophyll content under salt stress is still unclear, and this could be because chlorophyll content may be correlated with water content (Varshney & Bijal, 1979; Parakash & Prathapasenan, 1990), or because the plants grow well under a good nutrient supply. Particularly magnesium seems to have luxury consumption; although the NaCl treatment caused a significant decrease in Mg^{2+} content (Table 5.4) compared to the untreated plants, but the reduction didn't reach deficiency levels.

Fresh and dry mass of the shoots and dry mass of roots were markedly reduced under salt stress in both wheat cultivars (Table 5.5). These results are in agreement with previous studies on barley plants (Adam. 1996). This reduction in growth may result from salt effects on biomass allocation, ion relations, water status, physiological processes, biochemical reactions, or a

combination of such factors (Shonjani 2002). Kumar & Singh (1996) reported a decrease in wheat growth and grain yield due to salt stress, but GA_3 treatment of seeds increased both the growth and grain yield under salinity conditions, and this agrees with the results in Table 5.5 where the GA_3 treatment increased the fresh mass and dry mass of Flameks plants under moderate salinity (200 mM). This is in contrast to the Drommedaris plants where GA_3 caused a significant decreases in the shoot dry mass. Ashraf *et al.* (2002) suggested that increases in dry matter production and plant height, by application of GA_3 to wheat plants were due to the increases in photosynthetic activity, and they found that GA_3 treatment caused an increase in water use efficiency and intrinsic water use. Similar results of photosynthesis and its relationship with growth and dry matter production were found by Ashraf & O'Leary. (1996). Shonjani (2002) found that increase of salt in the growth media caused water potential decreases, the pressure potential of plant cells declined, and cells ultimately ceased to grow. And under these water stress conditions, in general, stomata close, resulting in the reduction of photosynthesis. Protein breakdown is enhanced and plants show poor growth. Rivelli *et al.*, (2002) found a reduction in the biomass of four wheat genotypes under salt stress and a reduction in stomatal conductance was found in the four wheat genotypes, which resulted in decreases in the photosynthetic rate.

Endogenous auxin and gibberellin have been shown to decline under water stress in some plants (Aharoni *et al.*, 1977; Guinn & Brummet, 1988). So it is possible that under saline conditions there is a decrease in the production of plant hormones and exogenous application of phytohormones could ameliorate the inhibitory affect of NaCl on plant growth. GA_3 may promote growth by accelerating the transcription of some mRNAs, since the receptors located on the plasma membranes may send signals in the form of DNA-

binding proteins (Alhadi *et al.* 1999). Accumulation of metabolites (proline, polyols, quaternary amines, sugars, ions, polyamines, etc.) and osmotic adjustment under water stress, may be related to regulatory functions of the phytohormones (Drolet *et al.*, 1986; Smirnoff & Cumbes, 1989; Mckersie & Leshem, 1994).

The water content decreased under salinity stress in the shoots of both cultivars (Table 5.5). The decrease in water content was a result of increases in salinity level and water deficit (Pearce, 2003; Ekanayake *et al.*, 1993) and the reduction occurred because the water potential in plant tissue is usually less negative than in the soil solution under salinity conditions. The reduction in water content in plant tissue leads to increases in water stress and water deficits limit the growth and distribution of natural vegetation. The salinity affects the performance of cultivated plants more than any other environmental factors (Kramer, 1983). Closure of stomata with decrease of water content in plant leaves disturbs the supply of CO_2 for photosynthesis (Tanaka *et al.*, 1990), and decreases the transpiration rate (Adam, 1996), which causes a reduction in water uptake. The closure of stomata may be the result of the increase in abscisic acid (Wright & Hiron, 1969; Zee Vart, 1971). Decreases in water content under salinity stress were also found in other studies (Gadallah, 1999; Zhang, 1997).

The proline content markedly increases under salinity stress in shoots and roots of the wheat cultivars (Table 5.5 and 5.6). It is well known that proline accumulates in stressed plant tissue because of drought stress, salt stress or even nutrient deficiency (Stewart *et al.*, 1996). Singh *et al.* (1972) showed that drought resistant barley cultivars accumulated many fold higher free proline than susceptible cultivars, and as it is 300 times more soluble in water than any other amino acid, it can act as a non-toxic osmolyte. Proline is one of

the important components of the defense reactions of plants to salinity (Sakhabutdinova *et al.*, 2003). Colmer *et al.*, (1995) found that proline levels were highest in the oldest leaf and progressively lower in youngest ones. The role of proline in the adaptation of non-halophytes to salinity is even less clear than that of other organic solutes (Greenway and Munns, 1980; Rabe, 1990). Tables 5.4 and 5.5 showed a strong correlation between Na^+ and proline content (shoot Na proline r =0.96, P<0.001. Roots Na proline r = 0.86, P<0.001) Table 5.6 showed the same in roots of both wheat cultivars. When the Na^+ decreased the proline also decreased. This agrees with the finding of Colmer *et al.* (1995). The reduction in the proline content as a result of GA_3 treatment seems to be related to the reduction in the Na^+ content that was caused by hormone treatment (Table 5.4-5.6). Pearson (1974) suggested that proline levels could be used as an indicator of the degree of water stress. So in this study, the reduction of proline content by GA_3 treatment, under salinity stress, could have occurred as a response to the role of the hormone in alleviation of salt effects (decreases of toxic ions).

Therefore, GA_3 enhanced the salt tolerance of the both wheat cultivars in several cases (decreased Mg^{2+}, Na^+, Cl^-, Na/K ratio, and increased Ca^{2+}, K^+, and also fresh and dry mass in some cases) (Tables 5.4-5.6).

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Summary

There is global alarm due to two abiotic stresses (drought and salinity); salinity is the topic of this study. The importance of salt-affected soils can be explained by their wide distribution on all continents, mostly in arid and semi-arid regions. To overcome the shortage in food supply as the result of land deterioration and the progressively increasing population, the utilization of saline soils and low quality water resources for agriculture is becoming more essential. However, the salinity of those saline soils and water sources typically exceeds the limit tolerated by conventional crop plants. One approach to this problem is to increase the salt tolerance of crop plants.

It is clear that the demands for the use of salinized land for agricultural purposes and the reinstatement of these lands for agricultural use, in the present time and in the future, requires a better understanding of the nature of salt resistance and salt sensitivity during seed germination and early seedling growth.

It is a fact that much work done in the last century, in many countries, has increased our understanding of the genetics and physiology of salt tolerance of plants. Plants responses to salt stress are made up of a number of complex and interrelated morphological, physiological and

biochemical processes. However, the underlying mechanisms of salt tolerance are still not completely understood. Many genetics studies, using traditional and modern methods of plant breeding, have led to the development and release of a number cultivars with improved salt tolerance.

