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BRIEF COMMUNICATION

Effects of foliar application of nitrogen on the photosynthetic performance and growth of two fescue cultivars under heat stress

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Abstract

The effects of nitrogen fertilization on the growth, photosynthetic pigment contents, gas exchange, and chlorophyll (Chl) fluorescence parameters in two tall fescue cultivars (*Festuca arundinacea* cv. Barlexas and Crossfire II) were investigated under heat stress at 38/30 °C (day/night) for two weeks. Shoot growth rate of two tall fescue cultivars declined significantly under heat stress, and N supply can improved the growth rates, especially for the Barlexas. Chl content, leaf net photosynthetic rate, stomatal conductance, water use efficiency, and the maximal efficiency of photosystem 2 photochemistry (F_v/F_m) also decreased less under heat stress by N supply, especially in Crossfire II. Moreover, cultivar variations in photosynthetic performance were associated with their different response to heat stress and nitrogen fertilization, which were evidenced by shoot growth rate and photosynthetic pigment contents.

Additional key words: chlorophyll fluorescence parameters, *Festuca arundinacea*, intercellular CO_2 concentration, stomatal conductance, tall fescue, transpiration, urea, water use efficiency.

Heat stress can affect many of the processes involved in the inhibitions of growth, enzyme activities, photosynthesis (Berry and Björkman 1980, Paulsen 1994, Marcum 1998, Karim *et al.* 1999, Xu and Huang 2000a, Murkowski 2001, Todorov *et al.* 2003, Wang *et al.* 2004), and acceleration of leaf senescence (Kuroyanagi and Paulsen 1988, Huang *et al.* 2001). In addition, heat stress may also lead to direct inhibitions of nutrient uptake or even to the change of contents of basic nutrients such as N and P in plant tissues (Gur and Shulman 1979, Huang and Xu 2000), thus it is important to understand how heat stress may be alleviated by the supply of macronutrients.

Nitrogen is required in greatest quantities as it is an essential component of chlorophyll (Chl), amino acids, protein, nucleic acids, enzymes, *etc*. (Esteban *et al.* 2004, Haque *et al.* 2006). Thus N fertilization promotes the growth and photosynthesis in many plants (*e.g.* Cechin and de Terezinha 2004). Recent research about heat stress is focused on its effects on activities of antioxidant enzymes (Fu and Huang 2003, Larkindale and Huang 2004). However, there is little information available on the influence of N on photosynthetic and Chl fluorescence parameters in turfgrass plants under heat stress. Therefore, the objectives of this paper were: *1*) to determine the changes in contents of photosynthetic pigments, Chl fluorescence, gas exchange and growth induced by heat stress and by foliar N application, and *2*) to improve understanding of physiological mechanisms of the two cultivars thermotolerance. We hypothesized that foliar application of optimum N fertilization would overcome or alleviate the adverse effects of heat stress.

Healthy seeds of tall fescue (*Festuca arundinacea* Schreb. cvs. Barlexas and Crossfire II) were collected from field plots at the Flower Cultivation Center, Nanjing University. Grass was grown in plastic pods (12 cm in

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Abbreviations: Car - carotenoids, Chl - chlorophyll; c_i - intercellular CO_2 concentration; E - transpiration rate; F_m - maximum fluorescence yield; F_0 - minimal fluorescence yield; F_v/F_m - maximum quantum efficiency of photosystem 2 photochemistry; F_v'/F_m' - effective quantum yield of PS 2 reaction centres; g_s - stomatal conductance; HT - high temperature; P_N - net photosynthetic rate; qNP - non-photochemical quenching; qP - photochemical quenching; R_D - dark respiration; WUE - water use efficiency.

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diameter, 24 cm long) filled with sand and clay (2/1, v/v) in the greenhouse. Turfgrass was mowed weekly to a 7-cm height with an electric clipper, watered daily until water drained from the bottom of pots, fertilized weekly with 50 cm^3 of half-strength Hoagland's nutrient solution for 60 d, and then transferred to growth chamber (14-h photoperiod, irradiance of 400 μmol m^{-2} s^{-1}, relative humidity of 75 ± 10 %, day/night temperature of 22/16 °C). The positions of pots in the growth chamber were arranged randomly and exchanged daily. Three weeks later, the plats were sprayed twice (with one week interval) with 5 mM urea or distilled water until the leaves were wet. Then half of treated plants were transferred into another growth chamber with a day/night temperature of 38/30 °C and the same irradiation and humidity for two weeks. Thus, we got the experiment with three factors (two grass species, two nitrogen fertilization levels, and two temperature treatments) and four replications (pots) arranged in a completely randomized design.

