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Full Length Research Paper

Silicon improves growth and antioxidative defense system in salt-stressed Kentucky bluegrass (*Poa pratensis* L.), 'Perfection' and 'Midnight'

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After 400 mM of NaCl treatment, 0.1 and 1.0 mM of silicon (Si) were added to Kentucky bluegrass, 'Perfection' and 'Midnight,' to identify the effect of Si on the antioxidant defense mechanisms and inorganic ions as a parameter in the salt stress on grass. Compared to the control, the NaCl treatment caused a significant decrease in the shoot length and the fresh and dry weight of shoot and root of the both types of Kentucky bluegrass. Adding Si after the NaCl treatment increased the growth and dry and fresh weight of shoot and root in the grass. In both types, the Na^+ concentration significantly increased after the NaCl-only treatment compared to control and decreased dramatically after 0.1 and 1.0 mM Si were added following the NaCl treatment compared to the NaCl-only treatment. K^+ and Si concentrations remarkably increased in the shoot and root when Si was added after NaCl treatment. There was a significant reduction in the oxygen radical absorption capacity and the total phenolic compounds in the both types. Compared to the NaCl-only treatment, higher glutathione and lower proline concentrations were observed in the plant treated with Si after NaCl treatment. These results suggest that, even though Si is not generally classified as 'essential element', Si may have a significant involvement in the antioxidant defense mechanisms and inorganic ions in the salt stress on grass.

Key words: diphenyl-1-picrylhydrazyl (DPPH), total phenolic concentration, proline, salt stress.

INTRODUCTION

Among the plant species belonging to the Gramineae family, grass with a relatively excellent adaptability to various environments is often used to cover barren soil and with an increasing public interest in the quality of life and green environment, the use of grass has been extended in terms of range and areas. Due to the increased use and plantation of grass, there is a need to establish improvement measures with respect to the problematic factors that degrade the quality of grass. In regards to salt stress, which is one of the causes of reduced grass quality, damage is caused by high salinity arising from continuous fertilization and irrigation using underground water. Also, because grass is a perennial plant, it is difficult to improve or change the plantation soil once it is planted. In regards to the management aspect, there are difficulties to maintain normal growth of grass planted in an inappropriate soil environment (Kim et al., 2008). In particular, the soil of sites near reclaimed or coastal areas has lower soil fertility and higher salt concentration compared to general soil and thus, causes nutritional imbalances due to toxicity (mainly Na^+ and Cl^-), reduced water potential and inhibition of ion uptake and transport, leading to growth and physiological impairment (Munns and Termaat, 1986).

Generally, various environmental stresses, including

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salt stress, impedes growth by reducing the photosynthetic ability of plants and a reduction in the carbon assimilation rate increases the flow of photosynthetic electrons to oxygen (O_2), which is known to exert an oxidative stress on plants by promoting the production of reactive oxygen species (ROS) (Bartosz, 1997). ROS with high oxidizing power is known to destroy proteins, lipids, pigments and genes if not removed quickly. Thus, most plants have a defensive restoration mechanism to respond to oxidative damages and develop enzymatic and non-enzymatic (carotenes, ascorbate, glutathione, etc., which are known as antioxidant molecules) antioxidant defense mechanisms in response to ROS to avoid oxidative damage (Baier and Dietz, 1998).

As such, the production of H_2O_2 is increased in different compartments of plant cells due to the enzymatic and non-enzymatic reactions that occur when plants are under stress (Foyer et al., 1997). Thus, an increase in the concentration of H_2O_2 when plant is under salt stress may be a signal of an adaptive response to stress (Foyer et al., 1997; Van Breusegem et al., 2001). With respect to the defensive mechanisms against oxidative stress, glutathione is involved in the ascorbate glutathion pathway and the redox state of protein thiol (Alscher et al., 1997), and protects plants against salt stress by maintaining the redox state (Shalata et al., 2001). Glutathione is also known as a major antioxidant in plants that is involved in protecting proteins from harmful oxidative effects.

When salt stress is applied to a plant, various substances are synthesized in the plant cells. Major substances include amino acids such as glutamine, proline and arginine involved in the synthesis of polyamine and polyamines including putrescine, spermine and spermidine (Krishnamurthy and Bhageat, 1989). In addition, phenolic compounds and sugars such as sorbitol, mannitol and arabitol have been reported to be synthesized under salt stress (Kakani et al., 2003). These substances increase the osmotic pressure and water potential in cells to help maintain the appropriate function of enzymes. Accordingly, there has also been research on the effect of external addition of these substances on the salt reduction effect (Lutts et al., 1996).

Among these substances, proline is an amino acid that typically accumulates under low water potential conditions such as in a dry condition or under salt stress. It not only acts as an osmotic regulator but also protects proteins from thermodynamic instability caused by dehydration. It is also reported to account for more than 2/3 of the total amino acid content (Voetberg and Sharp, 1991). Accumulation of proline is an adaptive response to salt stress and water shortage in plants and has been reported as a factor used to determine the salt tolerance of plants (Ramajulu and Sudhakar, 2000). However, there are also reports that proline accumulation has no correlation with the degree of salt tolerance and only appears as stress-induced damage (Liu and Zhu, 1997).

K^+ plays an important role when plant is under stress as it is involved in cell proliferation (Schachtman et al., 1997), maintenance of membrane stability (Rengel, 1992), osmotic stress control (Mansfield et al., 1990) and effect of K^+/Na^+ selectivity (Schachtman et al., 1991). High concentration of salt uptake leads to competition with the uptake of other nutrient ions, especially K^+, which in turn gives rise to K^+ deficiency (Fox and Guerinot, 1998). Salt treatment leads to an increase of Na^+ and Cl^- and a decrease in the Ca^{2+}, K^+ and Mg^{2+} concentrations in many of the plants (Khan et al., 2000).

