Preconditioning Responses of Salt-tolerant and Salt-sensitive Provenances of *Acacia tortilis* (Forsk.) Hayne to High Salinity

by

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A thesis submitted in conformity with the requirements for
the degree of Master of Science in Forestry at the
University of Toronto.

ABSTRACT

TITLE: Preconditioning responses of salt-tolerant and salt-sensitive provenances of *Acacia tortilis* (Forsk.) Hayne to high salinity.

Andrew David Park, MScF Thesis, December, 1995.

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Growth and nutrient responses to NaCl preconditioning treatments were studied in two seed provenances of *Acacia tortilis* (Forsk.) Hayne from saline (Sigor) and non-saline (Kitui) areas of Kenya. Seedlings were exposed to NaCl preconditioning treatments at 15, 21, and 28 days after germination. Subsequently, all seedlings were exposed to 200 millimolal NaCl for a further 39 days to test the efficacy of the different preconditioning schedules in hardening seedlings of different ages to high salinity.

Growth of Kitui was inhibited in proportion to the duration of pre-treatment, while growth of Sigor was equally inhibited by all NaCl treatments. Pre-treated seedlings grew at the same provenance-specific rates upon subsequent exposure to 200 millimolal NaCl, irrespective of pre-treatment. However, Sigor was distinguished from Kitui by having higher relative growth rates, faster rates of stem elongation and dry matter accumulation, lower root:stem and root:shoot ratios, and higher unit leaf rates. Higher rates of stem growth and elongation appear to be the key morphological feature that distinguishes Sigor from Kitui. The potential advantages of this trait include, the dilution of toxic ions in the transpiration stream, reduced self-shading by leaves and stem photosynthesis. Sigor maintained similar growth advantages over Kitui in control

treatments, suggesting that fast growth may be an inherent characteristic of salt tolerant provenances of *Acacia tortilis*.

Nutrient analysis showed that Sigor initially partitioned a greater proportion of total Na^+ uptake to roots than shoots. Both provenances had similar contents of macro-nutrients and Na^+ by the final harvest of the experiment. However, all ions were more concentrated in Kitui than Sigor, probably as a combined result of Kitui's lower growth and less efficient partitioning of Na^+. Multivariate ordinations confirmed the sensitivity of leaf abscission and shoot growth as variables associated with salt-tolerance, and suggested that the effects of Na+ were felt as much through disturbances in nutrient metabolism as they were through direct-ion toxicity.

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ABBREVIATIONS

ABA	absissic acid
gs	stomatal conductance
PCA	Principle Components Analysis
Pn	net photosynthesis
RDA	Redundancy analysis
RGR	Relative Growth Rate
RRGR	Root Relative Growth Rate
SRGR	Shoot Relative Growth Rate
ULR	Unit Leaf Rate
WUE	Water Use Efficiency

GLOSSARY

Eigenvalues (in multivariate ordination): a number between zero and one representing the fraction of the total sum of squares of a transformed data set extracted by an ordination axis.

Scores (of species, sample or other variables in multivariate ordination): Transformed weighted averages of the data set for a particular variable, or of variable values from a particular data set that are used to plot explanatory graphs and to interpret the eigenvalues of ordination axes.

Secondary salinization: salinization of a soil profile caused by irrigating crops with saline water, the capillary rise of saline ground water when the over-watering of crops is accompanied by high volumes of subsequent evaporation, and the exposure of saline mineral soil through soil erosion (Szabolcs, 1989). On pasture lands and wooded savannas, salinity may be the result of reduced evapotranspiration from over-grazed grass lands and from tree cover that has been over-harvested for fuelwood (Greenwood, 1986).

ACKNOWLEDGEMENTS

I owe the completion of this thesis to the advice, assistance and support of many people. Without their help, it could not have been written, and would have been a good deal less enjoyable to prepare.

I gratefully acknowledge the academic advice and material assistance I received from my supervisor, **Professor Terry Blake** during the course of my masters research. I would also like to thank **Professor Vic Timmer** for his patient advice on vector analysis and interpretation, and for the use of his laboratory facilities for plant nutrient analysis. **Doctor Alan Darlington** advised me on experimental design and statistical analysis, and **Vikram Malik** assisted me in the use of SAS. Thanks are also due to **Professor Terry Carleton** who freely donated his time and expertise to assist me in the planning and interpretation of multivariate analyses.

My good friends, **Mina Arakawa, Vera Borsos-Matovina, Christine Grekos, Natasha Suvorova** and **Dave Tamblyn** were indispensible for helping during various phases of plant harvesting, changing nutrient bottles and analyzing plant nutrients. Thanks to them for making a tough job that much easier and enjoyable to do.

DEDICATION

I dedicate this thesis to my mum, **Joanne Park**, who was the first to inspire me with a love of Nature and the Earth, and who has always encouraged me to do the things I loved to do. Way to go, Mum!

1

GENERAL INTRODUCTION

1.1 - BACKGROUND.

Chronic salinity stress to crop plants is probably as old as agriculture itself. The ancient Sumerians permanently salinized much of the fertile riparian lands bordering the Euphrates river, and their principle mistakes (overwatering, poorly timed watering and inadequate drainage of irrigated land), are being repeated on a global scale today (McWilliam, 1986).

The populations of arid and semi-arid lands are often caught in a triple crisis. Prime agricultural land is at a premium due to sedentarization and population growth, reduced fallows are reesulting in more intense land-use (Kanani and Torres, 1986), and fuelwood supplies are being depleted at alarming rates (El-Lakany, 1986). In the future, it is likely that forage crops and fuel wood plantations will be relegated to infertile and saline lands (Dudal and Purnell, 1986; Kanani and Torres, 1986; National Research Council, 1990), necessitating the evaluation and improvement of salt tolerant tree species (El-Lakany, 1986; Midgley et al., 1986).

Leguminous trees, such as *Acacia* and *Prosopis* spp., are widespread in arid and semi-arid lands that have natural- or induced-saline soils. For example, at least 18 tree species grow on the salt affected lands of Kenya, including six species of *Acacia* that provide forage for goats and camels, building materials, charcoal, and medicines to local people (Kanani and Torres, 1986). *Acacia* and *Prosopis* are biologically diverse

genera, with representatives that can tolerate extremes of drought and salinity (Fagg and Stewart, 1994), and are therfore likely candidates for planting on salt-affected lands.

1.2 - Objectives, Experiments And Hypotheses

The laboratory experiments reported in this thesis used seeds of **Kitui** (salt-sensitive), and **Sigor** (salt-tolerant) provenances of *Acacia tortilis* whose relative salt-tolerance had previously been determined (Muturi, 1993). The overall aim of the work was to assess whether *Acacia tortilis* provenances of known salt-tolerance could be preconditioned to moderately high, chronic levels of soil salinity. Preconditioning of plants to a variety of environmental stresses can be accomplished through preliminary exposure to moderate levels of that stress (Levitt, 1980), and recent studies have provided evidence that crop plants can be preconditioned to high levels of salinity (Amzallag et al., 1990; Amzallag et al., 1993; Seligmann et al., 1993). Lack of adequate conditioning has also been cited as a leading reason for the failure of outplanted seedlings in arid areas (Zumer-Linder, 1986). Therefore, conditioning tree seedlings to salinity could potentially increase their survival in the field, and possibly enhance their tolerance of other environmental stresses (O' Connor et al., 1991). *Acacia tortilis* has already shown promise as a salt-tolerant tree in Turkana, Kenya (Zumer-Linder, 1986), and may therefore be a good candidate for conditioning at the seedling stage.

Two experiments were performed:

Experiment 1 used growth analysis to investigate responses of the two selected provenances to three NaCl preconditioning schedules. The goals of the experiment were, (a) - to investigate age-specific responses of Sigor and Kitui provenances of *Acacia tortilis* (Forsk.) Hayne to NaCl pre-treatment, and (b) - to assess the relative efficiency of the preconditioning schedules by subjecting pre-treated seedlings to a longer period of chronic salinity.

Experiment 2 investigated nutrient distribution and salt partitioning as possible markers for conditioning to saline conditions. Salt and nutrient element distributions amongst plant organs were analyzed using ANOVA, vector analysis and multivariate ordination to assess differences in nutrient allocation amongst seedling organs and interactions between elements in seedlings exposed to different pre-treatment schedules.

The thesis is divided into five chapters. Following this general introduction, chapter two discusses the extent of global salinity, reviews the current literature on salinity tolerance, and discusses the possibility of preconditioning plants to saline conditions. Chapters three and four are devoted to experiments one and two respectively, while chapter five integrates the research findings and offers a general conclusion.

Table 1.1. Seed provenances of Acacia tortilis and site conditions of origin (from Muturi, 1993).

Provenance	Soil Description	Climatic Zone*	KEFRI batch #
Kitui	Non-Saline loamy sand/ sandy loam	III	038/015/86 (collected 1994)
Sigor	Strongly calcareous saline to mod. sodic stony loam.	VI	038/022/90 (collected 1994)

* Indicates aridity, increasing from III - VI based on rainfall: evapotranspiration ratio.

2

LITERATURE REVIEW

2.1 - INTRODUCTION

In the closing years of the twentieth century, many countries in both the developing and industrialized world face a growing environmental burden of soil salinization. Rates of secondary salinization are increasing (Szabolcs, 1994), and expanding human populations are increasingly exploiting naturally saline lands for agriculture, animal pasture and tree harvesting. Planting multi-purpose trees in saline areas is a rational use for saline lands that are unsuitable for arable crops, and may help to alleviate imminent fuelwood shortages in developing countries. However, selection criteria for salt-tolerant tree species are poorly developed. Despite advances in understanding the cellular and sub-cellular mechanisms of salt-tolerance, their significance for the adaptation of whole plants is poorly known.

This chapter reviews the literature on cellular, molecular, and whole plant responses to salinity. The somewhat sparser literature on preconditioning plants to high salinity is also reviewed. To date, such preconditioning has been studied in agricultural species and some halophytes, but never on trees. If it could be demonstrated in trees, preconditioning to salinity could be used to increase the survival and growth of tree seedlings on sites suffering from either natural or induced salinity.

2.2 - The Problem Of Secondary Soil Salinity.

History, causes and distribution of salinity problems.

Early agricultural civilizations caused the secondary salinization of fertile agricultural land in ancient Mesopotamia, the Indus Valley, and large areas of China and South America (Szabolcs, 1989). Today, estimates of the total land area affected by secondary salinization vary from 53 million (El-Lakany, 1986) to as many as 950 million hectares worldwide (Luangjame, 1990, Szabolcs, 1989). When naturally saline areas are added to these estimates, the total area of saline soils rises to 13.2 billion hectares, or about 7% of the global land base (Dudal and Purnell, 1986). The most chronically affected soils are in the irrigated riparian zones of major rivers flowing through arid lands. Chronic salinization has affected the valleys of the Tigris and Euphrates (Syria and Iraq), the Indus (Pakistan), Ganges (India), Mekong (northern Thailand), the Huang (North China Plain), and the Murray-Darling basin (Australia) (McWilliam, 1986). The Aral basin in (former) Soviet Central Asia has suffered extensive salinization as a result of contamination with recycled, saline irrigation water and wind-blown salts from the exposed bed of the drying Aral Sea (Prekoda, 1991).

The fuelwood crisis.

Expensive sub-surface drainage networks are the only way to permanently reclaim saline lands (McWilliam, 1986). Alternatively, promising food, fuel, and fodder plants could be screened for salt-tolerance, and then outplanted onto salt-affected land. Fuelwood crops could be particularly important on saline soils in developing countries, where over 1.5 billion people depend on wood for cooking and heating (Midgeley et

al., 1986). Of these, it was estimated (in 1980) that 100 million were unable to satisfy their basic fuelwood needs (250 - 1500 kg/person/year), while 1.11 billion could only do so by depleting naturally existing supplies (Dudal and Purnell, 1986). El-Lakany (1986) predicted the development of a fuel wood deficit of 15 x 10^9 m^3/yr. over the next twenty years if these trends continue. The deficit will have to be made good with a five-fold increase in tree planting, and since population pressures are forcing the complete development of the available high quality agricultural land for food crops, tree plantations will increasingly have to be nurtured from infertile or saline soils (Midgley et al., 1986).

Tree species for saline sites.

Salt-tolerance has been observed in many tree genera, including *Acacia, Casuarina, Eucalyptus, Melaleuca,* and *Prosopis.* (Felker et al., 1981; Clemens et al., 1983; National Research Council, 1990). The genus *Tamarix* has many salt-tolerant species, some of which can survive irrigation with sea water. Others, such as the Neem tree (*Azadirachta indica*), and mesquite (*Prosopis sp.*) can fix nitrogen, and can be established on infertile soil (National Research Council, Washington, DC, 1990). Differences in the salt-tolerance of tree species have been demonstrated (Blake, 1981; Aswathappa and Bachelard, 1986; Marcar and Termaat, 1990). Provenance-specific differences have also been observed (Sands, 1981, Midgley et al., 1986, Muturi, 1993), but are not always related to the soil salinity of the sites of origin (Dafni and Negbi, 1978; Waisel, 1972).

Silviculture on saline sites is still experimental, with a few species of known tolerance forming the basis of most reclamation programs. *Prosopis* and *Eucalyptus* are often preferred for planting in saline-arid and semi-arid areas, *Prosopis* for its nitrogen-fixing abilities, and *Eucalyptus* for its drought-tolerance and ability to lower saline water tables (National Research Council, 1990).

While exotic tree species are often preferred because of their fast growth and well known silvics, multi-purpose native species are now being tested because they may be more socially and ecologically appropriate in some areas. In Botswana, for instance, *Acacia tortilis* was planted following the failure of *Eucalyptus camaldulensis* (Tietma and Merkesdal, 1986), while in Turkana, Kenya, a mix of indigenous species was preferred by local people for fruit and fodder, while fast growing exotics (e.g. *Prosopis*), were used for commercial poles (Zumer-Linder, 1986). In Kenya, Kanani and Torres (1986) identified nine trees and five shrubs that commonly grew on saline sites. Collectively, these species were used for building materials, charcoal production, fodder, fuelwood, and the provision of medicines.

2.3 - Overview Of Salinity Effects On Higher Plants.

Although dominant elements in salt affected soils include magnesium, calcium and acid sulphate salts, in addition to NaCl (Szabolcs, 1989), NaCl is the most commonly used experimental salt (Munns and Termaat, 1986). In experimental systems, Na^+ has been the most investigated agent of toxicity and elicitor of plant responses. Sodium inhibits cell wall elongation during growth, and competitively inhibits the uptake of Ca^{++} (Rengel, 1992) and K^+ (Rains and Epstein, 1966; Jeschke, 1984). Chloride has

been thought of as being tolerated over a wide range of concentrations (Akita and Capuslay, 1990). However, Cl^- is known to inhibit the uptake of NO_3^- (Aslam et al., 1984), decrease nitrate-reductase in leaves and, possibly, inhibit the activity of the K^+-malate shuttle (Cramer et al., 1995). Low rates of Cl^- and Na^+ transport are associated with salt-tolerance in Rangpur lime (Storey, 1995), .

Sodium chloride can affect the physical properties of soil-water and cell-sap, and the physiological responses of plants exposed to saline environments. Soil-water (Luangjame, 1990) and apoplastic (Binzel et al., 1988) water potentials are reduced, necessitating cytosolic accumulation of organic solutes or tolerance to cytosolic NaCl (some halophytes) (Adams, 1993) to protect cell turgor.

The relationships between NaCl uptake and that of other nutrients, particularly K^+ and Ca^{++} are complex. Millimolar concentrations of Ca^{++} are essential for efficient cation uptake by roots (Epstein, 1961). Calcium, because of its structural role in cell membranes (Yeshem, 1992), may play a general role in their protection and the reversal of Na^+ mediated inhibition of K^+ uptake (Lauchli and Schubert, 1989; Rengel, 1992). Field and laboratory studies have shown that adding Ca^{++} to nutrient solutions or soil ameliorates the effects of salinity on growth and nutrient uptake (Gorham et al., 1988; Hansen and Munns, 1988 (a) and (b); Marcar and Termaat, 1990; Subbarao et al., 1990).

Growth responses to salinity vary between monocots and dicots, halophytes and glycophytes, and different species and families of plants. In glycophytes, they may include reduced leaf expansion (Munns and Termaat, 1986, Flowers and Yeo, 1989),

reduced shoot elongation (Marcar and Termaat, 1990), altered root/shoot ratios (Termaat and Munns, 1986; Marcar and Termaat, 1990; Gorham et al., 1988), and the abscission of older leaves (Gorham et al., 1988; Muturi, 1993). Slower growth is also observed in salt-conditioned halophytes (Adams, 1993), and some apparently having an obligate requirement for NaCl accumulation (Glenn et al., 1994).

2.4 Cellular And Molecular Responses To Salt-Stress.

The root cortex, endodermis, stele, xylem and phloem can be thought of as integrated subsystems of whole plants, while cell walls, plasmalemmae and tonoplasts regulate ion fluxes through these sub-systems and the ion-selectivity of plant tissues. The flux of hydrated ions across hydrophilic membrane bilayers is determined by a thermodynamic gradient made up of an electrical gradient and a gradient of chemiosmotic potential (Niu et al., 1995). Cell cortices are negatively charged to about -100mV relative to the outside of the cell (Barrett-Lennard, 1986). This condition results in passive inward fluxes of cations, and the necessity for active transport of anions. The maintenance of membrane potentials also consumes metabolic energy, as shown by membrane depolarization in anaerobic conditions (Contardi and Davis, 1978).

Competitive inhibition of ion uptake.

Much of the literature on salt-stress concentrates on the effects of Na^+ on the macronutrients Ca^{++} and K^+, which perform a variety of important physiological roles within plants(Lauchli and Schubert, 1989; Rengel, 1992). Potassium activates starch synthesis enzymes, regulates guard cell turgor through ABA-mediated trans-membrane

fluxes, and, because of its abundance, acts as a general osmoticant (Salisbury and Ross, 1985: p108). Calcium stabilizes integral membrane proteins (Yeshem, 1992), and the breakage and reformation of double covalent Ca^{++} bonds is responsible for cell wall extensibility during growth (Salisbury and Ross, 1985).

While the precise route by which Na^+ enters roots is unknown (Niu et al., 1995), it is known to competitively inhibit K^+ uptake (Jeschke, 1984), probably via the low K^+-affinity system 2 of selective trans-membrane K^+ transport (Rains and Epstein, 1967). The low K^+/Na^+-selectivity of system 2 probably permits a passive inward ionic flux at high concentrations of Na^+ (Maathuis and Sanders, 1993). However, energy-dependent ionic fluxes are coupled to H^+ flows along a thermodynamic gradient and the hydrolysis of Ca^{++}-ATPases in tonoplasts (Niu et al., 1995, Erdei and Kuiper, 1980).

Calcium may inhibit Na^+ flows through system 2 of K^+ uptake (Schroeder et al., 1994), and has been proposed as a general stress-signal transducer (Rengel, 1992) because of its key role as a "second messenger" that mediates many physiological processes (Evans et al., 1991; Schulte-Buckloh and Fromm, 1993). The NaCl-mediated depolarization of trans-membrane potentials results from displacement of cell-wall and plasma-membrane Ca^{++} fractions (Cramer et al., 1985), and is reversible by the addition of 4 mM Ca^{++} (Lauchli and Schubert, 1989). Membrane depolarization is also linked to non-competitive inhibition of NO_3^- uptake in wheat (Hawkins and Lewis, 1993), and NO_3^- uptake has been correlated with Ca^{++} status in Faba beans (Cordovilla et al., 1995). Chloride also inhibits NO_3^- uptake by competitive inhibition of the NO_3^-

transporter (Aslam et al., 1984). Thus, membrane depolarization could be the initial event in a cascade of nutrition-related physiological events.

Compartmentation of ions and cell turgor maintenance.

Sodium and Cl^- are thought to be actively excluded from the cytoplasm of higher plant cells, although Cheeseman (1988) argues that we do not know the concentration at which cytoplasmic Na^+ becomes unacceptable. Nevertheless, active transport and ion-selective channels in plasmalemmae and tonoplasts compartmentalize ions and modify the contents of cytoplasm .

The uptake of Na^+ is passive (Cramer et al., 1987), but the active efflux of Na^+ is mediated by Na^+/H^+ antiporters (Niu et al., 1995). A Ca^{++} dependent Na^+/H^+ antiporter in tonoplasts of *Beta vulgaris* concentrates Na^+ against its own concentration gradient (Barkla and Blumwald, 1991). Calcium, in association with calmodulin, also initiates the activities of a tonoplast Na^+ uniport and a Ca^{++}/Na^+ antiport (Yeshem, 1992).

Clearly, the intracellular location of ions will affect turgor maintenance within salt-stressed cells. For example, high Na^+ concentrations in cell walls would reduce cell wall water potential, placing the cytoplasm under osmotic stress (Bingham et al., 1968). Vacuolar compartmentation of Na^+ and Cl^- could contribute to cell turgor. However, since tonoplast Na^+/H^+-antiports are reversible, maximum rates of transport will be limited by the slopes of Na^+ and H^+ gradients between vacuoles and cytoplasm (Reinhold et al., 1989). Thus, tonoplast transport molecules of halophytes that accumulate Na^+ and Cl^- in leaf vacuoles may have a higher Vmax than those of

glycophytes which depend heavily upon the synthesis of organic osmotica, such as sugars, for turgor control (Adams, 1993).

2.5 - Whole Plant Responses To Salinity.

Whole plant regulation of ion uptake.

The rigors of terrestrial living complicate the extrapolation of salt effects from cell cultures to whole plants,. Additionally, whole plants may display different tissue and organ responses to salinity, leading to different degrees of tolerance in calluses or cell lines and whole plants (Perez-Alfocea et al., 1994; Flowers et al., 1989).

Ion compartmentation differs between tissues and organs. For example, Na^+ is partitioned away from non-vacuolated root meristem cells of *Hordeum distichum*, and has increasing deposition rates with distance from root tips (Jeschke and Stelter, 1976). Similarly, the ameliorative effects of increased Ca^{++} supply may be chiefly associated with plasma membranes of meristematic tissues (Zhong and Läuchli, 1994).

The integration of ion transport between plant organs is modified by salinity. Sodium may be reabsorbed into the xylem parenchyma, exported from shoots to roots via the phloem, and then excreted (Jeschke, 1984). Potassium is also preferentially translocated away from senescent tissues to growing roots and newly expanding leaves (Jeschke and Wolf, 1993), where it acts as an osmoticant and is closely associated with maximal protein deposition rates (Silk et al., 1986). Conversely, the operation of the K-malate shuttle in NO_3^- transport is inhibited by NaCl, and NO_3^- is loaded into the xylem in amino acids (Cramer et al., 1995).

