Growth Response and Gene Expression in Antioxidant-related Enzymes in Two Bermudagrass Genotypes Differing in Salt Tolerance

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ABSTRACT. Plant adaptation to salt stress may be associated with morphological, physiological, and gene expression alterations. The objective of this study was to investigate the effect of salt stress on morphological and antioxidant enzyme changes and its gene expressions in bermudagrass (Cynodon dactylon). Salt-tolerant ‘C43’ and salt-sensitive ‘C198’, previously determined in our preliminary study, were subjected to four salinity levels: 0 mM (control), 100 mM (low), 200 mM (moderate), and 400 mM (high) NaCl for 21 days. Salt stress decreased turf quality and canopy height, especially in ‘C198’. Salt stress increased root length, root number, root fresh weight, and root/shoot length ratio, to a greater extent in salt-tolerant genotype. Salt stress increased Na⁺ and decreased K⁺ content, which resulted in a higher Na⁺/K⁺ ratio in bermudagrass, to a great extent in shoot and root of ‘C198’. Moderate (200 mM) and high (400 mM) NaCl concentration increased malondialdehyde and hydrogen peroxide content in old leaves of ‘C198’. ‘C43’ exhibited a greater activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and dehydro-ascorbate reductase (DHAR) than ‘C198’ in old leaves subjected to 200 and 400 mM NaCl. Antioxidant gene expressions were upregulated in new leaves and downregulated in old leaves with increasing salinity levels for both genotypes. Salt-tolerant genotypes exhibited a relatively greater antioxidant gene expression than salt-sensitive ones when exposed to the same level of salt stress. These results suggested that SOD, CAT, APX, and DHAR might be involved in scavenging salt stress-induced reactive oxygen species in bermudagrass at the level of gene expression. Salt tolerance might be attributed to the development and maintenance of a more extensive root system under saline conditions and induced antioxidant gene expressions, leading to more efficient enzyme stimulation and protection in bermudagrass.

Salt stress is one of the major abiotic factors that affects plant growth. Shoot and root growth reduction is a common response to salt stress because plant growth is one of the most important agricultural indicators of salt stress tolerance (Huluri et al., 2007). Plant adaptation to salt is a complex phenomenon that may involve growth changes as well as physiological and biochemical processes (Hare et al., 1997). Salinity injury to plants was attributed to lower osmotic potential ($\psi_s$) and ion effect (Munns, 2002). Lower $\psi_s$ reduces the ability of the plant to absorb water and induces physiological drought (Munns and Tester, 2008). The excessive uptake of Na⁺ or Cl⁻ can limit the uptake of other nutritional ions such as K⁺, Ca²⁺, and Mg²⁺; cause adverse effects on ion homeostasis, which lead to premature leaf aging; less cell division and elongation; and reduces leaf and root growth (Munns, 2002; Zhu, 2001).

In addition, salinity also results in oxidative stress in plants as a result of the overproduction of reactive oxygen species (ROS) such as the super oxide radical ($\mathrm{O}_2^-$), hydrogen peroxide ($\mathrm{H}_2\mathrm{O}_2$), and hydroxyl radical ($\cdot\mathrm{OH}$). The oxygen species are detrimental to membrane lipids, proteins, and nucleic acids (Murillo-Amador et al., 2006). To minimize the adverse effects of ROS, plants have evolved an efficient enzymatic and non-enzymatic antioxidant system (Abdul-Jaleel et al., 2006). In the enzymatic system, superoxide dismutase catalyzes the dismutation of $\mathrm{O}_2^-$ into $\mathrm{H}_2\mathrm{O}_2$ and $\mathrm{O}_2$ (Siguaud-Kutner et al., 2002). Catalase, AOX, and DHAR decompose $\mathrm{H}_2\mathrm{O}_2$ to H₂O at different cellular locations (Edreva, 2005). The mechanisms regulating the activity and gene expression of different antioxidant enzymes are complex because the genes respond to environmental stress differentially (Sen Gupta et al., 1993). A higher level of these antioxidant enzyme activities is considered as one of the salt tolerance mechanisms in most plants (Ashraf, 2009). Previous studies demonstrated that salt-tolerant genotypes generally have a higher constitutive or an enhanced antioxidant enzyme activity under salt stress than the sensitive ones (Amor et al., 2006; Hu et al., 2012a; Mhadhbi et al., 2011). However, the response of plant antioxidant systems to salt varied for different plant species and the tissues (Mittova et al., 2003).

