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THE ALLEVIATION OF SALINITY INDUCED STRESS WITH THE APPLICATION
OF SILICON IN SOILLESS GROWN *Lactuca sativa* L. 'Eish!'

by

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CHAPTER ONE

INTRODUCTION

This thesis is divided into five chapters: Chapter 1: Introduction, Chapter 2: Literature Review, Chapter 3: The alleviation of salinity induced stress with the applications of silicon in soilless grow *Lactuca sativa* L. 'Eish!', Chapter 4: Salinity induced changes in oxidative stress and antioxidant status as affected by applications of silicon in lettuce (*Lactuca sativa* L. 'Eish!', Chapter 5: General Discussion and Conclusion, and Chapter 6: References. Chapter 3 and 4 are prepared in publishable format, and the layout is that of a journal article.

In addition to these foundation chapters, material that has been deemed relevant and complementary to this thesis, but incompatible with the articles, has been included at the end under the heading 'Appendices'.

Chapter 2 covers all aspects of salinity stress, silicon and antioxidants, the core subjects of this thesis and studies found herein. Salinity stress is introduced with the appreciation of the extent of salinity, the salts causing salinity, with the chemistry of this ancient problem being discussed in more detail in following paragraphs. The focal salt, NaCl, is then discussed, with the role of its cation Na^+ and anion Cl^- in relation to plant nutrition and toxicity, examined.

The basic chemistry of Si is examined next, with the role of this element, especially with regards to its beneficial, ameliorative properties. An emphasis is placed on silicon's more recent quasi-essential status in plant nutrition, as opposed to its previous non-essential status. This discussion focuses on the ability of Si to mitigate salinity stress, specifically NaCl induced salinity stress, and the mechanisms that have been proposed by various authors.

ROS and antioxidants are discussed, centred on the antioxidants that were investigated in chapter 4, namely CAT, GSH (and GSSG) and polyphenolics. The chemistry, role and basic function of these antioxidants are discussed in a manner appropriate for a broad understanding. This understanding is further utilised to describe the relationship between stress, particularly salinity stress, antioxidants and ROS. Malondialdehyde (MDA), a product of ROS damage is discussed towards the close of the review. Chapter 2 concludes with the converging of the core subjects of this thesis; salinity stress, Si and antioxidants.

DECLARATION

I, Christopher Jodi Milne, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

_____   _____
Signed   **Date**

ABSTRACT

This article based thesis includes two individual studies evaluating the role of silicon (Si) in mitigating the negative effects that are associated with sodium chloride (NaCl) induced toxicity in lettuce (*Lactuca sativa* L. 'Eish!').

The objectives of the studies were to evaluates the effects Si on NaCl stressed lettuce. This was done by assessing yield parameters, including fresh and dry root and shoot weights, chlorophyll content, Si, sodium (Na) and chlorine (Cl) concentrations in plant tissues, total polyphenolics (TP), oxidative radical absorbance capacity (ORAC), thiobarbituric acid reactive substance (TBARS), catalase (CAT), glutathione (GSH), and oxidised glutathione (GSSG). NaCl was applied at two different concentrations; 30 and 60 mM, and Si at four different concentrations; 0, 1, 2 and 4 mM.

Notable results include a significant increase in fresh root and shoot weight with additions of 2 mM Si compared to that of the 60 mM NaCl control, with notable, yet insignificant increases with Si concentrations of 1 and 4 mM. Dry root and shoot weight with additions of 1 mM Si, and in shoot weight with additions of 2 mM Si, significantly increased when compared to the 60 mM NaCl control. Increases in dry root and shoot weight of the other Si additions lead to clear, yet insignificant increases in dry weights. Few differences in Cl content were evident amongst treatments, besides a significant increase in root content and a significant decrease in shoot content with applications of 4 mM Si exposed to 60 mM NaCl. Na shoot content of 1, 2 and 4 mM Si at 60 mM NaCl showed a significant decrease compared to that of the control. Applications of 2 and 4 mM Si showed increased shoot Si when compared to both the 30 and 60 mM NaCl controls.

Other findings included increases in ORAC values, significantly with 4 mM Si at both 30 and 60 mM NaCl. Additionally, GSH concentrations significantly increased in 2 mM Si treated plants when compared to the 30 mM NaCl control, with increases in both GSH and GSSG at 1 and 4 mM Si treated plants when compared to the 60 mM NaCl control. CAT activity decreased with applications of Si, significantly with 1 and 4 mM NaCl when compared to the 30 mM control. TP concentrations stayed constant throughout treatments, with only marginal differences between the two controls.

It can be concluded that the applications of Si is of definite benefit to lettuce plant growth. These benefits are especially evident at higher NaCl concentrations. Although the regular inclusion of Si in nutrient solutions can be recommended at the NaCl concentrations that

CHAPTER FOUR: SALINITY INDUCED CHANGES IN OXIDATIVE STRESS AND ANTIOXIDANT STATUS AS AFFECTED BY APPLICATIONS OF SILICON IN LETTUCE (*Lactuca sativa* L. 'Eish!').

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APPENDIX B: Paper published in The International Journal of the Physical Sciences.

APPENDIX C: Paper accepted by the International Journal of Agriculture and Biology.

CHAPTER TWO

LITERATURE REVIEW

2.1   Salinity and its effects on plants

Salinity is defined as the quantity of NaCl, or as the quantity of total dissolved salts in a unit of water (Ghassemi et al., 1995). The dominant salts causing saline condition are chlorides, sulphates and bicarbonates of sodium, calcium and magnesium (Carter, 1981; Ghassemi et al., 1995). Of these salts, NaCl, as expressed by the definition, is the most common salt causing salinity (Harris, 1992:215; Epstein & Bloom, 2005:332-334).

Salts are found naturally in all soils (National Research Council, 1990), but the build-up of these salts in water and soil, known as salinisation (Ghassemi et al., 1995), is an immense problem - early stages of salinisation reduce soil productivity, and advanced stages destroy vegetation completely, thus ruining fertile land and diminishing biodiversity (Ghassemi et al., 1995).

Salinisation may be caused by long-term, natural processes, such as accumulation of weathered material, rain water containing dissolved solids or the appearance of the ocean over land (Ghassemi et al., 1995). These primary causes of salinisation are associated with conditions wherever evaporation exceeds precipitation (Epstein & Bloom, 2005:330-333). Salinisation, due to natural causes, is worldwide, occurring on all continents (Ghassemi et al., 1995).

Saline conditions caused by human activities greatly contribute to the salinisation problem (Epstein & Bloom, 2005:330-333). This human, secondary salinisation, is caused by the mobilisation of salts stored in the soil or groundwater by the introduction of water through activities, like irrigation or land clearing (Ghassemi et al., 1995). The addition of water through practices, such as irrigation, increases the water table, and upon reaching the surface evaporates leaving the salts behind. The irrigation water itself will certainly contain dissolved solutes, substantially contributing to salinisation (Epstein & Bloom, 2005:330-333; Ghassemi et al., 1995). Saline conditions are by no means a recent problem, with the decline of the Mesopotamian civilizations associated with the salinisation of previously fertile land (Lerner et al., 1993:39). Since then, many factors have increased salinisation, including an increase in population leading to increased food demand, increases in water usage and a subsequent over utilisation of groundwater (Ghassemi et al., 1995).

With the increase in soluble salts dissolved in water there will be an increase in the ability of the water to conduct electricity (Resh, 1995:109). This electrical conductivity (EC) is measured using an EC device, with EC expressed in milliSiemens per cm (mS cm^{-1}). With an increase in the EC, there is an increase in the osmotic potential of the soil solution, and because osmosis is the principal way by which water enters plants (Stern, 2006:151-152) it will have a direct effect on plant-water availability (Carter, 1981). Resh (1995:109) states that salt levels above 4 mS cm^{-1} leads to wilting, suppressed growth and fruit cracking, with desired levels for plant growth between 2 – 4 mS cm^{-1}. This correlates with Ghassemi et al. (1995) who confirms that when a saturated soil extract exceeds 4 mS cm^{-1}, it is considered saline. Relatively high EC levels, leading to osmotic stress, is the primary cause of decreased growth and productivity in plants growing in saline conditions (Carter, 1981; Ghassemi et al., 1995). Although preferred EC levels are known, as stated by Resh (1995:109), tolerable EC can differ from one plant to the next (Maas & Hoffman, 1977; Shannon & Grieve, 1999). Above this salinity threshold level, there is a noted decrease in plant growth and size with an increase in salinity. Tolerable levels range, for example in strawberries, with a low threshold tolerance of 1 mS cm^{-1}, to cotton with a high threshold tolerance of 7.7 mS cm^{-1} (Maas & Hoffman, 1977). Observable symptoms due to osmotic stress usually include stunting of growth, the most common symptom, and dark green, thicker and more succulent leaves (Maas & Hoffman, 1977). Affected plants could also show the same symptoms as drought stress, due to the decreased availability of water (Carter, 1981).

As discussed earlier, NaCl is the most common salt causing saline conditions, leading to an abundance of available Na$^+$ and Cl$^-$ ions. Cl is known to be an essential micronutrient in plants (Epstein & Bloom, 2005:210-211; Stern, 2006:161), and some argue that Cl should be classified as a macronutrient, contributing as much as 1% of the dry weight of some plants (Muckle, 1993). Na, on the other hand, is required by only a few plants (Epstein & Bloom, 2005:231-232). High concentrations of both of these ions are associated with leaf scorching (Shannon & Grieve, 1999), necrosis and defoliation (Maas & Hoffman, 1977). Additionally, excess NaCl has been associated with decreased photosynthesis and chlorophyll content (Moradi & Ismail, 2007; Savvas et al., 2009). Epstein & Bloom (2005:332-333), summarise the fundamental characteristics of salt-plant relations, and state that when Na is plentiful, above the micromolar range, it can displace and disrupt the functioning of K.

Plant species, cultivars and even individual plants of the same cultivar, differ in their tolerance to saline conditions (Rush & Epstein, 1978; Flowers & Yeo, 1981; Barroso & Alvarez, 1997). Lettuce, the crop used in this plant study, has an EC threshold tolerance of 1.3 mS cm^{-1}, and is subsequently classified as moderately salt sensitive (Mass & Hoffman,

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1977). Salinity, as in other plants, has shown to decrease growth, photosynthesis and chlorophyll in lettuce (Han & Lee, 2005; Yan-fang & Jun-yu, 2008).

2.2 Silicon and salinity stress mitigation

Si, the second most abundant element on earth, second only to oxygen (Marshak, 2005:43), is ever-present in plants (Epstein & Bloom, 2005:227-230), in quantities equal or more than many macro nutrients (Epstein, 1999). Si is found as silicic acid (H_4SiO_4) in the soil solution (Epstein, 1999), and when absorbed, it is transported to shoots and polymerises into solid amorphous silica ($SiO_2 \cdot nH_2O$), called opal, found mainly in cell walls (Epstein & Bloom, 2005).

The benefits of Si range from enhancement of plant growth and mechanical strength to improvement of mineral nutrition and resistance to abiotic and biotic stresses (Epstein, 1994). Despite this, Si does not qualify as essential under the widely accepted (yet disputed) definition of plant nutrient essentiality by Arnon & Stout (1939). Epstein (1999), rejects this definition and argues that Si qualifies as quasi-essential, because many plants grown with a deficiency in Si show irregularities in their growth, development or reproduction when compared to plants grown with the element in abundance.

There is a profusion of literature evaluating the benefits of Si. Included in this, is its well documented ability to mitigate stresses associated with salinity in plants, specifically, stresses associated with high levels of NaCl. This is evident in a wide range of plant species, including, but not limited to, wheat (Ahmad, 1992) barley (Liang, 1999), tomato (Zhu, 2004a) cucumber (Zhu, 2004b), zucchini (Savvas, 2009) grapevine (Soylemezoglu, 2009) and sugarcane (Ashraf, 2010).

Mechanisms of mitigation are at variance in the literature, with the majority of studies concluding that Si reduces the uptake, accumulation or concentration of Na in plant tissues (Ahmad *et al.*, 1992, Savvas et al., 2009, Soylemezoglu et al., 2009 and Hashemi, et al. 2010). Romero-Aranda et al. (2006), conclude that the mitigating properties are due to a Si induced increase in water storage capacity, thus diluting Na in plant tissues. Ma et al. (2001:31), conclude that silicon's beneficial affects stem from the decreased Na influx through a Si induced decrease in transpiration.

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2.3 Antioxidants and salinity stress

ROS are highly reactive chemical species that, although have recently been shown to have beneficial signalling properties, can damage, impair the function of cells or even cause cell death. They are ubiquitous species in plants and are ever present in cells in aerobic conditions, where O_2 is utilised for energy production (Mittler, 2002; Koolman & Rohm, 2005) as all plant do (Halliwell & Gutteridge, 1999). Examples of ROS include superoxide (O_2^-), Hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-) (Murray et al., 2003). Due to the high levels of molecular oxygen in the early atmosphere, leading to the inevitable formation of ROS, living organisms have had to evolve mechanisms to counteract the deleterious effects of ROS billions of years ago, in the form of antioxidants (Ndhala et al., 2010). In addition to standard levels of ROS, abiotic stresses, including salinity stress, together with salinity induced osmotic stress, is known to increase the concentrations of ROS (Dat et al., 2000; Xiong & Zhu, 2002).

Antioxidants can simply be defined as any substance that hinders or prevents oxidation of an oxidizable substrate, such as a protein or lipid (Halliwell & Gutteridge, 1999). There are various different antioxidants found in plants, falling into one of two categories; i.e. enzymatic or non-enzymatic (Ndhala et al., 2010). CAT, an indispensible enzymatic antioxidant, and a major antioxidant in plants (Mittler, 2002) is responsible for the conversion of the free radical hydrogen peroxide which is found in high concentrations in chloroplasts and leaf peroxisomes. CAT which is also found predominantly in peroxisomes, understandable due to the high concentrations of H_2O_2, converts H_2O_2 to water and oxygen, expressed by the following equation (Mittler, 2002; Feierabend, 2005:101):

$$2H_2O_2 \rightarrow 2H_2O + O_2$$
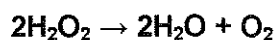
In terms of salinity stress, CAT has shown to decrease in tomato (Zhu et al., 2004a), cucumber (Zhu et al., 2005b), *Plantago media* (Sekmen et al., 2007) both increase and decrease in two different studies on lettuce (Eraslan et al., 2007; Leyva et al., 2011), and increase in *Beta maritime* (Bor et al., 2003) and *Jatropha curcas* (Gao et al., 2008) when plants were exposed to various levels of NaCl. These studies suggest that the effects of NaCl on CAT production varies from one species to the next, however, Mittler (2002) states that abiotic stresses generally lead to the production of CAT.

Phenolics are organic compounds found in all higher plants, comprised of a hydroxyl group bonded to an aromatic hydrocarbon. Gallic acid is a common example, and is used as a representation for total polyphenolic quantifications in some phenolics assays (Figure 2.1).

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Polyphenolics on the other hand, are defined by large amounts of these phenol units, and have only comparatively recently been recognized as antioxidants, with their antioxidant properties due to their electron donating activities (Grace, 2005:151-153). They have the ability to scavenge ROS directly due to being less reactive than oxygen, and therefore do not promote further redox reactions.

TP in plants subjected to NaCl salinity stress show a clear tendency to increase over time. Parida et al. (2002) and Parida et al. (2004), studying the effects of NaCl on *Bruguiera parviflora* and *Aegiceras corniculatum*, species of mangrove, reported significant increases in polyphenol concentrations over time. Mangroves are plants that inhabit saline conditions, and consequently have various mechanisms for salt tolerance. An increase in TP concentration with NaCl stress over time in mangroves is therefore a good indication that polyphenolics play a vital role in salinity stress mitigation. Increases in TP with salinity stress is not only reported in the salt tolerant mangroves, but also reported in important agricultural crops, such as maize. Hichem et al. (2009), report vast increases in TP concentrations with increasing NaCl salinity, and conclude that TP play an important physiological role in maize salinity tolerance.

GSH, a molecule with various functions, and like CAT, is a widely known antioxidant (Tausz et al., 2004). GSH reacts with many different ROS, and it plays an essential role in the ascorbate-glutathione cycle (Foyer et al. 2005:1-2). The ascorbate-glutathione cycle results in the naturalisation of H_2O_2, whereby APX reduces H_2O_2 to water, using ascorbate (vitamin C). GSH plays a later role in this pathway by being oxidised to GSSG, allowing the completion of the cycle with the reduction of dehydroascorbate to ascorbate (Smirnoff, 2005:65).

As stated previously, salinity stress results in increased concentrations of ROS, with an increase in GSH known to infer protection against ROS (Tausz et al., 2004). It is therefore no surprise that salinity stress is widely reported to result in an increase in GSH concentration (Saqib et al., 2008; Leyva et al. 2011), an appropriate response to the salinity induced increase in ROS. Furthermore, Tausz et al. (2004) stated that in addition to the salinity induced increase in GSH concentration, there is a prior increase in the GSH:GSSG ratio, which could act as signals allowing for appropriate cellular reactions (Foyer et al., 2005:1).

2.4 Antioxidant interactions with silicon and salinity stress

As previously discussed, Si has the ability to mitigate the negative effects associated with NaCl induced salinity stress. In addition to the NaCl related amelioration of salinity stress, which is commonly attributed to silicon's influence on Na, studies have indicated that Si can influence antioxidant activity. The effects of Si on antioxidants have been the focus of only a handful of studies. Important crop species that have been studied include; barley (Liang, 1999; Liang et al., 2003), tomato (Zhu, 2004a), cucumber (Zhu, 2004b), maize (Moussa, 2006) spinach (Eraslan et al., 2008) wheat (Saqib, 2008) and alfalfa (Wang et al., 2011).

Investigations in almost all of the studies considering the effects of Si on CAT concentrations in salinity stressed plants, lead to an increase in CAT (Liang et al. 2003; Moussa 2006; Eraslan et al, 2008; Wang et al. 2011), with Zhu et al., (2004a), concluding that Si counteracted the deleterious effects of NaCl by means of increases in CAT. Zhu et al., (2004b), however, found a general decrease in CAT concentrations when investigating cucumber. Gong et al. (2005), investigating applications of Si to ameliorate drought stress, a primary limitation for plants growing in saline conditions in the form of an increase in the soil solution's osmotic potential (Marschner, 1995:662-663), found that applications of Si lead to significantly decreased levels of CAT.

Liang et al., (2003), and Saqib et al., (2008), are two of the studies that examined the influence of GSH on salinity toxicity on barley and wheat respectively, as affected by Si applications. Both found that GSH concentrations increased over time compared to NaCl treatments alone. A higher concentration of GSH is advantageous and an appropriate response with regards to stress as additional GSH would result in superior antioxidative defence (Tausz et al., 2004). It follows that a better defence against ROS would result in a decrease in salinity induced production of ROS, and therefore the alleviation of salinity stress (Foyer et al., 2005:1-2,16-18).