Regulatory responses in whole plants.

Plant strategies for the regulation of tissue ion concentrations may, in part, be environmentally determined. Halophytes grow in conditions in which Na^+ and Cl^- are the dominant soil elements, and employ these elements to maintain turgor. They accomplish better ion regulation than glycophytes using thicker casparian strips that restrict apoplastic flow (Waisel, 1972) and more efficient tonoplast ionic pumps (Erdei, Stuiver and Kuiper, 1980). Regulatory options for glycophytes growing in saline environments include limiting transpiration (and therefore, the transport of toxic ions), regulation of the ionic contents of the transpiration stream, dilution of ionic concentration through growth, and the use of organic solutes as osmotica (Flowers and Yeo, 1989).

Differences in the distribution and strength of ionic sources and sinks could explain the regulatory responses of different species to salinity stress (Flowers and Yeo, 1986). For example, halophytes that accumulate Na^+ and Cl^- can maintain cell sap osmotic potentials two to three times higher than that of the soil solution, which confers tolerance to salinities as high as 1160 mol. m^{-3} (Glenn et al., 1994). Perennial glycophytes may divert excess ions to mature leaves, where they are either concentrated in the apoplast (dehydration) or the symplast (ion toxicity) (Flowers and Yeo, 1986). Eventually, abscission of presenescent leaves on the lower stem may occur, resulting in decreased leaf biomass and area, but postponing the onset of stress in the upper canopy (Flowers and Yeo, 1989). This pattern has been observed in *Casuarina equisetifolia* (Aswathappa and Bachelard, 1986), *Leucaena leucocephala* (Gorham et al., 1988), *Eucalyptus* (Marcar, 1989) and *Acacia tortilis* (Muturi, 1993).

Growth responses of whole plants

Although ion exclusion and partitioning help to protect growing tissues of plants inn saline environments, they are not completely effective. As salt concentrations increase in expanding leaves, cell volumes and elongation rates decrease (Singh et al., 1989), DNA and RNA synthesis may cease, and protein synthesis is inhibited (Aspinall, 1986).

Growth inhibition is not always related to reductions of cell turgor. Glycophyte cells that respond to NaCl-induced physiological drought by restoring cell turgor grow more slowly than unstressed cells (Singh et al., 1989). Munns and Termaat (1986) found that artificially restoring leaf turgor by applying air pressure to roots failed to increase leaf expansion in five crop plants. Although iso-osmotic macro-nutrient and NaCl solutions failed to inhibit leaf elongation, PEG and mannitol at the same osmotic potential reduced leaf expansion rates by 40 - 50% (Termaat and Munns, 1986). These data have been used to argue against growth reduction by NaCl through osmotic stress, and for more indirect effects, such as the proven inhibitory effects of NaCl on nutrient uptake (Munns and Termaat, 1986). By contrast, Cramer et al. (1994b) found no correlations of nutrient status or NaCl accumulation with shoot growth of salt-tolerant and salt-sensitive maize hybrids, arguing instead for the primacy of osmotic effects.

Increased root: shoot ratios under NaCl stress have been reported in many species (Luangjame, 1990; Hurkman and Tanaka, 1987; Aspinall, 1986, Termaat and Munns, 1986; Munns and Termaat, 1986). Root growth may also be inhibited, depending on the concentrations of NaCl and the species involved (Poljakoff-Mayber and Lerner,

1994). Either of these conditions could be the result of Cl^- interactions with NO_3^-. Trewavas, (1985) suggested that increased shoot C: N ratios as a result of NO_3^- inhibition and carbohydrate accumulation in shoots could stimulate root growth at the expense of shoots. Root growth may be inhibited by reduced protein synthesis in barley roots (Hurkman and Tanaka, 1987), either as a result of Cl^- inhibition of NO_3^-uptake, Na^+ inhibition of K^+ uptake, or both. *Hordeum vulgare* and *Ricinus communis* both support continued protein synthesis through the extensive retranslocation of K^+ from leaves and shoots to roots (Jeschke and Wolf, 1993). If roots sequester NO_3^-before it can reach the shoots (Termaat and Munns, 1986), K^+ is exported to roots, it is not surprising that leaf growth is inhibited, and that sugars accumulate in leaves as a result.

Long term exposure to salinity could reduce carbohydrate reserves below maintenance levels (Munns and Termaat, 1986). Reduced rates of leaf expansion, together with the abscission of older leaves, may account for much of the increase in root: shoot ratio (Gorham et al., 1988) and could eventually limit the carbon available for growth, as the demands of the continually growing root system outstrip the capacity of the canopy to supply them (Munns and Termaat, 1986). Growth reduction of spinach was proportional to decreased stomatal conductance, suggesting a possible limitation on carbon assimilation (Cheeseman, 1988). Alternatively, since phloem unloading is positively correlated with high Ca^{++} concentrations in sieve element cell walls and cytoplasm, (Schulte-Baukloh and Fromm, 1993), Na^+ competition with Ca^{++} uptake could result in carbohydrate build-up in some tissues and starvation of others. Therefore, the inability to transport carbohydrate, rather than to manufacture it, could starve the roots and initiate further reductions in water and mineral supply.

Life-cycle and morphological differences.

Differences in longevity, morphology and habitat help to determine the specificity of plant responses to salinity. The requirement of Na^+ and Cl^- as osmotica in some halophytes vs. their active exclusion in glycophytes provides the most obvious example of a habitat-based difference in response to salinity. Leaf abscission may only be a viable response to salinity in the case of perennial glycophytes (Adams, 1993), since annuals are likely to have seasonally-induced deterministic growth, and will therefore be unable to replace lost photosynthetic capacity.

Flowers and Yeo (1989) suggested that the dilution of cytoplasmic salt through growth could characterize some salt-tolerant species. Such dilution has been reported in the mangrove *Rhizophora mucronata* which maintains constant Na^+, Cl^- and water contents at constant levels throughout its leaf life-cycle (Atkinson et al., 1967). However, the quantity of ions reaching the shoots is probably a key determinant of growth (Flowers and Yeo, 1986). Higher growth rates would therefore demand higher rates of ion flow through the SPAC (R. L. Jefferies, personal communication) accompanied by the efficient extraction or exclusion of Na^+ and Cl^- from the transpiration stream.

Trees grown in saline soils are known to suffer leaf necrosis during hot, dry weather (Bernstein et al., 1972), and over several years, fruit trees may accumulate toxic levels of salts from relatively non-saline soils (Bernstein, 1980). Some leguminous trees and shrubs, such as *Acacia* and *Prosopis* sp. (Sprent, 1987) and *Tamarix aphylla* (Waisel, 1972), avoid the consequences of such long-term exposure by developing

deep tap roots that exploit non-saline water tables (Adams, 1993). Species with deep tap roots are therefore likely to be most vulnerable to salt-damage at the seedling stage, and therefore, fast-growing seedlings may be favoured, as has been observed in halophytic ecotypes of *Prosopis farcta* (Waisel, 1972).

2.6 - PRECONDITIONING PLANTS TO SALINITY.

General remarks

Preconditioning, the phenotypic modification of plants in response to an environmental stress, can be accomplished through preliminary exposure of plants to moderate levels of that stress (Levitt, 1980). In considering the measurement of preconditioning to salinity, Poljakoff-Mayber and Lerner (1994) use either enhanced relative growth rates or the completion of life-cycle (including reproduction) as measurements of successful conditioning. In perennial plants, growth enhancement may be inadequate, in the absence of efficient ion-partitioning and osmotic control, for ensuring long-term survival of saline conditions. Cellular mechanisms for maintaining ion homeostasis and osmotic adjustment must therefore exist (Niu et al., 1995) and possess the phenotypic flexibility that is necessary for conditioning to take place.

Evidence for salt-preconditioning

Work on cell physiology and genetics suggests that preconditioning of some genotypes to salinity is possible, although a degree of inherent salt-tolerance may be necessary. The induction of tonoplast ATPase dependent Na^+/H^+ antiport activity could only be stimulated in salt-tolerant *Plantago maritima* by prior exposure to 50mM NaCl (Staal et al., 1991). Furthermore, NaCl induces closure of tonoplast Na/K ion channels

in *Plantago* sp. (Maathuis and Prins, 1990). In *Lophopyrum elongatum* and wheat, messenger RNA's known to accumulate during early salt stress are activated by salinity, osmotic stress and external Ca^{++} (Galvez et al., 1993). That salinity induces specific phenotypic responses is suggested by the induction of different regions in two *Arabidopsis* genes by different stresses: namely, salt, drought and low temperature (Yamaguchi-Shinozaki and Shinozaki, 1994).

Preconditioning, measured by improved growth, growth recovery and survival at very high salinities, has been observed in whole plants of *Sorghum bicolor* and finger millet (*Eleusine coracana*). Seedlings of *Sorghum bicolor* (L.) Moench, cv. 610 preconditioned with 150mM NaCl (applied in 25mM/ day increments commencing 8 - 21 days after germination) grew faster, on subsequent exposure to 300 mM NaCl, than plants subjected to a 75 mM pretreatment (Amzallag et al., 1990). Plants of *Eleusine coracana*; plants survived at 400mM. NaCl following pretreatment with 200mM NaCl. (Uma et al., 1993).

Amzallag et al. (1993) showed that the preconditioning of *Sorghum bicolor* was age-dependent, the best adaptation occurring if preconditioning began five days after germination. The latest age for effective pretreatment was 12 days after germination (Seligmann et al., 1993). Salt-tolerance also improves with age in Muskmelon (*Cucumis melo*) (Shannon, 1985) and *Tamarindus indica* (Pongskull et al., 1988). The rate at which salinity is applied can also affect subsequent salt-tolerance in different species, some requiring slow, others needing rapid addition of NaCl (Hamza, 1978).

Other observations

Cross-tolerance between salt and other stresses has also been observed. A tissue culture derived, salt-tolerant semaclonal variant of flax (*Linum usitatissimum* L.) was more heat-tolerant than the parent plant after salt preconditioning (O' Connor et al., 1991). Preconditioning of cotton by heat shock prevents some adverse effects of subsequent salinity (Kuznetsov et al., 1992), and exposing the roots of six week old Spinach cv. Monnopa seedlings to 300 mM. NaCl increased frost hardiness by 2.4°C (Hincha, 1994).

Preconditioning of trees.

While the responses of trees to salinity have a genetic basis and are responsive to altered nutrition (e.g. Gorham et al, 1988, Marcar and Termaat, 1990), it is not known whether these responses can be improved by preconditioning. However, trees can be physiologically and morphologically conditioned to environmental stress. For example, sugar accumulation and osmotic adjustment occur in *Picea glauca* in response to cyclic droughts (Zwiazak, 1991). Drought preconditioning also reduces the effects of transplanting shock in seedlings of Corsican pine and Cedar of Atlas (Kaushal and Aussenac, 1990). Specific morphological responses to salinity that may represent conditioning in woody plants include the development of (presumed) transfer cells in the roots of hydro-cultured *Prosopis farcta* (Winter, 1988) and the modification of xylem structure in *Populus euphratica* (Waisel, 1972).

2.7 MEASURING SALT-TOLERANCE.

Tolerance can be measured in many ways, including survival (Blake, 1981), growth (Amzallag et al., 1990), tissue element concentration (Gorham et al., 1988; Marcar and Termaat, 1990), exclusion of Na^+ or Cl^-, or the maintenance of high Na^+/K^+ ratios (Shannon, 1985). However, while survival and growth recovery can be used to measure salt-tolerance at very high salinities, conditioning to the moderately saline conditions typically encountered in the field may be harder to discern using the available measures (Flowers and Yeo, 1986).

***Table 2.1** Summary of evidence for preconditioning to salinity.*

CATEGORY	SPECIES	RESEARCH FINDINGS	AUTHORS
Growth and Morphology	*Eleusine coracana*	Increased growth & survival at 400mM. NaCl following 200mM. NaCl preconditioning treatment.	Uma et al., 1993
	Sorghum bicolor	Increased growth & survival at 300 mM. NaCl following 150mM preconditioning treatment.	Amzallag et al., 1990
		Developmentally perturbed leaves associated with successful adaptation to salinity.	Seligman et al., 1993
Age-dependent responses	*Sorghum bicolor*	Superior survival when preconditioning initiated five (but not 12) days after germination.	Amzallag et al., 1990
	Muskmelon (Cucumis melo)	Increasing tolerance with age due to ontogenetic drift.	Shannon, 1985
	Tamarindus indica	Older saplings more salt-tolerant than younger seedlings.	Pongskull et al., 1988
Induction of CAM	*Mesembryanthemum crystallinum*	Transition from C3 to CAM mediated by salt but not other stresses.	Vernon et al., 1993
photosynthetic pathway		CAM induction depends on organized leaf tissues (not seen in isolated chloroplasts)	Adams et al., 1992
Cross tolerance induced by salt	Spinach cv. Monnopa	Frost hardiness increased by salt stress.	Hincha, 1994
	Cotton	Heat shock mitigates salinity induced reduction of [proline], enhances [putrescine]	Kuznetsov et al., 1992
	Flax	Salt-tolerant semaclonal variant was also more heat-tolerant.	O' Connor et al., 1991
Role of ABA	*Sorghum bicolor*	Exposure of leaves to exogenous ABA decreases the preconditioning period required for adaptation.	Seligmann et al., 1993
	Eleusine coracana	Addition of 10 µM. ABA confers tolerance to 600 mM. NaCl.	Uma et al., 1993
Indirect evidence	*Plantago media*	Only salt adapted tonoplast vesicles displayed Na/H antiport activity.	Staal et al., 1991
	Lophopyrum elongatum	Accumulation of specific mRNA's by salt stress.	Galvez et al., 1993

Munns and Termaat (1986) observed that transpiration declines and salt flux increases only slowly at high salinities, and suggested that growth reduction was the cause, not the result, of high tissue salt concentrations. However, better growth under salinity does not automatically translate into a conditioning response with long-term survival value. High growth rates can serve to maintain constant salt concentrations in plant organs (Atkinson et al., 1968), but if transpiration is high, and unless harmful ions are excluded from the transpiration stream, salts may still reach lethal concentrations in leaves. It seems, therefore, that for their long-term survival, glycophytes must balance growth and transpiration with the selective absorption and transport of ions (Flowers and Yeo, 1989).

Glycophyte species differ in their ability to selectively translocate Na^+ and nutrient ions. For example, the inclusion of Na^+ in the laminae of older leaves of barley is balanced by the massive import of K^+ away from mature tissues to growing leaves and roots (Jeschke and Wolf, 1993), presumably to maintain protein synthesis, turgor, and stomatal functioning. An alternative strategy, the active exclusion of Na^+ associated with translocation of K^+ from canopy to roots, confers a somewhat lower level of tolerance in *Ricinus communis* (Jeschke and Wolf, 1988). Referring back to the long term 'assimilate starvation' hypothesis of Munns and Termaat (1986), we could speculate that when root growth and Na^+ exclusion is maintained by exporting K^+ from the canopy, the ability of the canopy to supply K^+ will eventually be depleted. As root K^+ concentrations fell, root growth rates would also fall, and ion exclusion mechanisms might break down, resulting in death of the whole plant.

Results of investigations into the effects of salinity on net photosynthesis (Pn) and transpiration have been contradictory. In Kenaf (*Hibiscus cannabinus*), an industrial fibre crop, Pn and stomatal conductance (gs) remained high under salinity, despite elevated tissue ion concentrations and declining leaf water content (Curtis and Lauchli, 1986). Summarizing a number of reports, Cheeseman (1988) states that percentage growth reductions under salinity generally exceed reductions of Pn. On the other hand, in *Puccinellia* and *Suaeda* spp., WUE increased with increasing salinity. Salinity/environment interactions must also be considered; while WUE increased under salinity in glycophyte and halophyte species, lower atmospheric humidity (i.e. high SVPD) lowered WUE at any salinity level (Pitman, 1984).

2.8 Discussion: Preconditioning Of Trees And Its Measurement

Members of many tree genera have been screened for salt-tolerance, and provenance variations in tolerance have been established for a few of them, including *Acacia tortilis* (Muturi 1993) and *Eucalyptus camaldulensis* (Sands, 1981).

If afforestation of saline sites is to be successful, reliable physiological or morphological markers for salt-tolerance must be identified. Differences in plant growth, dry matter allocation amongst plant organs, the uptake of essential mineral nutrients, synthesis of certain proteins, and plant water status are frequently used measures of salt-tolerance. Changes in water use efficiency, stomatal connductance, and net photosynthesis under salinity have also been used, though less frequently or conclusively than other measures.

It is likely that a long term balance between growth, ion accumulation and compartmentation, and transpiration, are key determinants of salt-tolerance in whole plants. Age-dependent variability of growth rates may therefore explain why salt-tolerance varies with age, as observed in *Tamarindus indica* (Pongskull et al., 1988) and *Prosopis flexuosa* (Catalán et al, 1994). On the other hand, the early developmental window for preconditioning *Sorghum bicolor* (Amzallag et al., 1993) indicates the occurrence of a genetic shift towards salt-tolerance that is manifested externally as developmentally perturbed leaves (Seligmann et al., 1993).

Preconditioning of trees to salinity has yet to be accomplished. However, the possibility of preconditioning trees to saline environments is suggested by the fact that tolerance to heat, cold and drought can be successfully induced, and cross-tolerance to a variety of stresses is common. Morphological responses to salinity (e.g. altered root/shoot ratios and increasing leaf-succulence (Luangjame, 1990)), also suggest that preconditioning in trees may be possible.

Knowledge of the inherent tolerance of tree seedlings to different environmental stresses will contribute to successful nursery management and outplanting success. The use of appropriate preconditioning regimes to harden trees to specific stresses, could further increase growth and survival after outplanting. Salt-preconditioning of trees, if demonstrated, could provide a valuable tool to prepare seedlings for outplanting on saline sites.

3

PAPER 1

GROWTH RESPONSES OF TWO PROVENANCES OF *Acacia tortilis* (Forsk.) Hayne TO NaCl PRE-TREATMENT.

ABSTRACT

Seedlings of salt-sensitive and salt-tolerant provenances of *Acacia tortilis* (Forsk.) Hayne were exposed to NaCl preconditioning treatments, at 15, 21, and 28 days after germination, to test age-specific responses to NaCl pre-treatments. Although the timing of pre-treatment produced significant differences in a number of growth parameters in Kitui provenance, there were no significant effects in Sigor, for which growth was equally retarded by all treatments. Growth responses of both provenances to the subsequent application of 200 millimolal NaCl were also unaffected by the timing of pre-treatment. Sigor maintained higher growth rates than Kitui throughout the experiment. Sigor seedlings exposed to NaCl grew 38 - 86% faster than Kitui, while in controls, Sigor maintained a 21% growth rate advantage, confirming the inherently superior salt tolerance of this provenance. These data suggest that fast growth may be an inherent characteristic of salt tolerant *Acacia tortilis*.

3.1 INTRODUCTION

Secondary salinization: a global problem

Secondary salinization of agricultural soils and the expansion of populations into naturally saline lands are global problems. Such lands may cover 13.2 billion hectares worldwide (Dudal and Purnell, 1986). In developing countries, growing salt-tolerant trees on such lands may be a rational land-use alternative to the growing of food crops. Since 1.5 billion people worldwide depend on wood for cooking and heating, (Midgeley et al., 1986), and since 1.11 billion of them do so by depleting existing reserves (Dudal and Purnell, 1986), salt-tolerant tree crops could be used to offset some of this deficit.

Salt-tolerant trees

Many tree genera display salt-tolerance, including *Acacia, Casuarina, Eucalyptus, Melaleuca,* and *Prosopis.* (Felker et al., 1981; Clemens et al., 1983; National Research Council, 1990). Different species within genera (Blake, 1981; Aswathappa and Bachelard, 1986; Marcar and Termaat, 1990), and provenances within species (Sands, 1981, Midgley et al., 1986, Muturi, 1993), may have different responses to saline conditions.

Preconditioning plants to high salinity

Seedlings for agroforestry and reclamation projects in developing countries are grown beneath shade trees or in outdoor nurseries, often in uncontrolled conditions (Zumer-Linder, 1986). If a knowledge of seedling stress-tolerance or the use of salt-

preconditioning could be incorporated into nursery practices, seedling growth and survival after outplanting might be increased. However, although plants can be conditioned to a number of environmental stresses (Levitt, 1980), including salinity (Poljakoff-Mayber and Lerner, 1994), a literature review failed to locate any papers that dealt directly with salt-preconditioning in trees or shrubs.

Preconditioning to high levels of salinity, measured by growth recovery and survival at 400 - 600mM NaCl, has been observed in *Sorghum bicolor* (Amzallag et al., 1990) and *Eleusine coracana* (Uma et al., 1993). Amzallag et al. (1993) demonstrated that preconditioning in *Sorghum bicolor* was age-dependent, the best adaptation occurring when salt was applied five days after germination. In woody plants, wood structure of *Populus euphratica* is developmentally altered under saline conditions from diffuse to ring porous xylem type (Waisel, 1972), and *Prosopis farcta* grown in 10mM NaCl develop hypodermal transfer cells that may contribute to K^+:Na^+ selectivity(Winter, 1988). This evidence suggests that woody perrennials can be conditioned to saline conditions during the course of their development. However, older saplings of *Tamarindus indica* are more salt-tolerant than younger seedlings (Pongskull et al., 1988), which may mean either that the conditioning process is slow one, or that there is an age-specific component to salt-tolerance in woody plants.

The current study compared the effects of NaCl preconditioning on the growth of Kitui (salt-sensitive) and Sigor (salt-tolerant) provenances of *Acacia tortilis* of different size. Sigor (salt-tolerant) provenance grows in calcareous saline or mildly sodic loams,

while Kitui (salt-sensitive) grows on non-saline loams or loamy sands. Their relative salt-tolerances were previously determined in LD50 tests by Muturi (1993).

My null hypothesis was that the duration of preconditioning treatments (of 50 - 150 millimolal NaCl) were applied would have no effect on growth in salt tolerant and salt sensitive provenances of *Acacia tortilis* (Forsk.) Hayne when they were subsequently exposed to higher levels of salinity. The hypotheses was tested using growth analysis, assuming that more tolerant plants would grow faster, have longer shoots (Muturi, 1993), and maintain higher leaf areas relative to plant dry weight. Root to shoot ratios might also be lower, which may signal better mineral nutrition or better distribution of metabolites from the canopy to the roots (Munns and Termaat, 1986). Preconditioning treatments were applied to seedlings of three different ages, the aim being to test variations in age-specific timing of pre-treatment against a subsequent treatment with a single, higher level of salinity.