There have been reports that Si treatment in dicotyledons, plants belonging to the Gramineae family in particular, promote growth and development, increase photosynthetic activities, reduces pest infection, maintains the shoot in an erect position and alleviates salt stress (Ma, 2004). In particular, these effects are not only produced in barley (Liang et al., 2005), rice (Matoh et al., 1986) and wheat (Ahmad et al., 1992), belonging to the plant family, Gramineae, but also in tomato (Al-Aghabary et al., 2004) and cucumber (Zhujun et al., 2004). When cucumber and tomato under salt stress was treated with Si, there was reduction of lipid peroxidation, an increase in antioxidant enzyme activity and improved non-enzymatic antioxidant substances (Al-Aghabary et al., 2004; Zhujun et al., 2004).

The aim of this study was to identify the effect of Si, which is known to have to be effective in increasing the tolerance of some Gramineae plants, on the antioxidant defense mechanisms and inorganic ions as a parameter in the salt stress on grass.

MATERIALS AND METHODS

Plant materials and treatments

This study was conducted from November 2008 to April 2009. The plants used in this study were the Kentucky bluegrass (*Poa pratensis* L.), 'Perfection' and 'Midnight,' and 0.13 g of seeds of each type were sowed in a sand-filled pot with a diameter of 10.5 cm. The germination temperature was set at 24±1.5°C and the seeds were watered twice a day until germination and covered with a shade net for three weeks. During the seed germination period at four weeks after the seed-sowing, the head of the plants were watered twice a day, fertigated once a week with Hoagland nutrient solution (Hoagland and Amon, 1950). During the experiment, the highest temperature was 30 ±1.5°C, while the lowest temperature was 12±1.5°C. After 80 days of growth, the plants were moved to a plant growth chamber. The temperature in the plant growth chamber was maintained at 25°C day and night, light conditions at 12 h and light intensity at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. NaCl and Si treatments were performed on February 2, 2009 and Na_2SiO_3 was used as the source of Si. The plant pots were irrigated from the bottom of the pots at every 2-day intervals for 1 h. The experimental design consisted of a control (only Hoagland nutrient solution), 400 mM NaCl, and two Si treatments (0.1 and 1.0 mM Si in combination with 400 mM NaCl). The pH of the solution was adjusted to 5.5 using H_2SO_4. The plant growth was investigated and analyzed at days 5, 10 and 15. The treatments were done in completely randomized design with six replications and, for statistical analysis; Duncan Multiple Range Test was performed using SAS (SAS Institute, Cary, NC) to analyze the differences between the treatments.

Measurement of plant growth and the relative water content

The shoot length and fresh and dry weight of shoot and root were measured to determine the effect of Si treatment on salt stress. The shoot and root were dried for 48 h at 80°C and weighed. In order to determine the relative water content, the fresh weights (FW) of shoot and root were measured in accordance with the Barr and Weatherley (1962) technique and the total weight (TW) was measured after the shoot and root were left at room temperature for 4 h. The shoot and root were dried for 48 h at 80°C and weighed to determine the dry weight (DW). The relative water content was calculated by substituting the following equation:

Relative water content (%) = (FW-DW) / (TW-DW) × 100

Analysis of inorganic ion concentrations

To determine the concentrations of inorganic ions, 0.2 g of dry samples of root and short were added to 10 ml of lysis solution (sulfuric acid: hypochlorous acid: distilled water = 2:9:5) for wet digestion and the concentrations of sodium, potassium, calcium, magnesium and iron were determined using inductively coupled plasma spectrometer [ICP spectrometer (OPTIMA 4300DV/5300DV (Perkin Elmer)].

To determine the concentration of Si, 0.5 g of dry sample was decomposed using the H_2SO_4-$HClO_4$ method according to the ammonium molybdate blue technique. Distilled water was added to a 300 ml polyethylene beaker containing 25 ml of the lysis solution until the volume was adjusted to approximately 200 ml. 5 ml of sodium fluoride was added and the mixture was heated in a water bath for 30 min before being cooled down. It was then transferred into a 250 ml graduated cylinder and distilled water was added up to the grid. Exactly 50 ml of the mixture was transferred into a 100 ml volumetric flask and water was added to adjust the volume to 80 ml. 10 ml of hydrochloric acid was added while shaking the flask and 5 ml of ammonium molybdate solution was added before adding distilled water to the grid. The solution containing a color developing reagent was shaken well to allow color development for 30 min. The absorbances of the standard solution and the blank solution were measured at a wavelength of 420 nm and were multiplied by 0.4674 (coefficient) to determine the amount of Si.

Measuring the oxygen radical absorption capacity and antioxidant activity

The Electron Donating Ability (EDA) test using DPPH (α,α-diphenyl-β-picrylhydrazyl), Sigma-Aldrich chemical, Co. USA) was performed on the extract to determine its antioxidant capacity. To be specific, 16 mg of DPPH reagent was dissolved in 100 ml of absolute ethanol to prepare 4×10^4 M DPPH solution and the absorbance of the blank ethanol solution was adjusted to 0.95-0.99 at 525 nm. Next, 3 ml of ethanol was added to the extract with the sample solution prepared in mg/ml to 0.2 ml and the solution was mixed with 0.8 ml DPPH reagent before being powerfully shaken for 10 s and left at room temperature for 10 min. The absorbance was measured at a wavelength of 525 nm using a spectrophotometer (Uvikon, Italy) and the difference between the absorbance of the group containing the sample and that of the group without the sample was indicated as a percentage (%) to measure the EDA. The calculation method is as follows:

EDA (%) = [1-ABS/ABC] × 100

Where, ABS is the absorbance of the group containing sample, ABC is the absorbance of the group without sample.