Lipid peroxidation is a chain reaction that can lead to continuous tissue injury. This process provides a continuous supply of free radicals, resulting in recurring radical damage (Figure 2.3). To control lipid peroxidation, plants use antioxidants and therefore prevent the otherwise continual tissue damage from this reaction. MDA, a compound resulting only from the degradation of lipids with three or more double bonds, is used as a marker of lipid peroxidation (Murray et al., 2003).

MDA concentrations in NaCl stressed plants show significant increases compared to those without NaCl stress (Liang 1999; Zhu et al., 2004a; Moussa 2006). As could be

hypothesised, applications of Si on salt stressed plants results in a decrease in MDA content, reflecting a decrease in lipid peroxidation and therefore an alleviation of salinity toxicity (Liang 1999; Liang et al., 2003; Zhu et al., 2004; Moussa 2006).

Gathering and considering the information discussed in this literature review, the subject of this thesis was lettuce, one of the most important vegetable crops (Hemy, 1984:104) yet a plant species that to the author's knowledge has not been studied in relation to Si and NaCl salinity stress. The chapters that follow are the results of these investigations.

Figure 2.1: Gallic Acid

GSSG reductase

GSSG

GSH

Dehydroascorbate reductase

ASCORBATE

H_2O_2

Ascorbate peroxidase

H_2O

Dehydroascorbate   Monodehydroascorbate
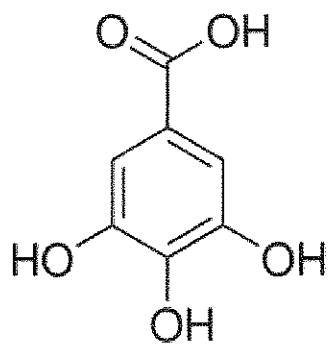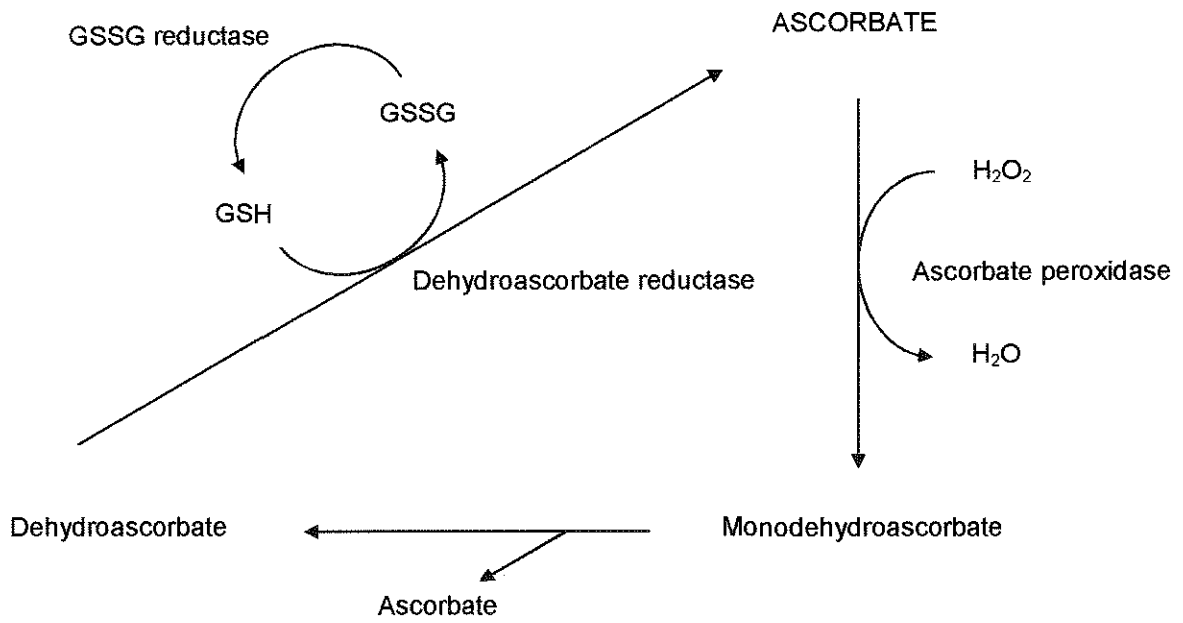
Ascorbate

Figure 2.2: The ascorbate-glutathione cycle, demonstrating the role of GSH in this pathway. and 4mM Si (Adapted and simplified from Smirnoff, 2005).
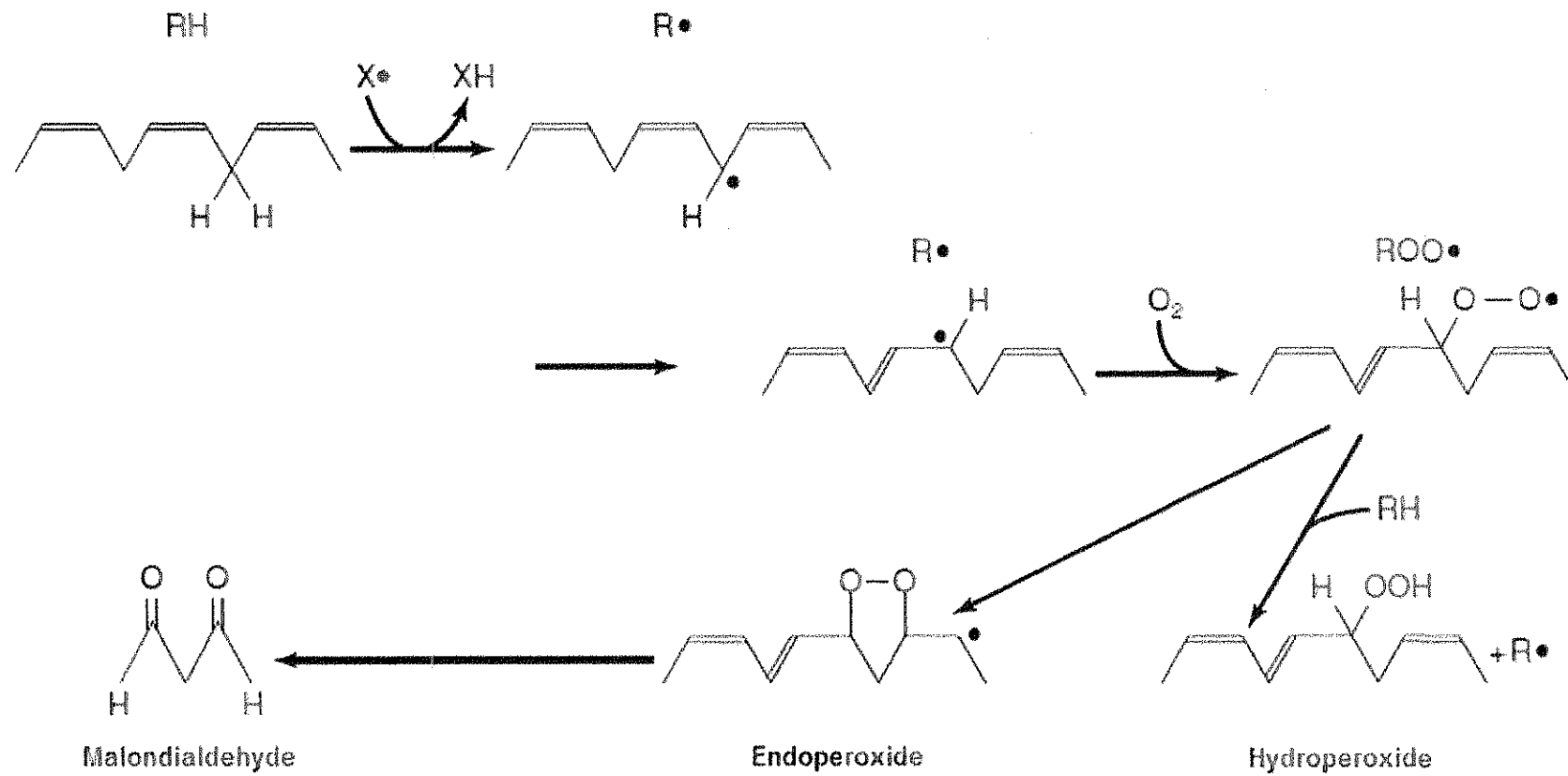
Figure 2.3: Lipid peroxidation. In this example, the reaction is initiated by a free radical (X). The process results in Malondialdehyde (MDA) (Adapted from Murray *et al.*, 2003).

CHAPTER THREE

THE ALLEVIATION OF SALINITY INDUCED STRESS WITH THE APPLICATION OF SILICON IN SOILLESS GROWN *Lactuca sativa* L. 'Eish!'.

3.1   ABSTRACT

The effects of 30 and 60 mM NaCl on Lettuce (*Lactuca sativa* L. 'Eish!'), grown in soilless culture, with additions of 0, 1, 2 and 4 mM Si was evaluated. Height, leaf number, weight, chlorophyll content and elemental analysis of plants were examined. Fresh root and shoot weight significantly increased with additions of 2 mM Si compared to that of the 60 mM NaCl control, with notable increases with Si treatments of 1 and 4 mM. Dry root and shoot weight with additions of 1 mM Si, and in shoot weight with additions of 2 mM Si, significantly increased when compared to the 60 mM NaCl control. Increases in dry root and shoot weight of the other Si additions lead to clear, yet insignificant increases in dry weights. Few differences in Cl content were evident amongst treatments, besides a significant increase in root content and a significant decrease in shoot content with applications of 4 mM Si exposed to 60 mM NaCl. Na shoot content of 1, 2 and 4 mM Si at 60 mM NaCl showed a significant decrease compared to that of the control. Applications of 2 and 4 mM Si showed increased shoot Si when compared to both the 30 and 60 mM NaCl controls. With clear evidence for a reduction in Na content in shoots with applications of Si at higher NaCl concentrations, and further increases in plant weights, it can be stated that applications of Si are of a distinct benefit for the growth of lettuce.

Key Words: Lettuce, Si, NaCl, Salt, Sodium chloride, Hydroponics

3.2   INTRODUCTION

Silicon [Si], an element whose abundance in the earth's crust is second only to oxygen (Marshak, 2005:43), can contribute as much as 0.1 - 10% of the dry matter of plants. These levels are equal to or exceeding those of essential macro nutrients (Epstein & Bloom, 2005:52). Si has in the past been classified as a non-essential element for plant nutrition, that is, one which does not meet the criteria for essentiality in the classic definition of essentiality proposed by Arnon & Stout (1939). More recently Si has been

classified as either a beneficial element (Marschner, 1995:417), or as a quasi-essential element in plants (Epstein, 1999).

Contributing to its classification as a beneficial or quasi-essential element in plants is its role as a plant stress ameliorator (Epstein, 1994; Belanger et al., 1995; Epstein, 1999). An important aspect of this is the ability of Si to ameliorate salt toxicity, defined as conditions of high salt build-up, with sodium chloride [NaCl] being the most common cause of saline conditions (Harris, 1992; Epstein & Bloom, 2005:332-334).

Salinity has two methods of causing abnormalities in plant growth. The first, which is independent of the type of salt, is by creating a higher osmotic potential in the medium surrounding the roots, and because osmosis is the principle method of water absorption in plants (Stern, 2006:151-152), it results in a decrease in plant-water availability (Carter, 1981). The second, which is salt dependent, is caused by an excess of available ions. In the case of NaCl salinity, the ions in question are Sodium [Na^+] and Chloride [Cl^-]. Cl is known to be an essential micronutrient in plants (Epstein & Bloom, 2005:57; Stern, 2006:161), and some argued that Cl should be classified as a macronutrient, contributing as much as 1% of the dry weight of some plants (Muckle, 1993). Na, on the other hand, is required by only a few plants (Epstein & Bloom, 2005:231-232), and has shown to be the primary cause of growth suppression in lettuce (Tas et al., 2005). In general, high concentrations of both Na and Cl are associated with leaf scorching (Shannon & Grieve, 1999), necrosis and defoliation (Maas & Hoffman, 1977).

The mitigation of NaCl induced toxicity with the application of Si has been demonstrated in many important agricultural and ornamental crops, including Alfalfa (Wang et al., 2011), Tomato (Romero-Aranda et al., 2006), Wheat (Ahmad et al., 1992), Zucchini (Savvas et al., 2009), Grapevine (Soylemezoglu et al., 2009), Mesquite (Bradbury & Ahmad, 1990), Roses (Savvas et al., 2007; Reezi et al., 2009) and several others. The present study investigated the ability of Si to alleviate NaCl toxicity in Lettuce (*Lactuca sativa* L. 'Eish!').

3.3   MATERIALS AND METHODS

The experiment took place within the experimental greenhouse located at the Cape Peninsula University of Technology in Cape Town, South Africa - 33° 55' 58.27"S, 18° 25' 57.04 E. The temperature within the greenhouse ranged from 9°C to 29°C, the mean being 17.6°C and the humidity ranged from 44% to 99%, the mean being 77%.

Lactuca sativa L. 'Eish!' seeds (Hygrotech, South Africa) were sown in vermiculite, and germinated under 40% shade-cloth. Once all seeds had germinated (8 days after sowing) they were fertilised daily with half strength CHEMICULT (Starke Ayres, South Africa) nutrient solution, and increased gradually to full strength (Harris, 1992:55-62). Once the seedlings were fully established (40 days after sowing) 80 plants were randomly selected and transplanted into individual 12.5 cm pots, containing expanded clay as the medium, and placed within the soilless growing system. The sub-irrigation, closed, soilless growing system consisted of a nutrient solution holding tray, delivering a constant ± 1 cm of circulating nutrient solution to each treatment. Using a randomised block design, 10 plants were placed within eight individual soilless culture units, allowing for 10 repeats of each.

3.3.1 Basal Nutrient Solution

Analytical grade, Potassium nitrate [KNO_3], Calcium nitrate [$(Ca(NO_3)_2 \cdot 4H2O$], Ammonium dihydrogen phosphate [$NH_4H_2PO_4$], and Magnesium sulphate [$MgSO_4 \cdot 7H2O$], were used to add 16 mM N, 6 mM K, 4 mM Ca, 2 mM P, 1 mM S and 1 mM Mg to the nutrient solution, which was based on the modified Hoagland solution presented by Epstein & Bloom (2005:31). Micro nutrients were supplied by using HYGROPLEX (Hygrotech, South Africa), adding 58 µM Boron [B], 11.4 µM Manganese [Mn], 7.6 µM Zinc [Zn], 1 µM Copper [Cu], 0.65 µM Molybdenum [Mo] and 31.9 µM Iron [Fe] to the nutrient solution. The complete basal nutrient solution was prepared using deionised water and had an electrical conductivity (EC) of 1.9 mS cm^{-1}.

3.3.2 Silicon and NaCl Treatments

Four levels of Si, 0 mM, 1 mM, 2 mM and 4 mM, were supplied by adding AGRISIL K50 (PQ Silicas, South Africa) - Potassium silicate [K_2SiO_3], each of which were combined with two levels of NaCl, 30 mM and 60 mM, making a total of 8 treatments. Treatments, which were applied in a single dose, commenced one week after transplantation. Newly prepared nutrient solutions containing 30 mM NaCl had a mean EC of 5.20 mS cm^{-1} and solutions containing 60 mM NaCl had a mean EC of 8.10 mS cm^{-1}. These EC levels were maintained daily. Potassium [K] was balanced in all treatments by subtracting additional K from KNO_3, and supplementing lost Nitrate [NO_3] by adding Nitric Acid [HNO_3]. The nutrient solution, which was replaced weekly, was maintained daily at a pH of 6.0 using Hydrochloric acid [HCl] and Sodium hydroxide [NaOH].

3.3.3 Plant Growth Parameters

Plant growth, in terms of plant height and leaf number, of all treatments, were measured at weekly intervals. Plant height was measured with a measuring tape, from the surface of the medium to the tip of the tallest leaf.

Five plants per treatment were harvested after 11 weeks from sowing (Hadfield, 2001:110), and separated into roots and shoots. Roots were rinsed once with tap water and twice with deionised water before being patted dry with paper towel. After the fresh weight had been recorded, plants were immediately placed within a specimen oven at 60°C until constant weight, and the dry weight recorded.

3.3.4 Determination of chlorophyll content in plant leaves

Extraction of chlorophyll by Dimethyl sulphoxide [DMSO] was done following a modified method described by Hiscox and Israelstam (1979). This method allows for the extraction of chlorophyll without plant tissue maceration. Five lettuce plants per treatment were lyophilised and ground to a fine powder using a mortar and pestle and stored at -80°C until analysed. 100 mg of the powder was placed in a 15 mL vial containing 7 mL DMSO and incubated at 65°C for 20 minutes. After the incubation period, an additional 3 mL of DMSO was added to the vial, and stored at 4°C. After a 24 hour storage period, the extract was centrifuged at 3220 g for 10 minutes, after which the supernatant was transferred to a new 15 mL vial. Before spectrophotometrical analysis, the supernatant was diluted to a factor of 15 of which 1 mL was placed in a cuvette and absorbance values of 645 and 663 nm recorded. Absorbance values were used in the equation proposed by Arnon (1949), to determine total leaf chlorophyll content against DMSO blank, expressed as mg g^{-1} dry weight.

3.3.5 Elemental analysis

Dried plant material was used to analyse for Na, Cl and Si. Na and Si was analysed by ashing the ground sample and dissolving the ash in HCl. Elemental concentrations were determined using an inductively-coupled plasma (ICP) emission spectrophotometer. Cl was analysed by digesting the dried, ground sample with HNO_3. An excessive quantity of Silver nitrate [AgNO3] was added, followed by back titration with Potassium thiocyanate

[KSCN]. Elemental concentrations of Na, Cl and Si are expressed as mg g^{-1} of dried plant material (A. Van Deventer, Bemlab, South Africa, Personal Communication).

3.3.6   Statistical Analysis

Statistical analysis was performed by using two–way independent analysis of variance (ANOVA) followed by the Bonferroni post test, with Si and NaCl concentrations being the factors assessed. Computations were executed with the software program SPSS (Urdan, 2005:120-170).

