CHAPTER III*

INVOLVEMENT OF ANTIOXIDANTS AND LIPID PEROXIDATION IN THE ADAPTATION OF TWO COOL-SEASON GRASSES TO LOCALIZED DROUGHT STRESS

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ABSTRACT

In natural environments, drought often occurs in surface soil while water is available deeper in the soil profile. The objective of the study was to examine the involvement of antioxidant metabolism and lipid peroxidation in the responses of two cool-season grasses to surface soil drying. Kentucky bluegrass (*Poa pratensis* L) and tall fescue (*Festuca arundinacea* Schreb.) were grown in split tubes, consisting of two sections (each 10 cm in diameter and 20 cm long). Grasses were subjected to three soil moisture regimes: a) well-watered control: whole soil profile was watered; b) surface drying: surface 20 cm of soil was dried by withholding irrigation and the lower 20 cm of soil was watered; c) full drying: whole soil profile was dried. Surface drying had no effects on relative water content (RWC) and chlorophyll content (Chl) for both grasses and caused only a slight reduction in shoot growth for tall fescue. Superoxide dismutase (SOD) activity increased, while catalase (CAT) and peroxidase (POD) activities remained unchanged during most periods of surface drying. Malondialdehyde (MDA) content was unaffected by surface drying for tall fescue, but increased initially and then decreased to the control level for Kentucky bluegrass. Under full drying, RWC, Chl content, and shoot dry weight decreased, but MDA content increased in both grasses; SOD and POD activities initially increased transiently and then decreased; CAT remained unchanged until 25 d and then decreased. These results suggested that both Kentucky bluegrass and tall fescue were capable of surviving localized drought stress. This capability could be related to increases in antioxidant activities, particularly SOD and CAT. However, full drying suppressed antioxidant activities and induced lipid peroxidation.

ABBREVATIONS:

RWC, relative water content; Chl, chlorophyll content; SOD, Superoxide dismutase; CAT, catalase; POD, peroxidase; MDA, Malondialdehyde.

INTRODUCTION

Drought stress near the soil surface is extremely common in the field, whereas water may be sufficient for plant uptake deeper in the soil profile. Some studies have reported that shoot growth and stomatal conductance decreased but leaf water status sustained when a portion of the root system is in drying soil (Blackman and Davies, 1985; Henson et al., 1989; Saab and Sharp, 1989; Zhang and Davies, 1990). Other studies found that shoot growth was unaffected by partial drying of the root system (Sadras et al., 1993; Gallardo et al., 1994; Melkonian and Wolfe, 1995; Zhang and Kirkham, 1995; Huang et al., 1997). Plant responses to localized soil drying may vary with the extent of drought resistance. Huang et al. (1997) and Huang (1999) found that shoot growth and leaf water status were not affected by surface soil drying for relatively drought-resistant buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], and seashore paspalum (*Paspalum vaginatum* Swartz.) but were reduced for relatively drought-sensitive zoysiagrass (*Zoysia japonica* Steud.) and bermudagrass [*Cynodon dactylon* (L.) Pers.]. Plant adaptability to localized soil drying has been attributed mainly to maintenance of water status by utilizing available soil water deeper in the soil profile by deep roots (Huang, et al., 1997; Huang, 1999) and by chemical signaling (Blackwell and Davies, 1985; Neales et al., 1989; Zhang and Davies, 1990). However, the biochemical mechanisms underlying plant adaptation to localized soil drought stress are understood poorly.

Increasing evidence suggests that drought induces oxidative stress through the production of active oxygen species during stress (Elstner, 1982; Smirnoff, 1993; Zhang et al., 1995; Perdomo, 1996). Active oxygen species including superoxide (O_2^-),

hydrogen peroxide (H_2O_2), hydroxyl free radical (OH^-), and singlet oxygen ($^1O_2$) form in the electron transport systems in chloroplasts and mitochondria. They are highly toxic and can damage many important cellular components, such as lipids, protein, DNA, and RNA (Smirnoff, 1993; Foyer et al., 1994a, b). Plant cells normally are protected against the detrimental effects of active oxygen by a complex antioxidant system (Elstner 1982; Smirnoff, 1993); active oxygen species can be scavenged by both enzymatic and nonenzymatic detoxification mechanisms (Breusegem, 1998). Some species that adapt to mild to moderate drought stress exhibit increases in activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). For example, Jagtap and Bhargava (1995) reported that SOD and CAT activities increased in drought-tolerant cultivars of maize (*Zea mays* L.). In wheat (*Triticum aestivum* L.), SOD activity increased or remained unchanged in the early phase of drought but decreased with further water stress (Zhang et al. 1995). Severe drought stress may cause damage to cells by inducing active oxygen production or by rupting the scavenging systems that quench active oxygen and eliminate the detrimental effects (Breusegem et al., 1998). Oxidative stress as indicated by lipid peroxidation can occur when the scavenging of active oxygen species is overwhelmed by the production.

