Journal of Experimental Botany, Vol. 52, No. 355, pp. 341–349, February 2001

Journal of Experimental Botany

Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses

Yiwei Jiang[1] and Bingru Huang[2,3]

[1] *Department of Horticulture, Forestry and Recreational Resources, Kansas State University, Manhattan, KS 66506, USA*

[2] *Department of Plant Science, Rutgers University, New Brunswick, NJ 08901, USA*

Received 31 March 2000; Accepted 5 September 2000

Abstract

Calcium (Ca^{2+}) may be involved in plant tolerance to heat stress by regulating antioxidant metabolism or/and water relations. This study was designed to examine whether external Ca^{2+} treatment would improve heat tolerance in two C_3, cool-season grass species, tall fescue (*Festuca arundinacea* L.) and Kentucky bluegrass (*Poa pratensis* L.), and to determine the physiological mechanisms of Ca^{2+} effects on grass tolerance to heat stress. Grasses were treated with $CaCl_2$ (10 mM) or H_2O by foliar application and then exposed to heat stress (35/30 °C) in growth chambers. Some of the Ca^{2+}-untreated plants were maintained at 20/15 °C as the temperature control. Heat stress reduced grass quality, relative water content (RWC), and chlorophyll (Chl) content of leaves in both species, but Ca^{2+} treatment increased all three factors under heat stress. The Ca^{2+} concentration in cell saps increased with heat stress and with external Ca^{2+} treatment in both species. Osmotic potential increased with heat stress, but external Ca^{2+} treatment had no effect. Osmotic adjustment increased during short-term heat stress, but then decreased with a prolonged period of stress; it was not influenced by Ca^{2+} treatment. The activity of superoxide dismutase (SOD) in both species increased transiently at 12 d of heat stress and then remained at a level similar to that of the control. External Ca^{2+} treatment had no effect on SOD activity. The activities of catalase (CAT), ascorbate peroxidase (AP), and glutathione reductase (GR) of both species decreased during heat stress. Plants treated with Ca^{2+} under heat stress had higher CAT, GR and AP activities than untreated plants. Lesser amounts of malondialdehyde (MDA) accumulated in Ca^{2+}-treated plants than in untreated plants during extended periods of heat stress. The results suggested that exogenous Ca^{2+} treatment enhanced heat tolerance in both tall fescue and Kentucky bluegrass. This enhancement was related to the maintenance of antioxidant activities and a decrease in membrane lipid peroxidation, but not to the regulation of osmotic potential and osmotic adjustment.

Key words: Antioxidant enzymes, Ca^{2+}, tall fescue, Kentucky bluegrass, osmotic adjustment.

Introduction

Heat stress is a major factor limiting growth of cool-season plant species in many areas. Growth suppression in cool-season grasses under heat stress involves many physiological and biochemical changes, including water deficit and oxidative stress (Tajima *et al*., 1976; Lehman and Engelke, 1993; Liu and Huang, 2000), which are detrimental to plant survival under high temperature conditions. Previous studies have elucidated the importance of maintaining a favorable antioxidative level and water status in plant adaptation to heat stress (Ahmad *et al*., 1989; Graves *et al*., 1991; Ashraf *et al*., 1994; Jagtap and Bhargava, 1995; Gong *et al*., 1997; Kurganova *et al*., 1997).

[3] To whom correspondence should be addressed. Fax: +1 732 932 9441. E-mail: huang@aesop.rutgers.edu

Abbreviations: RWC, relative water content; Chl, chlorophyll content; SOD, superoxide dismutases; CAT, catalase; AP, ascorbate peroxidase; GR, glutathione reductase; MDA, malondialdehyde.

Several studies have showed that Ca^{2+} is involved in the regulation of plant responses to various environmental stresses, including heat (Bramm, 1992; Biyaseheva *et al*., 1993; Colorado *et al*., 1994). Increasing cytosolic Ca^{2+} content under heat stress (Biyasheva *et al*., 1993; Gong *et al*., 1998) may alleviate heat injury and enable plant cells to better survive (Bamberg *et al*., 1998; Gong *et al*., 1998). However, excessive Ca^{2+} released into the cytosol and sustained high cytosolic Ca^{2+} concentration might be cytotoxic (Hepler and Wayne, 1985; Biyasheva *et al*., 1993; Wang and Li, 1999).

Limited research has been conducted concerning effects of exogenous Ca^{2+} on heat tolerance, and available results are contradictory. It was found that treatment of maize (*Zea mays* L.) seeds with 15 and 20 mM Ca^{2+} solution enhanced intrinsic heat tolerance of seedlings (Gong *et al*., 1997). External Ca^{2+} (5–10 mM) also reduced high temperature-induced membrane leakage in roots of beet (*Beta vulgaris* L.) (Toprover and Glinka, 1976; Cooke *et al*., 1986). In contrast, it has been reported that heat-induced growth retardation could not be alleviated by external Ca^{2+} treatment (1.9 mM) in excised coleoptiles of wheat (*Triticum aestivum* L.) (Onwueme and Laude, 1972).

The role of Ca^{2+} in the regulation of heat tolerance is still unclear. Some suggest that it may be involved in signal transduction (McAinsh *et al*., 1996) and gene expression (Bramm, 1992; Trofimova *et al*., 1999) under oxidative and heat stress. Others (Gong *et al*., 1997) found that Ca^{2+} increases antioxidant enzyme activities and reduces lipid peroxidation of cell membranes. Calcium has also been shown to regulate guard-cell turgor and stomatal aperture (Mansfield *et al*., 1990; Webb *et al*., 1996). Cell turgor maintenance depends on the accumulation of compatible, osmotically active solutes (osmotic adjustment) (Hare *et al*., 1998). External Ca^{2+} may interfere with cellular Ca^{2+} and affect osmotic adjustment of cells under stress conditions.

The objectives of this study were to investigate the involvement of Ca^{2+} in heat tolerance in cool-season grasses, and to examine the effects of external Ca^{2+} treatment on antioxidant enzymes and osmotic adjustment under heat stress. Two grass species, tall fescue and Kentucky bluegrass were examined to address these objectives.