Colorimetric assay was performed according to the Folin-Denis method (Amerine and Ough, 1980) to analyze the total phenolic compound. 0.3 g of the fresh sample was homogenized in 80% methanol and centrifuged for 15 min at 10,000 x *g* at 4°C. 1 ml of Folin-Ciocalteu reagent (Sigma-Aldrich Chemical Co., U.S.A.) was added to 1 ml of the supernatant and mixed. Then, 1 ml of 10% Na_2CO_3 was added to the mixture after 3 min before being shaken in an agitator and left at room temperature for 1 h. The absorbance was measured using a spectrophotometer (Uvikon, Italy) at a wave length of 650 nm. At this time, varying concentrations of gallic acid (Sigma-Aldrich Chemical Co., U.S.A.) were prepared as a standard substance and a standard curve was generated for calculation. The result was converted to the content for 100 g fresh sample.

To determine the concentration of glutathione (GSH), 0.3 g of the fresh sample was homogenized in 5% trichloroacetic acid (TCA) solution according to the method used by Guri (1983) and centrifuged for 20 min at 12,000 x *g* at 4°C. 0.5 ml of distilled water, 2 ml of 0.2 mM potassium phosphate buffer (pH 7.0) and 0.1 ml of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were added 0.5 ml of supernatant and the absorbance was measured at a wavelength of 412 nm using a spectrophotometer (Uvikon, Italy).

To determine the concentration of proline, 0.3 g of the fresh sample was homogenized in 3% sulfosalicylic acid solution according to the method used by Bates et al. (1973) and centrifuged for 15 min at 12,000 x *g* at 4°C. 0.5 ml of sulfosalicylic acid, 1 ml of acid-ninhydrin and 1 ml of acetic acid were added and mixed with 0.5 ml of supernatant and the mixture was heated at 95 to 100°C for 1 h before ice-cooled. After the reaction was complete, 2 ml of toluene was added to the reactants, which were then powerfully shaken for 15-20 s and allowed to settle for 5 min at room temperature. The chromophores were separated from the water-enriched layer. To determine the quantity of proline, the absorbance was measured at a wavelength of 520 nm using a spectrophotometer (Uvikon, Italy) and toluene as a blank and was compared with the standard curve. The quantity of free proline was calculated using the following equation;

Free proline (mg/g FW) = (mg proline × 5 (amount of extraction solution) / 0.3 (sample weight)

RESULTS AND DISCUSSION

The NaCl treatment caused a significant decrease in the shoot length and the fresh and dry weight of shoot and root compared to the control group (Table 1). Applying 0.1 and 1.0 mM Si after the NaCl treatment increased the shoot length of 'Perfection' by 48 and 43%, respectively, and that of 'Midnight' by 28 and 23%, respectively, compared to the NaCl treatment group. Also, applying 0.1 mM and 1.0 mM Si increased the fresh weight of the shoot of 'Perfection' by 72 and 66%, respectively, and dry weight by 78 and 61%, respectively. Both types with 0.1 and 1.0 mM Si after the NaCl treatment showed an increase in the relative water content of the shoot and the root and there were no significant differences in the relative water content between the control and Si treatment (data not shown).

The Na^+ concentration significantly increased after the NaCl-only treatment in both varieties compared to control and decreased dramatically when 0.1 and 1.0 mM Si were added after the NaCl treatment compared to the NaCl-only treatment (Table 2). On the other hand, the group treated with 0.1 and 1.0 mM Si after the NaCl treat-

Table 1. Effects of Si on the growth of NaCl pre-treated Kentucky bluegrass, 'Perfection' and 'Midnight'.

Treatment[z)] (mM)	Shoot length (cm)	Shoot (g/plant)		Root (g/plant)	
		Fresh weight	Dry weight	Fresh weight	Dry weight
Perfection					
Non-treatment	13.3a[y)]	0.172a	0.042a	0.023a	0.008a
NaCl	9.0b	0.100b	0.023c	0.016b	0.004b
NaCl+Si0.1	13.3a	0.172a	0.041a	0.023a	0.009a
NaCl+Si1.0	12.9a	0.166a	0.037b	0.022a	0.006ab
Midnight					
Non-treatment	11.0a	0.165a	0.035a	0.033a	0.012a
NaCl	8.6c	0.082c	0.025c	0.012c	0.003b
NaCl+Si0.1	11.0a	0.164a	0.035a	0.033a	0.011a
NaCl+Si 1.0	10.6b	0.147b	0.030b	0.028b	0.010a

[z)]NaCl 400 mM and Si 0.1 and 1.0 mM .[y)]Mean separation within columns by Duncan's multiple range test, $P = 0.05$.

Table 2. Effects of Si (mM) on the shoot mineral concentration of 400 mM NaCl pre-treated kentucky bluegrass 'Perfection' and 'Midnight'.

