The effect of exogenous growth regulators on salinity tolerance in *Erucastrum strigosum*.

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The effect of exogenous growth regulators on salinity tolerance in *Erucastrum strigosum.*

Nomagugu Gxaba

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A thesis submitted in partial fulfillment of the requirements for the degree of Magister Scientiae in the Department of Biodiversity and Conservation Biology, Faculty of Natural Science, University of the Western Cape.

Supervisors: Prof. L. M. Raitt
Mr. J. Aalbers

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Abstract:

Randomized block experiments were conducted to examine the putative amelioratory effects of kinetin or gibberellic acid at concentrations (0, 4, 12.5, 40, and 125 μM) in *Erucastrum strigosum* plants subjected to a salinity series (0, 100, 200, 300, and 400 mM NaCl) in the greenhouse. When the highest salinity concentration (increased stepwise) was reached, growth effects in relation to water and cation content of the plants were evaluated. Growth and water content were reduced progressively with salinity treatments. Na^+ concentration accumulated with salinity treatments to levels that were much higher than that of other cations (K^+, Ca^{2+} and Mg^{2+}) in both organs. However, it is noteworthy that Na^+ distribution was more in shoots than in roots. In kinetin treated plants, shoot growth decreased whilst root growth increased with moderate hormonal treatments. In gibberellin treated plants, shoot growth was increased and root growth remained unaffected. In plants, treated simultaneously with kinetin and salinity, inhibitory effects due to disrupted water and ionic balance persisted. Gibberellic acid, on the other hand, enhanced growth by improving water stress but the ionic stress effects persisted. These results suggest that treatments with growth regulators improve salt-induced growth inhibition by partially reducing water stress, one of the tolerance responses.

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Declaration:

"I declare that **the effect of growth regulators on salinity tolerance in *Erucastrum strigosum*** is my own work and it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references."

Nomagugu Gxaba Date:

Signed .....

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Chapter 1

Salinity, plants and plant hormones.

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1.1 Introduction

Salinity is the occurrence of salt in the land surface, soil, rocks or water. Three types of soil salinity have been identified. Firstly, soil salinity occurs when water-soluble sodium salts, notably chlorides, sulphates and carbonates accumulate in the soil (Blaylock, 1994 and Queensland Government, 2002). This type of salinity is measured in decisiemens per meter (dS/m), achieved by passing an electrical current through a soil solution made from a soil sample. This measurement is referred to as electric conductivity (Kotuby-Amacher *et al.*, 1997). Salinity is common in arid and semi arid regions of the world. In some areas, soil salt-effects are attributed to high exchangeable sodium known as sodic soils. Sodic soils are measured as Sodium Adsorption Ratio (SAR). This is a unitless ratio of a portion of cationic (positive) charges deposited to a soil sample by sodium in relation to that contributed by calcium and magnesium. In some areas, soil salt-effects may arise from the combination of excess salts and exchangeable sodium and they are referred to as saline sodic soils (Cardon & Mortvedt, 1994).

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The main focus of this study is on soil salinity induced by water-soluble salts. The development of saline soils may be related to the rising of watertables (or ground water). Groundwater is the water in the soil and underlying strata (DWAF, 2001). Salinity from rising watertables can be grouped into dryland, irrigation and urban salinity. Dryland salinity occurs in non-irrigated areas, usually as result of a rising watertable. The rising of watertables in turn may result from particular land use practices such as overclearing, urban development, river regulation, irrigation or cultivation of crops. Irrigation salinity originates from over irrigation, inefficient water use and poor drainage. Urban salinity

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results from a combination of dryland salinity processes and overwatering in towns and urban areas (DLWC, 2000). Also contributing to urban salinity is surface mobilization of pollutants from mining and industrial operations (DEAT, 1999). In some areas, salinity is a natural phenomenon. Natural causes of salinity include soil formation and water movement (for example inland salt pans, brackish streams, coastal salt marshes and naturally saline soils) (Blaylock, 1994). Regardless of where salinity comes from, soluble materials released to the soil will increase salinity.

Salinity is a worldwide problem, and each year about 10 million hectares are lost to salinity. Salinity affects both aquatic and terrestrial biodiversity. For example, in the western part of Western Australia, 450 species of plants, insect and bird life are threatened by salinity. However, the impacts of salinity on a landscape are separated both in time and space from its causes. This means that in Australia salinity problems are expected to continue increasing due to past and present activities (Australian Academy of Science, 1998). For example, the National Land and Water Resource Audit (2000) estimated that 5.4 million hectares are developing dryland salinity with damages totaling to Australia of $270 million a year and it has been predicted that this will rise to 17 million hectares by 2050. In South Africa, a hydrologist reported that the rise of contaminated groundwater lakes would be the cause of salinity in the next 10 to 100 years. In support of this view, the head of research at the Water Research Commission, Meiring du Plessis, was quoted as saying, "Groundwater lakes will decant at some point and will have a very high salinity, low pH and will also contain high concentrations of dissolved metals such as iron and manganese" (Paton, 2002). However, for the time

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being, salinity in South Africa is not of major concern, as it falls within the accepted limits (Noor-Jehan, 2002). On a worldwide scale, it is estimated that the 60 million hectors of irrigated land affected by salinity will double by 2050 (Zhang & Blumwald, 2001). This increase of salinity creates major challenges for governments, industries and communities to develop management approaches that are aimed at protecting environments and human assets that are at risk, address the problems of rising water tables and make productive use of saline resources. In Australia, a conference attended by local and international delegates was called to address salinity which is probably the biggest future threat to food production, salinity (Trench, 2001).

During the conference it emerged that salinity can be managed through irrigation, whereby salts that have accumulated in the soil surface are leached down to the subsoil layers (Kotuby-Amacher *et al.*, 1997). Accompanying this approach is the construction of evaporation basins, dams and reservoirs. For example, the Breede River in South Africa has been proclaimed a drainage basin for water from irrigated areas (Kirchner *et al.*, 1997). In Australia, a Darling-Murray drainage basin was constructed. Through these reservoirs salt inputs are drawn out of the irrigation water and reused for industrial purposes, before water is returned to the rivers. However, it has been pointed out that recycling of water requires a great deal of careful management, in order to avoid further increase of salt concentrations (Mannion, 1991). Furthermore, salt management, even though it seems to have high reclamative potential, in practice is limited by economical and environmental constraints, attached to it.

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An alternative to the above approach, is the selection and domestication of wild varieties of plants that can grow and survive under high saline environmental conditions. The interest in this approach is two fold (O' Leary, 2001). One possible way is to extract salt tolerant genes that could be conventionally bred into crop plants (Hale and Orcutt, 1987) e.g. barley: salt-tolerant strains, grown under salt stress conditions, have been isolated and propagated for generations (Epstein *et al.*, 1980). However, conventional breeding of salt tolerance into crop plants has been met with little success (Hale and Orcutt, 1987). Furthermore, it has been established that crop plants lack fundamental biological compatibility with saline conditions (Epstein, 1976). Secondly, one can choose plants that selectively adsorb environmental contaminants such as sodium (halophytes). There are two options as to what can be done with these plants, incinerate and disposed of the ashes or harvest salts for industrial purposes from them (Hinchman *et al.*, 1998). On the other hand, these halophytic plants can be screened for productive uses (Glenn *et al.*, 1998). These uses include fodder (*Atriplex numularia*), food (*Distichlis* sp.), oilseed (*Salirconia bigelovii*), soil protection from erosion (*Spartina alterniflora*), wastewater purification and biodiversity enhancement (Reeve, 2003). Most well-known crop plants that we utilize today, started out as wild plants, e.g. Zea *mays* and *Triticum aestivum*, and were domesticated for human consumption (Raven *et al.*, 1992). However, whether the world will ultimately turn to this alternative approach, depends largely on future food needs, economics and the extent to which freshwater ecosystems are withheld from further agricultural developments. This plant domestication approach has encouraged the constitution of dedicated networks to come together and coordinate research (Reeve, 2003).

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1.2 Plant responses to salinity

The response of sessile plants to environments that are frequently challenged by abiotic factors such as salt stress, have long been of interest to plant physiologists. Bernstein (1975), found that when he compared salt affected plants with normal ones; leaf burn, chlorosis, leaf bronzing and necrotic lesions were the manifestations of acute damage by salt stress on leaves. However, the extent of these effects in plants is governed by the sensitivity or (tolerance) of a species, cultivar and root stock, the concentration of salinity in the growth medium, duration of exposure, plant species, stage of development, plant organ, and environmental conditions (Marschner, 1995 and Ashraf *et al.*, 2001). For example, sugar beet, known to be highly tolerant to salt stress during most of its life cycle, is sensitive during germination (Marschner, 1995). In contrast, the salt sensitivity of rice (Lutts, *et al.*, 1995), tomato, wheat and barley (Marschner, 1995) is observed at the seedling stage. In maize, salt sensitivity is higher at the tasseling stage than at the grain filling stage (Maas *et al.*, 1983). However, it has been observed that even though, some cultivars in a given species (such as barley) may demonstrate sensitivity to salt stress at seedling stage and tolerance at maturity, some cultivars may show an opposite pattern (Marschner, 1995). Long-term exposure of plants to salinity may be associated with ion toxicity, water deficit and shortage of carbohydrates in younger leaves. However, not all plants growing under saline conditions deteriorate, there are plants that flourish.

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1.2.1 The effects of salinity on plant water content

The accumulation of salts in the rhizosphere, generates a negative osmotic potential that lowers the soil water potential (Salisbury and Ross, 1992). This modifies the overall water balance of plants and the leaves need to develop yet more negative water potentials to maintain a downhill gradient of water potential between the soil and the leaves. This effect of dissolved solutes is equivalent to that of drought stress. The immediate physiological response to such unpleasant conditions is a depression of growth and lowering of the photosynthetic rate, which is often followed by wilting and ultimately death. In some plants, a constant salt concentration is maintained by means of dilution; accomplished by absorption of enough water to prevent excessive concentration of salt in plant tissues. This dilution mechanism predominantly occurs in wet saline environments where water is not a limiting resource. This mechanism is often limited by the dilution capacity of plant tissues (Hanson *et al.*, 1993). These plant tissues have evolved thick, fleshy succulent structures such as those found in *Atriplex* species (Kozlowski, 1997) and *Mesembryanthemum crystallinum* (ice plant) (Salisbury and Ross, 1992). In some plants, salt tolerance is accomplished by production of osmotically active organic solutes called compatible solutes. These compatible organic solutes, which include proline, glycine, and betaine, counterbalance high salinity in plants with that of the growth media without posing inhibitory effects on the functions of enzymes and membranes (Munns *et al.*, 1983). For example, accumulation of mannitol in celery, pinitol in ice plants, and proline in *Vigna sinesis* and *Zea mays* seem to assure "osmotic adjustment". In addition, Saneoka *et al.* (1999) speculated that the accumulation of glycinebetaine in *Atriplex* sp., *Limonium*

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sp. and tropical grass species, in correlation with increasing salinity levels, was involved in the protection of photosynthetic enzymes. A related line of research examined the involvement of a low molecular weight protein called *osmotin* in response to salt stress (Salisbury and Ross, 1992). It has been observed that these proteins are involved in the protection against stress. Some species acquire salt tolerance by shifting from C_3 photosynthesis to CAM. As a result the transpiration is decreased drastically and the transport of salts to the shoots is also decreased. This was observed in facultative halophytes such as *Mesembryanthemum crystallinum* (Cushman *et al.*, 1990). However, in terms of energy demand, osmotic adjustment by means of osmoprotectants, as well as shifting to a better-adapted photosynthetic mechanism, can be expensive and it can cause low growth rates (Marschner, 1995).

1.2.2 The effects of salinity on plant ionic content

Saline substrates dominated by Na^+ and Cl^- ions depress growth by toxic ion effects in non-halophytic species. In many herbaceous crop species, grapevine and many fruit trees, growth inhibition and leaf injury has been traced to chloride and sodium ion toxicity. Chloride toxicity has been observed in deciduous woody trees such as *Picea omorika* and *Tilia* sp. as well as many leguminous species, including peanut and soybeans (Maas, 1993). With the exception of graminaceous species, many crop species suffer from sodium toxicity. In describing mechanisms of salt toxicity, it has been suggested that it stems from an inhibition of enzyme reactions and inadequate compartmentation between vacuoles and cytoplasm (Marschner, 1995). According to Oertli (1968), salt toxicity may be attributed to salt accumulation in the leaf apoplasm. For example, in the leaf

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apoplasm, Na^+ concentrations can accumulate up to levels as high as 500mM causing dehydration of leaf cells (Flowers *et al.*, 1991).

The other potential problem for plants growing under saline conditions is the disruption of ionic equilibria (Pardo *et al.*, 1998). This problem arises from the inhibition of nutrient uptake, transport or partitioning within a plant, as well as utilization in plants (Marschner, 1995). For example, in barley plants, exposure to a medium containing low manganese and high NaCl concentrations was followed by depressed growth, and this was due to an inhibited manganese uptake (Cramer and Nowak, 1992). High chlorine is often associated with inhibition of NO_3^- uptake, but induction of nitrogen deficiency is not likely to be the cause of growth depression. Growth depression under these conditions is due to high soil salinity. It has been established that salinity stress has both stimulatory and inhibitory effects on the uptake of some nutrients. For example, exposure of plants to high phosphorus and NaCl salinity, enhanced phosphorus uptake and depressed plant growth mainly by phosphorus toxicity (Roberts *et al.*, 1984). In contrast, Martinez and Läuchli (1991) found that growing cotton plants under low phosphorus and high NaCl salinity conditions resulted in depressed growth and they traced it to a depressed uptake and translocation of phosphorus. In tomato, the utilization efficiency of leaf phosphorus was depressed with increasing salinity concentrations (Marschner, 1995). Similarly, Chow *et al.* (1990) found that in spinach leaves of plants growing growing under saline habitats, the demand for potassium was higher than that in non-saline habitats. It is a well-known phenomenon that in plants, K^+ is required for optimal photosynthetic capacity.

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To circumvent these ionic effects, plants have evolved two defense mechanisms. Firstly, there is salt exclusion, a sodium-potassium pump system that maintains the sodium concentrations at levels that will allow metabolic activities to be carried out. Simultaneously, it ensures that potassium ions are supplied sufficiently to the plant. In some species, the pump operates primarily in the root zone, retranslocating Na^+ back to the surroundings and potassium into the roots (Raven *et al.*, 1992). This is the predominant strategy of salt tolerance in glycophytes (Greenway and Munns, 1980). However, the classification of glycophytes as excluders, is a relative term because it refers to salt uptake at much lower levels (Marschner, 1995). For example, in maize, it was observed that the levels of chloride and sodium in the shoots were relatively low and potassium and calcium were high when exposed to salinity. Retranslocation of Na^+ from shoots to roots has also been observed in beans plants. It has also been observed that genetic diversity in a species may contribute significantly to salt tolerance by controlling the rates of salt transport to shoots and selection of K^+ over Na^+ ions (Saneoka *et al.*, 1999). For example, higher salt tolerance of certain cultivars of wheat (Rivelli *et al.*, 2002), barley (Greenway and Munns, 1980) and citrus (Maas, 1993) is associated with effective restriction of Na^+ and Cl^- transport to the shoots.

According to plant agronomists and plant physiologists, plant resistance to salinity stress can be facilated by addition of fertilizers (Cengiz *et al.*, 2003). These fertilizers should enhance nitrate nutrition to delete the effects of high chloride concentration in the soil and water. Likewise, plant nutrition enriched with potassium, has been established as an efficient method of combating sodium-induced effects for many plants (Achilea, 2002).

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These findings suggest that the two techniques can be combined by application of multi-K (potassium nitrate). It has been shown that application of multi-K fertilizer to greenhouse-grown vegetables (tomato, lettuce and Chinese cabbage), field grown sweet corn and perennial citrus, combated sodium-induced stress and enhanced crop performance under saline conditions (Agrisupport, 2000). Also found to contribute to the supply of potassium to plants was the addition of calcium ions to the growth medium (Raven *et al.*, 1992). Calcium participates in the control of K^+ selection over Na^+. To substantiate this, Song and Fujiyama (1996), conducted a hydroponic study that was aimed at investigating the effects of the addition of calcium on the growth and cation uptake of rice and tomato plants subjected to Na-salinization. They found that in rice, Ca^{2+} improved growth by decreasing Na^+ uptake and increasing K^+ and Ca^{2+} uptake and in tomato, Ca^{2+} suppressed Na^+ transport to the tops. These findings suggest that there is an antagonistic relationship between Ca^{2+} and Na^+, affecting the K^+ selection to promote growth.

The second mechanism is referred to as salt inclusion where sodium is absorbed by the roots and efficiently sequestered into vacuoles of either the stems or the leaves. For example, in *Zea mays*, electron probe microanalysis observations suggested that salt was taken care of through salt inclusion (Yeo *et al.*, 1976). In other plants, specialized salt glands are developed on the leaf surface. These anatomical modifications of plants excrete salt at high concentrations. It is a common feature among halophytes but it has not been reported from crop plants (Glen *et al.*, 1998).

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It has become apparent that whatever the strategy is, the adaptations of plants to salty, inhospitable conditions, can be determined by a number of responses (Flowers *et al.*, 1977). Thus, the restriction of Na^+ ions across the plasma membrane, facilitation of Na^+ and Cl^- sequestration into vacuoles, and mediation of osmolytes and osmoprotectant production and accumulation all have a role. Most of these mechanisms require specialized properties of the plasma membrane, which are responsible for regulating the transportation of salt ions. Membrane composition can affect membrane fluidity, permeability and membrane protein activity. However, despite the implications that many studies devoted to salt tolerance have been centered around the membrane characteristics, it has not been established to what extent and whether growth regulators play any pivotal role (Salisbury & Marinos, 1985).

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1.3 Growth regulators and salt stress

Traditionally, growth regulators are chemical messengers that are involved in the regulation of the physiological aspects of plant growth and development, including cell division, apical dominance, formation of parthenocarpic fruit, senescence and promotion of germination (Raven *et al.*, 1992 and Hare *et al.*, 1997). When plants produce them endogenously they are often referred to as phytohormones (plant hormones). Plant hormones are synthetic or naturally occurring compounds that when applied directly to a target plant area, alter its life processes and structure; they elicit a response that will change a plants quality and may increase its yield. The terms plant growth regulators and phytohomones can be used interchangeably, particularly when referring to auxins, cytokinins, gibberellins, ethylene and abscisic acid.

The use of growth regulators in the betterment of the agricultural industry has increased substantially during recent years (Khalid, 2001). Salinity, like any abiotic stress, modifies the hormonal balance in plants (Benzoini *et al.*, 1974). For example, it has been established that under salt stress, the endogenous levels of abscisic acid are markedly increased. This occurrence is often correlated with increased water use efficiency whereby there is a decrease in the stomatal conductance, highlighting its importance in the regulation of the stomatal aperture and hence its contribution to the reduction of leaf desiccation under saline conditions (Bernstein, 1975 and Aldesuquy & Ibrahim, 2001). Furthermore, the accumulation of ABA in salt stressed plants alleviates stress by facilitating the production of low molecular weight compounds (*osmotins*) and uptake of compensatory ions such as calcium (Salisbury and Ross, 1992). Based on this, it was

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found that ABA featured prominently in studies on salt stress as it does in so many other osmotic related stress situations. Even though, plants are capable of synthesizing their own phytohormones and sometimes absorb those that are generated by soil microorganisms, they may respond to exogenously applied hormones at certain growth stages and under specific environmental conditions. Recently, it was discovered that the idea of using ABA in the alleviation of salt stress did not hold, the reason being as soon as plants are relieved of the stress, ABA starts posing detrimental effects on plants (Asch *et al.*, 1995 and Hare *et al.*, 1997).

Cytokinins are root-borne phytohormones that are immobilized and decreased in the aerial parts of salt stressed plants (Werner *et al.*, 2001). It has been established that under sub-optimal climatic and environmental conditions, plants may not synthesize enough endogenous phytohormones to maintain their optimal growth and development. There is a considerable amount of evidence suggesting that proper exogenous application of these growth regulators, may enhance plant growth and regulate some of the physiological aspects that become affected under these conditions. Nemat Alla and colleagues (2002) found that the foliar application of kinetin to waterlogged or salinity treated *Vigna sinesis* and *Zea mays* plants, reduced the inhibitory effects imposed on the contents of proline, anthocyanin and phenolic compounds, as well as the enzymatic activities in phenolic metabolism. As reviewed by Zhao and his co-workers (1986) it was demonstrated that the exogenous application of cytokinins promoted seed germination and growth in *Eleusine corocana*. In accord with these findings, Bozcuk (1981) found that germination was promoted in salt-affected seeds of tomato, barley, and cotton. Furthermore, they

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prevented salt-induced necrotic lesions in tobacco plants (Benzoini *et al.*, 1974). There is good evidence supporting the involvement of cytokinins in ion uptake by plants. Jacoby and Dagan (1970) found that in bean plants, treatment with kinetin accentuated the selective uptake of K^+ over Na^+. These findings suggest that exogenous application of kinetin alleviates salt stress by eliciting physiological responses that are used in salt tolerant species.

Gibberellins on the other hand, are produced in shoots and they are favoured indirectly by environmental factors (water and nutrient supply) involved in shoot growth (Marschner, 1995). Under saline conditions, whereby growth is reduced through water and nutrient deficits in plants, the synthesis of GA_3 is affected. It has been demonstrated that the growth inhibiting effects of salinity can be reduced, either partially or completely, by exogenous application of gibberellins (Aldesuquy & Ibrahim, 2001). For example, when gibberellins were applied at low concentrations to salt-treated cotton plants, they attenuated the salt induced effects (Stark *et al.*, 1975). Furthermore, in bean plants, they counteracted the salts injurious effects on growth, photosynthesis and translocation of assimilates. Because of constant attempts to convert halophytic species into crops, the promotive role of these growth regulators was examined in halophytes (Zhao *et al.*, 1986 and Glenn *et al.*, 1998). Boucard and Ungar (1976) found that treatment of *Suaeda* species with gibberellins stimulated growth and overcame salt induced dormancy. However, the mechanism by which gibberellic acid maintains growth under saline conditions is not well understood, but it has been demonstrated that in rice it may involve α-amylase and leaf carbohydrate metabolism (Lin and Kao, 1995). Hasan (2002) believes

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that the primary mode of action of GA_3 might reside with the cell membrane properties. In support of this view, Kawasaki *et al.* (1978) presented evidence demonstrating that gibberellins maintain growth by acting synergistically with either K^+ or Na^+ ions. The relationship between gibberellins and K^+ is beneficial for glycophytes growing under salt stress conditions as it improves their osmoregulation and thereby photosynthesis (Chow *et al.*, 1990). On the other hand, the relationship between gibberellins and Na^+ benefits salt tolerant species (halophytes and C_4 species), as it is important for nutritional purposes, whereas for glycophytes it is harmful (Marschner, 1995). These findings suggest that the exogenous application of GA_3 to salt-stressed plants might improve plant growth, but its role on salt tolerance is not clearly understood. Therefore, the involvement of gibberellins in salt tolerance requires further elucidation.

In conclusion, plant growth regulators next to fertilizers, pesticides and herbicides, may become the new generation of agro-chemicals (Khalid, 2001). It has become apparent that growth regulators, especially when supplied at extremely low concentrations, control normal plant growth and development throughout ontogeny, even under unfavorable environmental conditions such as salt stress.

In the current study, pot experiments were performed on *Erucastrum strigosum,* a member of the Brassicaceae family. Even though this plant has no economical value, it has characteristics in common in common with *Arabidopsis,* which has had much attention from plant physiologists and biochemists. The shift to this genus was due to the fact that it can provide sufficient material for various analyses to be carried out. The main

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objective in the present study was to examine the role of exogenous growth regulators, especially under salt stress conditions.

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1.4 References

ACHILEA O (2002) Alleviation of salinity-induced stress in cash crops by Multi-K (Potassium nitrate), five cases typifying the underlying pattern. Acta Horticulture 572: 43-48.

AGRISUPPORT (2000) Alleviation of salinity stress by Multi-K. http://www.agrisupportonline.com/salinity.htm downloaded on 29/05/03.

AL-ATTAR M (2002) The role of biosaline agriculture in managing freshwater resources shortages and improving water securities. http://www.worldfoodprize.com/Symposium/2002presentations/Al-Attar.pdf downloaded on 09/06/03.

ALDESUQUY H S, IBRAHIM A H (2001) Water relations, abscisic acid and yield of wheat plants in relation to the interactive effect of seawater and growth bioregulators. Journal of Agronomy and Crop Science 187: 97-104.

ASCH F, DÖRFFLING K, DINGKUHN M (1995) Response of rice varieties to soil salinity and air humidity: A possible involvement of root-borne ABA. Plant and Soil 177: 11-19.

ASHRAF M, NAZIR N, MCNEILLY T (2001) Comparative salt tolerance of amphidiploid and diploid *Brassica* species. Plant Science 160: 683-689.

AUSTRALIAN ACADEMY OF SCIENCE (1998) Monitoring the white death-soil salinity. http://www.science.org.au/nova/032/032key.htm downloaded on 09/06/03.

BENZOINI A, MIZRAHI Y, RICHMOND A E (1974) Effect of kinetin on plant's response to salinity. New Phytologist 73: 315-319.

BERNSTEIN L (1959) Salt tolerance of vegetable crops in the West. United States

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etd.uwc.ac.za

UNIVERSITY of the
WESTERN CAPE

etd.uwc.ac.za

Department of Agriculture Information Bulletin 205: 55-59.

BERNSTEIN L (1964) Salt tolerance of vegetable crops in the West. United State Department Agriculture Information Bulletin 283: 23-46.

BERNSTEIN L (1975) Effects of salinity and sodicity on plant growth. Annual Review of Plant Pathology 13: 295-312.

BLAYLOCK A D (1994) Soil salinity, salt tolerance and growth potential of horticultural and landscape plants. http://www.uwyo.edu/ces/PUBS/Wy988.pdf downloaded on 28/08/02.

BOUCAUD J, UNGAR I A (1976) Influence of hormonal treatment on growth of two halophytic species. American Journal of Botany 63: 694-699.

BOZCUK S (1981). Effects of kinetin and salinity on germination of tomato, barley and cotton seeds. Annals of Botany 48: 81-84.

CARDON G E, MORTVEDT J J (1994) Salt-affected soils. http://www.ext.colostate.edu/pubs/crops/00503.pdf downloaded on 06/060/03

CENGIZ K, HIGGS D, INCE F, AMADOR B, CAKIR A, SAKAR E (2003) Ameliorative effects of potassium phosphate on salt stressed pepper and cucumber. Journal of Plant Nutrition 26: 807-821.

CHOW W S, BALL M C, ANDERSON J M (1990) Growth and photosynthetic response of spinach to salinity: implications of K^+ nutrition for salt tolerance. Australian Journal of Plant Physiology 17: 563-578.

CRAMER G R, NOWAK R S (1992) Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt stressed barley. Physiologia Plantarum 84: 600-605.

etd.uwc.ac.za

UNIVERSITY of the
WESTERN CAPE

etd.uwc.ac.za

CUSHMAN J C, MICHALOWSKI C B, BOHNERT H J (1990) Development control of Crassulacean acid metabolism inducibility by salt stress in the common ice plant. Plant Physiology 94: 1137-1142.

DEAT (1999) Major water quality problems in South Africa. http://www.ngo.grida.no/soesa/nsoer/issues/water/state4.htm downloaded on 15/01/03.

DLWC (2000) Salinity in NSW. http://www.dlwc.nsw.gov.au/care/salinity/ downloaded on 06/06/03.

DWAF (2001) Water quality management. http://www.dwaf.gov.za/Dir_WQM/wqm.htm downloaded on 15/01/03.

EPSTEIN E (1976) Genetic potentials for solving problems of mineral stress: Adaptation of crops to salinity. In M. J. Wright (ed) Plant adaptation to mineral stress in problem soils. Cornell Univ. Press, Ithaca, N. Y. pp 73-82.

EPSTEIN E, NORLYN J D, RUSH D W, KINGSBURY R W, KELLEY D B, CUNNINGHAM G A, WRONA A F (1980) Saline culture of crops: a genetic approach. Science 210: 399- 404.

FLOWERS T J, HAJIBAGHERI M A, YEO A R (1991) Ion accumulation in the cell wall of rice plants growing under saline conditions: evidence for the Oertli hypothesis. Plant and Cell Environment 14: 319-325.

FLOWERS T J, TROKE A R, YEO A R (1977) The mechanism of salt tolerance in Halophytes. Annual Review of Plant Physiology 28: 89-121.