Materials and methods

Plant materials

Sod pieces of tall fescue (cv. Rebel Jr.) and Kentucky bluegrass (cv. Kenblue) were collected from field plots at the Rocky Ford Turfgrass Research Center, Kansas State University. Grasses were grown in polyvinylchloride tubes (10 cm in diameter, 60 cm long) filled with topsoil (fine, montmorillonitic, mesic, aquic arquidolls) in the greenhouse for 60 d and then transferred to growth chambers with a temperatures of 20/15 °C (day/night), a 14 h photoperiod, and a photosynthetically active radiation of 600 μmol m^{-2} s^{-1} at the canopy level. Grasses were well-watered and maintained at growth chamber conditions for 15 d to allow adaptation before heat stress was imposed.

Heat stress and Ca^{2+} treatment

The experiment included two temperatures regimes: optimum, control temperatures (20/15 °C, day/night) and high temperatures (35/30 °C). Each temperature regime was replicated in four growth chambers. A 20 ml $CaCl_2$ solution (10 mM) was sprayed uniformly on foliage at 10.00 h using a spray bottle once daily during a 3 d period immediately before heat stress was imposed. No leaf burn was observed after Ca^{2+} spray. Control plants were sprayed with 20 ml deionized water. Each treatment consisted of four replicates and lasted for 30 d. The Ca^{2+}-treated and untreated plants were arranged randomly in high-temperature growth chambers.

Analysis of variance was based on the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC). Least significance difference (LSD) at a 0.05 probability level was used to detect the differences between treatment means.

Measurements

Grass quality, a criterion commonly used to evaluate physiological health and appearance, was rated visually as an integral of grass color, uniformity, and density on the scale of 0 (desiccated, brown leaves) to 9 (turgid, green leaves) (Turgeon, 1999). The minimum acceptable level was 6.

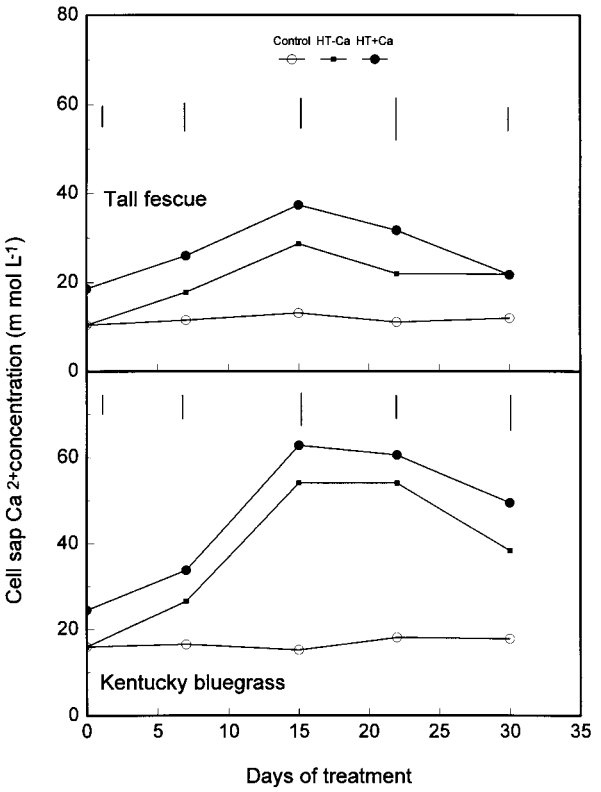

Fig. 1. Cell Ca^{2+} concentration as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

Leaf relative water content (RWC) was calculated as follows (Barrs and Weatherley, 1962): RWC=(FW−DW)/(SW−DW)×100, where FW is the leaf fresh weight, DW is leaf dry weight at 85 °C for 3 d, and SW is turgid weight of leaves after soaking in water for 4 h at room temperature (approximately 20 °C).

Leaves were frozen and pressed with a hydraulic press to collect cell sap for Ca^{2+} analysis and measurement of osmotic potential. The Ca^{2+} concentration was assayed at a 1:100 (v/v) dilution of cell sap using an inductively coupled plasma spectrophotometer (Fisons Instruments Inc., Beverley, MA). Leaf osmotic potential of stressed, dehydrated ($\psi\pi_o$), and fully rehydrated ($\psi\pi_{100}$) leaves was measured using a vapour pressure osmometer (Wescor, Inc., Logan, UT). Osmotic adjustment was calculated as the difference in osmotic potential at full turgor ($\psi\pi_{100}$) between control and stressed plants ($\psi\pi_o$) (Blum, 1989; Blum and Sullivan, 1986). Leaf chlorophyll (Chl) was extracted by soaking 0.05–0.1 g leaves in 20 ml dimethyl sulphoxide in the dark for 72 h (Hiscox and Israeltem, 1979). Absorbance of extracted Chl was measured at 663 and 645 nm using a spectrophotometer (Spectronic Instruments, Inc., Rochester, NY).

For enzyme extracts and assays, 0.2 g leaves were frozen in liquid nitrogen and then ground in 4 ml solution containing 50 mM phosphate buffer (pH 7.0), 1% (w/v) polyvinylpolypyrrolidone, and 0.2 mM ascorbic acid. The homogenate was centrifuged at 15 000 *g* for 30 min, and the supernatant was collected for enzyme assays.

The activity of SOD was measured as described previously (Giannopolities and Ries, 1977). The assay medium contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μm *p*-nitro blue tetrazolium chloride (NBT), 2 μm riboflavin, 0.1 mM EDTA, and 20–50 μl enzyme extract. Riboflavin was added last and the test tubes were placed under two 15 W fluorescent lamps. The reactions were terminated after 10 min by removal from light source. The absorbance was read at 560 nm. A non-irradiated reaction mixture did not develop colour and served as control. The reaction mixture lacking enzyme developed maximum colour as a result of maximum reduction of NBT. One unit of enzyme activity was determined as the amount of the enzyme to reach an inhibition of 50% NBT reduction rate.

The activity of CAT was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 (Change and Maehly, 1955). The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2.

The activity of AP was measured as a decrease in absorbance at 290 nm for 1 min (Nakano and Asada, 1981). The assay mixture consisted of 0.5 mM ASA, 0.1 mM H_2O_2, 0.1 mM EDTA, 50 mM sodium phosphate buffer (pH 7.0), and 0.15 ml enzyme extract.