Treatment[z)] (mM)	Shoot (mg•g-1DW)				
	Na	K	Ca	Mg	Fe
Perfection					
Non-treatment	4.27d[y)]	45.51a	9.92b	5.76a	0.21a
NaCl	149.93a	28.16d	11.49a	5.46b	0.00a
NaCl+Si 0.1	26.71c	37.06b	10.01b	5.18c	0.29a
NaCl+Si 1.0	33.14b	32.97c	7.97c	4.38d	0.06a
Midnight					
Non-treatment	4.23c	45.37a	9.04ab	5.42a	0.00a
NaCl	147.97a	27.17c	10.01a	4.72b	0.12a
NaCl+Si 0.1	28.85b	35.45b	9.68a	5.37a	0.26a
NaCl+Si 1.0	31.05b	34.36b	8.78b	4.31c	0.00a

[z)]NaCl 400 mM and Si 0.1 and 1.0 mM . [y)]Mean separation within columns by Duncan's multiple range test, $P = 0.05$.

ment showed an increase in the Na^+ concentration compared to the control group. The K^+ concentrations in both types dramatically decreased in the NaCl-only treatment group and increased after the addition of 0.1 and 1.0 mM Si following the NaCl treatment compared to the NaCl-only treatment group. However, the K^+ concentrations in the groups treated with 0.1 and 1.0 mM Si following the NaCl treatment were lower compared to the control group.

In both types, the Na^+ concentration significantly increased after the NaCl-only treatment compared to control and decreased dramatically after 0.1 and 1.0 mM Si were added following the NaCl treatment compared to the NaCl-only treatment (Table 3). On the other hand, the group treated with 0.1 and 1.0 mM Si after the NaCl treatment showed an increase in the Na^+ concentration compared to the control group. As for the K^+ concentrations, there were noticeable decreases in both types in the NaCl-only treatment group, but there were no differences in concentrations after the addition of 0.1 and 1.0 mM Si following the NaCl treatment compared to the NaCl-only treatment group. Meanwhile, the K^+ concentrations in the group treated with 0.1 and 1.0 mM Si following the NaCl treatment were lower compared to the control group.

The concentration of Si decreased in both groups after the NaCl-only treatment compared to control, and showed a remarkable increase after the addition of 0.1 and 1.0 mM Si following the NaCl treatment compared to the NaCl-only treatment group (Table 4). In the group treated with 0.1 and 1.0 mM Si after the NaCl treatment, both varieties showed an increase in the Si concentration compared to the control group. Treating the plants with 1.0 mM Si after the NaCl treatment resulted in a higher Si concentration compared to the treatment with 0.1 mM Si.

Greenway and Munns (1980) reported that when there is a high concentration of NaCl in the soil, there is high

Table 3. Effects of Si (mM) on the root mineral concentration of 400 mM NaCl pre-treated kentucky bluegrass 'Perfection' and 'Midnight'.

Treatment[z)] (mM)	Root (mg•g-1DW)				
	Na	K	Ca	Mg	Fe
Perfection					
Non-treatment	6.94c[y)]	16.64a	7.66a	3.05a	1.07c
NaCl	101.46a	6.97b	6.12b	2.33b	4.63b
NaCl+Si 0.1	23.78b	7.77b	7.05a	2.39b	8.53a
NaCl+Si 1.0	27.92b	5.35b	5.30b	2.06b	7.50ab
Midnight					
Non-treatment	6.18b	11.29a	6.46a	2.86a	0.75b
NaCl	67.28a	6.07b	4.51b	2.45a	3.59b
NaCl+Si 0.1	13.38b	6.92b	6.64a	2.99a	11.46a
NaCl+Si 1.0	17.58b	5.55b	6.54a	2.45a	10.53a

[z)]NaCl 400 mM and Si 01 and 1.0 mM. [y)]Mean separation within columns by Duncan's multiple range test, $P = 0.05$.

Table 4. Effects of Si (mM) on the Si concentration of 400 mM NaCl pre-treated kentucky bluegrass 'Perfection' and 'Midnight'.

Treatment[z)] (mM)	Si concentration (mg•g-1DW)			
	Perfection		Midnight	
	Shoot	Root	Shoot	Root
Non-treatment	699.9c[y)]	323.3c	622.5c	301.1c
NaCl	340.0d	158.9d	282.2d	134.3d
NaCl+Si 0.1	990.2b	461.5b	935.7b	425.3b
NaCl+Si 1.0	1260.5a	601.2a	1196.3a	573.8a

[z)]NaCl 400 mM and Si 01 and 1.0 mM. [y)]Mean separation within columns by Duncan's multiple range test, P=0.05.

concentration of Na^+ in the water absorbed by the shoot and thus, Na^+ accumulates in cells. According to Palmgren (1990), an increase in Na^+ concentration in cells inhibits growth by interfering with various enzymatic activities in the plasma or chloroplasts, while K^+ is characterized by the fact that it does not interfere with cellular activities and helps maintain a high osmotic pressure by competing with Na^+. The Na^+ accumulation and K^+ decrease caused by NaCl is reported to take place in numerous plants (Greenway and Munns, 1980).

There have been reports of K^+ reduction in cucumber (Alpaslan and Gunes, 2001) with an accumulation of Na^+ following a treatment with NaCl, which were consistent with the results of this study. Levent et al. (2008) reported that the Na^+ concentration decrease while the K^+ and Si concentrations increased in the shoot and root when Si was added to wheat treated with 100 mM NaCl. There have been findings of a reduction in the Na^+ concentration and a significant increase in the K^+ concentration in the shoot when Si was added to rice (Matoh et al., 1986) and wheat (Ahmad et al., 1992) after salt stress and an increase in Si concentration in both the shoot and the root after Si treatment (Gao et al., 2004).