Bermudagrass is one of the most widely used warm-season turfgrass species in temperate and tropical regions, which has shown good tolerance to salinity and can survive in saline soil (Mancino and Pepper, 1994). To our knowledge, most previous studies examined in bermudagrass under salinity conditions were at either morphological or physiological levels (Adavi et al., 2006; Akram et al., 2006; Alshammary et al., 2008; Hameed et al., 2010; Lu et al., 2007; Marcum et al., 2005; Marcum and Pessarakli, 2006; Shahba, 2010). Little research has investigated antioxidant response to salinity at the level of gene expression and enzyme activity. The objective of this study was to investigate the effects of different salinity levels on the growth as well as the antioxidant defense system at the enzyme and gene expression levels in two genotypes of bermudagrass differing in salt tolerance.
Photosynthetically active radiation levels of 1100 μmol·m⁻²·s⁻¹ were used as the crude extract for SOD, CAT, APX, and DHAR assay as we described before (Hu et al., 2012a, 2012b). Soluble proteins were quantified by the method of Bradford (1976) with bovine serum albumin as the standard. All spectrophotometric analyses were conducted using a spectrophotometer [ultraviolet-2600; UNICO (Shanghai) Instruments Co., Shanghai, P.R. China].

For determination of SOD activity, the 3-mL reaction solution contained 50 mM sodium phosphate buffer (pH 7.8), 195 mM methionine, 3 μM EDTA, 1.125 mM nitro blue tetrazolium (NBT), 60 μM riboflavin, and 100 μL of enzyme extract with non-enzyme solution as a control. The reaction mixtures were illuminated under 200 μmol·m⁻²·s⁻¹ irradiance for 20 min. Absorbance changes at 560 nm were monitored for 3 min by a spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to inhibit the NBT reduction by 50%.

For the measurement of CAT activity, the 3-mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 45 mM H₂O₂. The reaction was initiated by adding the 100 μL of enzyme extract, and the decrease in absorbance at 240 nm was recorded every 1 min for 3 min. One unit of CAT activity was defined as the absorbance changes of 0.01 units/min.

APX activity was determined by monitoring the rate of ascorbate oxidation at 290 nm. Briefly, the 3-mL reaction mixture contained 50 mM sodium-acetate buffer (pH 5.8), 5 mM H₂O₂, 10 mM ascorbate, 3 μM EDTA, and 100 μL of enzyme extract. The decrease in absorbance was recorded at 290 nm at an interval of 10 s up to 90 s. One unit APX was defined as the absorbance change of 0.01 units per minute.

DHAR activity was determined by measuring increase in absorbance at 265 nm as a result of the reduction of dehydroascorbate according to the method of Nakano and Asada (1981) with slight modifications. Briefly, the reaction mixture consisted of 0.5 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.1 mL of 1 mM EDTA, 0.1 mL of 5 mM reduced glutathione (GSH), and 0.1 mL of enzyme extract. The reaction was initiated by the addition of 0.2 mL of 0.06 mM dehydroascorbate. The increase in absorbance at 265 nm was monitored for 5 min. DHAR activity was calculated as nanomoles ascorbic acid (ASA) per minute per milligram protein.

Malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) reaction using the method of Heath and Packer (1968) with slight modifications as we described before (Hu et al., 2012a). Briefly, 1 mL of enzyme extraction was mixed with 1 mL of reaction solution containing 20% (v/v) trichloroacetic acid and 0.5% (v/v) TBA. The mixture was heated in a water bath at 95 °C for 30 min and then cooled to room temperature and centrifuged at 10,000 g, for 10 min. Absorbance of the supernatant was measured at 532 and 600 nm. The content of MDA was calculated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm and calibrated by using the extinction coefficient of 155 mmol⁻¹·cm⁻¹ (Heath and Packer, 1968).
The content of H$_2$O$_2$ was determined using the method described by Jena and Choudhuri (1981) with minor modifications. Briefly, 1 mL of supernatant was mixed thoroughly with 1 mL of 0.1% titanium sulphate in 20% H$_2$SO$_4$ (v/v), and the mixture was then centrifuged at 6000 g$_n$ for 15 min at room temperature. The absorbance of the yellow color of the supernatant was measured at 410 nm. The H$_2$O$_2$ level was calculated from the standard curve generated with known concentrations of H$_2$O$_2$ and calibrated with the extinction coefficient of 0.28 mmol$^{-1}$cm$^{-1}$.

For Na$^+$ and K$^+$ determination, the plant materials were dried in an oven at 80 °C, finely ground, and passed through a 2-mm mesh sieve. Samples were digested in 5 mL of 98% H$_2$SO$_4$ and 3 mL of 30% H$_2$O$_2$ for 5 h. The Na$^+$ and K$^+$ content of the mineral extract was determined using a flame photometer.

Total RNA was extracted from fresh tissues by using Trizol-reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. After extraction, the RNA pellet was dissolved in 100 µL of RNase-free water. RNase-free DNase I was added to the total RNA to remove DNA contamination. The total RNA concentration was then determined by absorbance at 260 nm and RNA quality was evaluated on a 0.8% agarose gel.