The activity of GR was determined by following the decrease in absorbance at 340 nm for 1 min due to the glutathione-dependence of NADPH (Cakmak *et al.*, 1993). The reaction mixture contained 1 mM EDTA, 0.5 mM GSSG, 0.15 mM NADPH, 100 mM sodium phosphate buffer (pH 7.8), and 0.15 ml enzyme extract.

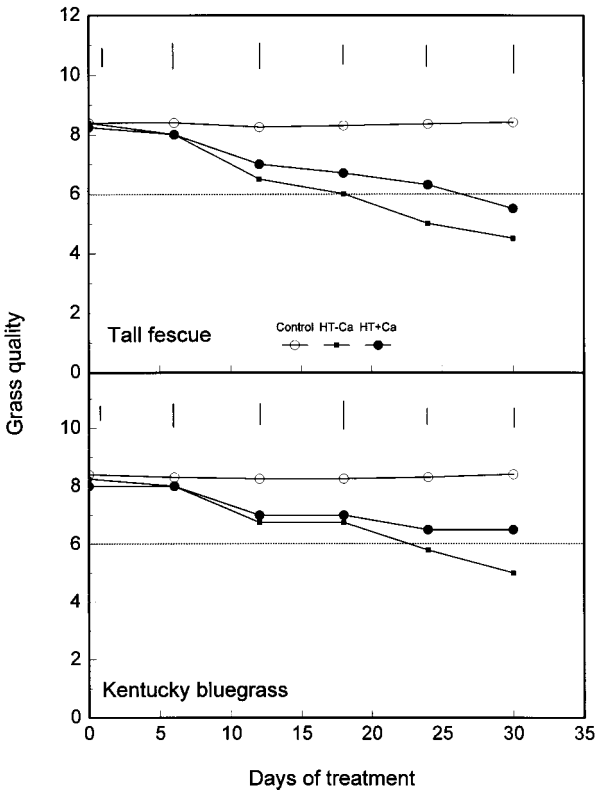

Fig. 2. Grass quality as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT+Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P=0.05$) for treatment comparison at a given day of treatment. Quality rated from 0 (worst) to 9 (Best). The dotted lines indicate acceptable level of grass quality.

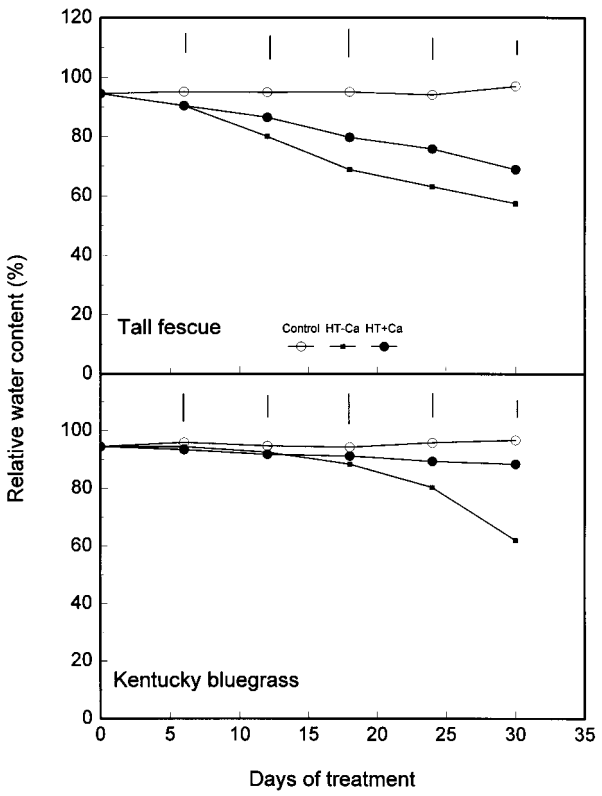

Fig. 3. Relative water content (RWC) as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT+Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P=0.05$) for treatment comparison at a given day of treatment.

Enzyme activities were expressed on the basis of per unit protein weight. Protein content was determined using bovine serum albumin as a standard (Bradford, 1976).

Lipid peroxidation was measured in terms of MDA content (Dhindsa *et al*., 1981). A 1 ml aliquot of supernatant was mixed with 4 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid. The mixture was heated at 100 °C for 30 min, quickly cooled, and then centrifuged at 10 000 *g* for 10 min. The absorbance of the supernatant was read at 532 nm. The unspecific turbidity was corrected by A_{600} subtracting from A_{530}. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer, 1968).

Results

Cell sap Ca^{2+} concentration

A rapid increase in Ca^{2+} concentration in the cell sap was observed during the first 15 d of heat stress in both species (Fig. 1). After 15 d, heat-stressed plants still maintained higher Ca^{2+} concentration than the controls. External Ca^{2+} treatment increased Ca^{2+} concentration in the cell sap before and after heat stress was imposed in both species, except for tall fescue at 30 d of heat stress.

Grass quality, chlorophyll content and water relations

Heat stress reduced grass quality to below the control (20/15 °C) level for both species, starting at 12 d (Fig. 2). Plants treated with Ca^{2+} maintained a higher quality than untreated plants after exposure to heat stress for 18 d in tall fescue and for 24 d in Kentucky bluegrass. At 30 d of heat stress, Ca^{2+}-treated Kentucky bluegrass still had acceptable quality (6.0), whereas quality of untreated plants had declined to below the acceptable level.

Leaf RWC decreased to below the control level, starting at 12 d of heat stress for tall fescue and 24 d for Kentucky bluegrass (Fig. 3). The Ca^{2+}-treated plants had higher RWC than untreated plants after 18 d and 24 d for tall fescue and Kentucky bluegrass, respectively. Kentucky bluegrass treated with Ca^{2+} under heat stress maintained RWC similar to the level of control plants without Ca^{2+} treatment during most of the experimental period, except at 30 d of heat stress.

Leaf Chl content increased to above the control level at 6 d of heat stress in both species and then decreased to below the control level at 24 d for tall fescue and 18 d for Kentucky bluegrass (Fig. 4). The Ca^{2+}-treated plants had higher Chl content than untreated plants after 24 d and 30 d for tall fescue and Kentucky bluegrass, respectively.