Shoot growth rate was recorded by the difference of canopy height [mm] in a one-day period using a ruler. Leaf pigments were extracted with 96 % ethanol and absorbance was determined with a *Beckman DU-65* spectrophotometer (*Beckman*, Fullerton, CA, USA) at wavelengths of 470, 649, and 665 nm (Bragina *et al.* 2004). Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), intercellular CO_2 concentration (c_i) and dark respiration rate (R_D) were measured with an open gas analysis system (*Li-Cor 6400*, Lincoln, NE, USA) with a 6-cm^2 chamber on the fully expanded penultimate leaf. Short-term water use efficiency (WUE) was calculated by dividing P_N by E. The measurements were recorded when both P_N and g_s were stable. Ambient temperature and humidity were used (also recorded). An average was taken from five readings spaced evenly 120 s. Readings were taken at 1200 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) provided by LED. R_D was recorded on the same leaf after 5 min of dark acclimation (Reynolds *et al.* 2000).

Chlorophyll fluorescence parameters were recorded on the fully expanded penultimate leaf with a portable fluorometer (*Hansatech Instruments*, King's Lynn, Norfolk, UK). The maximum efficiency of photosystem 2 (PS 2) photochemistry was determined as F_v/F_m, where $F_v = F_m - F_0$ and F_m and F_0 are the maximum and minimum fluorescence yield, respectively, of a dark-adapted leaf. F_0 was measured in the completely dark-adapted state using a dark-adaptation clip (DLC-8) for a period of 15 min, and F_m was determined after a 0.8 s saturating flashes at 8 000 mol m^{-2} s^{-1} in dark-adapted leaves. In addition, effective quantum yield of PS 2 reaction centres of irradiated sample, F_v'/F_m', was recorded and the coefficients of photochemical quenching (qP) and the no-photochemical quenching (qNP) were calculated by equations of Karim *et al.* (2003).

The values in figures are the means of all the measurements (n = 4 - 15). Sample variability was given as the standard deviation (SD) of the means and significantly different means ($P < 0.05$) were identified by the least significant difference (LSD) after the presence of significant effects had been confirmed by one-way *ANOVA*.

Temperature and N nutrition are two of the most important factors limiting plant growth and development (Paulsen 1994, Panković *et al.* 2000). The shoot growth rate of tall fescue cvs. Barlexas (FB) and Crossfire II (FC) declined from 5.1 to 1.0 and from 5.8 to 1.7 mm d^{-1}, respectively, under heat stress. However, N supply doubled shoot growth rates of FB and slightly increased the growth rate of FC (from 1.7 to 1.8 mm d^{-1}) in high temperature treatment (HT). CF had higher growth rate under HT than FB, these might result from its higher Chl content.

By foliar N fertilization, the contents of leaf photosynthetic pigments (Chl *a*, Chl *b*, and Car) increased in the two cultivars while they largely decreased under HT (Fig. 1). The pigment content in FC was higher than that in FB. Chl *a*/*b* ratio was remarkable decreased in HT by foliar N fertilization. High Chl a/b may be the proof of damaged light-harvesting complex 2 (LHC 2) because a considerable amount of Chl *b* is localized in LHC 2 (Lawlor 1993). Chloroplast is centre of photosynthetic response. Heat stress damaged chloroplast membrane and therefore can lead to the decrease of photosynthesis in many plants (Berry and Björkman 1980, Reynolds *et al.* 2000).

Leaf gas exchange parameters P_N, g_s and WUE showed a significant decrease under HT, and they mostly increased a little by foliar N fertilization (Fig. 1). Values of these parameters in FC were higher than those in FB under the same treatment. By N fertilization in HT, P_N increased by 50 % approximately in FB and by 67 % in FC, respectively, WUE increased by 73 % in FC which was more increase than that in FB (only 5 % increase), and g_s increased by 43 % in cv. FB. In contrast, c_i, E, and R_D in the two cultivars increased in response to HT (Fig. 1). Under HT, severe reduction in P_N has been found in many plant species (Xu and Huang 2000b) and a decrease of P_N is often accompanied by an increase in R_D (Xu and Huang 2000b). Sugiharto *et al.* (1990) found a significant positive correlation between the photosynthetic capacity of leaves and their N nutrition suggesting that most of the N is used for synthesis of components of the photosynthetic apparatus (Pandey and Tyagi, 1999). Significant differences between FB and FC were found in changes of P_N, g_s, E, R_D, and WUE which indicated lower damage of photosynthetic apparatus in FC than FB in response to heat stress, especially under N supply.

Chl fluorescence parameters F_0, F_m and qP did not vary significantly among treatments. We found a trend of decrease in the maximal efficiency of PS 2 photochemistry (F_v/F_m) and effective quantum yield of PS 2 reaction centres of irradiated sample (F_v'/F_m') in leaves of the two cultivars under HT, which was in agreement with results of other studies on plants under HT (Huang *et al.* 2001, Gesch *et al.* 2003). The lower decrement of F_v/F_m

and F_v'/F_m' by N supply may result from the lower decrease of Chl content in leaves of the two cultivars pretreated with N. Furthermore, N supply can directly modify the F_v/F_m ratio as well as the qP and qNP (Ciompi *et al.* 1996). The decrease in F_v/F_m may be a result of an increase in protective no-radiative energy dissipation, the