For reverse transcription–polymerase chain reaction, cDNA was prepared from 2 µg of total RNA with oligo(dT)$_{12-18}$ primer using a cDNA synthesis kit (Fermentas, Burlington, Ontario, Canada) according to the user manual. The resulting cDNA was stored in aliquots at −20 °C until further use. Gene-specific primers (Table 1) were synthesized based on a previously identified expressed sequence tag sequence in bermudagrass. Actin gene was used to confirm equal template loading. General polymerase chain reaction (PCR) was conducted with the following program: inactivated the reverse transcriptase at 94 °C for 3 min and then followed with 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s to 35 cycles. The PCR products were evaluated on a 1.3% agarose gel in 1 × Tris-acetate-ethylenediaminetetraacetic acid and stained with ethidium bromide. The band intensity was quantified by using imaging software (Tanon 2500; Tanon Science and Technology Co., Shanghai, P.R. China).

**Statistical analysis.** All data were subjected to analysis of variance by using the general linear model procedure of SAS (Version 9.0 for Windows; SAS Institute, Cary, NC) to determine the treatment effects and genotypic variations in growth and physiological response. Treatment means and genotype differences were separated using the least significant difference test at $P < 0.05$. The allometric relationship between shoot and root was examined by regressing shoot height over root length by fitting quadratic polynomials using SigmaPlot 10.0 (Systat Software, Richmond, CA).

### Results

**Growth response to salt stress.** Visual TQ significantly decreased with increasing salinity levels in both genotypes, to a greater extent in salt-sensitive ‘C198’ vs. the respective control level (Fig. 1A). There was no difference in TQ between two genotypes subjected to low (100 mM) salt stress. The 200- and 400-mM-treated plants had a greater level of TQ than ‘C198’. Relative canopy height (as a percent of control) decreased with increasing salinity levels in both genotypes (Fig. 1B). However, there was no difference in relative canopy height between two genotypes regardless of salinity levels.

Root growth (i.e., relative root length and number to the control) of ‘C43’ and ‘C198’ responded differentially to increasing salinity levels (Fig. 2). The NaCl-treated ‘C43’ had a relative longer RL and more RN than the control plants during the whole experimental period. ‘C198’ exhibited a relative longer RL (Fig. 2A) and more RN (Fig. 2B) only in 100- and 200-mM regimes. When subjected to a high dose of NaCl (i.e., 400 mM), ‘C198’ had a shorter RL and fewer RN than the control level. ‘C43’ had a greater root growth than ‘C198’ regardless of salinity levels during the whole experimental period.

The salinity usually increased root fresh weight (RFW) of both genotypes when compared with the respective control level, except for 400-mM-treated ‘C198’ that had a similar RFW to the control (Fig. 3A). There was no significant difference in

### Table 1. The gene-specific primer sequence for polymerase chain reaction used for detecting gene expression in leaves of bermudagrass.

| Accession | Gene name | Primer sequence (5′-3′) |
|-----------|-----------|------------------------|
| BG322311  | APX       | TCCGTGTAAGTAAAGAGTTGTC |
|           |           | CAGATGGGCTTAGTGAT       |
| DN987297  | CAT       | GCCGTGGTCTCTTGGTTTT     |
|           |           | TCCGGTTCAAGTGGCTG       |
| JK340747.1| DHAR      | GTTGGTGAAGCTTCTCTGG     |
|           |           | GTGATGGCAAGTGGATT       |
| JK340708.1| Cu/Zn SOD | TCTCCACACAGGACTTCC      |
|           | ACTCACGCACATTCCAGCATAG |

APX = ascorbate peroxidase; CAT = catalase; DHAR = dehydroascorbate reductase; Cu/Zn SOD = copper/zinc superoxide dismutase.

Fig. 1. Effects of salt stress on turf quality (A) and canopy height (B) in two bermudagrass genotypes (‘C43’ and ‘C198’) at 21 d. Turf quality was visually assessed on a 1 to 9 scale. Letters marked by the same letters are not significant at $P < 0.05$ (Tukey’s test) for the comparison of salt concentration in a genotype; × indicates significant difference at $P < 0.05$ (Tukey’s test) for the comparison of genotypes in a salt concentration (n = 6).
RFW between two genotypes subjected to 100 mM NaCl. Root fresh weight, however, was greater in ‘C43’ vs. ‘C198’ under moderate (200 mM) to high (400 mM) salt exposure.

The root/shoot length ratio was 0.3 to 0.47 under control level and similar between ‘C43’ and ‘C198’ (Fig. 3B). Salt stress increased root/shoot length ratio for both genotypes. When salinity increased from 0 to 400 mM NaCl, the ratio of root to shoot growth increased by twofold in ‘C198’ and ninefold in ‘C43’. The root/shoot length ratio was greater in ‘C43’ vs. ‘C198’ in 200- and 400-mM regimes.

**IONIC RESPONSE TO SALT STRESS.** The accumulation of Na⁺ in shoots and roots of both bermudagrass genotypes significantly increased with increasing salinity level, but to a great extent in salt-sensitive ‘C198’ (Figs. 4A and 5A). K⁺ content decreased with increasing salinity level in shoot and root of both genotypes (Figs. 4B and 5B). However, there was no difference for K⁺ content between two genotypes at each salt concentration.