3.2 Materials And Methods

Germination, growth and salinity treatment.

Seeds of Sigor (n = 853) and Kitui (n = 855) provenances of *Acacia tortilis* (Forsk.) Hayne (supplied by Kenya Forestry Research Institute, Nairobi, Kenya) were removed from cold (4^{o}C) storage and acclimatized at room temperature for two days. Seeds were individually scarified by scraping through the thick outer seed coat with medium grade sand paper, then immersed in distilled water to approximately three times their collective volume, and incubated for 48 hours in a germination chamber under the following conditions: 12 hours of day/night; temperature, 25^{o}C day, 20^{o}C

night. The swollen seeds were planted in 45 x 30 cm. seedling trays filled to a depth of four cm. with industrial quartz (Unimin 2010, Toronto, 10% retained on a number 20 mesh), and returned to the germination chamber to germinate.

Four days after planting, the rapidly germinating seedlings were transplanted into 5 x 1.5 x 1.5 inch Spencer Lamaire tubes (Spencer Lamaire Industries, Edmonton, Alberta) filled to within two cm. of their tops with Unimin 2010 quartz. Sixteen healthy seedlings of both provenances were allocated at random to each of 16 free-draining boxes. About 100 of seeds of each provenance were also planted in sand to provide a baseline sample for the calculation of relative growth rates. Seedlings were watered daily by direct immersion of the seedling boxes in a bowl containing quarter strength Hoagland's solution. On the eighth day after planting, all seedling boxes were transferred to the Faculty of Forestry greenhouse, where they were allocated at random amongst 16 eight litre bowls. These were connected by tubes to a sub-irrigation system comprising eight 18 litre Nalgene bottles, three Hagen aquarium pumps, a three-way solenoid, and a digital timer that delivered one 15 minute irrigation cycle daily (Appendix 1).

On the 10th day after planting, the nutrient concentration was increased to half strength Hoagland's solution, with Ca^{++} (as $Ca(NO_3)_2$ $4H_20$) modified to 4 mM (Appendix 2). Preconditioning treatments were begun on day 15 (see table 3.1 for schedule), and consisted of salinity treatments in half strength modified Hoagland's solution buffered to pH 5.4 - 5.6 with 0.1 molar KOH (between 0.1 and 0.25 ml l^{-1}., depending on the concentration of NaCl). Electrical conductivities of fresh solutions

were checked with a Hanna digital conductivity meter and pH was measured with a Corning pH meter. Solutions were changed every seven days, and their conductivities and pH remeasured (Appendix 2). The experiment was designed as a randomized complete block, with four replicates of each treatment and provenances nested within replicates.

Three harvests were made to measure changes in growth rate and nutrient distribution over the course of the experiment. On day 15, 32 seedlings of each provenance were harvested in order to measure initial differences in the dry weights of plant organs, stem lengths, leaf areas, and root:shoot, root:leaf, and root:stem ratios.

As an intermediate harvest, eight plants per provenance in each replicated treatment were harvested between days 43 and 47. The final harvest (of eight plants per provenance per replicate) was conducted after 37 days of treatment with 200 millimolal NaCl. Plants in the intermediate and final harvests were divided into roots, old leaves (defined as the first three sets of compound leaves initiated after germination), new leaves (all other leaves), and stems. Leaf areas were measured using a Licor 3000 Leaf Area Meter, and weights were determined to three significant figures using a Sartorius Basic electronic balance. Data from the three harvests were used to calculate relative growth rates, root relative growth rates, stem relative growth rates, leaf area ratios and unit leaf rates for each replicate treatment (Evans, 1972) (Appendix 3).

Growth parameters were analyzed by two way ANOVA using the Proc ANOVA and Proc GLM sub-routines of the SAS statistical program (SAS Institute, Cary, NC, USA). Growth data was analyzed as a split plot with salt-pretreatment as the main plot

and provenance as the subplot (Kuehl, 1994, p. 478). Provenance and salt means were separated at a 0.05 significance level using least significant differences (LSD). Planned orthogonal comparisons were used to determine provenance-specific differences between salt pre-treatments and differences between the two provenances in the control treatments.

3.3 Results

Growth parameters and dry weights

Initial differences between provenances.

Seeds of Kitui were 71% heavier, on a fresh weight basis, than those of Sigor. At day 15, Kitui was 27.7% larger in terms of dry weight ($p \leq 0.00001$) and had a 68.8% advantage in leaf area ($p \leq 0.0001$), but had shorter stems ($p \leq 0.00001$) and lower root:shoot ratios ($p \leq 0.05$) (Appendix 4).

Overall growth patterns.

By the time of the intermediate harvest, Sigor had developed more rapidly than Kitui in 12 out of 14 growth parameters (Tables 3.2 and 3.3). Between-provenance differences in stem length and dry weight, leaf area, relative growth rate and unit leaf rate became more pronounced under the high (200 millimolal) NaCl treatment. Root:stem and root:shoot ratios were the exception to this pattern. Although both ratios were higher in Sigor at day 15, they rose sharply in Kitui during pre-treatment, but fell equally sharply between the intermediate and final harvests, a pattern seen in both treated and untreated seedlings. Leaf and root dry weights did not differ significantly between provenances in either the intermediate or final harvests.

Therefore, changes in root:shoot and root:stem ratios were due to differences in stem growth (Appendix 5.1 and 5.2).

Although leaf areas and leaf dry weights were the same in each provenance in both harvests, unit leaf rates (the measure of dry weight gain per unit leaf area) were significantly higher in Sigor . Lower stem dry weight and the greater succulence of Kitui's tissues contributed to its lower unit leaf rates. The shorter stems of Kitui also produced a 'bushy' growth habit which would have resulted in self-shading of the foliage and a possible lowering of net photosynthesis.

Kitui was generally more sensitive to the length of pre-treatment than was Sigor. Significant ($p \leq 0.05$) orthogonal contrasts arose for 9 out of 14 growth parameters for Kitui (Table 3.2), and growth was more retarded in NaCl 1 than in shorter pre-treatments. In contrast, Sigor was not as affected by differences in pre-treatment schedule, significant orthogonal contrasts being observed only for stem length and stem relative growth rate.

Intermediate harvest treatment effects.

Pre-treatment with NaCl caused significant ($p \leq 0.05$) reductions stem length, RGR and SRGR, relative to controls. Least Significant Difference (LSD) tests indicated that NaCl 1 caused lower growth than later pre-treatments. Sigor maintained higher stem dry weights and unit leaf rates, while root:stem and root:shoot ratios were greater for Kitui. Salt x provenance interactions in leaf area, dry weight, and relative growth rate arose as a result of Kitui's greater sensitivity to the duration of pre-treatment (Table 3.2). There were also significant effects of block for several

parameters, and LSD showed this to be due to differences between block one and blocks two, three and four.

Final harvest and changes in growth parameters

In the final harvest, significant treatment occurred in all parameters except leaf area ratio, while provenance differences were seen in all variables except leaf dry weight, leaf area, dry weight and leaf area ratio. Treatment differences were due to significantly lower growth and higher leaf abscission in treated seedlings, relative to controls. However, with the exception of percentage dry weight in Kitui, the growth differences produced by different pre-treatment schedules were no longer observed. In one variable, leaf area ratio, responses of treated and untreated plants were similar, indicating a constant relationship between leaf area and total dry weight. However, there were clear treatment differences in unit leaf rates, with dry weight increasing relative to leaf area in the order NaCl 3 < NaCl 2 < NaCl 1 < controls. These differences were not due to differences in leaf abscission (measured on day 67), which were significant between provenances, but not between salt treatments. Final harvest RGRs for Sigor were 38 to 86% higher than those for Kitui in salt treatments, and 21.3% higher in untreated seedlings. Thus, Sigor had inherently greater growth and salt-tolerance, as measured by growth, than Kitui.

3.4 DISCUSSION

Sigor and Kitui provenances of *Acacia tortilis* (Forsk.) Hayne appear to have different inherent tolerances to NaCl. Growth of Kitui proved to be very sensitive to the duration of NaCl pre-treatment. Between the intermediate and final harvests,

untreated Sigor seedlings grew 21.3% faster than untreated Kitui, and 38 - 86% higher than Kitui during 5 weeks exposure to 200 millimolal NaCl. Thus Sigor maintained a considerable growth advantage in saline conditions and a less marked advantage in the absence of NaCl. However, the absence of significant orthogonal contrasts or salt x provenance interactions in the final harvest suggests that seedlings of both provenances responded similarly to 200 millimolal NaCl, irrespective of their previous pre-treatment schedule. Therefore, varying the timing and duration of pre-treatment failed to alter the tolerance of either provenance to a 5 week treatment with 200 millimolal NaCl.

Significant orthogonal contrasts from the intermediate harvest indicated that 9 out of 14 growth parameters for Kitui were sensitive to the duration of NaCl pre-treatment (Figure 3.1 and Table 3.2). For Sigor, by contrast, only stem lengths displayed significant between-treatment differences. These observations may be interpreted as indicating that some inherent salt-tolerance mechanisms were activated by day 15, with growth differences between provenances simply reflecting different thresholds of sensitivity. Alternatively, salt-tolerance in Sigor may be rapidly induced upon first exposure to 50 millimolal NaCl, whereas conditioning in Kitui could be somewhat slower.

The faster growth of Sigor under all treatments lends some support to Munns and Termaat's (1986) argument that salt in the cytoplasm could be rendered less toxic if diluted through faster growth. However, higher growth rates would also require proportionately higher nutrient supply, and therefore, higher rates of transpiration (R. L. Jefferies, personal communication). Therefore, it is likely that high growth rates in

Sigor were maintained with the help of efficient Na^+ partitioning mechanisms, a speculation supported by significantly higher rates of lower leaf abscission in Sigor than Kitui. Faster growth, accompanied by high rates of leaf expansion and photosynthesis was also associated with drought-tolerance in *Eucalyptus grandis*, in which the ability to draw water from large volumes of soil appears to be more important than limiting transpiration (Blake et al., in press). Thus, rapid growth may be a general marker for inherent tolerance of trees to a variety of environmental stresses. A known advantage of rapid growth for *Acacia* spp. is the development of deep tap roots that enable trees to exploit deep water tables (Sprent, 1987).

The character of early growth advantages should also be considered. Kitui allocated more dry matter to roots than Sigor after day 15, even in untreated seedlings (Fig. 3.4). Salt-treated Sigor seedlings, on the other hand, only showed a slow increase in root dry matter relative to shoots and stems after the intermediate harvest. In all cases, Kitui maintained higher root:shoot and root:stem ratios than Sigor, suggesting that Sigor either coped with Nacl-induced physiological drought better than Kitui, or was more selective than Kitui at nutrient uptake.

Differences in stem dry weights explained Sigor's lower root:stem and root:shoot ratios. In contrast, LAR,s for both provenances were similar, indicating a similar relative investment in photosynthetic machinery. This result contrasts with the sensitivity of LAR to different NaCl treatments in Kenaf (Curtis and Läuchli, 1986) and its value in distinguishing salt-tolerant rice cultivars (Akita and Capuslay, 1990). However, unit leaf rates were higher in Sigor than Kitui (Fig. 3.2), indicating greater

photosynthetic efficiency in Sigor. In part, Kitui's lower unit leaf rates may have been due to its greater relative investment in roots. Luangjame (1990) noted evidence that roots must use more assimilate per unit growth in dry matter than do other organs. Therefore, Sigor's greater relative investment in stem growth may have made more efficient use of available assimilate.

Higher rates of stem growth and elongation (Figure 3.3) could result in a number of adaptive advantages. A higher rate of leaf initiation could be associated with rapid stem elongation. However, in peas, inhibition of stem growth resulted in a shortening of the internodes rather than a reduction in their number (Poljakoff-Mayber and Lerner, 1994). Visual observations suggested that Kitui suffered from reduced internode length, which led to a bushy growth habit, and perhaps, reduced photosynthesis as a result of self-shading. An additional advantage of long stems in Sigor, prior to lignification, may have resided in an enhanced photosynthetic surface area (Evans, 1972). (Jeschke and Wolf (1993) observed the attenuation of Na^+ and K^+ concentrations with height in the transpiration stream of *Hordeum vulgare*. Could rapid stem growth play a role in diluting salt concentrations in the transpiration stream?

Visual observations (Figure 3.5) showed NaCl-treated Sigor root systems to have greater numbers of fine roots than those of NaCl-treated Kitui, which were often thick, club-shaped, and had fewer fine lateral roots. It may be that reduced soil water potential in NaCl treatments caused disturbance of lateral root-development in Kitui, a possible droughting effect suggested by Alam (1994). Alternatively, disturbance of membrane-bound Ca^{++} by Na^+ (Cramer et al., 1985) may have inhibited cell elongation

and growth in Kitui. Similar symptoms (root thickening, constrictions and root-tip curvature) were observed in *Pisum sativum* L., and were reversible by the addition of 10 mM Ca^{++} (Solomon et al., 1989).

Both provenances developed a degree of tissue succulence between the intermediate and final harvests (Tables 3.2 and 3.3). Succulence is caused by increased elongation of palisade mesophyll cells, and results in both dilution of internal ion concentrations and reduced leaf surface area. The development of succulence in *Acacia tortilis* may therefore have been a transpiration-limiting response (higher leaf volume:area), or a conditioning response that permitted more salt storage in larger leaf vacuoles.

3.5 Conclusions

Different preconditioning schedules did not affect within-provenance responses of either Kitui or Sigor provenances of Acacia tortilis (Forsk.) Hayne to their subsequent treatment with 200 millimolal NaCl. Significant between-provenance growth differences in Nacl treatments and controls implied that relative salt-tolerance was determined by genetic potential rather than phenotypic modification by any one pre-treatment. Sigor maintained lower root:shoot and root:stem ratios, higher unit leaf rates and higher leaf-abscission than Kitui. Sigor's greater investment in above-ground biomass was mainly in the form of stem tissue. However, the adaptive advantage of longer stems in Sigor is speculative rather than established.

Faster stem elongation in Sigor may facilitate the attenuation of salt concentrations in the transpiration stream, increase the photosynthetic area of early seedlings or avoid

self-shading by leaves. Kitui, on the other hand, experienced large reductions in stem length and dry weight. The resultant mutual shading of leaves, together with the increased succulence that represented a longer term preconditioning response, would have reduced transpiration and net photosynthesis in Kitui. Distortion of root development may have reduced either root Na^+ /macro-nutrient selectivity or nutrient-uptake capacity in Kitui, providing another potential explanation of Kitui's lower growth in saline conditions.

Figure 3.1 Relative growth rates (± standard errors, n=4) of Kitui and Sigor provenances of Acacia tortilis exposed to NaCl preconditioning treatments.

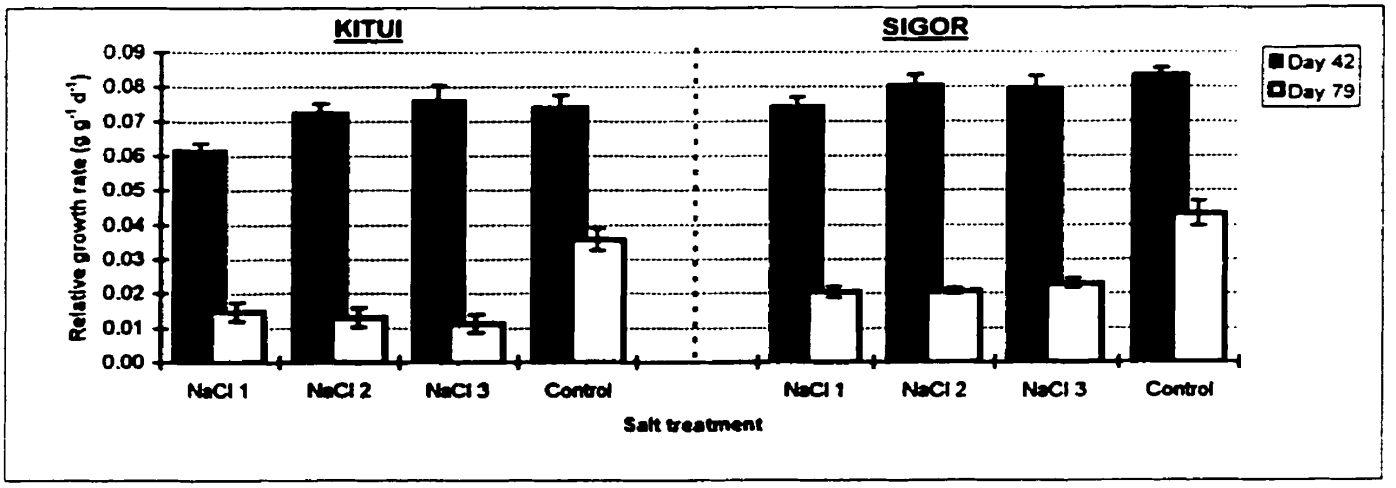


Figure 3.2 Unit leaf rates (± standard errors, n=4) of Kitui and Sigor provenances of Acacia tortilis exposed to NaCl preconditioning treatments.

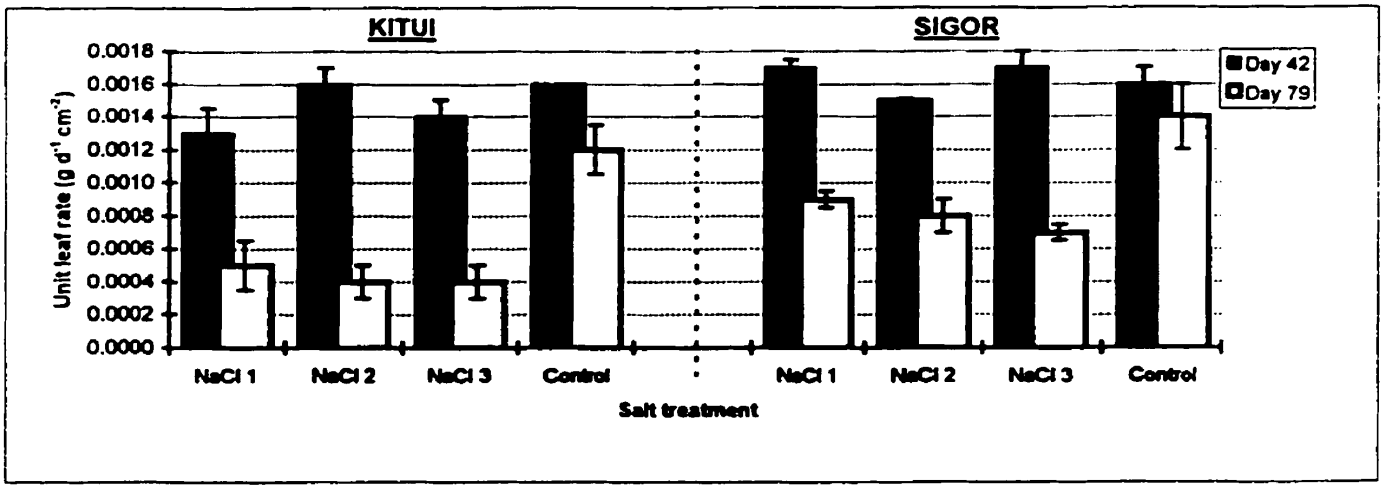


Figure 3.3 Changes in stem lengths of Kitui and Sigor provenances of Acacia tortilis (± standard errors, n=4).

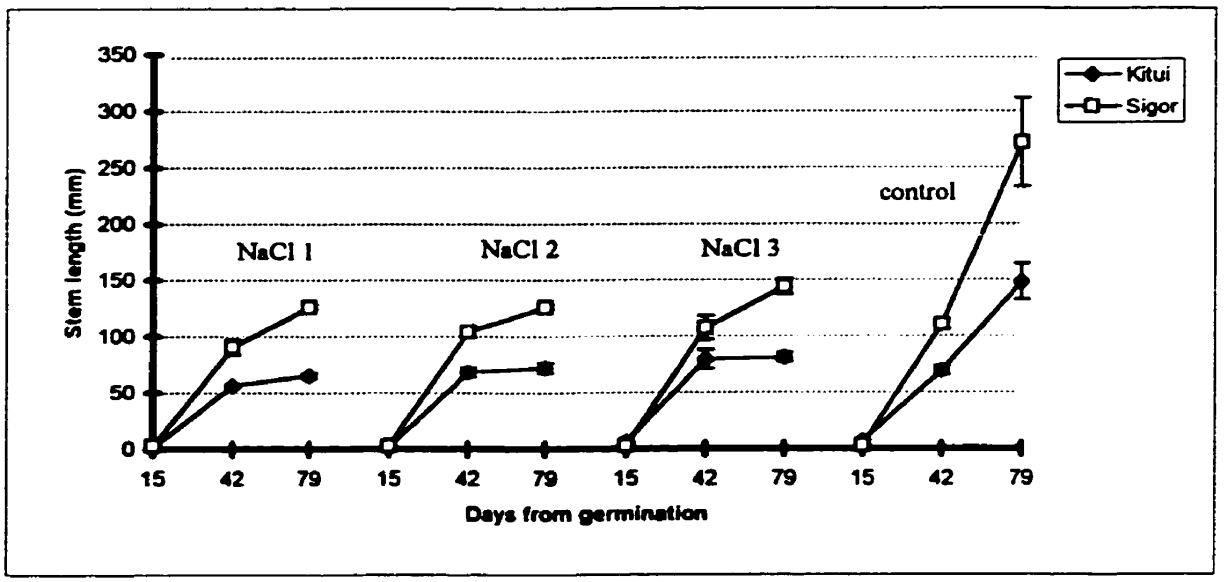


Figure 3.4 Changes in root:stem ratios of Kitui and Sigor provenances of Acacia tortilis (± standard errors, n=4).