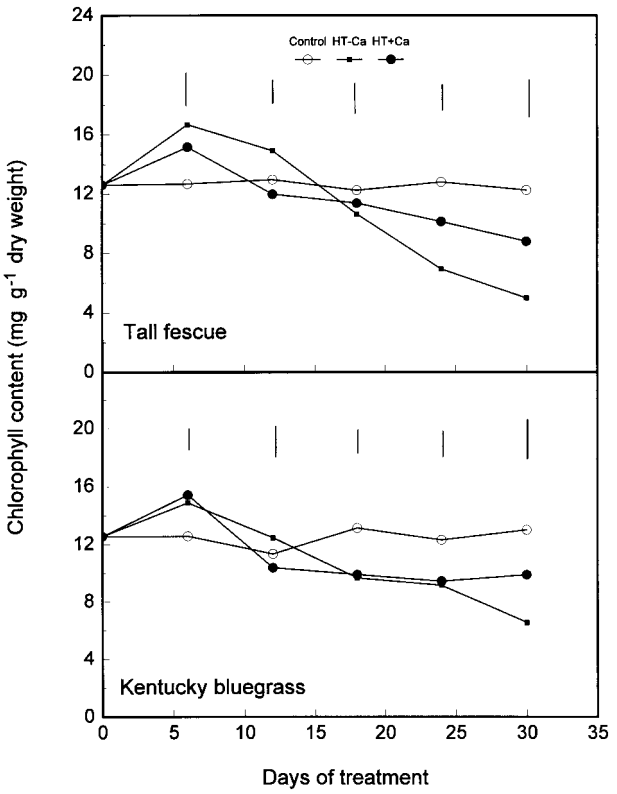

Fig. 4. Chlorophyll content as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

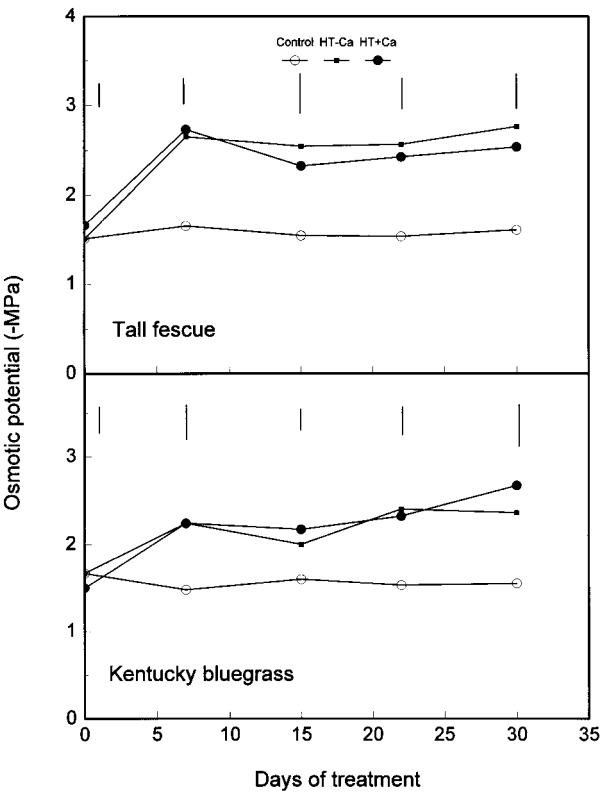

Fig. 5. Osmotic potential as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

Heat stress significantly increased leaf osmotic potential in both species, starting at 7 d (Fig. 5). Osmotic adjustment increased within the first 15 d of heat stress and then gradually decreased in both species (Fig. 6). External Ca^{2+} treatment had no significant effects on osmotic potential and osmotic adjustment (Figs 5, 6).

Antioxidant enzyme activities

The activity of SOD increased to above the control level at 12 d of heat stress in both species and then decreased to a level similar to that of the control (Fig. 7). The Ca^{2+} treatment had no effects on SOD activity under heat stress. Unlike SOD, CAT decreased to below the control level, beginning at 12 d of heat stress for tall fescue and 18 d for Kentucky bluegrass (Fig. 8). The activity of GR without Ca^{2+} treatment decreased to below the control level, starting at 6 d of heat stress for tall fescue and 12 d for Kentucky bluegrass (Fig. 9). The Ca^{2+}-treated plants had higher CAT and GR activities than untreated plants after 24 d of heat stress for both species. The AP activity decreased to below the control level after 18 d of heat stress for both species without Ca^{2+} treatment (Fig. 10). The Ca^{2+} treatment enhanced AP activity of heat-stressed plants to a level similar to that of control plants without Ca^{2+} treatment.

Lipid peroxidation

The MDA content in heat-stressed plants was higher than that in control plants, starting at 12 d in tall fescue and 18 d in Kentucky bluegrass (Fig. 11). The Ca^{2+} treatment considerably reduced MDA contents under heat stress at 12 d and 18 d for tall fescue and 18, 24 and 30 d for Kentucky bluegrass. The inhibitory effect of Ca^{2+} on MDA accumulation was more dramatic and lasted longer in Kentucky bluegrass than in tall fescue.

Discussion

Heat stress caused significant declines in grass quality, RWC and leaf Chl content. External Ca^{2+} application alleviated heat injury, as manifested by increases in these three factors compared to untreated plants. The responses of both cool-season species to heat stress and Ca^{2+} application followed the same pattern. These results are consistent with those reported for several other species. Application of 5–10 mM Ca^{2+} reduced thermal damage in beet root (Cooke *et al*., 1986) and potato (*Solanum tuberosum* L.) tubers (Coria *et al*., 1998). Similar concentrations of external Ca^{2+} also increased drought resistance in *Vigna catjang* (Mukherjee and Choudhuri, 1985), soybean (*Glycine max* L.) (Yang *et al*., 1993), and

