Physiological factors involved in positive effects of elevated carbon dioxide concentration on Bermudagrass tolerance to salinity stress

Jingjin Yu [a],[*],[1], Lihong Sun [a],[1], Ningli Fan [a], Zhimin Yang [a], Bingru Huang [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

ARTICLE INFO

ABSTRACT

Salinity stress due to increased use of non-potable water sources for irrigation imposes major limitations on plant growth in salt-affected soils. However, rising atmospheric CO_2 concentration may counteract the negative effects of salinity stress. The objective of this study was to determine whether elevated CO_2 mitigates salinity stress by influencing physiological activities and/or ion (Na^+ and K^+) balance in Bermudagrass (Cynodon dactylon cv. 'Tifway'). Plants were exposed to either ambient CO_2 concentration ($400\,\mu mol\,mol^{-1}$) or elevated CO_2 concentration ($800\,\mu mol\,mol^{-1}$) and maintained well-watered (control) with fresh water or subjected to salinity stress by irrigating plants with NaCl solution (200 mM). Salinity stress caused reduction in turf quality (TQ), leaf relative water content (RWC), leaf net photosynthetic rate (P_n), transpiration rate, stomatal conductance, and cellular membrane stability. Elevated CO_2 concentration alleviated the depression of those physiological parameters and promoted osmotic adjustment through accumulation of soluble sugars, proline, and glycine betaine (GB) under salinity stress, but had no significant effects on the ratio of Na^+ to K^+. Our results demonstrated that elevated CO_2 concentration was effective in alleviating physiological damages of salinity stress in Bermudagrass, suggesting that C_4 grasses may benefit from the rising atmospheric CO_2 concentration associated with global climate changes. The positive physiological effects of elevated CO_2, as manifested by improved TQ, RWC, P_n and cell membrane stability could be related to the maintenance of cellular hydration associated with osmotic adjustment due to the accumulation of soluble sugars, proline and GB, and the suppression of Na^+ accumulation independent of changes in K^+ accumulation.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Salinity stress in salt-affected soils or with increased use of non-potable water sources for irrigation can be detrimental to plant growth. Excessive salinity in the soil can cause osmotic and ionic stresses, leading to various growth and physiological damages to plants (Munns et al., 1999; Pessarakli, 2007, 2010). The atmospheric CO_2 concentration has been rising and is expected to double by the year 2100, which may mitigate the adverse effects of various abiotic stresses, including salinity stress (Kirkham, 2011; Munns et al., 1999) for various plant species, including several cool-sason turfgrass species (Song et al., 2014; Yu et al., 2012a,b, 2014). However, mechanisms of elevated CO_2 affecting plant responses to

salinity stress are not completely understood. Understanding how elevated CO_2 may mitigate salinity stress is important for developing plant germplasm adapted to increasing levels of salinity stress under the scenario of climate change.

Salinity tolerance of plants involves various mechanisms, including maintenance of cellular water status through osmotic adjustment with active accumulation of compatible solutes to reduce osmotic stress, increasing water use efficiencies coupled to lowering rates of water loss, or by maintaining ion balance, such as lower Na^+/K^+ ratios to lessen ionic damages of salinity (Munns et al., 1999; Pessarakli, 2007, 2010). Plants grown under salinity stress are subjected to ionic stress resulting from the accumulation of Na^+ in leaves (James et al., 2002; Yeo, 1998). Salinity-tolerant plants are able to increase K^+ uptake and minimize Na^+ uptake to maintain ion balance, detoxifying the adverse ionic effects of Na^+ (Munns et al., 1999). Osmotic adjustment is associated with the accumulation of osmolytes (i.e., sugars, amino acids, glycine betaine, and proline) (Hasegawa et al., 2000; García-Sánchez and Syvertsen, 2006). Elevated CO_2

* Corresponding authors. Tel.: +1 8489326390; fax: +1 7329329441.
E-mail addresses: jingjin_yu@126.com (J. Yu), huang@aesop.rutgers.edu (B. Huang).
[1] These authors had equal contribution to this work.