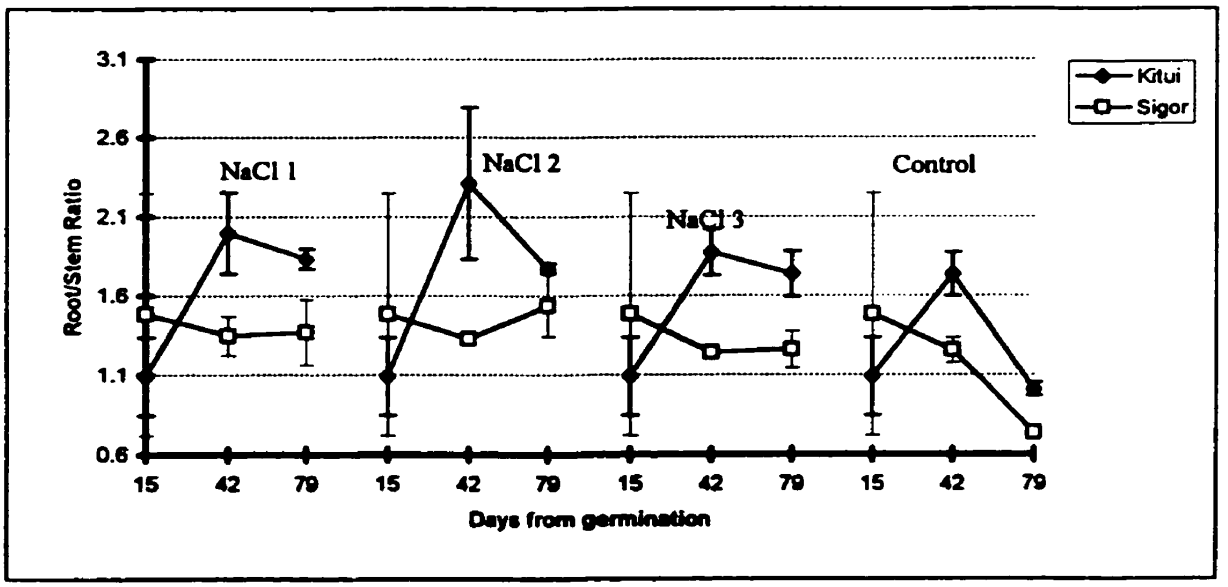


***Figure 3.5** - Roots of representative salt-treated Kitui and Sigor seedlings (from NaCl 3, block 3), clearly showing 'clubbing' and a paucity of fine roots in Kitui .*

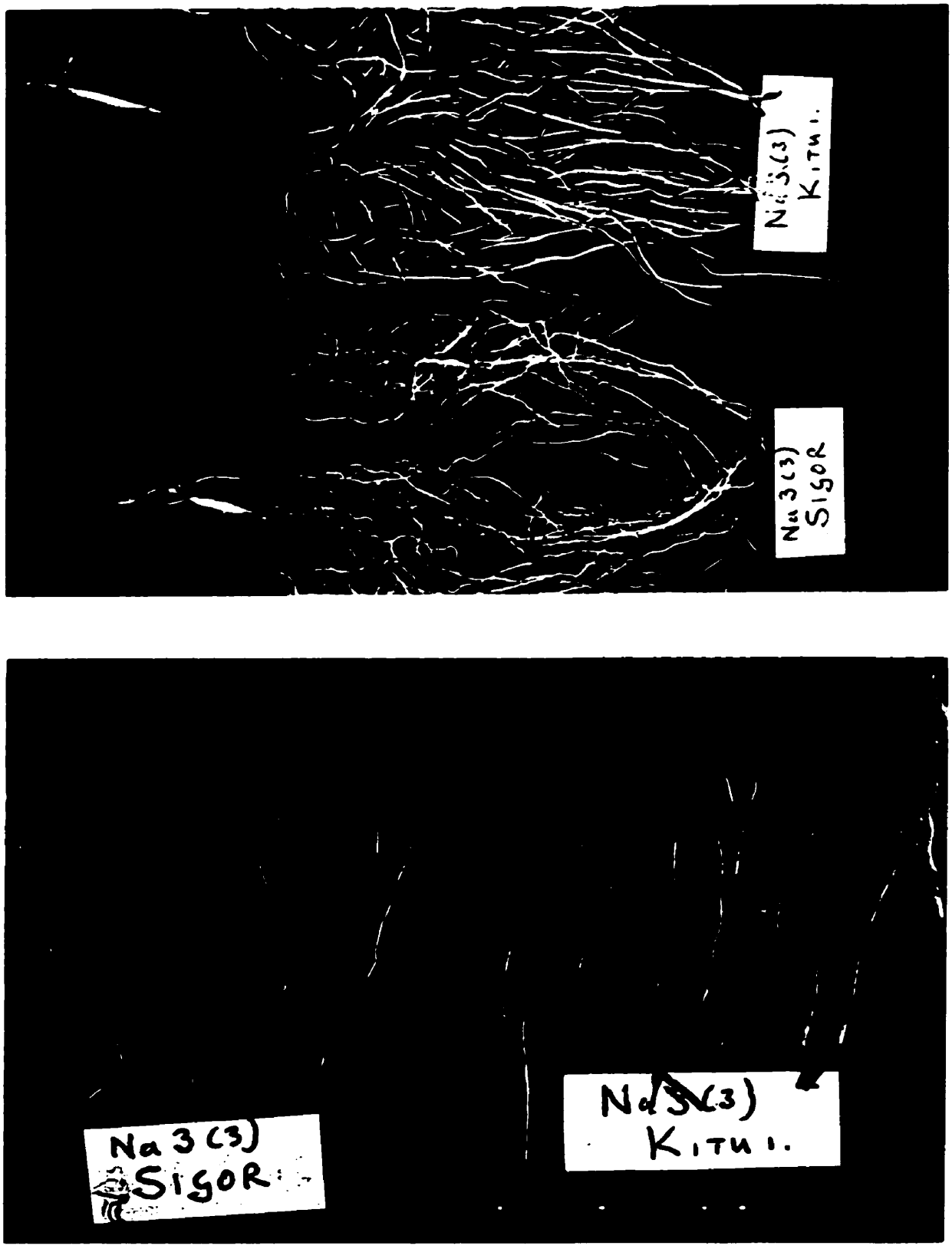


***Table 3.1** - Preconditioning schedules and final salinity treatments applied to Sigor and Kitui provenances of Acacia tortilis.*

	Day of treatment and concentration of NaCl (millimolal)					
Day →	**15**	**22**	**29**	**36**	**40**	**44 - 79**
NaCl 1	50	100	150	150	150	200
NaCl 2	0	50	100	100	150	200
NaCl 3	0	0	50	100	150	200
Control	0	0	0	0	0	0

Table 3.2 Growth parameters for intermediate harvest. Planned comparisons were performed between treatments for each provenance and between provenances in the control treatment. Treatments with different lower case letters and provenance groups in controls with different upper case letters, are significantly different ($p \leq 0.05$, $n=4$).

		Plant growth parameters					Ratios and totals				Growth rates (28 days)				
Provenance	Treatment	Stem length (mm)	Leaf area (cm3)	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	% Dry Wt.	Total dry weight (g)	Root: shoot ratio	Root: stem ratio	Leaf area ratio ($cm^2 g^{-1}$)	Relative growth rate (RGR) (d^{-1})	Unit leaf rate ($g\ cm^{-2} d^{-1}$)	Root RGR	Stem RGR
KITUI	NaCl 1	55.8	13.537	0.127	0.048	0.090	18.6	0.266	0.707	1.993	52.526	0.0615	0.0013	0.0885	0.0669
		a	a	a	a	a	a	a	a	a	a	a	a	a	a
KITUI	NaCl 2	68.0	18.550	0.168	0.069	0.139	19.9	0.376	0.880	2.323	51.131	0.0725	0.0016	0.1000	0.0796
		a	b	b	ab	a	a	bc	a	a	a	bc	b	b	b
KITUI	NaCl 3	79.0	22.567	0.184	0.089	0.135	20.4	0.409	0.780	1.872	54.482	0.0761	0.0014	0.1022	0.0856
		a	c	b	b	a	a	c	a	a	a	c	ab	bc	b
KITUI	CON	69.2	18.018	0.191	0.070	0.114	21.0	0.375	0.607	1.734	47.197	0.0739	0.0016	0.0971	0.0810
		A	A	A	A	A	A	A	A	A	A	A	A	A	A
SIGOR	NaCl 1	90.2	16.930	0.171	0.072	0.107	20.1	0.350	0.654	1.560	48.020	0.0776	0.0017	0.0995	0.0909
		a	a	a	a	a	a	a	a	a	a	a	a	a	a
SIGOR	NaCl 2	103.5	19.468	0.165	0.083	0.108	20.2	0.355	0.664	1.327	55.092	0.0805	0.0015	0.1010	0.0989
		ab	a	a	a	a	a	a	a	a	b	a	a	a	b
SIGOR	NaCl 3	107.2	17.370	0.162	0.085	0.102	21.5	0.349	0.637	1.239	50.543	0.0797	0.0017	0.0993	0.0993
		b	a	a	a	a	a	a	a	a	ab	a	a	a	b
SIGOR	CON	110.2	20.215	0.191	0.088	0.107	21.2	0.386	0.560	1.257	53.308	0.0833	0.0016	0.1011	0.1004
		B	A	A	A	A	A	A	A	A	A	B	A	A	B

Table 3.3 Growth parameters for final harvest. Planned comparisons were performed between treatments for each provenance and between provenances in the control treatment. Treatments with different lower case letters and provenance groups in controls with different upper case letters, are significantly different ($p \leq 0.05$, $n=4$).

		------Plant growth parameters------					----------Ratios and totals----------						-----Growth rates (37 days)-----			
Provenance	Treatment	Stem length (mm)	Leaf area (cm3)	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	% Dry Wt.	Total dry weight (g)	Root: shoot ratio	Root: stem ratio	% leaf fall	Leaf area ratio ($cm^2 g^{-1}$)	Relative growth rate (RGR) (d^{-1})	Unit leaf rate ($g\ cm^{-2} d^{-1}$)	Root RGR	Stem RGR
KITUI	NaCl 1	65	14.31	0.192	0.108	0.192	17.1	0.491	.682	1.830	62.5	27.941	0.0148	0.0005	0.0187	0.0205
		a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
KITUI	NaCl 2	72	24.38	0.261	0.148	0.251	16.9	0.658	.649	1.769	46.9	37.454	0.0131	0.0004	0.0139	0.0191
		a	a	a	a	a	a	a	a	a	ab	a	a	a	a	a
KITUI	NaCl 3	81	20.58	0.238	0.169	0.262	18.9	0.668	.723	1.735	37.5	31.042	0.0123	0.0004	0.0167	0.0165
		a	a	a	a	a	a	a	a	a	bc	a	a	a	a	a
KITUI	CON	148	53.15	0.733	0.428	0.399	23.8	1.560	.359	1.006	15.6	34.715	0.0357	0.0012	0.0310	0.0455
		A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
SIGOR	NaCl 1	126	20.47	0.243	0.192	0.238	20.2	0.670	.587	1.369	78.1	30.635	0.0204	0.0009	0.0254	0.0286
		a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
SIGOR	NaCl 2	126	22.29	0.289	0.203	0.290	21.4	0.782	.619	1.538	75.0	28.481	0.0207	0.0008	0.0243	0.0230
		a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
SIGOR	NaCl 3	144	27.70	0.300	0.242	0.291	20.9	0.833	.562	1.259	65.6	32.093	0.0229	0.0007	0.0278	0.0275
		a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
SIGOR	CON	272	70.12	0.897	0.691	0.483	25.4	2.069	.304	0.736	37.5	34.988	0.0433	0.0014	0.0388	0.0531
		B	A	A	B	A	A	A	A	A	B	A	A	A	A	A

4

PAPER 2

EFFECTS OF NACL PRE-TREATMENT ON NUTRIENT ALLOCATION AND UPTAKE IN TWO PROVENANCES of *Acacia tortilis* (Forsk.) Hayne.

ABSTRACT

The effects of salt uptake on nutrient distribution in seedling organs could be used to measure the success of salt preconditioning treatments. Nutrient and salt uptake were examined in Kitui and Sigor provenances of *Acacia tortilis* (Forsk.) Hayne exposed to three preconditioning schedules in order to understand the conditioning process and the effects of NaCl on seedling nutrition. Relative to controls, Sigor allocated more Na^+ to old leaves than Kitui. Earlier pre-treatment of Sigor also resulted in significantly to lower Na^+ accumulations in expanding foliage after exposure to 200 millimolal NaCl. Multivariate ordinations confirmed that leaf abscission and stem growth were sensitive indicators of salt-tolerance, and revealed close correlations between SRGR and Mg^{++} and K^+ concentrations, and negative correlations between RGR and concentrations of Na^+ and P . Nutrient uptake of whole seedlings was similar in both provenances, but nutrient concentrations were higher in Kitui. Although final harvest patterns of macro-nutrient and Na^+ accumulation were similar in both provenances, inherently higher growth enabled Sigor to limit Na^+ concentration in tissues, while Na^+ became more concentrated in Kitui because of lower growth and less efficient root retention.

4.1 INTRODUCTION

NaCl/ nutrient interactions.

Plants may respond to saline environments by accumulating organic solutes that reduce tissue water potentials (Adams, 1993), excluding salts at the root:soil interface, maintaining low Na^+/K^+ ratios in tissues (Shannon, 1985), and perhaps, diluting salts through rapid tissue expansion (Flowers and Yeo, 1989). Sodium may be sequestered in root cell vacuoles and its entry into the xylem restricted (Jeschke, 1984). In glycophytes, Na^+ commonly accumulates in mature leaves, which may be lost through abscission (Adam, 1993), while K^+ is preferentially transported to developing and transpiring leaves (Greenway, 1962; Jeschke and Wolf, 1985).

However, salt exclusion is never completely effective. As salt concentrations increase in expanding leaves, cell volumes and elongation rates decrease in some species (Singh et al., 1989), DNA and RNA synthesis is reduced, and protein synthesis is inhibited (Aspinall, 1986). Thus, for long-term survival, glycophytes must balance growth, transpiration, nutrient transport and Na^+ restriction (Flowers and Yeo, 1989).

Preconditioning and plant nutrition

Age-dependent preconditioning to high levels of salinity, measured by growth recovery and survival at over 300 mM NaCl has been observed in *Sorghum bicolor* (Amzallag et al., 1990). In *Eleusine coracana* (finger millet), plants preconditioned by 200 mM NaCl are able to tolerate 64 hours of potentially lethal (400 mM NaCl) salt-stress (Uma et al., 1993). Through phenotypic modification, these annual cereals are able to survive high salinities long enough to flower and set seed. However, perennial

glycophytes must sustain the flow of solutes to expanding leaf tissues while excluding salts from the root or sequestering them in cell vacuoles and older leaves (Flowers and Yeo, 1986) for long-term growth to take place.

Salt partitioning amongst seedling organs has been correlated with salt-tolerance in trees (Sands, 1981; Prat and Fathi-Ettai, 1990). Translocation of Na^+ to older leaves, followed by their abscission, occurs in salt-tolerant *Eucalyptus* spp. (Marcar, 1989), *Leucaena leucocephala* and *Sesbania bispinosa* (Gorham et al., 1988), and *Casuarina equisetifolia* (Aswathappa and Bachelard, 1986), and salt-tolerant provenances of *Acacia tortilis* (Muturi 1993).

Some woody species restrict the upward flow of salts by restricting transpiration (Moezel et al., 1989), which must inevitably alter nutrient supplies and growth rates of shoots. Others (e.g. *Prosopis glandulosa*) are able to sustain high rates of transpiration without leaf Na^+ concentrations appreciably increasing (Nilsen et al., 1986), and must therefore either exclude Na^+, or be able to maintain leaf expansion in spite of Na^+ accumulation. Therefore, slower growth of one species relative to another may not reflect their abilities to survive saline environments, but may simply be a consequence of different tolerance strategies. Factors such as the number and biomass of leaves, age-specific growth rates, and relative development of seedling organs are likely to interact with nutrition and salt-partitioning to affect levels of early salt-tolerance and the conditioning of seedlings to saline environments.

In experiment two, NaCl and nutrient partitioning were examined in *Acacia tortilis* (Forsk.) Hayne seedlings that had received NaCl preconditioning treatments at different

ages. I assumed that conditioned plants would sequester higher concentrations of NaCl in roots and older leaves, relative to controls. If salt is diverted to older leaves, younger plants with less developed shoots might be less successfully conditioned than larger ones, because abscission of older leaves would represent the loss of a relatively greater proportion of total leaf area. Therefore, the following hypothesis was considered:

The success of conditioning, and allocation of nutrients and NaCl in seedlings of salt-tolerant (Sigor) or salt-sensitive (Kitui) provenances of *Acacia tortilis* (Forsk.) Hayne will vary inversely with the age at which they are exposed to NaCl preconditioning treatments.

4.2 Materials And Methods

Seedlings of *Acacia tortilis* (Forsk.) Hayne that were the subjects of growth analysis in Experiment one were used for nutrient analysis in this experiment. Seed preparation, germination and propagation methods are described in Chapter three.

Nutrient analysis

Nutrient and NaCl concentrations were determined in seedlings from both the intermediate and final harvests. Samples for the final harvest consisted of plant organs from eight plants per replicate (n = 4). For the intermediate harvest, pairs of replicates were pooled (n = 2) because of limitations on laboratory availability. Tissues were ground in either a coffee grinder followed by a Wiley mill (shoots) or by hand with a pestle and mortar (roots and leaves). Samples of the ground tissue (0.3 ● 0.0005 g) were acid digested in a digestion block for two hours at 350°C in 4.4 ml of a 98%

H_2SO_4/30% H_2O_2 digestion mixture, following which they were diluted to 50ml volume with double distilled water. Tissue concentrations of Na^+, K^+, Ca^+, and Mg^{++} were measured (in ppm) with a Perkins Elmer atomic absorption spectrophotometer. Total N was measured with a Technicon AA2 Auto-Analyzer by comparing strip chart peaks with peaks from a range of known standards. Phosphorus was measured using a Pye Unicam SP6550 UV/Vis spectrophotometer that had been calibrated at 650nm with a set of known standards. Chloride was measured directly from acid digested samples, to which 1 ml of low level ionic adjuster (5M $(NH_4)_2.SO_4$) had been added, using an Orion combination chloride electrode (calibrated with a range of NaCl solutions prepared as for acid digestion) connected to a Corning pH meter.

Statistical analysis

Nutrient data were analyzed by ANOVA, multivariate ordination and vector analysis. ANOVAS were run on nutrient concentrations and total contents in the intermediate (n = 2) and final (n = 4) harvests (Appendix 6.5 - 6.8). They were run as split-split plots, with provenances as sub-plots within treatment whole plots, plant organs (old leaves, new leaves, stems and roots) as sub-sub-plots within provenances.

Data were further analyzed using vector analysis (Timmer, 1991; Haase and Rose, 1995) and multivariate ordination (Ter Braak, 1988). Vector analyses of ion contents and concentrations, normalized to percentages of the equivalent contents and concentrations in control plants, can be used to interpret changes in plant nutritional status due to concentration, dilution, toxicity, or antagonism (Timmer, 1991). Vector analysis was used to examine the affects of NaCl treatments on macro-nutrient

distribution amongst new leaves, old leaves and roots, and accumulation patterns of Na+ in all seedling organs.

Three closely related multivariate ordination techniques were used to distinguish the respective contributions of variance in growth parameters, nutrient concentrations and the experimental design to the total variance of plant growth parameters. Principle Components Analysis (PCA), an unconstrained ordination technique, was used to identify the growth variables that had the most influence on the individual sample scores calculated by the ordination algorithm. Redundancy Analysis (RDA), a canonical form of PCA was used to select linear combinations of nutrients and Na^+ that yielded the smallest residual sums of squares when regressed against the sample scores computed in the PCA (Jongman et al., 1987). A partial RDA was then calculated, incorporating the experimental treatments as nominal variables, in order to extract the proportion of total variance that could be explained by the experimental design alone.

The results of the multivariate ordinations are expressed as eigenvalues, which are the fractions of the total sum of squares in a data set extracted by each ordination axis. Differences between the eigenvalues for the PCA and RDA revealed the fraction of variance in growth that was explained by nutrient concentrations, while differences between the RDA and the partial RDA illustrated the contribution of experimental design covariables to variance in the growth data. The residual variance seen in the partial RDA represented the unique contribution of nutrient concentrations to the growth response of plant tissues (Prof. T. J. Carleton, personal communication, Ter Braak, 1988).

All inorganic ions, except Cl^-, were incorporated into the analysis. Growth variables were relative growth rate (RGR), unit leaf rate (ULR), percentage of plants suffering leaf fall by the end of the experiment, stem relative growth rate (SRGR), and root relative growth rate (RRGR). Individual samples consisted of nutrient concentrations or growth variable data from plant organs from provenances within replicates. Experimental design factors were treated as predictable covariables.

4.3 Results

Growth rates and general observations.

Growth of Kitui, as measured by eight different variables, was reduced in proportion to the duration of pre-treatment, while in Sigor, only SRGR and LAR showed significant differences between pre-treatments. Growth of Kitui was more inhibited than that of Sigor throughout the experiment (Chapter 3). Leaf chlorosis and abscission occurred in all treatments and both provenances, but by day 66 was significantly higher in Sigor (38 - 78% of seedlings) than in Kitui (16 - 63% of seedlings). Leaf fall was also significantly greater in NaCl than in NaCl 2 and 3.

Analysis of variance

Sodium accumulated in direct proportion to the duration of pre-treatment in both provenances, a pattern that was particularly noticeable in leaves (Figure 4.1). Although total uptake of Na^+ and macro-nutrients was the same in both provenances, significant provenance x salt x tissue interactions in Na^+, K^+ and N were indicative of different patterns of accumulation. Sigor was able to maintain selective uptake of K^+ and N to

new leaves as foliar Na^+ accumulated, while uptake of these elements to new leaves of Kitui was more sensitive to the timing of pre-treatment (Figure 4.1). In both provenances, the proportion of total Na^+ uptake allocated to new and old leaves increased with the duration of pre-treatment, while Na^+ in stems decreased slightly (Figure 4.3a). This observation suggests either the saturation of root storage capacity or the progressive breakdown of physiological mechanisms restricting the loading of Na^+ into the xylem.

Final harvest contents of all elements, including Na^+, were the same in treated seedlings of both provenances, indicating similar nutrient uptake capacities. However, Na^+, Ca^{++}, Mg^{++} and P were between 14% (Na^+) and 21% (Ca^{++}) more concentrated in Kitui than Sigor. These differences in concentration were probably due to the greater growth inhibition, especially in SRGR and stem dry weight, suffered by Kitui during NaCl treatment. By contrast, planned comparisons showed that uptake, but not concentration, of all nutrients, except Cl^-, was significantly higher in Sigor controls, reflecting inherently higher growth rates in this provenance (Figure 4.2). This means that NaCl treatment reduced nutrient uptake more in Sigor than Kitui, relative to the potential uptake in untreated seedlings.