The Na⁺/K⁺ ratio was calculated from the Na⁺ and K⁺ content. Salt treatment significantly increased the Na⁺/K⁺ ratio in shoot and root of both genotypes, to a great extent in ‘C198’ than in ‘C43’ (Figs. 4C and 5C).

**LIPID PEROXIDATION AND H₂O₂ LEVEL.** No significant difference was observed in MDA content of new leaves for two bermudagrass genotypes subjected to 100 and 200 mM NaCl when compared with the respective control (Fig. 6A). MDA content in new leaves of ‘C198’ had a 62% higher level than those of ‘C43’ when exposed to 400 mM NaCl. Salt stress increased MDA content by 2.7 and 7.2 times in old leaves of ‘C198’ at moderate (200 mM) and high (400 mM) salt concentration, respectively, when compared with the control level (Fig. 6B). However, salt stress had no effects on MDA content in old leaves of ‘C43’ when compared with the control level.

There was no significant effect of salt on H₂O₂ level in new leaves of both genotypes, except at high (400 mM) salt exposure (Fig. 6C). ‘C43’ had a higher H₂O₂ level in new leaves at mild (100 mM) salt exposure and control conditions. A significant increase in the accumulation of H₂O₂ was observed in the old leaves of ‘C198’ at moderate (200 mM) and high (400 mM) salt concentration when compared with control plants (Fig. 6D).

**RESPONSES OF ANTIOXIDANT ENZYMES TO SALT STRESS.** Moderate salt stress (200 mM) induced a greater level of SOD activity in new leaves of both genotypes when compared with the respective control level (Fig. 7A). No difference in SOD activity was observed for new leaves between two genotypes regardless of salinity levels (Fig. 7A). ‘C43’ old leaves had a greater level of SOD activity when subjected to the mild (100 mM) and moderate (200 mM) salt stress and then declined to the control level at high (400 mM) salt exposure in old leaves (Fig. 7B). The SOD activity increased under mild salt stress but decreased under high salt stress in ‘C198’ when compared with the controls. ‘C43’ exhibited a greater level of SOD activity than ‘C198’ in old leaves under moderate and high salt exposure (Fig. 7B).

The CAT activity in new leaves increased under mild (100 mM) salt stress for both genotypes, then declined to the control level for ‘C43’, and declined to a level lower than the control for ‘C198’ under moderate and high salt stress (Fig. 7C). ‘C198’ had a higher level of CAT activity than ‘C43’ in new leaves subjected to the control, mild, and moderate salt stress. The CAT activity in old leaves increased under mild salt stress but decreased under high salt stress in ‘C198’ when compared with the controls (Fig. 7D). Exposure to moderate (200 mM) salt stress resulted in an increase in CAT activity in
old leaves of ‘C43’. ‘C43’ had 38% and 66% higher CAT activity than ‘C198’ in old leaves under moderate or high doses of salt, respectively (Fig. 7D).

No difference in APX activity of new leaves was observed for two bermudagrass genotypes subjected to 100 and 200 mM NaCl when compared with the control (Fig. 8A). ‘C198’ new leaves had a 37% higher level of APX activity than ‘C43’ when exposed to 400 mM NaCl. Salt stress increased APX activity by 50%, 67%, and 71% in old leaves of ‘C43’ at mild (100 mM), moderate (200 mM), and high (400 mM) salt concentrations, respectively, when compared with the control level (Fig. 8B). Mild (100 mM) and moderate (200 mM) salt concentration increased the DHAR activity in old leaves of ‘C198’ (Fig. 8D). ‘C43’ had a 37% higher level of DHAR activity than ‘C198’ in old leaves under control conditions and a 46% higher activity at high salt (400 mM) exposure. No difference in old leaves of DHAR activity was observed between two genotypes at mild (100 mM) and moderate (200 mM) salt exposure (Fig. 8D).

**Antioxidant Enzyme Gene Expression.** The abundance of Cu/Zn SOD transcripts increased in new leaves and decreased in old leaves with increasing salinity levels in both genotypes when compared with the respective control (Fig. 9). No difference in Cu/Zn SOD transcripts was observed in new leaves of ‘C43’ vs. ‘C198’, however, salinity induced a 47% and 72% increase at 200 and 400 mM NaCl, respectively. Salt stress had no effects on DHAR activity in old leaves of ‘C43’ (Fig. 6D). Mild (100 mM) and moderate (200 mM) salt concentration increased the DHAR activity in old leaves of ‘C198’ (Fig. 8D). ‘C43’ had a 37% higher level of DHAR activity than ‘C198’ in old leaves under control conditions and a 46% higher activity at high salt (400 mM) exposure. No difference in old leaves of DHAR activity was observed between two genotypes at mild (100 mM) and moderate (200 mM) salt exposure (Fig. 8D).