FLOWERS TJ, YEO A R (1995) Breeding for salinity resistance in crop plants: Where next? http://www.hrwallingford.co.uk/projects/TPTRID/grid/g7breed.htm

etd.uwc.ac.za

UNIVERSITY of the
WESTERN CAPE

etd.uwc.ac.za

downloaded on 05/09/02.

GUILLET A, RIPANDELLI D (2002) Partnership initiative information sheet. http://www.johannesburgsummit.org/html/sustainable_dev/p2_managing_resources/1608_sust_biotechnology.pdf downloaded on 08/06/03.

GLENN E P, BROWN J J, O' LEARY J W (1998) Irrigating crops with seawater. Sci. Amer. 279: 57-61.

GREENWAY H, MUNNS R (1980) Mechanisms of salt tolerance in non-halophytes. Annual Review Plant Physiology 31: 149-190.

HALE M G, ORCUTT D M (1987) The physiology of plants under stress. John Wiley and Sons Inc. New York.

HARE P D, CRESS W A, VAN STADEN J (1997) The involvement of cytokinins in plants response to environmental stress. Plant Growth Regulation 23: 79-103.

HANSON B, GRATTAN S, FULTON A (1993) Crop, water and salinity. http://lawr.ucdavis.edu/irrigation/manuals/93-10.html downloaded on 02/06/03.

HASAN H A H (2002) Gibberellin and auxin production by plant root fungi and their biosynthesis under salinity-calcium interaction. Rostlinná Výroba 48: 101-106.

HINCHMAN R R, NEGRI M C, GATLIFF G E (1998) Phytoremediation: Using green plants to clean up contaminated soil, groundwater and wastewater. http://www.ipd.anl.gov/biotech/pdfs/phyto98.pdf downloaded on 28/08/02.

JACOBY B, DAGAN J (1970) Effect of N^6-benzyladenine on primary leaves of intact bean plants and their sodium absorption capacity. Physiologia Plantarum 23: 397-400

KAWASAKI H, TAKADA H, KAMISAKI S (1978) Requirement of sodium

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UNIVERSITY of the
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etd.uwc.ac.za

chloride for the action of gibberellic acid in stimulating hypocotyl elongation of a halophyte, *Salicornia herbacia* L. Plant & Cell Physiology. 19 : 1415-1425.

KHALID M (2001).A new approach to improve maize yield. http://www.dawn.com/2001/10/22/ebr14.htm downloaded on 07/10/02.

KIRCHNER J, MOOLMAN J H, DU PLESSIS H, REYNDERS A G (1997) Causes of salinity in the Breede River Valley, South Africa. Hydrology Journal 5: 98-108.

KOTUBY-AMACHER J, KOENIG R, KITCHEN B (1997) Salinity and plant Tolerance. http://extension.usu.edu/publica/agpubs/salini.htm downloaded on 07/10/02.

KOZLOWSKI T T (1997) Response of woody plants to flooding and salinity. http://heronpublishing.com/tp/monograph/kozlowski.pdf downloaded on 04/11/02.

LIN C C, KAO C H (1995) NaCl stress effects in rice seedlings: starch mobilization and the influence of gibberellic acid on seedling growth. Botanical Bulletin Academia Sinica 36: 169-173.

LUTTS S, KINET J M, BOUHARMONT J (1995) Changes in plants response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. Journal of Experimental Botany 46: 1843-1852.

MANNION A M (1991) Global Environmental change: A natural and cultural environmental history. Addison Wesley Longman, England.

MARSCHNER H (1995) Mineral nutrition of higher plants. 2nd ed Academic Press. San Francisco.

MARTINEZ V, LÄUCHLI A (1991) Phosphorus translocation in salt stressed cotton. Physiologia Plantarum 83: 627-632.

etd.uwc.ac.za

UNIVERSITY of the
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etd.uwc.ac.za

MAAS E V (1993) Salinity and citriculture. Tree Physiology 12: 195-216.

MAAS E V, HOFFMAN G J, CHABA G D, POSS J A, SHANNON M C (1983) Salt sensitivity of corn at various growth stages. Irrigation Science 4: 45-57.

MUNNS R, GREENWAY H, KIRST G O (1983) Halotolerant Eukaryotes. In: O. L. Lange, P. S. Nobel, C. B. Osmond and Ziegler, (eds). Encyclopedia of Plant Physiology. Springer- Verlag, Berlin, pp 59-135.

MUNNS R (1988) Effect of high external NaCl concentrations on ion transport within the shoot of *Lupinus albus*. I. Ions in xylem sap. Plant, Cell and Environment 11: 283-289.

NATIONAL LAND AND WATER RESOURCE AUDIT (2000) Australian dryland salinity assessment 2000. http://audit.ea.gov.au/ANRA/land/docs/national/Salinity_Intro.html downloaded on 09/06/03

NEMAT ALLA M M, YOUNIS M E, EL-SHIHABY O A, EL- BASTAWISY M (2002) Kinetin regulation of growth and secondary metabolism in waterlogging and salinity treated *Vigna senensis* and *Zea mays*. http://www.zfr.pan.krakow.pl/ang/app/2002no1/1.html downloaded on 15/01/03

NOOR-JEHAN Y B (2002) Acid mine water to be put to good use. http://allafrica.com/stories/200209120548.html downloaded on 08/06/03.

OERTLI J J (1968) Extracellular salt accumulation, a possible mechanism of salt injury in plants. Agrochimica 12: 461-469.

O'LEARY J W (2001) Water relations and stress physiology, especially use of halophytes to study adaptive responses to salinity.

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http://ag.arizona.edu/pls/faculty/oleary.html downloaded on 20/08/02

PARDO J M, REDDY M P, YANG S, MAGGIO A, HUH G -H, MATSUMOTO T, COCA M A, PAINO-D'URZO M, KOIWA H, YUN D–J, WATAD A A, BRESSAN R A, HASEGAWA P M (1998) Stress signaling through Ca^{2+} / calmodulin- dependent protein phosphatase calcineurin mediates salt adaptation in plants. Proceedings of National Academy of Science of the United State of America 95: 9681-9686.

PATON C (2002) Underground cesspools threaten Joburg. http://www.suntimes.co.za/2002/09/01/news/news29.asp downloaded on 08/06/03

QUEENSLAND GOVERNMENT (2002) Salinity. http://www.nrm.qld.gov.au/land/management/salinity.html downloaded on 15/01/03

RAVEN P H, EVERT R F, EICHHORN S E (1992) Biology of plants. 5th edn. Worth Publishers. New York.

REEVE T (2003) The role halophytes can have in saving commercial agriculture. http://jrscience.wcp.muohio.edu/FieldCourses99/MarineEcologyArticles/Therolehalophytescanhavei.html downloaded on 16/15/03.

RIVELLI R A, JAMES R A, MUNNS R, CONDON A G T (2002) Effect of salinity on water relations and growth of wheat genotypes with contrasting sodium uptake. Functional Plant Biology 29: 1065-1074.

ROBERTS J K M, LINKER C S, BENOIT A G, JARDETZKY O, NIEMAN R H (1984) Salt accumulation of phosphate uptake in maize root tips studied by ^{31}P nuclear magnetic resonance. Plant Physiology 75: 947-950.

SALISBURY F B, MARINOS N G (1985) The ecological role of plant growth Substances. In: R. P. Pharis and D. M. Reid (eds) Encyclopedia of plant physiology.

etd.uwc.ac.za

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etd.uwc.ac.za

Springer- Verlag, Berlin, pp 59-135.

SALISBURY F B, ROSS CW (1992) Plant physiology. 4th edn. Wadsworth Publishing Company. Belmont.

SANEOKA H, SHIOTA K, KURBAN H, CHAUDHARY M I, PREMACHANDRA G S, FUJITA K (1999) Effect of salinity on growth and solute accumulation in two wheat lines differing in salt tolerance. Soil Science and Plant Nutrition. 45: 873-880.

SONG J Q, FUJIYAMA H (1996) Differences in response of rice and tomato plants subjected to sodium salinization and to the addition of calcium. Soil Science and Plant Nutrition 42: 503-510.

STARCK Z, KARWOWSKA R, KRASZEWSKA E (1975) The effects of several stress conditions and growth regulation on photosynthesis and translocation of assimilates in the bean plant. Acta Societatis Botanicorum Polaniae 44: 567-588.

TRENCH Z (2001) Councils act on salinity. http://www.abc.net.au/rural/vic/stories/s330789.htm downloaded on 09/06/03.

TURAN M, SEZEN Y (2002) Effect of salt stress on plant nutrition. http://www.toprak.org.tr/isd/can_73htm downloaded on 05/09/02.

WERNER T, MOTYKA V, STRNAD M, SCHMÜLLING T (2001) Regulation of plant growth by cytokinin. Proceedings of National Academy of the Science of United State of America 98: 10487-10492.

YEO A R D, KRAMER A, LAÜCHLI A, GULLASCH J (1976) Ion distribution in salt stressed mature *Zea mays* roots in relation to ultra structure and retention of sodium. Journal of Experimental Botany 24: 1729-

ZHANG H – X, BLUMWALD E (2001) Transgenic salt tolerant tomato plants

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accumulate salt in foliage but not in fruit. Nature Biotechnology 19: 765- 768.

ZHAO K-F, LI M-L, LIU J-Y L (1986) Reduction by GA_3 of NaCl-induced inhibition of growth and development of *Suaeda usseriensis*. Australian Journal of Plant Physiology 13: 547-551.

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Chapter 2

The effects of exogenous cytokinins on the growth of salt stressed *Erucastrum strigosum* plants.

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2.1 Introduction

Salinity has become one of the world's major limitations to crop production. It has been estimated that 2 million km^2 of the land reserved for crop production and over 40% of the irrigated land, particularly that of the Mediterranean basin, California and Southern Asia has become saline (Lee *et al.*, 1999). Salinity by definition, is the occurrence of soluble sodium salts in the soil, water and consequently in plant tissues. Salinity deposition originates from various sources, thus, improper agricultural practice, including saline irrigation water, inadequate leaching, and poor drainage and naturally occurring saline soils, all add to the problem. In some areas, salinity stems from industrial practice and the use of seawater in the mining of heavy metals. The most common effects of salt stress are loss of dry matter, leaf discoloration and retardation of protein synthesis. A well-known phenomenon is that substrate salinity frequently results in acute damage to plants, manifested by necrotic lesions and marginal burn of leaves (Benzoini *et al.*, 1974 and Marschner, 1995).

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Although not a final decisive factor, salt-induced slow growth in plants may be attributable to changes in leaf water status. It has been shown that changes in leaf water status affect leaf turgidity (Munns & Termaat, 1986) and cell wall extensibility (Lynch *et al.*, 1988). Leaf turgidity is the pressure of water inside the cell pushing against the cell wall and cell wall extensibility measures how much the wall will stretch permanently when a force is applied (Raven *et al.*, 1992). However, it has been observed that the relationship between leaf growth, root pressure and cell wall extensibility, during salt stress, needed to be reviewed (Lerner *et al.*, 1994). This was acknowledged because in a

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living system turgor changes are not associated with cell wall extensibility. To substantiate this, Termaat *et al.* (1985) found that the application of pressure, to raise the leaf water potential of salt stressed plants, failed to prevent the decline in leaf expansion in wheat and barley. These findings suggest that turgor, although presumably necessary for growth, does not alone regulate shoot growth, but may be overridden by other factors (Marschner, 1995).

There is a possibility that the status of roots may regulate shoot growth via a chemical message (Termaat *et al.*, 1985). Such a message could be the supply of growth regulators. Cytokinins, which are synthesized in the roots and transported to aerial parts via the xylem, have been implicated in regulating shoot growth of water-stressed plants (Bernstein, 1975). This stems from their involvement in the mediation of physiological aspects of growth and differentiation, including cell division, apical dominance, senescence, nutrient mobilization, chloroplast development and flowering (Hare *et al*, 1997 and Van Staden & Davey, 1979). The use of growth regulators in overcoming salt stress, particularly if the viewpoint is crop production, has long been studied (Bernstein, 1975). It was observed that the exogenous application of growth regulators at appropriate levels may be used to alter their endogenous levels (Marschner, 1995). Kinetin is one of the cytokinins that have received much attention in the literature. For example, in a greenhouse study by Naqvi *et al.* (1982), it was demonstrated that treatment of salt stressed wheat seedlings with kinetin enhanced growth, which was indicated by leaf and root length measurements. Similarly, Amzallag and his co-workers (1991) measured dry weight production and found that simultaneous applications of kinetin and NaCl, partially

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reversed the inhibitory effects of salt stress. However, it has been pointed out that different plant parts vary in their response to exogenous treatment with kinetin. It was further observed that the promotive effects of kinetin on roots are more pronounced than in leaves, particularly at low salt levels. From a different perspective, a study was conducted to investigate the role of treatment with kinetin on saline-induced visible injurious effects (necrosis). The findings revealed that kinetin reversed these effects (Benzoini *et al.*, 1974). These results suggest that the addition of kinetin to the uptake solution counteracted the inhibitory effects of salt stress to a certain extent.

Based on these findings, it was deduced that the beneficial use of kinetin in alleviating some of the inhibitory effects of salt stress in plants, is not well understood and therefore further elucidation was required. In the present study, the aim was to investigate the effects of salinity and kinetin on the growth of *Erucastrum strigosum.*

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2.2 Materials and methods

2.2.1 Plant material and growth conditions

A randomized block design experiment with 5 kinetin by 5 salt treatments, replicated two times, was conducted under greenhouse conditions. Five seeds of *Erucastrum strigosum* were sown in each of 75 (10cm) pots that were filled with silica sand. The pots were watered twice a week with a full-strength chemicult solution (complete nutrient solution) or in alternate weeks with enough tap water ($100cm^3$) to flush out the system until treatments commenced. The chemicult used was a commercial hydroponics nutrient salt mix from Kompel, composed of 6.5%N; 27%P; 13%K; 7%Ca; 2.2%Mg; 7.5%S; 0.15%Fe; 0.024%Mn; 0.024%B; 0.005%Zn; 0.002%Cu and 0.001%Mo. Twenty-eight days after sowing, the seedlings were thinned out to two plants per pot and the kinetin-salt treatments were commenced. The five-kinetin concentrations used were 0; 4; 12.5; 40; and 125μM per liter. The salt used was incremented by 100mM of NaCl per week to provide salt stress at the following concentrations 0, 100, 200, 300 and 400mM NaCl per liter. The treatment solutions were incorporated into the growth medium twice a week. Sufficient solution was used to flush each pot of sand completely of the previous treatments.

Stress was allowed to prevail for thirty-five days in order to provide enough plant material for experimental analysis and to maintain the plants for a week at the highest concentration. Photographs were taken to record conspicuous injurious stress effects encountered during the period of growth. The plants were harvested and separated into roots and shoots; the roots were gently rinsed with tap water to remove all the sand and

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were then dried with paper toweling. The shoots were weighed for fresh mass determination. The shoots and roots were dried in an oven for a week at 70^0C to constant mass.

2.2.2. Statistical analysis

In the collected data, differences between means of shoot growth (g), root growth (g) and shoot water content (% of dry weight) were tested for level of significance (P0, 05) at N=10 using a student's t-least significant difference (LSD) test. This was performed with plants that were treated singly with salinity and kinetin. However, in plants that were treated with salinity-kinetin in combination, the data of the growth parameters of tissues and shoot water content was subjected to analysis of variance. The statistic analysis was carried out using SAS computer statistical programme version 8.2 (SAS, 1999).

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2.3 Results

2.3.1 The effects of salinity on plant growth.

The effects of salinity on plant growth were determined through fresh and dry mass production of shoots and roots of control plants and salinity treated plants at various concentrations. Data presented in Table 2.6.1, shows that the addition of salinity to the treatment solutions with weekly stepwise increments, had dramatic effects ($P<0.05$) on the fresh and dry mass production of shoots of *Erucastrum strigosum* plants. A negative correlation was observed between the shoot dry and fresh mass production, and the NaCl concentrations of the growth medium. Thus, the shoot growth of control and low saline treated plants, was higher than those of higher saline treated plants. The water content was not significantly affected by increasing external salinity (Table 2.6.1). In roots, only dry mass was considered, and it was observed that a pattern similar to that of shoots growth was shown. Thus, the plants exhibited an inversely proportional relationship with substrate salinity (Table 2.6.1). The photographs taken revealed that plants grown at high salt concentrations appeared smaller than the ones grown in control media (Figure 2.6.1). There were no prominent injurious effects observed in association with the effects of salt stress (Figure 2.6.1).

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2.3.2 The effects of kinetin on plant growth.

The effects of kinetin on growth responses of *Erucastrum strigosum* plants was measured at various concentrations and shown in Table 2.6.2. In shoots, the fresh and dry mass productions of control and kinetin treated plants, were examined. The statistical analysis of the compiled data (of fresh and dry mass production) revealed that the addition of kinetin to the treatment solution had no significant effects on shoot growth of *E. strigosum* plant. There were no significant differences observed between the water contents of control plants and that of kinetin treated plants (Table 2.6.2). In root growth, the examination of dry mass production revealed that the treatment of the solution with moderate kinetin levels (4-12.5μM) increased growth (Table 2.6.2). Thus, in control plants, growth was lower than that of kinetin treated plants but at the highest concentration, growth started to decrease. The kinetin treated plants appeared shorter than the control plants (Figure 2.6.2). There were no visible injurious effects observed in association with growth inhibitory effects of kinetin treatments (Figure 2.6.2).

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2.3.3 The interactive effects of salinity and kinetin on plant growth.

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The salt-hormonal effects on root and shoot growth, are shown in Figures 2.6.3, 2.6.4 and 2.6.6. It was observed that the dramatic effects of salt stress imposed on shoot fresh and dry mass production, persisted regardless of kinetin treatments (Figures 2.6.3 and 2.6.4). This means that the lower the salinity treatments, the higher the fresh and dry mass productions, and the higher the salinity treatments, the lower the shoot growth at all kinetin treatments. It was observed that the water content was not affected when salinity

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and kinetin treatments were applied concomitantly (Figure 2.6.5). In roots, the dry mass production assumed a pattern similar to that of shoot fresh and dry mass production (Figure 2.6.8). Thus, addition of kinetin on saline treated uptake solutions, had no counteractive effects on the reduction of root growth by salinity. The photos taken revealed that control plants, kinetin treated plants, and salt treated plants appeared taller and healthier than the ones treated simultaneously with salt and kinetin (Figures 2.6.6 and 2.6.7). The injurious effects were more prominent at higher concentrations than at lower concentrations, in plants treated simultaneously with kinetin and salinity.

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2.4.Discussion

The results of the present study clearly show that the growth of shoots and roots of *Erucastrum strigosum* plants was significantly reduced ($P<0.05$) with increasing substrate salinity. This reduction in growth suggests that these plants are sensitive to salt stress and similar responses were observed in other non-halophytic plants (Benzoini *et al.*1974, Marschner, 1995, Kozlowski, 1997 and Tozlu *et al.*, 2000). Munns and Termaat (1986) observed that plants respond to salt stress by means of a two-phased model. According to them, growth reduction is primarily caused by water stress effects (a decrease in soil water potential), followed by specific ionic effects (namely salt injury in old leaves), which die back when their vacuoles fail to sequester more salt. Marschner (1995) decided to divide the latter constraint into ion toxicity and nutrient imbalance. In this regard, the uptake and shoot transport of essential nutrients becomes disturbed, while the toxic elements such as Na^+ and Cl^- accumulate. However, in the present study it was demonstrated that reduction of growth was not accompanied by loss of water in leaves, or by injurious effects. These findings suggest that even though water stress is sometimes the primary cause of salt stress (Salisbury & Ross, 1992; Taiz & Zeiger, 1991 and Marschner, 1995), *E. strigosum* responded by displaying toxic and nutritional effects. Likewise, Lazof & Läuchli (1991) found that the salt stress induced slow growth of *Lactuca sativa*, exposed for weeks to salinity, and was due to nutritional disturbances that were observed in shoot apical meristems. It has therefore; become apparent that the precise mechanism by which substrate salinity inhibits growth in non-halophytes, is complex and controversial (Cheesemen, 1989).

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Treatment of plants with kinetin had no significant effects on shoot growth of the studied plants (Table 2.6.2). The failure of kinetin treatments to promote shoot growth has long been of interest to plant physiologists (Bernstein, 1959, Naqvi *et al* 1982, and Amzallag *et al.*, 1991). It has been demonstrated that plant response to treatment with growth regulators depends on several factors, such as sensitivity of the tissue, amount of exogenously applied hormones and site of action (Davies, 1995 and Hopkins, 1999). Salisbury and Ross (1992) found that the shoot growth of sunflower plants was retarded by exogenous application of cytokinins. Similar conclusions were drawn from the studies of Benzoini *et al.*, 1974 and Naqvi et al., 1982. In the present study, the failure of kinetin to promote shoot growth may be attributed to tissue sensitivity to kinetin treatments. It has long been demonstrated that in shoots, cytokinins are not as effective as in roots (Chen *et al.*, 1985). The insensitivity of shoots to kinetin treatment, on the other hand, may be attributed to degradation of cytokinins by shoot enzymes. In support of this view, our results indicate that root growth was stimulated by exogenous application of kinetin (Table 2.6.2). The stimulatory effects of kinetin in roots are controlled by the amount of kinetin exogenously applied. This was demonstrated by a reduction of root growth when the concentration of kinetin was increased. Similar effects were reported in a study by Amzallag *et al.*, (1991). The findings suggest that the efficiency of kinetin in growth stimulation is observed at low concentrations.

The reductions of shoot and root growth with salinity treatments suggest that the addition of kinetin to the saline uptake solution had no amelioratory effects on salt stressed *E. strigosum* plants. In line with these findings, Hare *et al.*, (1997) found that the addition of

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kinetin to bean plants and N^6 Benzladenine to a salt sensitive variety of barley, inhibited plant growth. In addition, Benzoini and his colleagues (1974) found that even though kinetin treatments markedly reduced salt damage to tobacco plants, growth was inhibited at higher salinity levels used. Bernstein (1975) found that inhibition of growth in kinetin treated plants could be attributed to an insufficient supply of cytokinins to shoots, one of the well-known salinity effects. However, the lack of a sufficient supply of kinetin to shoots, in association with a decreased stomatal aperture and its persistence, has recently been questioned by Kabar (1987). Plants growing under saline conditions do not appear kinetin-deficient. Thus, older leaves in salt treated plants do not senesce as early as those of non-saline controls. These findings suggest that the kinetin present in shoots could be conjugated into an inactive state. Moreover, it has been demonstrated that the decrease of stomatal aperture, appears to be generally persistent in salt affected plants (Naqvi *et al.*, 1982). The observations made in the present study are consistent with Kabar's (1987) findings because it was also found that there were no symptoms of kinetin-deficiency in *E. strigosum* throughout the treatment period. The injuries found could only be traced to salinity stress, even at higher concentrations of kinetin. The findings suggest that a sound explanation might only emerge through investigations of toxic and nutritional effects, because there were no osmotic effects found; meaning the stomatal aperture might not have been decreased. It is also important to note that the reversal effects of kinetins on salt stress are limited only to salt tolerant species (Bozcuk, 1981 and Kabar, 1987). It has become apparent that exogenous application of kinetin exacerbates salinity effects in salt sensitive species.

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2.5 References

AMZALLAG G N, LERNER H R, POLJAKOFF-MAYBER A (1991) Interaction between mineral nutrients, cytokinins and gibberellic acid during growth of Sorghum at high NaCl salinity. Journal Experimental Botany 43: 81-87.

BENZOINI A, MIZRAHI Y, RICHMOND A E (1974) Effect of kinetin on plants response to salinity. New Phytologist 73, 315-319.

BERNSTEIN L (1959) Salt tolerance of vegetable crops in the West. United States Department of Agriculture Information Bulletin 205: 55-59.

BERNSTEIN L (1975) Effects of salinity and sodicity on plant growth. Annual Review of Plant Pathology 13: 295-312.

BOZCUK S (1981) Effects of kinetin and salinity on germination of tomato, barley and cotton seeds. Annals of Botany 48: 81-84.

CHEN C-M, ERTL J R, LEISNER S M, CHANG C-C (1985) Localization of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiology. 78: 510-513.

CHEESEMEN J M (1989) Mechanism of salinity tolerance in plants. Plant Physiology. 87: 547-550

DAVIES J P (1995) Plant hormones: Physiology, biochemistry and molecular biology. 2nd edn. Kluwer Academic Publishers. Boston.

HARE P D, CRESS W A, VAN STADEN J (1997) The involvement of cytokinins in plants response to environmental stress. Plant Growth Regulation 23: 79-103.

HOPKINS G W (1999) Introduction to plant physiology. 2nd edn. John Wiley & Sons, Inc. New York.

UNIVERSITY of the WESTERN CAPE

etd.uwc.ac.za

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WESTERN CAPE

etd.uwc.ac.za

KABAR K (1987) Alleviation of salinity stress by plant growth regulators on seed germination. Journal of Plant Physiology 128:179–183.

KOZLOWSKI T T (1997) Response of woody plants to flooding and salinity. http://heronpublishing.com/tp/monograph/kozlowski.pdf downloaded on 04/11/02

LAZOF D, LÄUCHLI A (1991) The nutritional status of the apical meristem of *Lactuca sativa* as affected by NaCl salinization: an electron probe microanalytic study. Planta 184: 334-342.

LEE J H, VAN MONTAGU M, VERBRUGGEN N (1999) A highly conserved kinase is an essential component for stress tolerance in yeast and plant cells. Proceedings of National Academy of Science of the United State of America 96: 5873-5877.

LERNER HR, AMZALLAG N, FRIEDMAN Y, GOLOUBINOFF P (1994) The response of plants to salinity: from turgor adjustment to genome modification. Israel Journal of Plant Science 42: 285-297

LYNCH J, THIEL G, LÄUCHLI A (1988) Effects of salinity on the extensibility and Ca availability in the expanding region of growing barley leaves. Botanica Acta 101: 355- 361.

MARSCHNER H (1995) Mineral nutrition of higher plants. 2nd ed. Academic Press. San Francisco.

MUNNS R, TERMAAT A (1986) Whole plant response to salinity. Australian Journal Plant Physiology 13: 142-160.

NAQVI S S M, ANSARI R, KHANZADA A N (1982) Response of salt stressed wheat seedlings to kinetin. Plant Science Letters 26: 279-283.

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UNIVERSITY of the
WESTERN CAPE

etd.uwc.ac.za

RAVEN P H, EVERT R F, EICHHORN S E (1992) Biology of plants. 5th edn. Worth Publishers. New York.

SALISBURY F B, ROSS CW (1992) Plant physiology. 4th ed. Wadsworth Publishers. California.

SAS (1999) Sas/Stat User's guide, version 8.2. 1st printing. Volume 2. Sas institute. Cary, North Carolina

TERMAAT A, PASSIOURA J B, MUNNS R (1985) Shoot turgor does not limit shoot growth of NaCl affected wheat and barley. Plant Physiology 77: 869-872.

TAIZ L, ZEIGER E (1991) Plant Physiology. Benjamin Cummings. Redwood City. California.

TOZLU I, MOORE G A, GUY C L (2000) Effects of increasing NaCl concentration on stem elongation, dry mass production and macro- and micro nutrient accumulation in *Poncirus trifolita.* Australian Journal of Plant Physiology 27: 35-42.

VAN STADEN J, DAVEY E (1979) The synthesis, transport and metabolism of endogenous cytokinins. Plant, Cell and Environment 2:93-106.

