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High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility

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ABSTRACT. High temperature stress is a major limiting factor in the growth and development of tall fescue (*Festuca arundinacea*) in transitional and warm climatic regions. In this study, we evaluated the photosynthesis, PSII functionality, and antioxidant activity of two tall fescue cultivars, Jaguar 3 brand (heat-tolerant) and TF 66 (heat-sensitive) in response to high temperature stress. High temperature stress caused a net photosynthetic rate reduction in the two plants due to stomatal and non-stomatal limitations, photoinhibition increase, and Rubisco activity reduction. High temperature stress modified PSII functionality in leaves of the two plants, manifested by lower variable chlorophyll fluorescence yield (Fv), maximum photochemical efficiency of photosystem II in dark adapted leaves (Fv/Fm), and efficiency of the open reaction centre in light ($\Phi PSII_{open}$) in the two heat shocked cultivars. Heat stress led to reductions in the chlorophyll a+b and chlorophyll/carotenoid ratios and to an increase in the chlorophyll a/b ratio for the two stressed cultivars. Moreover, high temperature stress significantly increased lipid peroxidation, decreased cell membrane thermostability, and changed the activities of ascorbate peroxidase (APX) and superoxide dismutase (SOD) in the leaves of both plants. All the above effects induced by high temperature were more expressed in the TF 66 than in Jaguar 3 brand. Our results contain some insights which may prove useful in the selection and breeding of heat-tolerant tall fescue turfgrass cultivars.

Keywords: Antioxidant enzyme; Cell membrane integrity; *Festuca arundinacea*; High temperature stress; Photosynthetic characteristics; PSII photochemical efficiency.

Abbreviations: $\mathbf{A}_{max\text{-}CO2}$, maximum net photosynthesis under maximum Ci; $\mathbf{A}_{max\text{-}light}$, maximum net photosynthesis at maximum PPFD level; **AOS**, active oxygen species; **APX**, ascorbate peroxidase; $\mathbf{A}_{sat\text{-}CO2}$, CO_2-saturated net photosynthetic rate; $\mathbf{A}_{sat\text{-}light}$, light saturated net photosynthetic rate; **CE**, carboxylation efficiency; **Chl**, chlorophyll; **Ci**, internal CO_2 concentration; **E**, transpiration rate; **EL**, electrolyte leakage; **Fv**, variable chlorophyll fluorescence yield; **Fv/Fm**, maximum photochemical efficiency of photosystem II in dark adapted leaves; $\mathbf{g}_s$, stomatal conductance; **LCP**, light compensation point; **LSP**, light saturating point; **MDA**, malondialdehyde; **Pn**, net photosynthetic rate; **PPFD**, photosynthetic photo flux density; **PSII**, photosystem II; **Rd**, light respiration; **SOD**, superoxide dismutase; **WUE**, water use efficiency; **Γ**, CO_2 compensation point; **Φ**, apparent quantum efficiency; $\mathbf{\Phi PSII}_{open}$, efficiency of the open reaction centre in light.

INTRODUCTION

High temperature stress is one of the major factors limiting use of cool-season grasses in transitional and warm climatic regions (Carrow, 1996; Beard, 1997). High temperature for prolonged periods during midsummer in these regions can inhibit cool-season grass growth (Martin and Wehner, 1987), decrease turf quality, and reduce photosynthetic rate (Huang and Gao, 2000).

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Photosynthesis, one of the most heat sensitive processes, can be completely inhibited by high temperature before other symptoms of the stress are detected (Camejo et al., 2005). This photosynthesis decrease could result from structural and functional disruptions of chloroplasts and reduction of chlorophyll accumulation under high temperature stress (Xu et al., 1995; Dekov et al., 2000). Moreover, Higuchia et al. (1999) reported that photosynthesis showed heat inhibition of both stomatal limitation and non-stomatal limitation in cherimoya while Camejo et al. (2005) suggested that there was no stomatal limitation in tomato.

The reduction of photosynthesis by high temperature stress is also related to inactivation of many chloroplast enzymes, mainly induced by oxidative stress (Dekov et al., 2000). Oxidative stress can cause cause lipid peroxidation and consequently membrane injury, protein degradation, and enzyme inactivation (Sairam et al., 2000; Meriga et al., 2004). Therefore, the ability to maintain cell membrane integrity and diminish oxidative stress have been proposed as good indicators of thermotolerance in plants (Liu and Huang, 2000; Huang et al., 2001).

Tall fescue (*Festuca arundinacea*) is one of the most widely used cool-season species on golf putting greens. The optimum temperatures for tall fescue cultivation are between 15 and 20°C. However, temperatures of 30 to 35°C in the transitional and warm climatic regions during midsummer are common and can heavily reduce turf quality and cultivar growth. Therefore, this work aims to determine how two tall fescue grasses that differ in their tolerance to high temperature stress respond to it in their photosynthesis, PSII functionality and antioxidant ability to diminish oxidative stress. Based on these comparisons, insight into the mechanism responsible for differences in thermotolerance for tall fescue cultivars should be possible.