3.4   RESULTS

Leaf number showed no significant differences between Si treatments, whether compared to the control, containing 0 mM Si, or to each other (data not shown). Plant height showed similar insignificance between treatments and controls, besides the expected general increase in height over the growing period (Figure 3.1). There was a significant interaction between salt concentration and silicon treatments on fresh root weight (P ≤0.05) and fresh shoot weight (P ≤0.1). Main effect analysis showed a significant decrease in fresh root weight and fresh shoot weight (P ≤0.1 and P ≤0.05 respectively) between the two controls (Figure 3.2 and 3.3). Fresh root weight and fresh shoot weight showed no significant differences at Si levels of 1 mM and 4 mM whether grown with 30 mM or 60 mM NaCl, when compared to their respective control. The treatment of 2 mM Si exposed to 60 mM did however show a significant increase in fresh root (P ≤0.05) and shoot weight (P ≤0.1) when compared to the control. Although only 2 mM Si showed a significant effect on fresh root and shoot weight, there was a marked, yet insignificant, increase in fresh root and shoot weight of 4 mM Si at 30 mM NaCl, and 1 mM and 4 mM Si at 60 mM when compared to the control (Figure 3.2 and 3.3).

There was also a significant interaction between salt concentration and silicon treatments on dry root weight (P ≤0.1) and dry shoot weight (P ≤0.05). Main effect analysis showed that dry root weight significantly decreased (P ≤0.1) between the control of 30 mM NaCl and that of 60 mM NaCl. Besides a significant increase (P ≤0.1) in dry root weight between 1mM Si exposed to 60 mM NaCl and that of the control, the other treatments showed no significant differences, even though there was again a marked increase in weight, especially when compared to the 60 mM NaCl control (Figure 3.4). Dry shoot

weight showed no significant increase between controls. However, there were significant increases (P ≤0.05) in weight of 1 mM and 2 mM Si when compared to the 60 mM NaCl control. Again, although other treatments resulted in insignificant differences, there was a marked increase in root and shoot dry weight when compared to that of the control (Figure. 3.5).

Although there was a significant interaction between salt concentration and silicon treatments on root (P ≤0.001) and shoot (P ≤0.05) Cl content, main effect analysis showed that Cl concentrations only increased significantly in roots of the 4 mM Si with 60 mM NaCl treated plants and the shoots of 2 mM Si plants. This in turn corresponded to a significant decrease (P ≤0.1) in shoot Cl when compared to the control at 60 mM NaCl (Table 3.1) When comparing Cl content of plants treated with the same level of Si, 2 mM Si at 60 mM NaCl showed a significant decrease in shoot Cl content (P ≤0.05) compared to the plants treated with 30 mM NaCl. Contrarily, plants treated with 4 mM at 60 mM NaCl showed a significant increase in root Cl content (P ≤0.01) when compared to the plants treated with 30 mM NaCl (Table 3.2).

There was a significant interaction between salt concentration and silicon treatments on shoot Na content (P ≤0.001) but not on root Na content. Although no significant differences in root Na content were obtained, there was a marked decrease in Na root concentration at 60 mM NaCl (Figure 3.6). Main effect analysis showed that Na shoot content at 30 mM NaCl mirrored the results of that of the roots, and showed no significant differences between treatments. However, Na shoot content at 60 mM NaCl with Si levels of 1, 2 and 4 mM all resulted in a significant decrease in shoot Na content compared to that of the control (Table 3.1). When comparing Na content of plants treated with the same level of Si, root Na significantly increase between the controls of 30 and 60 mM NaCl (P ≤0.001) and shoot Na significantly increase (P ≤0.001) with 60 mM NaCl when compared to 30 mM NaCl at all Si levels (Table 3.2).

There was a significant interaction between salt concentration and silicon treatments on Si shoot content (P ≤0.001), but not on Si root content. Main effect analysis showed that there was however a significant decrease (P ≤0.05) of root Si between treatments at 30 and 60 mM NaCl receiving 1mM Si. Si shoot concentrations of 2 and 4 mM, at both 30 and 60 mM NaCl showed a significant increase when compared to the control. When the two NaCl levels at the same 4 mM Si treatment were compared, a significant decrease (P ≤0.01) in Si was observed in the shoots. This was apparent in the 4 mM treatment alone (Table 3.2).

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There was a significant interaction between salt concentration and silicon treatments on chlorophyll content (P ≤0.05). Main effect analysis revealed that, when comparing Si treated plants to the controls, there was only a significant decrease in chlorophyll with applications of 2 mM Si at 30 mM to that of the control (Figure 3.7).

3.5   DISCUSSION

The results obtained from this study support the fundamental findings of previous studies, that Si has beneficial mitigating properties with regards to NaCl induced salinity stress. These findings indicate some benefits of Si applications at 30 mM NaCl, however, the benefits of Si seem to be more apparent with the higher experimental concentrations of 60 mM NaCl. This is evident when looking at the significant increase in fresh root and shoot weight at 2 mM Si at 60 mM NaCl, the significant increases in dry root, at 1 mM Si, dry shoot weight at 1 and 2 mM, and the overall marked increases in fresh and dry weights when comparing all Si treatments to that of the 60 mM NaCl control (Figure 3.2, 3.3, 3.4 and 3.5). These findings are supported by the literature which states that dry shoot and root weights of plants increase with applications of Si compared to a range of NaCl concentrations lacking Si (Romero-Aranda et al., 2005; Tuna et al., 2008; Ashraf et al., 2010).

Chlorophyll content showed no significant differences, with only one exception with an application 2 mM Si at 30 mM NaCl (Figure 3.7). This is in line with Tas et al. (2005), who although did not investigate Si, reported negligible effects of NaCl on chlorophyll content on lettuce. However, in a study conducted by Hashemi et al. (2010), evaluating the mitigating effects of Si on Canola, a vast increase in chlorophyll content was found.

A notable oddity with regards to elemental analysis was the exceptional accumulation of Cl in the roots of the plants treated with 4 mM Si at 60 mM (Table 3.1 and 3.2). This, when compared to the control, corresponded to a significant decrease in Cl accumulation in shoots. Savvas et al. (2007), and Savvas et al. (2009), found similar results, reporting a significant decrease in shoot Cl content with Si applications. However, Romero-Aranda et al. (2005), and Soylemezoglu et al. (2009), reported a slight increase in Cl shoots concentration with applications of Si.

A significant finding with regards Na content was the reduction of Na within shoots when Si treated plants exposed to 60 mM NaCl when compared to the control (Table 3.1). The

decrease of Na content in Si treated plants has been a major finding when evaluating the ability of Si to mitigate NaCl salinity stress. Many studies that evaluate Si and NaCl relate NaCl mitigation to a reduction in Na shoot concentrations (Saquib et al., 2008; Tuna et al., *2008;* Savvas et al., 2009; Ashraf *et al.* 2010), the reduction of which may be linked to Si reducing transpiration, as concluded by Ma et al. (2001). Savvas (2007), also reported a decrease in Na with applications of Si, but did not conclude this to be factor influencing the ability of Si to mitigate salinity stress.

Other mechanisms of salinity mitigation by Si have been suggested, including the ability of Si to improve water storage within plant tissues. Due to the higher growth rate that this would allow, it follows that salt concentrations would decrease through dilution, and therefore ameliorate salt toxicity (Romero-Aranda et al., 2005). Anti oxidants have also been a topic of investigation, with Liang (1999), Zhu et al. (2004a) and Zhu et al. (2004b), investigating the interaction of Si with antioxidants in salt stressed barley, cucumber and tomato respectively, with promising results.

Although transpiration and anti oxidants were not factors investigated in this study, plant weight increases, and subsequent decreases in Na shoot concentrations, leads to the conclusion that mitigation of NaCl induced salinity with applications of Si was at least partially due to the reduction of shoot Na content. Additions of Si to plants under salinity stress can be recommended, especially at higher salinity levels, as shown by the results of this paper.

3.6   ACKNOWLEDGMENTS

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3.7.   REFERENCES

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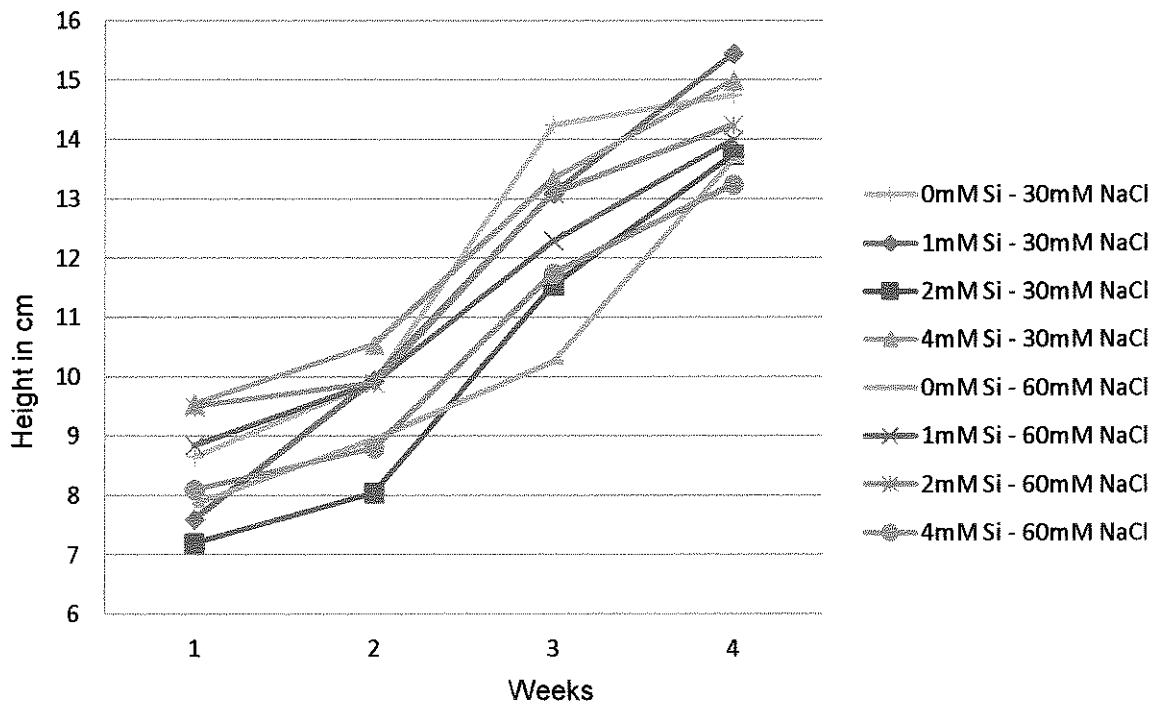
Figure 3.1: The effect of 30 and 60 mM NaCl on plant height, with additions of 0,1, 2 and 4 mM Si. Measurements were take weekly from 27 May (week 1) to 17 June (week 4) 2011.
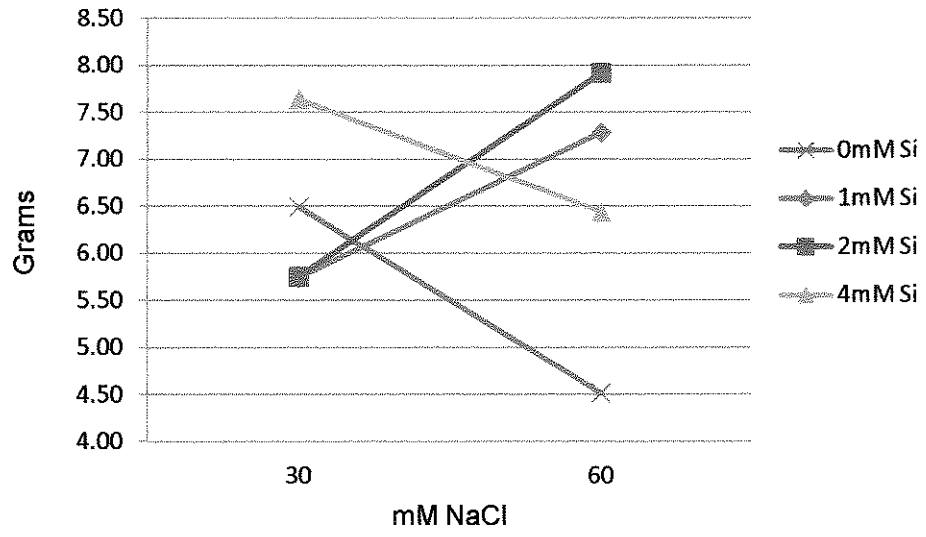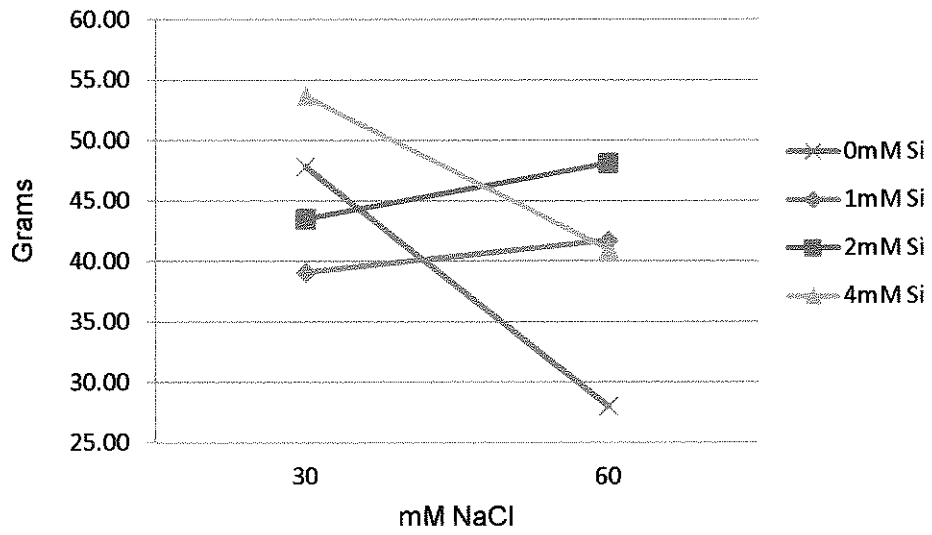
Figure 3.3. The effect of 30 and 60 mM NaCl on fresh shoot weight, with additions of 0, 1, 2 and 4 mM Si.
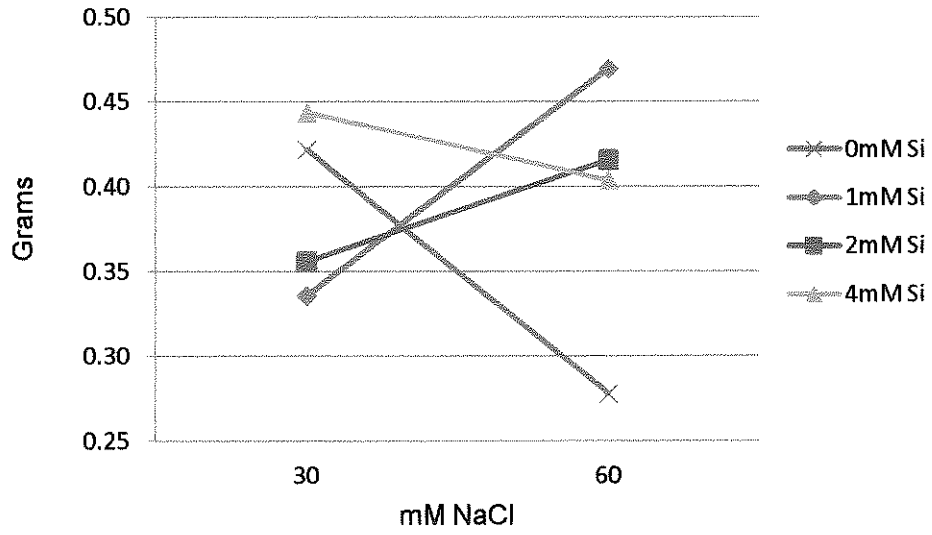
Figure 3.4. The effect of 30 and 60 mM NaCl on dry root weight, with additions of 0, 1, 2 and 4 mM Si.
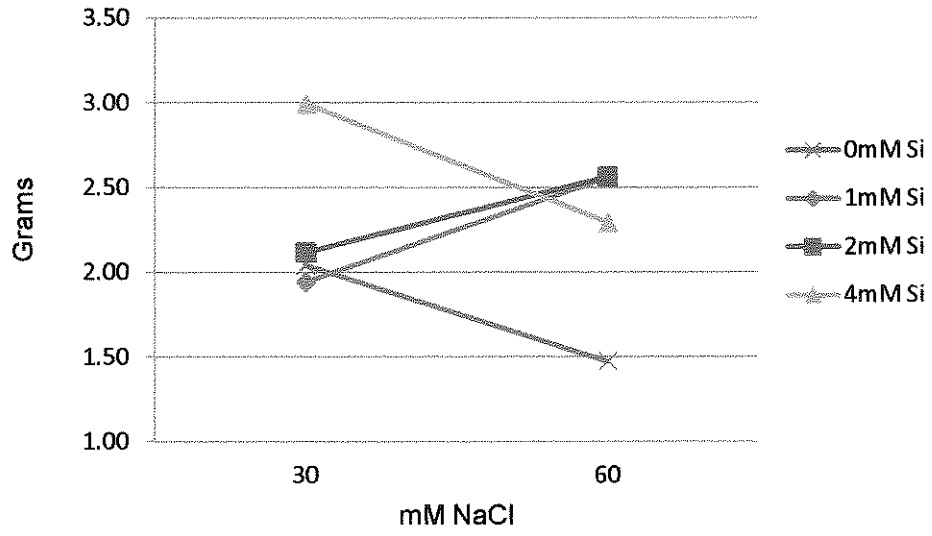
Figure 3.5. The effect of 30 and 60 mM NaCl on dry shoot weight, with additions of 0, 1, 2 and 4 mM Si
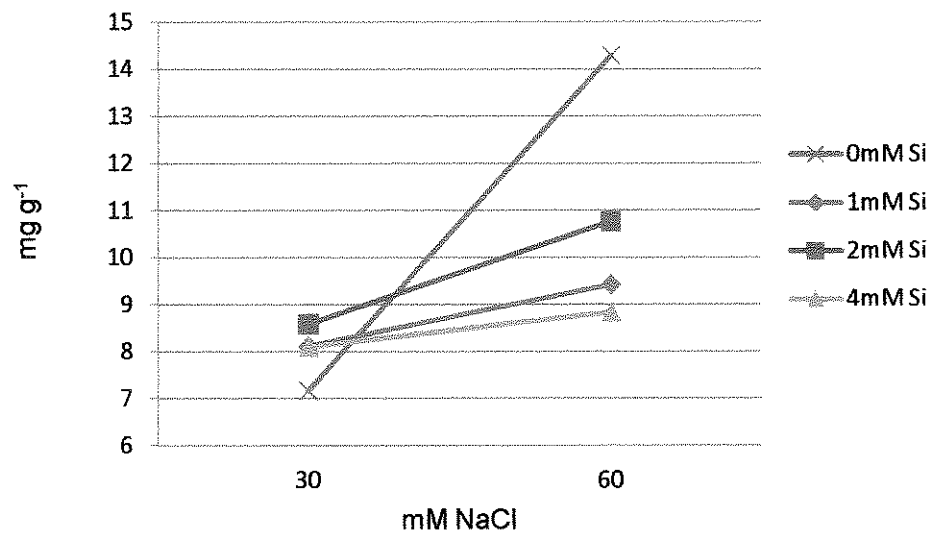
Figure 3.6. The effect of 30 and 60 mM NaCl on Na root content, with additions of 0, 1, 2 and 4 mM Si
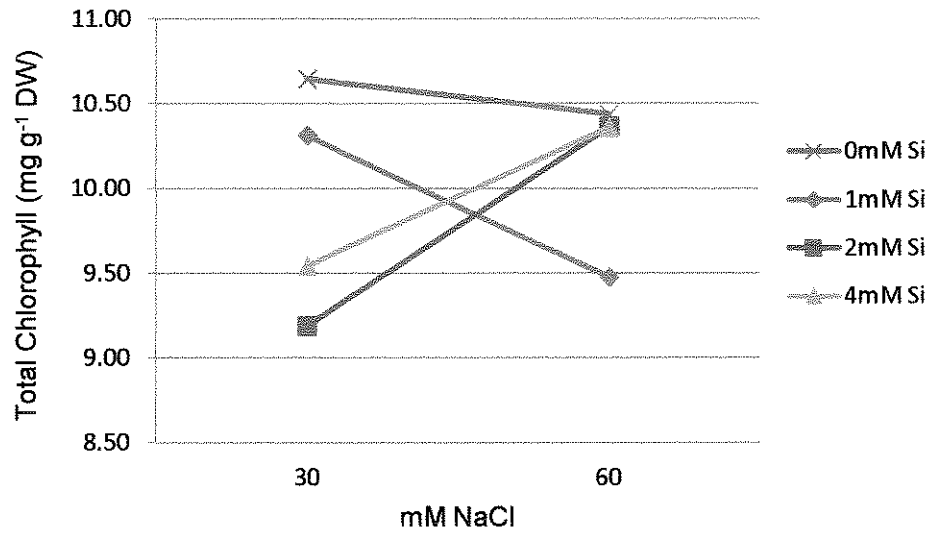
Figure 3.7. The effect of 30 and 60 mM NaCl on total chlorophyll content with additions of 0, 1, 2 and 4 mM Si.