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2.6.Appendix

Table 2.6.1. The effect of salinity on root and shoot growth; and water content (% of dry mass) of *Erucastrum strigosum* plants. Differences between treatment means of the measured parameters were tested for level of significance (P=0.05) at N=10 using LSD; means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of NaCl (mM)					
		0	**100**	**200**	**300**	**400**	**LSD**
Shoots	**Fresh mass (g)**	**15.50 A**	**11.84 B**	**9.47 BC**	**7.14 C**	**8.30 C**	**3.12**
	Dry mass (g)	**2.05A**	**1.52B**	**1.38B**	**1.12B**	**1.20B**	**0.49**
	Water content (%of d. m.)	**677.24 A**	**770.22 A**	**582.98 A**	**583.01 A**	**604.81 A**	**210.31**
Roots	**Dry mass (g)**	**1.76A**	**0.64B**	**0.25B**	**0.20B**	**0.26B**	**0.46**

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5.1 Introduction

It has become apparent that NaCl-induced growth inhibition in most crop plants is likely to revolve around their inability to effectively regulate ionic and osmotic effects (Marschner, 1995). It is well established that strategies, employed by salt-tolerant species to regulate these parameters, depend largely on changes that occur in membranes and cytosolic processes. One of the most common features of salinity tolerance in crop plants is the use of a K/Na discrimination mechanism whereby the accumulation of Na^+ ions is blocked by the influx of K^+ into xylem tissues. It has been established that accumulation of Ca^{2+} in the cytoplasm controls the K^+ selective channels (Blatt & Theil, 1993 and Song & Fujiyama, 1996). In addition, Raven *et al.* (1992) reported that this phenomenon may be accomplished by retranslocating Na^+ ions back to the medium while replacing them with potassium i.e. a Na^+-K^+ antiport. For example, Reid and Smith (2000) reported that treatment of the NaCl-containing medium with calcium has been shown to improve root elongation and shoot growth as well as the abolishment of nutrient deficiencies. Furthermore, Ca^{2+} improved growth of NaCl-stressed *Fusarium oxysporum* fungus by elevating its gibberellic acid content (Hasan, 2002). The decrease of gibberellins under salt stress conditions is now a well-known phenomenon. The exogenous application of growth regulators to alter the status of phytohormones has long been practiced for agricultural and horticultural purposes.

Gibberellic acid is one of the growth regulators that have been implicated in the activation of proton pumping proteins. Gibberellic acid has been shown to influence the uptake and accumulation of cations such as calcium. The link between calcium and

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gibberellic acid was reported on in studies that were performed on barley aleurone cells (Schuurink *et al.*, 1996). In these cells GA_3 increased [Ca^{2+}] by increasing the influx of Ca^{2+} at the plasma membrane. These effects have also been shown for other cereal aleurones such as rice, wheat and wild oats. However, these effects have not been reported for other cations and they have not been well explored for other non-graminaceous plant species. Furthermore, it is not well understood whether these effects occur even under sub-optimal growth conditions, such as salinity stress. The present study is aimed at investigating the effects of exogenous application of gibberellic acid on the cation concentration of salt-stressed *Erucastrum strigosum* plants.

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5.2. Materials and methods

5.2.1 Plant material and growth conditions (refer to 4.2.1)

5.2.2 Chemical analysis (refer to 3.2.2) (Allen *et al.*, 1986)

5.2.2. Statistical analysis.

Statistical analysis was carried out using the SAS version 8.2 computer package (SAS, 1999). In plants treated with GA_3 and NaCl, a student's t- Least Significant Differences (LSD) test was performed to estimate the level of significance between the means at P= 0.05. In salt-gibberellin treated plants, the data obtained was subjected to analysis of variance (ANOVA).

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5.3 Results.

5.3.1. The effects of salinity on the cation concentrations of roots and shoots of *Erucastrum strigosum* plants.

The effects of NaCl concentrations of the treatment solution on cation contents of roots and shoots of *E. strigosum* were measured in $mg.kg^{-1}$ of dry mass (Table 5.6.1). It was generally observed that the sodium concentration in shoots was significantly higher than that of roots. Thus, the shoots of the control plants and that of NaCl treated plants accumulated more sodium than the roots. The comparison made between treatments showed that the sodium concentration increased with increasing substrate salinity for both roots and shoots. The distribution pattern of potassium in shoots was similar to that of sodium. However, when comparison was made between treatments it was observed that the potassium concentration of the control plants was higher than that of saline treated plants for both roots and shoots. The calcium concentration was greater in roots and lower in shoots. However, the root and shoot calcium concentrations of control plants was higher than of saline treated plants. In saline treated plants, calcium concentration for both roots and shoots was more or less the same at all treatments. The shoot magnesium concentration was higher than that of roots. Comparison between treatments revealed that the shoot magnesium concentration of control plants was higher than that of saline treated plants. In saline treated plants, the shoot magnesium concentration was more or less the same at all treatments. The root magnesium concentration, on the other hand, was not significantly affected by increasing substrate salinity.

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5.3.1. The effects of gibberellic acid on cation concentrations of roots and shoots of *Erucastrum strigosum* plants.

The effects of a gibberellic acid series on cations of roots and shoots were measured at $mg.kg^{-1}$ of dry mass and summarized in Table 5.6.2. Generally, shoots contained more sodium than roots. Thus, the sodium accumulated more in shoots than in roots at all levels of gibberellic acid. However, there were no significant differences observed between treatments, on both shoot and root sodium concentrations. The shoot potassium concentration was also higher than roots. Nevertheless, the shoot potassium was decreased with gibberellic acid treatments. However, with root potassium concentrations, there were no significant differences between treatments. The shoot calcium concentration was maintained at levels lower than those of roots. Furthermore, both shoot and root calcium concentrations were not significantly affected by treatments with gibberellic acid. Magnesium concentration was higher in shoots than in roots but it was also not significantly affected by treatments with gibberellins. However, it was observed that the root magnesium concentration decreased with increasing gibberellic acid.

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5.3.2. Interactive effects of salinity and gibberellic acid on cation concentrations of roots and shoots of *Erucastrum strigosum* plants.

The interactive effects of salinity and gibberellic acid on cations of *E. strigosum* roots and shoots measured in $mg.kg^{-1}$ of dry mass are shown in Figures 5.6.1 to 5.6.8. It was observed that salinity treatment increased the shoot sodium concentration to levels that were much higher than those of roots throughout the gibberellic acid treatments. At lower salinity, sodium concentration was smaller than that at higher treatments irrespective of gibberellic acid added to the uptake solution. Potassium concentration was higher in shoots than in roots (Figures 5.6.2 and 5.6.7). Comparison between treatments showed that at lower salinity treatments, the shoot potassium concentration was higher than at higher salinity treatments. These observations were made throughout gibberelic acid concentrations of the treatment solution. The calcium concentration was higher in shoots than in roots at lower levels of the treatments. At higher treatments it was higher in roots and lower in shoots. Simultaneous application of salinity and gibberelic acid decreased shoot calcium concentration at all salinity treatments. The shoot magnesium concentration was higher than that of roots. The magnesium concentration was decreased by substrate salinity regardless of treatments with gibberellic acid. In roots, the sodium concentration increased with increasing salinity at all gibberelic acid treatment levels (Figure 5.6.5). Potassium (Figure 5.6.6) and magnesium (Figure 5.6.8) concentrations increased with salinity treatments but addition of gibberellic acid decreased these concentrations throughout the treatments. Calcium concentration increased with salinity treatments but decreased with addition of gibberellic acid at all treatment levels.

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5.4 Discussion.

The results of this study demonstrated that the nutritional status of glycophytes is affected by salt stress. These effects were expressed more clearly in shoots than in roots. It has been demonstrated that the onset of salt stress in non-halophytes can be attributed to the accumulation of toxic elements such as Na^+ and Cl^+ in shoots. Furthermore, it has been reported that glycophytes resist salinity stress by blocking the uptake and /or transport of saline ions from the root zone to aerial parts (Greenway and Munns, 1980). Accordingly Zandstra-Plom *et al.* (1998) found that plants that resist salinity by using this mechanism accumulate Na^+ in their roots, pith cells and lower parts of the stem. In the present study, it was observed that the shoot Na^+ content was higher than that of roots. These findings suggest that in the *E.strigosum* plant, the mechanism that controls influx of Na^+ into the xylem was lacking or unable to cope, hence the accumulation of Na^+ in shoots (Table 5.6.1). Moreover, they suggest that glycophytes such as *E.strigosum* are unable to maintain an appropriate K/Na discrimination. Greenway and Munns (1980) found that under saline conditions the concentration of K^+ in many glycophytes is severely reduced. This was the case for *E. strigosum.* There seem to be an undeniable correlation between the decrease of K^+ and inappropriate K/Na discrimination. Marschner (1995) reviewed that there is a carrier protein that regulates the uptake and transport of K^+ and Na^+ at the membranes. It is therefore feasible to deduce that in the present study the accumulation of Na^+ and the decrease of K^+ resulted in a lowered K/Na ratio.

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Ca^{2+} has been reported to play a role in salt stress alleviation (Hasan, 2002). However, for calcium to function effectively it has been demonstrated that it should be present in the cytoplasm at elevated amounts (Reid and Smith, 2000). Calcium ameliorates salt stress by promoting the selectivity for K^+ (Raven *et al.*, 1992). Because calcium was not found at elevated amounts, the selectivity for K^+ over that of Na^+ may be impaired (Table 5.6.1).

The initial decrease of Mg^{2+} concentration with increasing substrate salinity in shoots was expected (Table 5.6.1). The hypothesis that salinity affects the homeostasis of Mg^{2+} has also been proposed by Tozlu *et al.* (2000). In their results they demonstrated that Mg^{2+} deficiency is manifested by leaf chlorosis. Furthermore, symptoms of salt stress are similar to those of ion deficiency and they are usually traced to the inability of essential ions to compete with Na^+. However, in this study the accumulation of Na^+ was not accompanied by symptoms of Mg^{2+} deficiency. These findings suggest that the Mg^{2+} concentration was not deficient.

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It was observed that shoot Na^+ concentrations were much higher than that of roots, regardless of treatments with gibberellic acid (Table 5.6.2). The shoot K^+ concentration on the other hand, decreased with increasing gibberellic acid. These results suggest that the K/Na selection at the xylem entry was affected by hormonal treatments. Ca^{2+} concentration was only affected in shoots and Mg^{2+} was affected in roots. Both cations were decreased by hormonal treatments. It has been reported that calcium in plants is often found in a steady state (Bradley, 1999). The decrease of Ca^{2+} in shoots suggest that

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the response of calcium to gibberellic acid is either species specific or at the whole plant level and it requires something else to restore the steady state of calcium. In barley aleurone, GA teamed up with ABA, was reported to effectively reset the steady state of calcium (Gilroy and Jones, 1992). These findings suggest that the efficiency of gibberellic acid in resetting the steady state of calcium, is not well known.

It has been established that higher plants tolerate salt stress by using two types of mechanisms (Tozlu *et al.*, 2000). In the first mechanism, plants exclude toxic ions such as Na^+ and Cl^- from the leaves. In the second mechanism, toxic ions are absorbed by the cells and sequestered into vacuoles. The use of tolerance patterns has enabled scientists to discriminate between salt tolerance and salt sensitivity in species and cultivars. In the present study, treatment of salt stressed plants with GA_3, exacerbated the ionic effects of salinity. Thus, there was accumulation of Na^+ accompanied by decrease of other cations (K, Ca and Mg). These findings suggest that GA_3 failed to stimulate a mechanism that blocks the uptake and transport of Na^+. It has been postulated that these blockage mechanism can either operate at root membranes or the xylem entry, and it is referred to as the K/Na selection (Raven *et al.*, 1992). It discriminates between Na^+ and K^+ (Marschner, 1995). However, it is possible that the tolerance mechanism, stimulated by treatment with gibberellic acid, was the sequestering of these toxic ions into vacuoles. This is possible because Na^+ accumulation in shoots and roots paralleled growth enhancement particularly at low concentrations of GA_3. Nevertheless, there has not been much study on this possibility.

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5.5 References

ALLEN S E, GRIMSHAW H M, ROWLAND A P (1986) Chemical analysis. In: Moore, P D and Chapman, S B (eds) Methods in plant ecology. Blackwell Scientific publications, London, pp 285-337.

BLATT M R, THEIL G (1993) Hormonal control of ion channel gating. Annual Review of Plant Physiology and Plant Molecular Biology 44: 543-567.

BRADLEY P (1999) Nutrients in hydroponics. http://www.hydrogarden.com/index.html downloaded on 12/11/02.

GILROY S, JONES R L (1992) Gibberellic acid and abscisic acid coordinately regulate cytoplasmic calcium and secretory activity in barley aleurone protoplast. Proceedings of National Academy of Science of the United State of America 89: 3591-
3595.

GREENWAY H, MUNNS R (1980) Mechanisms of salt tolerance in non-halophytes. Annual Review of Plant Physiology 31: 149-190.

HASAN H A H (2002) Gibberellin and auxin production by plant root-fungi and their biosynthesis under salinity-calcium interaction. Rostlinná Výroba 48: 101-106.

MARSCHNER H (1995) Mineral nutrition of higher plants. 2nd ed. Academic Press, San Francisco.

RAVEN P H, EVERT R F, EICHHORN S E (1992) Biology of plants. 5th edn. Worth Publishers. New York.

REID R J, SMITH F A (2000) The limits of sodium/ calcium interactions in plant

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growth. Australian Journal of Plant Physiology 27: 709-715.

SAS (1999) SAS/STAT User's guide, version 8.2, 1st printing, Volume 2 SAS Institute Inc. SAS Campus drive, Cary, North Carolina 27513.

SCHUURINK R C, CHAN P V, JONES R L (1996) Modulation of calmodulin mRNA and protein levels in barley aleurone. Plant physiology 111: 371-380.

SONG J Q, FUJIYAMA H (1996) Differences in response of rice and tomato subjected to sodium salinization to the addition of calcium. Soil Science and Plant Nutrition 42: 503-510.

TOZLU I, MOORE G A, GUY C L (2000) Effects of increasing NaCl concentration on stem elongation, dry mass production and macro- and micro nutrient accumulation in *Poncirus trifolita.* Australian Journal of Plant Physiology 27: 35-42.

ZANDSTRA-PLOM M, VOGELRANG S A, VEEN B N (1998) Sodium fluxes in sweet pepper exposed to varying sodium concentrations. Journal of Experimental Botany 49: 1863-1868.

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5.6. Appendix

Table 5.6.1. The effect of salinity on the cation status (mg.kg^{-1} of dry mass) of *Erucastrum strigosum* shoots and roots. The differences between the treatment means were tested for level of significance (P=0.05) at N=10 using LSD; means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of NaCl (mM)					
		0	100	200	300	400	LSD
Shoots	[Na]	5482 C	64468 B	81626 A	87045 A	95252 A	13633
	[K]	65582 A	25407 B	19938 C	17873 C	17418 C	3064
	[Ca]	14037 A	11045 B	10265 CB	10571 CB	9295 C	1392
	[Mg]	4592 A	2081 B	1592 C	1523 C	1524 C	319
Roots	[Na]	2047 D	13706 C	21067 B	29177 A	24535 BA	6503
	[K]	20869 BA	15042 B	24142 A	20308 BA	20692 BA	6299
	[Ca]	10495 B	13909BA	11635 B	19684 A	14856 BA	7746
	[Mg]	816 BC	595 C	1073 BA	1212 A	1021 BA	366

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Table 5.6.2. The effect of GA_3 on the cation status (mg. kg^{-1} of dry mass) of *Erucastrum strigosum* shoots and roots. The differences between the treatment means were tested for level of significance (P=0.05) at N=10 using LSD; means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of Gibberellic acid (μM)					
	Cations ($mg.kg^{-1}$)	**0**	**4**	**12.5**	**40**	**125**	**LSD**
Shoots	**[Na]**	**64707 A**	**70263 A**	**60027 A**	**69424 A**	**69453 A**	**13633**
	[K]	**30595 A**	**30324 BA**	**29493 BA**	**27430 B**	**28377 BA**	**3064**
	[Ca]	**11466 A**	**12011 A**	**10659 BA**	**11074 BA**	**10002 B**	**1392**
	[Mg]	**2344 A**	**2307 A**	**2150 A**	**2229 A**	**2383 A**	**319**
Roots	**[Na]**	**18978 A**	**20710 A**	**15479 A**	**16907 A**	**18458 A**	**6504**
	[K]	**23493 A**	**20011 A**	**19603 A**	**20595 A**	**17351 A**	**6299**
	[Ca]	**18210 A**	**15889 A**	**11691 A**	**11106 A**	**13682 A**	**7746**
	[Mg]	**1153 A**	**1001 BA**	**782 B**	**982 BA**	**799 BA**	**366**

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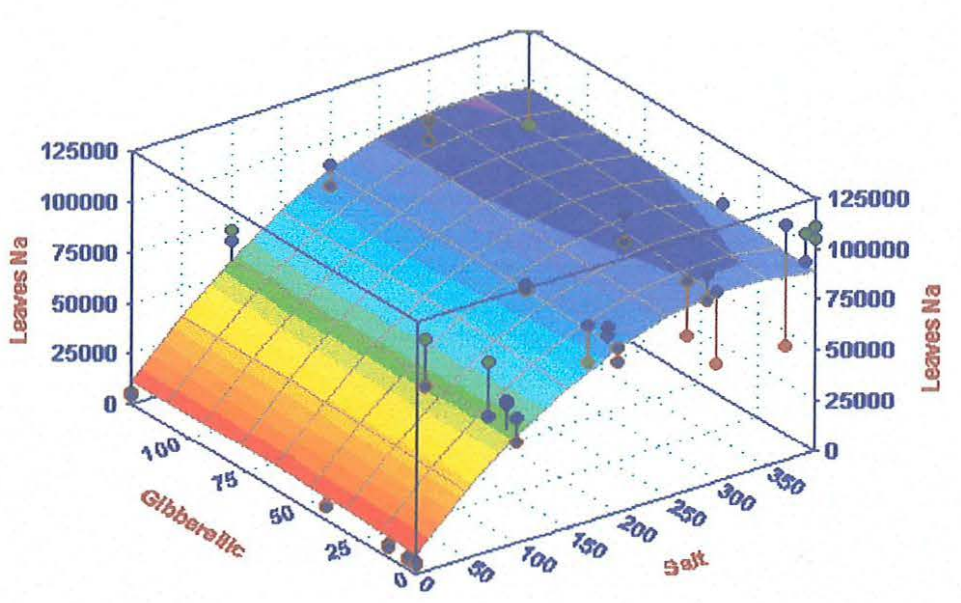

Figure 5.6.1. The interactive effects of salinity (m M) and GA_3 (µM) on the shoot Na^+ concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

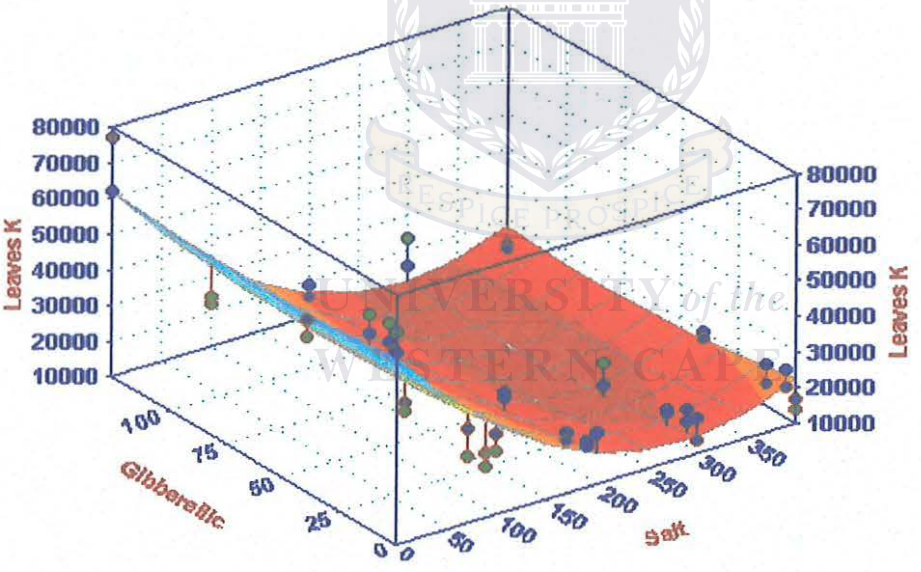

Figure 5.6.2. The interactive effects of salinity (mM) and GA_3 (µM) on the shoot K^+ concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

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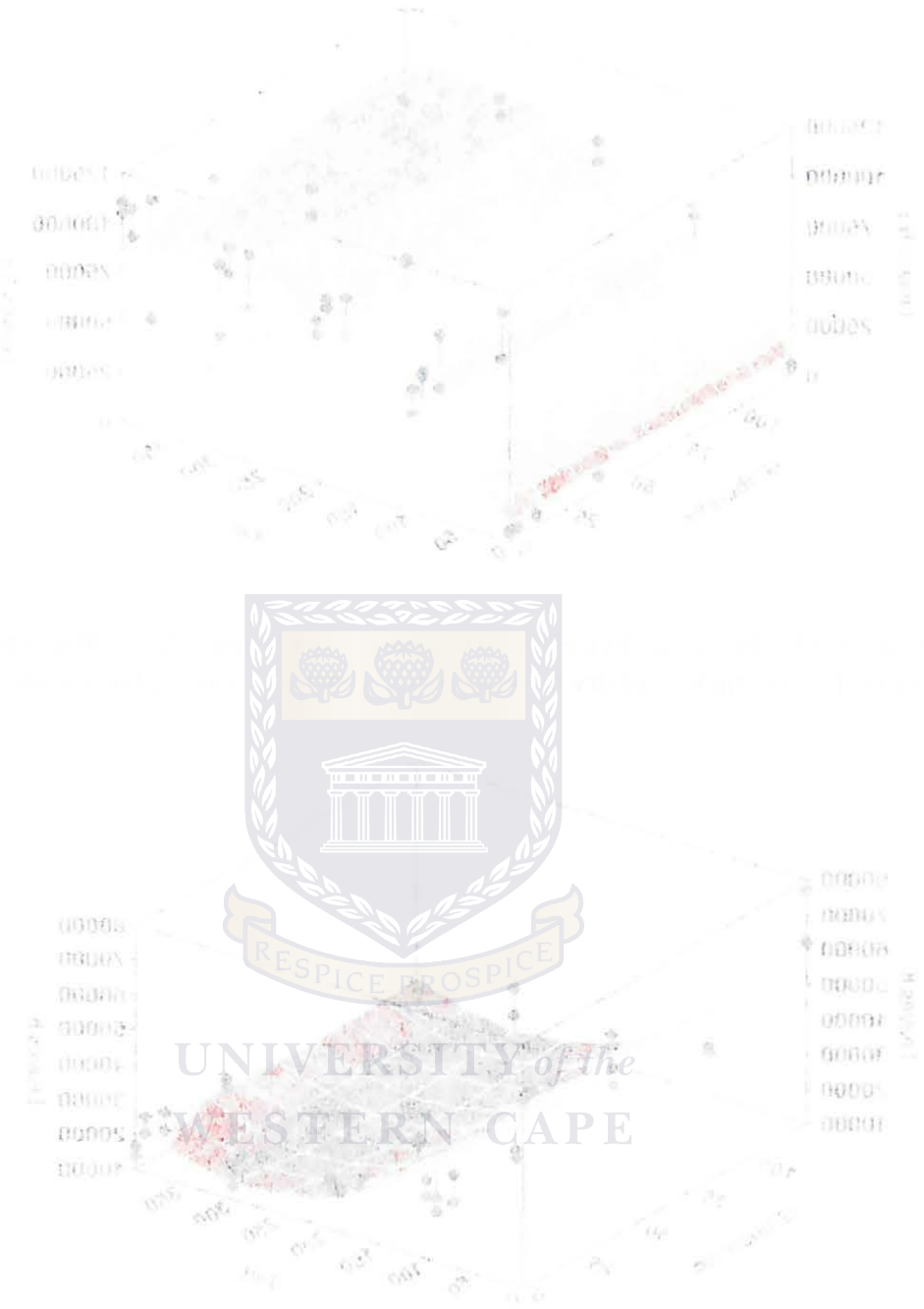

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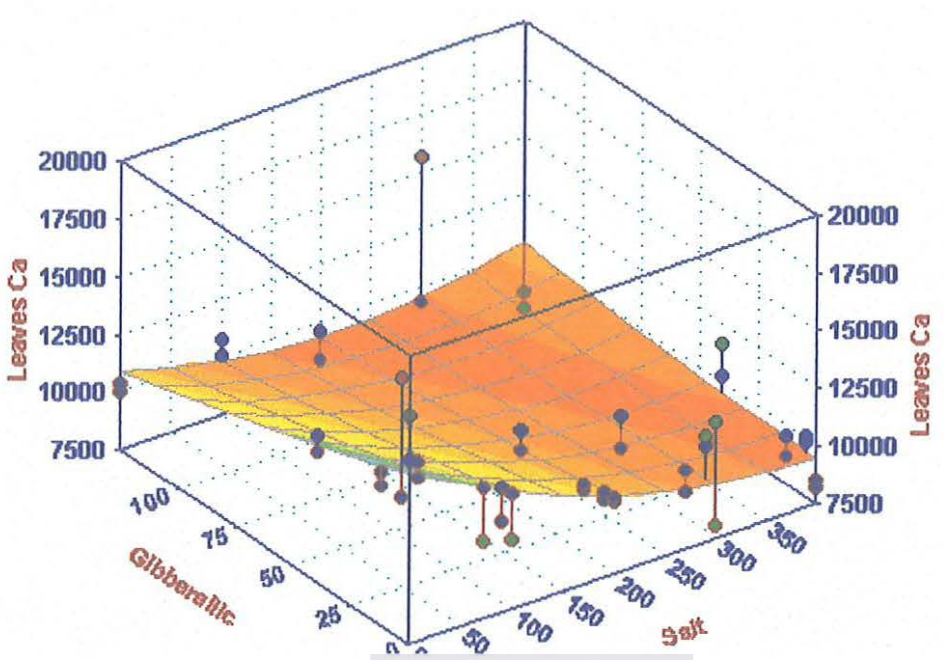

Figure 5.6.3. The interactive effects of salinity (mM) and GA_3 (µM) on the shoot Ca^{2+} concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

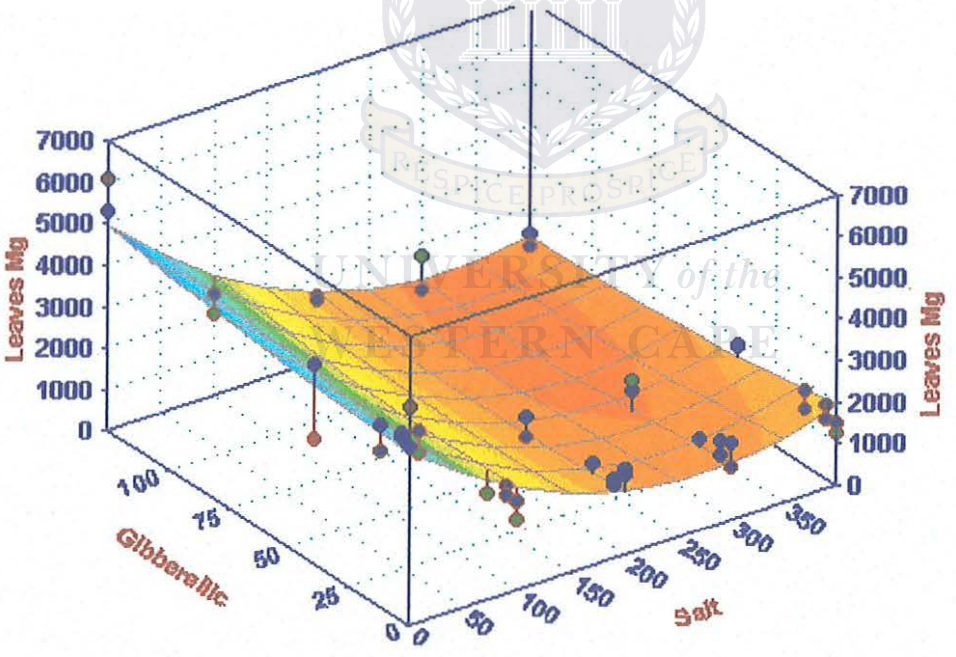

Figure 5.6.4. The interactive effects of salinity (mM) and GA_3 (µM) on shoot Mg^{2+} concentration ($mg.kg^{-1}$ of dry mass) of Erucastrum strigosum plants, using ANOVA.