The significant treatment x organ interactions of cation contents in both harvests, and P and N in the final harvest, arose as a result of treatment induced re-allocation of similar absolute quantities of ions (see Figures 4.1 - 4.2). Cation contents, with the exception of Ca^{++}, were reduced more in leaves and stems than roots. Calcium was up to 50% more concentrated in old leaves than new leaves, reflecting lack of phloem

mobility. Phosphorus became more concentrated in leaves and shoots of treated plants, as did N in stems. Chloride contents and concentrations remained generally very low compared to those of Na^+, and no main effects were observed for the chloride ion throughout the experiment.

During pre-treatment, root Na^+, as a percentage of Na^+ in new leaves, declined from 300% (NaCl 3) to 130% in Kitui (NaCl 1) and 176% in Sigor (NaCl 1). By final harvest, root Na^+ contents rose to between 170 and 200% in Kitui, and 249 and 218% in Sigor (NaCl 1 and NaCl 3, respectively).

The overall pattern of Na^+ accumulation in treated plants seemed to be that of a rapid initial accumulation in shoots that eventually stabilized at 50 - 55% of total Na^+ during treatment with 200 millimolal NaCl (Figures 4.3a and b). Control plants, in contrast, reduced the allocation of Na^+ to leaves between initial and final harvests, but increased Na^+ in stems and (in Kitui only) roots.

Vector analysis

Vector analyses confirmed that Na^+ accumulated in proportion to the duration of NaCl pre-treatment (Figure 4.4), with new and old leaves experiencing the greatest accumulations amongst seedling organs, relative to controls. Final harvest vector analyses showed dramatic between-provenance differences in the allocation of Na^+. New leaves of Kitui had concentrated Na^+ up to 38,000% higher than controls (357% higher than old leaves and 150% higher, relative to controls, than new leaves of Sigor). Old leaves of Sigor experienced Na^+ accumulations and concentrations up to 700% higher than new leaves of equivalent treatments (Figure 4.4). This high relative

accumulation reflects both the increasing importance of Na^+ allocation to old leaves and the fact that final harvest old leaves of Sigor control seedlings contained less Na^+ (0.4% of the total) than those from the intermediate harvest (7.6%) (Figure 4.3a and b).

Vector analysis also illustrated differential reductions of macro-nutrient uptake amongst seedling organs as a result of Na^+ antagonism (Figures 4.5 and 4.6). Relative to controls, cation contents were reduced by up to 45% in the intermediate harvest of both provenances, and were further reduced by the final harvest, with root Ca^{++} contents falling as low as 6% in Sigor. Patterns of accumulation were similar in both provenances, although Sigor experienced 33 - 37% lower relative concentrations of Mg^{++} and 8 - 16% higher relative concentrations of K^+, relative to Kitui. Nitrogen was also 8 - 14% more concentrated, relative to controls, in Sigor than in Kitui.

Phosphorus concentrations, but not contents, were between 38 and 212% higher in NaCl treatments than in controls in the final harvest, the highest concentrations occurring in old leaves. Phosphorus uptake therefore appears to be more limited by growth (uptake capacity) than by any specific effect of NaCl treatment.

Multivariate analysis.

Multivariate analyses indicated discreet responses in growth parameters of seedling organs of Kitui and Sigor to concentrations of specific elements, but failed to separate the effects of specific NaCl treatments (Figure 4.7). However, they did reveal relationships between growth and nutrition that could help to explain Provenance-specific differences in salt-tolerance.

The percentage of plants suffering leaf fall was by far the largest influence on eigenvalues for the first set of multivariate analyses (Table 4.1 a, b), in which 99% of the variance was partitioned into the first eigenvector. Potassium, Na^+, Ca^{++}, and Mg^{++} were correlated with rates of leaf fall.

Since the influence of percentage leaf fall obscured the effects of other growth factors, a second set of ordinations were run with this variable removed from the data set. In these analyses (Table 4.2 a, b), variance was distributed between three axes. Relative growth rate and SRGR were now the most influential growth variables, and Mg^{++}, Na^+, and Ca^{++} were the most influential nutrients.

The biplot of nutrient and sample scores (Figure 4.7) illustrates the major influence of K^+ and Mg^{++} on the first axis and Na^+ on the second. The sample score plots show that SRGR was influenced by the first axis, while ULR's in new and old leaves were heavily influenced by Na^+. Old leaves of Kitui (NaCl 1) and Sigor (NaCl 1 and 2) had higher scores on the Na^+ axis than other treatments, suggesting a somewhat closer correlation with Na^+. Root RGR scores for Sigor were negatively correlated with Na^+, perhaps as a result of their higher root Na^+ concentrations, while the equivalent scores for Kitui roots were closely associated with Ca^{++}, N and P. Control values for stems were closely related to K^+ and Mg^{++} biplot scores.

In the three dimensional ordination plot of nutrient scores and growth variable scores (Figure 4.7), the vector for SRGR was also closely correlated with vectors for K^+ and Mg^{++}, but poorly with vectors for Na^+ and P. Relative growth rate had an almost perfectly negative correlation with vectors for Na^+ and P, and weak positive

K^+ and Mg^{++}, but poorly with vectors for Na^+ and P. Relative growth rate had an almost perfectly negative correlation with vectors for Na^+ and P, and weak positive correlations with K^+ and Mg^{++}. Root RGR was closely correlated with vectors for N and Ca^{++}, and had weak negative correlations with both Na^+ and RGR, with K^+ and Mg^{++}.

4.4 Discussion

In *Acacia tortilis* (Forsk.) Hayne, Sigor and Kitui provenances responded to 200 millimolal NaCl by increasing Na^+ partitioning to older leaves and continuing to store 45 -55% of total Na^+ in roots. Although Na^+ accumulation in foliage was proportional to the duration of pre-treatment in both provenances, Kitui accumulated shoot Na^+ more rapidly than Sigor. Sigor maintained selectivity of K^+ and N transport to new leaves during the accumulation of Na^+, a factor that may have contributed to higher ULR's than in Kitui (Figure 3.2). By the final harvest, a greater proportion of Sigor seedlings were experiencing abscission of lower leaves, and it is likely that these had even higher concentrations of Na^+ than old leaves that were retained on the stem. Treated seedlings experienced rapid shoot accumulation of Na+ during pre-treatment that stabilized at about 50% of total Na^+ by the time of final harvest. However, control seedlings of both provenances substantially reduced the proportion of Na+ allocated to leaves, suggesting that control of Na^+ movements between plant organs may improve with age in both provenances (Figure 4.3a and b).

In controls, final harvest contents of macro-nutrients and Na^+ were higher in Sigor than Kitui. However, this difference was eliminated in treated seedlings, while Sigor's

Lower rates of growth may have allowed Na^+ to become more concentrated in Kitui foliage, resulting in further growth inhibition. On the other hand, Sigor's inherently higher growth, particularly of stems (tables 3.2 and 3.3) may have assisted it in maintaining lower shoot Na^+ concentrations, further enhancing its growth rate advantages over Kitui.

By the time of the final harvest, seedlings of Sigor (NaCl 1) excluded Na^+ from new leaves more efficiently than other pre-treatment groups, an observation supported by significant ($p \leq 0.05$) planned comparisons for Sigor (NaCl 1 vs. NaCl 2 and 3). Sample scores for old leaves in Kitui (NaCl 1) and Sigor (NaCl 1 and 2) were also more closely associated with the Na+ biplot score in the RDA (Figure 4.7). This reversal of intermediate harvest Na^+ distribution patterns suggests that earlier pre-treated Sigor seedlings may have been better conditioned to 200 millimmolal NaCl than those exposed to later pre-treatments. However, there were no treatment-specific differences in other response variables, and it seems likely that differences in conditioning between pre-treatment schedules were minor.

Vector analysis showed that NaCl reduced macro-nutrient uptake (Figures 4.5 and 4.6). Multivariate analysis (Figures 4.7 and 4.8) suggested that disturbed nutrition may have been as important in limiting seedling growth as the potentially toxic effects of Na^+ accumulation. Stem RGR, especially of control plants, was positively correlated with Mg^{++} and K^+. Root RGR scores for Kitui, but not Sigor, were correlated with Ca^{++}, P and N. Unit leaf rate scores were also associated with N and P. Relative growth rate was negatively associated with P, as well as Na^+.

Phosphorus was the element least affected by NaCl treatment, and the only element to become more concentrated in NaCl-treated organs than in controls. High P uptake induces Zn deficiency, resulting in stunted shoot growth, chlorosis and dwarfed leaves (Teng and Timmer, 1990), visual symptoms that may be confounded with those of Na^+-toxicity. Phosphorus concentration has also been observed in members of the Triticeae grown in saline media (Gorham et al., 1986), and Zn deficiency has been noted in salt-stressed mesquite (Jarrell and Virginia, 1990). However, P contents were lower in treated seedlings than controls, suggesting that growth reduction was more likely to be the cause than the effect of high P concentrations. Therefore, the negative correlation of RGR with P (Figure 4.8) was probably an incidental result of P uptake being less inhibited than that of other macro-nutrients.

The positive correlation of SRGR with K^+ and Mg^{++} nutrition may have resulted from the multiple roles these elements play in plant growth. Potassium is critically involved in several aspects of protein synthesis as well as tissue elongation. Magnesium, in addition to being the central atom in chlorophyll, is required for protein synthesis, and as a co-factor in enzymes that use Mg-ATP as substrates (Wyn Jones and Pollard, 1983).

Root Ca^{++} contents and concentrations fell to very low levels in pre-treated seedlings, and were reduced between intermediate and final harvests, while root Ca^{++} in control seedlings increased. Sodium displaces cell wall bound Ca^{++} (Cramer et al., 1985; Läuchli and Schubert, 1989), and selective K^+ uptake is also dependent on adequate calcium nutrition (Jeschke, 1984). Reduced root growth rates, especially in

Kitui (Figure 4.7) could therefore be explained by Na^+ inhibited root Ca^{++} uptake. However, although root Ca^{++} was lower in Sigor than Kitui, the weak correlations of Sigor RRGR with the Ca^{++} biplot vector of Figure 4.7 do not support a limiting role for Ca^{++} nutrition in determining root growth in Sigor. *Brassica* species differing in salt-tolerance also display differing responses to similar Na^+/Ca^{++} ratios (Ashraf and Naqvi, 1992). Differences in Ca^{++} distribution between root vacuoles, tonoplasts and plasma membranes may be of greater importance to ion regulation than the bulk Ca^{++} concentration (Zhong and Läuchli, 1994), and may help to explain provenance-specific differences in salt-tolerance in *Acacia tortilis*.

4.5 Conclusion

Superior growth, Na^+ partitioning and the shedding of salt-saturated lower leaves characterized differences in salt-tolerance between Sigor and Kitui provenances of *Acacia tortilis* (Forsk.) Hayne. Concentrations, but not contents, of nutrient elements were significantly higher in Kitui, suggesting that more efficient nutrient metabolism, more effective nutrient allocation, or a combination of both, were supporting Sigor's higher growth rates. Earlier pre-treated seedlings of Sigor also maintained lower new leaf and stem relative concentrations of Na^+ when exposed to 200 millimolal NaCl than seedlings exposed to later pre-treatments, suggesting that they may have been better conditioned to high salinity. However, other nutrition parameters did not indicate significant between-treatment differences, and the effect of different pre-treatment schedules on the overall conditioning process was probably minimal.

Multivariate analyses confirmed that the percentage of plants suffering leaf fall was a highly sensitive predictor of reaction to salt treatment, with SRGR being a second predictor. Abscission of older leaves accompanied by rapid stem elongation and high RGR's may therefore provide good predictors of salt-tolerance in different provenances of *Acacia tortilis*. Secondary disturbances in nutrient uptake and distribution, such as P induced deficiency in Zn, altered distribution of N, and disruption of K^{+}, Ca^{++} and Mg^{++} metabolism may also contribute to differences in salt-tolerance between *Acacia tortilis* provenances.

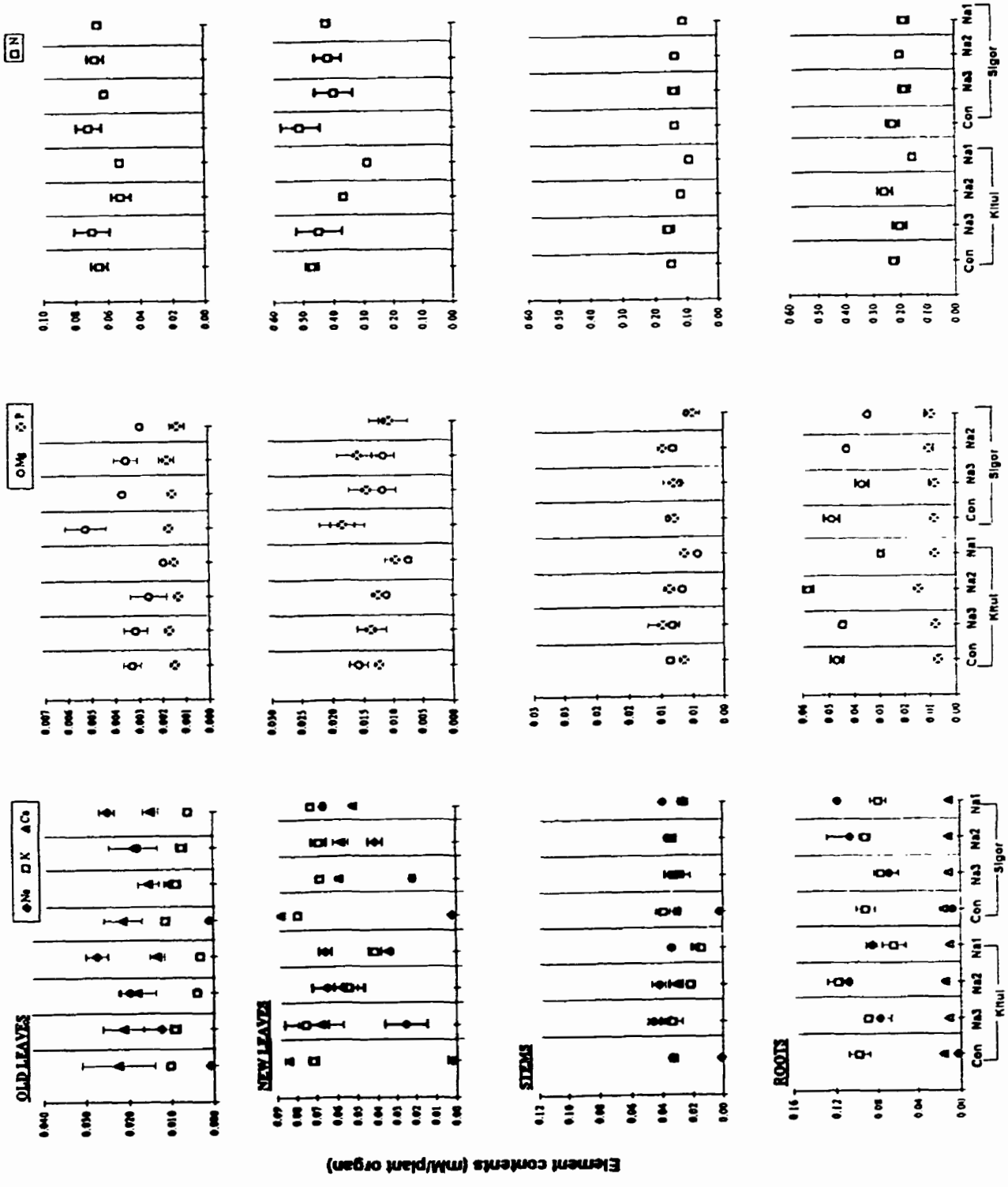


Figure 4.1 Sodium and macro-nutrient contents of Kitui and Sigor provenances of Acacia tortilis after pre-treatment (intermediate harvest). Bars represent standard errors of the mean. Axis legend: Con= control, Na1 - Na3 = NaCl1 - NaCl3, respectively.

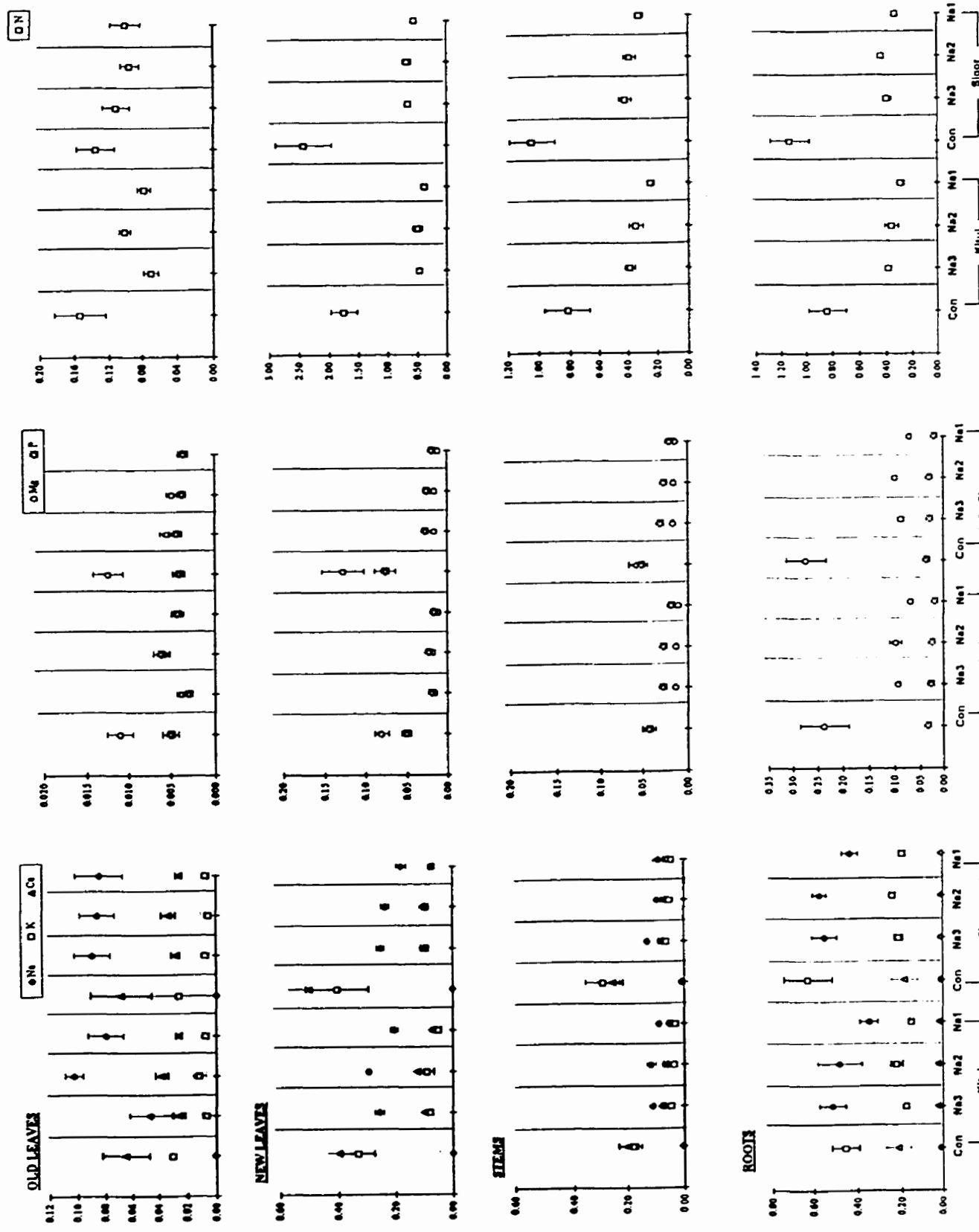


Figure 4.2 Sodium and macro-nutrient contents of Kitui and Sigor provenances of Acacia tortilis after 37 days of treatment with 200 millimolal NaCl (final harvest).Bars represent standard errors of the mean; axis codes as in Figure 4.1.

