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Photosynthesis and protein metabolism associated with elevated CO_2-mitigation of heat stress damages in tall fescue

Jingjin Yu[a,b,1], Zhimin Yang[a,b,1], David Jespersen[b], Bingru Huang[a,b,*]

[a] College of Agro-Grassland Science, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08901, USA

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ABSTRACT

Heat stress is a primary factor limiting the growth of cool-season (C_3) perennial grass species during summer months. Elevated CO_2 may alleviate heat stress damage in C_3 plants. The objective of this study was to investigate mechanisms underlying elevated CO_2-mitigation of adverse effects due to heat stress in C_3 perennial grass species by examining effects of elevated CO_2 on major photosynthetic components and proteins for tall fescue (*Festuca arundinacea*) subjected to heat stress. Plants of tall fescue (cv. 'Rembrandt') were grown under ambient CO_2 (400 μmol mol^{-1}) or elevated CO_2 (800 μmol mol^{-1}) and subjected to ambient temperature (25/20 °C day/night) or heat stress (35/30 °C day/night). Elevated CO_2 enhanced photosynthetic rate under both ambient temperature and heat stress in tall fescue. The improved photosynthesis under elevated CO_2 was associated with the increase in the abundance of proteins involved in photosynthetic light reactions (chlorophyll a–b binding protein), electron transport carrier molecule (ferredoxin), and ATP generation enzyme (adolase), as well as higher carbon assimilation efficiency and carboxylation enzyme activities of the Calvin cycle [higher carbon:nitrogen ratio (C:N), maximal rate of photosynthetic electron transport (J_{max}), Rubisco activity and Rubisco activation]. Elevated CO_2 also induced the accumulation of proteins involved in antioxidant metabolism (ascorbate peroxidase and 2-Cys peroxiredoxin). Elevated CO_2 induced stomatal closure and chlorophyll content decline under both ambient temperature and heat stress, which could have limited the positive effects of elevated CO_2 on the photosynthetic capacity. It would be useful to select cultivars of C_3 perennial grass species with decreased stomatal sensitivity to elevated CO_2 to achieve maximal benefits of elevated CO_2 on photosynthesis and whole-plant growth. Our results suggested that the increased photosynthetic efficiency and activities, as well as protein abundance involved in photosynthesis and antioxidant metabolism could play important roles in elevated CO_2-mitigation of heat stress damage in C_3 perennial grass species.

1. Introduction

Elevated temperature and CO_2 concentration can have significant impact on plant growth. During this century, global temperatures are predicted to rise by 2–5 °C (IPCC, 2007). The optimal temperature for shoot and root growth of cool-season C_3 plant species is 18–24 °C but during summer months the ambient temperature can be 10 °C higher or more than the temperature requirement driving optimal growth in cool-season plants, which leads to various cellular and physiological damages (DiPaola and Beard, 1992). Photosynthesis is one of the most sensitive responses to increasing temperatures, and heat-inhibition of photosynthesis contributes to the decline in the availability of carbohydrates for the supply of energy and carbon skeletons for plant growth (Bencze et al., 2005; Crafts-Brandner and Salvucci, 2000; Liu and Huang, 2008; Salvucci and Crafts-Brandner, 2004). Along with increasing temperature, atmospheric CO_2 concentration has increased by 100 μmol mol^{-1} since the beginning of the industrialized era and the concentration is predicted to continue rising at a rate of approximately 2 μmol mol^{-1} per year (IPCC, 2007). Changes in these global climate factors will likely lead to plant exposure to combined elevated temperature and CO_2, which may have interactive effects on plant growth.

Previous research has shown that elevated CO_2 promotes plant growth under optimal growing temperatures in various plant species, particularly C_3 species (Hamerlynck et al., 2000; Kirkham, 2011; Prasad et al., 2002, 2009; Qaderi et al., 2006). The physiological and biochemical effects of elevated CO_2 on various plant species grown under optimal growth temperatures have been well

* Corresponding author at: Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08901, USA. Tel.: +86 2584396359; fax: +1 7329329441.
E-mail address: huang@aesop.Rutgers.edu (B. Huang).
[1] These authors contributed equally to this work.

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documented (Bunce, 2001; Griffin and Seemann, 1996; Stitt and Krapp, 1999; Woodward, 2002). The enhancement of plant growth by elevated CO_2 under optimal temperature conditions has been associated with promotion of photosynthesis and inhibition of dark respiration and photorespiration rates, as well as increased carbohydrate accumulation (Leakey et al., 2006; Gonzàlez-Meler et al., 1996; Reddy et al., 2010). However, a limited number of studies have examined the interactive effects of elevated CO_2 and heat stress or the beneficial effects of elevated CO_2 on plant growth in relation to abiotic stress tolerance (Hamilton et al., 2008; Prasad et al., 2002, 2009). Few available studies have demonstrated that elevated CO_2 can mitigate the adverse effects of heat stress on photosynthesis, water use, and overall plant growth in different plant species (Idso and Idso, 1994; Hamilton et al., 2008; Prasad et al., 2009), including C_3 perennial grass species (Yu et al., 2012a,b), but the mechanisms of enhanced heat tolerance on photosynthesis due to elevated CO_2 are unclear.