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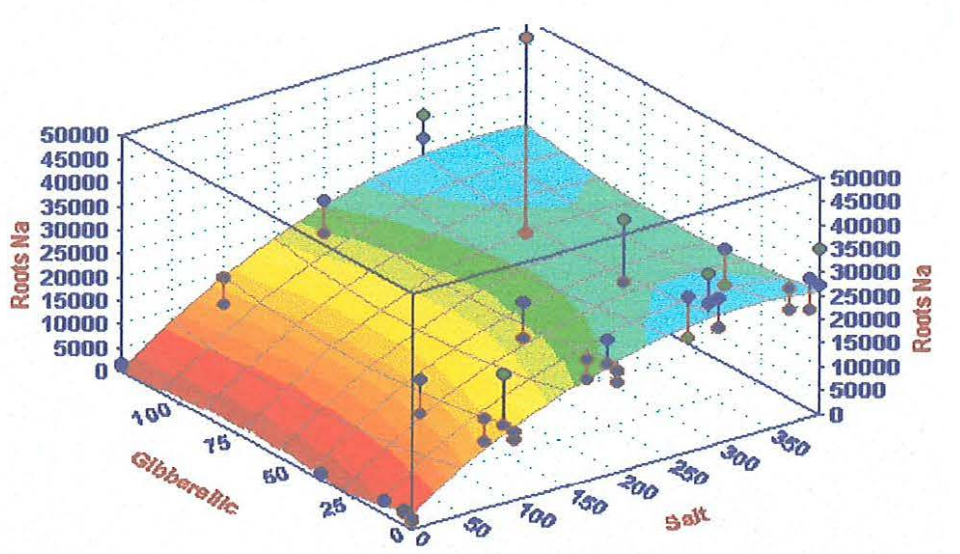

Figure 5.6.5. The interactive effects of salinity (mM) and GA_3 (µM) on root Na^+ concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

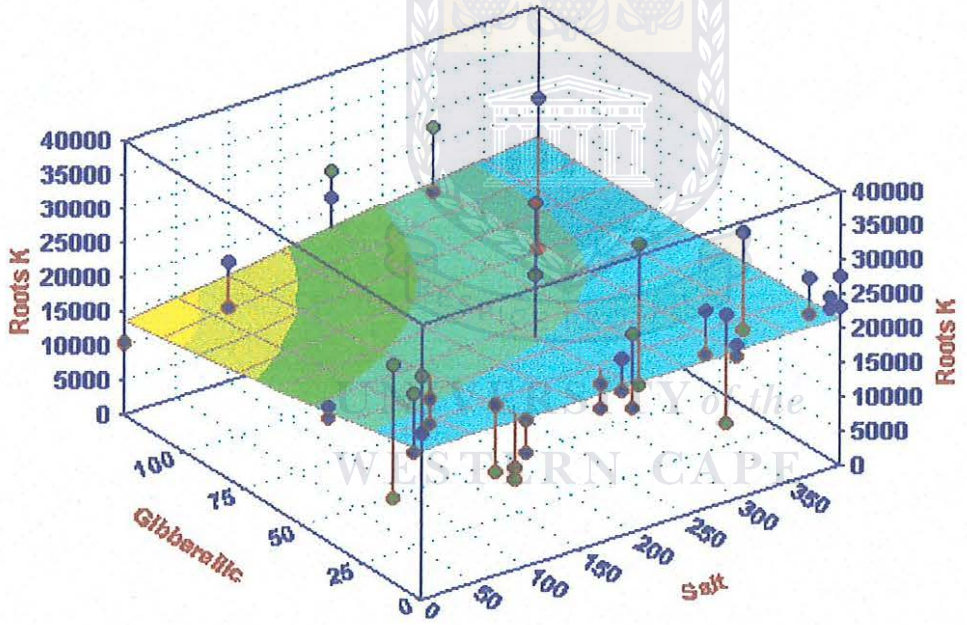

Figure 5.6.6. The interactive effects of salinity (mM) and GA_3 (µM) on root K^+ concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

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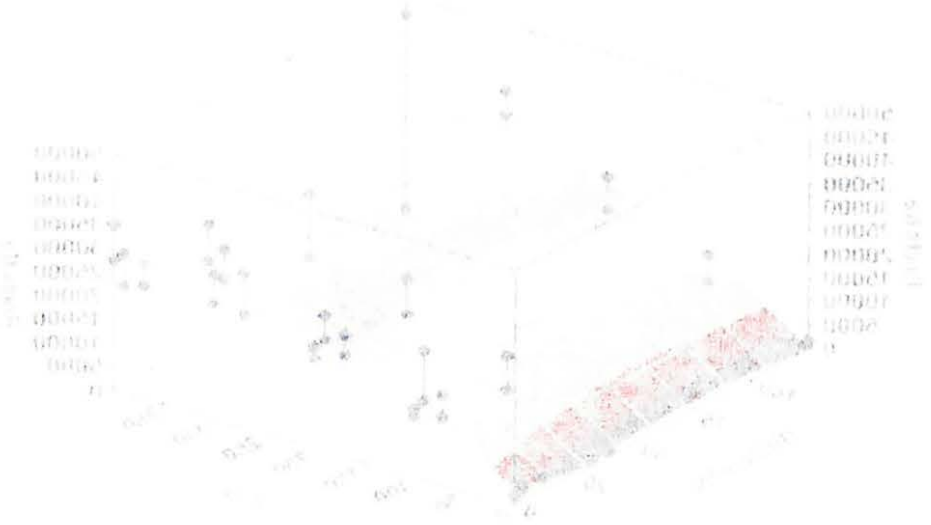

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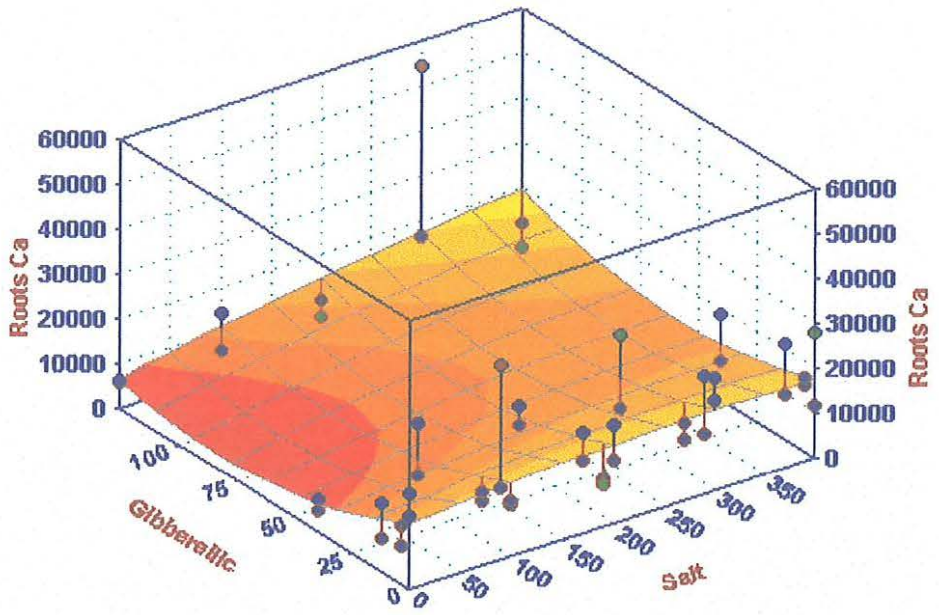

Figure 5.6.7. The interactive effects of salinity (mM) and GA_3 (µM) on root Ca^{2+} concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

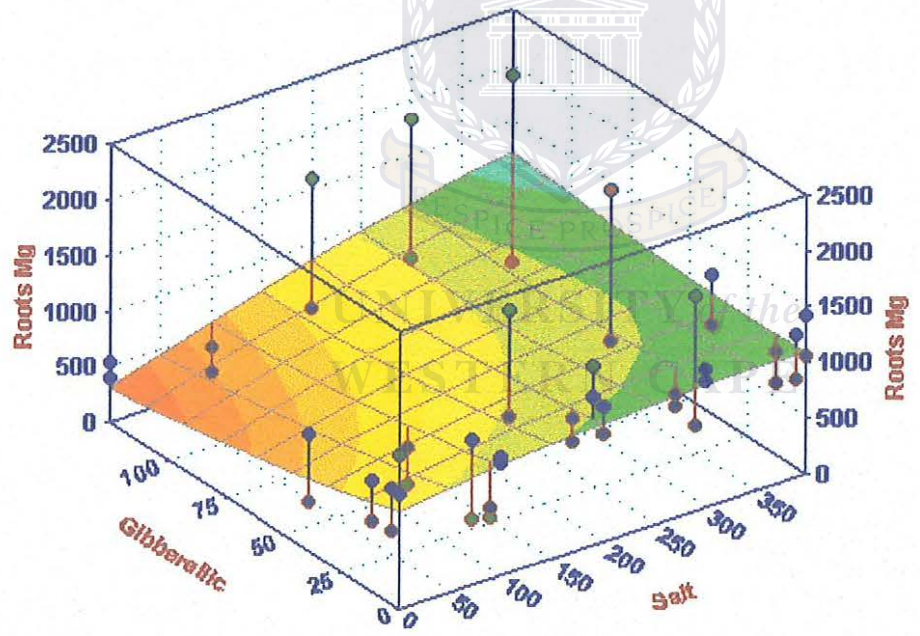

Figure 5.6.8. The interactive effects of salinity (mM) and GA_3 (µM) on root Mg^{2+} concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

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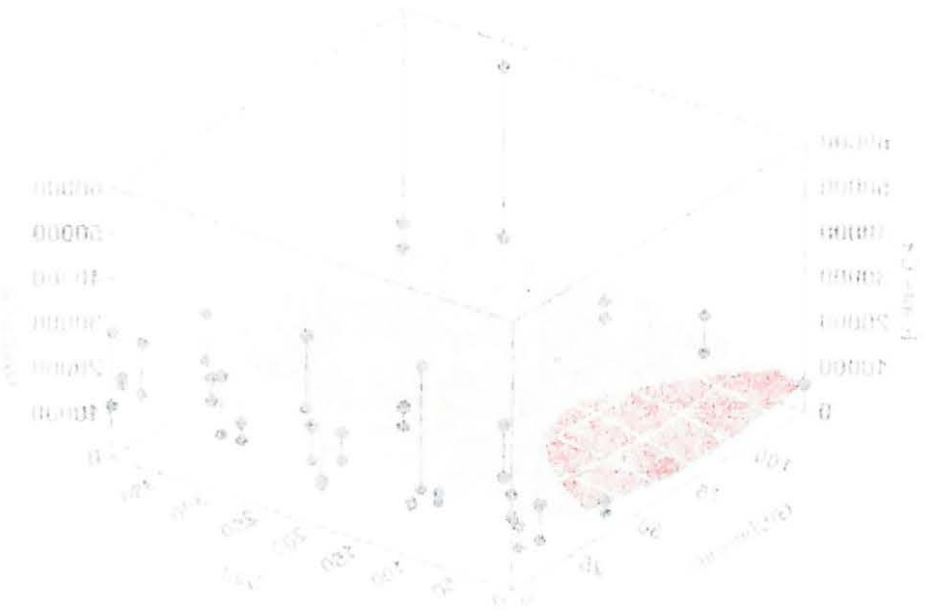

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Chapter 6:

Summary and conclusion

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The increased substrate NaCl suppressed both shoot and root growth of *E. strigosum* plants (Tables 2.61 and 4.6.1). It is well established that growth response to salinity stress can be described in terms of a two-phased model; osmotic and salt specific responses (Munns & Termaat, 1986). This was also the case for the response of *E. strigosum* plants. Thus, the factors observed, included leaf water loss (Table 4.6.1), accumulation of sodium ions to toxic levels and depression of shoot potassium, calcium and magnesium (Tables 3.6.1 and 5.6.1). The leaf water content decreased with increasing substrate salinity; a well known predominant effect of salinity. Thus, from concentrations as low as 100 mM, the shoot water content was reduced. These observations clearly indicate that *E. strigosum* plants are sensitive to salt stress. Injurious effects such as necrosis, marginal leaf burns and a crinkly leaf appearance also confirmed the sensitivity of this plant to salt stress. The toxic effects of salinity were demonstrated by the accumulation of Na^+ (Tables 3.6.1 and 5.6.1). Nutrient imbalances, on the other hand, were observed as lowered shoot concentrations of K^+, Ca^{2+} and Mg^{2+}. The other factors of salinity stress, reported in the literature, such as effects on stages of development and changes in the growth medium, were not evaluated in the present study.

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The salt sensitivity of *E. strigosum* plants could also be explained by their inability to maintain K selectivity, a tolerance response of glycophytes (Young *et al.*, 1990; Ashraf *et al.*, 2001 and Shirazi *et al.*, 2002). Thus, the shoot K^+ concentration started to decrease when the Na^+ content was increased significantly, relative to controls (Tables 3.6.1 and 5.6.1). The inability to maintain K selectivity under salt stress seems to revolve around the replacement of K^+ with Na^+(Tozlu *et al.*, 2000). In some plants this is controlled by

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addition of calcium to the growth medium (Blatt & Theil, 1993 and Song & Fujiyama, 1996). In the present study, it has been demonstrated that there was not a sufficient supply of calcium to trigger the selection of K^+ (Table 5.6.1). The decrease of K^+ in shoots is also correlated with a plants osmoticum (Marschner, 1995).

Growth depression via the decrease of shoot K^+, has also been observed in kinetin treated plants (Table 3.6.2). Apart from its involvement in leaf osmotic adjustment, K^+ has been found to play a synergistic role with kinetin on growth response. Thus, the inhibitory effects observed in shoots of plants treated with increasing levels of kinetin, were accompanied by depression of the K^+ concentration. It was also observed that in roots, growth was triggered by treatments with kinetin and so was the increase of K^+ content. These findings are in line with the view that cytokinin in shoots is not as effective as those in roots (Chen *et al.*, 1985). Thus, the sensitivity of shoots in response to cytokinins is controlled by K^+ ions. Hence, the kinetin stimulation of root growth paralleled accumulation of potassium in roots of *E. strigosum* (Table 3.6.2). Several hypotheses proposed for the mechanism of action of K^+ ions and kinetins include factors of osmotic adjustment. These findings suggest that the inhibition of growth during exposure to kinetin might be due to a decrease of K^+ ions in shoots.

The exposure of salt stressed plants to kinetin was aimed at evaluating the efficiency of kinetin in the improvement of salinity-induced effects. It was observed that treatments of salt stressed *E. strigosum* plants with kinetin, decreased growth (Table 2.6.1) and exacerbated ionic effects (Table 3.6.1). The salinity-induced ionic effects include the

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accumulation of potentially toxic sodium ions and the decrease of potassium, calcium and magnesium ions (Figures 3.6.2.1-4). Thus, the selection mechanism was not in favour of K^+. These findings suggest that both tolerance mechanisms used during exposure to salinity, by many glycophytes, were not enhanced by addition of kinetin in the studied plant. These observations are in agreement with those of Benzoini *et al.* (1974) and Aldesuquy & Ibrahim (2001).

Gibberellic acid, on the other hand, managed to enhance growth in both salinated and salt free plants. Even though gibberellic acid enhanced growth in these media, it was observed that at higher concentrations it started to reduce growth (Figures 4.6.1-3). These observations suggest that GA_3 is effective only at low concentrations. It is interesting to note that ionic-specific effects such as Na^+ accumulation and decrease of K^+, Ca^{2+} and Mg^{2+} persisted regardless of treatments with GA_3 (Figures 5.6.1-4). Kawasaki and his coworkers (1978) reviewed that GA_3 and K^+ or Na^+ act synergistically in promoting the elongation of lettuce hypocotyls segments. However, this cannot be used to explain shoot growth in *E. strigosum* plants because Na^+ in this plant has no nutritional value. Na^+ is nutritionally important in selected halophytes and some C_4 species (Raven *et al.*, 1992).

The depression of K^+ in the shoots of kinetin and gibberellin treated salanized plants, whether followed by growth or not, suggests that treatment of *E. strigosum* plants with these hormones failed to stimulate a salt exclusion mechanism. Thus, it has failed to induce K^+ selection in shoots. These observations are in contrast with those of Aldesuquy & Ibrahim (2001) who found that when *Triticum aestivum* plants were pretreated with

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GA_3, IAA and ABA, before it was exposed to seawater, GA_3 effectively increased the absorption of K^+. Their findings were based on the assumptions that GA increased the absorption of K^+ by plants. It is possible that in the case of GA_3-treated plants, a salt inclusion mechanism was stimulated. Marschner (1995) postulated that plants utilizing a salt inclusion mechanism, grow under salt stress conditions. In the present study, gibberellic acid enhanced growth and accumulation of Na^+ ions in both shoots and roots. It is therefore recommended that this be further explored. Moreover, one should evaluate the osmotic adjustment effects by measuring the induction of osmoprotectants such as proline, glycine, betaine (trimethylglycine) and a low weight protein, osmotin, when salt stressed plants are treated with kinetin and GA_3. It has already been observed that salt tolerance of kinetin treated *Vigna sinensis* and *Zea mays*, is associated with regulation of proline, and other osmoprotectants (Nemat Alla *et al.*, 2002). Whether this can be used to explain growth stimulation by GA_3 in *E. strigosum* plants remains unknown at the moment.

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References

ALDESUQUY H S, IBRAHIM A H (2001) Water relations, abscisic acid and yield of wheat plants in relation to the interactive effect of seawater and growth bioregulators. Journal of Agronomy and Crop Science 187: 97-104.

ASHRAF M, NAZIR N, MCNEILLY (2001) Comparative salt tolerance of amphidiploid and diploid *Brassica* species. Plant Science 683-689.

BENZOINI A, MIZRAHI Y, RICHMOND A E (1974) Effect of kinetin on plant's response to salinity. New Phytologist 73: 315-319.

BLATT M R, THEIL G (1993) Hormonal control of ion channel gating. Annual. Reviews of Plant Physiology and Plant Molecular Biology 44: 543-567.

CHEN C-M, ERTL J R, LEISNER S M, CHANG C-C (1985) Localization of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiology 78: 510-513.

KAWASAKI H TAKADA H, KAMISAKA S (1978) Requirement of sodium chloride for the action of gibberellic acid in stimulating hypocotyls elongation of a alophyte, *Salicornia herbacea* L. Plant & Cell Physiology. 19: 1415-1425

MARSCHNER H 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, San Francisco.

MUNNS R, TERMAAT A (1986) Whole plant response to salinity. Australian Journal of Plant Physiology 13: 142-160.

NEMAT ALLA M M, YOUNIS M E, EL-SHIHABY O A, EL- BASTAWISY M (2002) Kinetin regulation of growth and secondary metabolism in waterlogging and salinity

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treated *Vigna senensis* and *Zea mays*.

http://www.zfr.pan.krakow.pl/ang/app/2002no1/1.html downloaded on 15/01/03

RAVEN P H, EVERT R F, EICHHORN S E (1992) Biology of plants. 5th edn. Worth Publishers. New York.

SHIRAZI M U, KHANZANDA B, ALI M, U-ISLAM E, MUJTABA S M, ANSARI R ALAM S M, KHAN M A, ALI M (2002) Response of three wheat genotypes grown under saline medium to low/ high potassium levels. Acta Physiologia Plantarum 24: 157- 161.

SONG J Q FUJIYAMA H (1996) Differences in response of rice and tomato subjected to sodium salinization to the addition of calcium. Soil Science and Plant nutrition 42: 503-510.

TOZLU I, MOORE G A, GUY C L (2000) Effects of increasing NaCl concentration on stem elongation, dry mass production and macro- and micro nutrient accumulation in *Poncirus trifolita*. Australian Journal of Plant Physiology 27: 35-42.

YOUNG, Y. W. NEWTON, R. J. & MILLER, F. R. 1990. Salinity tolerance in sorghum. I. Whole plant response to sodium chloride in *Sorghum bicolor* and *Sorghum halepense*. Crop Science 30: 775- 78

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Figure 2.6.1. Reduced growth of *Erucastrum strigosum* due to salinity: left 0 mM NaCl and 0µM kinetin and right 400mM NaCl and 0µM kinetin treated plants.

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Table 2.6.2. The effect of Kinetin on roots and shoot growth; and water content (% of dry mass) of *Erucastrum strigosum* plant. Differences between treatment means of the measured parameters were tested for level significance (P=0.05) at N=10 using LSD; means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of Kinetin (µM)					
		0	**4**	**12.5**	**40**	**125**	**LSD**
Shoots	**Fresh mass (g)**	**11.15A**	**10.61A**	**11.03A**	**9.78 A**	**9.68 A**	**3.12**
	Dry mass (g)	**1.48 A**	**1.46 A**	**1.46 A**	**1.39 A**	**1.47 A**	**0.49**
	Water content (% of d. m.)	**636.33 A**	**650.03 A**	**735.42 A**	**605.47 A**	**591.01 A**	**210.31**
Roots	**Dry weight (g)**	**0.43A**	**0.64C**	**0.86C**	**0.50 C**	**0.69 C**	**0.46**

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Figure 2.6.2. Reduced growth of *Erucastrum strigosum* due to kinetin: (left) 125 µM kinetin + 0 mM NaCl and (right) 0 µM kinetin + 0 mM NaCl treated plants.

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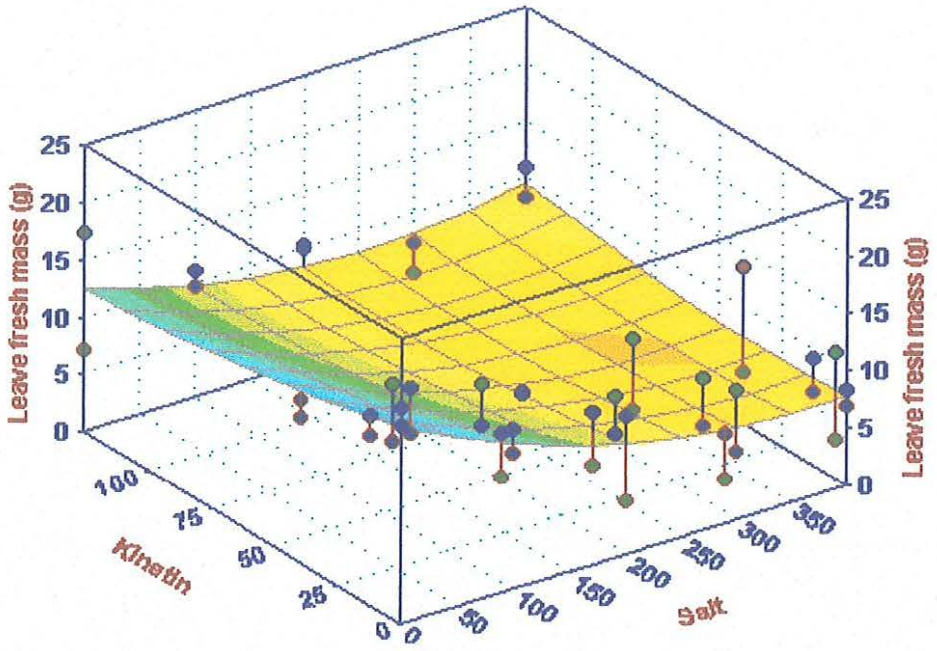

Figure 2.6.3. The interactive effects of salinity (mM) and kinetin (µM) on shoot fresh mass production (g) of *Erucastrum strigosum* plants, using ANOVA

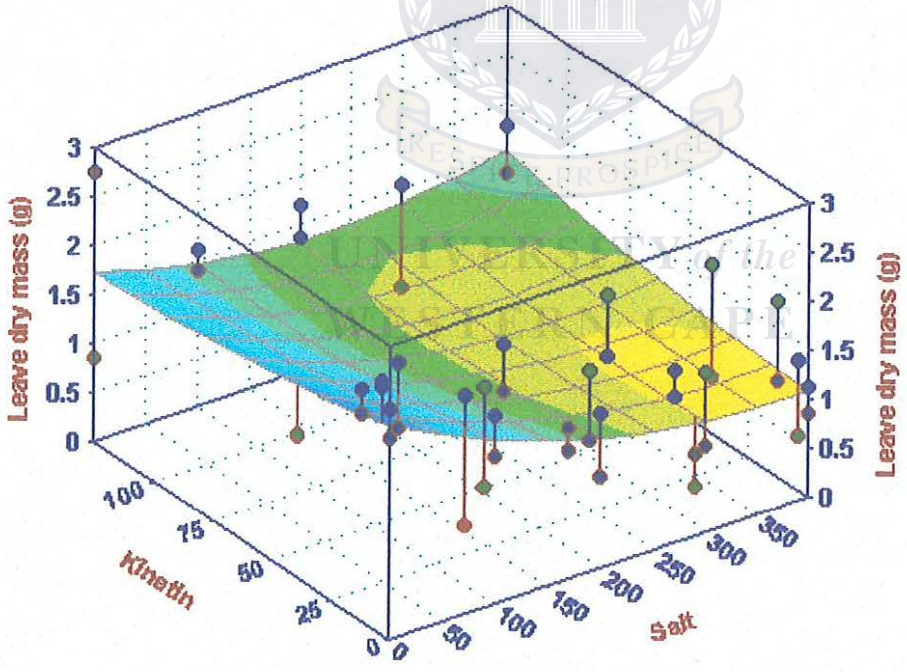

Figure 2.6.4 The interactive effects of salinity (mM) and kinetin (µM) on shoot dry mass production (g) of *Erucastrum strigosum* plants, using ANOVA.

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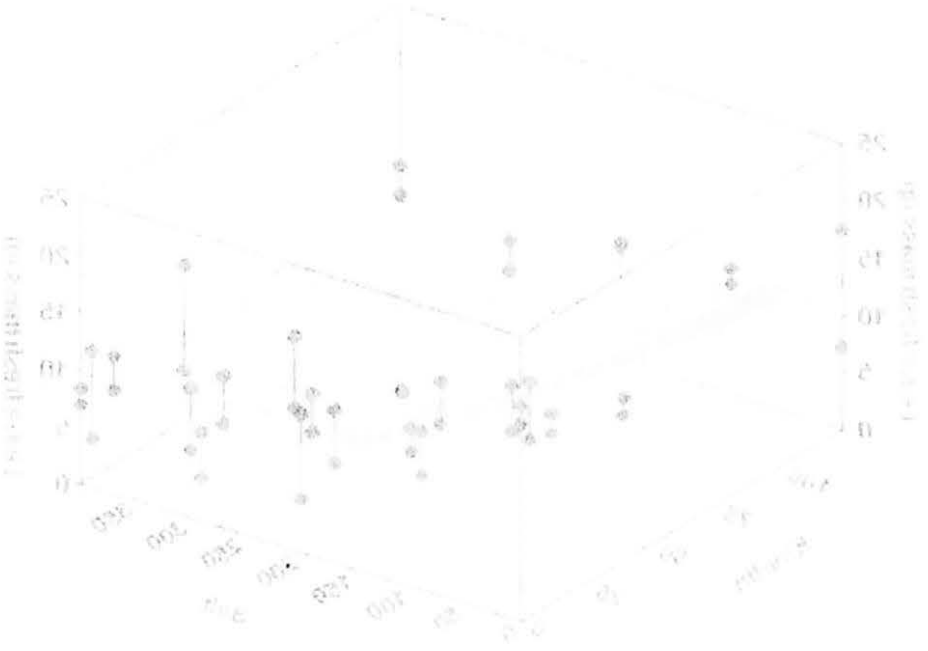

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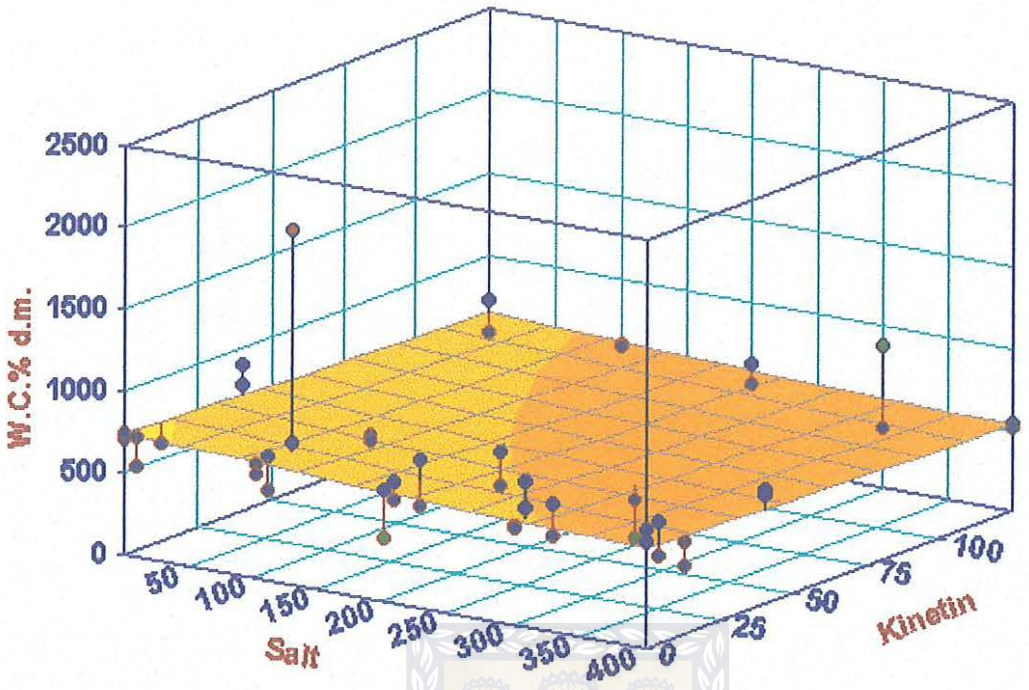

Figure 2.6.5. The interactive effects of salinity (mM) and kinetin (µM) on shoot water content (% of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

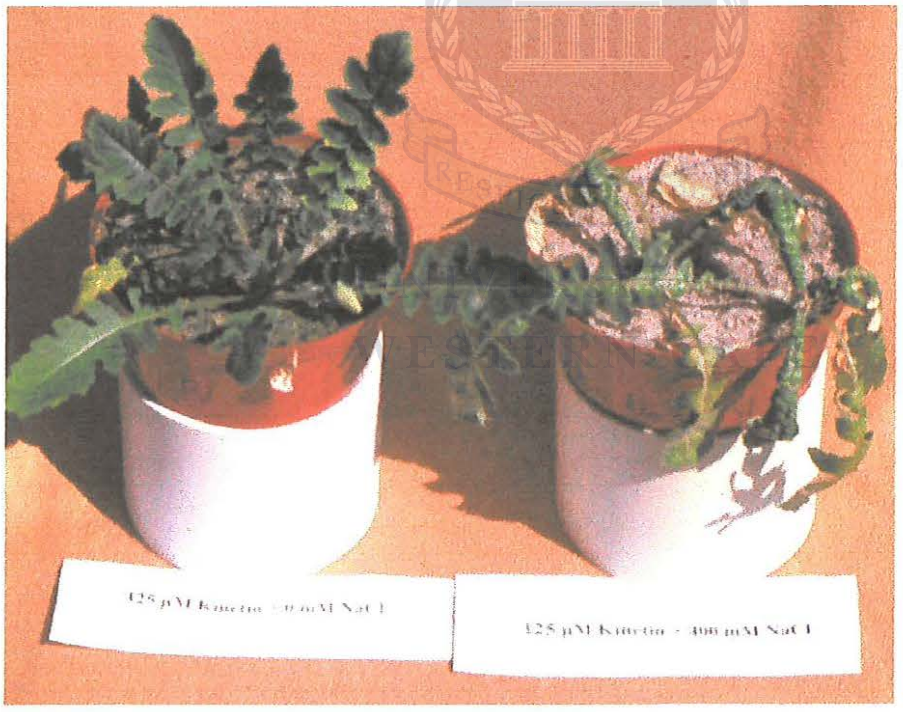

Figure 2.6.6. Reduced growth of *Erucastrum strigosum* due to simultaneous application of salinity and kinetin; (left) 125 µM kinetin + 0 mM NaCl and (right) 125 µM kinetin + 400 mM NaCl treated plants.