Figure 4.3a. *Sodium contents, expressed as a percentage of total seedling uptake in Kitui and Sigor provenances of Acacia tortilis, in the intermediate harvest of experiment 2.*

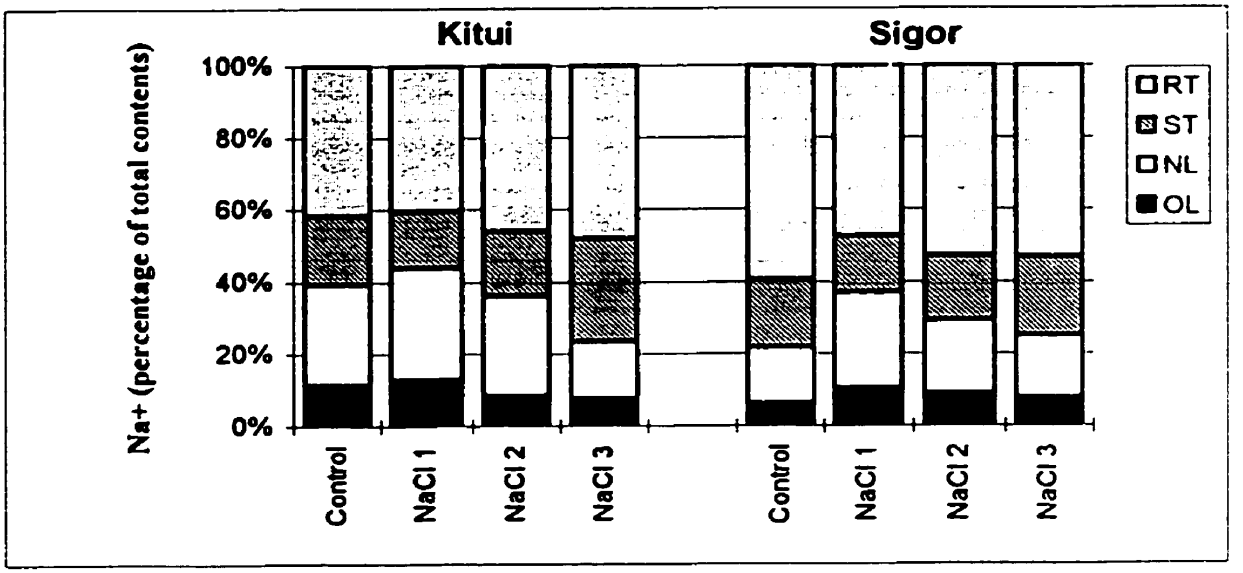


Figure 4.3b. *Sodium contents, expressed as a percentage of total seedling uptake in Kitui and Sigor provenances of Acacia tortilis, in the final harvest of experiment 2.*

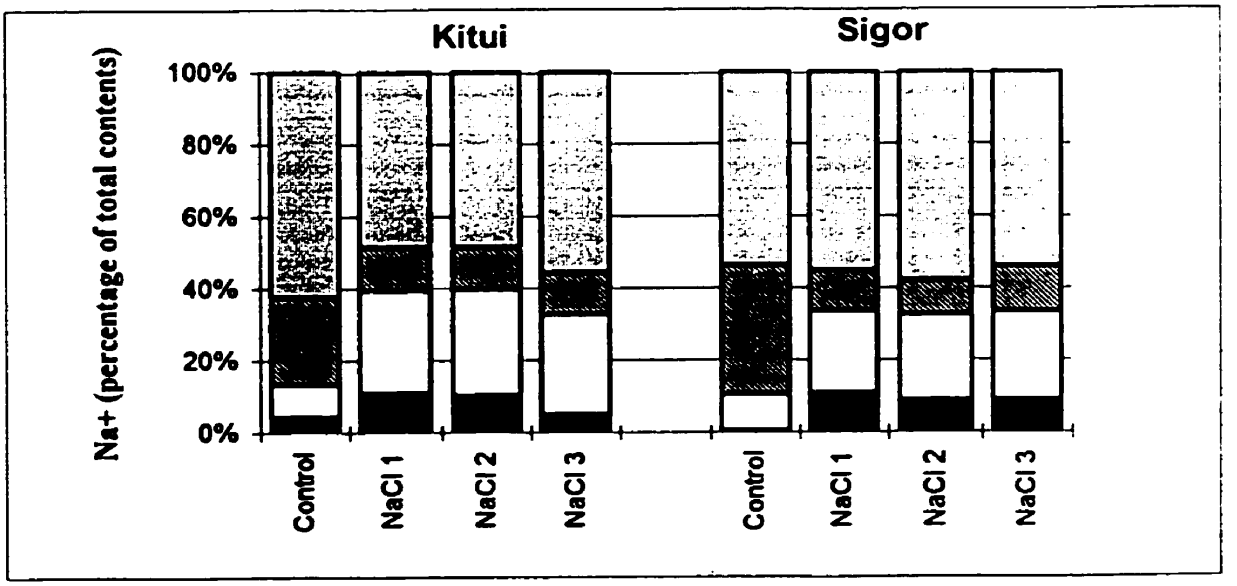
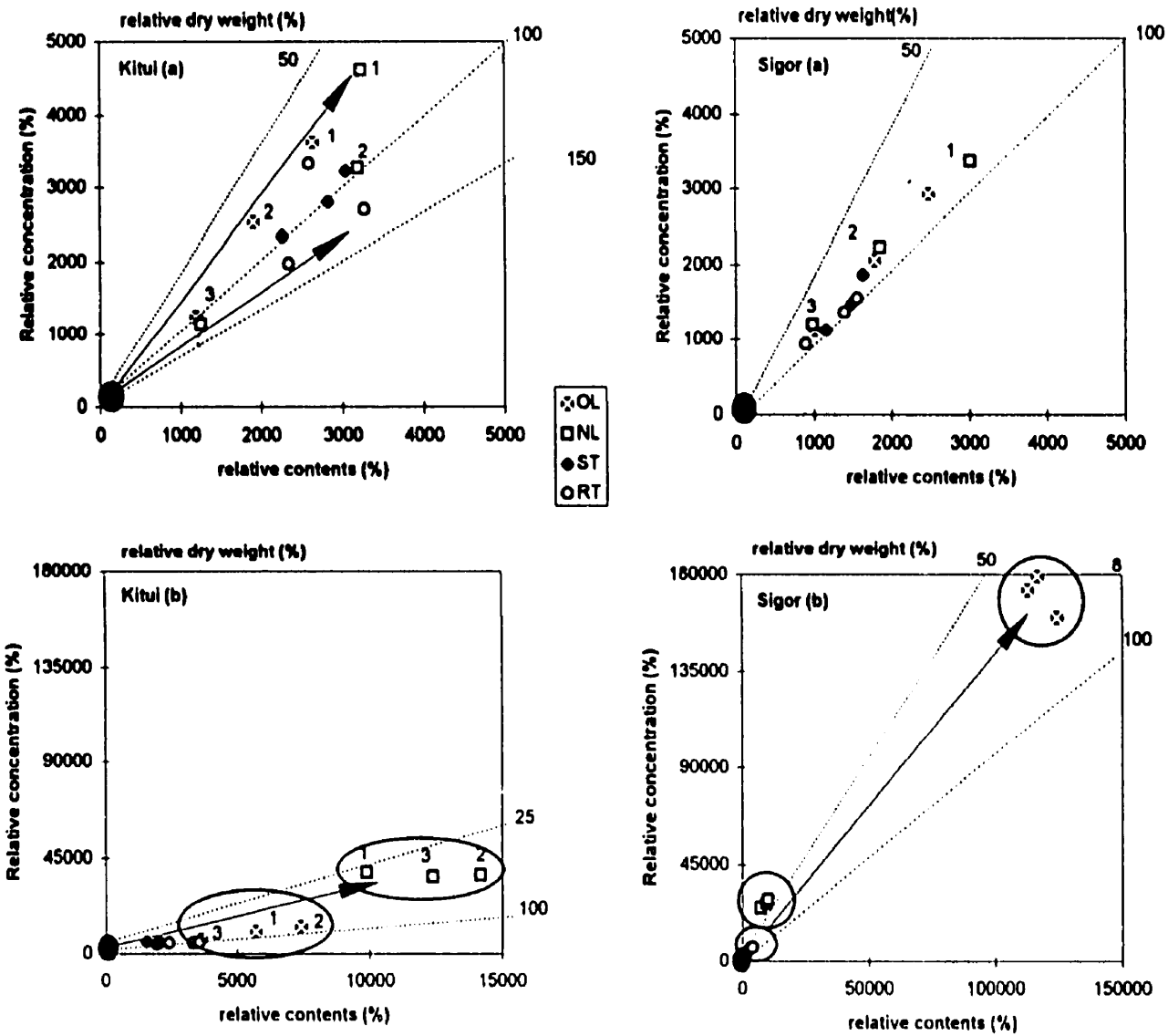


Figure 4.4 - Vector analysis of Na+, relative to controls, in intermediate (a) and final harvest (b) tissues of Acacia tortilis (legend: OL = old leaves, NL = new leaves, ST = stems, RT = roots; numbers are NaCl pre-treatments, 1 = longest, 3 = shortest).



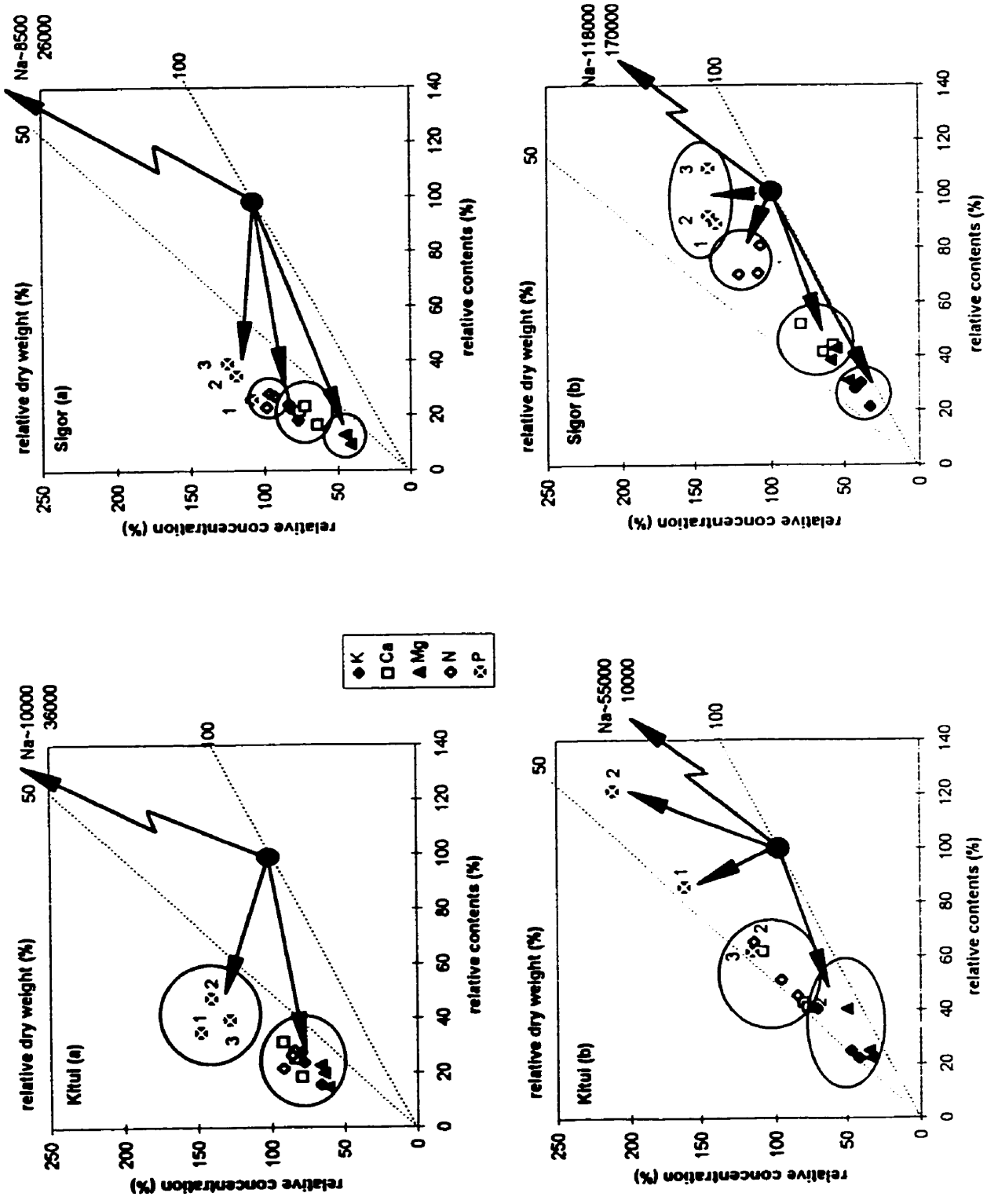


Figure 4.5 - Vector analysis of final harvest nutrient contents of new leaves (a) and old leaves (b) of Kitui and Sigor provenances of Acacia tortilis, relative to controls (numbers on chart refer to NaCl pre-treatments 1 - 3).

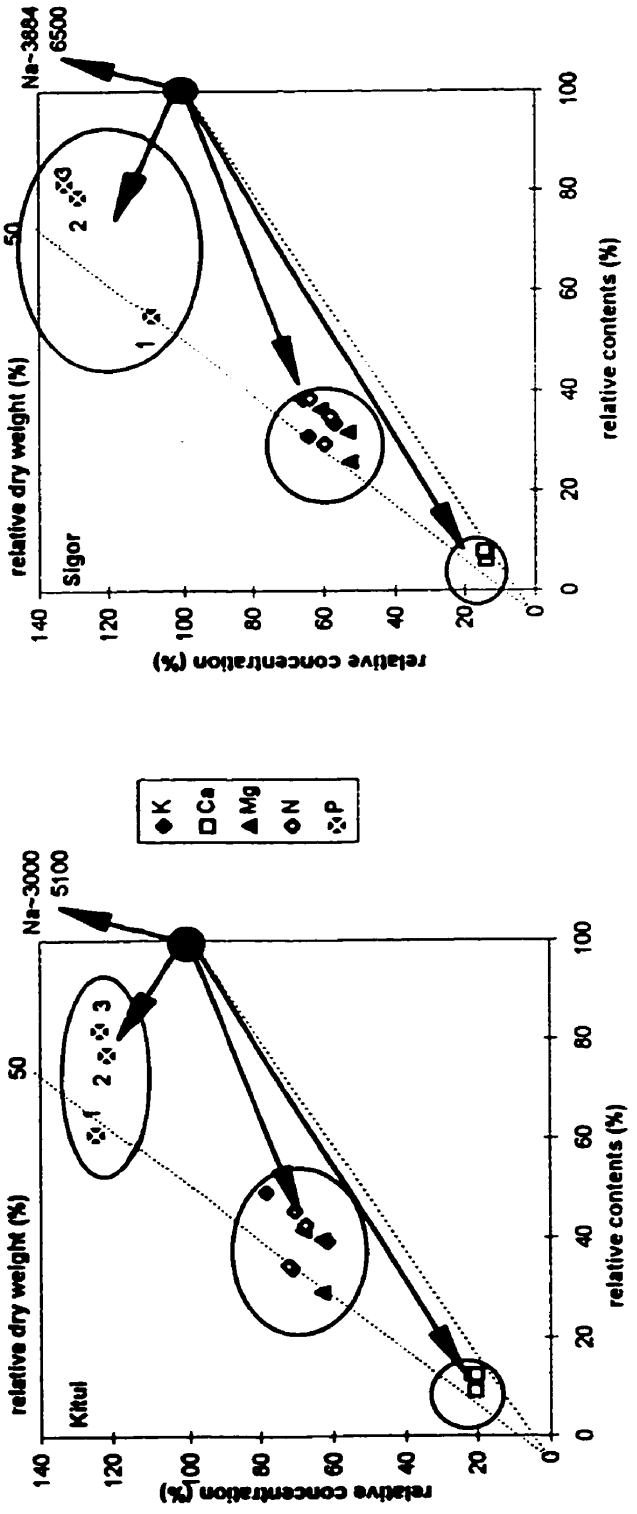


Figure 4.6- *Vector analysis of final harvest nutrient contents of roots of Kitui and Sigor provenances of <u>Acacia tortilis</u>, relative to controls (numbers on chart refer to NaCl pre-treatments 1 - 3).*

Figure 4.7 *Sample scores that are linear fuunctions of nutrient elements and values for nutrient biplot from redundency analysis. Nutrient scores are multiplied by 3 (for clarity). Angles between nutrient vectors and sample scores for tissues display approximate correlations.Codes: KC and SC refer to controls for Kitui and Sigor, respectively; S or K1, 2 and 3 refer to NaCl pre-treatments 1, 2 and 3.*

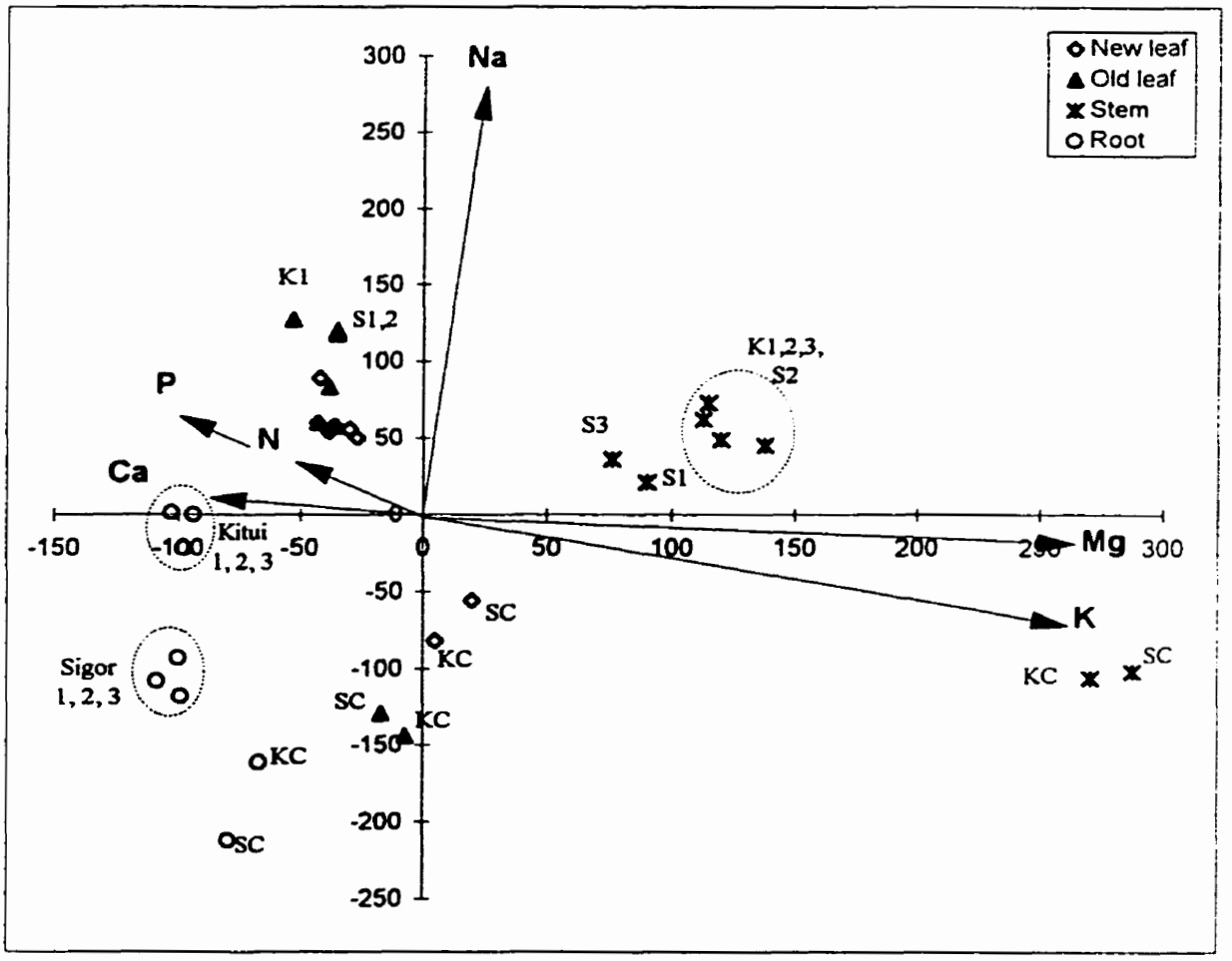


Figure 4.7 *Nutrient biplot and growth variable scores from redundency analysis.*

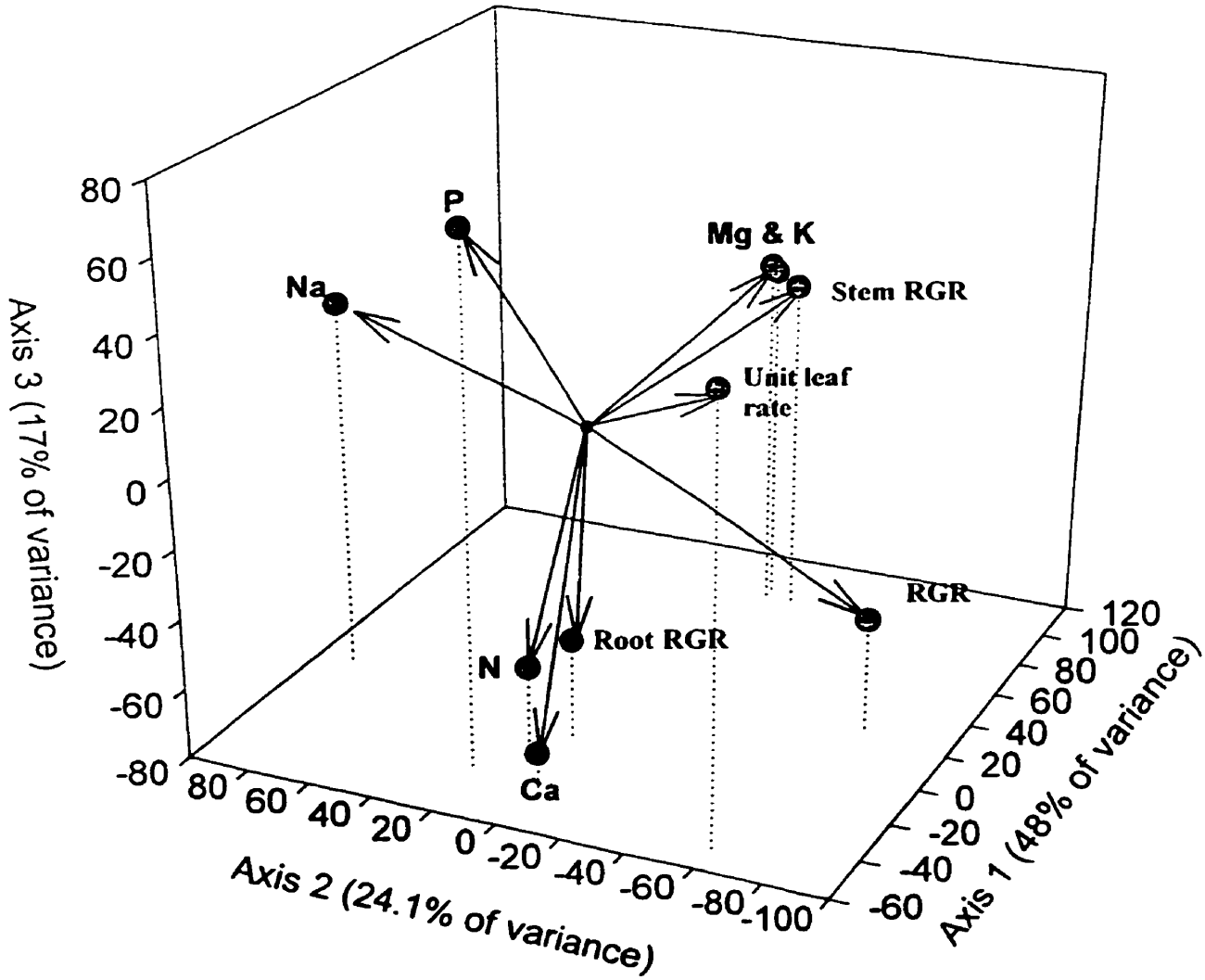


Table 4.1 (a), Eigenvectors and variance for first set of multivariate analyses, (b), growth variable scores for first two eigenvectors.

(a)	Eigenvalues and Variance				
	Axis 1	**Axis 2**	**Axis3**	**Axis 4**	**Sum**
1) PCA	0.993	0.004	0.002	0.000	**1.000**
1) RDA	0.848	0.003	0.001	0.001	**0.853**
3) Partial RDA	0.110	0.001	0.000	0.000	**0.111**
				Unique contribution of growth (1 - 2)	**0.147**
				Unique contribution of experimental design (2 - 3)	**0.742**

(b)		
Growth variable	**Axis 1**	**Axis 2**
RGR	-95	211
ULR	-271	-135
% leaf fall	1000	0
SRGR	-274	915
RRGR	-287	-614

Table 4.2 (a), Eigenvectors and variance for second set of multivariate analyses, (b), growth variable scores for first three eigenvectors.