Almost all research on drought stress injury or resistance in relation to antioxidant metabolism and lipid peroxidation has concerned drought stress in the whole soil profile or the entire root system of a plant. How antioxidant metabolism is involved in plant adaptation to localized drought stress has not been documented, despite of the fact that soil moisture is highly uneven in natural environments. Knowledge of antioxidative metabolic responses to localized drought stress will further our understanding of the

biochemical mechanisms of drought resistance. Therefore, the objective of the present study was to investigate whether antioxidants and lipid peroxidation are involved in the adaptation to localized drought stress for two cool-season grasses, Kentucky bluegrass (*Poa pratensis* L.) and tall fescue (*Festuca arundinacea* Schreb.). Physiological responses were assessed by evaluating leaf water status, chlorophyll content, and shoot dry matter production. Antioxidant responses were assessed by measuring activities of enzymatic antioxidants, including CAT, SOD, and POD. In addition, production of malondialdehyde (MDA) was measured to evaluate the level of lipid peroxidation.

MATERIALS AND METHODS

Plant materials and growth conditions

Plants of 'Livingston' Kentucky bluegrass and 'Falcon II' tall fescue each with five uniform tillers were collected from 3-year-old turfgrass plots at the Rocky Ford Research Center, Kansas State University, Manhattan, Kansas. Grasses were transplanted into split polyvinylchloride (PVC) tubes consisting of two sections (each 20 cm long, 10 cm in diameter) filled with fritted clay (Profile, ALMCOR, Deerfield, IL) and kept in a greenhouse. Fritted clay is a granular material made by firing coarsely milled, dry clay in a rotary kiln. It has a relatively low dry-bulk density and retains a large quantity of plant-available water (Van Bavel et al., 1978). The field capacity of fritted clay is 25% (v/v). Sections of soil columns were separated with waxed paper supported by a nylon screen coated with Vaseline. The split segments were taped externally with duct tape to hold the columns in place. Four drainage holes (5 mm in diameter) were drilled on the side wall at the bottom of each section to allow drainage of excess water and soil aeration.

Plants were grown in the PVC tubes for about 60 d, allowing roots to penetrate the barrier and become established in the bottom section of the split tubes before treatments were imposed. By the end of the experiment, approximately 20% of the roots were found in the lower 20-cm layer and 80% of the roots in the upper 20-cm layer for each grass species. During the 60-d period, plants were watered on alternate days until water drained freely from the drainage holes at the bottom of each section and fertilized weekly with full-strength Hoaglands solution (Hoagland and Arnon, 1950). Plants were maintained in a greenhouse with daily maximum/minimum temperatures of 24°C/18°C and a 16-h photoperiod. The light regime was supplemented with 1 kw metal halide

lamps. Light intensity on a horizontal plane just above the canopy at 12:00 h averaged 900 μmol m^{-2} s^{-1}.

Treatments

The experiment consisted of three soil moisture treatments: control, surface drying, and full drying. In the well-watered control, plants were watered on alternate days until water drained freely. Each soil layer was irrigated separately using a drip irrigation system, with tubes positioned about 2 cm beneath the soil surface in each layer. Irrigation was automated using a pressure and flow controller. In the surface drying treatment, the surface 20 cm of soil was allowed to dry down by withholding irrigation, while the lower 20 cm of soil was well watered on alternate days. At the end of treatment, the surface soil was very dry, with a water content of only about 5% (v/v), whereas water content was maintained at about 80% of field capacity in the bottom 20 cm of soil. In the full drying treatment, the whole soil profile (40 cm) was allowed to dry down by withholding irrigation. At the end of this treatment, soil water contents in both layers were only 5% (v/v). All three treatments lasted for 40 d. The barrier between the top and bottom soil layers allowed root penetration but minimized water exchange. This technique also provided a suitable system for simulating the field situation in which only the surface soil layers dry down, but still enabled plant response to soil drying to be examined under controlled conditions.

Measurements

Leaf relative water content (RWC) was calculated based on leaf fresh weight, dry weight, and weight at full turgor after soaking leaves in water for 24 h. Leaf chlorophyll

(Chl) content was determined using the method of Hiscox and Israelstam (1979). Chlorophyll was extracted by soaking leaves in dimethyl sulfoxide solution for 48 h. Absorbance of extracts was measured at 635 and 645 nm with a spectrophotometer (Spectronic Instruments, Inc. New York). At the end of the experimental period, shoots were harvested and dried in an oven at 85°C for 72 h. Shoot dry weight then was determined.

For the assays of SOD, CAT, POD and MDA, about 0.2-g samples of young, fully expanded leaves were collected at 11:00 h at 0, 11, 18, 25, and 32 days of treatment. After determination of fresh weight, the samples were frozen immediately at –70 C until use. For extraction of enzymes, frozen leaves were homogenized with 5 ml of 50 mM buffer solution, which contained 0.07 % of $NaH_2PO_4.2H_2O$ and 1.6 % $Na_2HPO_4.12H_2O$, crushed with a mortar and pestle, and centrifuged at 20000 ×g for 25 min in a refrigerated centrifuge. The supernatant was collected in a bottle for the determination of soluble protein content, enzymes activities, and MDA content.

The SOD activity was determined according to the method of Giannopolitis and Ries (1977) with some modifications (Chowdhury and Choudhuri, 1985; Zhang et al., 1995). A 3 ml reaction mixture contained 63 μM NBT, 1.3 μM riboflavin, 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), and 20 μl of enzyme extract. The test tubes containing the mixture were placed under light at 4000 lux for 10 min, and absorbance at 560 nm was recorded. A nonirradiated reaction mixture that did not develop color served as the control, and its absorbance was subtracted from A_{560} of the reaction solution. One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the rate of NBT reduction at 560 nm.