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Figure 2.6.7. Reduced growth *Erucastrum strigosum* due to simultaneously application of salinity and kinetin: (left) 0 µM kinetin + 400 mM NaCl and (right) 125 µM kinetin + 400 mM NaCl treated plants.

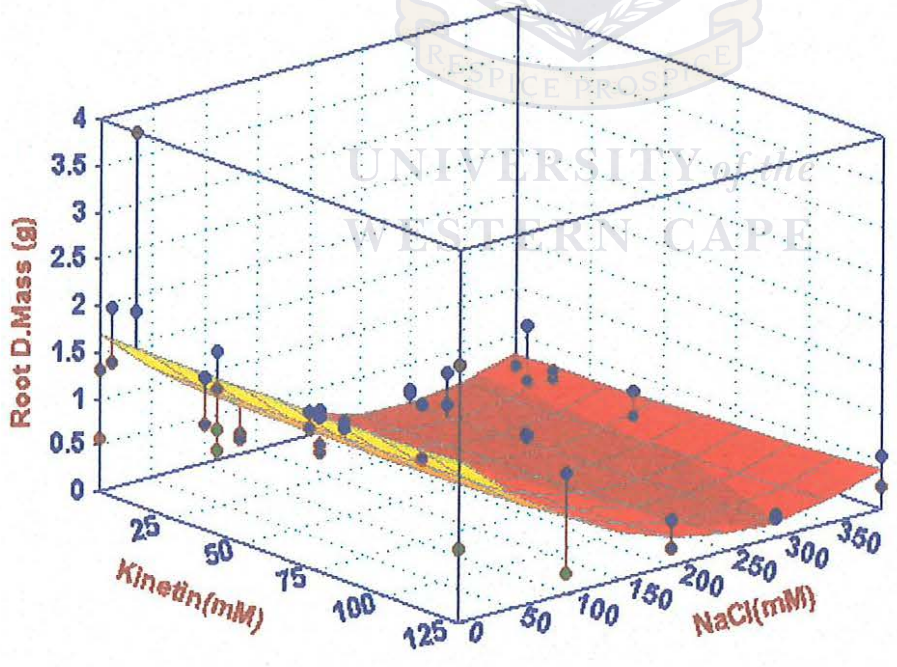

Figure 2.6.8. The interactive effects of salinity (mM) and kinetin (µM) on root dry weight production (g) of *Erucastrum strigosum* plants, using ANOVA.

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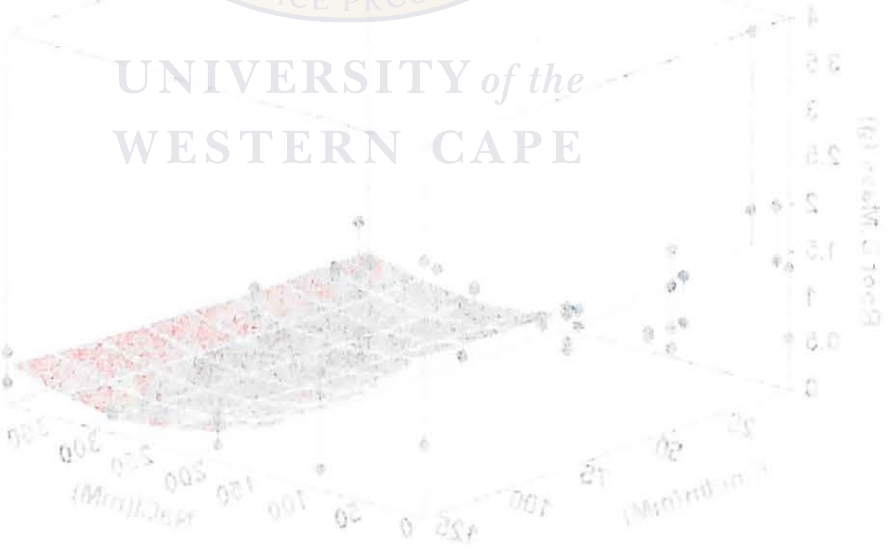

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Chapter 3

The effects of exogenous kinetin application on the cationic status of salt stressed *Erucastrum strigosum* plants.

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3.1 Introduction.

The salt-induced slow growth of plants can also arise from disturbances of ionic status. This is a two way model. At first, salt stressed plants accumulate Na^+ and Cl^- to levels that are considered toxic. These levels are considered toxic because they are often followed by necrosis, marginal leaf burns and leaf bronzing (Bernstein, 1975). Secondly, salinity affects the ability of plants to take up and transport essential mineral nutrients to the aerial parts (Marschner, 1995 and Turan & Sezen, 2002). However, there seems to be a close correlation between ion toxicity and nutritional imbalances (Hale & Orcutt, 1987). For example, it has been observed that Cl^- and Na^+ ions compete with NO_3^- and K^+ ions respectively at the site of uptake (Marschner, 1995). NO_3^- in plants facilitates the reduction of nitrogen by activating nitrate reductase. K^+ on the other hand, functions in osmotic adjustment, the activation of respiratory and photosynthetic enzymes, and the promotion of starch and protein synthesis (Hopkins, 1999). It has therefore become apparent, that the disturbance in the uptake and transport of these ions to aerial parts during salinity stress result in depressed ionic activities. Sodium and chlorine-induced depression of nutrient activities produce extreme ratios of Na^+/ Ca^{2+}, Na^+/ K^+, Ca^{2+}/ Mg^{2+} and Cl^-/ NO_3^-, that are often accompanied by susceptibility to injury (Turan & Sezen, 2002).

It has long been shown that plant hormones are involved in ion transport, even under nutrient deficient conditions (Benzoini *et al.*, 1974; Dhakal & Erdei, 1986 and Vodnik *et al.*, 1999). The decrease in the nutrient supply of plants results in a decreased production

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of cytokinins. Therefore, nutrient retention in plants should be ameliorated by exogenous application of cytokinins. For example, it has been observed that increases in cell plasticity and cell enlargement of fusciccon, auxin and kinetin treated plants, were accompanied by proton extrusion, K^+ uptake, and an increase in the transmembrane electric potential, possibly creating an osmotic gradient that improves the water status of a plant (Marre *et al.*, 1974). Kuiper and colleagues (1989) found that restriction of mineral nutrient supply in *Plantago major* ssp. *pleiosperma* plants, was partially alleviated by the exogenous application of benzyadenine. Van Stevenick (1976) observed that when conditions are favourable, plant hormones influence ion uptake and transport through membrane properties, as well as its transport processes. In accordance with this, Dhakal and Erdei (1986) demonstrated that the application of cytokinins under optimal growth conditions, alters the selectivity for ions in a membrane via Ca^{2+} modulation. Furthermore, it was observed that treatment with cytokinins, together with mychorrhizal fungi, influenced the uptake and transport of K, Ca, Na and P, by increasing the plants membrane fluidity (Vodnick *et al.*, 1999). These findings suggest that under both nutrient limiting and nutrient sufficient conditions, exogenous application of cytokinins modifies the nutritional status of plants by altering membrane properties and transport processes.

There is an enormous amount of evidence that exogenous cytokinins affect the ion transport in plants. However, Benzoini and his colleagues (1974) found that Na^+ accumulation was accompanied by depression of K^+, characteristics of salt stress, which persisted even in kinetin treated plants. The efficiency of cytokinins in the improvement of salt induced nutritional disorders requires further elucidation. The present study

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therefore aimed at investigating the role of kinetins on the cationic status of salt-stressed *Erucastrum strigosum* plants.

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3.2. Materials and methods

3.2.1. Plant material and growth conditions (refer to 2.2.1)

3.2.2. Chemical analysis

The oven-dried material was finely ground by pestle and mortar or in a Wiley Intermediate mill, depending on the amount of sample available. In plants with high yields, 0.4g of each sample's ground material was weighed, enfolded in cigarette paper and transferred to a digestion tube. In plants with small yield, whatever was contained in the sample bottles, was weighed and used. A H_2SO_4 - H_2O_2 digestion mixture was prepared and 4.4 cm^3 added to each digestion tube (Allen *et al.*, 1986). The digestion tubes were transferred to the digestion block where they were heated until a colorless or straw-coloured solution was obtained. The digestion tubes were removed from the digestion block, distilled deionised water added to the tubes and the solutions were allowed to cool down. The solutions were then filtered through Scheicher and Schull #595 filter papers and made up to 100cm^3 in volumetric flasks with distilled deionised water. A Pye UniCam SP9 Solaar M Series Atomic Spectrophotometer was used in the flame mode to determine the concentration of the four major cations (Ca^+, Mg^+, Na^+ and K^+) in the plant digest (Allen *et al.*, 1986).

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3.2.3. Statistical analysis

The statistical analysis of the data was carried out using SAS version 8.2 computer package (SAS, 1999). The difference between the means of the cation concentrations of shoots and roots was tested for LSD at the P=0.05 level. Whereas, the data collected from plants treated simultaneously with kinetin and salinity were subjected to analysis of variance.

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3.3. Results

3.3.1. The effects of salinity on the cation status of *Erucastrum strigosum* plants.

The effects of NaCl, applied to the growth medium at varying concentrations, on the cation content of *E. strigosum* plants, were determined in shoots and roots and expressed as $mg.kg^{-1}$ of dry mass and summarized in Table 3.6.1. Generally, the sodium concentration in shoots was significantly higher ($P<0.05$) than that of roots. Thus, both control and NaCl treated plants accumulated more sodium in shoots than in roots. Comparisons between treatments showed that the sodium concentrations increased progressively with salinity treatments. The distribution pattern of potassium to shoots was similar to that of sodium. However, the shoot potassium concentration of control plants was higher than that of saline treated plants. In saline treated plants, the potassium concentration was higher at lower treatments than at higher treatments. Because the protein pump responsible for the transportation of potassium and sodium to the shoots is the same, it is important to note that in control plants the sodium concentration is maintained at levels lower than those of potassium. However, when NaCl is added to the treatment solution, sodium concentration became higher than the potassium concentration. These findings suggest that the sodium-potassium pump is altered by salinity treatments of the medium.

The shoot calcium concentration of control plants and 100 mM NaCl treated plants was higher than that of roots. At higher salinity treatments roots accumulated more calcium

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than shoots. However, there were no significant differences found for calcium concentration in the shoots when comparing control plants with treated plants, and between treatments. In roots, the calcium concentration was proportional to the salinity treatments. Thus, it was lower at lower salinity treatments and higher at higher treatments.

In shoots, the magnesium concentration of control plants was significantly more than that of roots. Nevertheless, there was a significant progressive decrease with increasing salinity in shoot magnesium concentrations, but this did not fall to levels lower than those of roots. In roots, the magnesium concentration was not significantly affected by treatment with salinity. Thus, the magnesium concentration remained more or less the same at all treatments.

3.3.2 The effects of kinetin on the cation status of *Erucastrum strigosum* plants.

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The effect of kinetin treatment in the growth medium on the cationic content of the *E.strigosum* plants was measured in roots and shoots in $mg.kg^{-1}$ of dry mass and summarized Table 3.6.2. It was generally observed that sodium accumulation was more in shoots than in roots. Thus shoot sodium was higher than that of roots in both treated and untreated plants. Comparison between treatments revealed that the shoot sodium concentrations increased consistently with increasing kinetin treatments. The shoot potassium concentration on the other hand, decreased with kinetin treatments.

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Nevertheless, shoot potassium was maintained at levels higher than that of roots. These findings suggest that the sodium-potassium pump responsible for the passage of potassium and sodium, favoured sodium ions at all kinetin treatments. However, in roots, there were no significant differences observed in the selectivity of potassium and sodium between the treatments. Thus, sodium and potassium concentrations were more or less the same at all the treatments. The shoot and root calcium concentration were not affected by different levels of kinetin treatment. Similar effects were observed for magnesium concentration.

3.3.3.1 The interactive effects of salinity and kinetin on the cation status of *Erucastrum strigosum* plants.

The interactive effects of kinetin and salinity treatments, at varying concentrations, on the distribution of cations within *E. strigosum* were measured in roots and shoots in $mg.kg^{-1}$ of dry mass (Figures 3.6.1 to 3.6.8). Generally, shoots accumulated more sodium than roots at all salinity levels, regardless of kinetin treatment (Figures 3.6.1 and 3.6.5). The same distribution pattern was observed for potassium (Figures 3.6.2 and 3.6.6). Comparing Figure 3.6.1 with Figure 3.6. 2, shows that sodium increased and potassium decreased with increasing salinity treatments, even in kinetin treated plants. These findings show that at lower salinity treatments the sodium-potassium pump was in favour of potassium at all kinetin levels and at higher treatments it favoured sodium, irrespective of kinetin treatments. Therefore, treatments with kinetin did not trigger a tolerance response to salt stress. The calcium concentration, on the other hand, was greater in roots

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than in shoots at higher salt treatments (Figures 3.6.3 and 3.6.7). However, at lower salt treatments, the shoot calcium concentration was more than that of the roots. A comparison made across the treatments reveals that the shoot calcium concentration was not affected by treatment with kinetin and salinity (Figure 3.6.3). Magnesium was generally higher in shoots than in roots at all salinity-kinetin treatments (Figures 3.6.4 and 3.6.8). The shoots magnesium concentration was higher at lower salt treatments than at higher treatments.

In roots, both sodium (Figure 3.6.5) and potassium (Figure 3.6.6) concentrations increased with increasing treatments. The sodium concentration was highest at the highest salinity-kinetin treatments. On the other hand, it was observed that the potassium concentration was highest at lower kinetin and highest salinity treatments. In comparison of the two figures, the sodium-potassium pump favoured potassium at lower salinity-kinetin concentrations but at higher treatments the pump favoured sodium. Root calcium concentration increased with moderate kinetin treatments and with salinity treatments at all salinity-kinetin levels (Figure 3.6.7). The highest root calcium concentration was observed at lower kinetin and higher salinity treatments. These findings suggest that at higher salinity treatments, addition of kinetin to the uptake solution, reduced the root calcium concentration. Root magnesium concentration was not affected.

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3.4 Discussion.

It has once more emerged, that growth depression by high salt concentrations also arises from ion toxicity as well as nutrient imbalances. According to Marschner (1995), ion toxicity is the excessive uptake of Na^+ and Cl^-, and nutrient imbalance is the depression in the uptake and transport of essential nutrients. In the present study, it was observed that in shoots, Na^+ accumulation was significantly greater ($P<0.05$) than in roots at all salt concentrations other than in control plants (Table 3.6.1). This suggests that there was no blocking-exclusion mechanism preventing the accumulation of sodium in shoots of *E. strgosum* plants. It is a well-known phenomenon that some glycophytes tolerate salinity by restricting the transfer of sodium from roots to shoots (Greenway & Munns, 1980).

Transport processes, such as K/Na selection, have been isolated and characterized in many plants (Young *et al.*, 1990; Ashraf *et al.*, 2001 and Shirazi *et al.*, 2002). In this mechanism the influx of K^+ into xylem tissues is accomplished by blocking of Na^+ (Jeschke & Nassery, 1981). However, in the present study, the decrease of potassium in shoots with increasing substrate salinity suggests that K/Na selection had a reduced affinity for Na^+ extrusion. This characterization of sodium and potassium profiles in shoots of *E. strigosum* revealed that a similar mechanism might have been in operation. In plants that utilize salt inclusion as the tolerance strategy, growth may be enhanced by NaCl salinity and is often accompanied by an increase in the shoot levels of Cl^- and Na^+ and a decrease in that of K^+ and Ca^{2+} (Marschner, 1995). Growth in the studied plants was reduced (Table 2.6.1 and Figure 2.6.1), suggesting that it was sensitive to salt stress. Tattini (1994) and Lutts *et al.* (1995) reported similar findings for other plants. According

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to Marschner's (1995) classification, the *E. strigosum* plants fall under group B. In this classification, four groups have been identified depending on whether the K^+ ions are exchangeable with Na^+ ions or not. This exchange of potassium with sodium suggests that functions such as cell expansion, osmoregulation and cellular and whole plant homeostasis, operating under both saline and non-saline conditions, may be impaired (Tozlu *et al.*, 2000).

Ca^{2+}, on the other hand, are known to influence the K/Na selectivity of higher plants during exposure to salt stress. Calcium plays a crucial role in holding the plasma membrane's integrity intact and subsequently controls the membranes permeability in plants. In the current study, the results clearly indicate that the K/Na selectivity mechanism was in favour of Na^+, and the Ca^{2+} concentration was concomitantly either decreased or not affected at all in both treated and control plants. These findings are consistent with the hypothesis that the efficiency of the K/Na selection mechanism is controlled by the accumulations of Ca^{2+} in the cytoplasm. Therefore, it seems reasonable to conclude that the injurious effects of salt stress are primarily due to the inefficiency of the tolerance mechanism and a lack of Ca^{2+}. However, Ca^{2+} deficiency is often associated with tip burns in leaves (Marschner, 1995) but in the present study there were no traces of leaf tip burn in salt stressed plants. These findings suggest that even though Ca^{2+} failed to induce tolerance to salinity, its uptake and transport was not negatively affected by salt stress (Figures 3.6.3 and 3.6.7). Thus, the cell membrane integrity might not have been affected in the present study.

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The Mg^{2+} concentration in shoots, on the other hand, decreased, but no signs of Mg^{2+} deficiency were observed (Figures 2.6.1 and 3.6.4). Mg^{2+} deficiency is usually associated with chlorophyll degradation, which is manifested as leaf chlorosis (Tozlu *et al.*, 2000).

Plant growth response to kinetin treatment may be attributed to a synergistic relationship between K^+ supply and kinetin treatments (Green & Muir, 1979). In support of this view, Davies (1995) found that changes in a plants hormonal homeostasis are sometimes accompanied by lowered K/Na selectivity. In the present study, it was observed that the slight decrease in shoot growth with kinetin treatments (Table 2.6.2) paralleled the depression of shoot K^+ concentration (Table 3.6.2). In consistence with these findings, Mengel and Arneke (1982) found in their study that the decrease in shoot growth was due to K^+ deficiency. Potassium deficiency in plants is often associated with lowered turgor, cell size and leaf area and subsequently loss of water, which are common features of reduced plant growth (Marschner, 1995). However, the correlation between K^+ concentration and kinetin treatments, was not shown in roots (Table 3.6.2). This lack of correlation between kinetin treatments and root K^+ concentration suggests that it might have been overruled by other factors such as sensitivity of an organ to kinetin treatments (Davies, 1995 and Hopkins, 1999). Whether there is correlation between K^+ supply from roots to shoots and organ sensitivity to kinetin is not known at the moment.

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The results obtained from the simultaneous application of kinetin and salinity to *E. strigosum* plants clearly show that at higher treatments, the Na^+ content was raised to levels that were much higher than those of other cations in both roots and shoots. These findings suggest that the salinity induced toxic ionic effects persisted irrespective of kinetin addition. Furthermore, there was a depression of K^+ and Mg^{2+} concentrations accompanying Na^+ accumulation in treated plants. These findings suggest that transport processes, such as K/Na selectivity, operating at the entry of xylem tissue, were not improved by treatment with kinetin. The observations that kinetin-salinity treatment increases Na^+ and depresses K^+ and Mg^{2+} are in line with those of Benzoini *et al.* (1974). However, in their study, salt induced injurious effects were partially reduced by kinetin. Because K^+ are involved in osmoregulation, and subsequently in the increase in cell growth, its reduction is therefore associated with decreased plant growth (Bradley, 1999). These findings suggest that kinetin failed to stimulate a mechanism that could take care of Na^+, hence the persistence of injurious effects such as marginal leaf burns, necrotic lesions and a crinkly appearance (Figure 2.6.6). These results are similar to those mentioned by Marschner (1995) in which it was demonstrated that the increase of Na^+ content in leaves is associated with the appearance of necrotic lesions as well as leaf burns. The curling appearance of leaves, on the other hand, may be due to Cl^- accumulation. The curling suggests that both Na^+ and Cl^- accumulation influence injurious effects of salt-stressed plants.

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3.5 References

ALLEN S E, GRIMSHAW H M, ROWLAND A P (1986) Chemical analysis. In: Moore, P. D. and Chapman, S. B. (eds) Methods in plant ecology. Blackwell Scientific publications. London pp 285-337.

ASHRAF M, NAZIR N, MCNEILLY T (2001) Comparative salt tolerance of amphidiploid and diploid *Brassica* species. Plant Science 160: 683-689.

BENZOINI A, MIZRAHI Y, RICHMOND A E (1974) Effect of Kinetin on plant response to salinity. New Phytologist 73: 315-319.

BERNSTEIN L (1975) Effects of salinity and sodicity on plant growth. Annual Review of Plant Pathology 13: 295-312.

BRADLEY P (1999). Nutrients in hydroponics. http://www.hydrogarden.com/index.html downloaded on 12/11/02

DAVIES P J (1995) Plant hormones: Physiology, biochemistry and molecular biology. 2nd edn. Kluwer Academic Publishers. Netherlands.

DHAKAL M R, ERDEI L (1986) Long-term effects of plant hormones on K^+ levels and transport in young wheat plants of different K^+ status. Physiologia Plantarum 68: 632-636

GREEN J F, MUIR R M (1979) Analysis of the role of potassium in the growth effects of cytokinin, light and abscisic acid on cotyledon expansion. Physiologia Plantarum 46: 19-24.

GREENWAY H, MUNNS R (1980) Mechanisms of salt tolerance in non-halophytes. Annual Review of Plant Physiology 31: 149-190.

HALE M G, ORCUTT D M (1987) The physiology of plants under stress. John

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Wiley and Sons Inc. New York. Pp 93-100.

HOPKINS W G (1999) Introduction to plant physiology. 2nd edn. John Wiley & Sons, Inc. New York.

JESCHKE W D, NASSERY H (1981) K^+-Na^+ selectivity in roots of *Triticum, Helianthus,* and *Allium.* Physiologia Plantarum 52: 217- 224.

KUIPER D, KUIPER P J C, LAMBERS H, SCHMIT J, STAAL M (1989) Cytokinin concentration in relation to mineral nutrition and benzyladenine treatment in *Plantago major* L. ssp. *pleiosperma.* Physiologia Plantarum 75: 511-517.

LUTTS S, KINET J M, BOUHARMONT J (1995) Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. Journal of Experimental Botany 46: 1843-1852.

MARRE E, COLOMBO R, LADO P, CALDOGNO R (1974) Correlation between proton extrusion and stimulation of cell enlargement: effects of fusciccon and of cytokinins on leaf fragments and isolated cotyledons. Plant Science Letters 2: 139-150

MARSCHNER H (1995) Mineral nutrition of higher plants. 2nd ed. Academic Press. San Francisco.

MENGEL K, ARNEKE W W (1982) Effects of potassium water potential, the osmotic potential, and cell enlargement in leaves of *Phaseolus vulgaris.* Physiologia Plantarum 54: 402- 408.

SAS (1999) Sas/Stat User's guide, version 8.2. 1st printing. Volume 2. Sas institute Cary, North Carolina.

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SHIRAZI M U, KHANZANDA B, ALI M, U-ISLAM E, MUJTABA S M, ANSARI R, ALAM S M, KHAN M A, ALI M (2002) Response of three wheat genotypes grown under saline medium to low/ high potassium levels. Acta Physiologia Plantarum 24: 157- 161.

TATTINI M (1994) Ionic relations of aeroponically-grown olive plants during salt stress. Plant and Soil 161: 251-256.

TOZLU I, MOORE G A, GUY C L (2000) Effects of increasing NaCl concentration on stem elongation, dry mass production and macro- and micro nutrient accumulation in *Poncirus trifolita*. Australian Journal of Plant Physiology 27: 35-42.

TURAN M, SEZEN Y (2002) Effect of salt stress on plant nutrition. http://www.toprak.org.tr/isd/can_73htm downloaded on 05/09/02

VAN STEVENICK R F M (1976). Effects of hormones and related substances on ion transport. *In* Lüttge U and Pitman M U (eds.), Encyclopedia of Plant Physiology, Springer-Verlog, Berlin. pp. 307-342.

VODNICK D, JENTSCHKE G, FRITZ E, GOGALA N, GODBOLD D L (1999) Root-applied cytokinin reduces lead uptake and affects its distribution in Norway spruce seedlings. Physiologia Plantarum 106: 75-81.

YOUNG Y W, NEWTON R J, MILLER F R (1990) Salinity tolerance in sorghum. I. Whole plant response to sodium chloride in *Sorghum bicolor* and *Sorghum halepense*. Crop Science 30: 775- 781.