Many factors have been found to be involved in salt tolerance mechanisms, such as morphological compartmentation, compatible solute production, regulation of transpiration, control of ion movement, membrane characteristics, toleration of high Na/K ratios in the cytoplasm and genetic traits.

Salinity affects plant growth from the early germination stage until late in seed production. Thirty-eight wheat cultivars were investigated in terms of salinity resistance at the germination and early seedling stages and the different cultivars were arranged in order of their salinity resistance. Considerable intervarietal differences between wheat cultivars were reported. The results of this study showed that increases in salinity levels up to 100 mM NaCl in the wheat germination media caused significant reduction in: seed germination, root length, shoot length, fresh weigh, dry weigh and accumulation of the toxic ions (Na^+, Cl^-), increased Na/K ratio and reduction in the Mg^{2+}, K^+, Ca^{2+} content were found. No pronounced changes in chlorophyll and water content were found.

The treatment of some wheat cultivars with plant hormones, particularly gibberellic acid (GA_3) could alleviate some damaging effects of high salt levels on seedlings and pre-reproductive growth and development of wheat plants. The role of GA_3 in alleviating the salt damage was reported in several spheres; decreases in Na^+, Cl^- and Mg^{2+} content and Na/K ratio and increases in fresh mass and Ca^{2+} contents. The reduction in proline content reported may be an indicator of a reduction in the stress, which is caused by high salt treatments. Our results with benzyl adenine were not promising.

A clarifying part of this study showed that the effects of GA_3 on wheat cultivars under saline conditions, were due to hormonal effects and not to the reduction of the pH of the germination media. This study recommends the use of GA_3 pretreatments of wheat seeds that will be sown and grown under high salt levels such as when plants are grown in brackish soil and those watered with water containing a high salt level. Further studies of the use of gibberellic acid to improve salt tolerance and involving a range of species are needed.

The study concluded that the salinity tolerance in wheat plants can be dramatically enhanced. Cultivar selection and/or the treatment with some biological active chemicals such as plant hormones, especially GA_3, can be in use to alleviate, salt effects and increase salt tolerance.

Appendix 1

Tables Captions

Table.1. Table of ANOVA (wheat cultivars, salinity and the interaction) for the various parameters measured on thirty eight wheat cultivars.

		Root Length		Col. Length		%Germination		Root Mass	
Source	d.f	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Trial	**5**	33.99	0.0001	6.884	0.0001	758.5	0.0001	0.0007	0.0001
Error (a)	**6**	0.33	0.8269	0.16	0.3785	93.41	0.0982	0	0.977
Cultivar	**39**	2.53	0.0001	1.2	0.0001	384.2	0.0001	0.0001	0.0001
Salinity	**4**	2722	0.0001	685.1	0.0001	27094	0.0001	0.0436	0.0001
Cul*Sal	**156**	2.47	0.0001	0.806	0.0001	132	0.0001	0.0001	0.0001
Error(b)	**239**	0.7		0.149		51.66		0.00002	

Table.2. Effects of three citric acid treatments on root length, shoot length (cm), root mass, shoot mass (g) and germination (%) of two wheat cultivars under three NaCl (mM) treatments.

Cultivar		Losper			Knoppies		
	NaCl / Citric	0	200	400	0	200	400
Root length	0	7.24	5.17 -*	0.66 -*	9.34	5.11 -*	0.51 -*
	40	8.40 n	4.19 n	0.67 n	9.84 n	4.15 n	0.52 n
	125	8.24 n	3.53 -*	0.78 n	7.20 -*	3.42 -*	0.20 n
Shoot length	0	3.00	0.86 -*	0.23 -*	4.24	1.19 -*	0.15 -*
	40	3.66 n	0.60 n	0.19 n	4.42 n	0.81 n	0.13 n
	125	3.75 n	0.54 n	0.21 n	3.62 n	0.64 n	0.30 n
Root mass	0	0.0366	0.0294 n	0.0039 -*	0.0390	0.0222 -*	0.0018 -*
	40	0.0479 n	0.0248 n	0.0028 n	0.0441 n	0.0193 n	0.0015 n
	125	0.0459 n	0.0157 -*	0.0045 n	0.0316 -*	0.0125 -*	0.0008 n
Shoot mass	0	0.0354	0.0100 -*	0.0018 -*	0.0440	0.0102 -*	0.0009 -*
	40	0.0412 n	0.0081 n	0.0021 n	0.0418 n	0.0072 n	0.0008 n
	125	0.0372 n	0.0111 n	0.0020 n	0.0361 -*	0.0069 n	0.0008 n
Germination %	0	95%	95% n	75% n	100%	100% n	90% n
	40	85% n	85% n	80% n	100% n	95% n	80% n
	125	40% -*	55% n	40% n	70% -*	70% -*	20% -*

n = Non Significant
+* = Positive Effects
-* = Negative Efficts

Table.3. Effects of three malic acid treatments on root length, shoot length (cm), root mass, shoot mass (g) and germination (%) of two wheat cultivars under three NaCl (mM) treatments.

Cultivar		Losper			Knoppies		
	NaCl / Malic	0	200	400	0	200	400
Root length	0	7.236	5.172 n	0.664 -*	9.335	5.105 -*	0.509 -*
	40	9.903 +*	4.997 n	0.777 n	9.516 n	4.014 n	0.493 n
	125	10.360 +*	4.317 n	0.693 n	6.631 -*	2.711 -*	0.500 n
Shoot length	0	3.003	0.859 -*	0.233 -*	4.235	1.185 -*	0.150 -*
	40	3.509 +*	0.955 n	0.169 n	4.527 n	0.878 n	0.129 n
	125	3.953 +*	0.800 n	0.229 n	3.594 n	0.800 n	0.150 n
Root mass	0	0.0366	0.0294 n	0.0039 -*	0.0390	0.0222 -*	0.0018 -*
	40	0.0623 +*	0.0304 n	0.0032 n	0.0476 n	0.0191 n	0.0014 n
	125	0.0761 +*	0.0227 n	0.0040 n	0.0406 n	0.0132 n	0.0019 n
Shoot mass	0	0.0354	0.0100 -*	0.0018 -*	0.0442	0.0102 -*	0.0009 -*
	40	0.0426 n	0.0110 n	0.0016 n	0.0448 n	0.0081 n	0.0013 n
	125	0.0584 +*	0.0088 n	0.0019 n	0.0385 n	0.0081 n	0.0006 n
Germination %	0	95%	95% n	75% n	100%	100% n	90% n
	40	70% n	85% n	70% n	95% n	95% n	70% n
	125	40% -*	30% -*	40% n	60% -*	45% -*	30% -*