Activities of CAT and POD were measured using the method of Chance and Maehly (1955). For CAT, the decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm for 1 min. The 3-ml reaction mixture contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2, and 0.1 ml enzyme extract, which initiated the reaction. For POD, the oxidation of guaiacol was measured by the increase in absorbance at 470 nm for 1 min. The reaction mixture contained 50 µl of 20 mM guaiacol, 2.8 ml of 10 mM phosphate buffer (pH 7.0), and 0.1 ml enzyme extract. The reaction was started with 20 µl of 40 mM H_2O_2.

For measurement of MDA content, 4 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid was added to 1-ml aliquot of the supernatant. The mixture was heated at 95 C for 30 min and then quickly cooled in an ice bath. After the tube was centrifuged at 10000 ×g for 10 min, the absorbance of the supernatant was read at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted from the 532 nm reading. The concentration of MDA was calculated using MDA's extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer, 1968).

Experimental design and statistical analysis

The experiment consisted of two factors (two grasses and three soil moisture treatments) with four replications arranged in a completely randomized design. Treatment effects were determined by analysis of variance according to the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC). Variation was partitioned into grass species and soil moisture as main effects and corresponding interactions. The comparison of moisture treatments within a grass clearly

showed performance of each grass under stress conditions. Thus, the emphasis was on comparing responses to soil moisture treatments within a grass. Differences among treatment means within a grass were separated by least significant difference at the 0.05 level of probability.

RESTULTS

Growth responses

Surface soil drying had no effect on shoot dry matter production for Kentucky bluegrass and caused only a slight reduction (19%) for tall fescue (Table 1). Under full drying, however, shoot dry matter was reduced significantly for both species (Table 1).

LRWC was not affected for both grasses during most of the experimental period; except after 17 d of surface soil drying for Kentucky bluegrass and 14 and 17 d for tall fescue, when LRWC of surface-dried plants was lower than that of well-watered control plants (Fig. 1).

No significant differences in leaf Chl a and Chl b contents were detected between surface-dried and control plants for both species (Table 2). Leaf Chl a and Chl b contents of fully dried plants for both grass species were significantly lower than those of the control plants after 25 and 18 days of drying respectively.

Antioxidant enzyme activity

No differences in SOD activity were observed between surface-dried and control plants within the first 12 d of treatment (Fig. 2). However, SOD activity of surface-dried plants increased to above the control level at 18 and 25 d for both species. A transient increase in SOD activity occurred in fully dried tall fescue after 11 d of treatment (Fig. 2); SOD activity of fully dried Kentucky bluegrass was unchanged initially. SOD activity declined dramatically to below the control level after 25 d for both species.

A transient increase in POD activity occurred after 11 d of full drying (Fig. 3), but no difference in POD activity between fully dried plants and control plants was observed

after 18 d. Activity of POD was unchanged in response to surface soil drying during the entire experimental period.

For both species in this study, surface soil drying had no effects on CAT activity (Fig. 4). The activity of CAT in fully dried plants was similar to that in control plants for both species before 25 days of treatment. Thereafter, fully dried plants had significantly lower CAT activity than control plants.

Lipid peroxidation

Leaf MDA content of fully dried plants was significantly higher than that of the control plants for both species, beginning at 18 days (Fig. 5). By 32 days, MDA content in fully dried plants was greater than that in control plants, about two times higher for Kentucky and 1.6 times higher for tall fescue.

Surface soil drying had no effects on MDA content for tall fescue. For Kentucky bluegrass, MDA content of surface-dried plants increased after 18 and 25 d but then decreased to the control level at 32 d.

DISCUSSION

Shoot growth, leaf RWC, and Chl content of both Kentucky bluegrass and tall fescue generally were not affected by surface soil drying. This suggested that both Kentucky bluegrass and tall fescue were capable of surviving surface soil drying and maintaining favorable water status and photosynthetic capacity by preserving photosynthetic pigments, even though most of their roots (80%) were exposed to drying soil.

Under full drying, leaf Chl content declined, to a greater extent for Chl a than Chl b for both species. Full drying also caused severe internal water deficit (50% RWC) after 28 days of treatment. Detrimental effects on chloroplast biochemistry or Chl fluorescence occur when RWC drops below 60% in tall fescue (Huang et al., 1998). Kaiser (1987) indicated that an irreversible decrease in plant photosynthetic capacity occurs as RWC declines below 30%, leading to cell death from membrane damage in chloroplasts. Drought-induced decreases in photosynthetic electron transfer and chlorophyll contents have been reported previously in various species (Zuily et al., 1990; Moran et al., 1994). The loss of Chl during full drying could also be related to photo-oxidation resulting from oxidative stress (Kato and Shimizu, 1985), as demonstrated by the decline in the activity of antioxidants and increased lipid peroxidation as discussed later.