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3.6 Appendix

Table 3.6.1. The effect of salinity on the cation status (mg. kg^{-1} d. m.) of *Erucastrum strigosum* shoots and roots. The differences between the means were tested for level of significance (P0.05) using LSD at N=10. Means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of NaCl (mM)					
	Cations (mg. kg^{-1})	0	100	200	300	400	LSD
Shoots	[Na]	4377 A	58879 B	75020 BC	92484 C	96968 C	18251
	[K]	52452 A	29758 B	25748 BC	26486 C	25034 BC	3701
	[Ca]	11357 A	11701 A	10474 A	11443 A	10994 A	6693
	[Mg]	8257 A	5226 B	4061 C	4285 BC	3964 C	938.35
Roots	[Na]	597 B	8266 B	27086 A	27088 A	25342 A	10439
	[K]	12486 B	13482 B	18416 BA	18886 BA	23497 A	7821
	[Ca]	5967 B	8380 B	14928 BA	19034 BA	23154 A	3259
	[Mg]	1444 A	4719 A	3702 A	3845 A	3136 A	3276

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Table 3.6.2. The effect of kinetin on the cation status (mg. kg^{-1} d. m.) of *Erucastrum strigosum* shoots and roots. The differences between the means were tested for level of significance (P0.05) using LSD at N=10. Means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of kinetin (µM)					
	Cations (mg. kg^{-1})	0	4	12.5	40	125	LSD
Shoots	[Na]	51126B	63425BA	64313BA	72752A	76112A	18251
	[K]	35179A	30368B	30536B	32344BA	29693B	3701
	[Ca]	11946 A	11859 A	10550 A	11022 A	10591 A	6693
	[Mg]	5372 A	5026A	4864A	5557A	4975A	938.35
Roots	[Na]	14733A	13923A	16520A	19996A	23206A	10439
	[K]	17224A	17027A	13415A	19154A	15385 A	7821
	[Ca]	16210A	16911A	10336 A	17275 A	10730 A	3259
	[Mg]	2823A	2453A	5139A	3362A	3069A	3276

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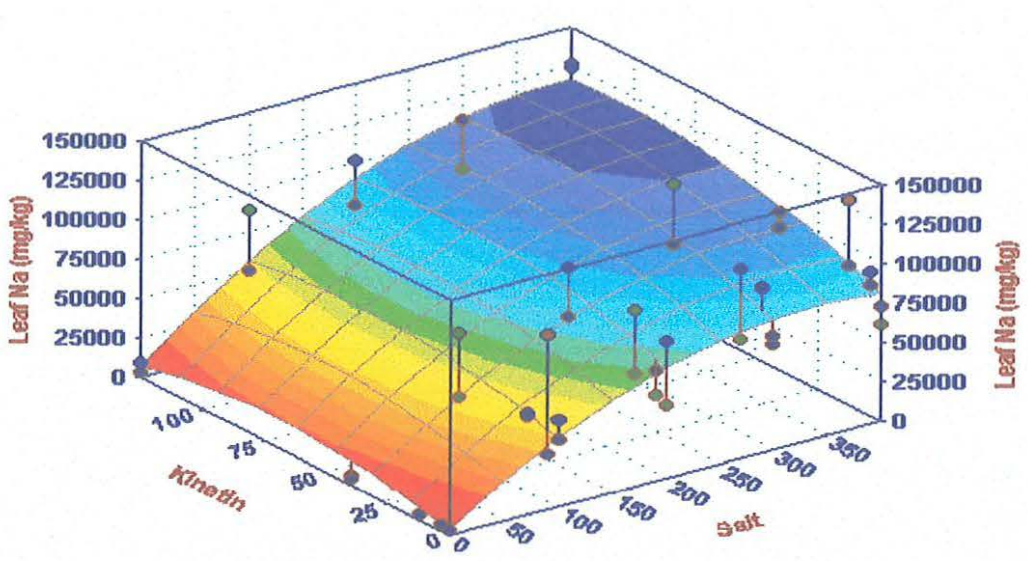

Figure 3.6.1. The interactive effects of salinity (mM) and kinetin (µM) on shoot Na^+ concentration (mg. Kg^{-1} of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

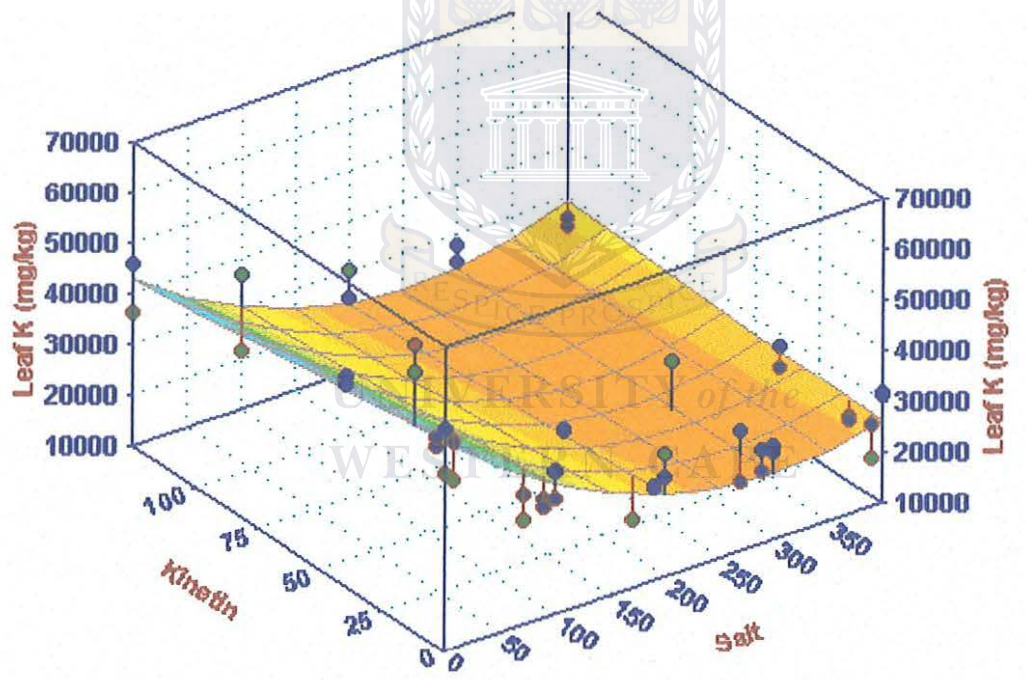

Figure 3.6.2. The interactive effects of salinity (mM) and kinetin (µM) on shoot K^+ concentration (mg. Kg^{-1} of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

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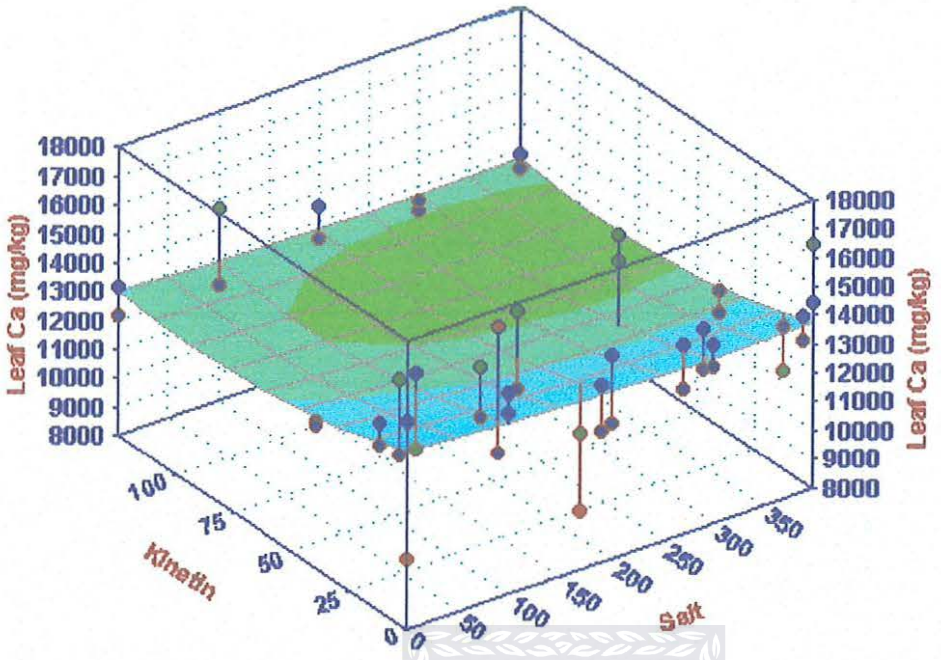

Figure 3.6.3. The interactive effects of salinity (mM) and kinetin (µM) on shoot Ca^{2+} concentration (mg. kg^{-1} of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

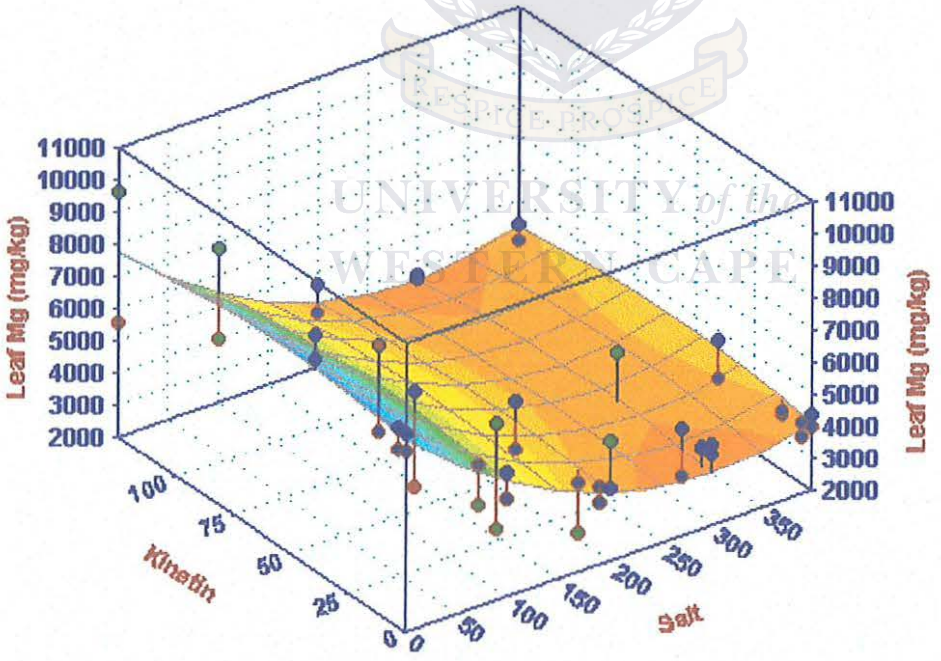

Figure 3.6.4. The interactive effects of salinity (mM) and kinetin (µM) on shoot Mg^{2+} concentration (mg. kg^{-1} of dry mass) *Erucastrum strigosum* plants, using ANOVA.

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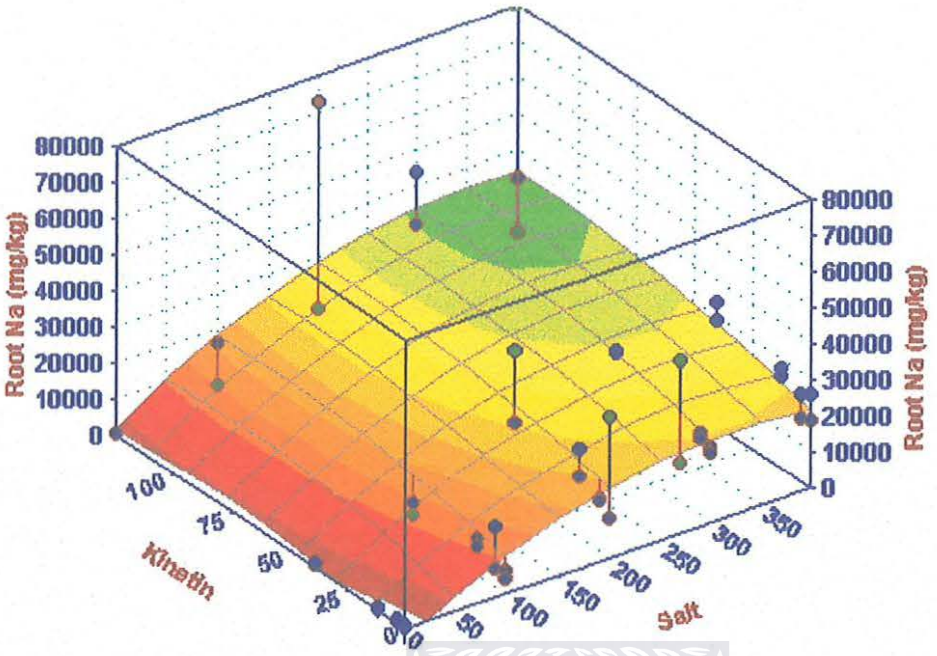

Figure 3.6.5. The interactive effects of salinity (mM) and kinetin (µM) on root Na^+ concentration (mg. kg^{-1} of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

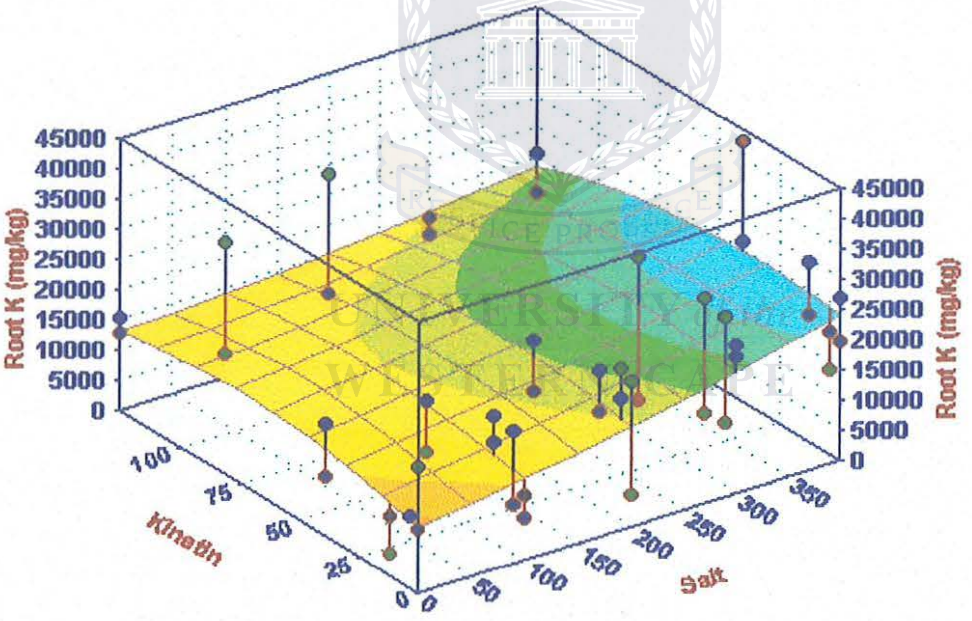

Figure 3.6.6. The interactive effects of salinity (mM) and kinetin (µM) on root K^+ concentration (mg. kg^{-1} of dry mass) *Erucastrum strigosum* plants, using ANOVA.

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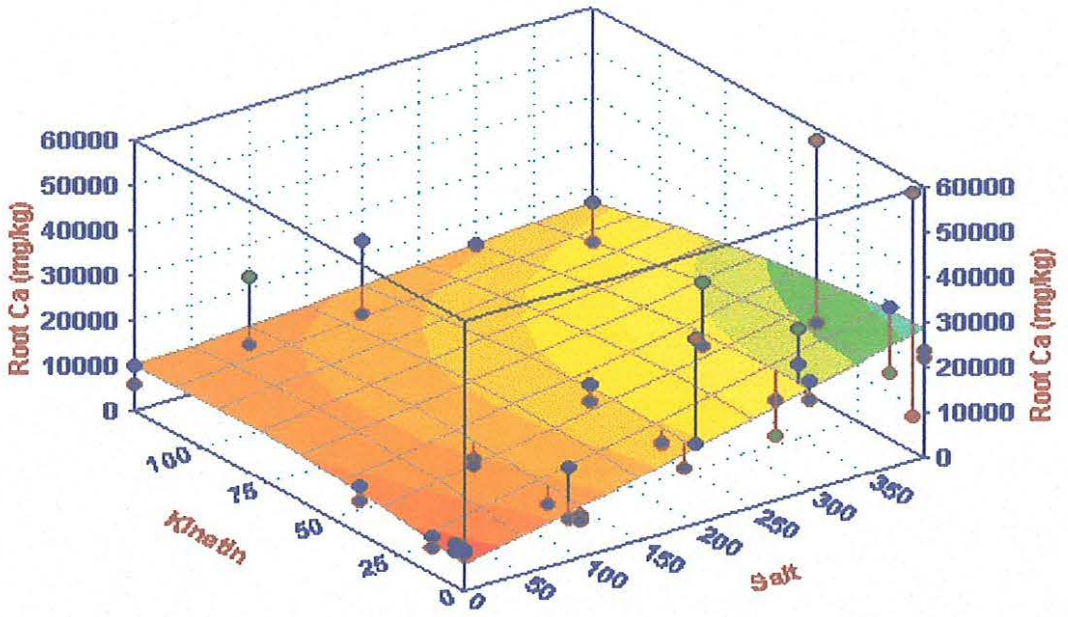

Figure 3.6.7. The interactive effects of salinity (mM) and kinetin (µM) on root Ca^{2+} concentration (mg. kg^{-1} of dry mass) *Erucastrum strigosum* plants, using ANOVA.

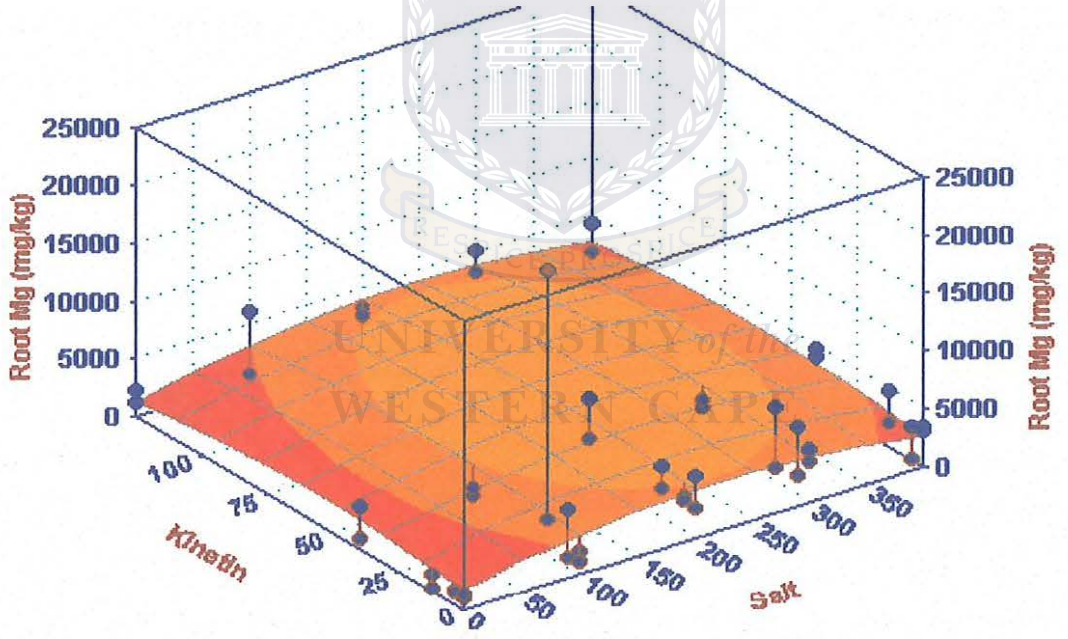

Figure3. 6.8. The interactive effects of salinity (mM) and kinetin (µM) on root Mg concentration (mg. kg^{-1} of dry mass) *Erucastrum strigosum* plants, using ANOVA.

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Chapter 4

The effects of exogenous gibberellic acid (GA_3) application on growth of salt stressed *Erucastrum strigosum* plants.

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4.1 Introduction.

Gibberellins are tetracyclic (or pentacyclic) diterpenoid naturally occurring plant growth factors that are also produced commercially from fungi for agricultural and horticultural industries (Davies, 1995). The first gibberellins were isolated from the *Gibberella fujikuroi* fungus in which they occur in large quantities as secondary metabolites. More than 78 gibberellins have been isolated (Raven *et al.*, 1992). It is now generally agreed that gibberellins are widely distributed throughout the plant kingdom (Hasan, 2002). In support of this view, Davies (1995) reported that not only do gibberellins occur in other species of fungus; but they have also been found in some ferns, gymnosperms and angiosperms. The characterized gibberellins vary in structure as well as in biological activitiy (Raven *et al.*, 1992). For convenience gibberellins have been differentiated from each other by assigning each 'a number'. The gibberellin that has received most attention is GA_3 (gibberellic acid). Hasan (2002) noted that this structure, isolated from *Gibberella fujikuroi*, is very important because of its high biological activity. This has been associated with the dramatic effects on stem and leaf elongation in intact plants, and on cell division (Cosgrove & Sovonick-Dunford, 1988).

Salinity is known to represent one of the most important factors exerting stress on plant cell metabolism. For example, salinity reduces shoot growth and development by inhibiting leaf elongation as well as cell division. These findings suggest that the presence of salinity in the growth medium interferes with the biological activities of

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gibberellic acid. It has been demonstrated that salinity accomplishes some of its effects by reducing the endogenous levels of gibberellic acid (Lerner *et al.*, 1994). It has been reported in the literature that exogenous application of plant hormones, particularly at low concentrations, might alter the endogenous levels and reduce salt-induced changes in plants. In agreement with this, Kaur *et al.* (1998) found that the exogenous GA_3, at levels as low as 6μM, reversed the salt-induced inhibitory effects imposed on germination and seedling growth in chickpea plants. Furthermore, it has been reported that growth is enhanced by addition of gibberellic acid to cotton plants and halophytic species grown under saline conditions (Agakishiev, 1964; Boucaud & Ungar, 1976 and Zhao *et al.*, 1986). Lin and Kao (1995) reported that stimulation of growth in salt-stressed rice plants by gibberellic acid is in part associated with α-amylase synthesis and leaf carbohydrate metabolism. Nevertheless, the evidence that GA_3 improved plant growth by acting on sugars is not very strong. Apparently, the possibility that other factors are involved in GA_3 growth stimulation of plants cannot be ruled out. It has long been known that in some plants GA_3 improves growth by acting on the osmotic potential, thereby increasing turgor pressure, raising wall stress and accelerating wall extension (Cosgrove & Sovonick-Dunford, 1988). However, the evidence for this hypothesis remains indirect. It has been observed that the viscoelastic properties of cell walls in the presence of GA_3 did not change; instead, it is suspected that turgor might have been altered (Kazama & Katsumi, 1983). Lerner *et* al. (1994) demonstrated that the relationship between plant growth, root pressure and cell wall extensibility, during salt stress, is overridden by other factors.

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These findings suggest that the mechanism of action of gibberellic acid on growth of salt-stressed plants is not fully understood. The aim of the present study was to investigate whether exogenous application of gibberellic acid would enhance growth of salt treated *Erucastrum strigosum* plants.

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4.2 Materials and methods

4.2.1 Plant material and growth conditions

A randomized block design experiment with 5 gibberellic acid (GA_3) concentrations by 5 salt (NaCl) treatments, replicated two times, was conducted under greenhouse conditions. Five seeds of *Erucastrum strigosum* were sown in each of 50 plastic pots (10cm diameter) filled with silica sand. The holes at the base of the pots were covered with glass wool to prevent loss of the sand. The pots were watered twice a week with $100cm^3$ of full-strength chemicult hydroponic solution or with tap water during alternate weeks. This was sufficient to completely flush the chemical contents out of the system to prevent salt build up and continued until the treatments commenced. The chemicult used was a complete commercial hydroponics nutrient salt from Kompel that is composed of 6.5%N; 27%P; 13%K; 7%Ca; 2.2%Mg; 7.5%S; 0.15%Fe; 0.024%Mn; 0.024%B; 0.005%Zn; 0.002%Cu and 0.001%Mo. Twenty-eight days after sowing, the seedlings were thinned out to two plants per pot and the GA_3 and salt treatments were commenced. The five GA_3 concentrations used were 0; 4; 12.5; 40 and 125μM. The salt used was increased by 100mM increments of NaCl per week to provide salt stress at the following concentrations 0, 100, 200, 300 and 400mM. The treatment solutions were added to the growth medium twice a week in sufficient quantities to displace all solution left in the sand filled pots.

Stress was allowed to prevail until the required salt concentration was reached and the highest concentration was maintained for one week. Photographs were taken to record conspicuous injurious stress effects encountered during the period of growth. The plants

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were harvested and separated into roots and shoots; the roots were gently rinsed with water to remove the sand and were then blotted with paper toweling. The shoots were weighed for fresh mass determination. The shoots and roots were dried in an oven for a week at 70^0C to constant mass.

4.2.2. Statistical analysis

Statistical analysis was carried out using the SAS version 8.2a computer package (SAS, 1999). Significant differences in the results of the means of fresh and dry mass production of organs (g) and water content (% of dry weight) at P=0.05 were tested using LSD. The data obtained from plants treated with a combination of salinity and kinetin was subjected to analysis of variance (ANOVA).

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4.3 Results.

4.3.1 The effects of salinity on root and shoot growth of *Erucastrum strigosum* plants.

The effects of salinity on plant growth were determined through fresh and dry mass production at different salt concentrations. It was observed that the increase in salinity of the treatment solutions had dramatic effects ($P<0.05$) on the fresh and dry mass production of shoots as well as on the dry mass production of roots of *Erucastrum strigosum* plants (Table 4.6.1). There was a negative correlation between the parameters considered for shoots and the substrate NaCl. Thus, the fresh and dry mass production of shoots of control plants and that of low saline treated plants was significantly greater than that of salt treated plants. The water content measured in shoots assumed a pattern similar to that of fresh and dry mass production (Table 4.6.1). In roots, the growth response was estimated through dry mass production. In control plants, root growth was significantly greater ($P<0.05$) than that of salt treated plants. In saline treated plants, root dry mass production decreased progressively with salinity treatments. Thus, at lower salinity treatments growth was higher and at higher salinity it declined (Table 4.6.1).

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4.3.2 The effects of gibberellic acid on root and shoot growth of *E. strigosum* plants.

The effects of gibberellic acid on growth responses of *Erucastrum strigosum* plants are presented (Table 4.6.2). In shoots, the fresh and dry mass productions were significantly increased by the increase in gibberellic acid of the treatment solution. Thus, GA_3 treated plants maintained their growth at levels higher than those of control plants. These observations persisted until 40 µM GA_3 was reached. At concentrations higher than 40 µM GA_3 a decline was observed. These results suggest that GA_3 stimulates shoot growth when applied at low concentrations. There were no significant differences observed for the water content of treated plants and that of control plants. In roots, there was no significant difference between the dry mass production of control plants and that of gibberellin treated plants.

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4.3.2 The interactive effects of salinity and gibberellic acid on root and shoot growth of *E. strigosum* plants.

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The effects of salt-hormonal treatments on growth were estimated either as fresh and dry mass productions (shoots) or dry mass productions (roots), (Figures 4.6.1 and 4.6.2). In shoots, it was observed that when gibberellic acid was added to the salinized treatment solutions, the fresh and the dry mass production increased. The water content was calculated as a % of the dry mass production. It was observed that the water content was

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not affected by saline-GA_3 treatments (Figure 4.6.3). In roots, gibberellic acid increased the dry mass production at all salinity concentrations (Figure 4.6.4). However, it is important to note that the promotive effects of gibberellic acid in both roots and shoots, were pronounced at moderate gibberellins levels (4-40 μM) and declined to levels relatively similar to those of control plants with further increase.

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4.4. Discussion.

The results obtained in the present study indicate that the presence of NaCl in the rooting medium caused a decrease in all growth parameters that were measured in *Erucastrum strigosum.* Thus, the fresh and dry mass productions of shoots were dramatically reduced by increase in NaCl (Table 4.61). Similar results were reported for other species, considered to be sensitive to salt stress, such as *Phaseolus vulgaris*, where the dry mass of shoots and roots were significantly reduced by increasing substrate salinity (Greenway and Munns, 1980, and Gouia *et al.*, 1994). Salt treatments also induced reduction in the leaf water content (Table 4.6.1). According to Katerji *et al.* (1997) loss of water in the leaves is associated with reduction of leaf extension. Thus, shoot growth inhibition in these plants was correlated to a loss of water, which in turn is caused by salt treatments. Similar effects were also observed in salt sensitive varieties of sugar beet (Ghoulam *et al.*, 2002).