Materials and Methods

Plant materials and treatments

Seeds of two tall fescue (*Festuca arundinacea* S.) cultivars, Jaguar 3 brand (heat tolerant), and TF 66 (heat sensitive) were sown and grown in a mixture of sand, vermiculite, and organic matter (3:1:1) in polyethylene pots (14 cm in height and 13 cm in diameter with 175 seeds in each pot). Each grass was planted in twelve pots. Plants were kept in growth chambers at 20/15°C (day/night), a photosynthetic photo flux density (PPFD) of 200 μmol m^{-2} s^{-1}, and a 14-h photoperiod for 70 d (including 8 d for germination) before they were subjected to high temperature stress. Then six pots of each grass were transferred to growth chambers with high temperature 35/30 °C (day/night) for 20 d, with the remaining pots kept under control conditions 20/15°C (day/night). Before and during the temperature treatments, grasses were mowed weekly to a height of 8 cm with electric hair clippers, watered daily, and fertilized weekly with a full Hoagland's nutrient solution (Hoagland and Arnon, 1950).

Gas-exchange measurements

Gas-exchange measurements were taken on healthy leaves using a portable Licor 6400 photosynthesis system (LI-6400, Li-Cor Inc., Lincoln NE, USA). The measurements were conducted under 20 or 35°C with 400 μmol CO_2 mol^{-1}air CO_2 concentration and 1000 μmol m^{-2} s^{-1} PPFD (supplied by a red-blue LED light source). Photosynthetic water use efficiency (WUE) was determined by the ratio of net photosynthetic rate (Pn) to transpiration rate (E).

Photosynthetic irradiance and CO_2 response

Measurements of leaf photosynthetic irradiance and CO_2 response curves were made using a Licor 6400 photosynthesis system (LI-6400, Li-Cor Inc., Lincoln NE, USA). Artificial illumination was supplied to the leaf from a red-blue LED light source, and ambient CO_2 partial pressure was supplied by 6400 CO_2 mixer. A photosynthetic irradiance response curve (A-PPFD curve) was monitored using the following procedures: healthy leaf was illuminated at a PPFD of 800 μmol m^{-2} s^{-1} until a steady state of net CO_2 fixation was reached at 400 μmol CO_2 mol^{-1}air CO_2 concentration. Irradiance was then changed in a stepwise reduction of PPFD ranging from 1800 to 0 μmol m^{-2} s^{-1} and measurements were made once the leaf attained a steady net CO_2 fixation rate. The asymptotic exponential equation of Prioul and Chartier (1977) was fitted by non-linear least square regression to the A-PPFD data. Fixed parameters of the model were estimated using a SPSS 10.0 statistical package.

Photosynthetic response to different internal CO_2 concentrations (Ci) (A-Ci curve) was measured for the photosynthetic irradiance response curves with the exception that once steady state was attained, ambient CO_2 partial pressure in the leaf chamber (Ca) was reduced in steps from 400 μmol CO_2 mol^{-1} air down to 0 μmol CO_2 mol^{-1} air, and then increased up to 1400 μmol CO_2 mol^{-1} air, with a saturated PPFD 1200 μmol m^{-2} s^{-1}. Net photosynthetic rate (Pn) of the leaves of the two grasses was measured at 20 or 35°C. A-Ci parameters were calculated by the model of Olsson and Leverenz (1994).

Pigment content determination

The content of chlorophyll (Chl) a, b and carotenoid in leaf segments was determined in 80% acetone following Lichtenthaler's (1987) method using a UV-VISIBLE Spectrophotometer (Hitachi U-3000).

Leaf photochemical efficiency measurements

Leaf photochemical efficiency was estimated with a plant photosynthesis efficiency analyzer (Hansatech Instrument LTD, Kings Lynn, England). Initial chlorophyll fluorescence yield, Fo, variable chlorophyll fluorescence yield, Fv, and maximum chlorophyll fluorescence yield, Fm, were read in the fluorometer. Attached leaves were covered in a leaf chamber and adapted in the dark for 20 min before measurements were conducted. Maximum photochemical efficiency of the photosystem II in dark adapted leaves (Fv/Fm) and efficiency of the open reaction centre in light ($\Phi PSII_{open}$) were determined using following equations:

$$Fv/Fm=(Fm-Fo)/Fm$$

$$\Phi PSII_{open}=Fv'/Fm'=(Fm'-Fo)/Fm'$$

Lipid peroxidation and membrane thermostability

The level of lipid peroxidation in leaf and root tissue was measured in terms of malondialdehyde (MDA) con-

tent, which was determined by the thiobarbituric acid reaction following the procedure of Špundová et al. (2003). Membrane thermostability was determined by measuring electrolyte leakage (EL). EL was measured by conductivity method according to Gulen and Eris (2004).