	Eigenvalues and Variance				
(a)	**Axis 1**	**Axis 2**	**Axis3**	**Axis 4**	**Sum**
1) PCA	0.515	0.311	0.173	0.000	**1.000**
1) RDA	0.484	0.241	0.139	0.000	**0.864**
3) Partial RDA	0.097	0.031	0.013	0.000	**0.141**
				Unique contribution of growth (1 - 2)	**0.136**
				Unique contribution of experimental design (2 - 3)	**0.723**

(b)			
Growth variable	**Axis 1**	**Axis 2**	**Axis3**
RGR	274	-723	-484
ULR	-76	35	-517
SRGR	928	-91	228
RRGR	-494	-604	438

5

General Discussion And Conclusions.

5.1 - Growth, Nutrition And Conditioning

Salt-tolerant (Sigor) and salt-sensitive (Kitui) provenances of *Acacia tortilis* (Forsk.) Hayne responded to salt preconditioning in very different ways. Kitui was sensitive to the timing of preconditioning, resulting in significantly lower growth rates and seedling size in earlier pre-treated seedlings. Growth of Sigor seedlings was equally retarded by all pre-treatments, indicating that seedling age at the time of pre-treatment made little difference to subsequent growth. Sigor allocated more dry matter to stems and roots than Kitui, although leaf dry weights were no different. Sigor, however, had high rates of leaf abscission, and had these been incorporated into the leaf dry weight calculations, it is likely that net leaf production would have been greater.

Sodium and macro-nutrient uptake was also more sensitive to the duration of pre-treatment in Kitui than in Sigor. The proportions of Na^+ accumulated in shoots increased in proportion to the duration of pre-treatment and to a greater degree in Kitui than Sigor. The difference in Na^+ concentrations between old and new leaves was smaller in Kitui than Sigor, suggesting poorer ion-partitioning ability in this provenance.

The long-term response to Na^+ accumulation in both provenances may have been the development of succulence (Table 3.3 and Appendix 5.2). Sigor was less succulent

than Kitui and had higher unit leaf rates, suggesting that transpiration and net photosynthesis were probably higher in this provenance. Kitui also experienced perturbed root growth under saline conditions, but this did not result in lower overall Na^+ or nutrient uptake. Therefore, it appears that growth reduction in Kitui rather than increased uptake of Na^+ or reduced macro-nutrient supply, led to the observed increases in Na^+ and macro-nutrient concentration. Kitui's greater succulence may have been a transpiration-limiting response that resulted in lower leaf area per unit of leaf dry weight (Iyengar and Reddy, 1994).

Although seedlings responded to pre-treatment with altered growth patterns and increased succulence, pre-treatment schedules made no difference to subsequent growth responses of either provenance to 200 millimolal NaCl. Therefore, no age-dependent differences in preconditioning could be inferred. Growth was used to distinguish hardened from sensitive plants of *Sorghum bicolor* (Amzallag et al., 1990) and *Eleusine coracana* (Uma et al., 1993). However, these are fast-growing cereals that must mature and set seed in a single growing season. Hardening to salinity may have been faster and more clearly expressed in growth rate differences than in *Acacia tortilis*. Furthermore, Ca^{++}, which ameliorates the effects of salt in a number of ways (Rengel, 1992), was maintained at an Na^+:Ca^{++} ratio of 30:1 in the experiments on *Sorghum bicolor* (Amzallag et al., 1990). Grieve and Maas (1988) found that adding Ca^{++} improved growth of several varieties of *Sorghum bicolor* under saline conditions. It may be that additional Ca^{++} is required before salt-hardening is expressed as improved growth, and the fact that I maintained Ca^{++} constant at 4 mM might explain the absence of treatment-specific growth responses at 200 millimolal NaCl.

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5.2 - Nutrient Distribution

Cheeseman (1988) suggested that the effects of Na^+ could only be understood at the level of whole plant responses to salt stress. ANOVA (Appendix 6.5 - 6.8) and multivariate analyses (Figures 4.6 and 4.7) suggested that interference by Na^+ with nutrient metabolism could have been more important than direct ion toxicity in affecting seedling growth in *Acacia tortilis*. Potassium, Mg^{++}, N, P and Na^+ differed significantly between provenances in their distribution amongst tissues. Orthogonal comparisons also showed significant ($p< 0.05$) differences in P (but not Na^+) between all Sigor salt treatments, suggesting that control of P uptake and distribution may be important for this provenance.

In Figure 4.7, the three major vectors are determined by different elements and growth parameters. Phosphorus and Na^+ are negatively associated with RGR, SRGR are positively correlated with K^+ and Mg^{++}, and RRGR was associated with N and Ca^{++}. The association of K^+ with stem growth was to be expected, since K^+ is essential for cell expansion (Flowers and Yeo, 1989). Differences in K^+ partitioning may explain greater stem elongation and unit leaf rates (which imply greater leaf expansion) that were observed in Sigor. Differences in K^+ partitioning were also associated with differential salt-tolerance of *Casuarina* spp. (Aswathappa and Bachelard, 1986). Magnesium increases in leaves and stems of salt-stressed *Leucaena*

Many studies support the role of Ca^{++} in improving salt-tolerance (e.g. Gorham et al., 1988, Subbarao et al., 1990), mainly through its beneficial effects on trans-membrane ion transport and exclusion (Rengel, 1992). Kent and Lauchli (1985) observed improved root growth in salt-stressed cotton in the presence of supplementary Ca^{++}. Membrane-bound Ca^{++} may reduce the efflux of carbohydrates under salt-stress (Hansen, 1984), helping to maintain root growth, and providing a possible explanation for the strong correlation of Ca^{++} with RRGR. The correlation of N with RRGR may have been incidental, since N is reduced in roots rather than leaves under saline conditions due to inhibition of the K^+-malate shuttle (Cramer et al., 1995). Alternatively, faster-growing roots may have been better supplied with amino acids and other forms of reduced nitrogen, as suggested by Termaat and Munns (1986). New leaf N contents were also higher in Sigor than Kitui, and since leaf dry weights were not significantly different, it may be that N was being incorporated into compatible osmotica (for example, proline).

5.3 Ecological Implications Of salt Preconditioning

Variations in the salt-tolerance of seven *Acacia tortilis* (Forsk.) Hayne provenances from Kenya were positively correlated with the soil salinity of seed source areas (Muturi, 1993.). In the current experiment, salt-tolerant Sigor grew faster than salt-sensitive Kitui in all treatments, strongly suggesting that faster growth is a marker for salt-tolerant *Acacia tortilis*. However, the interpretation of tolerance based on seed source may be complicated by extreme local variations in soil salinity (Epstein and Rains, 1987). For example, there was no correlation between salinity of seed source

and salt-tolerance in *Prosopis farcta* from Israel (Dafni and Negbi, 1978), and variation in salt-tolerance in *Prosopis flexuosa* from Argentina was greater between the progeny of individual trees than between provenances (Catalán et al., 1994).

Catalán et al. (1994) found that high rates of germination under saline conditions do not translate into improved salt-tolerance in older plants, perhaps because germination occurs after rainfall events that flush salts away from the soil surface. This raises the question of whether early salt-tolerance is relevant to the survival of *Acacia tortilis* in the field. Under these conditions, the larger seeded Kitui might have an advantage over the small-seeded Sigor, as suggested for large-seeded plants in general by Leishman and Westoby (1994) (Appendix 4). Another question is whether small seedlings, whether hardened to salinity or not, could withstand the accumulation of salt that would accompany prolonged exposure to saline conditions. In these conditions, Sigor would always have an advantage over Kitui because of its relative insensitivity to early exposure, higher growth rates, and its ability to partition salt into older leaves. Salt concentrations were generally lower in Sigor (Appendix 6.3 and 6.5), lending credence to the idea that tolerant plants may dilute high influxes of Na^+ through faster growth (Flowers and Yeo, 1989; Munns and Termaat, 1986).

In reviewing reforestation efforts in Turkana, Kenya, Zumer-Linder (1986) stated that long-term trials of replanting techniques were needed to ensure successful reforestation in harsh climates. Although the present study can not be extrapolated to all the conditions seedlings would encounter in the field, it does suggest some further lines of inquiry. For example, provenances of *Acacia tortilis* already known to be salt-

tolerant might be further hardened by growing them in mildly saline soil. However, silviculturalists may wish to strike a balance between early exposure of salt-tolerant seedlings to local saline soils, which may improve salt partitioning, and maintaining high growth rates that dilute those salts that are transported to leaves.

5.4 CONCLUSION

Superior growth, the partitioning of Na^+ to older leaves and higher unit leaf rates were the chief characteristics distinguishing salt-tolerant Sigor from salt-sensitive Kitui provenance of *Acacia tortilis* (Forsk.) Hayne. Although treated seedlings maintained higher root:shoot and root:stem ratios than controls, and developed greater succulence as a longer-term response to salinity, the duration and timing of pre-treatment produced no measurable differences in these responses.

Sodium contents were lower in earlier pre-treated Sigor than in later pre-treated seedlings. However, in the absence of other indicators of superior conditioning, such as higher growth rates, differences in the efficacy of pre-treatments cannot be concluded.

Multivariate analysis revealed complex interactions of salt, nutrition and growth variables. Leaf abscission was the major variable distinguishing Sigor from Kitui, and this characteristic may be a useful marker for inherent salt-tolerance in *Acacia tortilis*. Other growth characteristics were associated with specific nutrient elements. Particularly strong positive associations existed between K^+, Mg^{++} and SRGR, and N, Ca^{++}, and RRGR. Strong negative correlations were seen between Na^+, P, and RGR. These data imply that Na^+ inhibits growth in *Acacia tortilis* primarily by interfering in nutrient allocation and metabolism.

Further experiments should consider the possibility that perennial trees may not express superior conditioning through higher growth rates, or have a specific developmental window for conditioning, as has been seen in cereals. Future experiments should consider using longer intervals between pre-treatment schedules to test the effects of substantial differences in seedling size on conditioning. Preconditioning might also be attempted under a range of nutritional conditions in order to further explore the potential role of nutrient ions in the conditioning process.

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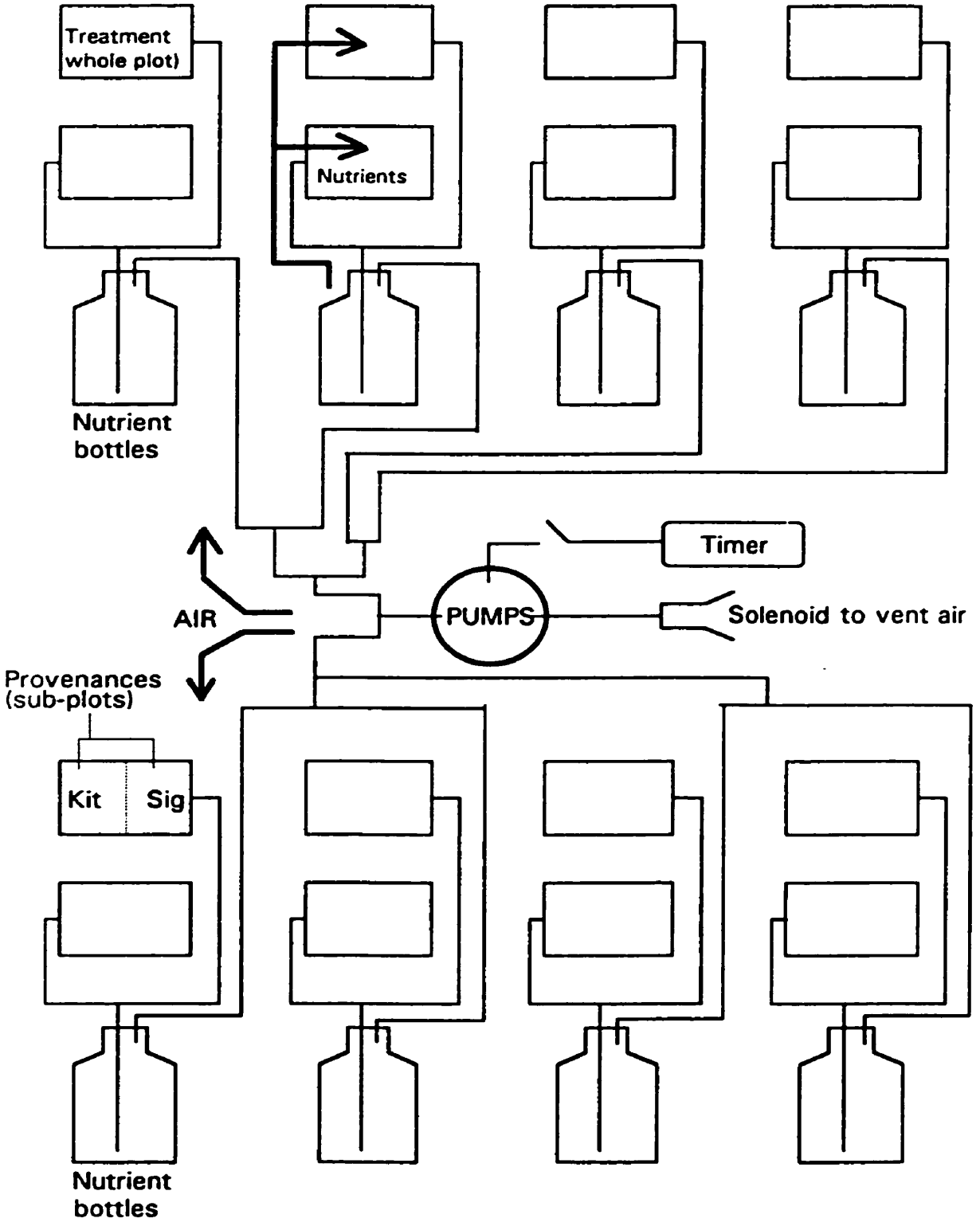
APPENDICES.

APPENDIX 1

Experimental apparatus used to deliver nutrients on a single daily cycle.



APPENDIX 2

Table A2.1 Modified Hoagland's number 2 solution.

ELEMENTS		PROPORTIONS in SOLUTION			
Formula	Molecular weight	Stock (g/l)	ml/l of full strength	ml/20 l. container	Major ion (millimol./l 1/2 str)
$NH_4H_2PO_4$	115	1M	1	20	0.50000
KNO_3	101.1	1M	6	120	3.01205
$Ca(NO_3)_24H_2O$	174.1	1M	8	160	4.00000
$MgSO_4.7H_2O$	246.4	1M	2	40	1.00000
* H_3BO_3	61.8	2.86	-	-	0.02314
* $MnCl_2.4H_2O$	197.9	1.81	-	-	0.00457
* $ZnSO_4.7H_2O$	287.5	0.22	-	-	0.00038
* $CuSO_45H_2O$	249.7	0.08	-	-	0.00016
* $H_2MoO_4H_20$	179.9	0.02	-	-	0.00006
*E.D.T.A.(5%)	366.9	1.835	-	-	0.00250

• Use 1ml micronutrient stock/l

Table A2.2 Example nutrient solution characteristics

		DAY 0			DAY 7	
Bottle #	Millimol - ality NaCl	Electrical conductivity (mS)	pH (day 0)	ml/l. KOH	Electrical conductivity (mS)	pH (day 7)
1	150	16.5	-	-	16.7	-
5	150	15.9	5.69	0.15	15.4	6.32
2	100	11.5	-	-	12.8	-
6	100	11.3	5.53	0.20	12.7	6.28
3	50	7.2	-	-	7.5	-
7	50	6.9	5.51	0.25	7.0	5.76
4	0	1.6	-	-	2.2	-
8	0	1.4	5.58	0.35	2.2	6.23
FINAL TREATMENT (31/03/95 - 7/04/95)						
1	200	21.6	-	0.10	22.5	-
2	200	22.1	5.62	0.10	22.6	6.51
3	200	21.6	-	0.10	23.1	-
4	0	1.5	5.62	0.35	1.7	6.36
5	200	22	-	0.10	22.9	-
6	200	22.1	-	0.10	23.3	-
7	200	21.6	5.56	0.10	23.4	6.5
8	0	1.6	-	0.35	1.8	-

APPENDIX 3

EQUATIONS USED FOR CALCULATING PLANT GROWTH PARAMETERS.

Relative Growth Rate (RGR)

$$\frac{\text{Log}_e DW_2 - \text{Log}_e DW_1}{T_2 - T_1}$$

.....where DW = dry weight of whole plant, shoots or roots and T = time

Unit Leaf Rate (E)

$$\frac{\partial DW}{\partial T} \times \frac{1}{L_A}$$

.....where L_A = Leaf area

Leaf Area Ratio

$$\frac{L_A}{DW}$$

(Reference: Evans, 1972.)

APPENDIX 4

Table A4.1 Differences In Seedling Growth At 15 Days. Seeds of Kitui weighed 1.192g/20 seeds and seeds of Sigor weighed 0.697g/ 20 seeds (n = 10).

	TOTAL DRY WEIGHTS		ROOT/SHOOT + LEAF		ROOT/SHOOT RATIO	
	KITUI	*SIGOR*	*KITUI*	*SIGOR*	*KITUI*	*SIGOR*
Mean	0.0461	0.0361	0.2806	0.3350	1.0890	1.4853
Variance	0.0001	0.0001	0.0117	0.0141	0.2412	2.3250
Observations	32	32	32	32	32	32
df	62		62		62	
t statistic	5.0487		-1.9184		-1.3997	
P(T<=t) one-tail	***		*		NS	
t Critical one-tail	1.6698		1.6698		1.6698	
P(T<=t) two-tail	***		NS		NS	
t Critical two-tail	1.9990		1.9990		1.9990	

	LEAF:SHOOT RATIO		SHOOT LENGTHS		LEAF AREA	
	KITUI	*SIGOR*	*KITUI*	*SIGOR*	*KITUI*	*SIGOR*
Mean	2.9298	2.7404	1.8172	2.6909	1.5678	0.9334
Variance	1.2208	3.1805	0.1714	0.4229	0.6021	0.2510
Observations	32	32	32	32	32	32
df	62		62		62	
t statistic	0.5108		-6.4115		3.8852	
P(T<=t) one-tail	NS		***		***	
t Critical one-tail	1.6747		1.6741		1.6741	
P(T<=t) two-tail	NS		***		***	
t Critical two-tail	2.0066		2.0057		2.0057	

Table A4.2 Paired sample t-tests for growth parameters of Kitui and Sigor provenances at day 15. Asterisks indicate significance levels (≤0.05, ** ≤0.01, *** ≤0.001).*

	TOTAL DRY WEIGHTS		ROOT/SHOOT + LEAF		ROOT/SHOOT RATIO	
	KITUI	*SIGOR*	*KITUI*	*SIGOR*	*KITUI*	*SIGOR*
Mean	0.0461	0.0361	0.2806	0.3350	1.0890	1.4853
Variance	0.0001	0.0001	0.0117	0.0141	0.2412	2.3250
Observations	32	32	32	32	32	32
df	62		62		62	
t statistic	5.0487		-1.9184		-1.3997	
P(T<=t) one-tail	***		*		NS	
t Critical one-tail	1.6698		1.6698		1.6698	
P(T<=t) two-tail	***		NS		NS	
t Critical two-tail	1.9990		1.9990		1.9990	

	LEAF:SHOOT RATIO		SHOOT LENGTHS		LEAF AREA	
	KITUI	*SIGOR*	*KITUI*	*SIGOR*	*KITUI*	*SIGOR*
Mean	2.9298	2.7404	1.8172	2.6909	1.5678	0.9334
Variance	1.2208	3.1805	0.1714	0.4229	0.6021	0.2510
Observations	32	32	32	32	32	32
df	62		62		62	
t statistic	0.5108		-6.4115		3.8852	
P(T<=t) one-tail	NS		***		***	
t Critical one-tail	1.6747		1.6741		1.6741	
P(T<=t) two-tail	NS		***		***	
t Critical two-tail	2.0066		2.0057		2.0057	

APPENDIX 5

SUMMARIES OF ANOVAS FOR MAIN EFFECTS AND INTERACTIONS IN GROWTH ANALYSIS (EXPERIMENT 1).

Example SAS ANOVA model

Data were analyzed as split-plots with salt treatments as main plots and provenances as sub-plots within treatments. The term "TEST H= SALT E=SALT*REP;" instructs SAS to use the salt x block mean square (df = 9) to calculate the F value for salt-treatment, rather than the experiment-wise error term (df = 12), a procedure demanded by the split-plot design.

```
DATA DW2I;
INFILE 'b:\EXP2\DW2FBSAS.PRN';
INPUT PROV SALT REP SHTLNTH LEAFAR ROOTPCT PCTDW DRYWT RATIO
LAR RGR ULR RRGR SRGR;
CARDS;
PROC GLM;
CLASSES PROV SALT REP;
MODEL SHTLNTH LEAFAR ROOTPCT PCTDW DRYWT RATIO LAR RGR ULR
RRGR SRGR= REP SALT REP*SALT PROV SALT*PROV;

TEST H= SALT E=SALT*REP;
MEANS REP SALT REP*SALT PROV PROV*SALT/LSD;

CONTRAST 'SALT 1 VS 2 IN PROV 2' SALT  0 1 -1 0 SALT*PROV 0 0 0 0 0 1 -1 0;
CONTRAST 'SALT 1 VS 2 IN PROV1'  SALT  0 1 -1 0 SALT*PROV 0 1 -1 0 0 0 0;
CONTRAST 'SALT 2 VS 3 IN PROV1'  SALT  0 0 1 -1 SALT*PROV 0 0 1 -1 0 0 0;
CONTRAST 'SALT 2 VS 3 IN PROV2'  SALT  0 0 1 -1 SALT*PROV 0 0 0 0 0 1 -1;
CONTRAST 'SALT 1 VS 3 IN PROV2'  SALT  0 1 0 -1 SALT*PROV 0 0 0 0 1 0 -1;
CONTRAST 'SALT 1 VS 3 IN PROV1'  SALT  0 1 0 -1 SALT*PROV 0 1 0 -1 0 0 0;
CONTRAST 'PROV 1 VS 2 IN SALT 0' PROV 1 -1  SALT*PROV 1 0 0 0 -1 0 0 0;
RUN;
```

*Table A5.1 F values and probabilities for intermediate harvest growth data (probability of F: * = 0.05, ** = 0.01, *** = 0.001).*

SOURCE OF VARIATION	df		SHOOT LENGTH (mm)	LEAF AREA (cm^3)	LEAF DRY WEIGHT (g)	SHOOT DRY WEIGHT (g)	ROOT DRY WEIGHT (g)	TOTAL % Dry Wt.	TOTAL DRY WEIGHT	ROOT: SHOOT RATIO	ROOT: STEM RATIO	LEAF AREA RATIO	RELATIVE GROWTH RATE (d-1)	UNIT LEAF RATE ($g\ cm^{-2}\ d^{-1}$)	ROOT RGR	SHOOT RGR
Block	3	F	5.07	3.84	5.00	2.90	1.83	0.44	5.10	0.10	0.09	1.59	16.19	3.57	7.32	6.86
		P	**	*	*	ns	ns	ns	*	ns	ns	ns	***	*	**	**
Salt Treatment	4	F	6.64	5.62	5.36	5.48	1.99	1.62	5.13	1.86	0.97	0.93	12.66	1.03	2.04	12.37
		P	**	*	*	*	ns	ns	*	ns	ns	ns	***	ns	ns	***
Salt x Block	9	F	1.27	3.67	3.09	1.49	0.91	0.58	2.08	1.18	0.94	1.46	1.98	0.83	0.55	1.41
		P	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Provenance	1	F	103.84	0.14	0.30	7.43	2.89	1.40	0.06	7.66	14.89	0.07	62.99	5.36	2.21	122.38
		P	***	ns	ns	*	ns	ns	ns	*	**	ns	***	*	ns	***
Provenance xSalt	3	F	0.58	4.60	3.36	1.67	2.31	0.28	4.10	0.76	0.60	3.08	4.88	3.24	1.80	1.50
		P	ns	*	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns	ns
Salt (using salt x Block MS as error term)	3	F	5.22	1.53	1.73	3.69	2.20	2.81	2.47	0.10	1.03	0.64	6.38	1.24	3.68	8.77
		P	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	**

Table A5.2 F values and probabilities for final harvest growth data (probability > F: . <= 0.05, .. <= 0.01, ... <= 0.001).