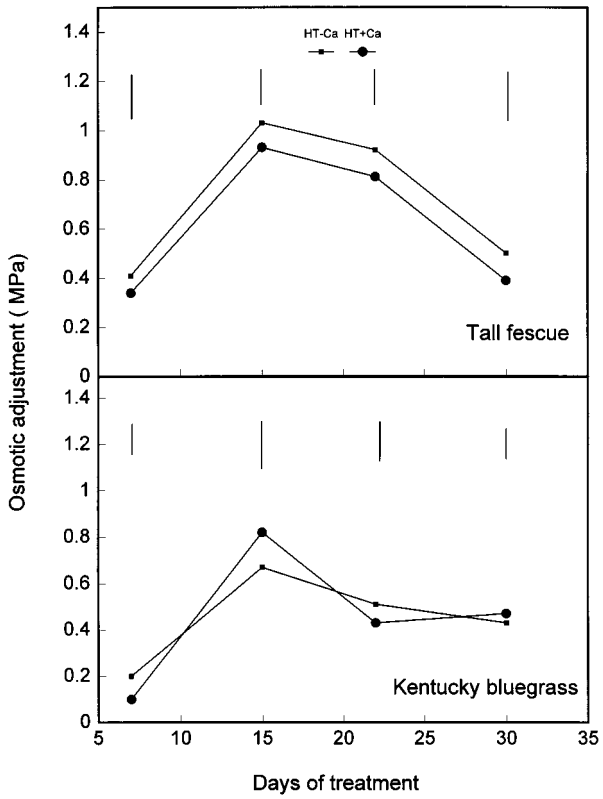

Fig. 6. Osmotic adjustment as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

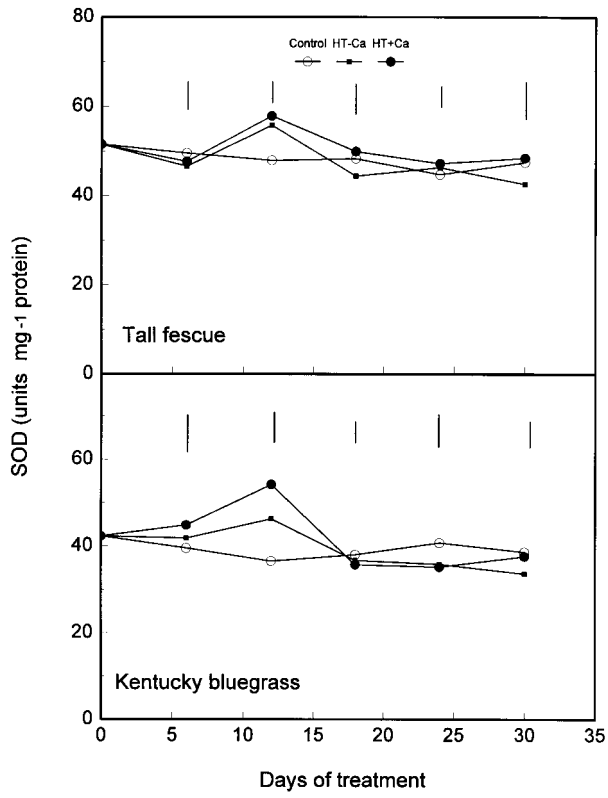

Fig. 7. SOD activity as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

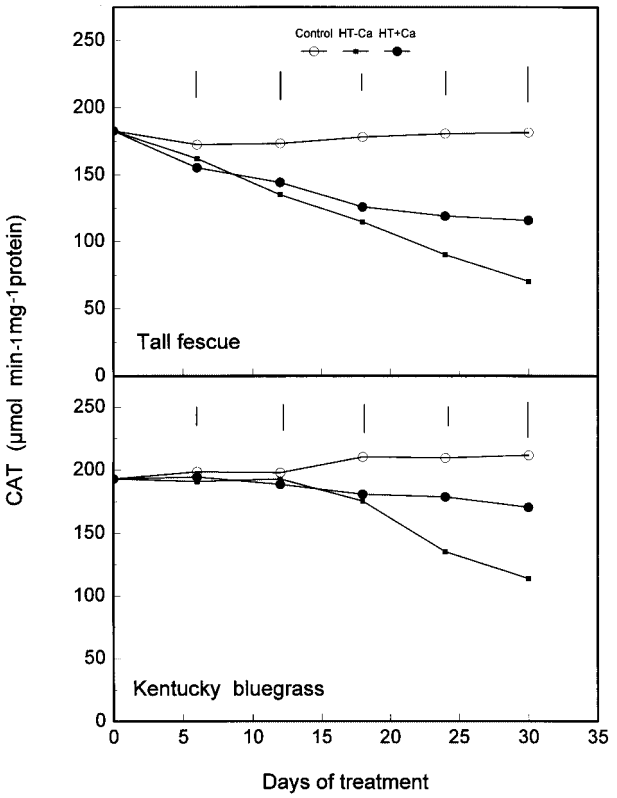

Fig. 8. CAT activity as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) on under heat stress (H) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

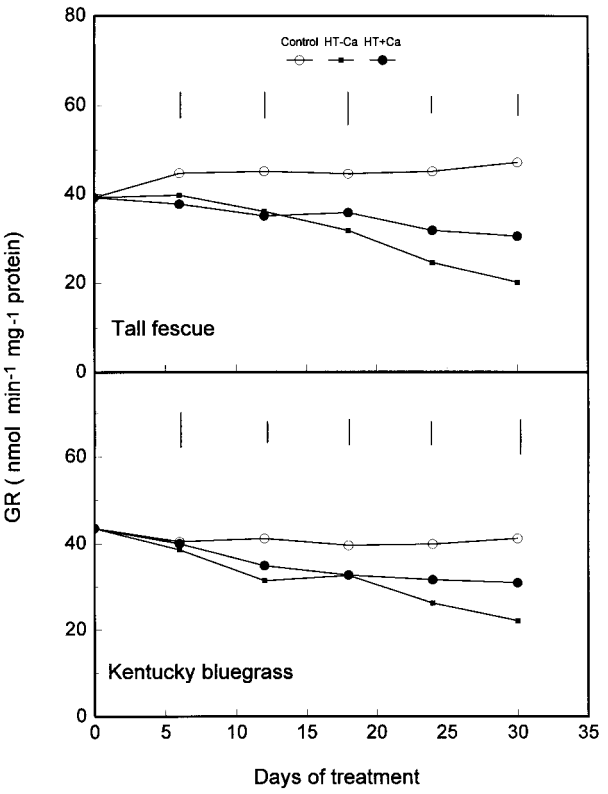

Fig. 9. GR activity as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

cotton (*Gossypium hirsutum* L.) (Cheng *et al.*, 1997), and increased salinity tolerance in bean (*Phaseolus vulgaris* L.) roots (Cachorro *et al.*, 1993). Heat stress significantly increased Ca^{2+} concentration in cell sap of both tall fescue and Kentucky bluegrass, especially during the early period of stress. Accumulation of cytosolic free Ca^{2+} also has been found under heat shock in other species (Klein and Ferguson, 1987; Biyaseheva *et al.*, 1993; Gong *et al.*, 1998; Wang and Li, 1999).