Enzyme extraction and enzyme assays

A 0.2 g of leaf tissue was harvested into liquid nitrogen at various times throughout the study and stored at -80 °C. On removal from the -80°C freezer, the samples were immediately placed in liquid nitrogen, removed, and immediately ground with a micropestle in an eppendorf in 0.25 mL of cold phosphate buffer (50 mmol/L, pH 7.0) containing 0.2 mmol/L ascorbate and 1% polyvinylpolypyrrolidone. The ground tissue was then spun at 0°C for 15 min in a microfuge. The resulting extract was stored on ice for as little time as possible prior to taking readings.

All of the enzyme assays were measured by the procedures of Larkindale and Huang (2004). The assay for ascorbate peroxidase (APX) (EC 1.11.1.11) was done by monitoring the rate of oxidation of ascorbate at 290 nm using the spectrophotometer blanked against an aliquot of buffer (50 mmol/L phosphate buffer, pH 7.0, 0.5 mmol/L ascorbate, 0.1 mmol/L EDTA).

Superoxide dismutase (SOD) (EC 1.15.1.1) was measured at 560 nm, and 1 unit of activity was defined as the amount of enzyme required to inhibit 50% of the NBT reduction rate in the controls containing no enzyme.

All samples were corrected for the amount of total protein in the extract, which was measured according to the method of Bradford (1976).

Statistical analysis

One-way analysis of variance was performed using the SPSS computer package (SPSS Inc. 1999) for all sets of data, and means were compared using Duncan's multiple comparison test at $P = 0.05$.

RESULTS

The effect of high temperature on photosynthetic rate and WUE

Net photosynthetic rate (Pn) and stomatal conductance (g_s) in the two treated cultivars were reduced after high temperature stress compared to controls (Figure 1A, B). Transpiration rate (E) in both plants increased under heat stress (Figure 1C). Under this stress condition, no significant variations in the internal CO_2 concentrations (Ci) for the two cultivars were measured (Figure 1D). Water use efficiency (WUE) in both treated cultivars fell after high temperature stress compared to controls (Figure 2). Pn in Jaguar 3 brand was always higher than in TF 66. The difference in the g_s between the control plants of Jaguar 3 brand and TF 66 was not remarkable while the g_s in Jaguar 3 was higher than in TF 66 after 10 d stressed treatment and then lower after 20 d. Compared to TF 66, Jaguar 3 had lower E under control conditions and a higher E under high temperature stress. Under high temperature stress, the rates of increase in E for Jaguar 3 were significantly higher than those in TF 66. WUE in Jaguar 3 brand was higher than in TF 66 under control conditions, but lower under stressed conditions.

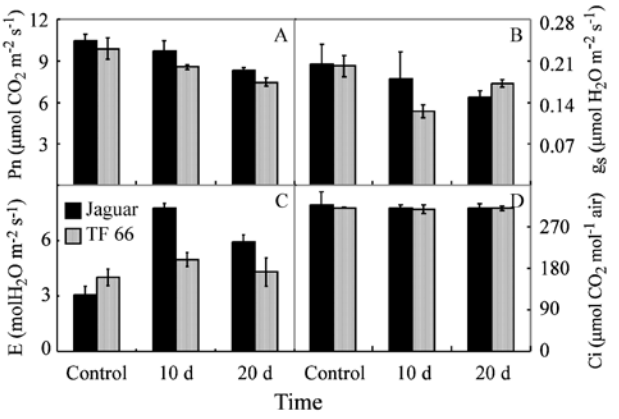

Figure 1. Net photosynthetic rate, Pn (A), stomatal conductance, g_s (B), transpiration rate, E (C) and internal CO_2 concentrations Ci, (D) in leaves of two tall fescue (*Festuca arundinacea*) cultivars (Jaguar 3 brand and TF 66) grown under normal and high temperature conditions. Measurements were conducted at 20 or 35°C. Each point is the mean ± S.E. of 3-6 leaves.

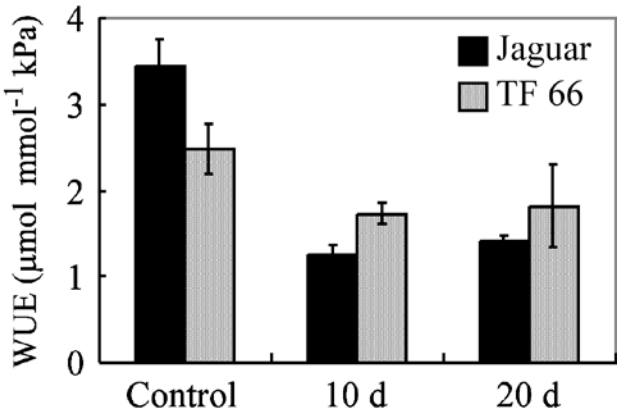

Figure 2. Water use efficiency (WUE) in leaves of two tall fescue (*Festuca arundinacea*) cultivars (Jaguar 3 brand and TF 66) grown under normal and high temperature conditions. Measurements were conducted at 20 or 35°C. Each point is the mean ± S.E. of 3-6 leaves.