n = Non Significant
+* = Positive Effects
-* = Negative Efficts

Appendix 2

Figures Captions

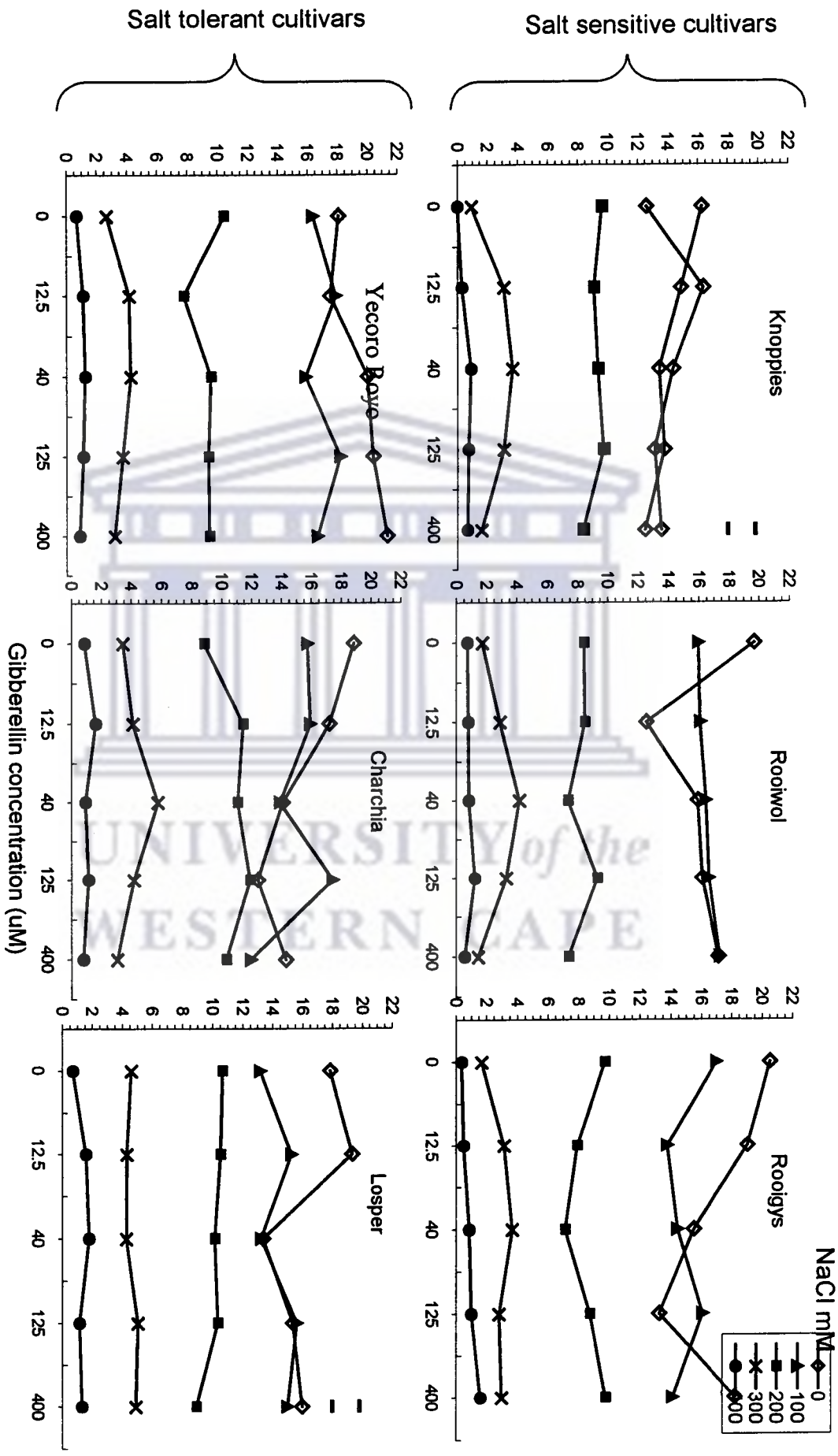

Fig.1. Effects of gibberellin and salinity (mM) on the root length (cm) of six wheat cultivars.