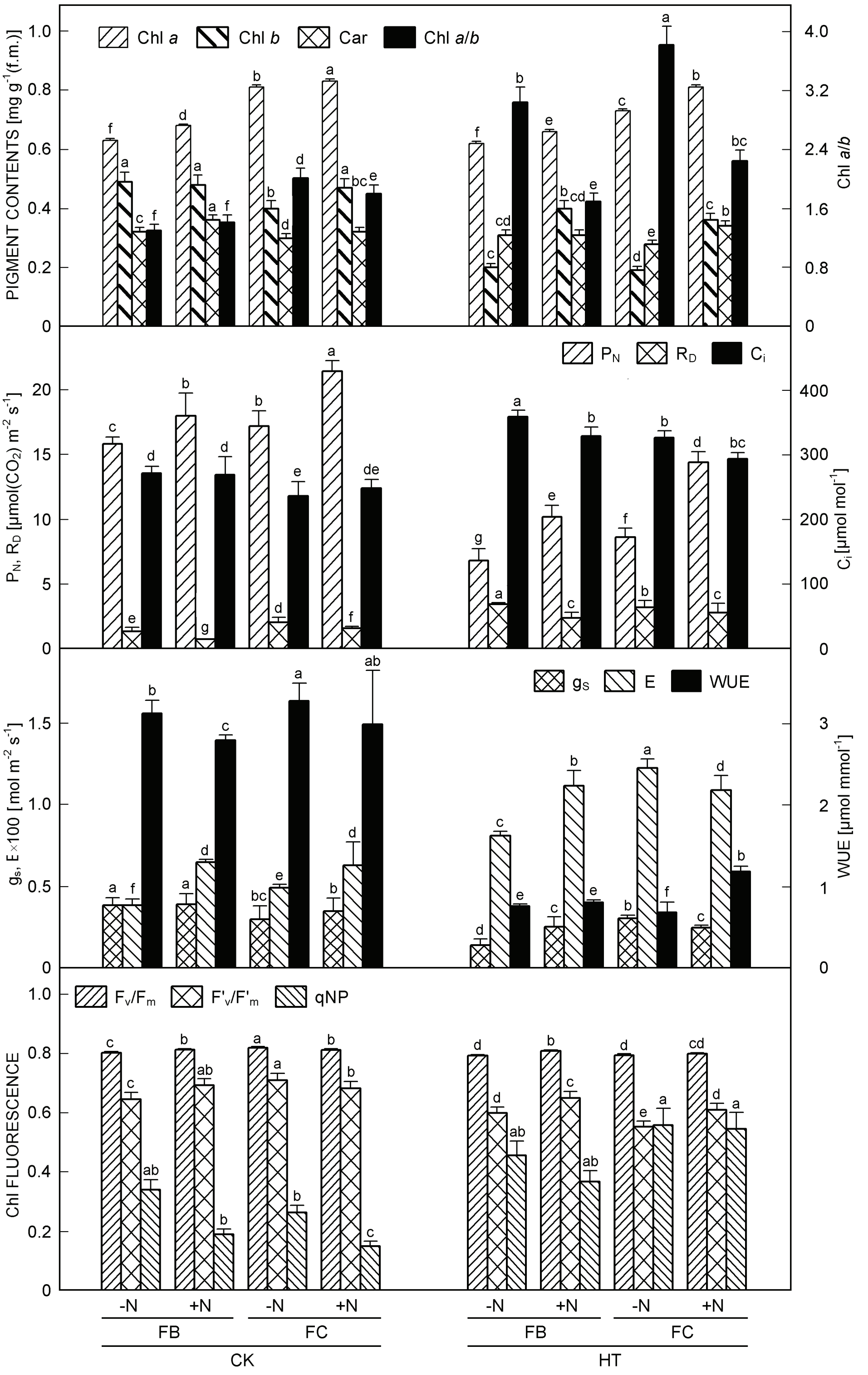

Fig. 1. Changes of pigment contents, photosynthetic parameters and chlorophyll fluorescence parameters in leaves of two tall fescue cultivars by foliar nitrogen fertilization under heat stress. Within the same series, values over standard bar with the same letters are not significantly different ($P > 0.05$) following one-way *ANOVA*. FB - cv. Barlexas; FC - cv. Crossfire II; -N - no N fertilization; +N - N fertilization; CK - control; HT - heat stress.

non-photochemical quenching (qNP). Under heat stress, qNP in leaves of the two cultivars increased, and can maintained lower by N pretreatment. The lower qNP indicated that lower proportion of absorbed photons was lost as heat generation in PS 2 reaction centres instead of being used to drive photosynthesis (Ciompi *et al.* 1996, Karim *et al.* 2003). Therefore, to some extent, photosynthetic ability was maintained higher for each cultivar under HT by N supply, which may result from lower decrease of PS 2 activity and Chl content by N fertilization, especially for FC, the heat-tolerating cultivar.

In conclusion, we demonstrated that foliar application of N fertilizer alleviated partially the adverse effects of heat stress on growth and photosynthetic performance in the two tall fescue cultivars. According to the changes in the growth, gas exchange parameters, and Chl fluorescence of the two cultivars, cv. Crossfire II was more heat tolerant than cv. Barlexas and especially foliar application of N fertilizer contributed to its higher heat tolerance.

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