In plants treated with gibberellic acid, it was observed that the growth response of the parameters considered for *Erucastrum strigosum* was related to organ sensitivity. Thus, the fresh and dry mass productions of shoots were dramatically increased with increasing GA_3, particularly at low concentrations, while the root dry mass production remained unaffected (Table 4.6.2). However, the growth response of shoots to GA_3 cannot be attributable to organ sensitivity alone, because it has been demonstrated that gibberellic acid's enhancement of longitudinal growth can also be concentration dependent. In support of this view, Salisbury and Ross (1992), presented experimental evidence that implicated the likely involvement of concentration differences in the stimulation of plant

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growth after gibberellic acid treatment. Thus, dwarf rice responded to levels as low as 3,5 x 10^{-12}g of GA_3. In the present study, it was observed that in controls (non- GA_3 treated plants) plants were shorter than in 40 μM GA_3 -treated plants. It was further observed that increasing GA_3 concentrations to levels that were higher than 40 μM was followed by a decline in growth. These results seem to suggest the idea that low concentrations of hormones are likely to cause growth responses in treated plants, still holds. However, as soon as the concentration of hormones in the treatment solution increases, growth starts to decrease. Amzallag and his coworkers (1991) also reported on this phenomenon. What is the mechanism of action by which gibberellic acid promotes growth? In response to this question, a hypothesis that gibberellins increase growth by changing a plants osmotic potential or the influx of water, has been formulated (Beals *et al.*, 1999). According to this hypothesis, gibberellins release solutes into the cell and the growth rate of the plant would increase. In the present study, it was found that the water content of plants treated with gibberellic acid was not significantly different to that of control plants. In another study, it was also found that there were no effects on hydraulic conductivity, as well as the solute reflection coefficient, when gibberellic acid was added. If the influx of water into the cell is not responsible for growth promotion in gibberellin treated plants, then what is the cause? The logical hypothesis is that gibberellins promote growth via cell wall extensibility (Raven *et al.*, 1992). However, this effect of gibberellic acid on cell wall extensibility is believed to be indirect (Fry, 1980). In Fry's research, GA_3 enhanced growth by preventing the cross-linking of non-cellulose microfilaments. In explaining this further, Raven *et al.* (1992) reported that gibberellins may act by breaking polymers

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within the cell wall or increase the synthesis of cell wall polymers. Cosgrove (2000) found evidence that agrees with the Fry's cross-linking hypotheses.

It has been reported that gibberellic acid reduces NaCl-induced growth inhibition in some plants (Agakishiev, 1964; Boucaud & Ungar, 1976; Zhao *et al.*, 1986 and Lin & Kao 1995). In the present study, it was observed that addition of GA_3 to *Erucastrum strigosum*, counteracted NaCl-inhibition of shoot growth more prominently than that of root growth (Figures 4.6.1, 4.6.2 & 4.6.4). These findings are in line with the idea that the sensitivity of an organ to growth regulators, controls growth of that particular tissue, even if the plant is exposed to sub-optimal conditions such as salt stress. It was observed that the water content of the plants treated with salinity and gibberellic acid was relatively similar to that of the control plants (Figure 4.6.3). These findings suggest that gibberellic acid did not counteract the salt-induced inhibitory effects on growth by altering the plant's water content. However, it is possible that gibberellins reverse salinity effects by increasing the cell wall's extensibility in order to allow the movement of water into the plant, as a result growth was enhanced. Thus, the increase of growth depends on the extensibility of the cell wall, as well as the permeability to water, regardless of environmental conditions (Beals *et al.*, 1999). Similar conclusions were drawn in other studies, including those involving members of the Brassicaceae family e.g. *Brassica campestris* exposed to water stress (Banyal & Rai, 1983) and which responded to GA_3.

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4.5. References.

AGAKISHIEV, D (1964) Effects of gibberellin on cotton under conditions of salinization. Fiziologiya Rastenii 11:210-205.

AMZALLAG G N, LERNER H R, POLJAKOFF-MAYBER A (1991) Interaction between mineral nutrients, cytokinins and gibberellic acid during growth of Sorghum at high NaCl salinity. Journal of Experimental Botany 43: 81-87.

BANYAL S, RAI V K (1983) Reversal of osmotic stress by gibberellic acid in *Brassica campestris*. Recovery of hypocotyls growth, protein and mRNA levels in the presence of GA. Physiologia Plantarum 59: 111-114.

BEALS M, GROSS L, HARREL S (1999) Plant cell expansion and plant hormones. http://www.tiem.utk.edu/~harrell/webmodules/cellwallexpansion.html downloaded on 13/05/03

BOUCAUD J, UNGAR I A (1976) Influence of hormonal treatment on the growth of two halophytic species. American Journal of Botany 63: 694-699.

COSGROVE D J (2000) Loosening of plant cell walls by expansins. Nature 407: 321-326.

COSGROVE D J, SOVONICK-DUNFORD S A (1989) Mechanism of gibberellin-dependent stem elongation in peas. Plant Physiology 89: 184-191.

DAVIES P J (1995) Plant hormones: Physiology, biochemistry and molecular biology. 2nd edn. Kluwer Academic Publishers. Netherlands.

FRY C S (1980) Gibberellin-controlled pectic acid and protein secretion in growing cells. Phytochemistry 19: 735-740.

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UNIVERSITY of the
WESTERN CAPE

etd.uwc.ac.za

GHOULAM C, FOURSY A, FARES K (2002) Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet varieties. Environmental and Experimental Botany 47: 39-50.

GREENWAY H, MUNNS R (1980) Mechanisms of salt tolerance in non-halophytes. Annual Review of Plant Physiology 31: 149-190.

GOUIA H, GHORBAL MH, TOURAINE B (1994) Effects of NaCl on flows of N and mineral ions and on NO_3^- reduction rate within whole plants of salt-sensitive bean and salt-tolerant cotton. Plant Physiology 105: 1409–1418.

HASAN H A H (2002) Gibberellin and auxin production by plant root-fungi and their biosynthesis under salinity-calcium interaction. Rostlinná Výroba.48: 101-106.

KATERJI N, VAN HOORN J W, HAMDY A, MASTRORILLI M, MOU KARZEL E (1997) Osmotic adjustment of sugar beets in response to soil salinity and its influence on stomatal conductance, growth and yield. Agricultural Water Management 34: 57–69.

KAUR S, GUPTA A K, KAUR N (1998) Gibberellin A (3) reverses the effects of salt stress in chickpea (*Cicer arietinum* L.) seedlings by enhancing amylase activity and mobilization of starch in cotyledons. Plant Growth Regulation 26: 85-90.

KAZAMA H, KATSUMI M (1983) Gibberellins induced changes in the water absorption, osmotic potentials and starch content of cucumber hypocotyls. Plant, Cell Physiology. 24: 1209- 1216.

LERNER H R, AMZALLAG N, FRIEDMAN Y, GOLOUBINOFF P (1994) The response of plants to salinity: from turgor adjustment to genome modification. Israel Journal of Plant Science 42: 285-297

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LIN C C, KAO C H (1995) NaCl stress effects in rice seedlings: starch mobilization and the influence of gibberellic acid on seedling growth. Botanical Bulletin of the Academia Sinica 36: 169-173.

RAVEN P H, EVERT R F, EICHHORN S E (1992) Biology of plants. 5th edn. Worth Publishers. New York.

SALISBURY FB, ROSS CW (1992) Plant physiology. 4th ed. Wadsworth Publishing Company. California.

SAS (1999) Sas/Stat User's guide, version 8.2. 1st printing. Volume 2. Sas institute, Cary, North Carolina.

ZHAO K-F, LI M-L, LIU J-Y (1986) Reduction by GA_3 of NaCl-inhibition of growth and development in *Suaeda ussuriensis*. Australian Journal of Plant Physiology 34: 547-551.

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4.6.Appendix

Table 4.6.1. The effects of salinity on root and shoot growth; and water content (% of dry mass) of *Erucastrum strigosum*. Differences between treatment means were tested for level of significance (P=0.05) at N=10 using LSD; means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of NaCl (mM)					
		0	100	200	300	400	LSD
Shoots	Fresh mass (g)	15.08 A	12.67 BA	10.35 BC	7.68 DC	7.06 D	2.91
	Dry mass (g)	1.30A	1.15BA	1.12BA	0.87BC	0.79C	0.49
	Water content (%)	1319.69 A	1051.7 BA	880.24 B	772.32 B	814.06 B	431.39
Roots	Dry mass (g)	0.44 A	0.24 B	0.22 B	0.15 B	0.14 B	0.174

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Table 4.6.2. The effects of GA_3 on root and shoot growth and water content (% of dry mass) of *Erucastrum strigosum*. Differences between treatment means were tested level of significance (P=0.05) at N=10 using LSD; means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of GA_3 (µM)					
		0	4	12.5	40	125	LSD
Shoots	Fresh mass (g)	9.53BC	10.53BAC	11.73BA	12.46 A	8.59C	2.91
	Dry mass (g)	0.90 B	0.97 BA	1.20 BA	1.27 BA	0.88 B	0.32
	Water content (% of d.m.)	948.30 A	1176.71A	890.34 A	940.26 A	882.44 A	431.39
Roots	Dry mass (g)	0.13 A	0.20 A	0.29 A	0.30 A	0.28 A	0.174

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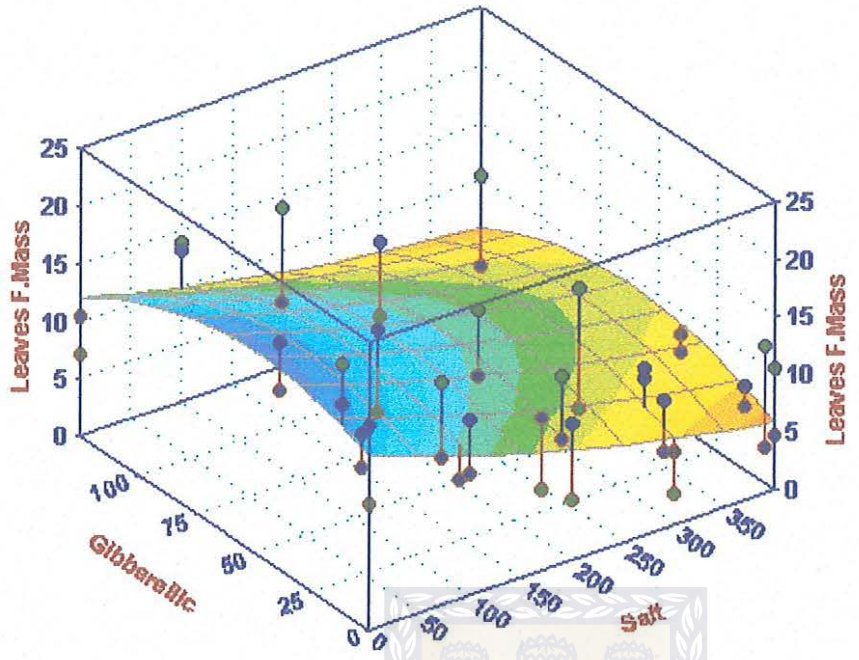

Figure 4.6.1. The interactive effects of salinity (mM) and gibberellic acid (µM) on shoot fresh mass production (g) of *Erucastrum strigosum* plants, using ANOVA.

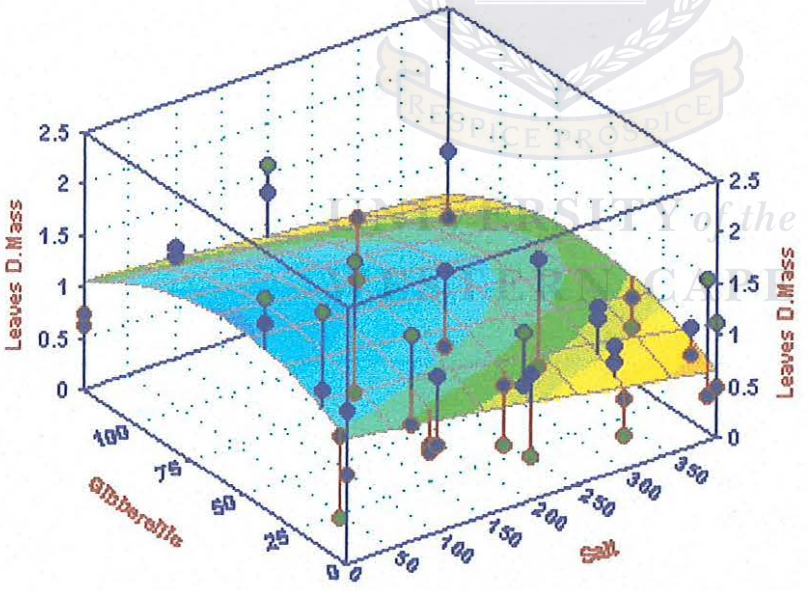

Figure 4.6.2. The interactive effects of salinity (mM) and gibberellic acid (µM) on shoot dry mass production (g) *Erucastrum strigosum* plants, using ANOVA.

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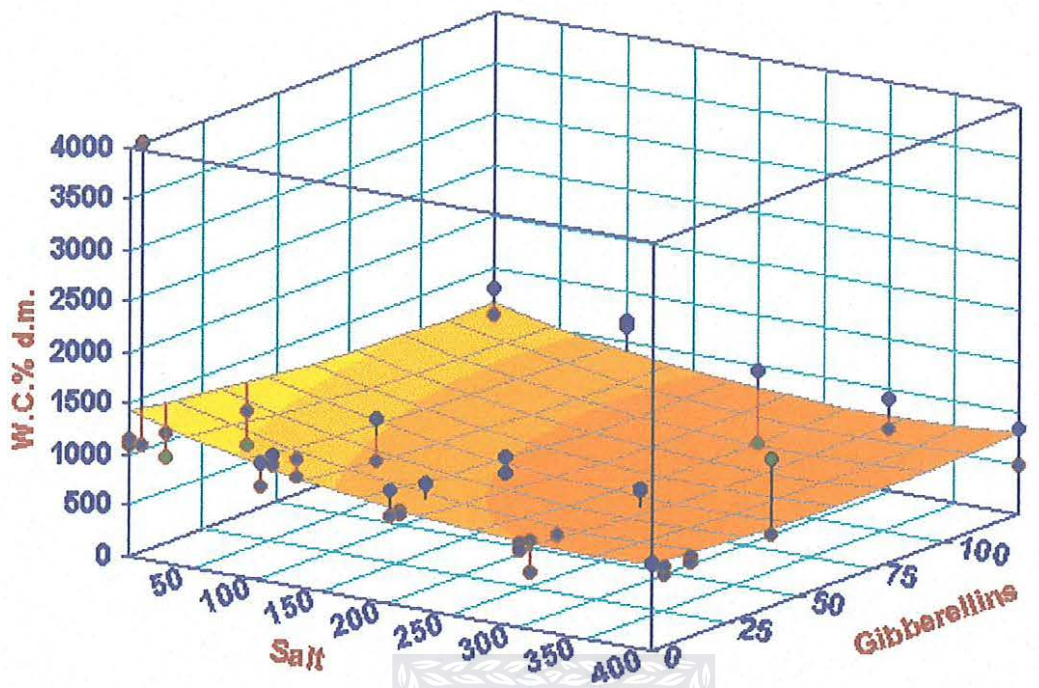

Figure 4.6.3. The interactive effects of salinity (mM) and gibberellic acid (µM) on shoot water content (% of dry mass) *Erucastrum strigosum* plants, using ANOVA.

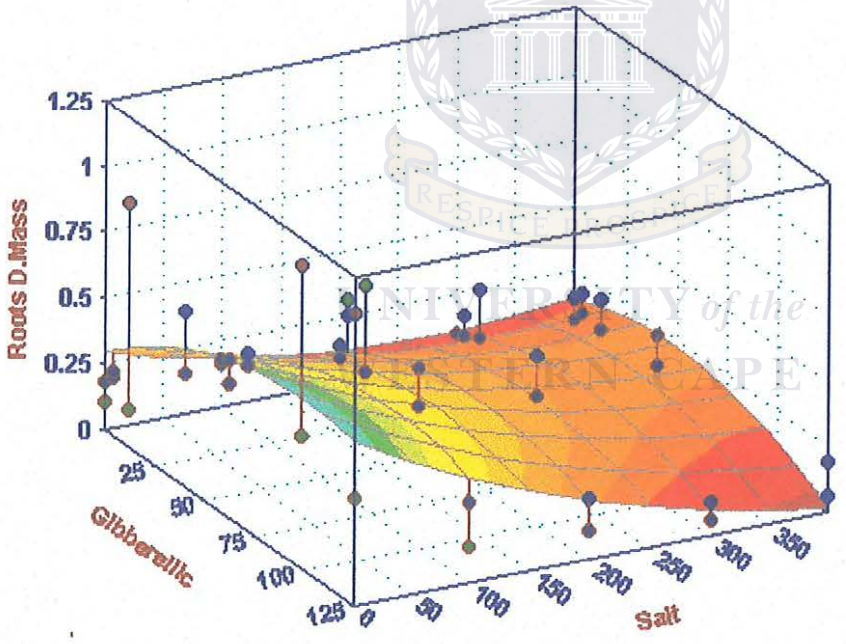

Figure 4.6.4. The interactive effects of salinity (mM) and gibberellic acid (µM) on root dry mass production (g) *Erucastrum strigosum* plants, using ANOVA.

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Chapter 5

The role of exogenous gibberellic acid on the cation content of salt stressed *Erucastrum strigosum* plants.

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5.1 Introduction

It has become apparent that NaCl-induced growth inhibition in most crop plants is likely to revolve around their inability to effectively regulate ionic and osmotic effects (Marschner, 1995). It is well established that strategies, employed by salt-tolerant species to regulate these parameters, depend largely on changes that occur in membranes and cytosolic processes. One of the most common features of salinity tolerance in crop plants is the use of a K/Na discrimination mechanism whereby the accumulation of Na^+ ions is blocked by the influx of K^+ into xylem tissues. It has been established that accumulation of Ca^{2+} in the cytoplasm controls the K^+ selective channels (Blatt & Theil, 1993 and Song & Fujiyama, 1996). In addition, Raven *et al.* (1992) reported that this phenomenon may be accomplished by retranslocating Na^+ ions back to the medium while replacing them with potassium i.e. a Na^+-K^+ antiport. For example, Reid and Smith (2000) reported that treatment of the NaCl-containing medium with calcium has been shown to improve root elongation and shoot growth as well as the abolishment of nutrient deficiencies. Furthermore, Ca^{2+} improved growth of NaCl-stressed *Fusarium oxysporum* fungus by elevating its gibberellic acid content (Hasan, 2002). The decrease of gibberellins under salt stress conditions is now a well-known phenomenon. The exogenous application of growth regulators to alter the status of phytohormones has long been practiced for agricultural and horticultural purposes.

Gibberellic acid is one of the growth regulators that have been implicated in the activation of proton pumping proteins. Gibberellic acid has been shown to influence the uptake and accumulation of cations such as calcium. The link between calcium and

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gibberellic acid was reported on in studies that were performed on barley aleurone cells (Schuurink *et al.*, 1996). In these cells GA_3 increased $[Ca^{2+}]$ by increasing the influx of Ca^{2+} at the plasma membrane. These effects have also been shown for other cereal aleurones such as rice, wheat and wild oats. However, these effects have not been reported for other cations and they have not been well explored for other non-graminaceous plant species. Furthermore, it is not well understood whether these effects occur even under sub-optimal growth conditions, such as salinity stress. The present study is aimed at investigating the effects of exogenous application of gibberellic acid on the cation concentration of salt-stressed *Erucastrum strigosum* plants.

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5.2. Materials and methods

5.2.1 Plant material and growth conditions (refer to 4.2.1)

5.2.2 Chemical analysis (refer to 3.2.2) (Allen *et al.*, 1986)

5.2.2. Statistical analysis.

Statistical analysis was carried out using the SAS version 8.2 computer package (SAS, 1999). In plants treated with GA_3 and NaCl, a student's t- Least Significant Differences (LSD) test was performed to estimate the level of significance between the means at P= 0.05. In salt-gibberellin treated plants, the data obtained was subjected to analysis of variance (ANOVA).

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5.3 Results.

5.3.1. The effects of salinity on the cation concentrations of roots and shoots of *Erucastrum strigosum* plants.

The effects of NaCl concentrations of the treatment solution on cation contents of roots and shoots of *E. strigosum* were measured in $mg.kg^{-1}$ of dry mass (Table 5.6.1). It was generally observed that the sodium concentration in shoots was significantly higher than that of roots. Thus, the shoots of the control plants and that of NaCl treated plants accumulated more sodium than the roots. The comparison made between treatments showed that the sodium concentration increased with increasing substrate salinity for both roots and shoots. The distribution pattern of potassium in shoots was similar to that of sodium. However, when comparison was made between treatments it was observed that the potassium concentration of the control plants was higher than that of saline treated plants for both roots and shoots. The calcium concentration was greater in roots and lower in shoots. However, the root and shoot calcium concentrations of control plants was higher than of saline treated plants. In saline treated plants, calcium concentration for both roots and shoots was more or less the same at all treatments. The shoot magnesium concentration was higher than that of roots. Comparison between treatments revealed that the shoot magnesium concentration of control plants was higher than that of saline treated plants. In saline treated plants, the shoot magnesium concentration was more or less the same at all treatments. The root magnesium concentration, on the other hand, was not significantly affected by increasing substrate salinity.

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5.3.1. The effects of gibberellic acid on cation concentrations of roots and shoots of *Erucastrum strigosum* plants.

The effects of a gibberellic acid series on cations of roots and shoots were measured at $mg.kg^{-1}$ of dry mass and summarized in Table 5.6.2. Generally, shoots contained more sodium than roots. Thus, the sodium accumulated more in shoots than in roots at all levels of gibberellic acid. However, there were no significant differences observed between treatments, on both shoot and root sodium concentrations. The shoot potassium concentration was also higher than roots. Nevertheless, the shoot potassium was decreased with gibberellic acid treatments. However, with root potassium concentrations, there were no significant differences between treatments. The shoot calcium concentration was maintained at levels lower than those of roots. Furthermore, both shoot and root calcium concentrations were not significantly affected by treatments with gibberellic acid. Magnesium concentration was higher in shoots than in roots but it was also not significantly affected by treatments with gibberellins. However, it was observed that the root magnesium concentration decreased with increasing gibberellic acid.

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5.3.2. Interactive effects of salinity and gibberellic acid on cation concentrations of roots and shoots of *Erucastrum strigosum* plants.

The interactive effects of salinity and gibberellic acid on cations of *E. strigosum* roots and shoots measured in $mg.kg^{-1}$ of dry mass are shown in Figures 5.6.1 to 5.6.8. It was observed that salinity treatment increased the shoot sodium concentration to levels that were much higher than those of roots throughout the gibberellic acid treatments. At lower salinity, sodium concentration was smaller than that at higher treatments irrespective of gibberellic acid added to the uptake solution. Potassium concentration was higher in shoots than in roots (Figures 5.6.2 and 5.6.7). Comparison between treatments showed that at lower salinity treatments, the shoot potassium concentration was higher than at higher salinity treatments. These observations were made throughout gibberelic acid concentrations of the treatment solution. The calcium concentration was higher in shoots than in roots at lower levels of the treatments. At higher treatments it was higher in roots and lower in shoots. Simultaneous application of salinity and gibberelic acid decreased shoot calcium concentration at all salinity treatments. The shoot magnesium concentration was higher than that of roots. The magnesium concentration was decreased by substrate salinity regardless of treatments with gibberellic acid. In roots, the sodium concentration increased with increasing salinity at all gibberelic acid treatment levels (Figure 5.6.5). Potassium (Figure 5.6.6) and magnesium (Figure 5.6.8) concentrations increased with salinity treatments but addition of gibberellic acid decreased these concentrations throughout the treatments. Calcium concentration increased with salinity treatments but decreased with addition of gibberellic acid at all treatment levels.

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5.4 Discussion.

The results of this study demonstrated that the nutritional status of glycophytes is affected by salt stress. These effects were expressed more clearly in shoots than in roots. It has been demonstrated that the onset of salt stress in non-halophytes can be attributed to the accumulation of toxic elements such as Na^+ and Cl^+ in shoots. Furthermore, it has been reported that glycophytes resist salinity stress by blocking the uptake and /or transport of saline ions from the root zone to aerial parts (Greenway and Munns, 1980). Accordingly Zandstra-Plom *et al.* (1998) found that plants that resist salinity by using this mechanism accumulate Na^+ in their roots, pith cells and lower parts of the stem. In the present study, it was observed that the shoot Na^+ content was higher than that of roots. These findings suggest that in the *E.strigosum* plant, the mechanism that controls influx of Na^+ into the xylem was lacking or unable to cope, hence the accumulation of Na^+ in shoots (Table 5.6.1). Moreover, they suggest that glycophytes such as *E.strigosum* are unable to maintain an appropriate K/Na discrimination. Greenway and Munns (1980) found that under saline conditions the concentration of K^+ in many glycophytes is severely reduced. This was the case for *E. strigosum.* There seem to be an undeniable correlation between the decrease of K^+ and inappropriate K/Na discrimination. Marschner (1995) reviewed that there is a carrier protein that regulates the uptake and transport of K^+ and Na^+ at the membranes. It is therefore feasible to deduce that in the present study the accumulation of Na^+ and the decrease of K^+ resulted in a lowered K/Na ratio.

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Ca^{2+} has been reported to play a role in salt stress alleviation (Hasan, 2002). However, for calcium to function effectively it has been demonstrated that it should be present in the cytoplasm at elevated amounts (Reid and Smith, 2000). Calcium ameliorates salt stress by promoting the selectivity for K^+ (Raven *et al.*, 1992). Because calcium was not found at elevated amounts, the selectivity for K^+ over that of Na^+ may be impaired (Table 5.6.1).

The initial decrease of Mg^{2+} concentration with increasing substrate salinity in shoots was expected (Table 5.6.1). The hypothesis that salinity affects the homeostasis of Mg^{2+} has also been proposed by Tozlu *et al.* (2000). In their results they demonstrated that Mg^{2+} deficiency is manifested by leaf chlorosis. Furthermore, symptoms of salt stress are similar to those of ion deficiency and they are usually traced to the inability of essential ions to compete with Na^+. However, in this study the accumulation of Na^+ was not accompanied by symptoms of Mg^{2+} deficiency. These findings suggest that the Mg^{2+} concentration was not deficient.

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It was observed that shoot Na^+ concentrations were much higher than that of roots, regardless of treatments with gibberellic acid (Table 5.6.2). The shoot K^+ concentration on the other hand, decreased with increasing gibberellic acid. These results suggest that the K/Na selection at the xylem entry was affected by hormonal treatments. Ca^{2+} concentration was only affected in shoots and Mg^{2+} was affected in roots. Both cations were decreased by hormonal treatments. It has been reported that calcium in plants is often found in a steady state (Bradley, 1999). The decrease of Ca^{2+} in shoots suggest that

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the response of calcium to gibberellic acid is either species specific or at the whole plant level and it requires something else to restore the steady state of calcium. In barley aleurone, GA teamed up with ABA, was reported to effectively reset the steady state of calcium (Gilroy and Jones, 1992). These findings suggest that the efficiency of gibberellic acid in resetting the steady state of calcium, is not well known.

It has been established that higher plants tolerate salt stress by using two types of mechanisms (Tozlu *et al.*, 2000). In the first mechanism, plants exclude toxic ions such as Na^+ and Cl^- from the leaves. In the second mechanism, toxic ions are absorbed by the cells and sequestered into vacuoles. The use of tolerance patterns has enabled scientists to discriminate between salt tolerance and salt sensitivity in species and cultivars. In the present study, treatment of salt stressed plants with GA_3, exacerbated the ionic effects of salinity. Thus, there was accumulation of Na^+ accompanied by decrease of other cations (K, Ca and Mg). These findings suggest that GA_3 failed to stimulate a mechanism that blocks the uptake and transport of Na^+. It has been postulated that these blockage mechanism can either operate at root membranes or the xylem entry, and it is referred to as the K/Na selection (Raven *et al.*, 1992). It discriminates between Na^+ and K^+ (Marschner, 1995). However, it is possible that the tolerance mechanism, stimulated by treatment with gibberellic acid, was the sequestering of these toxic ions into vacuoles. This is possible because Na^+ accumulation in shoots and roots paralleled growth enhancement particularly at low concentrations of GA_3. Nevertheless, there has not been much study on this possibility.

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5.5 References

ALLEN S E, GRIMSHAW H M, ROWLAND A P (1986) Chemical analysis. In: Moore, P D and Chapman, S B (eds) Methods in plant ecology. Blackwell Scientific publications, London, pp 285-337.

BLATT M R, THEIL G (1993) Hormonal control of ion channel gating. Annual Review of Plant Physiology and Plant Molecular Biology 44: 543-567.

BRADLEY P (1999) Nutrients in hydroponics. http://www.hydrogarden.com/index.html downloaded on 12/11/02.

GILROY S, JONES R L (1992) Gibberellic acid and abscisic acid coordinately regulate cytoplasmic calcium and secretory activity in barley aleurone protoplast. Proceedings of National Academy of Science of the United State of America 89: 3591-
3595.

GREENWAY H, MUNNS R (1980) Mechanisms of salt tolerance in non-halophytes. Annual Review of Plant Physiology 31: 149-190.

HASAN H A H (2002) Gibberellin and auxin production by plant root-fungi and their biosynthesis under salinity-calcium interaction. Rostlinná Výroba 48: 101-106.

MARSCHNER H (1995) Mineral nutrition of higher plants. 2nd ed. Academic Press, San Francisco.