Table 3.1. The effect of 30 and 60 mM NaCl on Cl, Na and Si root and shoot content, with additions of 0, 1, 2 and 4 mM Si.

| Treatments | | Cl (mg g^{-1}) | | Na (mg g^{-1}) | | Si (mg g^{-1}) | |
NaCl	Si	Roots	Shoots	Roots	Shoots	Roots	Shoots
30mM	0 mM	12.18	16.78	7.17	11.11	0.42	0.06
	1 mM	9.78	18.70	8.12	11.41	0.44	0.12
	2 mM	9.60	22.54	8.59	11.22	0.51	0.15**
	4 mM	14.42	13.74	8.10	11.64	0.43	0.43***
60 mM	0 mM	15.98	21.20	14.30	16.88	0.37	0.07
	1 mM	14.82	13.62	9.43	13.75***	0.29	0.11
	2 mM	7.66	14.20	10.77	15.40**	0.45	0.16***
	4 mM	74.8***	12.74*	8.83	14.00***	0.40	0.18***

Values presented are means ± SE, n = 5. *; **;*** = significant at $P \leq 0.1$*, $P \leq 0.05$** or $P \leq 0.01$***. Significance compared to that of 0 mM Si, with corresponding NaCl concentrations (Controls).

Table 3.2. The effect of 30 and 60 mM NaCl on Cl, Na and Si root and shoot content, with additions of 0, 1, 2 and 4 mM Si.

Treatments		Cl (mg g^{-1})		Na (mg g^{-1})		Si (mg g^{-1})	
Si	NaCl	Roots	Shoots	Roots	Shoots	Roots	Shoots
0mM	30 mM	12.18	16.78	7.17	11.11	0.42	0.06
0mM	60 mM	15.98	21.20	14.30***	16.88***	0.37	0.07
1mM	30 mM	9.78	18.70	8.12	11.41	0.44	0.12
1mM	60 mM	14.82	13.62	9.43	13.75***	0.29**	0.11
2mM	30 mM	9.60	22.54	8.59	11.22	0.51	0.15
2mM	60 mM	7.66	14.20**	10.77	15.40***	0.45	0.16
4mM	30 mM	14.42	13.74	8.10	11.64	0.43	0.43
4mM	60 mM	74.8***	12.74	8.83	14.00***	0.40	0.18***

Values presented are means ± SE, n = 5. *; **;*** = significant at P ≤ 0.1*, P ≤ 0.05** or P≤ 0.01***. Significance compared to that of the same Si concentrations, with different NaCl concentrations.

CHAPTER FOUR

SALINITY INDUCED CHANGES IN OXIDATIVE STRESS AND ANTIOXIDANT STATUS AS AFFECTED BY APPLICATIONS OF SILICON IN LETTUCE (*Lactuca sativa* L. 'Eish!').

4.1 ABSTRACT

The antioxidant content, capacity and certain oxidative stress parameters were investigated in salinity stressed *Lactuca sativa* L. 'Eish!' with the addition of exogenous silicon (Si). No significant changes were shown for total polyphenol concentrations in any of the experimental plants. Applications of 4mM Si significantly increased oxygen radical absorbance capacity (ORAC) values at both 30 and 60 mM NaCl. The redox status of glutathione was also improved with a significant increase in reduced glutathione (GSH) in 2mM Si treated plants at 30 mM NaCl. Increases in both GSH and oxidised glutathione (GSSG) were noted at 60 mM NaCl. The activity of catalase (CAT) was significantly decreased with the addition of 1 and 4 mM Si at 30 mM NaCl. Lipid peroxidation (measured as thiobarbituric acid reactive substances, TBARS) was not influenced by the different Si concentrations. These results suggest that applications of Si could be beneficial with regards to the modulation of oxidative stress in salinity stressed lettuce, and should be considered as an addition when cultivating lettuce.

Key Words: Potassium silicate, ROS, Hydroponics, Catalase, Lipid peroxidation, Glutathione, Polyphenolics, Oxygen Radical Absorbance Capacity, Thiobarbituric Acid Reactive Substances.

4.2 INTRODUCTION

Salinity is a significant plant stress factor and a major problem on all continents (Ghassemi et al., 1995). Saline conditions influence plant growth in two core ways; by increasing the osmotic potential of the soil solution, which has a direct effect on plant-water availability (Carter, 1981), and through ion toxicities, determined by the specific ions that are in excess (Marschner, 1995:662). In most cases, sodium (Na^+) and chloride (Cl^-) ions are the cause of saline conditions (Harris, 1992:215; Epstein & Bloom, 2005:332-

334). The effect of NaCl induced salinity on plants differ from plant to plant, but generally leads to a reduced growth rate, sometimes nutritional deficiencies, and at higher concentrations, the accumulation of Na^+ and Cl^-, resulting in leaf scorching, chlorosis and necrosis (Shannon & Grieve, 1999; Wahid et al., 1999). Along with these symptoms, salinity stress is known to result in an excess production of reactive oxygen species (ROS) (Jaspers et al., 2005), oxidative damage and a change in concentrations of antioxidants (Bor et al., 2003; Sekmen et al., 2007; Gao et al., 2008). Consequently, ROS are good cellular indicators of stress (Mittler, 2002).

Silicon (Si), a quasi-essential element for plants (Epstein, 1999), has proved to have significant ameliorative properties regarding NaCl toxicity in many important agricultural and ornamental crops. It has shown to repeatedly result in an increase in yield together with other beneficial growth parameters (Romero-Aranda et al., 2006; Savvas et al., 2007; Soylemezoglu et al., 2009). Silicon allows plants to grow more productively on saline land, leading to definite economic benefits, especially for developing nations (Shay, 1990).

The literature disagrees in the manner by which silicon mitigates NaCl toxicity. Si has the ability to influence Na absorption, distribution or transportation (Ahmad et al., 1992; Savvas et al., 2009; Soylemezoglu et al., 2009; Hashemi, et al. 2010), which is sometimes associated with an increase in potassium (K) concentration (Saqib et al., 2008; Tuna et al., 2008; Ashraf et al., 2010). Ameliorative properties of silicon have also been associated with the decrease in plant Na concentrations through dilution, thus mitigating salt toxicity (Romero-Aranda et al., 2006). Ma et al. (2001), suggested silicon's beneficial effects stems from the decreased Na influx through a Si induced decrease in transpiration. In addition to the Si – Na related amelioration of salinity stress, studies have indicated that Si can influence antioxidant activity. This has been reported in, amongst others, salt stressed barley, (Liang, 1999), cucumber, (Zhu et al., 2004a), tomato (Zhu et al., 2004b) and wheat (Saqib et al., 2008).

In this study the influence of Si in NaCl stressed *Lactuca sativa* L. 'Eish!' was investigated in order to assess whether the known Si induced yield increase can be associated with antioxidant concentrations and oxidative damage. Furthermore, it is to our knowledge that this is the first study examining Si applications influencing antioxidant status and oxidative damage in NaCl salinity stressed lettuce.

4.3 MATERIALS AND METHODS

4.3.1 Plant Conditions and Cultivation

Lettuce cultivation took place in the research greenhouse at the Cape Peninsula University of Technology (Cape Town, South Africa). Greenhouse temperatures ranged between a minimum of 9 and maximum of 29°C, and humidity between a minimum of 44 and a maximum of 99%. Seeds were sown in a 128 plug polystyrene seedling tray, with vermiculite as the sowing medium. Seeds germinated 8 days after sowing, and were fertilised daily with half strength NUTRIFEED (Starke Ayres, South Africa); a complete, water soluble fertiliser providing 9.3 mM N, 1.7 mM P, 6.7 mM K, 3.5 mM Ca, 1.8 mM Mg, 4.7 mM S, 53.7 µM Fe, 8.7 µM Mn, 44.4 µM B, 1.5 µM Zn, 6.3 µM Cu and 0.2 µM Mo. Four weeks after sowing, 80 seedlings were chosen at random, and transplanted into the soilless growing systems. Seedlings were transplanted into 12.5cm plastic pots with HYDROTON (Germany) expanded clay as the medium, and divided evenly amongst the 8 soilless growing systems, equating to 8 different treatments with 10 repeats of each. Each closed, sub irrigative, soilless growing system was comprised of a nutrient solution holding tray, delivering ±1cm of recirculating nutrient solution.

4.3.2 Basal Nutrient Solution

Salts used to add macro nutrients to the nutrient solution were of analytical grade; KNO_3, $Ca(NO_3)_2 \cdot 4H2O$, $NH_4H_2PO_4$, and $MgSO_4 \cdot 7H2O$ were used to include 16 mM N, 6 mM K, 4 mM Ca, 2 mM P, 1 mM S and 1 mM Mg respectively, based on the modified Hoagland solution as described by Epstein and Bloom (2005:31). Micro nutrients were supplied using HYGROPLEX (Hygrotech, South Africa); a complete commercially available micro nutrient mix, adding 58 µM B, 11.4 µM Mn, 7.6 µM Zn, 1 µM Cu, 0.65 µM Mo and 31.9 µM Fe to the nutrient solution. The basal nutrient solution, having had an electrical conductivity (EC) of 1.9 mS cm^{-1}, was formulated using deionised water.

4.3.3 Silicon and NaCl Treatments

Silicon was added to the basal nutrient solution using K_2SiO_3 (AGRISIL K50), at concentrations of 0 mM, 1 mM, 2 mM and 4 mM Si. Two concentrations of NaCl, 30mM and 60 mM, were applied to each of the 4 Si concentrations, making a total of 8

treatments. Treatments were applied in a single dose, implemented one week after transplantation. Newly prepared treatments containing 0, 1, 2 and 4 mM Si and 30 mM NaCl had a mean EC of 5.20 mS cm^{-1}, and treatments containing 0, 1, 2 and 4 mM Si and 60 mM NaCl, had a mean EC of 8.10 mS cm^{-1}. These EC levels were maintained daily. K that was added with the applications of K_2SiO_3, was balanced in all treatments by subtracting additional K from KNO_3, and supplementing lost NO_3 by adding HNO_3. The nutrient solution, which was renewed weekly, was maintained daily at a pH of 6.0 using HCl and NaOH.

4.3.4   Sampling and Harvesting

4.3.4.1 Antioxidant status and oxidative damage

The shoots of 5 plants per treatment were harvested 6 weeks after being transplanted into the soilless growing system. Once harvested, shoots were rinsed twice in deionised water and patted dry with paper towel. The shoots were then placed directly in an air tight plastic bag, and stored at -80°C. The shoots were transported on dry ice, and lyophilised at -80°C for 16 hours. Once the lyophilisation was complete, samples were ground to a powder using a mortar and pestle and stored at -80°C until analysed.

4.3.4.2 Plant yield and elemental analysis

Plant yield is expressed as fresh and dry weight of roots and shoot in grams. Five plants per treatments were separated into roots and shoots, rinsed twice in deionised water and patter dry with paper towel. After recording the fresh weights, the plant material were placed into paper bags and dried at 65°C. Once the weight of the dried plant material stayed constant, dry shoot and root weights were recorded.

Quantities of shoot Si of the 5 treatments were measured by dissolving the ground, ashed material in HCl. Concentrations, determined using an inductively-coupled plasma emission spectrophotometer, are expressed in mg g^{-1} of dried plant material.

4.3.5   Total protein analysis

For total protein extraction, 125 mg of lyophilised plant material was homogenised with 6mL of 25mM HEPES-KOH buffer containing 0.2mM EDTA and 2% PVP (pH 7.8), on ice. The homogenate was centrifuged in 2 mL microcentrifuge tubes at 15 000 g for 10 minutes at 4°C. The resulting supernatant was transferred to new 2mL microcentrifuge tubes and stored at -80°C until needed.

Protein content was determined using the PIERCE BCA protein assay kit, utilising the procedures accompanying the kit, with absorbance levels being measured at 562 nm. Total protein content (expressed as $\mu g\ mL^{-1}$) was used for catalase activity quantification.

4.3.6 Polyphenol antioxidant content and capacity

4.3.6.1 Total polyphenol analysis

For TP analysis, 125 mg of lyophilised plant material was homogenised with 6mL of methanol on ice, and centrifuged in 2mL microcentrifuge tubes at 15 000 g for 10 minutes at 4°C. 25 µL undiluted supernatant was then transferred, in triplicate, to a 96 well plate containing 125 µL of 10 times diluted Folin-Ciocalteu's phenol reagent (0.2 N). After 5 minutes, 100 µL of sodium carbonate (7.5%, w/v) was added and the resulting mixture incubated at room temperature for a further two hours. Absorbance values at 765 nm were recorded and compared to those of a Gallic acid standard. Results are expressed as mg GAE g^{-1}.

4.3.6.2 Oxygen radical absorbance capacity analysis

Extraction followed that of TP, with 12 µL of 10 times diluted supernatant being added to 138µL fluorescein and 50 µL AAPH, and analysed, in triplicate, using a fluorescence spectrophotometer until zero fluorescence occurred. The ORAC values were calculated by dividing the sample curve by that of the Trolox standard, and expressed as µmol TE g^{-1} (Ou et al., 2001).

4.2.7 Catalase analysis

Catalase (CAT) activity was measured, in triplicate, by placing 20 µL of the supernatant (obtained from protein analysis, refer 2.5) with 170 µL of a 50 mM Potassium phosphate

buffer (pH7.0) and 75 μL H_2O_2 in a 96 well micro plate. The disappearance of H_2O_2 was measured over a 2 minute period by measuring absorbance levels at 240 nm every 15 seconds. Catalase activity was calculated using the extinction coefficient of H_2O_2 and expressed as μmol min^{-1} $μg^{-1}$ protein (Aebi, 1984).

4.3.8 Glutathione analysis

For GSH extraction, 125 mg of lyophilised plant material was homogenised with 6 mL of 5% Trichloroacetic Acid buffer, on ice. The homogenate was centrifuged in 2 mL microcentrifuge tubes at 15 000 *g* for 10 minutes at 4°C. The resulting supernatant was transferred to new 2 mL microcentrifuge tubes and stored at
-80°C until needed.

Glutathione concentrations were measured, in triplicate, by placing 50 μL of 50 times diluted supernatant, with 50 μL of 0.3 mM DTNB (5,5'-Dithiobis(2-nitrobenzoic acid)) and 50μL Glutathione reductase (GR) solution (0.02U/μL) in a 96 well micro plate. After a 5 minute incubation period at room temperature, 50 μL of 1 mM NADPH was placed in each well. Absorbance levels at 412 nm were measured at 15 second intervals for 5 minutes. Absorbance levels were compared to those of a GSH standard curve, and total GSH expressed as μmol g^{-1} dried plant material.

GSSG extraction followed that of GSH, except for the addition of 4.8 mg of M2VP (1-Methyl-2-vinylpyridinium) within the homogenate buffer. Measurement of GSSG followed that of GSH, except a 25 times diluted supernatant was used, and absorbance levels were compared to those of a GSSG standard curve (Asensi et al., 1999).

4.3.9 Lipid peroxidation analysis

For thiobarbituric acid reactive substances extraction, the method by Yagi, 1984, adapted for plant samples, was utilised. 125 mg of lyophilised plant material was homogenised with 6mL of Methanol, on ice. The homogenate was centrifuged in 2 mL microcentrifuge tubes at 15 000 *g* for 10 minutes at room temperature. The resulting supernatant was transferred to new 2 mL microcentrifuge tubes and stored at -80°C until needed.

250 µL of undiluted supernatant, with 375 µL of 0.44 mM PCA (perchloric acid) and 125 µL of 42 mM TBA (2-Thiobarbituric acid) were place in 2 mL microcentrifuge tubes. After a 60 minute incubation period at 100°C, the 2 mL microcentrifuge tubes were centrifuged at 10 000 g for 5 minutes at room temperature. The resulting supernatant was placed, in triplicate, in a 96 well plate, and absorbance levels at 532 nm were compared to those of the malondialdehyde standard and expressed as µmol g^{-1} dried plant material (Yagi, 1984).

4.3.10 Statistical Analysis

Statistical analysis was performed using two–way analysis of variance (ANOVA), followed by the Bonferroni post test, with P values ≤ 0.05 considered significant. Pearson correlations coefficient was used to calculate correlations. Computations were executed with the software program SPSS (Urdan, 2005:120-170).

4.4 RESULTS

Plant weights showed no significant differences when treated plants were compared to the 30 mM NaCl control. However, there were a 12 and 17% increase in fresh shoot and root weight and a 46% increase in dry shoot weight when comparing the 4 mM Si treatment to the control (Table 4.1).