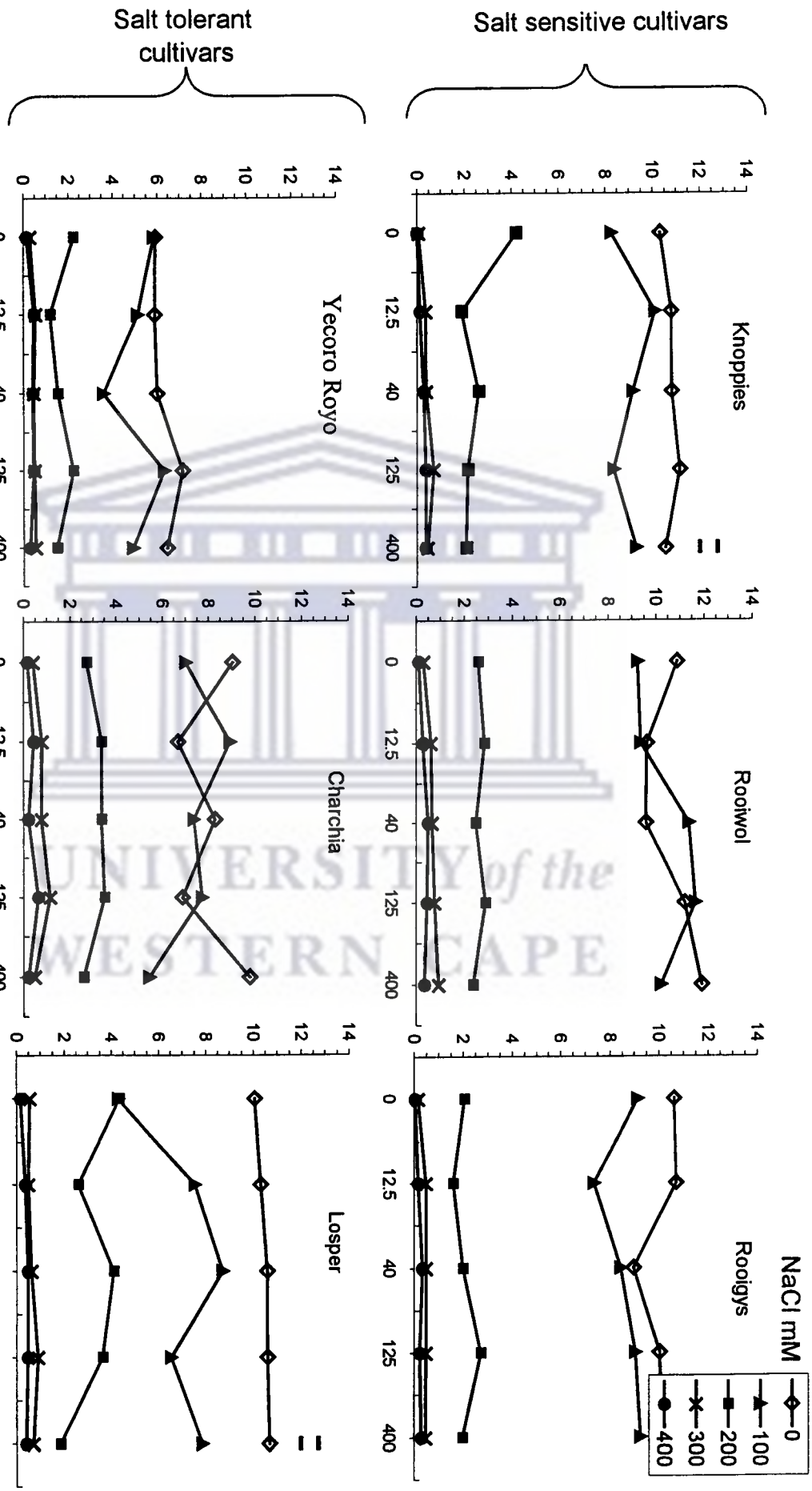

Fig.2. Effects of gibberellin and salinity (mM) on the shoot length (cm) of six wheat cultivars.

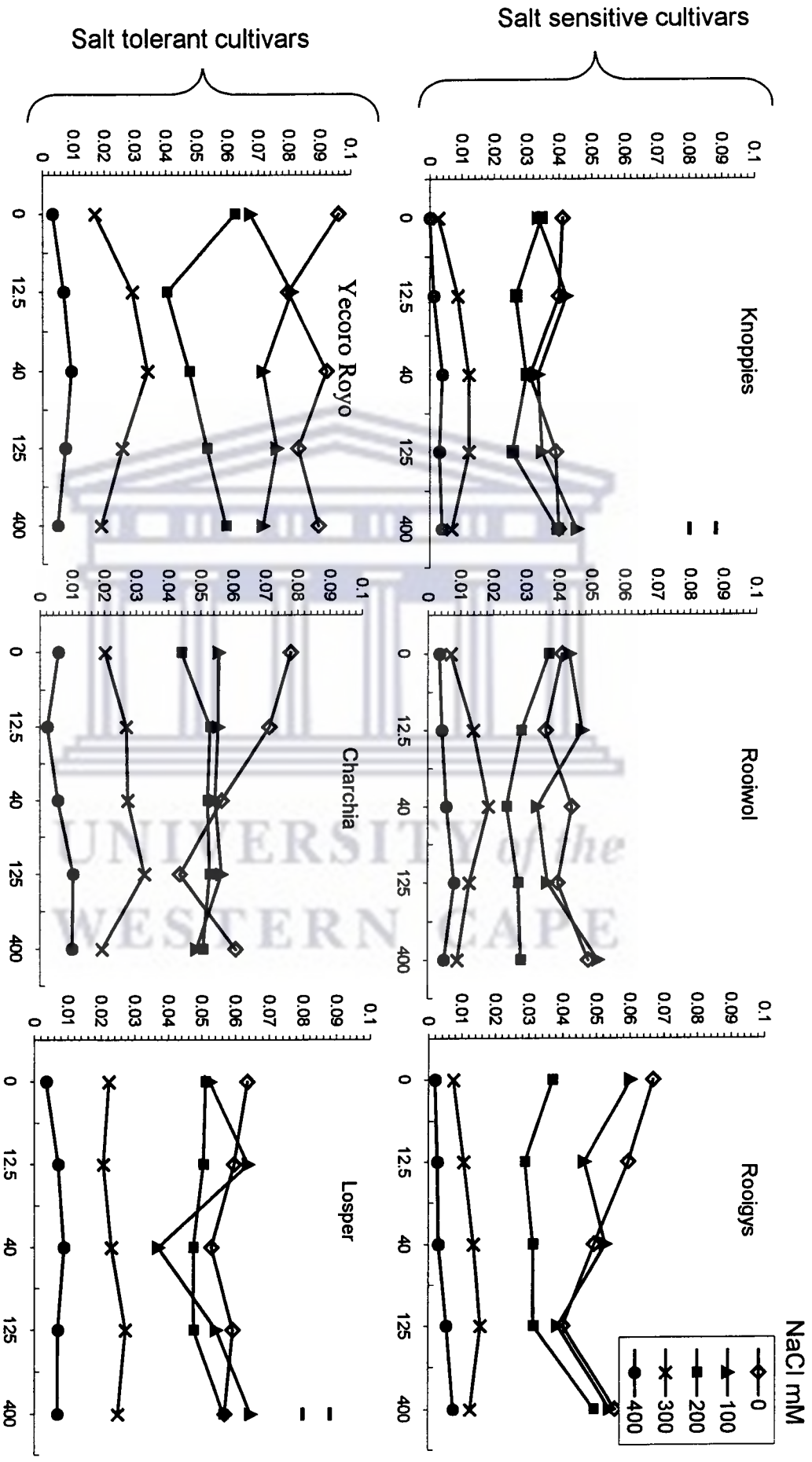

Fig.3. Effects of gibberellin and salinity (mM) on the root mass (g) of six wheat cultivars.