A-PPFD curves parameters

Analyses of A-PPFD curves showed that both apparent quantum efficiency (Φ), maximum net photosynthesis ($A_{max\text{-}light}$) at maximum PPFD, and light saturated net photosynthetic rate ($A_{sat\text{-}light}$) (PPFD = 800 μmol m^{-2} s^{-1}) for the two stressed cultivars were decreased when compared to each control (Figure 3, Table 1). The light compensa-

tion point (LCP) of the two cultivars decreased after 10 d treatment and then increased after 20 d treatment. In comparison to control plants, the light saturation point (LSP) of the two heat stressed cultivars increased (Table 1). Jaguar 3 brand had higher $A_{max\text{-}light}$, $A_{sat\text{-}light}$ and Φ than TF 66, whether treated or not. Differences of LCP and LSP between Jaguar 3 brand and TF 66 were not significant under normal or stressed conditions (Figure 3, Table 1).

A-Ci curves parameters

The curves, which characterize Pn, vs. Ci, were presented in Figure 4. Within the range of 12-560 μmol CO_2 mol^{-1} air Ci, essentially a single-line dependence of Pn was observed. The further increase of Ci concentration only slightly influenced the rate of Pn. Both maximum net photosynthesis under maximum Ci ($A_{max\text{-}CO2}$) and CO_2-saturated Pn ($A_{sat\text{-}CO2}$) (Ca = 400 μmol CO_2 mol^{-1} air) for the two cultivars were reduced by high temperature stress compared to the control plants (Figure 4, Table 2). Heat stress increased light respiration (Rd) and CO_2 compensation point (Γ) in the two treated plants. Compared to the control, carboxylation efficiencies (CEs) of the heat stressed plants were lower except for a slight increase for Jaguar 3 brand at 10 d heat stress (P<0.05) (Table 2). Jaguar 3 brand had higher $A_{max\text{-}CO2}$, $A_{sat\text{-}CO2}$ and CE than TF 66 under normal and stressed conditions while a reverse phenomenon was found for Rd and Γ, but the differences in $A_{sat\text{-}CO2}$ and CE were not reliable.

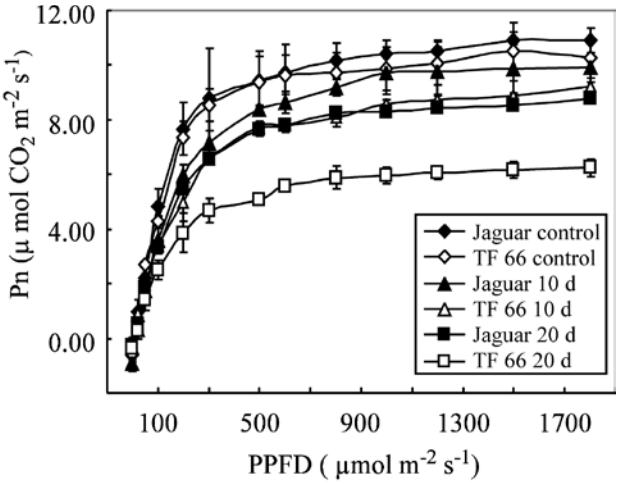

Figure 3. The relationship between net photosynthetic rate (Pn) and photosynthetic photon flux density (PPFD) in leaves of two tall fescue (*Festuca arundinacea*) cultivars (Jaguar 3 brand and TF 66) grown under normal and high temperature conditions. Measurements were conducted at 20 or 35°C, with a CO_2 concentration around 400 μmol CO_2 mol^{-1}air. Each point is the mean ± S.E. of 3-6 leaves.

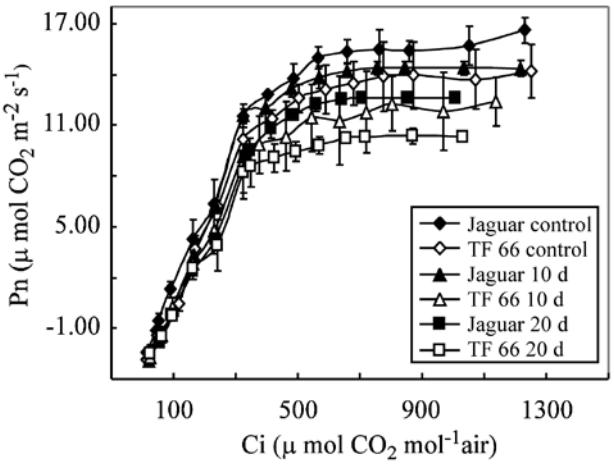

Figure 4. The relationship between net photosynthetic rate (Pn) and internal CO_2 concentrations (Ci) in leaves of two tall fescue (*Festuca arundinacea*) cultivars (Jaguar 3 brand and TF 66) grown under normal and high temperature conditions. Measurements were conducted at 20 or 35°C, with a saturated photosynthetic photon flux density (PPFD) 1200 μmol m^{-2} s^{-1}. Each point is the mean ± S.E. of 3-6 leaves.