However, plant weights showed a clear increase when treated plants were compared to the 60 mM NaCl control. Although 2 mM Si was the only treatment that resulted in a marginally significant increase (P ≤ 0.1) in fresh shoot weight, the increase was a considerable (72%). The fresh root weight of the 2 mM Si treated plants resulted in a significant increase (75%) when compared to the control. Dry shoot weights significantly increase by 74 and 75% with both 1 and 2 mM Si treated plants respectively when compared to the control. Although the 4 mM Si treated plants showed no significant difference when compared to the 60 mM NaCl control, there was a marked increase of 56%. Dry root weights showed a marginal significant increase of 68% with 1 mM Si when compared to the 60 mM NaCl control. Again, although dry root weights of 2 and 4 mM Si treated plants resulted in no significant differences when compared to the control, there was a noteworthy increase of 50 and 43% respectively.

Shoot Si concentrations of 1 mM Si treated plants showed no significant difference at both 30 and 60 mM NaCl when compared to their individual controls. Nevertheless, treatments of 2 and 4 mM Si at 30 mM NaCl resulted in a significant concentration increase of 150% and 616% respectively. Similarly, treatments of 2 and 4 mM Si at 60 mM NaCl resulted in a significant increase of 129% and 157% respectively (Figure 4.1.).

With regards to antioxidant status and oxidative stress between the two controls, there were no significant differences between any of the parameters, besides that of the TP controls. The 60 mM NaCl control expressed an 18% increase in TP concentrations when compared to that of the 30 mM NaCl control, resulting in a marginal difference (P ≤ 0.1) between the two controls. However, when Si treated plants were compared to that of the controls, TP concentrations showed no differences (Table 4.2). ORAC values showed a tendency to increase with applications of Si at all concentrations when compared to the controls. However, only 4 mM Si at both 30 and 60 mM NaCl showed significant increases when compared to controls, with a 28% and 21% increase respectively (Figure 4.2).

Catalase activities showed a clear tendency to decrease with applications of Si when plants were exposed to 30 mM NaCl, with 1 and 4 mM Si, a decrease of 29 and 25% respectively, being significant when compared to the 30 mM NaCl control. Conversely Si applications at 60 mM NaCl showed no tendency to decrease, and showed no significance when compared to that of the 60 mM control (Figure 4.3).

Concentrations of both GSH and GSSG, like that of ORAC, tended to increase with applications of Si. Concentrations of GSH at 2 mM Si with 30 mM NaCl, and both 1 and 4 mM Si with 60 mM NaCl showed significant increases of 138%, 106% and 99% respectively, when compared to their individual controls (Figure 4.4). Concentrations of GSSG showed a significant increase of 100% at 4 mM Si, and a marginally significance increase of 93% (P ≤ 0.1) at 1 mM Si when compared to the 60 mM NaCl control. The ratio of GSH:GSSG showed a limited trend to increase or decrease, with an application of 2 mM Si with 30 mM NaCl being the only treatment showing a significant increase of 33% (P ≤ 0.1) when compared to the control.

Like that of TP concentrations, TBARS values showed no significant differences when any of the treatments were compared to their respective controls. In addition to this, no correlations were found when comparing variables within treatments.

4.5 DISCUSSION

Plant stresses, including salinity stress, are known to disturb cellular homeostasis, enhancing the production of ROS (Dat et al., 2000). Additionally, osmotic stress, one of the foremost stresses associated with high salinity levels, has shown to cause the production of ROS (Xiong and Zhu, 2002).

Although ROS have roles as signalling molecules, active generation of which can be initiated by abiotic stresses (Desikan et al., 2005), ROS are traditionally thought of as species causing cell injury or death (Mittler, 2002). In the light of this, plants need ways to detoxify ROS. CAT is a major ROS scavenging enzyme seen as indispensable, with stress bringing about the production of CAT (Mittler, 2002). With ROS causing the production of CAT, it can be argued that lower concentrations of CAT, indicating lower levels of ROS, would be a sign of less oxidative stress. In this study, with the application of Si, there was an overall tendency for CAT concentrations to decrease, significantly with 1 and 4 mM at 30 mM NaCl. Zhu et al. (2004a), however, showed that Si raises CAT levels in salt treated tomatoes, while Zhu et al., (2004b) reported tendencies for CAT levels to decrease with applications of Si on salt treated plants. Bor et al., (2003), Gao et al., (2008) and Eraslan et al., (2007), although not investigating Si, found applications of NaCl to significantly increase CAT activities, supporting the idea that increased CAT activities could be indicative of oxidative stress. Gong et al. (2005) when investigating applications of Si to ameliorate drought stress, found that applications of Si lead to significantly decreased CAT activity. This is relevant to NaCl stress, as osmotic stress is a primary limitation for plants growing in saline conditions (Marschner, 1995:662).

Contrary to CAT activites, yet similarly indicating a decrease in oxidative stress, ORAC levels showed a tendency to increase, significantly at 4 mM Si with both 30 and 60 mM NaCl. ORAC, a hydrophilic antioxidant capacity assay, is one of the most commonly used methods for measuring antioxidative capacity in biology (Yeum et al., 2010). It is assumed that with an increase in TP concentrations, there would be a correlative increase in ORAC values. The level of TP in Si treated plants showed no significant differences between controls, and perhaps even a tendency to decrease. Liu et al. (2007) reported similar results, although with relatively higher TP concentrations, with no correlation to the DPPH assay, a procedure that has shown to be comparable to that of the ORAC assay (Thaipong et al., 2006), even though different mechanisms are involved (Ndhlala et al., 2010).

TBARS concentrations, a well-known indicator of lipid peroxidation (Wada et al., 2008), a process that can lead to cell death (Mittler, 2002), showed no significant differences between the treated and the control plants, and no trend to increase or decrease. This is in disagreement with Liang, (1999), Zhu et al., (2004a), and Zhu et al., (2004b) all finding applications of Si to decrease lipid peroxidation.

Increases in GSH concentrations are known to be associated with salinity stress (Ruiz & Blumwald, 2002; Leyva et al., 2011). The results from this study show that applications of Si tend to increase GSH concentrations, significantly with 2 mM Si at 30 mM NaCl and with 1 and 4 mM Si at 60mM NaCl, when compared to NaCl alone. These results are in agreement with Saquib et al. (2008), reporting that GSH increases with applications of Si in NaCl stressed wheat. Foyer et al., (2005:1) argue that the signal responses that could be brought about by the interaction between GSH and the GSH:GSSG ratio are linked to plant growth cessation. This is interesting when considering that, contrary to conventional thought, active plant growth cessation, as opposed to stress limiting growth, has been linked to surviving adverse environmental conditions (Harberd et al., 2009), including salinity stress (Magome et al., 2008). However, applications of Si are widely reported to increase plant growth parameters (Romero-Aranda et al., 2006; Soylemezoglu et al., 2009), which is in line with the data presented in this study. Therefore, applications of Si induced increases in GSH concentrations seem unlikely to be associated with a growth cessation stress response. GSH are well known antioxidants, and higher concentrations would infer superior antioxidative defence (Tausz et al., 2004), and would therefore logically result in a decrease in ROS concentrations brought about by salinity stress (Foyer et al., 2005:1-2,16-18). This approach would arguably provide more probable grounds for the increases in GSH with applications of Si.

From this study, the overall impression of Si applications in relation to antioxidant concentrations and oxidative stress modulation is that fertilisation and regular inclusion of Si in nutrient solutions, where Si is often limited, is of value to lettuce cultivation. Investigations into the effects of higher salinity concentrations would likely show a clear cut results with regards to the effects of Si applications on antioxidant status in salinity stressed lettuce, and is a recommended focus for future study. Additionally, more insight into the relationships between yield parameters and antioxidant status would bring light to the positive effects Si has on the antioxidative status of plants on a more practical, economically beneficial level.

4.6 ACKNOWLEDGMENTS

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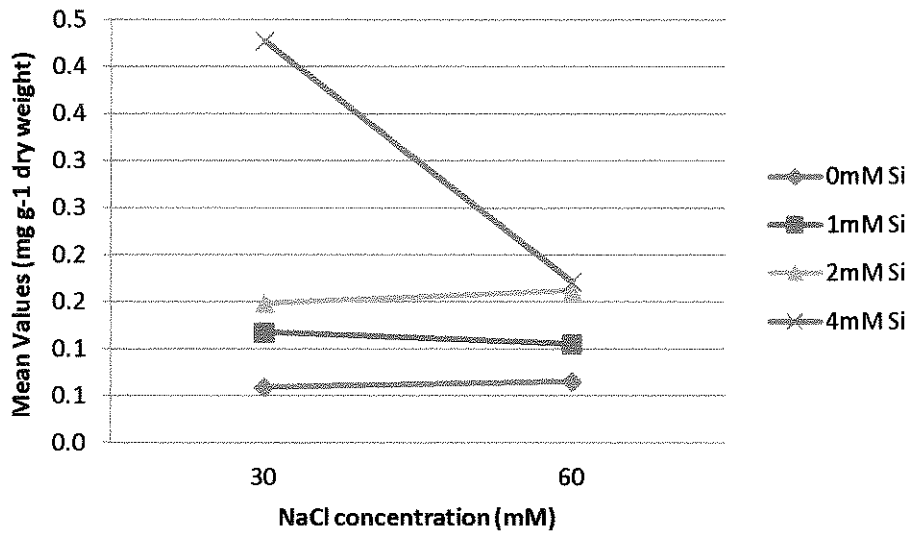
Figure 4.1: Effect of Si applications on Si shoot concentrations in salinity stressed lettuce.
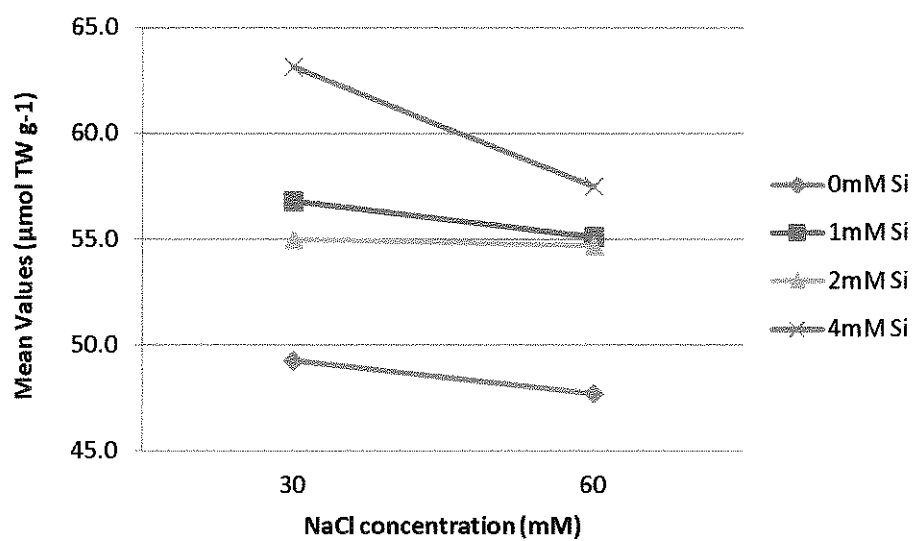
Figure 4.2: Effect of Si applications on Oxidative Radical Absorbance Capacity (ORAC) values in salinity stressed lettuce.
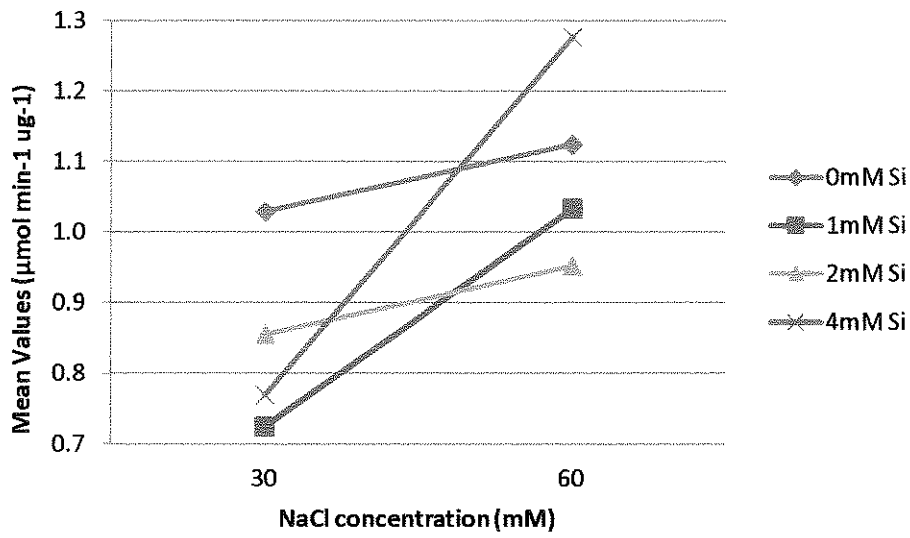
Figure 4.3: Effect of Si applications on Catalase (CAT) concentrations in salinity stressed lettuce.
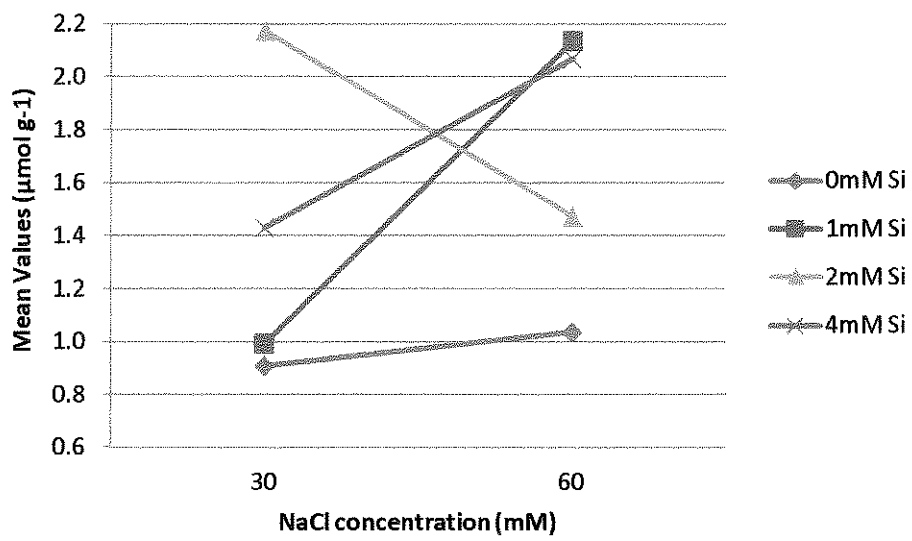
Figure 4.4: Effect of Si applications on Glutathione (GSH) concentrations in salinity stressed lettuce.

Table 4.1: Effects of Si applications on plant fresh and dry root and shoot weights in salinity stressed lettuce.

NaCl	Si	Fresh Weight (g)		Dry Weight (g)	
		Shoots	Roots	Shoots	Roots
30 mM	0 mM	47.85 ± 17.56	6.49 ± 1.83	2.04 ± 0.51	0.42 ± 0.1
	1 mM	39.1 ± 13.07	5.74 ± 1.81	1.94 ± 0.73	0.34 ± 0.1
	2 mM	43.52 ± 10.49	5.75 ± 1.54	2.12 ± 0.55	0.36 ± 0.09
	4 mM	53.70 ± 11.54	7.64 ± 1.60	2.99 ± 0.56	0.44 ± 0.09
60 mM	0 mM	28.05 ± 6.55	4.52 ± 1.27	1.47 ± 0.31	0.28 ± 0.1
	1 mM	41.73 ± 12.39	7.29 ± 2.38	2.56 ± 0.73**	0.47 ± 0.19*
	2 mM	48.12 ± 14.92*	7.92 ± 2.06**	2.57 ± 0.69**	0.42 ± 0.14
	4 mM	40.97 ± 9.83	6.44 ± 1.52	2.29 ± 0.59	0.40 ± 0.05

Mean values of treated plants were compared to that of the controls (0 mM Si). Significance levels are represented by *P ≤ 0.1, **P ≤ 0.05.

Table 4.2: Effects of Si applications on Total Polyphenolics (TP) and Oxidative Radical Absorbance Capacity, in salinity stressed lettuce.

NaCl	Si	TP (mg GAE g^{-1})	ORAC (μmol TE g^{-1})
30 mM	0 mM	5.05 ± 0.37	49.28 ± 3.93
	1 mM	4.23 ± 0.35	56.81 ± 4.23
	2 mM	4.71 ± 0.59	54.99 ± 4.07
	4 mM	5.19 ± 0.63	63.16 ± 5.83*
60 mM	0 mM	5.97 ± 0.58	47.72 ± 3.22
	1 mM	5.25 ± 0.59	55.12 ± 9.86
	2 mM	4.98 ± 0.50	54.70 ± 5.74
	4 mM	5.85 ± 0.65	57.51 ± 2.79**

Mean values of treated plants were compared to that of the controls (0 mM Si). Significance levels are represented by *$P \leq 0.1$, **$P \leq 0.05$, ***$P \leq 0.001$.

Table 4.3: Effects of Si applications on Thiobarbituric Acid Reactive Substances (TBARS), Glutathione (GSH), Glutathione disulfide (GSSG), GSH:GSSG ratio and Catalase (CAT), in salinity stressed lettuce.

NaCl	Si	TBARS (μM g^{-1})	GSH (μmol g^{-1})	GSSG (μmol g^{-1})	GSH:GSSG	CAT (μmol min^{-1} ug^{-1})
30 mM	0 mM	0.26 ± 0.03	0.91 ± 0.37	0.15 ± 0.06	5.96 ± 0.81	1.03 ± 0.09
	1 mM	0.26 ± 0.04	0.99 ± 0.2	0.19 ± 0.05	5.20 ± 0.27	0.73 ± 0.09***
	2 mM	0.24 ± 0.03	2.17 ± 0.24***	0.23 ± 0.08	7.94 ± 0.59*	0.86 ± 0.12
	4 mM	0.29 ± 0.04	1.43 ± 0.46	0.24 ± 0.07	5.91 ± 0.67	0.77 ± 0.11**
60 mM	0 mM	0.25 ± 0.02	1.04 ± 0.83	0.15 ± 0.1	6.94 ± 1.76	1.12 ± 0.10
	1 mM	0.28 ± 0.03	2.14 ± 0.37**	0.29 ± 0.07*	7.69 ± 1.87	1.03 ± 0.06
	2 mM	0.25 ± 0.03	1.47 ± 0.38	0.25 ± 0.06	5.87 ± 0.15	0.95 ± 0.11
	4 mM	0.26 ± 0.03	2.07 ± 0.28**	0.30 ± 0.05**	7.06 ± 1.01	1.28 ± 0.16

Mean values of treated plants were compared to that of the controls (0 mM Si). Significance levels are represented by *$P \leq 0.1$, **$P \leq 0.05$, ***$P \leq 0.001$.

