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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 4 mM Si significantly increased oxygen radical absorbance capacity analysis (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 2 mM 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; 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 throughion toxicities, determined by the specific ions that are in excess (Marschner, 1995; Qadir *et al*., 2001). 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 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

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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 *Lactucasativa* 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 9.3 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 12.5 cm 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 systems, delivering ±1cm of re-circulating nutrient solution. Each of these eight systems had a different treatment, equating to eight treatments with 10 plants (repeats) per treatment.

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 mMCa, 2 mM P, 1 mM S and 1 mM Mg respectively, based on the modified Hoagland solution as described by Epstein and Bloom (2005). 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 dS m^{-1}, was formulated using deionized water.

Silicon and NaCl treatments: Si was added to the basal nutrient solution using K_2SiO_3 (AGRISIL K50), at concentrations of 0, 1, 2 and 4 mM Si. Two concentrations of NaCl (30 & 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 dSm^{-1}, and treatments containing 0, 1, 2 and 4 mM Si and 60 mM NaCl, had a mean EC of 8.10 dSm^{-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.

Sampling and Harvesting

Antioxidant status and oxidative damage: The shoots of 5 of the repeats per treatment were harvested 6 weeks after being transplanted into the soilless growing system. Once harvested, shoots were rinsed twice in deionized 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 lyophilized at -80°C for 16 h. Once the lyophilization was complete, samples were ground to a powder using a mortar and pestle and stored at -80°C until analyzed.

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 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.

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

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

Polyphenol Antioxidant Content and Capacity

Total polyphenol (TP) analysis: For TP analysis, 125 mg of lyophilized plant material was homogenised with 6 mL of methanol on ice, and centrifuged in 2 mL microcentrifuge tubes at 15 000 *g* for 10 min 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 (0.2N). After 5 min, 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}.

Oxygen radical absorbance capacity analysis: Extraction followed that of TP, with 12 µL of 10 times diluted supernatant being added to 138 µL fluoresce in and 50 µL 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 20 µL of the supernatant (obtained from protein analysis, refer 2.5) with 170 µL of a 50 mM Potassium phosphate buffer (pH 7.0) and 75 µL H_2O_2 in a 96 well micro plate. The disappearance of H_2O_2 was measured over a 2 min period by measuring absorbance levels at 240 nm every 15 sec. 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, 125 mg of lyophilized plant material was homogenised with 6 mL of 5% Trichloroacetic Acid buffer, on ice. The homogenate was centrifuged in 2 mL micro centrifuge tubes at 15 000 *g* for 10 min at 4°C. The resulting supernatant was transferred to new 2 mL micro centrifuge 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 50 µL glutathionereductase (GR) solution (0.02 U/µL) in a 96 well micro plate. After a 5 minute incubation period at room temperature, 50 µL of 1 m MNADPH was placed in each well. Absorbance levels at 412 nm were measured at 15 sec intervals for 5 min. 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).

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

A 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 micro centrifuge tubes. After a 60 min incubation period at 100°C, the 2 mL micro centrifuge tubes were centrifuged at 10 000 *g* for 5 min at room temperature. The resulting supernatant was placed, in triplicate, in a 96 well plate, and absorbance at 532 nm 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 Bonferronipost 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).

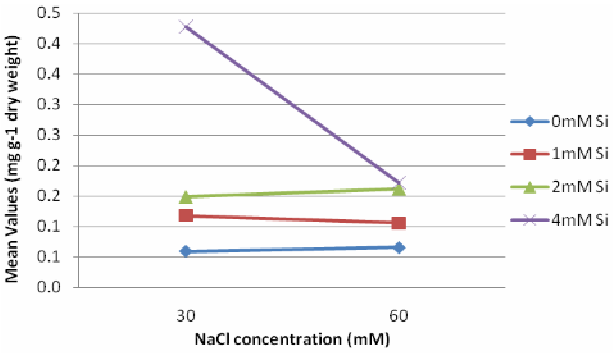
RESULTS

Plant weights showed no significant differences when treated plants were compared to the 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 I). 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 ($P \leq 0.1$) in fresh shoot weight, the increase was 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 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 marginally 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 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 (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 60 mM NaCl control showed a 18% increase in TP concentrations when compared to that of the 30 mM NaCl control, resulting in a marginal difference

Fig. 1: Effect of Si applications on Si shoot concentrations in salinity stressed lettuce



(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 4 mM Si at both 30 and 60 mM 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 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 (Table III). 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.

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. Concentrations of GSSG showed a significant increase of 100% at 4 mM Si, and a marginally significance increase of 93% ($P \leq 0.1$) 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% ($P \leq 0.1$) 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).

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
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 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})
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 ≤ 0.1, **P ≤ 0.05, ***P ≤ 0.001

Table III: Effects of Si applications onThiobarbituric 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 ≤ 0.1, **P ≤ 0.05, ***P ≤ 0.001

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).

Contrary to CAT activities, 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 & 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 2 mM Si at 30 mM NaCl and with 1 and 4 mM Si at 60 mM 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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