Table 1. Average parameters of A-PPFD curves for two tall fescue (*Festuca arundinacea*) cultivars (Jaguar 3 brand and TF 66) grown under normal and high temperature conditions.

	Jaguar 3 brand			TF 66		
	Control	10 d stress	20 d stress	Control	10 d stress	20 d stress
$A_{max\text{-}light}$	11.50±0.24^a	9.80±0.41^c	10.65±0.33^b	10.2±0.28^a	9.47±0.56^b	9.45±0.35^b
$A_{sat\text{-}light}$	10.16±0.45^a	9.19±0.30^b	8.24±0.19^c	9.71±0.73^a	8.07±0.35^b	5.87±0.45^c
Φ	0.080±0.003^a	0.048±0.009^c	0.063±0.005^b	0.061±0.003^a	0.044±0.001^c	0.054±0.006^b
LCP	7.35±0.52^b	6.72±0.55^b	13.68±1.16^a	8.72±0.75^b	7.16±0.63^c	12.9±2.59^a
LSP	303.1±10.2^c	372.1±20.2^a	330.3±18.6^b	284.3±11.7^b	324.7±20.2^a	358.0±18.1^a

These parameters include maximum net photosynthesis at maximum PPFD level ($A_{max\text{-}light}$) (μmol CO_2 $m^{-1}s^{-1}$), light saturated net photosynthetic rate ($A_{sat\text{-}light}$) (μmol CO_2 $m^{-1}s^{-1}$), apparent quantum efficiency (Φ) (μmol CO_2 μmol^{-1} photon), light compensation point (LCP) (μmol m^{-2} s^{-1}) and light saturating point (LSP) (μmol m^{-2} s^{-1}). Each value is the mean ± S.E. based on six determinations.

Table 2. Average parameters of A-Ci curves for two tall fescue (*Festuca arundinacea*) cultivars (Jaguar 3 brand and TF 66) grown under normal and high temperature conditions.

	Jaguar 3 brand			TF 66		
	Control	10 d stress	20 d stress	Control	10 d stress	20 d stress
$A_{max\text{-}CO2}$	16.60±0.75^a	14.36±0.47^b	12.62±0.18^c	14.19±1.58^a	12.38±0.45^b	11.43±0.25^c
$A_{sat\text{-}CO2}$	11.53±0.68^a	11.26±0.29^a	9.16±0.63^b	11.05±0.47^a	9.14±0.36^b	8.89±0.42^b
Rd	2.88±0.34^b	4.70±0.20^a	4.77±0.28^a	4.22±0.24^c	4.84±0.05^b	6.03±0.34^a
CE	0.043±0.001^b	0.047±0.002^a	0.037±0.003^c	0.042±0.003^a	0.039±0.008^a	0.032±0.003^b
Γ	15.03±0.86^c	17.18±1.15^b	24.29±1.43^a	20.15±3.88^b	22.28±2.16^b	28.97±1.25^a

These parameters include maximum net photosynthesis under maximum Ci ($A_{max\text{-}CO2}$) (μmol CO_2 $m^{-1}s^{-1}$), CO_2-saturated net photosynthetic rate ($A_{sat\text{-}CO2}$) (μmol CO_2 $m^{-1}s^{-1}$), light respiration (Rd) (μmol $m^{-2}s^{-1}$), CO_2 compensation point (Γ) (μmol mol^{-1}) and carboxylation efficiency (CE). Each value is the mean ± S.E. based on six determinations.

Chlorophyll and carotenoid content and leaf photochemical efficiency

Heat Stress altered chlorophyll (Chl) and carotenoid content and leaf photochemical efficiency in the two stressed plants (Figure 5). Both Chl a+b content and Chl/carotenoid ratio fell in the two heat stressed cultivars in relation to the control plants (Figure 5A, C). An increase in the Chl a/b ratio occurred in the two treated plants (Figure 5B), caused mainly by a higher decrease in Chl b content than Chl a content (data not shown). Both variable chlorophyll fluorescence yield (Fv) and maximum photochemical efficiency of photosystem II in dark adapted leaves (Fv/Fm), as well as efficiency of the open reaction centre in light ($\Phi PSII_{open}$), were reduced in the two stressed cultivars at the end of heat shock (Figure 5D, E, F). Jaguar 3 brand retained a significantly higher Chl a+b content, Chl/carotenoid ratio, and Fv and Fv/Fm ratio than did TF 66 during high temperature stress. The Chl a/b ratio in TF 66 was much higher than in Jaguar 3 brand after 10 d and 20 d treatment. Moreover, $\Phi PSII_{open}$ in Jaguar 3 brand was higher than in TF 66 at normal and stressed conditions, but the difference was not significant at 20 d treatment (P<0.05).