Superoxide dismutase, catalyses the dismutation of $O^{\cdot -}_2$ to H_2O_2 and O_2 (Bowler et al., 1992; Scandalios, 1993) and plays a key role in quenching active oxygen. The lack of effects of surface drying on SOD suggests that maintenance of physiological activities under surface drying conditions, as manifested by high water and Chl levels and shoot growth, could be related to the increases in SOD activity to scavenge active oxygen. The

transient increase in SOD during initial periods of drying might protect plants from oxidative injury. However, the decline in SOD after prolonged full drying indicated that the scavenging function of SOD was impaired with prolonged, severe drought stress. The decrease in SOD activity would favor accumulation of $O^{\cdot}_2$. This result indicated that under severe drought stress, the balance between active oxygen formation and the scavenging system could be disturbed (Breusegem, 1998).

Previous studies have shown that responses of SOD activity to water deficit have varied with drought severity, duration, and species. Zhang and Kirkham (1996) suggested that water stress did not influence SOD activity under moderate stress in sorghum [*Sorghum bicolor* (L.) Moench]. Jagtap and Bhargara (1995) reported that SOD activity increased in drought-tolerant cultivars of maize (*Zea mays* L.). In wheat (*Triticum aestivum* L.), SOD activity increased or remained unchanged in the early phase of drought and then decreased with further water stress (Zhang et al. 1995). Reddy and Vajranabhaiah (1993) observed that SOD activity in upland rice (*Oryza sativa* L.) decreased with osmotic stress.

Peroxidase catalyzes H_2O_2–dependent oxidation of substrate. Both grass species were able to maintain POD activity for detoxifying active oxygen in response to surface and full drying. Other studies have reported increases (Zhang et al., 1995), decreases (Zhang and Kirkham, 1996), and no changes (Fangmeier et al., 1994) in POD activity in response to drought stress.

Catalase eliminates H_2O_2 by breaking it down directly to form water and oxygen (Smirnoff, 1993; Winston, 1990). Prolonged full drying reduced CAT activity for both species. Decreases in CAT activity would result in the accumulation of H_2O_2, which can

react with O^{-}_2 to produce hydroxyl free radicals via the Herbert-Weiss reaction (Elstner, 1982; Bowler et al., 1992). Declines in CAT activity in response to prolonged drought have been reported for other species (Dwivedi et al., 1979; Chowdhury and Choudhuri, 1985). For both species in this study, surface soil drying had no effects on CAT activity. Zhang and Kirkham (1996) also reported that CAT activity was not affected by mild drought. These results indicated that the ability of CAT to quench active oxygen was maintained during initial stress but was limited during a prolonged period of full drying; however, plants were able to maintain some CAT activity even when water was available only in deep soil.

Lipid peroxidation indicates the prevalence of free radical reactions in tissues. The content of MDA often is used as an indicator of the extent of lipid peroxidation resulting from oxidative stress (Smirnoff, 1993). After, 32 days of full drying, MDA increased about two fold for both species, suggesting that prolonged full drying caused membrane lipid peroxidation, which could be attributed to the decreases in SOD and CAT activities. These decreased activities induced by severe drought stress favor accumulation of O^{-}_2 and H_2O_2, which can result in lipid peroxidation. Drought stress has been reported to damage cell membrane stability (Bandurska et al., 1995; Pastori and Trippi 1 993). C ell m embrane s tability was s hown t o b e a ffected b y l ipid p eroxidation caused by active oxygen species under various stress conditions (Levitt, 1980; Dhindsa et al., 1 981). T he i ncreases o bserved i n l eaf M DA c ontents o f f ully d ried plants o f b oth species after a prolonged period (18 d) were in agreement with results of other studies (Price and Hendry, 1991; Zhang et al. 1995). For both grasses, MDA generally was not affected during most of the experimental periods by surface soil drying. This indicated

that the capabilities of both grass species to adapt to surface soil drying could be related to a low degree of lipid peroxidation, which could result from to the maintenance of high activities of some antioxidant enzymes, particularly SOD.

In conclusion, both tall fescue and Kentucky bluegrass were capable of surviving surface soil drying, as manifested by maintenance of shoot growth and water and chlorophyll levels, although major proportions of roots were exposed to drying soil. This capability for adaptation to localized drought stress was related to the maintenance of or increases in the ability to detoxify superoxide radicals by antioxidant enzymes. Particularly, SOD played a key role in protecting plants from oxidative stress by increasing its activity. The detrimental effects of prolonged drying of the entire soil profile were related to oxidative stress, as demonstrated by increases in membrane lipid peroxidation and decreases in the activities of antioxidant enzymes, particularly SOD and CAT.

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Table 1. Shoot growth after 30 days of treatment as affected by surface and full drying for Kentucky bluegrass and tall fescue

Grasses	Soil moisture treatment	Shoot dry weight (g)
Kentucky bluegrass	Well-watered control	6.0 a
	Surface drying	5.9 a
	Full drying	4.8 b
	LSD	1.1
Tall fescue	Well-watered control	7.6 a
	Surface drying	6.1 b
	Full drying	5.1 b
	LSD	1.3

* Means within column followed by the same letters were not significantly different based on an LSD test at P=0.05.