CHAPTER FIVE
GENERAL DISCUSSION AND CONCLUSION

Silicon, in this study, and in the myriad of other studies investigating silicon's ability to ameliorate abiotic and biotic stress factors, has proved its status as a quasi-essential nutrient in plants. With salinisation being a widespread occurrence in many soils, and the accumulation of salts in soilless growing systems a regular incident, Si shows a remarkably cost efficient and environmentally friendly way of mitigating the deleterious effects that are associated with salinity stress in plants, ultimately increasing yield.

The studies herein confirm the beneficial effects of Si with regards to NaCl salinity stress, with the first study reporting on yield, chlorophyll, leaf numbers, height and elemental content of lettuce plants. Although leaf number, plant height and chlorophyll content showed limited or no significance when comparing the treated plants to the controls, fresh root and shoot weights showed clear increases when compared to the controls grown without Si, as did dry root and shoot weights.

Most noticeable, and in agreement with the majority of other studies evaluating Si and NaCl stress, Na tissue content showed a clear tendency to decrease with applications of Si, and this can be seen as the foremost reason for the increase in yield and therefore the mitigating properties associated with Si applications; the Si-Na interaction. Although the mechanism of this Si-Na interaction has only been the subject of a limited number of studies, it is probable that it is the result of Na dilution due to an increase in water use efficiency and therefore a decrease in Na toxicity (Romero-aranda et al., 2005), although data from this study cannot support this statement. This is supported by studies reporting positive effects of Si applications on drought stressed plants.

The antioxidant status and oxidative damage study showed positive results with applications of Si, although the interpretation of these results were subject to a higher degree of speculation than the more obvious growth parameters of the first study. Concentrations of both GSH and GSSG tended to increase with applications of Si. TBARS concentrations showed a surprisingly limited trend to increase or decrease with or without applications of Si, a decrease of which would have given a clear indication of oxidative damage mitigation. Most noticeable with regards to this study was the tendency for CAT activities to decrease with applications of Si. CAT is a major ROS scavenging enzyme seen as indispensable, with stress bringing about the production of CAT (Mittler, 2002). It follows that with increased CAT production during stress, a decrease of CAT would be a sign of less oxidative stress, as was

found with applications of Si in this study. Contrarily, and as noticeable, ORAC values increased with Si applications, indicating a higher capacity to scavenge ROS, and therefore a beneficial characteristic brought about by Si.

From these results, it can be concluded that Si has a significantly positive effect on yield, antioxidant status, oxidative damage and NaCl stress mitigation as a whole. However, the connection between the resultant antioxidant status to yield data, showed no correlation. Additionally, oxidative damage was somewhat unconvincing, therefore additions of higher concentrations of NaCl would possibly lead to more conclusive oxidative damage data, and perhaps other non-antioxidant related factors as well. These factors are important to consider for future studies.

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APPENDIX A: The subirrigative, closed soilless growing systems for the cultivation of *Lactuca sativa* L. 'Eish!'.

Lettuce seedlings 1 week after transplanting in the soilless growing systems.

Figure 3.7. Lettuce seedlings 4 week after transplanting in the soilless growing systems.

APPENDIX B:

Full Length Research Paper

The alleviation of salinity induced stress with applications of silicon in soilless grown *Lactuca sativa* L. 'Eish!'

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The effects of 30 and 60 mM NaCl on Lettuce (*Lactuca sativa* L. 'Eish!'), grown in soilless culture, with additions of 0, 1, 2 and 4 mM Si was evaluated. Height, leaf number, weight, chlorophyll content and elemental analysis of plants were examined. Fresh root and shoot weight significantly increased with additions of 2 mM Si compared to that of the 60 mM NaCl control, with notable increases with Si treatments of 1 and 4 mM. Dry root and shoot weight with additions of 1 mM Si, and in shoot weight with additions of 2 mM Si, significantly increased when compared to the 60 mM NaCl control. Increases in dry root and shoot weight of the other Si additions lead to clear, yet insignificant increases in dry weights. Few differences in Cl content were evident amongst treatments, besides a significant increase in root content and a significant decrease in shoot content with applications of 4 mM Si exposed to 60 mM NaCl. Na shoot content of 1, 2 and 4 mM Si at 60 mM NaCl showed a significant decrease compared to that of the control. Applications of 2 and 4 mM Si showed increased shoot Si when compared to both the 30 and 60 mM NaCl controls. With clear evidence for a reduction in Na content in shoots with applications of Si at higher NaCl concentrations, and further increases in plant weights, it can be stated that applications of Si are of a distinct benefit for the growth of lettuce.

Key words: Lettuce, Si, NaCl, salt, sodium chloride, hydroponics.

INTRODUCTION

Silicon [Si], an element whose abundance in the earth's crust is second only to oxygen (Marshak, 2005), can contribute as much as 0.1 to 10% of the dry matter of plants. These levels are equal to or exceeding those of essential macro nutrients (Epstein and Bloom, 2005). Si has in the past been classified as a non-essential element for plant nutrition, that is, one which does not meet the criteria for essentiality in the classic definition of essentiality proposed by Arnon and Stout (1939). More recently Si has been classified as either a beneficial element (Marschner, 1995), or as a quasi-essential element in plants (Epstein, 1999).

Contributing to its classification as a beneficial or quasi-essential element in plants is its role as a plant stress ameliorator (Epstein, 1994, 1999; Belanger et al., 1995). An important aspect of this is the ability of Si to ameliorate salt toxicity, defined as conditions of high salt build-up, with sodium chloride [NaCl] being the most common cause of saline conditions (Harris, 1992; Epstein and Bloom, 2005).

Salinity has two methods of causing abnormalities in plant growth. The first, which is independent of the type of salt, is by creating a higher osmotic potential in the medium surrounding the roots, and because osmosis is the principle method of water absorption in plants (Stern, 2006), it results in a decrease in plant-water availability (Carter, 1981). The second, which is salt dependent, is

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caused by an excess of available ions. In the case of NaCl salinity, the ions in question are sodium [Na$^+$] and chloride [Cl$^-$]. Cl is known to be an essential micronutrient in plants (Epstein and Bloom, 2005; Stern, 2006), and some argued that Cl should be classified as a macronutrient, contributing as much as 1% of the dry weight of some plants (Muckle, 1993). Na, on the other hand, is required by only a few plants (Epstein and Bloom, 2005), and has been shown to be the primary cause of growth suppression in lettuce (Tas et al., 2005). In general, high concentrations of both Na and Cl are associated with leaf scorching (Shannon and Grieve, 1999), necrosis and defoliation (Maas and Hoffman, 1977).

The mitigation of NaCl induced toxicity with the application of Si has been demonstrated in many important agricultural and ornamental crops, including Alfalfa (Wang et al., 2011), tomato (Romero-Aranda et al., 2006), wheat (Ahmad et al., 1992), zucchini (Savvas et al., 2009), grapevine (Soylemezoglu et al., 2009), mesquite (Bradbury and Ahmad, 1990), roses (Savvas et al., 2007; Reezi et al., 2009) and several others. The present study investigated the ability of Si to alleviate NaCl toxicity in lettuce (Lactuca sativa L. 'Eish!').

MATERIALS AND METHODS

The experiment took place within the experimental greenhouse located at the Cape Peninsula University of Technology in Cape Town, South Africa - 33° 55' 58.27"S, 18° 25' 57.04 E. The temperature within the greenhouse ranged from 9 to 29°C, the mean being 17.6°C and the humidity ranged from 44 to 99%, the mean being 77%.

L. sativa L. 'Eish!' seeds (Hygrotech, South Africa) were sown in vermiculite, and germinated under 40% shade-cloth. Once all seeds had germinated (8 days after sowing) they were fertilised daily with half strength CHEMICULT (Starke Ayres, South Africa) nutrient solution, and increased gradually to full strength (Harris, 1992). Once the seedlings were fully established (40 days after sowing) 80 plants were randomly selected and transplanted into individual 12.5 cm pots, containing expanded clay as the medium, and placed within the soilless growing system. The sub-irrigation, closed, soilless growing system consisted of a nutrient solution holding tray, delivering a constant ± 1 cm of circulating nutrient solution to each treatment. Using a randomised block design, 10 plants were placed within eight individual soilless culture units, allowing for 10 repeats of each.

Basal nutrient solution

Analytical grade, potassium nitrate [KNO$_3$], calcium nitrate [(Ca(NO$_3$)$_2$·4H$_2$O], ammonium dihydrogen phosphate [NH$_4$H$_2$PO$_4$], and magnesium sulphate [MgSO$_4$·7H$_2$O], were used to add 16 mM N, 6 mM K, 4 mM Ca, 2 mM P, 1 mM S and 1 mM Mg to the nutrient solution, which was based on the modified Hoagland solution presented by Epstein and Bloom (2005). Micro nutrients were supplied by using HYGROPLEX (Hygrotech, South Africa), adding 58 µM Boron [B], 11.4 µM manganese [Mn], 7.6 µM zinc [Zn], 1 µM copper [Cu], 0.65 µM molybdenum [Mo] and 31.9 µM iron [Fe] to the nutrient solution. The complete basal nutrient solution was prepared using deionised water and had an electrical conductivity (EC) of 1.9 mS cm^{-1}.

Silicon and NaCl treatments

Four levels of Si, 0, 1, 2 and 4 mM, were supplied by adding AGRISIL K50 (PQ Silicas, South Africa) - Potassium silicate [K$_2$SiO$_3$], each of which were combined with two levels of NaCl, 30 and 60 mM, making a total of 8 treatments. Treatments, which were applied in a single dose, commenced one week after transplantation. Newly prepared nutrient solutions containing 30 mM NaCl had a mean EC of 5.20 mS cm^{-1} and solutions containing 60 mM NaCl had a mean EC of 8.10 mS cm^{-1}. These EC levels were maintained daily. Potassium [K] was balanced in all treatments by subtracting additional K from KNO$_3$, and supplementing lost Nitrate [NO$_3$] by adding nitric acid [HNO$_3$]. The nutrient solution, which was replaced weekly, was maintained daily at a pH of 6.0 using hydrochloric acid [HCl] and sodium hydroxide [NaOH].

Plant growth parameters

Plant growth, in terms of plant height and leaf number, of all treatments, were measured at weekly intervals. Plant height was measured with a measuring tape, from the surface of the medium to the tip of the tallest leaf.

Five plants per treatment were harvested after 11 weeks from sowing (Hadfield, 2001), and separated into roots and shoots. Roots were rinsed once with tap water and twice with deionised water before being patted dry with paper towel. After the fresh weight had been recorded, plants were immediately placed within a specimen oven at 60°C until constant weight, and the dry weight recorded.

Determination of chlorophyll content in plant leaves

Extraction of chlorophyll by dimethyl sulphoxide [DMSO] was done following a modified method described by Hiscox and Israelstam (1979). This method allows for the extraction of chlorophyll without plant tissue maceration. Five lettuce plants per treatment were lyophilised and ground to a fine powder using a mortar and pestle and stored at -80°C until analysed. 100 mg of the powder was placed in a 15 ml vial containing 7 ml DMSO and incubated at 65°C for 20 min. After the incubation period, an additional 3 ml of DMSO was added to the vial, and stored at 4°C. After a 24 h storage period, the extract was centrifuged at 3220 g for 10 min, after which the supernatant was transferred to a new 15 ml vial. Before spectrophotometrical analysis, the supernatant was diluted to a factor of 15 of which 1 ml was placed in a cuvette and absorbance values of 645 and 663 nm recorded. Absorbance values were used in the equation proposed by Arnon (1949), to determine total leaf chlorophyll content against DMSO blank, expressed as mg g^{-1} dry weight.

Elemental analysis

Dried plant material was used to analyse for Na, Cl and Si. Na and Si was analysed by ashing the ground sample and dissolving the ash in HCl. Elemental concentrations were determined using an inductively-coupled plasma (ICP) emission spectrophotometer. Cl was analysed by digesting the dried, ground sample with HNO$_3$. An excessive quantity of silver nitrate [AgNO3] was added, followed by back titration with potassium thiocyanate [KSCN]. Elemental concentrations of Na, Cl and Si are expressed as mg g^{-1} of dried plant material (A. Van Deventer, Bemlab, South Africa, Personal Communication).

Statistical analysis

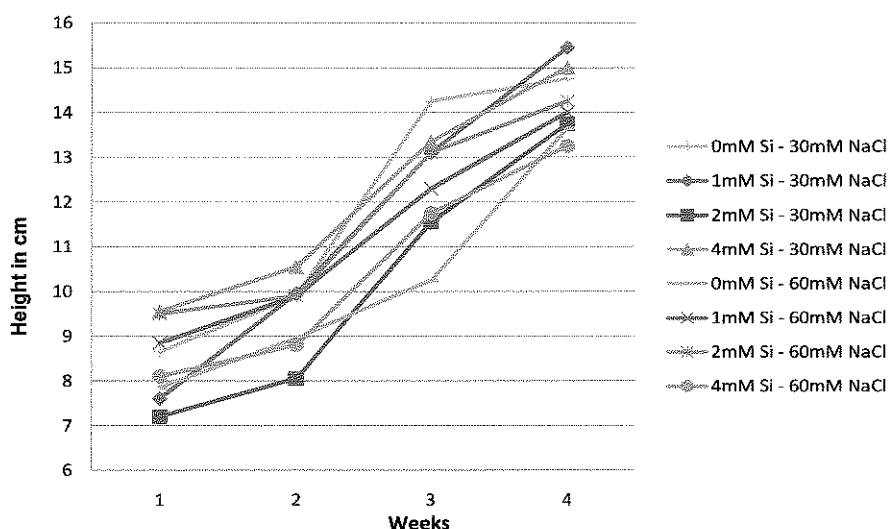Statistical analysis was performed by using two-way independent

Figure 1. The effect of 30 and 60 mM NaCl on plant height, with additions of 0, 1, 2 and 4 mM Si. Measurements were taken weekly from 27 May (1) to 17 June (4) 2011.
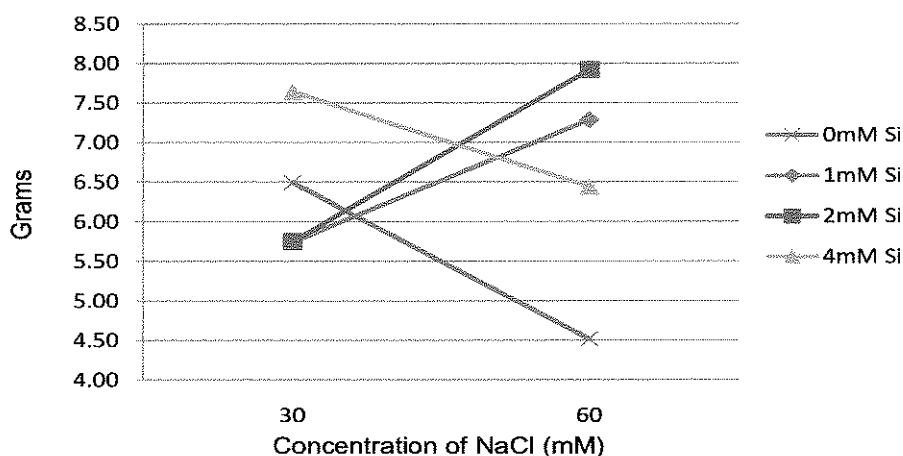

Figure 2. The effect of 30 and 60 mM NaCl on fresh root weight, with additions of 0, 1, 2 and 4 mM Si.

analysis of variance (ANOVA) followed by the Bonferroni post test, with Si and NaCl concentrations being the factors assessed. Computations were executed with the software program SPSS (Urdan, 2005).

RESULTS

Leaf number showed no significant differences between Si treatments, whether compared to the control, containing 0 mM Si, or to each other (data not shown). Plant height showed similar insignificance between treatments and controls, besides the expected general increase in height over the growing period (Figure 1). There was a

significant interaction between salt concentration and silicon treatments on fresh root weight ($P \leq 0.05$) and fresh shoot weight ($P \leq 0.1$). Main effect analysis showed a significant decrease in fresh root weight and fresh shoot weight ($P \leq 0.1$ and $P \leq 0.05$ respectively) between the two controls (Figures 2 and 3). Fresh root weight and fresh shoot weight showed no significant differences at Si levels of 1 and 4 mM whether grown with 30 or 60 mM NaCl, when compared to their respective control. The treatment of 2 mM Si exposed to 60 mM did however show a significant increase in fresh root ($P \leq 0.05$) and shoot weight ($P \leq 0.1$) when compared to the control. Although only 2 mM Si showed a significant effect on
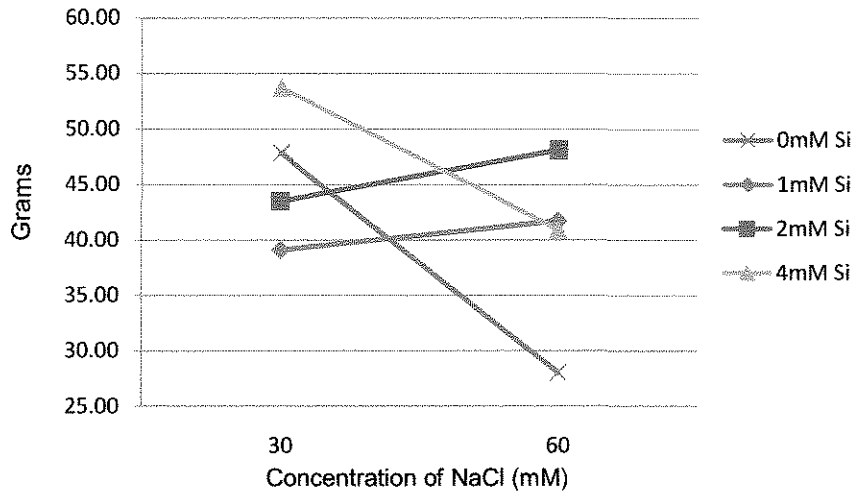
Figure 3. The effect of 30 and 60 mM NaCl on fresh shoot weight, with additions of 0, 1, 2 and 4 mM Si.
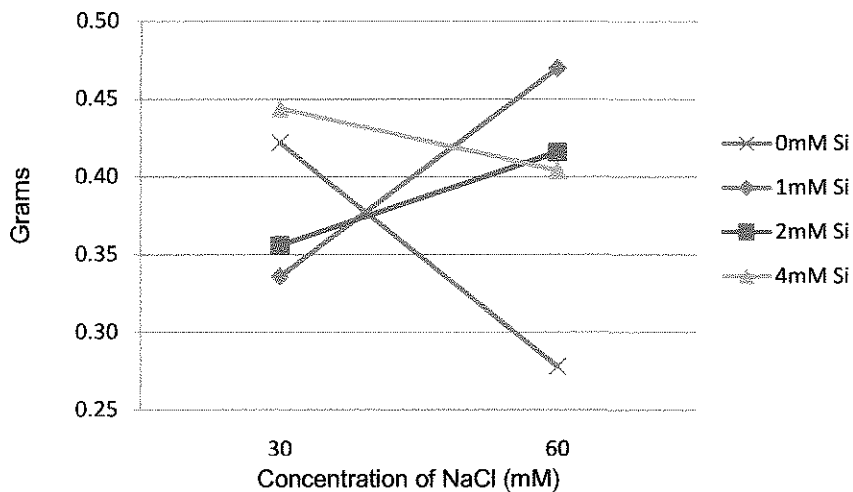
Figure 4. The effect of 30 and 60 mM NaCl on dry root weight, with additions of 0, 1, 2 and 4 mM Si.

fresh root and shoot weight, there was a marked, yet insignificant, increase in fresh root and shoot weight of 4 mM Si at 30 mM NaCl, and 1 mM and 4 mM Si at 60 mM when compared to the control (Figures 2 and 3).