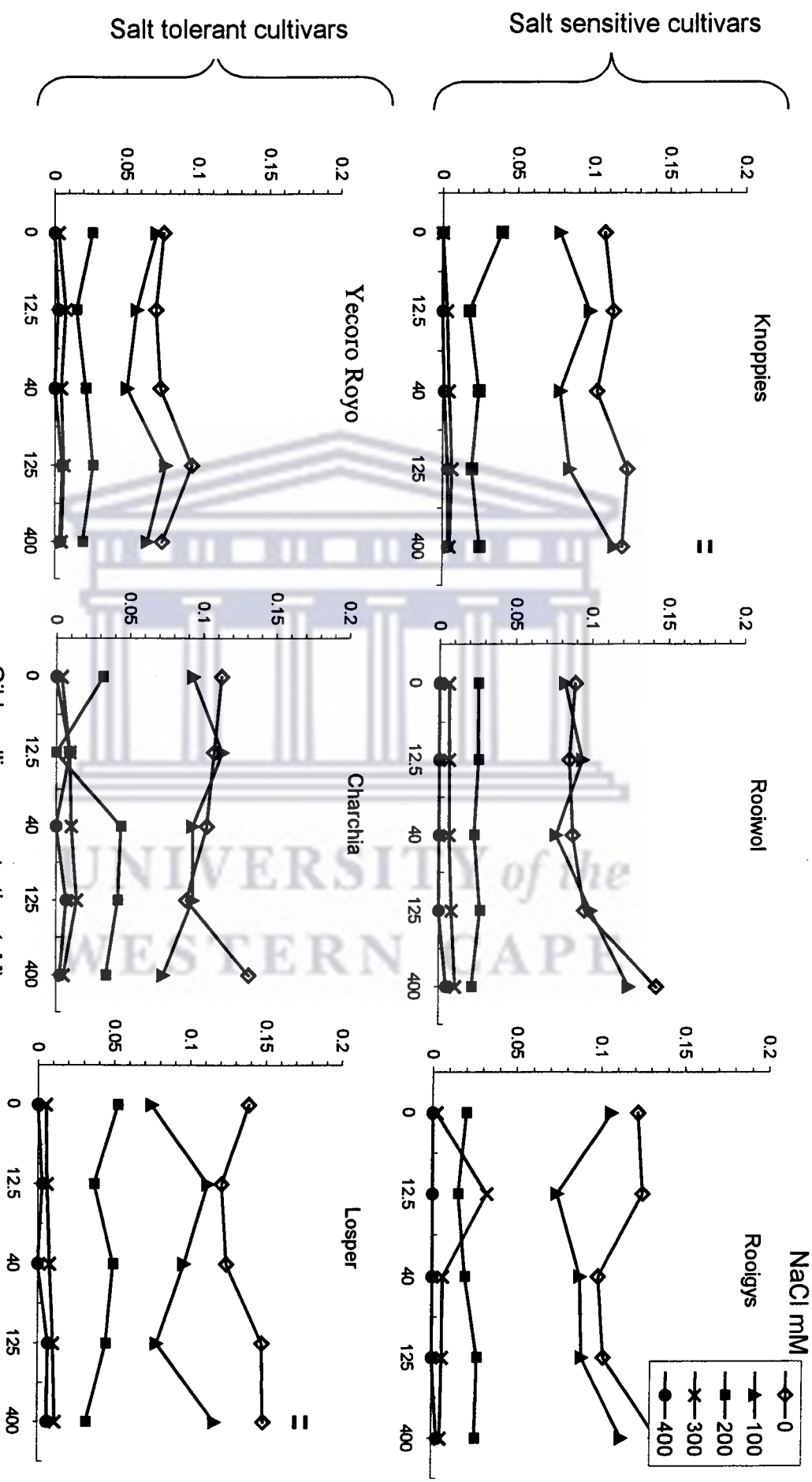

Fig.4. Effects of gibberellin and salinity (mM) on the shoot mass (g) of six wheat cultivars.

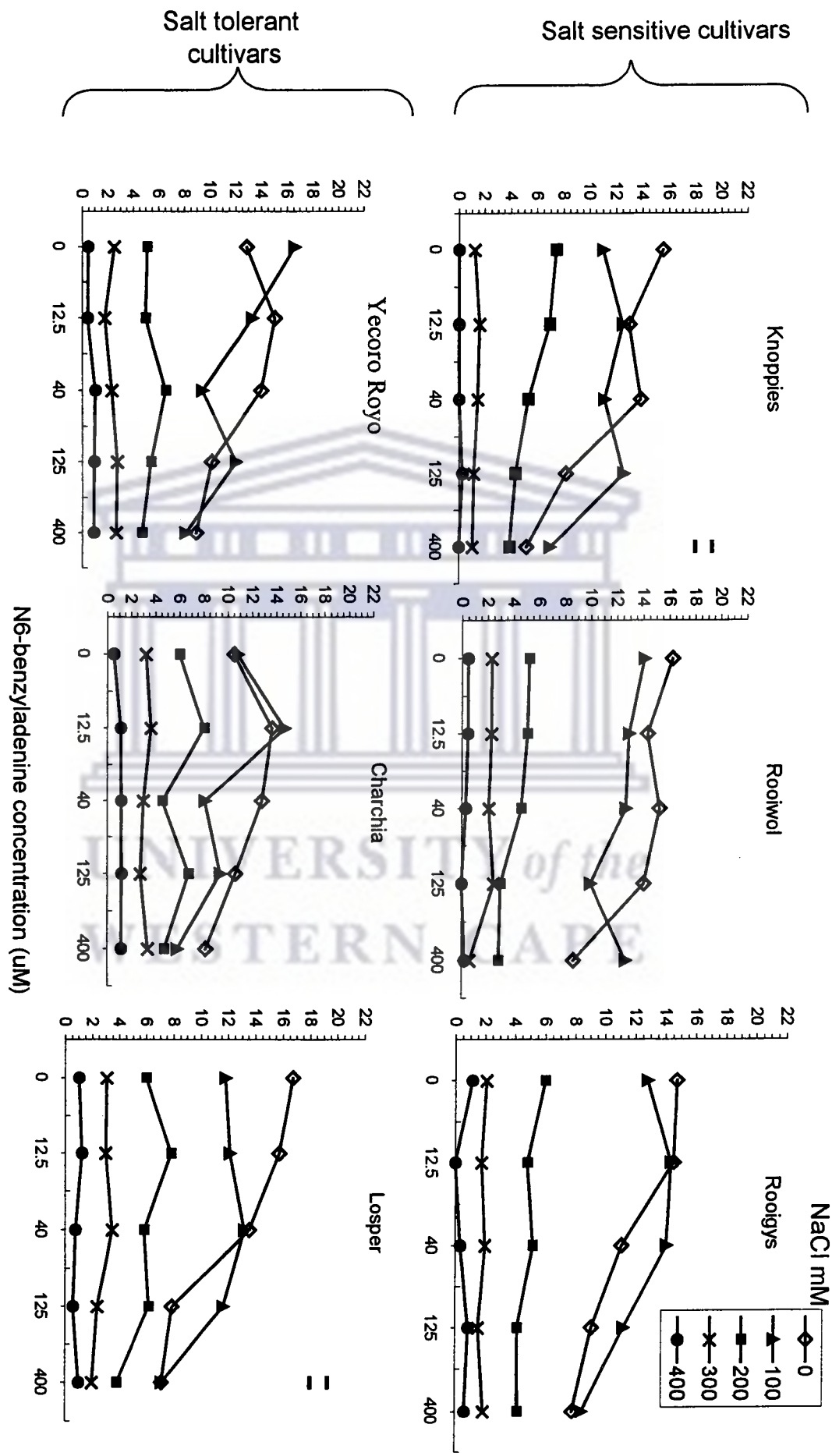

Fig.5. Effects of N6-benzyladenine and salinity (mM) on the root length (cm) of six wheat cultivars.