Table 2. Leaf Chl a and Chl b content as affected by surface and full drying for Kentucky bluegrass and tall fescue

Grasses	Soil moisture treatment	Chl a (mg g^{-1} dry wt)			Chl b (mg g^{-1} dry wt)		
		18 d	25 d	32 d	18 d	25 d	32 d
Kentucky bluegrass	Well watered control	8.57 a	7.91 a	8.76 a	1.28 a	1.14 a	1.38 a
	Surface dring	7.79 a	8.42 a	8.64 a	1.05 ab	1.25 a	1.29 a
	Full dring	7.89 a	6.51 b	5.93 a	0.83 b	0.77 b	0.84 b
	LSD	1.26	1.48	1.8	0.31	0.35	0.34
Tall fescue	Well watered control	9.37 a	9.66 a	9.86 a	1.30 a	1.42 a	1.51 a
	Surface dring	8.44 a	8.94 a	9.11 a	1.37 b	1.37 a	1.32 a
	Full dring	7.89 a	5.74 b	5.26 b	1.03 b	0.77 b	0.68 b
	LSD	1.82	1.63	1.42	0.26	0.48	0.44

* Means within column followed by the same letters were not significantly different based on an LSD test at P=0.05.

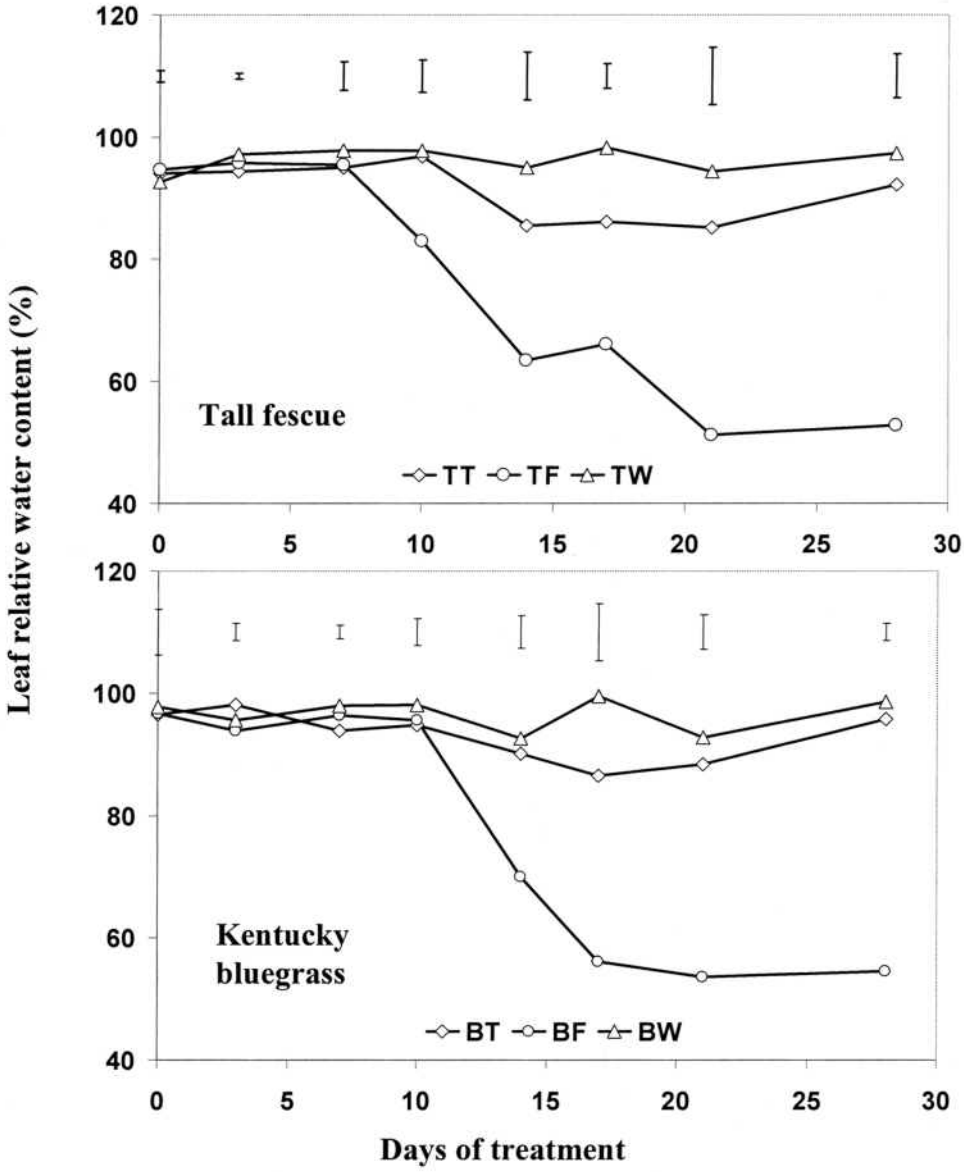

Fig. 1 Leaf relative water contents of Kentucky and tall fescue in response to drought stress. Bars indicate protected LSD (P=0.05) for treatment comparisons at a given day