The changes in the oxygen radical absorption capacity of the Kentucky bluegrass types, 'Perfection' and 'Midnight,' were investigated following a treatment with NaCl and Si. The results showed that there was a significant reduction in the oxygen radical absorption capacity of 'Perfection' over time after the NaCl-only treatment compared to control (Figure 1). When 0.1 and 1.0 mM Si was added after the NaCl treatment, there was an increase in the oxygen radical absorption capacity compared to the NaCl-only treatment group. The group treated with 0.1 and 1.0 mM Si after the NaCl treatment showed slightly lower oxygen radical absorption capacity compared to the control group. The changes in the oxygen radical absorption capacity of 'Midnight' showed similar tendencies as that of 'Perfection.'

Plants subjected to salt stress are susceptible to oxidetive damage and it also induces the production of active oxygen species in cells that interfere with the electron transport process in mitochondria and ultimately lead to cell destruction and death (Polle, 2001). However, the oxidative stress caused by salt can be mitigated through the antioxidant activities of plants. The antioxidant system consists of antioxidant enzymes and small molecular antioxidants (Foyer et al., 1994). According to Rios et al. (2008), an enhanced DPPH oxygen radical absorption capacity is part of a defense mechanism that takes place along with an increase of antioxidant substances to

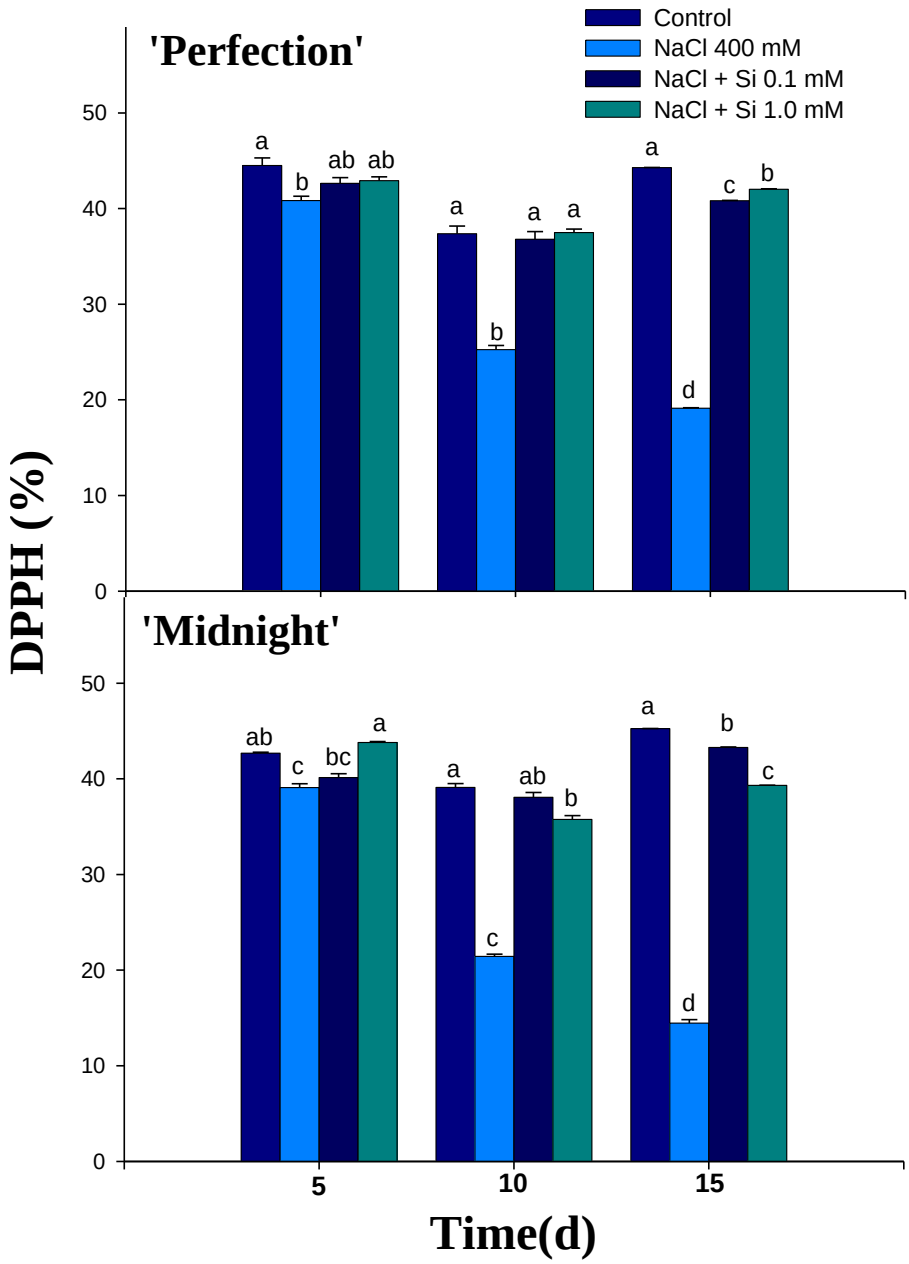

Figure 1. Changes of Si (mM) on the DPPH radical scavenging activities of 400 mM NaCl pre-treated Kentucky bluegrass 'Perfection' and 'Midnight'. Data are the mean ± S.D. of three replicates.

remove active oxygen species produced by oxidative stress and is used to determine the antioxidant capacity of plants.