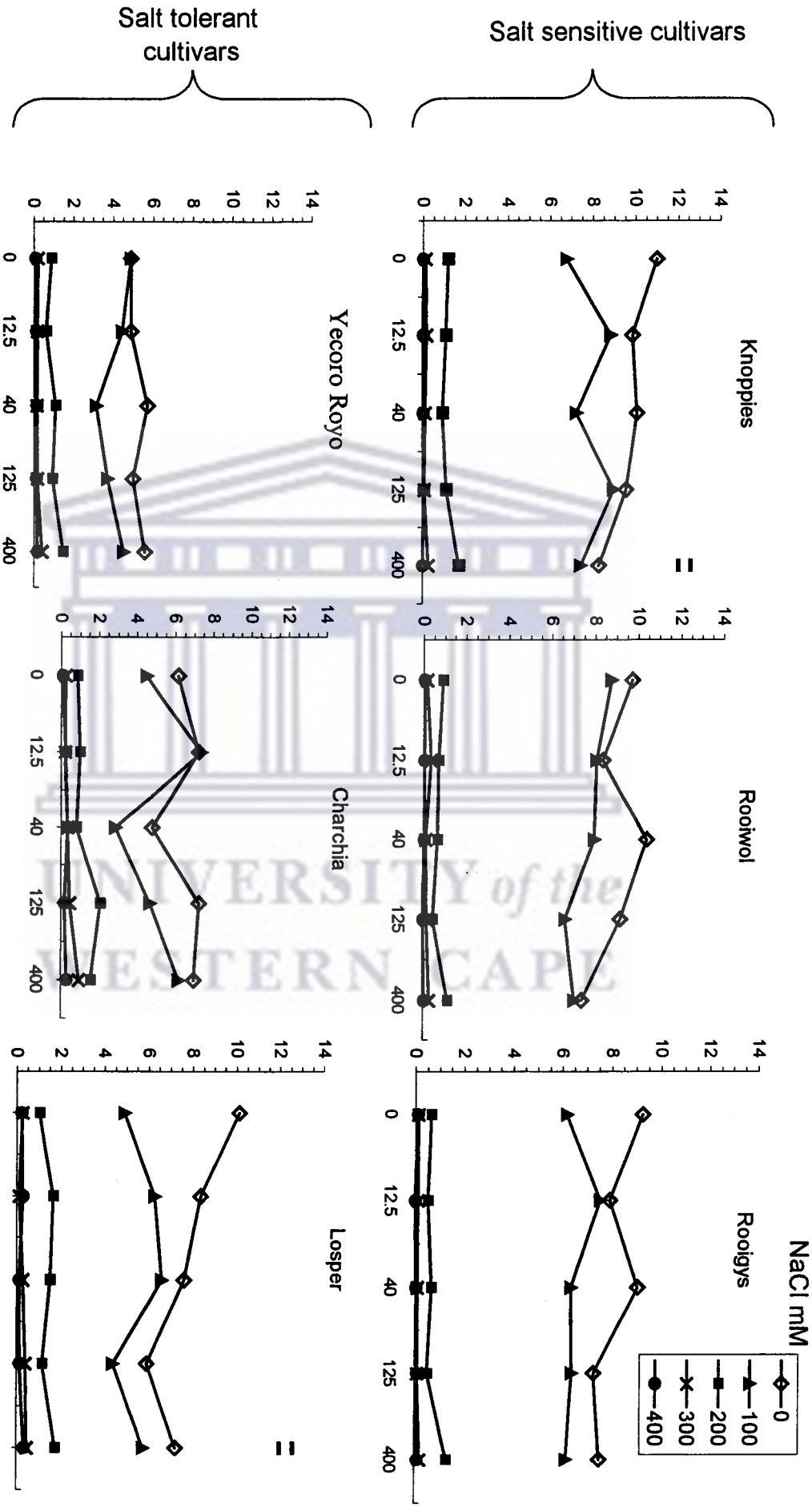

Fig.6. Effects of N6-benzyladenine and salinity (mM) on the shoot length (cm) of six wheat cultivars

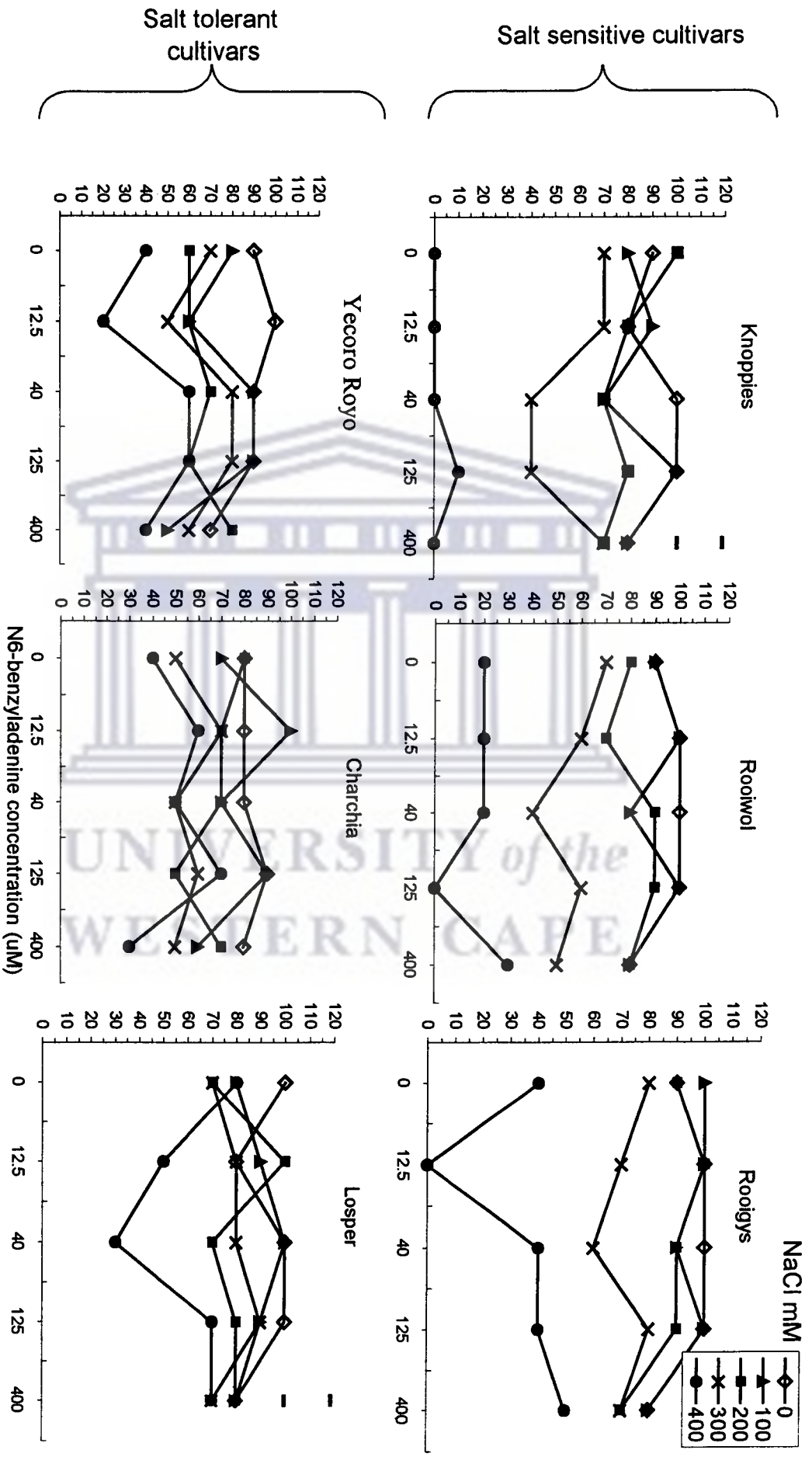

Fig.7. Effects of N6-benzyladenine and salinity (mM) on the germination (%) of six wheat cultivars.