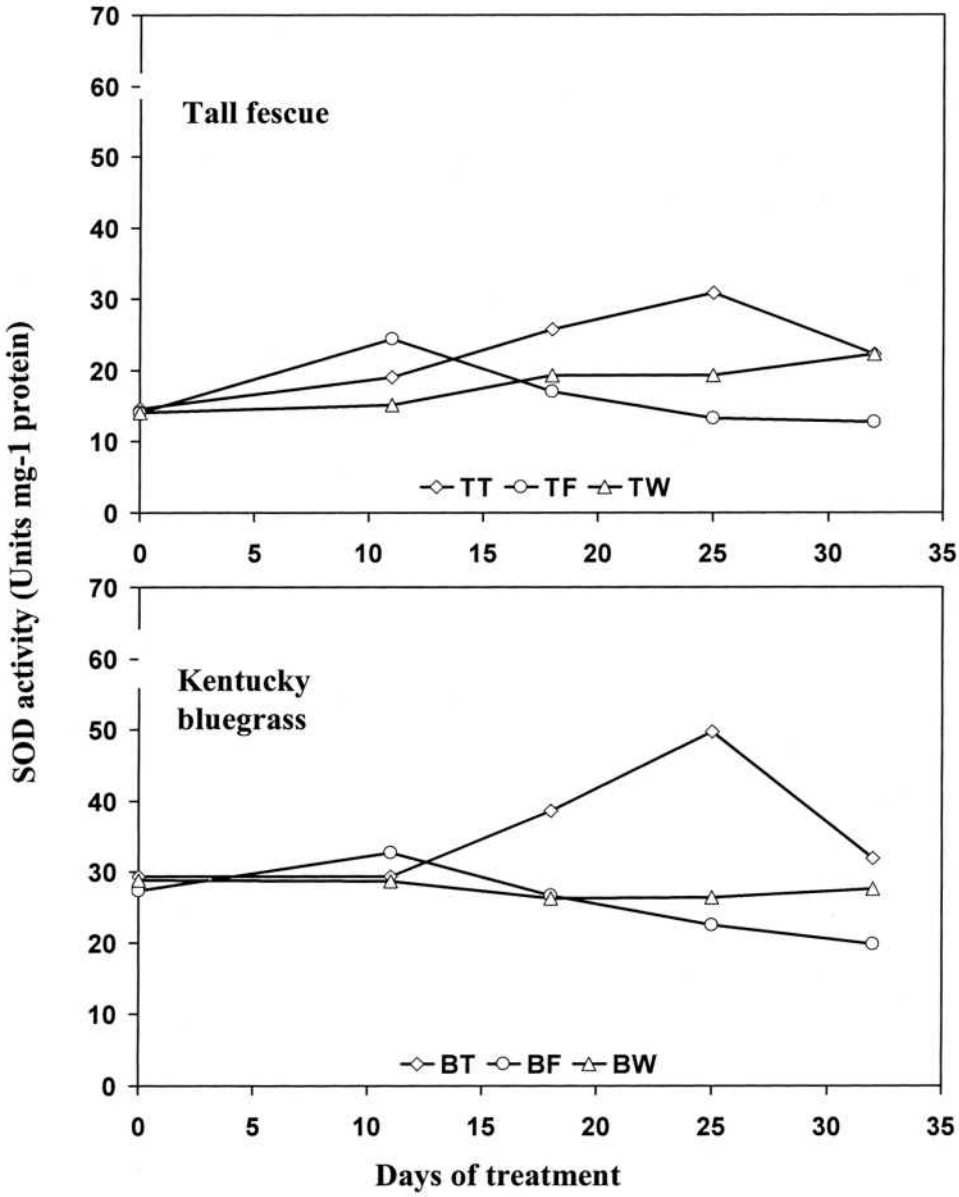

Fig. 2 Superoxide dismutase (SOD) of Kentucky bluegrass and tall fescue in response to drought stress. Bars indicate protected LSD (P=0.05) for treatment comparisons at a given day