There was also a significant interaction between salt concentration and silicon treatments on dry root weight (P ≤0.1) and dry shoot weight (P ≤ 0.05). Main effect analysis showed that dry root weight significantly decreased (P ≤ 0.1) between the control of 30 mM NaCl and that of 60 mM NaCl. Besides a significant increase (P ≤ 0.1) in dry root weight between 1 mM Si exposed to 60 mM NaCl and that of the control, the other treatments showed no significant differences, even though there was again a marked increase in weight, especially when compared to the 60 mM NaCl control (Figure 4). Dry shoot weight showed no significant increase between controls. However, there were significant increases (P ≤ 0.05) in weight of 1 and 2 mM Si when compared to the60 mM NaCl control. Again, although other treatments resulted in insignificant differences, there was a marked increase in root and shoot dry weight when compared to that of the control (Figure 5).

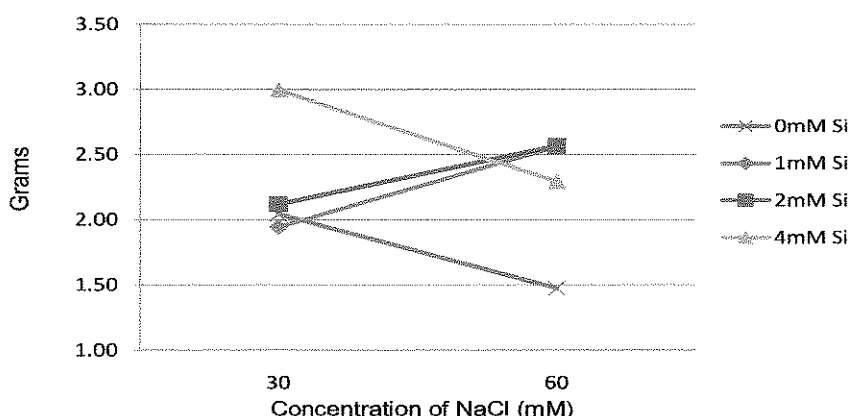Although there was a significant interaction between salt concentration and silicon treatments on root (P ≤0.001)

Figure 5. The effect of 30 and 60 mM NaCl on dry shoot weight, with additions of 0, 1, 2 and 4 mM Si.

Table 1. The effect of 30 and 60mM NaCl on Cl, Na and Si root and shoot content, with additions of 0, 1, 2 and 4mM Si.

Treatment		Cl (mg g^{-1})		Na (mg g^{-1})		Si (mg g^{-1})	
NaCl (mM)	Si (mM)	Root	Shoot	Root	Shoot	Root	Shoot
30	0	12.18	16.78	7.17	11.11	0.42	0.06
	1	9.78	18.70	8.12	11.41	0.44	0.12
	2	9.60	22.54	8.59	11.22	0.51	0.15**
	4	14.42	13.74	8.10	11.64	0.43	0.43***
60	0	15.98	21.20	14.30	16.88	0.37	0.07
	1	14.82	13.62	9.43	13.75***	0.29	0.11
	2	7.66	14.20	10.77	15.40**	0.45	0.16***
	4	74.8***	12.74*	8.83	14.00***	0.40	0.18***

and shoot (P ≤ 0.05) Cl content, main effect analysis showed that Cl concentrations only increased significantly in roots of the 4 mM Si with 60 mM NaCl treated plants and the shoots of 2 mM Si plants. This in turn corresponded to a significant decrease (P ≤ 0.1) in shoot Cl when compared to the control at 60 mM NaCl (Table 1). When comparing Cl content of plants treated with the same level of Si, 2 mM Si at 60 mM NaCl showed a significant decrease in shoot Cl content (P ≤ 0.05) compared to the plants treated with 30 mM NaCl. Contrarily, plants treated with 4 at 60 mM NaCl showed a significant increase in root Cl content (P ≤0.01) when compared to the plants treated with 30 mM NaCl (Table 2).

There was a significant interaction between salt concentration and silicon treatments on shoot Na content (P ≤0.001) but not on root Na content. Although no significant differences in root Na content were obtained, there was a marked decrease in Na root concentration at 60 mM NaCl (Figure 6). Main effect analysis showed that Na shoot content at 30 mM NaCl mirrored the results of

that of the roots, and showed no significant differences between treatments. However, Na shoot content at 60 mM NaCl with Si levels of 1, 2 and 4m M all resulted in a significant decrease in shoot Na content compared to that of the control (Table 1). When comparing Na content of plants treated with the same level of Si, root Na significantly increase between the controls of 30 and 60 mM NaCl (P ≤ 0.001) and shoot Na significantly increase (P ≤ 0.001) with 60 mM NaCl when compared to 30 mM NaCl at all Si levels (Table 2).

There was a significant interaction between salt concentration and silicon treatments on Si shoot content (P ≤0.001), but not on Si root content. Main effect analysis showed that there was however a significant decrease (P ≤ 0.05) of root Si between treatments at 30 and 60 mM NaCl receiving 1 mM Si. Si shoot concentrations of 2 and 4 mM, at both 30 and 60 mM NaCl showed a significant increase when compared to the control. When the two NaCl levels at the same 4 mM Si treatment were compared, a significant decrease (P ≤0.01)

Table 2. The effect of 30 and 60 mM NaCl on Cl, Na and Si root and shoot content, with additions of 0, 1, 2 and 4 mM Si.

Treatment		Cl (mg g^{-1})		Na (mg g^{-1})		Si (mg g^{-1})	
Si (mM)	NaCl (mM)	Roots	Shoots	Roots	Shoots	Roots	Shoots
0	30	12.18	16.78	7.17	11.11	0.42	0.06
	60	15.98	21.20	14.30***	16.88***	0.37	0.07
1	30	9.78	18.70	8.12	11.41	0.44	0.12
	60	14.82	13.62	9.43	13.75***	0.29**	0.11
2	30	9.60	22.54	8.59	11.22	0.51	0.15
	60	7.66	14.20**	10.77	15.40***	0.45	0.16
4	30	14.42	13.74	8.10	11.64	0.43	0.43
	60	74.8***	12.74	8.83	14.00***	0.40	0.18***

Values presented are means ± SE, n = 5. *; **; *** = significant at $P \leq 0.1^*$, $P \leq 0.05^{**}$ or $P \leq 0.01^{***}$. Significance compared to that of the same Si concentrations, with different NaCl concentrations.
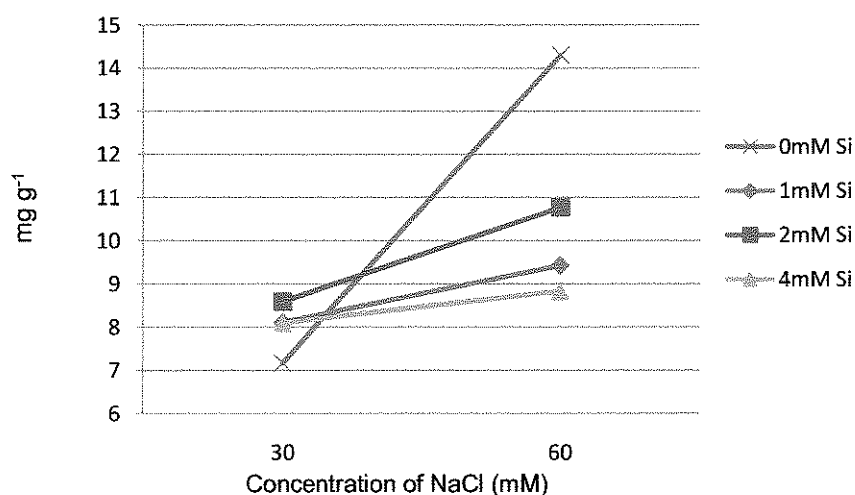
Figure 6. The effect of 30 and 60 mM NaCl on Na root content, with additions of 0, 1, 2 and 4 mM Si.

in Si was observed in the shoots. This was apparent in the 4 mM treatment alone (Table 2).

There was a significant interaction between salt concentration and silicon treatments on chlorophyll content (P ≤ 0.05). Main effect analysis revealed that, when comparing Si treated plants to the controls, there was only a significant decrease in chlorophyll with applications of 2 mM Si at 30 mM to that of the control (Figure 7).

DISCUSSION

The results obtained from this study support the

fundamental findings of previous studies, that Si has beneficial mitigating properties with regards to NaCl induced salinity stress. These findings indicate some benefits of Si applications at 30 mM NaCl; however, the benefits of Si seem to be more apparent with the higher experimental concentrations of 60 mM NaCl. This is evident when looking at the significant increase in fresh root and shoot weight at 2 mM Si at 60 mM NaCl, the significant increases in dry root, at 1 mM Si, dry shoot weight at 1 and 2 mM, and the overall marked increases in fresh and dry weights when comparing all Si treatments to that of the 60 mM NaCl control (Figures 2 to 5). These findings are supported by the literature which
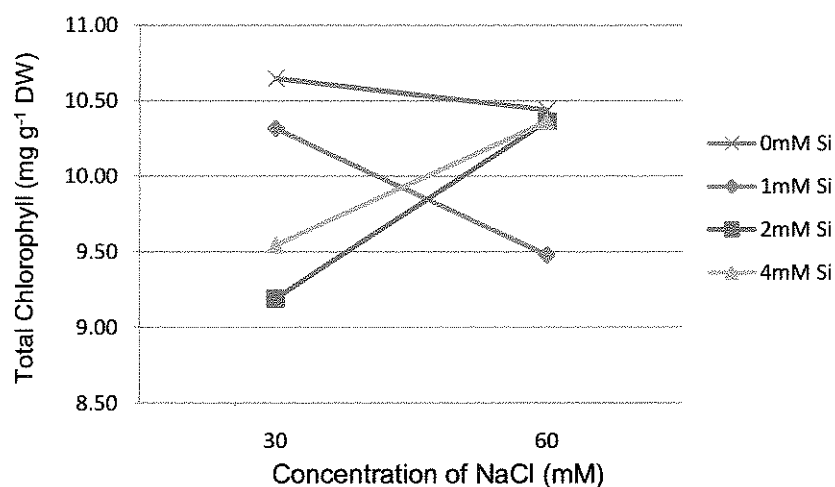
Figure 7. The effect of 30 and 60 mM NaCl on total chlorophyll content with additions of 0, 1, 2 and 4 mM Si.

states that dry shoot and root weights of plants increase with applications of Si compared to a range of NaCl concentrations lacking Si (Romero-Aranda et al., 2006; Tuna et al., 2008; Ashraf et al., 2010).

Chlorophyll content showed no significant differences, with only one exception with an application 2 mM Si at 30 mM NaCl (Figure 7). This is in line with Tas et al. (2005), who although did not investigate Si, reported negligible effects of NaCl on chlorophyll content on lettuce. However, in a study conducted by Hashemi et al. (2010), evaluating the mitigating effects of Si on Canola, a vast increase in chlorophyll content was found.

A notable oddity with regards to elemental analysis was the exceptional accumulation of Cl in the roots of the plants treated with 4 mM Si at 60 mM (Tables 1 and 2). This, when compared to the control, corresponded to a significant decrease in Cl accumulation in shoots. Savvas et al. (2007, 2009) found similar results, reporting a significant decrease in shoot Cl content with Si applications. However, Romero-Aranda et al. (2006), and Soylemezoglu et al. (2009), reported a slight increase in Cl shoots concentration with applications of Si.

A significant finding with regards Na content was the reduction of Na within shoots when Si treated plants was exposed to 60 mM NaCl when compared to the control (Table 1). The decrease of Na content in Si treated plants has been a major finding when evaluating the ability of Si to mitigate NaCl salinity stress. Many studies that evaluate Si and NaCl relate NaCl mitigation to a reduction in Na shoot concentrations (Saqib et al., 2008; Tuna et al., 2008; Savvas et al., 2009; Ashraf et al., 2010), the reduction of which may be linked to Si reducing transpiration, as concluded by Ma et al. (2001). Savvas (2007), also reported a decrease in Na with applications of Si, but did not conclude this to be factor influencing

the ability of Si to mitigate salinity stress.

Other mechanisms of salinity mitigation by Si have been suggested, including the ability of Si to improve water storage within plant tissues. Due to the higher growth rate that this would allow, it follows that salt concentrations would decrease through dilution, and therefore ameliorate salt toxicity (Romero-Aranda et al., 2006). Anti oxidants have also been a topic of investigation, with Liang (1999) and Zhu et al. (2004a, b), investigating the interaction of Si with antioxidants in salt stressed barley, cucumber and tomato respectively, with promising results.

Although transpiration and anti oxidants were not factors investigated in this study, plant weight increases, and subsequent decreases in Na shoot concentrations, leads to the conclusion that mitigation of NaCl induced salinity with applications of Si was at least partially due to the reduction of shoot Na content. Additions of Si to plants under salinity stress can be recommended, especially at higher salinity levels, as shown by the results of this paper.

ACKNOWLEDGEMENTS

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Salinity induced changes in oxidative stress and antioxidant status as affected by applications of silicon in lettuce (*Lactuca sativa*)

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Abstract

The antioxidant content, capacity and certain oxidative stress parameters were investigated in salinity stressed *Lactuca sativa* L. 'Eish!' with the addition of exogenous silicon (Si). No significant changes were shown for total polyphenol concentrations in any of the experimental plants. Applications of 4mM Si significantly increased oxygen radical absorbance capacity analysis (ORAC) values at both 30 and 60mM NaCl. The redox status of glutathione was also improved with a significant increase in reduced glutathione (GSH) in 2mM Si treated plants at 30mM NaCl. Increases in both GSH and oxidised glutathione (GSSG) were noted at 60mM NaCl. The activity of catalase (CAT) was

significantly decreased with the addition of 1 and 4mM Si at 30mM NaCl. Lipid peroxidation (measured as thiobarbituric acid reactive substances, TBARS) was not influenced by the different Si concentrations. These results suggest that applications of Si could be beneficial with regards to the modulation of oxidative stress in salinity stressed lettuce, and should be considered as an addition when cultivating lettuce.

Key Words: Potassium silicate, ROS, Antioxidants, Hydroponics

Introduction

Salinity is a significant plant stress factor and a major problem on all continents (Ghassemi et al., 1995). Saline conditions influence plant growth in two core ways; by increasing the osmotic potential of the soil solution, which has a direct effect on plant-water availability (Carter, 1981), and through ion toxicities, determined by the specific ions that are in excess (Marschner, 1995). In most cases, sodium (Na^+) and chloride (Cl^-) ions are the cause of saline conditions (Harris, 1992; Epstein & Bloom, 2005). The effect of NaCl induced salinity on plants differ from plant to plant, but generally leads to a reduced growth rate, sometimes nutritional deficiencies, and at higher concentrations, the accumulation of Na^+ and Cl^-, resulting in leaf scorching, chlorosis and necrosis (Shannon & Grieve, 1999; Wahid et al., 1999). Along with these symptoms, salinity stress is known to result in an excess production of reactive oxygen species (ROS) (Jaspers et al., 2005), oxidative damage and a change in

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concentrations of antioxidants (Bor *et al.*, 2003; Sekmen *et al.*, 2007; Gao *et al.*, 2008). Consequently, ROS are good cellular indicators of stress (Mittler, 2002).

Silicon (Si), a quasi-essential element for plants (Epstein, 1999), has proved to have significant ameliorative properties regarding NaCl toxicity in many important agricultural and ornamental crops. It has shown to repeatedly result in an increase in yield together with other beneficial growth parameters (Romero-Aranda *et al.*, 2006; Savvas *et al.*, 2007; Soylemezoglu *et al.*, 2009). Silicon allows plants to grow more productively on saline land, leading to definite economic benefits, especially for developing nations (Shay, 1990).

The literature disagrees in the manner by which Si mitigates NaCl toxicity. Si has the ability to influence Na absorption, distribution or transportation (Ahmad *et al.*, 1992; Savvas *et al.*, 2009; Soylemezoglu *et al.*, 2009; Hashemi, *et al.*, 2010), which is sometimes associated with an increase in potassium (K) concentration (Saqib *et al.*, 2008; Tuna *et al.*, 2008; Ashraf *et al.*, 2010). Ameliorative properties of silicon have also been associated with the decrease in plant Na concentrations through dilution, thus mitigating salt toxicity (Romero-Aranda *et al.*, 2006). Ma *et al.* (2001), suggested silicon's beneficial effects stems from the decreased Na influx through a Si induced decrease in transpiration. In addition to Si – Na related amelioration of salinity stress, studies have indicated that Si can influence antioxidant activity. This has been reported in, amongst others, salt stressed barley, (Liang, 1999), cucumber, (Zhu *et al.*, 2004a), tomato (Zhu *et al.*, 2004b) and wheat (Saqib *et al.*, 2008).

In this study the influence of Si in NaCl stressed *Lactuca sativa* L. 'Eish!' was investigated in order to assess whether the known Si induced yield increase can be associated with antioxidant concentrations and oxidative damage. Furthermore, it is to our knowledge that this is the first study examining Si applications influencing antioxidant status and oxidative damage in NaCl salinity stressed lettuce.