The results of this study showed enhanced oxygen radical absorption capacity in both varieties following a Si treatment and this is consistent with the findings of Liang et al. (2003), who reported that the use of Si on barley subjected to salt stress reduces lipid peroxidation and promotes the antioxidant enzymatic activities in the roots to remove the active oxygen species.

The NaCl and Si treatments had an impact on the total phenolic compounds of the Kentucky Bluegrass types, 'Perfection and 'Midnight' (Figure 2). There was a significant reduction of the total phenolic compounds in 'Perfection' over time after the NaCl-only treatment com-pared to control. When 0.1 and 1.0 mM Si were added following the NaCl treatment, there was a noticeable increase in the total phenolic compounds compared to the NaCl-only treatment. However, there were no differences between the control group and the group that was treated with 0.1 and 1.0 mM Si in terms of the total phenolic compounds. Phenolic compounds play a role in protecting plant cells

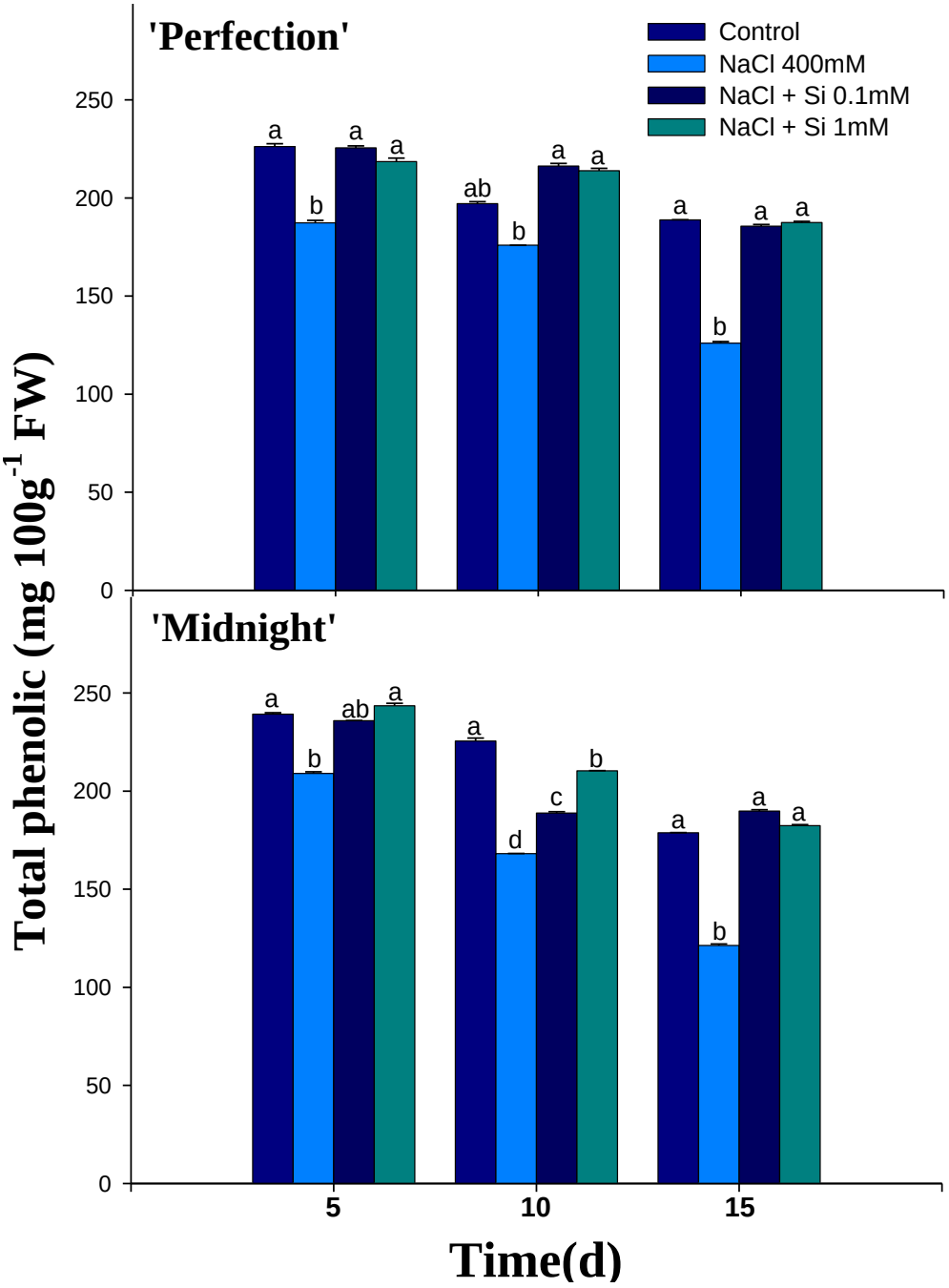

Figure 2. Changes of Si (mM) on the total phenol compound of 400 mM NaCl pre-treated kentucky bluegrass 'Perfection' and 'Midnight'. Data are the mean ± S.D. of three replicates.

(Allwood et al., 1999). According to Kakani et al. (2003),phenolic compounds are synthesized in plants due to oxi-dative stress. In this study, however, the phenolic com-pound content in the two varieties showed a noticeable decrease after NaCl treatment. Although the fluctuations cannot be understood, the reduction in the total phenolic compounds is thought to be caused by interference with biosynthesis or destruction of products due excessive NaCl stress.