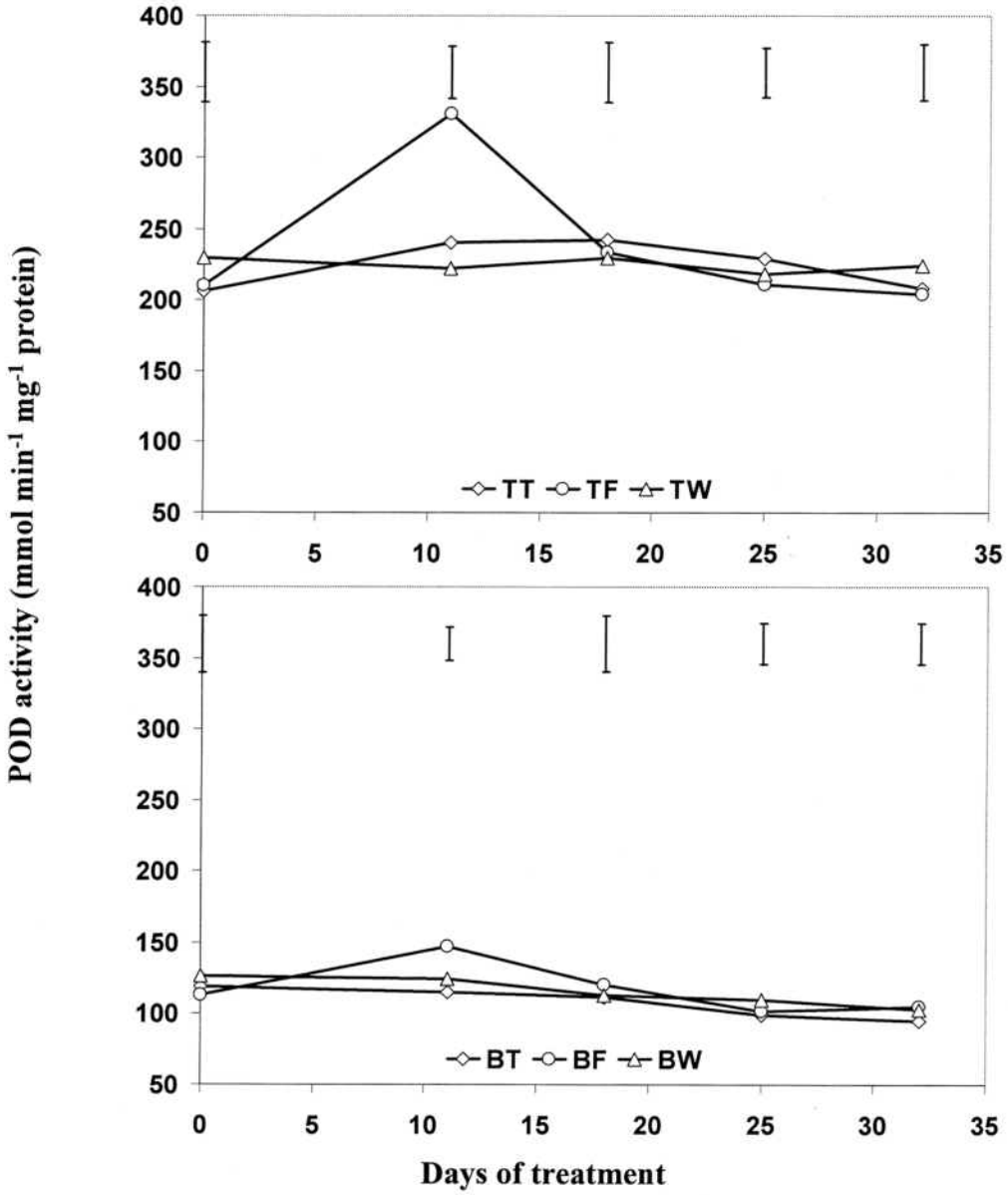

Fig.3 Peroxidase (POD) of Kentucky bluegrass and tall fescue in response to drought stress. Bars indicate protected LSD (P=0.05) for treatment comparisons at a given day