Osmotic adjustment is an important mechanism of plant tolerance to drought and heat stress (Smith *et al.*, 1989; Ludlow *et al.*, 1990). Osmotic adjustment increased during short-term heat stress in both species, but was not affected by external Ca^{2+} treatment under heat stress conditions. These results indicated that the enhancing effects of external Ca^{2+} on leaf water status and heat tolerance in both tall fescue and Kentucky bluegrass were not due to its regulation of osmotic potential and osmotic adjustment.

Short-term heat stress caused a transient increase in Chl content. However, prolonged periods of heat stress resulted in the loss of Chl and might have been related to damage to reaction centres (Kyle, 1987). External Ca^{2+} treatment inhibited the loss of chlorophyll under heat stress, possibly by its reducing photo-oxidation (Wise and Naylor, 1987) or maintaining membrane integrity (Coria *et al.*, 1998).

Heat stress induces oxidative injury and alters the activities of antioxidant enzymes including SOD, CAT, AP, and GR in many plant species (Burke and Oliver, 1992; Foyer *et al.*, 1994; Jagtap and Bhargava, 1995; Gong *et al.*, 1997). The SOD activity increased transiently during initial stress periods in tall fescue and Kentucky bluegrass. This increase might have been due to a rapid and large accumulation of Cu/Zn SOD mRNA, as was found in tobacco leaves during heat shock (Tsang *et al.*, 1991). The external Ca^{2+} treatment did not affect SOD activity under heat stress, suggesting that SOD was not related to the effects of Ca^{2+} on heat-induced oxidative stress. However, it has been found that external Ca^{2+} increased SOD activity in maize seedlings (Gong *et al.*, 1997). The increased SOD activity also might reflect the increased production of O_2^-. (Thompson *et al.*, 1987). Limited studies have suggested that calcium loading in root cells induces a dramatic increase in O_2^-. release during wound stress (Minibayeva *et al.*, 1998), but there is no direct evidence of the effect under heat stress.

Activity of CAT continued to decline with prolonged periods of heat stress in both species. Reduction in CAT activity also has been reported in other plants during heat

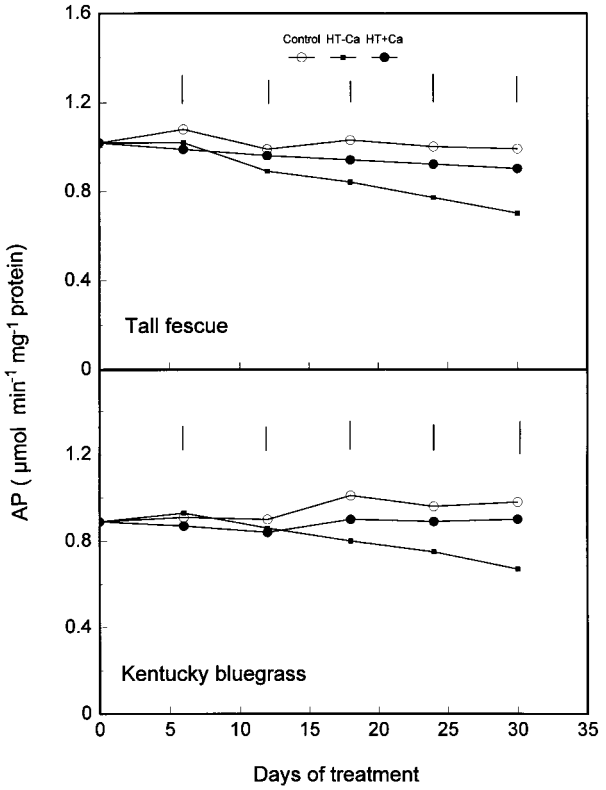

Fig. 10. AP activity as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

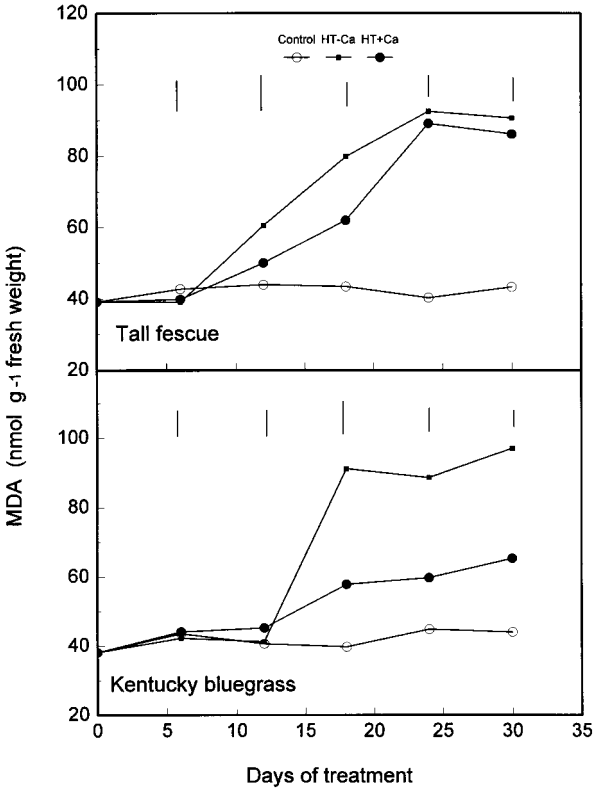

Fig. 11. Lipid peroxidation (MDA content) as affected by heat stress (HT-Ca) and Ca^{2+} treatment (HT + Ca) in tall fescue and Kentucky bluegrass. Vertical bars indicate LSD values ($P = 0.05$) for treatment comparison at a given day of treatment.