**Fig. 4.** Effects of salt stress on Na+ content (A), K+ content (B), and Na+/K+ ratio (C) in shoots of two bermudagrass genotypes (‘C43’ and ‘C198’) at 21 d. Bars marked by the same letters are not significant at $P < 0.05$ (Tukey’s test) for the comparison of salt concentration in a genotype; * indicates significant difference at $P < 0.05$ (Tukey’s test) for the comparison of genotypes in a salt concentration ($n = 3$).

**Fig. 5.** Effects of salt stress on Na+ content (A), K+ content (B), and Na+/K+ ratio (C) in roots of two bermudagrass genotypes (‘C43’ and ‘C198’) at 21 d. Bars marked by the same letters are not significant at $P < 0.05$ (Tukey’s test) for the comparison of salt concentration in a genotype; * indicates significant difference at $P < 0.05$ (Tukey’s test) for the comparison of genotypes in a salt concentration ($n = 3$).
compared with the control (Fig. 10). Salt-tolerant ‘C43’ exhibited a greater expression level in new leaves (Fig. 10A) and old leaves (Fig. 10B) under moderate (200 mM) and high (400 mM) salt treatment when compared with the ‘C198’.

**Discussion**

**Growth response to salt stress.** Salt stress showed a remarkable effect on the shoot and root growth of bermudagrass genotypes differing in salt tolerance. The reduction in canopy height under salt stress was associated with the increasing Na+, Na+/K+ ratio, and reduction of K+. These results suggested that growth inhibition could be related to the osmotic and ionic effects (nutritional deficiency and/or imbalance). Growth parameters such as shoot growth, root growth, and TQ have been reported to be excellent criteria to determine salinity tolerance in turfgrasses (Marcum, 1999; Marcum and Kopec, 1997). In our study, ‘C43’ maintained a relatively higher TQ and root growth under salinity conditions, indicating that the tolerance of ‘C43’ could be related to the development and maintenance of a more extensive root system under saline conditions.

Several investigators have demonstrated that shoot growth is more sensitive to salinity than root growth and leads to an increased root/shoot ratio (Bernstain et al., 2001; Qian et al., 2000). Our results also showed that the shoot of bermudagrass is more sensitive to salinity than the root system.
Compared with the shoot growth, the root growth of the two bermudagrass genotypes was less affected or even stimulated by salt stress, particularly in salt-tolerant 'C43'. This was in agreement with the previous reports on warm-season or more stress-tolerant grasses/halophytes (Marcum et al., 2005; Pessarakli and Kopec, 2004; Pessarakli et al., 2005; Sagi et al., 1997).

Higher resource allocation to root growth is a symptom of water stress and/or nonoptimal nutritional conditions in the root environment (Bell and O'Leary, 2003). In our study, the root/shoot length ratio was higher than the control level in two bermudagrass genotypes under salinity conditions, particularly in salt-tolerant 'C43', indicating greater allocation of metabolites and energy for root growth relative to shoot growth. Salt-stressed roots emphasize elongation growth, which helps them explore the soil to seek a more optimal environment from which to acquire water and minerals (McCarty and Dudeck, 1993). Bermudagrass allocates more resources to root growth relative to shoot growth under salt stress. It seems that the need to find deeper freshwater and nutrient in a saline habitat exceeds the need to increase the shoot growth for light and photosynthetic carbon gain. The proposed explanations for the root growth stimulation were attributed to the maintenance of a high photosynthetic rate despite retardation of shoot growth, reduced percentage defoliation resulting from shoot growth retardation, or a differential response of shoots and roots to phytohormones (Youngner and Lunt, 1967).

**PHYSIOLOGICAL RESPONSE TO SALT STRESS.**

The MDA content is generally used as an indicator of oxidative damages induced by salinity stress (Hernández and Almansa, 2002). Our results showed that a remarkable increase in leaf MDA content in salt-sensitive 'C198' under high (400 mM) salt stress indicated a higher rate of lipid peroxidation for 'C198' as a result of salt stress and salt-tolerant 'C43' might have better protection against oxidative damage. The lower oxidative damages in leaves of 'C43' compared with 'C198' may reflect a more efficient antioxidative system as evidenced by a higher activity of SOD, CAT, APX, and DHAR enzymes.

The increased, decreased, and unchanged antioxidant enzyme activities in the new and old leaves of bermudagrass indicate a different antioxidant metabolism in response to salt stress. SOD plays a key role for maintaining normal physiological processes and coping with oxidative stress by rapidly converting O$_2^\cdot$ to O$_2$ and H$_2$O$_2$ (Mittler, 2002). The effects of salt stress on SOD activity varied at interspecific and intraspecific levels and the age of tissue or organ (Ashraf, 2009). In our study, the activity of SOD was increased more in the old leaves of the tolerant genotype ('C43') and slightly increased or even decreased in SOD activity in the sensitive genotype ('C198'). Such a differential response to salt stress has been reported between cultivars of potato [Solanum tuberosum (Rahnama and Ebrahimzadeh, 2005)], millet [Setaria italica (Sreenivasulu et al., 2000)], wheat [Triticum aestivum (Sairam et al., 2002)], and strawberry [Fragaria vesca (Turhan and Gulen Erics, 2008)]. These results suggested that the SOD activities can be induced by ROS under salt stress and the salt-tolerant 'C43' might have better protection against oxidative damage. The lower oxidative damages in leaves of 'C43' compared with 'C198' may reflect a more efficient antioxidative system as evidenced by a higher activity of SOD, CAT, APX, and DHAR enzymes.
These results suggested that higher antioxidant defense existed in new leaves than in old leaves leading to new leaf survival and maintaining growth under salinity conditions.