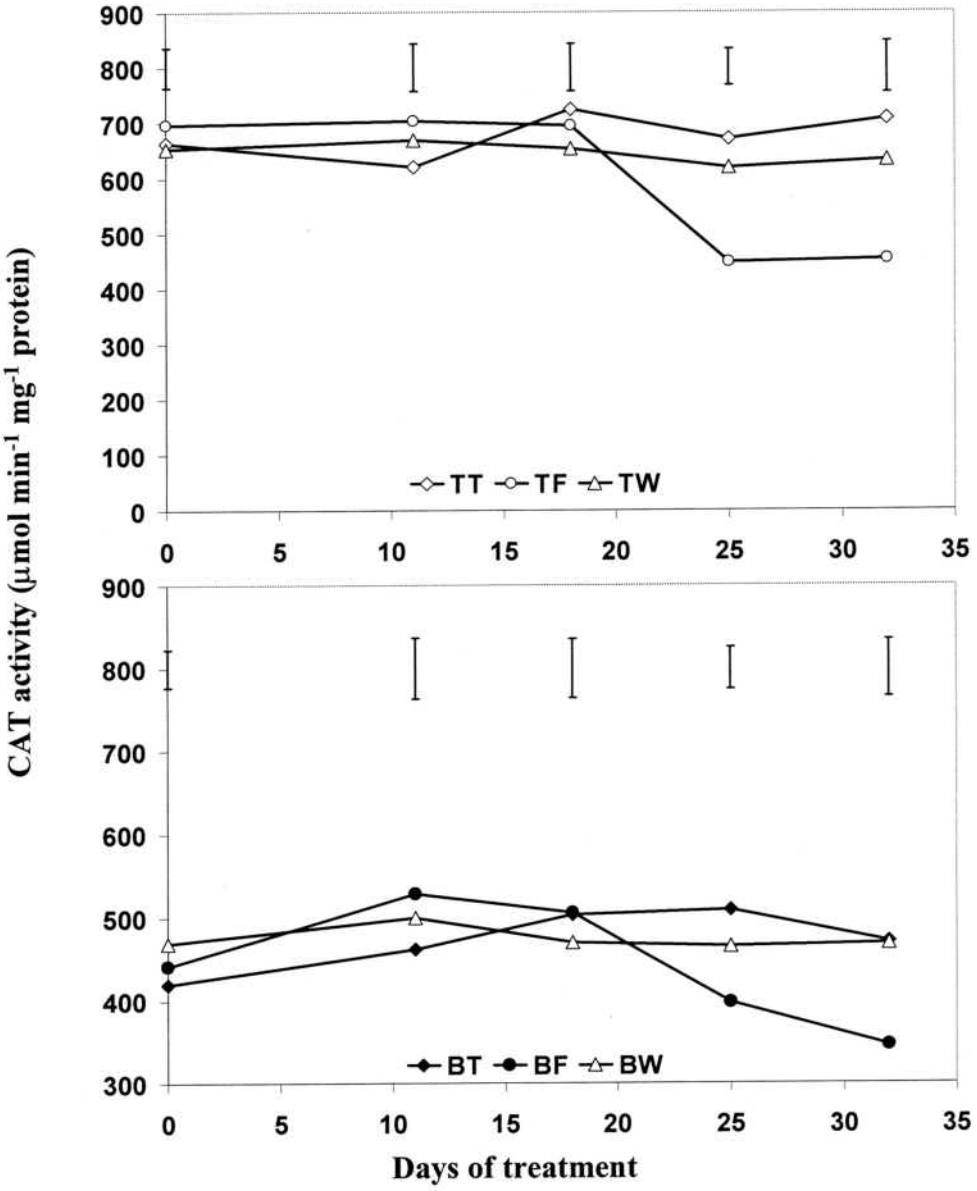

Fig.4 Catalase (CAT) of Kentucky bluegrass and tall fescue in response to drought stress. Bars indicate protected LSD (P=0.05) for treatment comparisons at a given day

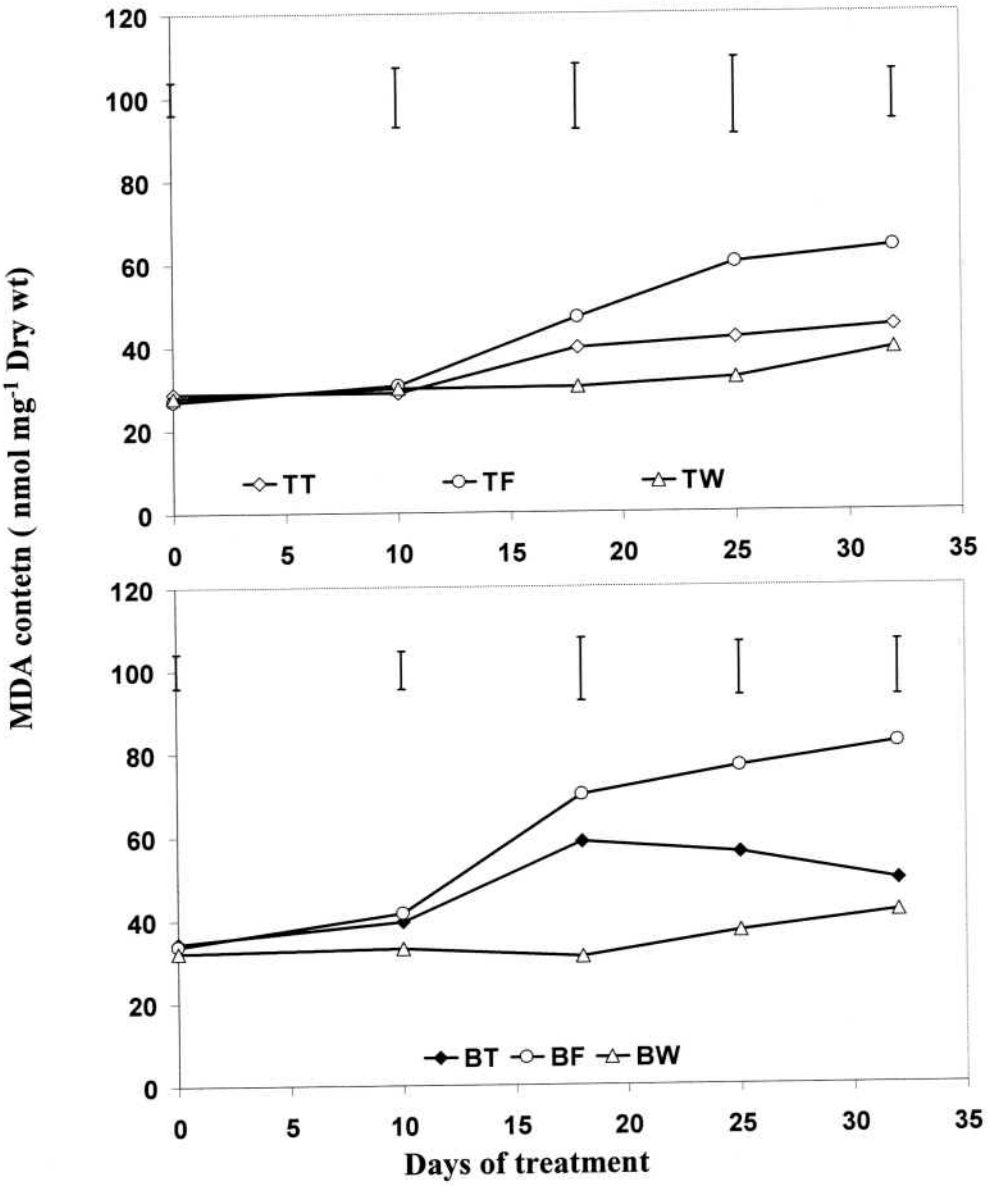

Fig.5 Catalase (CAT) of Kentucky bluegrass and tall fescue in response to drought stress. Bars indicate protected LSD (P=0.05) for treatment comparisons at a given day