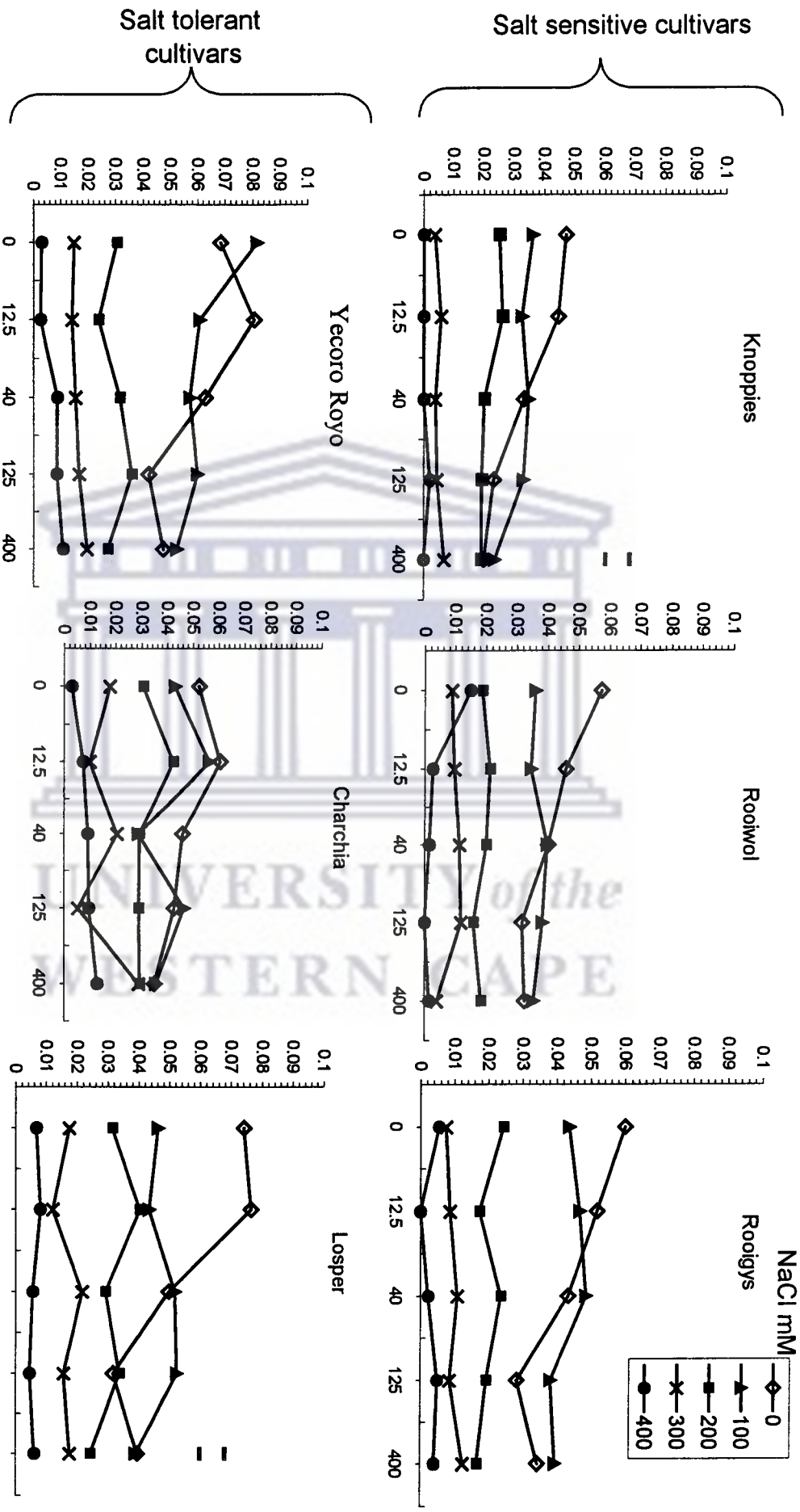

Fig.8. Effects of N6-benzyladenine and salinity (mM) on the root mass (g) of six wheat cultivars.