Materials and Methods

Plant Conditions and Cultivation

Lettuce was cultivated in a research greenhouse at the Cape Peninsula University of Technology (Cape Town, South Africa). Greenhouse temperatures ranged between a minimum of nine and maximum of 29°C, and humidity between a minimum of 44% and a maximum of 99%. Seeds were sown in a 128 plug polystyrene seedling tray, with vermiculite as the sowing medium. Seeds germinated eight days after sowing, and the seedlings were fertilized daily with half strength NUTRIFEED (Starke Ayres, South Africa); a complete, water soluble fertilizer providing 9.3mM N, 1.7mM P, 6.7mM K, 3.5mM Ca, 1.8mM Mg, 4.7mM S, 53.7μM Fe, 8.7μM Mn, 44.4μM B, 1.5μM Zn, 6.3μM Cu and 0.2μM Mo. Four weeks after sowing, 80 seedlings were chosen at random, and transplanted into 12.5cm plastic pots, with HYDROTON (Germany) expanded clay used as the medium, and then moved directly into the soilless growing systems. There were a total of eight, closed, sub irrigative, soilless growing

Polyphenol Antioxidant Content and Capacity

Total Polyphenol (TP) Analysis

For TP analysis, 125mg of lyophilized plant material was homogenised with 6mL of methanol on ice, and centrifuged in 2mL microcentrifuge tubes at 15 000 g for 10 minutes at 4°C. 25µL undiluted supernatant was then transferred, in triplicate, to a 96 well plate containing 125µL of 10 times diluted Folin-Ciocalteu's phenol reagent (0.2N). After 5 minutes, 100 µL of sodium carbonate (7.5%, w/v) was added and the resulting mixture incubated at room temperature for a further two hours. Absorbance values at 765nm were recorded and compared to those of a Gallic acid standard. Results are expressed as mg GAE g^{-1}.

Oxygen Radical Absorbance Capacity Analysis

Extraction followed that of TP, with 12µL of 10 times diluted supernatant being added to 138µL fluorescein and 50µL AAPH, and analyzed, in triplicate, using a fluorescence spectrophotometer until zero fluorescence occurred. The ORAC values were calculated by dividing the sample curve by that of the Trolox standard, and expressed as µmol TE g^{-1} (Ou et al., 2001).

Catalase Analysis

Catalase (CAT) activity was measured, in triplicate, by placing 20µL of the supernatant (obtained from protein analysis, refer 2.5) with 170µL of a 50mM Potassium phosphate buffer (pH7.0) and 75µL H_2O_2 in a 96 well micro plate. The disappearance of H_2O_2 was measured over a 2 minute period by measuring absorbance levels at 240nm every 15 seconds. Catalase activity was calculated using the extinction coefficient of H_2O_2 and expressed as µmol min^{-1} $µg^{-1}$ protein (Aebi, 1984).

Glutathione Analysis

For GSH extraction, 125mg of lyophilized plant material was homogenised with 6mL of 5% Trichloroacetic Acid buffer, on ice. The homogenate was centrifuged in 2mL microcentrifuge tubes at 15 000 g for 10 minutes at 4°C. The resulting supernatant was transferred to new 2mL microcentrifuge tubes and stored at -80°C until needed.

Glutathione concentrations were measured, in triplicate, by placing 50µL of 50 times diluted supernatant, with 50µL of 0.3mM DTNB (5,5'-Dithiobis(2-nitrobenzoic acid)) and 50µL glutathione reductase (GR) solution (0.02U/µL) in a 96 well micro plate. After a 5 minute incubation period at room temperature, 50µL of 1mM NADPH was placed in each well. Absorbance levels at 412nm were measured at 15 second intervals for 5 minutes. Absorbance levels were

compared to those of a GSH standard curve, and total GSH expressed as µmol g^{-1} dried plant material.

GSSG extraction followed that of GSH, except for the addition of 4.8mg of M2VP (1-Methyl-2-vinylpyridinium) within the homogenate buffer. Measurement of GSSG followed that of GSH, except a 25 times diluted supernatant was used, and absorbance levels were compared to those of a GSSG standard curve (Asensi et al., 1999).

Lipid Peroxidation Analysis

For thiobarbituric acid reactive substances extraction, the method by Yagi, (1984), adapted for plant samples, was utilised. 125mg of lyophilized plant material was homogenised with 6mL of Methanol, on ice. The homogenate was centrifuged in 2mL microcentrifuge tubes at 15 000 g for 10 minutes at room temperature. The resulting supernatant was transferred to new 2mL microcentrifuge tubes and stored at -80°C until needed.

250µL of undiluted supernatant, with 375µL of 0.44mM PCA (perchloric acid) and 125µL of 42mM TBA (2-Thiobarbituric acid) were place in 2mL microcentrifuge tubes. After a 60 minute incubation period at 100°C, the 2mL microcentrifuge tubes were centrifuged at 10 000 g for 5 minutes at room temperature. The resulting supernatant was placed, in triplicate, in a 96 well plate, and absorbance at 532nm were compared to those of the

malondialdehyde standard and expressed as μmol g^{-1} dried plant material (Yagi, 1984).

Statistical Analysis

Statistical analysis was performed using two–way analysis of variance (ANOVA), followed by the Bonferroni post test, with P values ≤ 0.05 considered significant. Pearson correlations coefficient was used to calculate correlations. Computations were executed with the software program SPSS (Urdan, 2005).

Results

Plant weights showed no significant differences when treated plants were compared to the 30mM NaCl control. However, there were a 12 and 17% increase in fresh shoot and root weight and a 46% increase in dry shoot weight when comparing the 4mM Si treatment to the control (Table I).

However, plant weights showed a clear increase when treated plants were compared to the 60mM NaCl control. Although 2mM Si was the only treatment that resulted in a marginally significant increase (P ≤ 0.1) in fresh shoot weight, the increase was considerable (72%). The fresh root weight of the 2mM Si treated plants resulted in a significant increase (75%) when compared to the control. Dry shoot weights significantly increase by 74 and 75% with both 1 and

2mM Si treated plants respectively when compared to the control. Although 4mM Si treated plants showed no significant difference when compared to the 60mM NaCl control, there was a marked increase of 56%. Dry root weights showed a marginally significant increase of 68% with 1mM Si when compared to the 60mM NaCl control. Again, although dry root weights of 2 and 4mM Si treated plants resulted in no significant differences when compared to the control, there was a noteworthy increase of 50 and 43% respectively.

Shoot Si concentrations of 1mM Si treated plants showed no significant difference at both 30 and 60mM NaCl when compared to their individual controls. Nevertheless, treatments of 2 and 4mM Si at 30mM NaCl resulted in a significant increase of 150% and 616% respectively. Similarly, treatments of 2 and 4mM Si at 60mM NaCl resulted in a significant increase of 129% and 157% respectively (Fig. 1).

With regards to antioxidant status and oxidative stress between the two controls, there were no significant differences between any of the parameters, besides that of the TP controls. The 60mM NaCl control showed a 18% increase in TP concentrations when compared to that of the 30mM NaCl control, resulting in a marginal difference (P ≤ 0.1) between the two controls. However, when Si treated plants were compared to that of the controls, TP concentrations showed no differences (Table II). ORAC values showed a tendency to increase with applications of Si at all concentrations when compared to the controls. However, only 4mM Si at both 30 and 60mM NaCl

showed significant increases when compared to controls, with a 28% and 21% increase respectively.

Catalase activities showed a clear tendency to decrease with applications of Si when plants were exposed to 30mM NaCl, with 1 and 4mM Si, a decrease of 29 and 25% respectively, being significant when compared to the 30mM NaCl control (Table III). Conversely Si applications at 60mM NaCl showed no tendency to decrease, and showed no significance when compared to that of the 60mM control.

Concentrations of both GSH and GSSG, like that of ORAC, tended to increase with applications of Si. Concentrations of GSH at 2mM Si with 30mM NaCl, and both 1 and 4mM Si with 60mM NaCl showed significant increases of 138%, 106% and 99% respectively, when compared to their individual controls. Concentrations of GSSG showed a significant increase of 100% at 4mM Si, and a marginally significance increase of 93% ($P \le 0.1$) at 1mM Si when compared to the 60mM NaCl control. The ratio of GSH:GSSG showed a limited trend to increase or decrease, with an application of 2mM Si with 30mM NaCl being the only treatment showing a significant increase of 33% ($P \le 0.1$) when compared to the control.

Like that of TP concentrations, TBARS values showed no significant differences when any of the treatments were compared to their respective controls. In addition to this, no correlations were found when comparing variables within treatments.

Discussion

Plant stresses, including salinity stress, are known to disturb cellular homeostasis, enhancing the production of ROS. (Dat *et al.*, 2000). Additionally, osmotic stress, one of the foremost stresses associated with high salinity levels, has been shown to cause the production of ROS (Xiong & Zhu, 2002).

Although ROS have roles as signalling molecules, active generation of which can be initiated by abiotic stresses (Desikan *et al.*, 2005), ROS are traditionally thought of as species causing cell injury or death (Mittler, 2002). In the light of this, plants need ways to detoxify ROS. CAT is a major ROS scavenging enzyme seen as indispensable, with stress bringing about the production of CAT (Mittler, 2002). With ROS causing the production of CAT, it can be argued that lower concentrations of CAT, indicating lower levels of ROS, would be a sign of less oxidative stress. In this study, with the application of Si, there was an overall tendency for CAT concentrations to decrease, significantly with 1 and 4mM at 30mM NaCl. Zhu *et al.* (2004a), however, showed that Si raises CAT levels in salt treated tomatoes, while Zhu *et al.* (2004b) reported tendencies for CAT levels to decrease with applications of Si on salt treated plants. Bor *et al.* (2003), Gao *et al.* (2008) and Eraslan *et al.* (2007), although not investigating Si, found applications of NaCl to significantly increase CAT activities, supporting the idea that increased CAT activities could be indicative of oxidative stress. Gong *et al.* (2005) when investigating applications of Si to ameliorate drought stress, found that applications of Si lead to significantly decreased CAT activity.

This is relevant to NaCl stress, as osmotic stress is a primary limitation for plants growing in saline conditions (Marschner, 1995).

Contrary to CAT activities, yet similarly indicating a decrease in oxidative stress, ORAC levels showed a tendency to increase, significantly at 4mM Si with both 30 and 60mM NaCl. ORAC, a hydrophilic antioxidant capacity assay, is one of the most commonly used methods for measuring antioxidative capacity in biology (Yeum et al., 2010). It is assumed that with an increase in TP concentrations, there would be a correlative increase in ORAC values. The level of TP in Si treated plants showed no significant differences between controls, and perhaps even a tendency to decrease. Liu et al. (2007) reported similar results, although with relatively higher TP concentrations, with no correlation to the DPPH assay, a procedure that has shown to be comparable to that of the ORAC assay (Thaipong et al., 2006), even though different mechanisms are involved (Ndhlala et al., 2010).

TBARS concentrations, a well-known indicator of lipid peroxidation (Wada et al., 2008), a process that can lead to cell death (Mittler, 2002), showed no significant differences between the treated and the control plants, and no trend to increase or decrease. This is in disagreement with Liang (1999), Zhu et al. (2004a & b), all finding applications of Si to decrease lipid peroxidation.

Increases in GSH concentrations are known to be associated with salinity stress (Ruiz & Blumwald, 2002; Leyva et al., 2011). The results from this study showed that applications of Si tended to increase GSH concentrations,

significantly with 2mM Si at 30mM NaCl and with 1 and 4mM Si at 60mM NaCl, when compared to NaCl alone. These results are in agreement with Saquib *et al.* (2008), reporting that GSH increases with applications of Si in NaCl stressed wheat. Foyer *et al.* (2005) argue that the signal responses that could be brought about by the interaction between GSH and the GSH:GSSG ratio are linked to plant growth cessation. This is interesting when considering that, contrary to conventional thought, active plant growth cessation, as opposed to stress limiting growth, has been linked to surviving adverse environmental conditions (Harberd *et al.*, 2009), including salinity stress (Magome *et al.*, 2008). However, applications of Si are widely reported to increase plant growth (Romero-Aranda *et al.*, 2006; Soylemezoglu *et al.*, 2009), which is in line with the data presented in this study. Therefore, applications of Si induced increases in GSH concentrations seem unlikely to be associated with a growth cessation stress response. GSH are well known antioxidants, and higher concentrations would infer superior antioxidative defence (Tausz *et al.*, 2004), and would therefore logically result in a decrease in ROS concentrations brought about by salinity stress (Foyer *et al.*, 2005). This approach would arguably provide more probable grounds for the increases in GSH with applications of Si.

The overall impression of Si applications in relation to antioxidant concentrations and oxidative stress modulation ROS is that fertilization and regular inclusion of Si in nutrient solutions, where Si is often limited, is of value to lettuce cultivation. Investigations into the effects of higher salinity concentrations would likely show clear cut results with regards to the effects of Si applications on antioxidant status in salinity stressed lettuce, and is a

recommended focus for future studies. In addition to this, an investigation into other antioxidant related factors such as ascorbate peroxidase, glutathione reductase and ascorbic acid could provide more insight into Si induced amelioration of oxidative stress.

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Table I:
Effects of Si applications on plant fresh and dry root and shoot weights in salinity stressed lettuce.

NaCl	Si	Fresh Weight (g)		Dry Weight (g)	
		Shoots	Roots	Shoots	Roots
30mM	0mM	47.85 ± 17.56	6.49 ± 1.83	2.04 ± 0.51	0.42 ± 0.1
	1mM	39.1 ± 13.07	5.74 ± 1.81	1.94 ± 0.73	0.34 ± 0.1
	2mM	43.52 ± 10.49	5.75 ± 1.54	2.12 ± 0.55	0.36 ± 0.09
	4mM	53.70 ± 11.54	7.64 ± 1.60	2.99 ± 0.56	0.44 ± 0.09
60mM	0mM	28.05 ± 6.55	4.52 ± 1.27	1.47 ± 0.31	0.28 ± 0.1
	1mM	41.73 ± 12.39	7.29 ± 2.38	2.56 ± 0.73**	0.47 ± 0.19*
	2mM	48.12 ± 14.92*	7.92 ± 2.06**	2.57 ± 0.69**	0.42 ± 0.14
	4mM	40.97 ± 9.83	6.44 ± 1.52	2.29 ± 0.59	0.40 ± 0.05

Mean values of treated plants were compared to that of the controls (0mM Si). Significance levels are represented by *$P \leq 0.1$, **$P \leq 0.05$.

Table II:
Effects of Si applications on Total Polyphenolics (TP) and Oxidative Radical Absorbance Capacity, in salinity stressed lettuce.

NaCl	Si	TP (mg GAE g^{-1})	ORAC (μmol TE g^{-1})
30mM	0mM	5.05 ± 0.37	49.28 ± 3.93
	1mM	4.23 ± 0.35	56.81 ± 4.23
	2mM	4.71 ± 0.59	54.99 ± 4.07
	4mM	5.19 ± 0.63	63.16 ± 5.83*
60mM	0mM	5.97 ± 0.58	47.72 ± 3.22
	1mM	5.25 ± 0.59	55.12 ± 9.86
	2mM	4.98 ± 0.50	54.70 ± 5.74
	4mM	5.85 ± 0.65	57.51 ± 2.79**

Mean values of treated plants were compared to that of the controls (0mM Si). Significance levels are represented by *$P \leq 0.1$, **$P \leq 0.05$, ***$P \leq 0.001$.

Table III:
Effects of Si applications on Thiobarbituric Acid Reactive Substances (TBARS), Glutathione (GSH), Glutathione disulfide (GSSG), GSH:GSSG ratio and Catalase (CAT), in salinity stressed lettuce.

NaCl	Si	TBARS (μM g^{-1})	GSH (μmol g^{-1})	GSSG (μmol g^{-1})	GSH:GSSG	CAT (μmol min^{-1} ug^{-1})
30mM	0mM	0.26 ± 0.03	0.91 ± 0.37	0.15 ± 0.06	5.96 ± 0.81	1.03 ± 0.09
	1mM	0.26 ± 0.04	0.99 ± 0.2	0.19 ± 0.05	5.20 ± 0.27	0.73 ± 0.09***
	2mM	0.24 ± 0.03	2.17 ± 0.24***	0.23 ± 0.08	7.94 ± 0.59*	0.86 ± 0.12
	4mM	0.29 ± 0.04	1.43 ± 0.46	0.24 ± 0.07	5.91 ± 0.67	0.77 ± 0.11**
60mM	0mM	0.25 ± 0.02	1.04 ± 0.83	0.15 ± 0.1	6.94 ± 1.76	1.12 ± 0.10
	1mM	0.28 ± 0.03	2.14 ± 0.37**	0.29 ± 0.07*	7.69 ± 1.87	1.03 ± 0.06
	2mM	0.25 ± 0.03	1.47 ± 0.38	0.25 ± 0.06	5.87 ± 0.15	0.95 ± 0.11
	4mM	0.26 ± 0.03	2.07 ± 0.28**	0.30 ± 0.05**	7.06 ± 1.01	1.28 ± 0.16

Mean values of treated plants were compared to that of the controls (0mM Si). Significance levels are represented by *$P \leq 0.1$, **$P \leq 0.05$, ***$P \leq 0.001$.
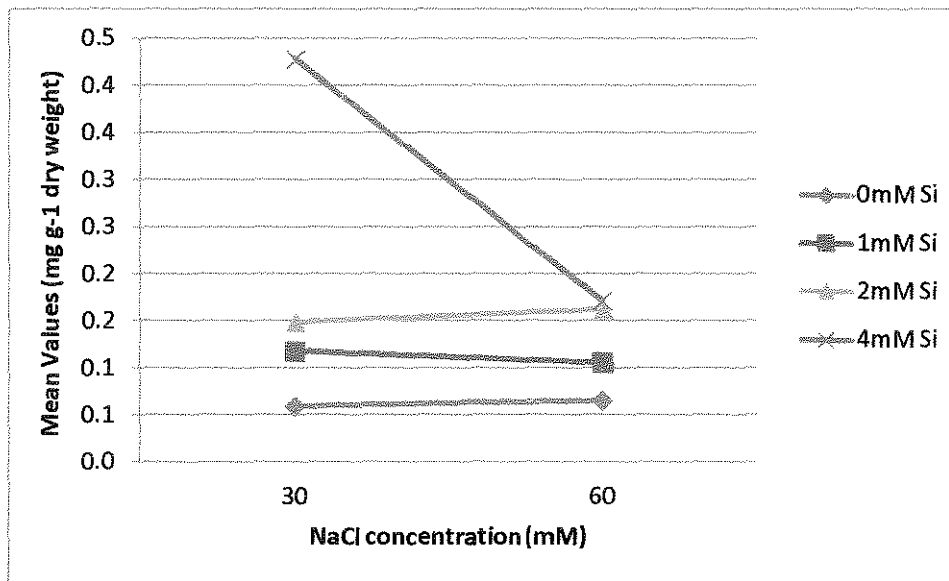
Figure 1. Effect of Si applications on Si shoot concentrations in salinity stressed lettuce.