RAVEN P H, EVERT R F, EICHHORN S E (1992) Biology of plants. 5th edn. Worth Publishers. New York.

REID R J, SMITH F A (2000) The limits of sodium/ calcium interactions in plant

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RESPICE PROSPICE
etd.uwc.ac.za

growth. Australian Journal of Plant Physiology 27: 709-715.

SAS (1999) SAS/STAT User's guide, version 8.2, 1st printing, Volume 2 SAS Institute Inc. SAS Campus drive, Cary, North Carolina 27513.

SCHUURINK R C, CHAN P V, JONES R L (1996) Modulation of calmodulin mRNA and protein levels in barley aleurone. Plant physiology 111: 371-380.

SONG J Q, FUJIYAMA H (1996) Differences in response of rice and tomato subjected to sodium salinization to the addition of calcium. Soil Science and Plant Nutrition 42: 503-510.

TOZLU I, MOORE G A, GUY C L (2000) Effects of increasing NaCl concentration on stem elongation, dry mass production and macro- and micro nutrient accumulation in *Poncirus trifolita*. Australian Journal of Plant Physiology 27: 35-42.

ZANDSTRA-PLOM M, VOGELRANG S A, VEEN B N (1998) Sodium fluxes in sweet pepper exposed to varying sodium concentrations. Journal of Experimental Botany 49: 1863-1868.

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5.6. Appendix

Table 5.6.1. The effect of salinity on the cation status (mg.kg^{-1} of dry mass) of *Erucastrum strigosum* shoots and roots. The differences between the treatment means were tested for level of significance (P=0.05) at N=10 using LSD; means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of NaCl (mM)					
		0	100	200	300	400	LSD
Shoots	[Na]	5482 C	64468 B	81626 A	87045 A	95252 A	13633
	[K]	65582 A	25407 B	19938 C	17873 C	17418 C	3064
	[Ca]	14037 A	11045 B	10265 CB	10571 CB	9295 C	1392
	[Mg]	4592 A	2081 B	1592 C	1523 C	1524 C	319
Roots	[Na]	2047 D	13706 C	21067 B	29177 A	24535 BA	6503
	[K]	20869 BA	15042 B	24142 A	20308 BA	20692 BA	6299
	[Ca]	10495 B	13909BA	11635 B	19684 A	14856 BA	7746
	[Mg]	816 BC	595 C	1073 BA	1212 A	1021 BA	366

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Table 5.6.2. The effect of GA_3 on the cation status (mg. kg^{-1} of dry mass) of *Erucastrum strigosum* shoots and roots. The differences between the treatment means were tested for level of significance (P=0.05) at N=10 using LSD; means followed by the same letter do not differ significantly.

Plant organs	Observations	Concentration of Gibberellic acid (μM)					
	Cations ($mg.kg^{-1}$)	**0**	**4**	**12.5**	**40**	**125**	**LSD**
Shoots	**[Na]**	**64707 A**	**70263 A**	**60027 A**	**69424 A**	**69453 A**	**13633**
	[K]	**30595 A**	**30324 BA**	**29493 BA**	**27430 B**	**28377 BA**	**3064**
	[Ca]	**11466 A**	**12011 A**	**10659 BA**	**11074 BA**	**10002 B**	**1392**
	[Mg]	**2344 A**	**2307 A**	**2150 A**	**2229 A**	**2383 A**	**319**
Roots	**[Na]**	**18978 A**	**20710 A**	**15479 A**	**16907 A**	**18458 A**	**6504**
	[K]	**23493 A**	**20011 A**	**19603 A**	**20595 A**	**17351 A**	**6299**
	[Ca]	**18210 A**	**15889 A**	**11691 A**	**11106 A**	**13682 A**	**7746**
	[Mg]	**1153 A**	**1001 BA**	**782 B**	**982 BA**	**799 BA**	**366**

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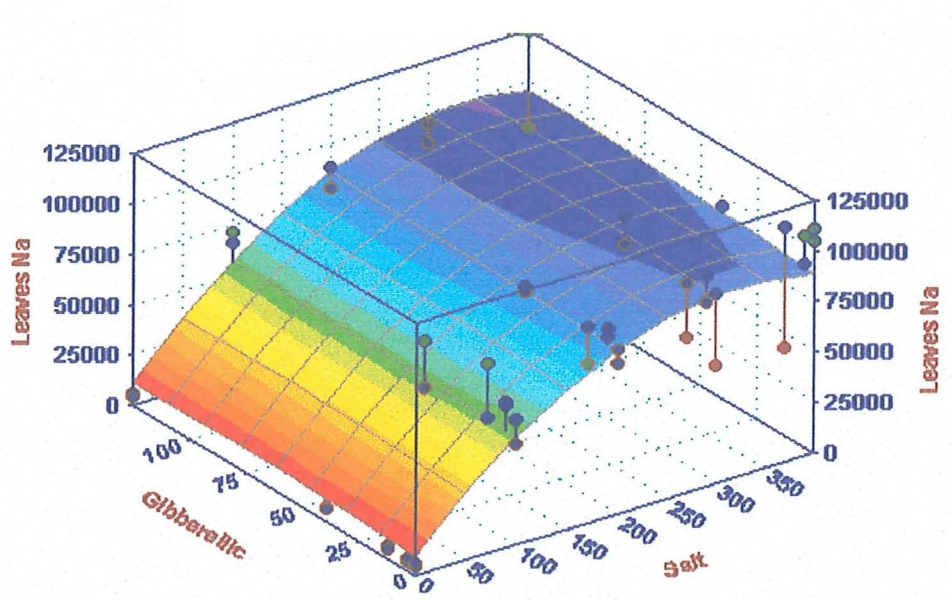

Figure 5.6.1. The interactive effects of salinity (m M) and GA_3 (µM) on the shoot Na^+ concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

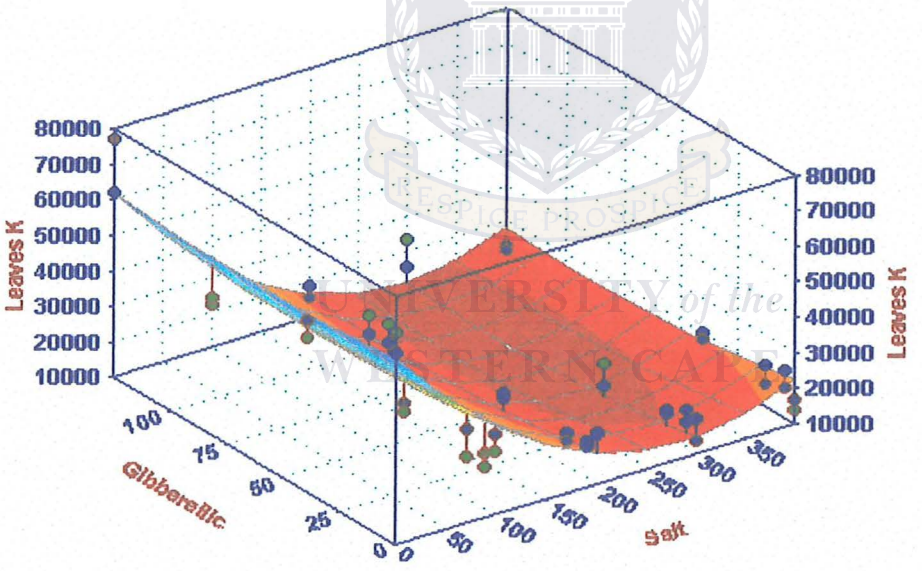

Figure 5.6.2. The interactive effects of salinity (mM) and GA_3 (µM) on the shoot K^+ concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

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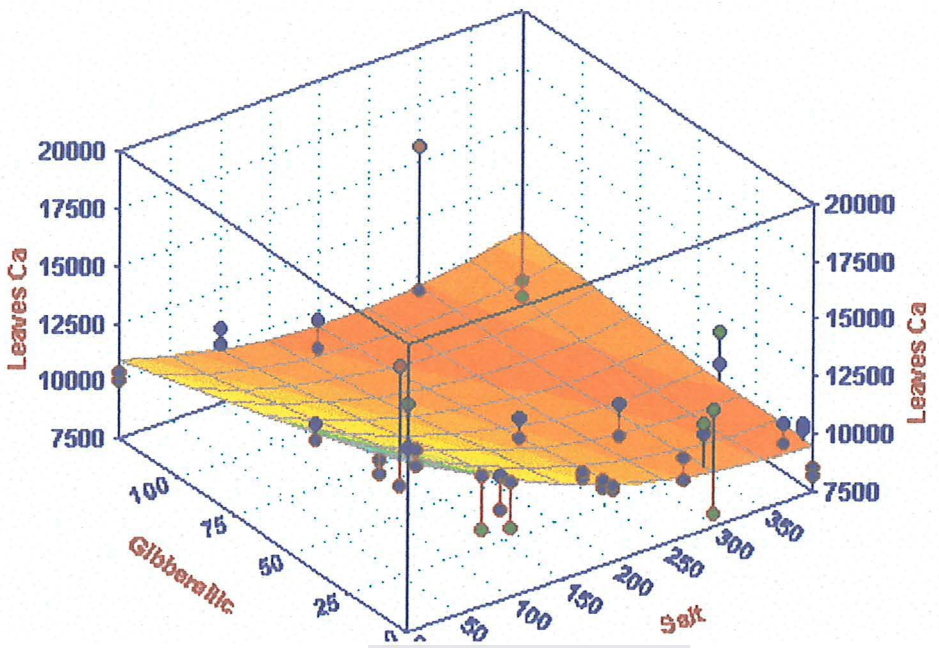

Figure 5.6.3. The interactive effects of salinity (mM) and GA_3 (µM) on the shoot Ca^{2+} concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

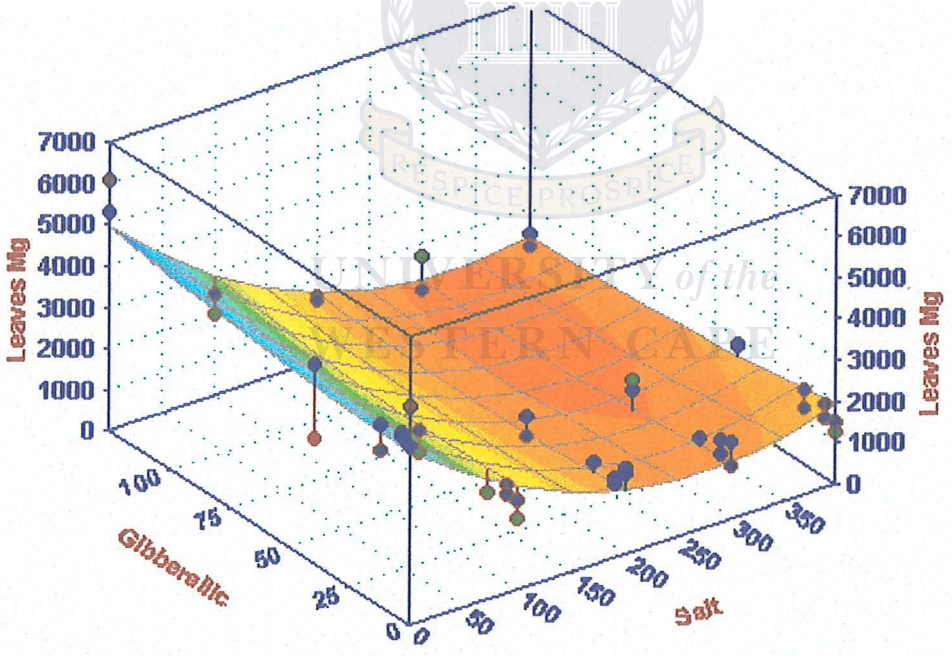

Figure 5.6.4. The interactive effects of salinity (mM) and GA_3 (µM) on shoot Mg^{2+} concentration ($mg.kg^{-1}$ of dry mass) of Erucastrum strigosum plants, using ANOVA.

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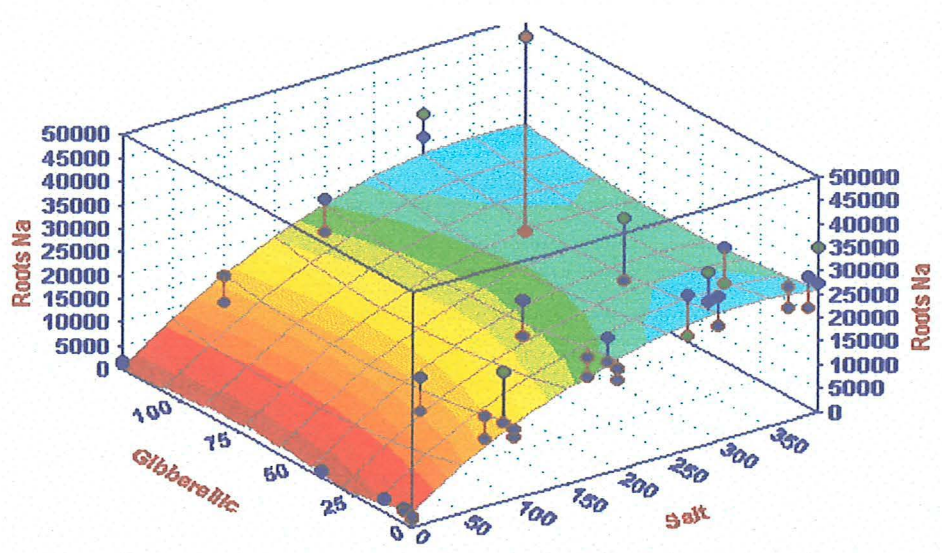

Figure 5.6.5. The interactive effects of salinity (mM) and GA_3 (µM) on root Na^+ concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

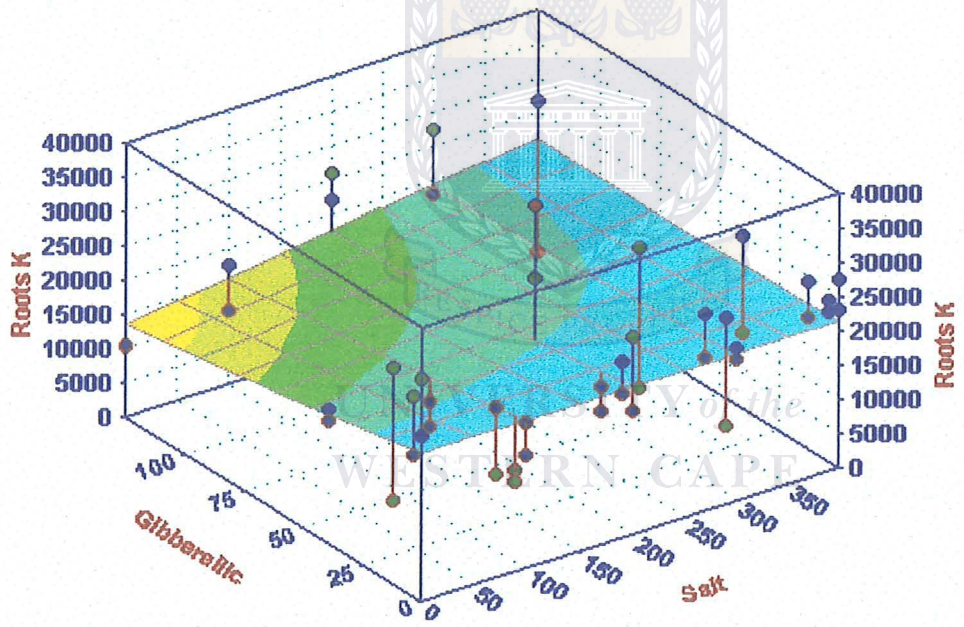

Figure 5.6.6. The interactive effects of salinity (mM) and GA_3 (µM) on root K^+ concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

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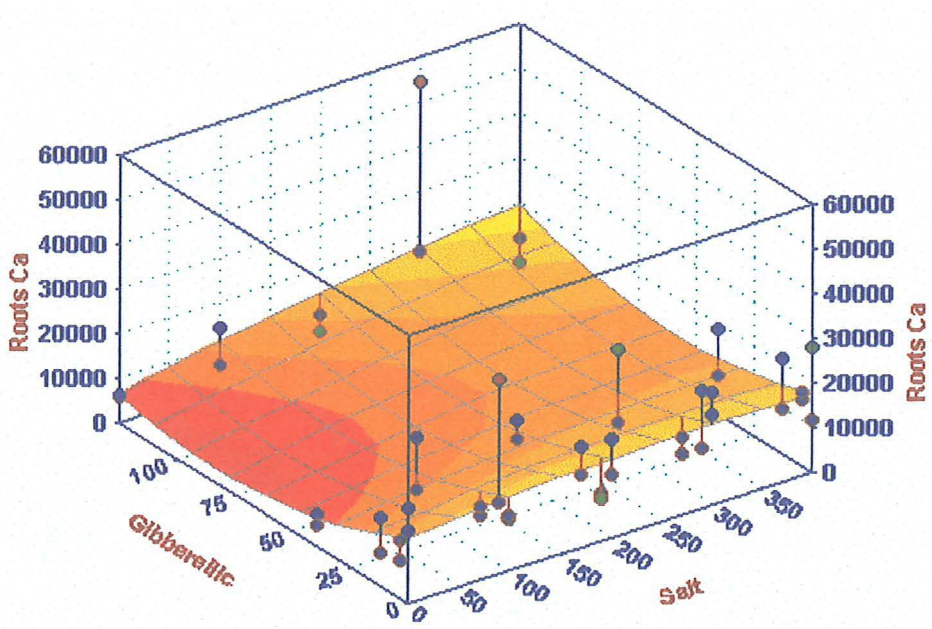

Figure 5.6.7. The interactive effects of salinity (mM) and GA_3 (µM) on root Ca^{2+} concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

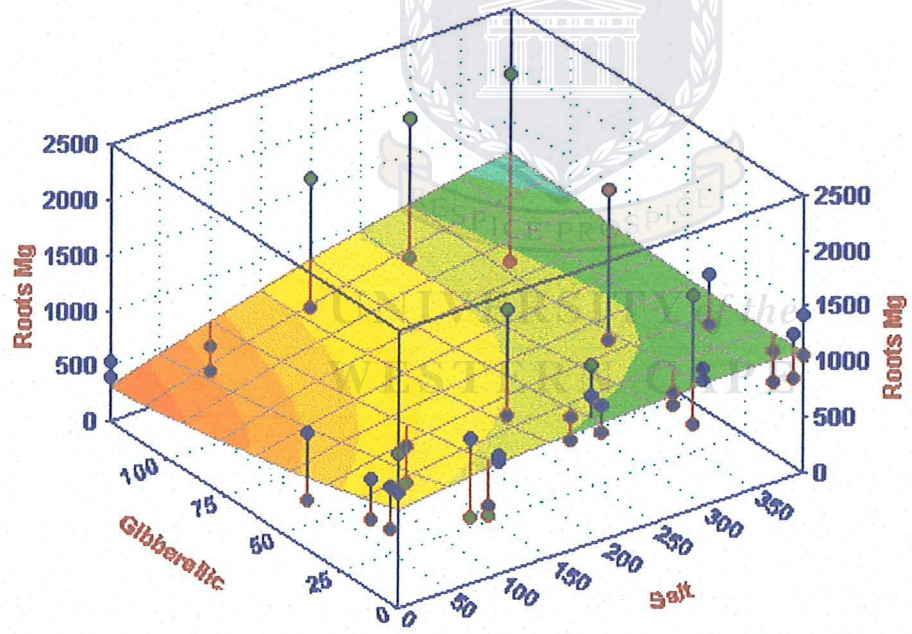

Figure 5.6.8. The interactive effects of salinity (mM) and GA_3 (µM) on root Mg^{2+} concentration ($mg.kg^{-1}$ of dry mass) of *Erucastrum strigosum* plants, using ANOVA.

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Chapter 6:

Summary and conclusion

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The increased substrate NaCl suppressed both shoot and root growth of *E. strigosum* plants (Tables 2.61 and 4.6.1). It is well established that growth response to salinity stress can be described in terms of a two-phased model; osmotic and salt specific responses (Munns & Termaat, 1986). This was also the case for the response of *E. strigosum* plants. Thus, the factors observed, included leaf water loss (Table 4.6.1), accumulation of sodium ions to toxic levels and depression of shoot potassium, calcium and magnesium (Tables 3.6.1 and 5.6.1). The leaf water content decreased with increasing substrate salinity; a well known predominant effect of salinity. Thus, from concentrations as low as 100 mM, the shoot water content was reduced. These observations clearly indicate that *E. strigosum* plants are sensitive to salt stress. Injurious effects such as necrosis, marginal leaf burns and a crinkly leaf appearance also confirmed the sensitivity of this plant to salt stress. The toxic effects of salinity were demonstrated by the accumulation of Na^+ (Tables 3.6.1 and 5.6.1). Nutrient imbalances, on the other hand, were observed as lowered shoot concentrations of K^+, Ca^{2+} and Mg^{2+}. The other factors of salinity stress, reported in the literature, such as effects on stages of development and changes in the growth medium, were not evaluated in the present study.

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The salt sensitivity of *E. strigosum* plants could also be explained by their inability to maintain K selectivity, a tolerance response of glycophytes (Young *et al.*, 1990; Ashraf *et al.*, 2001 and Shirazi *et al.*, 2002). Thus, the shoot K^+ concentration started to decrease when the Na^+ content was increased significantly, relative to controls (Tables 3.6.1 and 5.6.1). The inability to maintain K selectivity under salt stress seems to revolve around the replacement of K^+ with Na^+(Tozlu *et al.*, 2000). In some plants this is controlled by

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addition of calcium to the growth medium (Blatt & Theil, 1993 and Song & Fujiyama, 1996). In the present study, it has been demonstrated that there was not a sufficient supply of calcium to trigger the selection of K^+ (Table 5.6.1). The decrease of K^+ in shoots is also correlated with a plants osmoticum (Marschner, 1995).

Growth depression via the decrease of shoot K^+, has also been observed in kinetin treated plants (Table 3.6.2). Apart from its involvement in leaf osmotic adjustment, K^+ has been found to play a synergistic role with kinetin on growth response. Thus, the inhibitory effects observed in shoots of plants treated with increasing levels of kinetin, were accompanied by depression of the K^+ concentration. It was also observed that in roots, growth was triggered by treatments with kinetin and so was the increase of K^+ content. These findings are in line with the view that cytokinin in shoots is not as effective as those in roots (Chen *et al.*, 1985). Thus, the sensitivity of shoots in response to cytokinins is controlled by K^+ ions. Hence, the kinetin stimulation of root growth paralleled accumulation of potassium in roots of *E. strigosum* (Table 3.6.2). Several hypotheses proposed for the mechanism of action of K^+ ions and kinetins include factors of osmotic adjustment. These findings suggest that the inhibition of growth during exposure to kinetin might be due to a decrease of K^+ ions in shoots.

The exposure of salt stressed plants to kinetin was aimed at evaluating the efficiency of kinetin in the improvement of salinity-induced effects. It was observed that treatments of salt stressed *E. strigosum* plants with kinetin, decreased growth (Table 2.6.1) and exacerbated ionic effects (Table 3.6.1). The salinity-induced ionic effects include the

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accumulation of potentially toxic sodium ions and the decrease of potassium, calcium and magnesium ions (Figures 3.6.2.1-4). Thus, the selection mechanism was not in favour of K^+. These findings suggest that both tolerance mechanisms used during exposure to salinity, by many glycophytes, were not enhanced by addition of kinetin in the studied plant. These observations are in agreement with those of Benzoini *et al.* (1974) and Aldesuquy & Ibrahim (2001).

Gibberellic acid, on the other hand, managed to enhance growth in both salinated and salt free plants. Even though gibberellic acid enhanced growth in these media, it was observed that at higher concentrations it started to reduce growth (Figures 4.6.1-3). These observations suggest that GA_3 is effective only at low concentrations. It is interesting to note that ionic-specific effects such as Na^+ accumulation and decrease of K^+, Ca^{2+} and Mg^{2+} persisted regardless of treatments with GA_3 (Figures 5.6.1-4). Kawasaki and his coworkers (1978) reviewed that GA_3 and K^+ or Na^+ act synergistically in promoting the elongation of lettuce hypocotyls segments. However, this cannot be used to explain shoot growth in *E. strigosum* plants because Na^+ in this plant has no nutritional value. Na^+ is nutritionally important in selected halophytes and some C_4 species (Raven *et al.*, 1992).

The depression of K^+ in the shoots of kinetin and gibberellin treated salanized plants, whether followed by growth or not, suggests that treatment of *E. strigosum* plants with these hormones failed to stimulate a salt exclusion mechanism. Thus, it has failed to induce K^+ selection in shoots. These observations are in contrast with those of Aldesuquy & Ibrahim (2001) who found that when *Triticum aestivum* plants were pretreated with

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GA_3, IAA and ABA, before it was exposed to seawater, GA_3 effectively increased the absorption of K^+. Their findings were based on the assumptions that GA increased the absorption of K^+ by plants. It is possible that in the case of GA_3-treated plants, a salt inclusion mechanism was stimulated. Marschner (1995) postulated that plants utilizing a salt inclusion mechanism, grow under salt stress conditions. In the present study, gibberellic acid enhanced growth and accumulation of Na^+ ions in both shoots and roots. It is therefore recommended that this be further explored. Moreover, one should evaluate the osmotic adjustment effects by measuring the induction of osmoprotectants such as proline, glycine, betaine (trimethylglycine) and a low weight protein, osmotin, when salt stressed plants are treated with kinetin and GA_3. It has already been observed that salt tolerance of kinetin treated *Vigna sinensis* and *Zea mays*, is associated with regulation of proline, and other osmoprotectants (Nemat Alla *et al.*, 2002). Whether this can be used to explain growth stimulation by GA_3 in *E. strigosum* plants remains unknown at the moment.

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etd.uwc.ac.za

References

ALDESUQUY H S, IBRAHIM A H (2001) Water relations, abscisic acid and yield of wheat plants in relation to the interactive effect of seawater and growth bioregulators. Journal of Agronomy and Crop Science 187: 97-104.

ASHRAF M, NAZIR N, MCNEILLY (2001) Comparative salt tolerance of amphidiploid and diploid *Brassica* species. Plant Science 683-689.

BENZOINI A, MIZRAHI Y, RICHMOND A E (1974) Effect of kinetin on plant's response to salinity. New Phytologist 73: 315-319.

BLATT M R, THEIL G (1993) Hormonal control of ion channel gating. Annual. Reviews of Plant Physiology and Plant Molecular Biology 44: 543-567.

CHEN C-M, ERTL J R, LEISNER S M, CHANG C-C (1985) Localization of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiology 78: 510-513.

KAWASAKI H TAKADA H, KAMISAKA S (1978) Requirement of sodium chloride for the action of gibberellic acid in stimulating hypocotyls elongation of a alophyte, *Salicornia herbacea* L. Plant & Cell Physiology. 19: 1415-1425

MARSCHNER H 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, San Francisco.

MUNNS R, TERMAAT A (1986) Whole plant response to salinity. Australian Journal of Plant Physiology 13: 142-160.

NEMAT ALLA M M, YOUNIS M E, EL-SHIHABY O A, EL- BASTAWISY M (2002) Kinetin regulation of growth and secondary metabolism in waterlogging and salinity

etd.uwc.ac.za

treated *Vigna senensis* and *Zea mays*.

http://www.zfr.pan.krakow.pl/ang/app/2002no1/1.html downloaded on 15/01/03

RAVEN P H, EVERT R F, EICHHORN S E (1992) Biology of plants. 5th edn. Worth Publishers. New York.

SHIRAZI M U, KHANZANDA B, ALI M, U-ISLAM E, MUJTABA S M, ANSARI R ALAM S M, KHAN M A, ALI M (2002) Response of three wheat genotypes grown under saline medium to low/ high potassium levels. Acta Physiologia Plantarum 24: 157- 161.

SONG J Q FUJIYAMA H (1996) Differences in response of rice and tomato subjected to sodium salinization to the addition of calcium. Soil Science and Plant nutrition 42: 503-510.

TOZLU I, MOORE G A, GUY C L (2000) Effects of increasing NaCl concentration on stem elongation, dry mass production and macro- and micro nutrient accumulation in *Poncirus trifolita*. Australian Journal of Plant Physiology 27: 35-42.

YOUNG, Y. W. NEWTON, R. J. & MILLER, F. R. 1990. Salinity tolerance in sorghum. I. Whole plant response to sodium chloride in *Sorghum bicolor* and *Sorghum halepense*. Crop Science 30: 775- 78

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