shock (Willekens *et al.*, 1995; Foyer *et al.*, 1997; Dat *et al.*, 1998), which paralleled increases in H_2O_2 content (Dat *et al.*, 1998). External Ca^{2+} treatment helped maintain higher CAT activity under heat stress. This enhanced CAT activity could reduce the accumulation of H_2O_2 and alleviate the damage to cell membranes. Ascorbate peroxidase located in both the cytosol and the chloroplasts, can also remove H_2O_2 (Cakmak *et al.*, 1993). Gong *et al.* reported that external Ca^{2+} treatment increased activity of AP in maize seedlings during heat stress and this enhanced activity could be related to improvement of heat tolerance (Gong *et al.*, 1997). These results agreed with this observation in cool-season grasses. External Ca^{2+} treatment resulted in a transient increase in cytosolic Ca^{2+} concentration during heat stress (Gong *et al.*, 1998) and the inhibitors of AP modify H_2O_2-induced transients in cytosolic Ca^{2+} concentration (Price *et al.*, 1994), suggesting some relationship among AP activity, cytosolic Ca^{2+} and oxidative stress. Glutathione reductase also plays an important role in maintaining the cellular antioxidant/ prooxidant ratio. Heat stress decreased GR activity, but Ca^{2+}-treated plants had higher GR activity than untreated plants. Calcium deprivation strongly inhibited GR activity in cell culture of *Digitalis thapsi* and changed the redox state of cells (Paranhos *et al.*, 1999). Thus, the enhancement of GR activity by external Ca^{2+} treatment could protect the chloroplastic components against oxidation by H_2O_2.

The content of MDA often is used as an indicator of lipid peroxidation resulting from oxidative stress (Smirnoff, 1995). Heat stress increased MDA contents in both tall fescue and Kentucky bluegrass. The Ca^{2+} treatment reduced lipid peroxidation, as indicated by less accumulation of MDA compared to untreated plants. This result agreed with those for maize seedlings (Gong *et al.*, 1997). Calcium is known to reduce membrane permeability and maintain membrane integrity under heat stress (Cooke *et al.*, 1986; Coria *et al.*, 1998). External Ca^{2+} treatment not only could affect membrane structure, but also could be involved in oxidative signal transduction concomitant with the regulation of antioxidant enzymes under heat stress (McAinsh *et al.*, 1996; Gong *et al.*, 1997). The results show that application of Ca^{2+} would reduce loss of grasses in hot environments by mitigating oxidative stress.

Acknowledgements

The authors thank Dr Mary Beth Kirkham for reviewing the manuscript. Funds for this study were provided by Kansas

Turfgrass Foundation and Kansas Agricultural Experiment Station. Contribution No. 00-341-J from the KAES.

References

Ahmad S, Ahmad N, Ahmad R, Hamid M. 1989. Effect of high temperature stress on wheat reproductive growth. *Journal of Agricultural Research Lahore* **27,** 307–313.

Ashraf M, Saeed MM, Qureshi MJ. 1994. Tolerance to high temperature in cotton at initial growth stages. *Environmental and Experimental Botany* **34,** 275–283.

Bamberg JB, Palta JP, Peterson LA, Martin M, Krueger AR. 1998. Fine screening potato species germplasm for tuber calcium. *American Journal of Potato Research* **75,** 181–186.

Barrs HD, Weatherley PE. 1962. A re-examination of the relative turgidity techniques for estimating water deficits in leaves. *Australian Journal of Biological Science* **15,** 413–428.

Biyaseheva AE, Molotkovskii YG, Mamonov LK. 1993. Increase of free Ca^{2+} in the cytosol of plant protoplasts in response to heat stress as related to Ca^{2+} homeostasis. *Plant Physiology* **40,** 540–544.

Blum A. 1989. Osmotic adjustment and growth of barley genotypes under drought stress. *Crop Science* **29,** 230–233.

Blum A, Sullivan CY. 1986. The comparative drought resistance of landraces of sorghum and millet from dry and humid regions. *Annals of Botany* **57,** 838–846.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72,** 248–254.

Bramm J. 1992. Regulated expression of the calmodulin-related TCH genes in cultured *Arabidopsis* cells: induction by calcium and heat shock. *Proceedings of the National Academy of Sciences, USA* **89,** 3213–3216.

Burke JJ, Oliver MJ. 1992. Differential temperature sensitivity of pea superoxide dismutases. *Plant Physiology* **100,** 1595–1598.

Cachorro P, Ortiz A, Cerda A. 1993. Effects of saline stress and calcium on lipid composition in bean roots. *Phytochemistry* **32,** 1131–1136.

Cakmak I, Strbac D, Marschner H. 1993. Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. *Journal of Experimental Botany* **44,** 127–132.

Change B, Maehly AC. 1955. Assay of catalases and peroxidase. *Methods in Enzymology* **2,** 764–775.

Cheng LH, Tang LS, Zhang YG, Yan JY, Zhang H. 1997. The effect of $CaCl_2$ on the drought resistance of young cotton seedlings. *China Cottons* **24,** 17–18.

Colorado P, Rodriguez A, Nicolas G, Rodriguez D. 1994. Abscisic acid and stress regulate gene expression during germination of chick-pea seeds. Possible role of calcium. *Physiologia Plantarum* **91,** 461–467.

Cooke A, Cookson A, Earnshaw MJ. 1986. The mechanism of action on calcium in the inhibition on high temperature-induced leakage of betacyanin from beet root discs. *New Phytologist* **102,** 491–497.

Coria NA, Sarquis JI, Penalosa I, Urzua M. 1998. Heat-induced damage in potato (*Solanum tuberosum*) tubes: membrane stability, tissue viability, and accumulation of glycoalkaloids. *Journal of Agricultural and Food Chemistry* **46,** 4524–4528.

Dat JF, Lopez-Delgado H, Foyer CH, Scott IM. 1998. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiology* **116,** 1351–1357.

Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. 1981. Leaf senescence: correlated with increased leaves of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany* **32,** 93–101.

Foyer CH, Leandais M, Kunert KJ. 1994. Photooxidative stress in plants. *Physiologia Plantarum* **92,** 696–717.

Foyer CH, Lopez-Delgado H, Dat JF, Scott IM. 1997. Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signaling. *Physiologia Plantarum* **100,** 241–254.

Giannopolities CN, Ries SK. 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiology* **59,** 309–314.

Gong M, Chen SN, Song YQ, Li ZG. 1997. Effect of calcium and calmodulin on intrinsic heat tolerance in relation to antioxidant systems in maize seedlings. *Australian Journal of Plant Physiology* **24,** 371–379.