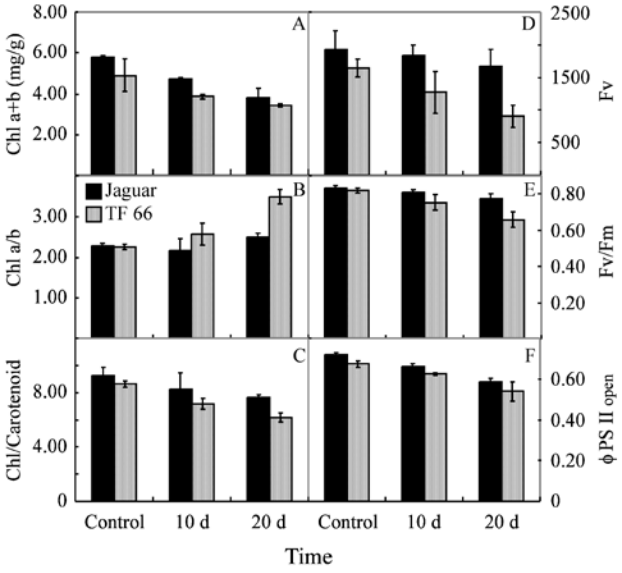

Figure 5. Chlorophyll a+b, Chl a+b (A), chlorophyll a/b ratio, Chl a/b (B), chlorophyll/carotenoid ratio, Chl/carotenoid (C), variable chlorophyll fluorescence yield, Fv (D), maximum photochemical efficiency of photosystem II in dark adapted leaves, Fv/Fm (E) and efficiency of the open reaction centre in light, $\Phi PSII_{open}$ (F) in leaves of two tall fescue (*Festuca arundinacea*) cultivars (Jaguar 3 brand and TF 66) grown under normal and high temperature conditions. Measurements were conducted at 20 or 35 °C. Each value is the mean ± S.E. based on six determinations.

Lipid peroxidation, membrane thermostability, and antioxidant enzyme activities

High temperature stress increased leaf membrane peroxidation and decreased membrane thermostability in the two stressed cultivars. Malondialdehyde (MDA) content and electrolyte leakage (EL) level increased significantly in both stressed cultivars (Figure 6A, B). During high temperature stress, TF 66 maintained a significantly higher content of MDA and level of EL than Jaguar 3 brand did (P<0.05).

Activity of ascorbate peroxidase (APX) in the two cultivars increased after 10 d heat stress while it decreased remarkably in TF 66 and further increased in Jaguar 3 brand after 20 d treatment compared to the control plants (Figure 6C). Superoxide dismutase (SOD) activity increased at 10 d stress and then decreased at 20 d treatment compared to that in control cultivars for both plants (Figure 6D). Compared to TF 66, Jaguar 3 brand had higher APX and SOD activities under normal or stressed regimes (P<0.05).

DISCUSSION

Photosynthesis, one of the most heat sensitive processes, shows heat inhibition of both stomatal limitation

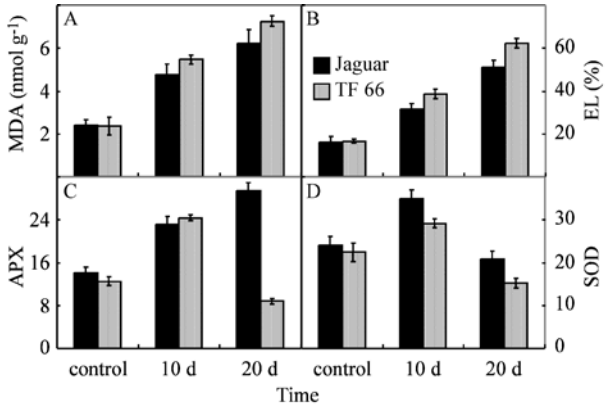

Figure 6. Malondialdehyde, MDA (A), electrolyte leakage, EL (B), ascorbate peroxidase activity (mmol mg^{-1} protein min^{-1}), APX (C) and superoxide dismutase activity (Unite mg^{-1} protein), SOD (D) in leaves of control and stressed plants of Jaguar 3 brand and TF 66. Measurements were conducted at 20 or 35°C. Each value is the mean ± S.E. based on four determinations.