SOURCE OF VARIATION	df		SHOOT LENGTH (mm)	LEAF AREA (cm^2)	LEAF DRY WEIGHT (g)	LEAF FALL (% plants on d. 67)	SHOOT DRY WEIGHT (g)	ROOT DRY WEIGHT (g)	TOTAL % Dry Wt.	TOTAL DRY WEIGHT	ROOT: SHOOT RATIO	ROOT/ STEM RATIO	LEAF AREA RATIO	RELATIVE GROWTH RATE (d-1)	UNIT LEAF RATE ($g\ cm^{-2}\ d^{-1}$)	ROOT RGR	SHOOT RGR
Block	3	F	1.18	0.73	0.43	0.57	0.94	1.77	2.18	0.79	0.54	0.22	0.44	0.40	1.56	1.18	0.37
		P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Salt Treatment	3	F	19.49	25.60	24.49	18.91	22.94	14.88	37.70	22.60	25.76	19.54	0.90	30.60	18.70	8.98	28.42
		P	***	***	***	***	***	***	***	***	***	***	ns	***	***	**	***
Salt x Block	9	F	0.55	0.67	0.23	2.43	0.44	0.66	1.00	0.35	1.62	1.58	1.08	0.65	1.19	6.74	0.77
		P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Provenance	1	F	37.37	3.10	1.77	25.04	8.71	3.84	38.95	4.13	7.88	19.30	0.25	15.31	13.53	14.72	9.18
		P	***	ns	ns	***	*	ns	***	ns	*	***	ns	**	**	**	*
Provenance x Salt	3	F	1.78	0.95	0.28	0.37	1.46	0.22	2.02	0.55	0.97	0.60	1.13	0.26	0.51	0.20	0.34
		P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Salt Treatment (using Salt x Block MS as error term)	3	F	35.38	38.26	106.58	7.78	51.80	22.52	37.53	65.05	25.76	12.36	0.83	46.76	15.56	12.17	36.89
		P	***	***	***	**	***	***	***	***	***	***	ns	***	***	**	***

APPENDIX 6

SUMMARIES OF PLANT NUTRITION DATA AND ANALYSES OF VARIANCE

Appendix A6.1 Average nutrient concentrations (mM g^{-1}) for intermediate harvest of experiment two (OL = old leaves, NL = new leaves, ST = stems, RT = roots).

	CONTROL							
	KITUI				SIGOR			
ION	OL	NL	SH	RT	OL	NL	ST	RT
Na	0.029	0.014	0.022	0.029	0.029	0.014	0.029	0.072
K	0.311	0.452	0.469	0.863	0.335	0.501	0.445	0.859
Ca	0.665	0.532	0.482	0.139	0.649	0.561	0.347	0.150
Mg	0.099	0.099	0.123	0.415	0.154	0.117	0.099	0.456
N	1.981	2.971	2.115	1.945	2.115	3.230	1.561	2.097
P	0.046	0.079	0.093	0.061	0.051	0.116	0.090	0.077
Cl	0.0010	0.0010	0.0012	0.0009	0.0015	0.0016	0.0013	0.0025
	NaCl 1							
Na	1.051	0.645	0.710	0.964	0.848	0.457	0.543	1.116
K	0.119	0.405	0.303	0.703	0.200	0.512	0.347	0.733
Ca	0.507	0.341	0.387	0.114	0.503	0.366	0.376	0.100
Mg	0.075	0.072	0.096	0.326	0.099	0.079	0.079	0.326
N	2.025	2.828	1.963	1.713	2.248	2.980	1.508	1.695
P	0.058	0.095	0.146	0.092	0.046	0.077	0.069	0.086
Cl	0.0010	0.0015	0.0013	0.0016	0.0017	0.0004	0.0016	0.0016
	NaCl 2							
Na	0.735	0.457	0.616	0.783	0.598	0.312	0.428	0.978
K	0.147	0.384	0.313	0.848	0.238	0.512	0.413	0.844
Ca	0.658	0.405	0.453	0.108	0.606	0.430	0.399	0.100
Mg	0.094	0.079	0.096	0.418	0.116	0.086	0.096	0.394
N	1.929	2.605	1.731	1.838	2.218	3.087	1.615	1.820
P	0.049	0.089	0.126	0.104	0.058	0.117	0.116	0.094
Cl	0.0016	1.0483	0.0236	0.0016	0.0018	0.0016	0.0021	0.0017
	NaCl 3							
Na	0.362	0.159	0.514	0.572	0.326	0.167	0.326	0.667
K	0.277	0.497	0.367	0.671	0.275	0.520	0.388	0.757
Ca	0.642	0.447	0.420	0.083	0.482	0.449	0.378	0.106
Mg	0.096	0.089	0.093	0.336	0.117	0.089	0.086	0.364
N	2.132	2.953	1.776	1.499	1.954	3.016	1.615	1.758
P	0.053	0.095	0.110	0.060	0.050	0.110	0.094	0.078
Cl	0.0015	0.0010	0.0011	0.0015	0.0013	0.0017	0.0015	0.0002

Appendix A6.2 Average nutrient contents (mM/plant organ) for intermediate harvest of experiment two (OL = old leaves, NL = new leaves, ST = stems, RT = roots).

	CONTROL							
	KITUI				SIGOR			
ION	OL	NL	SH	RT	OL	NL	ST	RT
Na	0.009	0.002	0.002	0.003	0.001	0.002	0.002	0.008
K	0.010	0.071	0.033	0.098	0.011	0.079	0.039	0.092
Ca	0.023	0.084	0.034	0.016	0.021	0.088	0.031	0.016
Mg	0.003	0.016	0.009	0.047	0.005	0.018	0.009	0.049
N	0.065	0.471	0.147	0.221	0.072	0.508	0.137	0.225
P	0.002	0.012	0.007	0.007	0.002	0.018	0.008	0.008
Cl	0.00004	0.00020	0.00009	0.00011	0.00005	0.00023	0.00018	0.00028
	NaCl 1							
Na	0.027	0.066	0.003	0.086	0.026	0.067	0.039	0.118
K	0.003	0.041	0.014	0.064	0.006	0.073	0.025	0.079
Ca	0.013	0.035	0.018	0.010	0.015	0.052	0.027	0.011
Mg	0.002	0.007	0.004	0.029	0.003	0.011	0.006	0.034
N	0.053	0.288	0.092	0.153	0.066	0.420	0.109	0.182
P	0.002	0.010	0.006	0.008	0.001	0.011	0.005	0.009
Cl	0.0004	0.00011	0.00007	0.00012	0.00005	0.00020	0.00012	0.00016
	NaCl 2							
Na	0.020	0.065	0.042	0.108	0.018	0.041	0.036	0.106
K	0.004	0.054	0.022	0.118	0.007	0.069	0.034	0.091
Ca	0.018	0.057	0.031	0.015	0.019	0.058	0.033	0.011
Mg	0.003	0.011	0.007	0.058	0.004	0.012	0.008	0.043
N	0.052	0.369	0.119	0.256	0.067	0.415	0.134	0.197
P	0.001	0.013	0.009	0.014	0.002	0.016	0.010	0.010
Cl	0.00005	0.15400	0.00170	0.00020	0.00005	0.00021	0.00017	0.00019
	NaCl 3							
Na	0.012	0.025	0.046	0.077	0.010	0.022	0.028	0.068
K	0.009	0.075	0.033	0.089	0.009	0.068	0.033	0.077
Ca	0.021	0.068	0.038	0.011	0.015	0.059	0.032	0.011
Mg	0.003	0.014	0.008	0.045	0.004	0.012	0.007	0.037
N	0.070	0.448	0.158	0.202	0.062	0.396	0.137	0.179
P	0.002	0.014	0.010	0.008	0.002	0.015	0.008	0.008
Cl	0.00005	0.00018	0.00011	0.00020	0.00004	0.00023	0.00013	0.00015

Appendix A6.3 Average nutrient concentrations (mM g^{-1}) for final harvest of experiment two (OL = old leaves, NL = new leaves, ST = stems, RT = roots).

	CONTROL							
	KITUI				SIGOR			
ION	OL	NL	ST	RT	OL	NL	SH	RT
Na	0.018	0.004	0.014	0.036	0.001	0.004	0.018	0.029
K	0.385	0.513	0.424	1.134	0.345	0.484	0.412	1.275
Ca	0.797	0.612	0.465	0.511	0.880	0.592	0.372	0.356
Mg	0.149	0.125	0.106	0.578	0.173	0.151	0.082	0.562
N	2.007	2.703	1.927	2.106	1.851	2.882	1.535	2.347
P	0.065	0.078	0.106	0.087	0.055	0.091	0.072	0.080
Cl	0.0031	0.0029	0.0029	0.0031	0.0025	0.0036	0.0036	0.0036
	NaCl 1							
Na	1.927	1.377	0.808	1.801	1.766	0.909	0.464	1.819
K	0.181	0.341	0.267	0.806	0.148	0.375	0.233	0.817
Ca	0.643	0.487	0.488	0.104	0.564	0.382	0.336	0.049
Mg	0.096	0.077	0.096	0.364	0.082	0.062	0.072	0.297
N	1.934	2.489	2.239	1.517	2.123	2.837	1.704	1.401
P	0.105	0.116	0.172	0.108	0.075	0.099	0.108	0.087
Cl	0.0041	0.0033	0.0031	0.0035	4.9771	0.0031	0.0027	0.0029
	NaCl 2							
Na	2.332	1.333	0.779	1.851	1.790	0.975	0.471	1.971
K	0.272	0.411	0.244	0.887	0.113	0.399	0.244	0.838
Ca	0.865	0.564	0.440	0.110	0.702	0.453	0.356	0.049
Mg	0.130	0.084	0.087	0.393	0.102	0.069	0.077	0.343
N	2.298	2.293	2.284	1.419	2.004	2.784	1.900	1.499
P	0.138	0.110	0.181	0.106	0.077	0.108	0.130	0.106
Cl	0.0353	0.0031	0.0033	6.5346	0.0031	0.0033	0.0029	0.0028
	NaCl 3							
Na	1.220	1.301	0.656	1.942	1.599	1.040	0.533	1.884
K	0.160	0.402	0.261	0.697	0.134	0.405	0.253	0.726
Ca	0.619	0.515	0.448	0.103	0.502	0.430	0.331	0.051
Mg	0.092	0.081	0.082	0.364	0.095	0.067	0.067	0.300
N	1.708	2.338	2.266	1.481	1.992	2.686	1.722	1.365
P	0.075	0.100	0.165	0.107	0.077	0.114	0.124	0.103
Cl	0.0035	0.0031	0.0032	0.0032	0.0035	0.0032	0.0033	0.0029

Appendix A6.4 Average nutrient contents (mM/plant organ) for final harvest of experiment two (OL = old leaves, NL = new leaves, ST = stems, RT = roots).

	CONTROL							
	KITUI				SIGOR			
ION	OL	NL	SH	RT	OL	NL	ST	RT
Na	0.001	0.002	0.006	0.015	0.000	0.003	0.009	0.013
K	0.030	0.332	0.178	0.453	0.025	0.403	0.294	0.628
Ca	0.065	0.398	0.198	0.217	0.068	0.501	0.256	0.186
Mg	0.011	0.080	0.039	0.238	0.012	0.128	0.057	0.273
N	0.154	1.756	0.819	0.843	0.135	2.422	1.040	1.131
P	0.005	0.050	0.049	0.034	0.004	0.076	0.051	0.037
Cl	0.0002	0.0018	0.0012	0.0012	0.0002	0.0003	0.0025	0.0017
	NaCl 1							
Na	0.079	0.205	0.087	0.349	0.084	0.180	0.089	0.433
K	0.008	0.052	0.029	0.154	0.007	0.074	0.044	0.195
Ca	0.026	0.074	0.053	0.020	0.025	0.084	0.063	0.012
Mg	0.004	0.012	0.010	0.069	0.004	0.012	0.014	0.071
N	0.078	0.379	0.243	0.292	0.100	0.555	0.321	0.333
P	0.004	0.018	0.019	0.021	0.003	0.019	0.020	0.021
Cl	0.0002	0.0005	0.0003	0.0007	0.0002	0.0007	0.0005	0.0007
	NaCl 2							
Na	0.103	0.294	0.116	0.482	0.086	0.236	0.095	0.573
K	0.012	0.091	0.036	0.223	0.005	0.090	0.050	0.242
Ca	0.038	0.125	0.065	0.027	0.033	0.109	0.072	0.014
Mg	0.006	0.018	0.013	0.099	0.005	0.016	0.016	0.099
N	0.101	0.497	0.341	0.360	0.095	0.675	0.387	0.435
P	0.006	0.024	0.027	0.026	0.004	0.026	0.027	0.031
Cl	0.0016	0.0007	0.0005	1.1630	0.0000	0.0008	0.0006	0.0008
	NaCl 3							
Na	0.046	0.256	0.109	0.513	0.089	0.252	0.128	0.550
K	0.007	0.079	0.044	0.178	0.008	0.096	0.062	0.211
Ca	0.025	0.101	0.075	0.027	0.028	0.118	0.080	0.015
Mg	0.004	0.016	0.014	0.094	0.005	0.016	0.016	0.087
N	0.070	0.462	0.384	0.384	0.111	0.655	0.419	0.396
P	0.003	0.020	0.028	0.028	0.004	0.028	0.030	0.030
Cl	0.0001	0.0006	0.0005	0.0009	0.0002	0.0008	0.0008	0.0008

Table A6.5 F values and associated probabilities for tissue nutrient concentrations in intermediate harvest (probabilities • <= 0.05, •• <= 0.01, ••• <= 0.001).

SOURCE OF VARIATION	df		Na	K	Ca	Mg	N	P	Cl
Block	1	F	2.44	1.20	2.38	0.07	3.11	0.70	1.05
		P	ns	ns	ns	ns	ns	ns	ns
Salt (using type III MS)	3	F	461.77	10.65	2.96	7.09	11.80	8.08	1.00
		P	•••	•	ns	ns	•	ns	ns
Salt x Block.	3	F	0.71	3.02	1.20	2.75	0.95	0.85	1.06
		P	ns	*	ns	ns	ns	ns	ns
Provenance (using type III MS)	1	F	10.75	26.69	5.57	14.74	1.26	0.02	0.99
		P	•	••	•	•	ns	ns	ns
Provenance x Salt (type III MS)	3	F	2.89	2.90	0.83	3.24	1.80	1.02	1.00
		P	ns	ns	ns	ns	ns	ns	ns
Provenance x Block (salt)	4	F	0.60	1.21	0.19	0.26	1.89	8.78	1.06
		P	ns	ns	ns	ns	ns	***	ns
Tissue	3	F	73.01	781.47	75.52	793.12	632.01	65.89	1.00
		P	***	***	***	***	***	***	ns
Salt x Tissue	9	F	10.06	8.47	0.72	3.87	4.30	2.19	1.00
		P	***	***	ns	**	**	ns	ns
Provenance x Tissue	3	F	9.81	1.70	0.80	2.75	28.05	8.07	1.00
		P	***	ns	ns	ns	***	***	ns
Provenance x Salt x Tissue	9	F	1.37	1.51	0.28	0.76	4.50	1.33	1.00
		P	ns	ns	ns	ns	**	ns	ns

Table A6.6 F values and associated probabilities for tissue nutrient contents in intermediate harvest (probabilities • <= 0.05, •• <= 0.01, ••• <= 0.001).

SOURCE OF VARIATION	df		Na	K	Ca	Mg	N	P	Cl
Block	1	F	4.41	2.24	3.89	2.12	2.86	0.53	1.03
		P	•	ns	ns	ns	ns	ns	ns
Salt (using type III MS)	3	F	51.81	6.44	9.91	7.11	13.16	1.21	1.00
		P	••	ns	•	ns	•	ns	ns
Salt x Block.	3	F	1.56	1.20	0.50	2.07	0.34	1.01	1.03
		P	ns	ns	ns	ns	ns	ns	ns
Provenance (using type III MS)	1	F	2.57	2.52	0.28	0.19	1.00	0.42	0.99
		P	ns	ns	ns	ns	ns	ns	ns
Provenance x Salt (type III MS)	3	F	33.03	9.99	9.57	13.00	11.09	2.94	1.00
		P	••	•	•	•	•	ns	ns
Provenance x Block (salt)	4	F	0.17	0.93	0.31	0.66	0.61	1.91	1.03
		P	ns	ns	ns	ns	ns	ns	ns
Tissue	3	F	42.21	151.14	116.26	204.98	189.77	56.72	1.01
		P	•••	•••	•••	•••	•••	•••	ns
Salt x Tissue	9	F	7.50	5.98	2.81	10.08	2.08	0.98	1.00
		P	•••	•••	•	•••	ns	ns	ns
Provenance x Tissue	3	F	2.18	0.50	0.62	0.09	0.85	1.56	1.00
		P	ns	ns	ns	ns	ns	ns	ns
Provenance x Salt x Tissue	9	F	3.14	2.71	0.86	5.82	1.45	0.82	1.00
		P	•	•	ns	•••	ns	ns	ns

*Table A6.7 F values and associated probabilities for tissue nutrient concentrations in final harvest (probabilities * <= 0.05, ** <= 0.01, *** <= 0.001).*

SOURCE OF VARIATION	df		Na	K	Ca	Mg	N	P	Cl
Block	3	F	3.06	2.32	7.62	0.06	0.80	1.10	1.96
		P	*	ns	***	ns	ns	ns	ns
Salt (using type III MS)	3	F	207.95	102.66	12.30	85.16	3.97	28.47	0.68
		P	***	***	**	***	*	***	ns
Salt x Block.	9	F	1.49	1.17	2.03	0.98	2.59	1.75	0.68
		P	ns	ns	*	ns	*	ns	ns
Provenance (using type III MS)	1	F	14.79	1.18	30.35	12.66	67.00	48.82	0.04
		P	*	ns	***	**	ns	***	ns
Provenance x Salt (type III MS)	3	F	4.23	2.07	0.73	1.90	0.02	5.56	1.33
		P	*	ns	ns	ns	ns	*	ns
Provenance x Block (salt)	12	F	0.81	1.07	0.71	1.10	1.85	1.31	1.00
		P	ns	ns	ns	ns	ns	ns	ns
Tissue	3	F	132.66	895.43	143.46	912.15	203.40	71.80	0.68
		P	***	***	***	***	***	***	ns
Salt x Tissue	9	F	17.68	12.88	5.28	21.78	17.32	4.49	1.10
		P	***	***	***	***	***	***	ns
Provenance x Tissue	3	F	2.72	3.92	0.24	4.37	32.69	19.13	1.31
		P	ns	*	ns	**	***	***	ns
Provenance x Salt x Tissue	9	F	1.93	1.51	0.84	0.54	3.20	1.95	0.09
		P	ns	ns	ns	ns	**	ns	ns

Table A6.8 F values and associated probabilities for tissue nutrient contents in final harvest (probabilities • <= 0.05, •• <= 0.01, ••• <= 0.001).

SOURCE OF VARIATION	df		Na	K	Ca	Mg	N	P	Cl
Block	3	F	4.25	6.57	10.36	2.83	3.53	3.53	1.35
		P	••	•••	•••	•	•	•	ns
Salt (using type III MS)	3	F	45.69	59.47	31.22	65.17	69.74	27.56	1.00
		P	•••	•••	•••	•••	•••	•••	ns
Salt x Block.	9	F	3.34	3.46	6.95	0.78	2.01	3.07	0.88
		P	••	•	•••	ns	•	••	ns
Provenance (using type III MS)	1	F	0.82	2.70	0.14	0.73	3.49	3.84	0.64
		P	ns	ns	ns	ns	ns	ns	ns
Provenance x Salt (type III MS)	3	F	0.32	0.85	0.26	2.64	0.62	1.15	1.12
		P	ns	ns	ns	ns	ns	ns	ns
Provenance x Block (salt)	12	F	1.36	7.75	8.20	3.64	5.32	3.16	1.00
		P	ns	•••	•••	•••	•••	••	ns
Tissue	3	F	231.20	245.91	107.85	214.55	126.69	165.85	0.88
		P	•••	•••	•••	•••	•••	•••	ns
Salt x Tissue	9	F	26.60	23.48	27.47	22.98	27.60	18.61	1.04
		P	•••	•••	•••	•••	•••	•••	ns
Provenance x Tissue	3	F	2.90	3.91	2.13	0.39	4.36	4.20	1.12
		P	•	•	ns	ns	••	••	ns
Provenance x Salt x Tissue		F	0.72	1.17	1.27	0.57	0.85	1.61	0.96
		P	ns	ns	ns	ns	ns	ns	ns