Catalase is one of the important enzymes that scavenge H$_2$O$_2$, the product of SOD-facilitated reactions, by directly breaking it down to H$_2$O and O$_2$ (Mittler, 2002). The CAT activity increased in salt-stressed tobacco \textit{(Nicotiana tabacum)} (Savoure et al., 1999), tomato \textit{(Lycopersicon pennellii)} (Rodriguez-Rosales et al., 1999), and mulberry \textit{(Morus alba)} ( Sudhakar et al., 2001). However, unchanged CAT activity under salt stress was also observed in potato (Benavides et al., 2000) and rice \textit{(Oryza sativa)} (Lin and Kao, 2000). Khorasavnejad et al. (2008) and Seckin et al. (2010) observed a rapid and continued increase in CAT activity of salt-tolerant barley \textit{(Hordeum vulgare)} cultivars under salt stress. In the present study, CAT activity increased under mild to moderate stress but declined at a high salt concentration for salt-sensitive ‘C198’ when compared with the control plants. These results indicated that the changes in CAT activity may depend on the plant species, the development and metabolic state of the plant as well as on the duration and intensity of the stress (Chaparzadeh et al., 2004). The level of H$_2$O$_2$ in new leaves was lower in ‘C198’ than ‘C43’ under control and mild salt exposure with a higher CAT activity in ‘C198’ than that of ‘C43’ and vs. in old leaves under moderate and high salt treatment. These results suggested that CAT may play a major role in scavenging H$_2$O$_2$ for bermudagrass under salt stress.

In addition to CAT, an alternative and more effective detoxification mechanism against H$_2$O$_2$ was the ascorbate–glutathione cycle (GSH-ASC) (Asada and Takahashi, 1987). The cycle maintains a ratio of reduced peroxidized ASA and GSH for proper scavenging ROS in plant cells (Mittler, 2002). APX and DHAR are major ascorbate-reducing enzymes in the GSH-ASC cycle that scavenges H$_2$O$_2$ (Parvaiz and Satyawati, 2008). Our results showed that APX activities increased in old leaves and did not change in new leaves of salt-tolerant genotype under salinity conditions. The enhancement of APX activities suggested that salt-triggered antioxidative capacity might be responsible for the removal of excessive H$_2$O$_2$ in old leaves. However, the DHAR activity in old or new leaves showed variations for two genotypes exposed to different salinity levels. These results indicated that the different responses of these enzyme activities to salt stress may depend on plant species, stress severity, and intensity of ROS production in different tissues (Bian and Jiang, 2009). Generally, the increased or unchanged APX and DHAR activities in the leaves of bermudagrass under salt stress may help maintain proper levels of ASA and GSH, the two important antioxidants against ROS toxicity (Foyer et al., 1994).

The comparison of the genotype-dependent response pattern for SOD, CAT, APX, and DHAR activities to salt stress indicated significant genotypic difference at different concentrations of salt exposure. The SOD, CAT, and APX seem to be
the key enzymes in determining salt tolerance in bermudagrass, because their activities were much higher in salt-tolerant leaves than the sensitive ones under medium to high salt stress. The decreased activities of SOD, CAT, and APX in old leaves of salt-sensitive genotype at high salt stress (400 mM NaCl) were probably the result of the harmful effect of overproduction of ROS or its poisonous ROS derivatives in old leaves over the new leaves or the tolerant genotype ‘C43’. The expression patterns of SOD, CAT, APX, and DHAR in old and new leaves were not totally consistent with the enzyme activities in both genotypes. These suggested that the abundance of a particular transcript does not always correlate with corresponding changes in antioxidant protein levels and/or enzyme activities (Mullineaux and Creissen, 1997).

In conclusion, salt stress (100, 200, and 400 mM NaCl) significantly inhibited shoot growth in both genotypes and stimulated root growth (except 400 mM for ‘C198’). Differences in growth response and salinity tolerance between the two genotypes was attributed largely to the development and maintenance of a more extensive root system under saline conditions and induced antioxidant gene expressions, leading to more efficient enzyme stimulation and protection in bermudagrass. These criteria might effectively be used in breeding programs to select and develop salt-tolerant bermudagrass cultivars.

**Literature Cited**

Abdul-Jaleel, C., R. Gopi, G.M.A. Lakshmanan, and R. Panneerselvam. 2006. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in Catharanthus roseus (L.) G. Don. Plant Sci. 171:271–276.