and non-stomatal limitation (Higuchia et al., 1999). In our case, decreases of both Pn and g_s (Figure 1A, B) under high temperature stress indicated that reductions in CO_2 assimilation observed in the two plants were partly attributable to stomatal limitation. The limitations to CO_2 assimilation imposed by stomatal closure may promote an imbalance between photochemical activity at photosystem II (PSII) and the electron requirement for photosynthesis, leading to an overexcitation and subsequent photoinhibitory damage of PSII reaction centers (Souza et al., 2004). Moreover, no significant variations of Ci (Figure 1D) in the two cultivars suggested the existence of a non-stomatal limitation of photosynthesis in the two stressed cultivars. Our findings were in agreement with the earlier report of Higuchia et al. (1999). However, Camejo et al. (2005) reported no stomatal limitation in tomato cultivars under high temperature stress. WUE in the two stressed cultivars decreased compared to the control plants. This decrease was mainly attributed to decrease in Pn and increase in E in the two treated cultivars. Under high temperature stress, Jaguar 3 brand had a significantly higher transpiration rate and a greater increase in transpiration rate than TF 66, which resulted in a higher WUE in TF 66 than in Jaguar 3 brand.

The decrease in Φ (Table 1) in the two stressed cultivars indicates that photoinhibition of photosynthesis occurred (Osmond, 1994). In general, the occurrence of photoinhibition signals an imbalance between light energy absorption and utilization in PSII. Under normal conditions, chloroplasts recover from irreversible photoinhibition (Colom and Vazzana, 2003). This result was in agreement with an earlier report that photoinhibition could be induced by high temperature stress (Xu et al., 1995; Sinsawat et al., 2004). Moreover, this finding could further explain the reductions of both $A_{max\text{-}light}$ and $A_{sat\text{-}light}$ in our case. Heat stress also changed LCP and LSP in the two cultivars, but differences between Jaguar 3 brand and TF 66 were not significant. Higher $A_{max\text{-}light}$, $A_{sat\text{-}light}$ and Φ in Jaguar 3 brand than in TF 66 probably showed that these characteristics can be associated with heat tolerance in tall fescue cultivars.

Photosynthesis in the two plants was significantly influenced by heat stress. This observation was also confirmed by the analysis A-Ci curves. High temperature stress decreased $A_{max\text{-}CO2}$ and $A_{sat\text{-}CO2}$, and increased Rd and Γ in the two treated plants compared to the control plants (Table 2). Reduction in CE in the two treated cultivars due to high temperature stress indicated that reduction in Rubisco activity by the stress in the two cultivars was a probable reason for low Pn. Significant differences in $A_{max\text{-}CO2}$, Rd and Γ of the two plants indicated that these parameters also probably characterised heat tolerance in tall fescue cultivars.

Both the Chl a+b content and Chl/carotenoid ratio decreased, and the Chl a/b ratio increased in the two heat stressed cultivars compared to the control plants (Figure 5A, B, C). Jaguar 3 brand retained a significantly higher Chl a+b content and Chl/carotenoid ratio and a lower Chl a/b ratio than TF 66 during high temperature stress. These results suggested that these characteristics could be used as indicators of heat tolerance and the physiological status of tall fescue cultivars under high temperature stress conditions. An increase in the Chl a/b ratio, resulting from Chl b's faster degradation, indicated a preferential decrease in light-harvesting chlorophyll a/b-binding proteins (LHC) associated with PSII (LHCII) to transfer excitation energy to the PS II core complex (Xu et al., 1995). The preferential decrease in LHCII could reduce the risk of photooxidative damage due to a relative decrease in the absorption cross-section of photosystems (Špundová et al., 2003). A decrease in LHCII in the two stressed cultivars was attributed to more pigment composition of the photosynthesis apparatus converting to sun-type chloroplast, which possesses less LHCII. Sun-type chloroplasts are known to possess a higher carotenoid content on a Chl basis than medium-light or low-light chloroplasts (Camejo et al., 2005), which could explain the lower Chl/carotenoid ratio in the two stressed plants.

High temperature stress reduced the Fv and Fv/Fm ratio, as well as $\Phi PSII_{open}$ (Figure 5D, E, F), indicating that a structural and functional disorder of the photosynthetic apparatus and damage to the PSII had occurred (Osmond, 1994; Pereira et al., 2000; Murkowski, 2001). Decrease in Fv indicated a reduction in the number of open PSII units. Reductions in the Fv/Fm ratio and $\Phi PSII_{open}$ under high temperature stress suggested an important portion of the PSII reaction centre was damaged in the two stressed cultivars. These damages were associated with structural modifications on PSII, especially in D1 protein, which in conditions of heat stress was phosphorylated and degraded afterwards (Asada et al., 1998). Jaguar 3 brand had higher Fv and Fv/Fm ratios and $\Phi PSII_{open}$ during high temperature stress than TF 66 did, indicating that the photosynthetic

apparatus in TF 66 was more susceptible to heat stress than in Jaguar 3 brand.