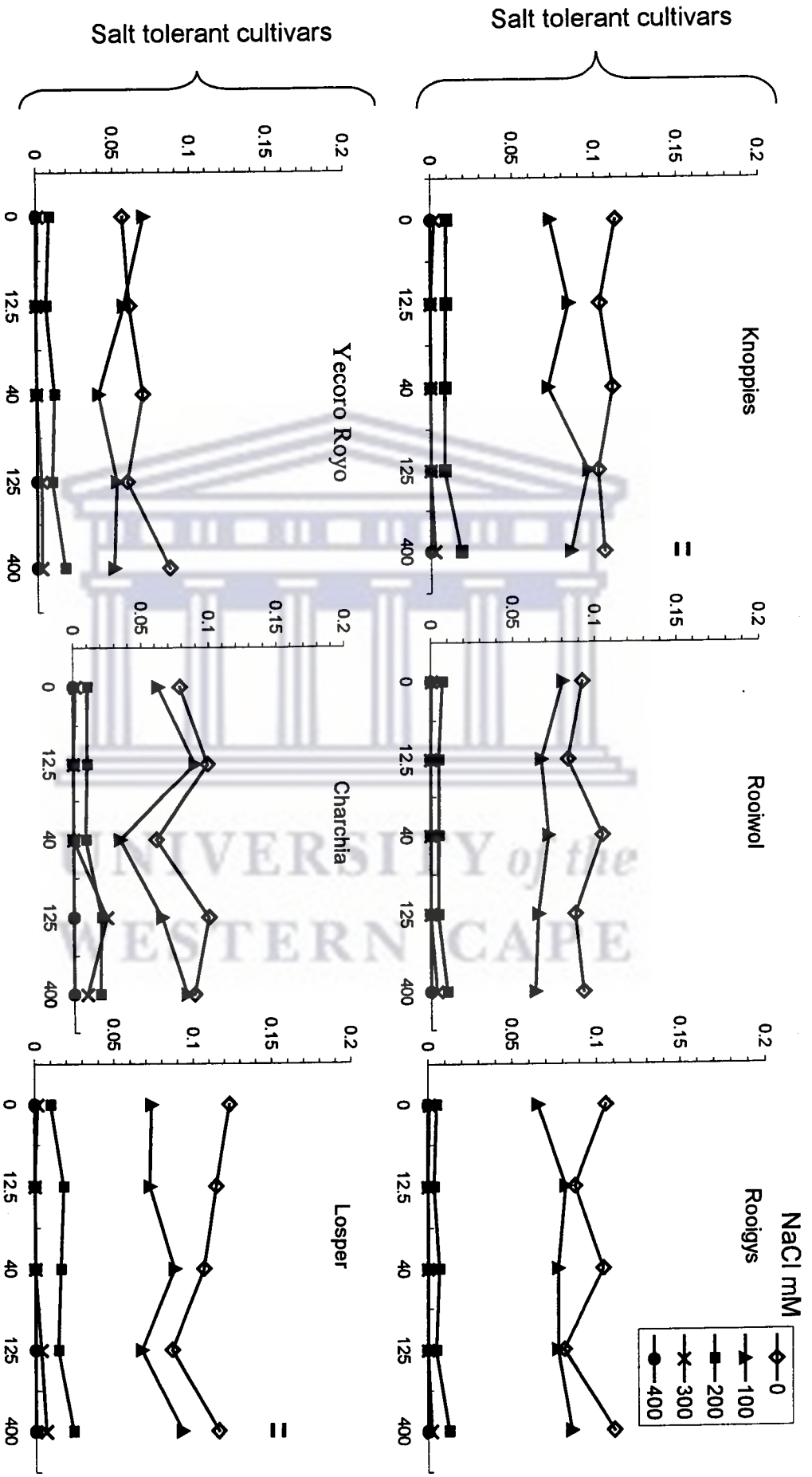

Fig.9. Effects of N6-benzyladenine and salinity (mM) on the shoot mass (g) of six wheat cultivars.

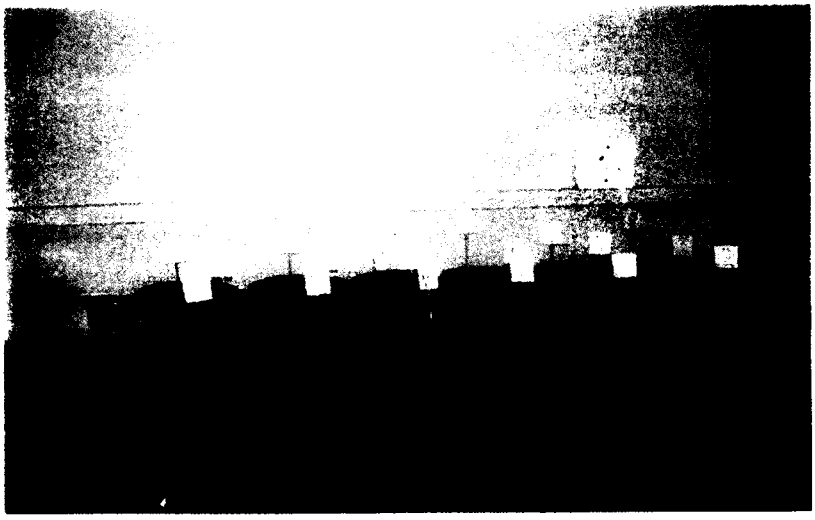

Fig. 10. Preparing pots and sand

Fig. 11. First day in the growth chamber

Fig. 12. Plants just before harvesting

Fig. 13 Control and 400 mM NaCl treated Flamek-
plants just before harvesting

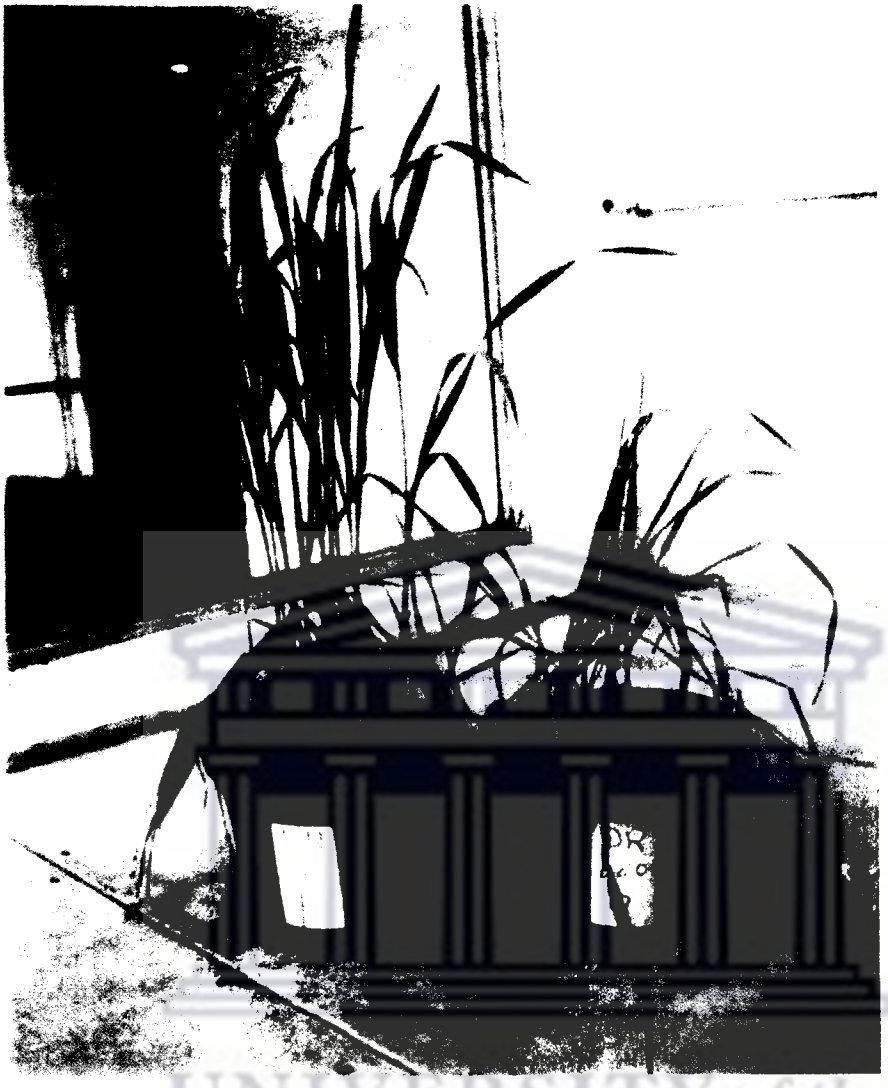

Fig 14 Control and 400 mM NaCl treated Drommedia[illegible] plants just before harvesting