Adavi, Z., K. Razmjoo, and M. Mobli. 2006. Salinity tolerance of bermudagrass (Cynodon spp. L.C. Rich) cultivars and shoot Na, K and Cl contents under a high saline environment. J. Hort. Sci. Biotechnol. 81:1074–1078.

Akrum, N.A., M. Shahbaz, H.R. Athar, and M. Ashraf. 2006. Morphophysiological responses of two differently adapted populations of Cynodon dactylon (L.) Pers. and Cenchrus ciliaris L. to salt stress. Pak. J. Bot. 38:1581–1588.

Alshammary, S.F., G. Hussain, and Y.L. Qian. 2008. Response of four warm-season grasses to saline irrigation and protection under arid climate. Asian J. Plant Sci. 7:619–627.

Amor, N.B., A. Jiménez, W. Megdiche, M. Lundqvist, F. Sevilla, and C. Abdelliy. 2006. Response of antioxidant systems to NaCl stress in the halophyte Calkile maritima. Physiol. Plant. 126:446–457.

Asada, K. and M. Takahashi. 1987. Production and scavenging of active oxygen in photosynthesis, p. 227–287. In: Kyle, D.J., C.B. Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. California Agr. Expt. Sta. Circ. 347.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125:189–198.

Hernández, J.A. and M.S. Almansa. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plant. 115:251–257.

Hoagland, D.R. and D.I. Armon. 1950. The water-culture method for growing plants without soil. California Agr. Expt. Sta. Circ. 347.

Hu, L., H. Li, H. Pang, and J. Fu. 2012a. Responses of antioxidant gene, protein and enzymes to salinity stress in two genotypes of perennial ryegrass (Lolium perenne) differing in salt tolerance. J. Plant Physiol. 169:146–156.

Hu, L., T. Hu, X. Zhang, H. Pang, and J. Fu. 2012b. Exogenous glycine betaine ameliorates the adverse effect of salt stress on perennial ryegrass (Lolium perenne L.). J. Amer. Soc. Hort. Sci. 137:38–46.

Huang, B. and L. Liu. 2009. Physiological responses of creeping bentgrass to heat stress affected by phosphate fungicide application. Intl. Turfgrass Soc. Res. J. 11:799–806.

Hulsi, K., B. Melike, Ø. Filiz, and T. Ismail. 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ. Exp. Bot. 60:344–351.

Jena, S. and M.A. Choudhuri. 1981. Glycolate metabolism of three submerged aquatic angiosperms during aging. Aquat. Bot. 12:345–354.

Khosravinejad, F., R. Heydari, and T. Farboodnia. 2008. Antioxidant responses of two barley varieties to saline stress. Res. J. Biol. Sci. 3:486–490.

Lin, C. and C. Kao. 2000. Effect of NaCl stress on H2O2 metabolism in rice leaves. Plant Growth Regulat. 30:151–155.

Lu, S., X. Peng, Z. Guo, G. Zhang, Z. Wang, C. Wang, C. Pang, Z. Fan, and J. Wang. 2007. In vitro selection of salinity tolerant variants from triploid bermudagrass (Cynodon transvaalensis × C. dactylon) and their physiological responses to salt and drought stress. Plant Cell Rpt. 26:1413–1420.

Mancino, C. and I.L. Pepper. 1994. Effects of wastewater on the turfgrass/soil environment, p. 121–135. In: Snow, J.T., M.P. Kenna, K.S. Erusha, M. Henry, C.H. Peacock, and J.R. Watson (eds.). Waste water reuse for golf course irrigation. Lewis Publisher, Chelsea, MI.

Marcum, K.B. 1999. Salinity tolerance mechanisms of grasses in the subfamily Chloridoideae. Crop Sci. 39:1153–1160.

Marcum, K.B. and D.M. Kopec. 1997. Salinity tolerance of turfgrasses and alternative species in the subfamily Chloridoideae (Poaceae). Intl. Turfgrass Soc. Res. J. 8:735–742.