The changes in the concentration of GSH (glutathione) in 'Perfection' and 'Midnight' showed similar tendencies after treatment with Si (Figure 3). Compared to control, the concentration of GSH showed a decrease after the NaCl-only treatment but then showed similar tendencies as the control group over time. When the plants were treated with 0.1 and 1.0 mM Si after NaCl treatment, the

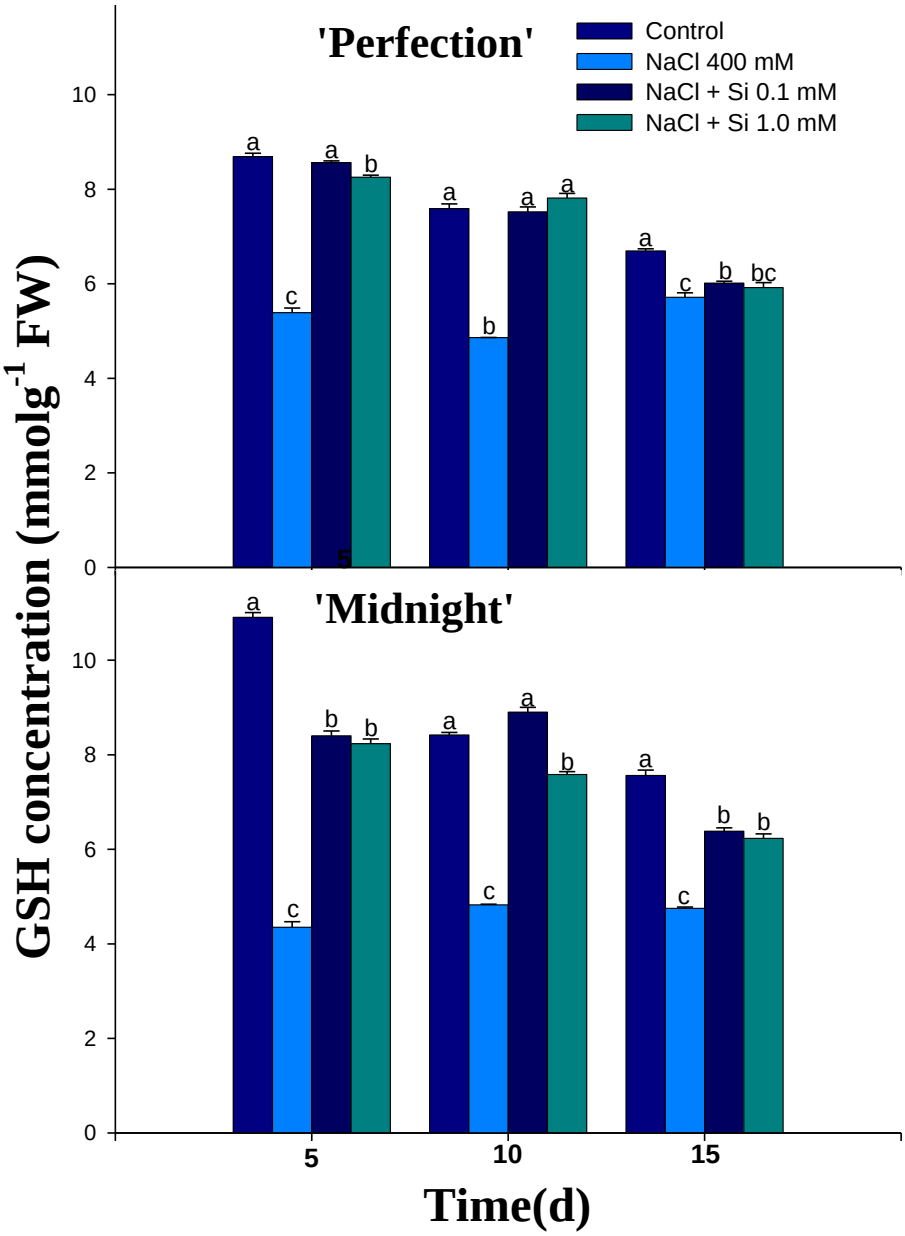

Figure 3. Changes of Si (mM) on the GSH concentration of 400 mM NaCl pre-treated Kentucky bluegrass 'Perfection' and 'Midnight'. Data are the mean ± S.D. of three replicates.

concentration of GSH was higher compared to the NaCl-only treatment and was slightly lower than the control group.

The high oxidizing power of the active oxygen species generated is known to destroy proteins, lipids, pigment molecules and genes and most plants develop enzymatic (superoxide dimutase, ascorbate peroxidase and glutathione reductase) and non-enzymatic (mainly known antioxidant molecules such as tocopherols, carotenes, ascorbate, glutathione, etc.) antioxidant defense mechanisms against the generated active oxygen species to avoid oxidative damage (Baier et al., 1998). Of the non-enzymatic antioxidant defense mechanisms, GSH, which is an important antioxidant substance in plants, is known to play a role in protecting proteins from oxidative effects (Kranner, 1996).