Moreover, reduction in the Fv/Fm ratio also suggested the occurrence of photoinhibition, also known as photodamage (Colom and Vazzana, 2003). When this is the case, accumulation of reduced electron acceptors may increase the generation of reactive radicals such as active oxygen species (AOS), which can induce oxidative injuries (Souza et al., 2004). These oxidative injuries could enhance Chl degradation or the inhibition of its biosynthesis (Papadakis et al., 2004), damage PSII components (Souza et al., 2004), inactivate many chloroplast enzymes, especially those participating in CO_2 assimilation (Dekov et al., 2000), and could further explain the reductions in Pn, Fv/Fm, and leaf Chl pigment content in the high temperature stressed plants in the present study.

The occurrence of photooxidative damage, mainly caused by active oxygen species (AOS), was further supported by significantly higher MDA and EL levels in heat stressed plants (Figure 6A, B). MDA, a product of the peroxidation of unsaturated fatty acids in phospholipids, and EL have been used as indicators of free radical damage to cell membranes and membrane thermostability under heat stress (Lin and Kao, 1998; Liu and Huang, 2000). Our results suggest that high temperature stress significantly increased membrane lipid peroxidation and decreased membrane thermostability in both cultivars, especially in heat-sensitive TF 66.

Along with the occurrence of oxidative damage during heat stress, the two plants responded by activation of antioxidant enzymes (Figure 6C, D). Variations of APX and SOD activities were different in Jaguar 3 brand and TF 66 under stressed conditions. In comparison to the control plants, activities of APX and SOD increased in the two plants after 10 d treatment, then decreased after 20 d treatment, except for a further increase of APX in Jaguar 3 brand. This suggests that the two stressed plants had an effective system for detoxifying active oxygen species at 10 d treatment, and this system gradually deteriorated after 20 d. Jaguar 3 brand had higher APX and SOD activities compared to TF 66. This result, along with the remarkable increase of MDA and EL levels, indicates that cell membranes in TF 66 were more susceptible to heat stress than in Jaguar 3 brand and that AOS scavenging ability in TF 66 was lower than in Jaguar 3 brand.

In conclusion, comparison of two tall fescue cultivars with different heat susceptibility permitted determining that the tolerance or sensitivity to high temperature was manifested throughout the photosynthetic activity. High temperature induced chlorophyll content deterioration and functional damage in PS II and further decreased photosynthetic activity. Maintenance of high photosynthetic activity is very important in enabling tall fescue cultivars to overcome high temperature. Stomatal and non-stomatal limitations and photoinhibition increase, as well as Rubisco activity decrease, also provoked CO_2 assimilation rate reduction in the two stressed cultivars. The function of PS II and the pigment content of the light harvesting complex were important aspects of the tolerance of tall fescue plants to high temperature. The preservation of higher antioxidant ability to curtail lipid peroxidation and cell membrane damage was also associated with the heat tolerance of tall fescue cultivars.

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高溫脅迫對兩個耐熱性不同的高羊茅品種光合作用，光系統 II 功能和抗氧化活性的影響

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高溫是高羊茅草坪草 (*Festuca arundinacea*) 在亞熱帶地區夏季生長的主要限制因數。本文以高羊茅草坪草耐熱型 “美洲虎 3 號” 和熱敏感型 “TF 66” 的葉片為材料，比較研究了高溫脅迫對高羊茅兩個品種的光合作用、光系統 II 功能和抗氧化活性的影響。結果表明，高溫脅迫通過誘發了葉片氣孔限制和非氣孔限制，促使光抑制增加和 Rubisco 活性下降而降低了高羊茅的淨光合速率 (Pn)。高溫 脅迫改變了高羊茅葉片光系統 II 的功能，表現為高溫脅迫下兩品種的葉片可變熒光 (Fv)、光系統 II 最大光化學量子產量 (Fv/Fm) 以及光下開放反應中心效率 ($\Phi PSII_{open}$) 都顯著降低。高溫脅迫極大地降低了兩品種葉綠素 a+b 含量和葉綠素/類胡蘿蔔素比率，增加了葉綠素 a/b 比率。 此外，兩供試品種受脅迫後葉片膜脂過氧化程度顯著增加，細胞膜熱穩定性顯著降低，抗壞血酸過氧化物酶 (APX) 活性和超氧化物岐化酶 (SOD) 活性也發生了明顯的改變。相同程度的高溫脅迫對 “TF 66” 的光合作用，光系統 II 功能和抗氧化活性的影響比對 “美洲虎 3 號” 的影響更大。本研究結果為日後進行耐熱型高羊茅品種的選育工作提供了一些新的指導。

關鍵詞：抗氧化酶；細胞膜完整性；高羊茅；高溫脅迫；光合特性；光系統 II 光化學效率。