Gong M, Van der Liut AH, Knight MR, Trewavas AJ. 1998. Heat-shock-induced changes in intracellular Ca^{2+} level in tobacco seedlings in relation to thermotolerance. *Plant Physiology* **116,** 429–437.

Graves WR, Joy RJ, Dana MN. 1991. Water use and growth of honey locust and tree-of-heaven at high root-zone temperature. *Hort Science* **26,** 1309–1312.

Hare PD, Cress WA, Van SJ. 1998. Dissecting the roles of osmolyte accumulation during stress. *Plant, Cell and Environment* **21,** 535–553.

Heath RL, Packer L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* **125,** 189–198.

Hepler PK, Wayne RO. 1985. Calcium and plant development. *Annual Review of Plant Physiology* **36,** 397–439.

Hiscox JD, Israeltem GF. 1979. A method for extraction of chlorophyll from leaf tissue without maceration using dimethyl sulfoxide. *Canadian Journal of Botany* **57,** 1332–1334.

Jagtap V, Bhargava S. 1995. Variation in antioxidant metabolism of drought-tolerant and drought-susceptible varieties of *Sorghum bicolor* (L.) Moench. exposed to high light, low water and high temperature stress. *Journal of Plant Physiology* **145,** 195–197.

Klein JD, Ferguson IB. 1987. Effect of high temperature on calcium uptake by suspension-cultured pear fruit cells. *Plant Physiology* **84,** 153–156.

Kurganova LN, Veselov AP, Goncharova TA, Sinitsyna YV. 1997. Lipid peroxidation and antioxidant system of protection against heat shock in pea (*Pisum sativum* L.) chloroplasts. *Russian Journal of Plant Physiology* **44,** 725–730.

Kyle DJ. 1987.The biochemical basis for photoinhibition of photosystem II. In: Kyle DJ, Osmond CB, Arntzen CJ, eds. *Photoinhibition*. Amsterdam: Elsevier, 197–226.

Lehman VG, Engelke MC. 1993. Heritability of creeping bentgrass shoot water content under soil dehydration and elevated temperature. *Crop Science* **33,** 1061–1066.

Liu XZ, Huang BR. 2000. Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Science* **40,** 503–510.

Ludlow MM, Santamaria JM, Fukai S. 1990. Contribution of osmotic adjustment to grain yields in *Sorghum bicolor* (L.) Moench under water-limited conditions. II. Water stress after anthesis. *Australian Journal of Agricultural Research* **41,** 67–78.

Mansfield TA, Hetherington AM, Atkinson CJ. 1990. Some current aspects of stomatal physiology.*Annual Review of Plant Physiology and Plant Molecular Biology* **41,** 55–75.

McAinsh MR, Clayton H, Mansfield TA, Hetherington AM. 1996. Changes in stomatal behavior and guard cell cytosolic

free calcium in response to oxidative stress. *Plant Physiology* **111,** 1031–1042.

Minibayeva FV, Kolesnikov OP, Gordon LK, Asard H, Navas P. 1998. Contribution of a plasma membrane redox system to supeoxide production by wheat root cells. *Protoplasma* **205,** 101–106.

Mukherjee SP, Choudhuri MA. 1985. Implication of hydrogen peroxide-ascorbate system on membrane permeability of water stressed *Vigna* seedlings. *New Phytologist* **99,** 355–360.

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology* **22,** 867–880.

Onwueme IC, Laude HM. 1972. Heat-induced growth retardation and attempts at its prevention in barley and wheat coleoptiles. *Journal of Agricultural Science* **79,** 331–333.

Paranhos A, Fernandez-Jarrago J, Corchete P. 1999. Relationship between active oxygen species and cardenolide production in cell cultures of *Digitalis thapsi*: effect of calcium restriction. *New Phytologist* **141,** 51–60.

Price AH, Taylor A, Ripley SJ, Driffiths A, Trewavas AJ, Knight MR. 1994. Oxidative signals in tobacco increase cytosolic calcium. *The Plant Cell* **6,** 1301–1310.

Smirnoff N. 1995. Antioxidant systems and plant response to the environment. In: Smirnoff N, ed. *Environment and plant metabolism: flexibility and acclimation*. Oxford: Bios Scientific Publishers. 217–243.

Smith MAL, Spomer AL, Skiles ES. 1989. Cell osmolarity adjustment in *Lycopersicon* in response to stress pretreatments. *Journal of Plant Nutrition* **12,** 233–244.

Tajima K, Akita S, Shimizu N. 1976. Effect of high soil temperature on growth and water balance of tall fescue and perennial ryegrass. *Journal of Japanese Society Grassland Science* **22,** 256–260.

Thompson JE, Legge RL, Barber RF. 1987. The role of free radicals in senescence and wounding. *New Phytologist* **105,** 317–314.

Toprover Y, Glinka Z. 1976. Calcium ions protect beet root cell membrane against thermally induced changes. *Physiologia Plantarum* **37,** 131–134.

Trofimova MS, Andreev IM, Kuznetsov VV. 1999. Calcium is involved in regulation of the synthesis of HSPs in suspension-cultured sugar beet cells under hyperthermia. *Physiologia Plantarum* **105,** 67–73.

Tsang WT, Bowler C, Herouart D, Van Camp W, Villarroel R, Genetello C, Van Montagu M, Inzé D. 1991. Differential regulation of superoxide dismutase in plants exposed to environmental stress. *The Plant Cell* **3,** 783–792.

Turgeon AJ. 1999. *Turfgrass management*. Englewood Cliffs, NJ: Prentice Hall.

Wang JB, Li RQ. 1999. Changes of Ca^{2+} distribution in mesophyll cells of pepper under heat stress. *Acta Horticulturae Sinica* **26,** 57–58.

Webb AAR, Mcainsh MR, Taylor JE, Hetherington AM. 1996. Calcium ions as intercellular second messengers in higher plants. *Advances in Botanical Research* **22,** 45–96.

Willekens H, Inzé D, Van Montagu M, Van Camp W. 1995. Catalase in plants. *Molecular Breeding* **1,** 207–228.

Wise RR, Naylor AW. 1987. Chilling-enhanced photo-oxidation. The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. *Plant Physiology* **83,** 278–282.

Yang GP, Gao AL, Jiang JH. 1993. The relation of calcium to cell permeability in water stressed soybean hypocotyls. *Plant Physiology Communication* **29,** 179–181.