The results of this study was consistent with that of Liang et al. (2003), who reported a significant in the GSH concentration of barley treated with 120 mM NaCl and an increase in the GSH concentration following Si treatment. The reduction of GSH due to stress is known to severely interfere with metabolic processes such as photosynthe-

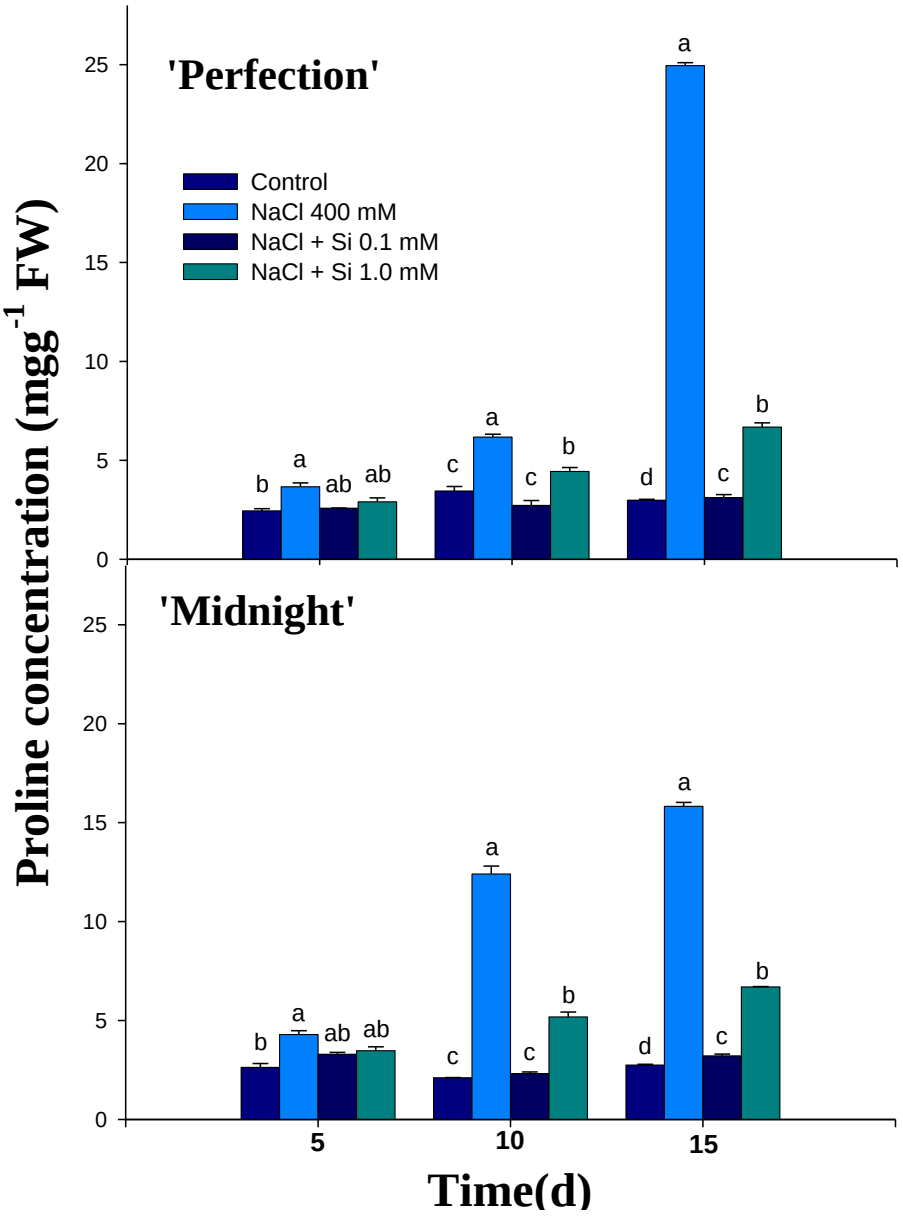

Figure 4. Changes of Si (mM) on the proline concentration of 400 mM NaCl pre-treated Kentucky bluegrass 'Perfection' and 'Midnight'. Data are the mean ± S.D. of three replicates.

tic CO_2 assimilation in leaves (Foyer et al., 1995; Noctor et al., 1996), and treating barley with Si following salt stress will result in simultaneous activation of GR and increase in the GSH concentration. Thus, an increase in GR activity in plants ultimately provides plants with resistance by inducing an accumulation of GSH (Liang et al., 2003). Figure 4 shows the changes in the proline concentrations of the Kentucky bluegrass types, 'Perfection' and 'Midnight,' following NaCl and Si treatments. In 'Perfection,' the proline concentration significantly increased over time in the group treated with NaCl only compared to the control group. When 0.1 and 1.0mM Si were added after NaCl treatment, the prolien concentrations decreased noticeably compared to the NaCl-only treatment. There were no significantdifferences in the proline concentration between the control group and the group treated with 0.1 mM following the NaCl treatment. On the other hand, the NaCl and 1.0 mM Si treatment group showed a slight increase in the proline concentration compared to the control group. The changes in the proline concentration of 'Midnight' showed similar tendencies as that of 'Perfection.'

Exposure to NaCl or water stress causes proline to accumulate in plants. Proline is reported to play a role in

osmotic protection to maintain the enzymatic activities related to osmotic stress adaptation as well as homeostasis within the cell membrane and organelles (Delauney and Verma, 1993) and remove hydroxyl radicals (Smironff and Cumbes, 1989). According to Dubey and Pessarakli (1995), rapid accumulation of amino acids caused by excess nitrogen due to salt stress increases the proline concentration, which in turn affects plant growth. According to Levent et al. (2008), treating wheat with 100 mM NaCl and then Si caused a reduction of proline, which was consistent with the results of this experiment.

In conclusion, the addition of Si after NaCl treatment significantly alleviated the growth inhibitory effect in the grass. In Si treatment, there was a significant reduction in the concentration of Na^+ and proline, the oxygen radical absorption capacity and the total phenolic compounds. Higher concentrations of K^+, Si, and glutathione were observed in the plant with Si application after NaCl treatment. These results suggest that, even though Si is not generally classified as 'essential element', Si may strongly be involved in the metabolic or physiological activity in salt-stressed grass.

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