Marcum, K.B. and M. Pessarakli. 2006. Salinity tolerance and salt gland excretion efficiency of bermudagrass turf cultivars. Crop Sci. 46:2571–2574.
Marcum, K.B., M. Pessarakli, and D.M. Kopec. 2005. Relative salinity tolerance of 21 turf-type desert saltgrasses compared to bermudagrass. HortScience 40:827–829.
McCarty, L.B. and A.E. Dudeck. 1993. Salinity effects on bengtgrass germination. HortScience 28:15–17.
Mhadhbi, H., V. Fotopoulos, P.V. Mylona, M. Jebara, M. Elarbi Aouani, and A.N. Polidoros. 2011. Antioxidant gene–enzyme responses in Medicago truncatula genotypes with different degree of sensitivity to salinity. Physiol. Plant. 141:201–214.
Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405–410.
Mittova, V., M. Tal, M. Volokita, and M. Guy. 2003. Up-regulation of the leaf mitochondrial and peroxisomal antioxidant systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species Lycopersicon pennelli. Plant Cell Environ. 26:845–856.
Mullineaux, P.M. and G.P. Creissen. 1997. Glutathione reductase: Regulation and role in oxidative stress, p. 667–714. In: Scandalios, J. (ed.). Oxidative stress and the molecular biology of antioxidant defenses. Cold Spring Harbor Laboratory Press, New York, NY.
Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25:239–250.
Munns, R. and M. Tester. 2008. Mechanism of salinity tolerance. Annu. Rev. Plant Biol. 59:651–681.
Munns, R. and M. Tester. 2008. Mechanism of salinity tolerance. Annu. Rev. Plant Biol. 59:651–681.
Murillo-Amador, B., H.G. Jones, C. Kaya, R.L. Guilar, J.L. Garcia-Hernandez, E. Troyo-Diegeuz, N.Y. Avila-Serrano, and E. Rueda-Puente. 2006. Effects of foliar application of calcium nitrate on growth and physiological attributes of cowpea (Vigna unguiculata L.) grown under salt stress. Environ. Exp. Bot. 58:188–196.
Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22:867–880.
Parvaz, A. and S. Satyawati. 2008. Salt stress and phyto–biochemical responses of plants—A review. Plant Soil Environ. 54:89–99.
Pessarakli, M. and D.M. Kopec. 2004. Growth responses of bermudagrass and Seashore Paspalum to different levels of FerroGrow multi-nutrient fertilizer. J. Food Agr. Environ. 2:284–286.
Pessarakli, M., K.B. Marcum, and D.M. Kopec. 2005. Growth responses and nitrogen-15 absorption of desert saltgrass (Distichlis spicata L.) to salinity stress. J. Plant Nutr. 28:1441–1452.
Qian, Y.L., M.C. Engelke, and M.J.V. Foster. 2000. Salinity effects on zoysiagrass cultivars and experimental lines. Crop Sci. 40:488–492.
Rahnama, H. and H. Ebrahimzadeh. 2005. The effect of NaCl on antioxidant enzyme activities in potato seedlings. Biol. Plant. 49:93–97.
Rodriguez-Rosales, M.P., L. Kerkeb, P. Bueno, and J.P. Donaire. 1999. Changes induced by NaCl in lipid content and composition, lipoxigenase, plasma membrane H+-ATPase and antioxidant enzyme activities of tomato (Lycopersicon esculentum Mill.) calli. Plant Sci. 143:143–150.
Saig, M., N.A. Savidov, N.P. L’vov, and S.H. Lips. 1997. Nitrate reductase and molybdenum cofactor in annual ryegrass as affected by salinity and nitrogen source. Physiol. Plant. 99:546–553.
Sairam, R.K., K.V. Rao, and G.C. Srivastava. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci. 163:1037–1046.
Savouré, A., D. Thorin, M. Davey, X.J. Hua, S. Mauro, M. Van Montagu, D. Inze, and N. Verbruggen. 1999. NaCl and CuSO 4 treatments trigger distinct oxidative defense mechanisms in Nicotiana plumbaginifolia L. Plant Cell Environ. 22:387–396.
Seckin, B., I. Turkan, A.H. Sekmen, and C. Ozfidan. 2010. The role of antioxidant defense systems at differential salt tolerance of Hordeum marinum Huds. (sea barleygrass) and Hordeum vulgare L. (cultivated barley). Environ. Exp. Bot. 69:76–85.
Sen Gupta, A.S., J.L. Heinen, A.S. Holaday, J.J. Burke, and R.D. Allen. 1993. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci. USA 90:1629–1633.
Shahba, M.A. 2010. Interaction effects of salinity and mowing on performance and physiology of bermudagrass cultivars. Crop Sci. 50:2620–2631.
Sigaud-Kutner, T.C.S., E. Pinto, O.K. Okamoto, L.R. Latorre, and P. Colepicolo. 2002. Changes in superoxide dismutase activity and photosynthetic pigment content during growth of marine phytoplankters in batch-cultures. Physiol. Plant. 114:566–571.
Sreenivasulu, N., B. Grimm, U. Wobus, and W. Weschke. 2000. Differential response of antioxidant compounds to salinity stress in salt tolerant and salt sensitive seedlings of foxtail millet (Setaria italica). Physiol. Plant. 109:435–442.
Sudhakar, C., A. Lakshmi, and S. Giridarakumar. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (Morus alba L.) under NaCl salinity. Proc. Biochem. 36:619.
Turan, E. and A. Gulen Erics. 2008. The activity of antioxidative enzymes in three strawberry cultivars related to salt-stress tolerance. Acta Physiol. Plant. 30:201–208.
Youngner, V.B. and O.R. Lunt. 1967. Salinity effects on roots and tops of bermudagrass. Grass Forage Sci. 22:257–259.
Zhu, J.K. 2001. Plant salt tolerance. Trends Plant Sci. 6:66–71.