Proteomic profiling combining two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) in combination with mass spectrometry (MS) is a powerful approach to identify and quantify stress-responsive proteins in various plant species (Abbasi and Komatsu, 2004; Hashimoto and Komatsu, 2007; Lee et al., 2007; Xu and Huang, 2008; Xu et al., 2010; Zhao et al., 2011). Heat stress alone alters protein metabolism, causing up-regulation or down-regulation of proteins involved in different metabolic processes, such as energy metabolism (photosynthesis and respiration), antioxidant stress defense, protein synthesis and storage, and protein stability (Ferreira et al., 2006). Elevated CO_2 also induces changes in protein expression, as shown by Bae and Sicher (2004) who identified six proteins responding to elevated CO_2 concentration under optimal temperatures in *Arabidopsis*, among which three proteins were involved in plant growth and development or stress defense, one protein (23 kDa subunit) of the oxygen evolving complex (OEC23) involved in photosynthesis and two proteins with unknown functions. The authors concluded that elevated CO_2 did not singly have a significant impact on protein expression in *Arabidopsis* because only a few proteins were responsive to CO_2 enrichment. Others have found potentially important changes in proteins related to plant physiological response to abiotic stress as discussed below. Several proteins involved in photosynthesis, such as Rubisco activase (van Oosten and Besford, 1995), carbonic anhydrase (Majeau and Coleman, 1996; Peet et al., 1986) and the chlorophyll a–b binding protein (Moore et al., 1998; van Oosten et al., 1994) decreased at the transcript level in response to elevated CO_2 compared to those under ambient CO_2 under optimal growth temperatures. However, few studies have investigated elevated CO_2-induced changes in proteins, particularly those involved in photosynthetic metabolism and stress defense under heat stress conditions which may be associated with CO_2-mitigation of heat stress.

Tall fescue is a widely utilized C_3 forage and turfgrass throughout cool temperate regions and is sensitive to heat stress, whereas tall fescue heat tolerance could be improved by doubling ambient CO_2 concentration (Yu et al., 2012a,b). Elevated CO_2 mitigated the adverse effects of heat and drought stress on tall fescue by enhancing plant water status, cellular membrane stability, and photosynthetic capacity, as well as suppressing carbon consumption through lowering respiration rates (Yu et al., 2012a). Doubling ambient CO_2 concentration also enhanced accumulation of metabolites involved in photosynthesis, respiration, and amino acid metabolism under heat stress (Yu et al., 2012b). Understanding changes to photosynthetic components and proteins in response to CO_2 increase and interaction with heat stress tolerance of tall fescue will provide further insights into the mechanisms underlying heat stress mitigation by elevated CO_2 in cool-season perennial grass species. Therefore, the objective of this study was to investigate CO_2-responsive photosynthetic components and proteins associated with the positive effects of elevated CO_2 concentration on heat tolerance in tall fescue. The ultimate aim was to reveal metabolic processes and molecular mechanisms underlying elevated CO_2 enhancement of heat tolerance in C_3 perennial grass species.

2. Materials and methods

2.1. Plant materials and growth conditions

Sod plugs of tall fescue (cv. 'Rembrandt') plants were collected from the turfgrass research farm at Rutgers University in Adelphia, NJ, and transplanted into plastic pots (10 cm diameter and 60 cm long) filled with a mixture of fine sand and soil (1:1, v/v). During this establishment period, plants were watered every other day and fertilized once a week with half-strength Hoagland's solution (Hoagland and Arnon, 1950). Plant leaves were trimmed once per week to maintain a copy height of 5–6 cm. Plants were maintained in a greenhouse with an average temperature of 21/16 °C (day/night) and 810 $\mu mol\,m^{-2}\,s^{-1}$ photosynthetically active radiation (*PAR*) in natural sun light, and 65% relative humidity for 50 d (May–June 2011) to fully establish the canopy and root system. After establishment, plants were moved to growth chambers with the temperature set at 25/18 °C (day/night), 70% relative humidity, *PAR* of 650 $\mu mol\,m^{-2}\,s^{-1}$ and a 12-h photoperiod to allow for acclimation of controlled-environment conditions for 7 d before elevated CO_2 and temperature treatments were imposed.

2.2. Experimental design and treatments

The experiment consisted of two factors, CO_2 concentration and temperature, each with two levels, which were arranged in a factorial design with four replicates per treatment. The CO_2 treatments included ambient CO_2 ($400 \pm 10\,\mu mol\,mol^{-1}$) and elevated CO_2 ($800 \pm 10\,\mu mol\,mol^{-1}$). Plants were grown at the two CO_2 levels in the growth chambers for 40 d before imposition of temperature treatments. Temperature treatments were controlled at two levels: 25/20 °C (day/night, optimal temperature control) and 35/30 °C (day/night, heat stress) for 28 d. Plants were well-watered to maintain soil water content at field capacity (28%, v/v) and were fertilized once a week with half-strength Hoagland's solution during the CO_2 and temperature treatment period.

The CO_2 and temperature treatment set-up in growth chambers followed the same designed as described in Yu et al. (2012a,b). Four growth chambers were maintained at the ambient CO_2 level with two of them at elevated temperature and the other two at optimal temperature. The ambient CO_2 treatment was conducted in four growth chambers from February to April in 2012. Four growth chambers were set to the elevated CO_2 level, with two at elevated temperature and two at the optimal temperature. The elevated CO_2 treatment was conducted from May to July in 2012. Plants were re-randomized among the growth chambers once per week to minimize confounding effects of possible environmental variations between different chambers. The concentration of CO_2 inside each growth chamber was maintained with an automated, open-chamber CO_2 control system connected to a gas tank containing 100% CO_2 (Airgas, Inc., Radnor, PA) (Yu et al., 2012a,b). The CO_2 levels were continuously monitored through an infrared gas analyzer (Li-820, LI-COR, Lincoln, NE) and controlled using an automatic system consisting of a programmable logic controller unit, solenoid valves, and a laptop computer with monitoring software accurate to within 10 $\mu mol\,mol^{-1}$ of the target levels (400 and 800 $\mu mol\,mol^{-1}$).