concentration has been found to improve plant tolerance to salinity in association with increases in the accumulation of proline and carbohydrates in halophyte *Aster tripolium* (Geissler et al., 2009). Elevated CO_2 concentration also increases leaf net photosynthetic rate, but reduces water loss through transpiration under optimal and stress conditions for some plant species, particularly in C_3 species (Kirkham, 2011). Plants grown under elevated CO_2 conditions could accumulate lower amounts of Na^+ due to lower transpiration rate which is the driving force for water uptake (García-Sánchez and Syvertsen, 2006; Maggio et al., 2002). Bowman and Strain (1987), however, found elevated CO_2 had no significant effects on Na^+ uptake despite increased water use efficiency and reduced water loss in C_4 halophytes. The relative importance of those aforementioned factors involved in elevated CO_2 mitigation of salinity stress may vary with plant species.

Bermudagrass (*Cynodon dactylon*) is a widely-used C_4 perennial forage and turfgrass species and has excellent drought and heat tolerance, but is sensitive to salinity stress (Fry and Huang, 2004). Salinity-sensitive grass species, such as Bermudagrass, may benefit greatly from the rising atmospheric CO_2 concentration based on the information of positive interactive effects of salinity and elevated CO_2 concentrations reported in salinity-tolerant halophytes (Ball and Munns, 1992; Geissler et al., 2009). However, physiological and metabolic factors responsive to elevated CO_2 concentrations that may contribute to the positive effects of elevated CO_2 on salinity tolerance, particularly for salinity-sensitive grass species, are not well documented. It was hypothesized that elevated CO_2 concentrations may increase salinity tolerance of Bermudagrass through the regulation of photosynthesis, water relations and ionic effects. The objective of this study was to determine how elevated CO_2 may mitigate salinity stress of Bermudagrass through influencing various physiological activities and/or ion (Na^+ and K^+) balance.

2. Materials and methods

2.1. Plant materials and growth conditions

Stolons of Bermudagrass (cv. 'Tifway') plants were collected from the research farm at Nanjing Agricultural University in Jiangsu province, China, and transplanted into pots (20 cm diameter and 20 cm long) filled with sand. Plants were maintained in a greenhouse at Nanjing Agricultural University, with average temperature of 30/22 °C (day/night) and natural light to establish canopy and roots for two months (August and September, 2013). During this establishment period, plants were irrigated once a week with half-strength Hoagland's solution (Hoagland and Arnon, 1950). Plants were trimmed once a week to maintain a canopy height of 5–6 cm. After establishment, plants were moved to growth chambers (Xubang, Jinan, Shandong province, China) with the temperature set at 35/30 °C (day/night), 70% relative humidity, photosynthetically active radiation of 650 $\mu mol\, m^{-2}\, s^{-1}$ and a 12 h photoperiod.

2.2. Experimental design and treatments

The experiment consisted of two factors (two CO_2 concentrations and two salinity concentrations), which were arranged in a split-plot design with CO_2 concentrations as the main plot and salinity as the sub-plot with four replicates for each treatment.

The CO_2 treatments included ambient CO_2 ($400 \pm 10\, \mu mol\, mol^{-1}$) and elevated CO_2 ($800 \pm 10\, \mu mol\, mol^{-1}$). The treatment set-up and assignment in growth chambers followed the same design as described in Yu et al. (2012a). Four chambers were maintained at the ambient CO_2 level and four chambers were set at

the elevated CO_2 level. 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 (Yu et al., 2012a).

Plants in each chamber were watered daily with water (control) or NaCl solution (salinity). For salinity treatment, soil in each pot was drenched with 250 mL NaCl solution at incremental electrical conductivity by $3\, dS\, m^{-1}$ per day until the final electrical conductivity reached $15\, dS\, m^{-1}$ (0 r 150 mM, approximately 30% of NaCl concentration in sea water based on 500 mM NaCl or EC about 42 dS/m in sea water) in order to protect plants from the initial salinity shock. Plants were exposed to the final salinity level for 28 d. The control and salinity treatment each had four replicates (pots, each pot with multiple plants), which were placed in four growth chamber set to the ambient CO_2 or the elevated CO_2 concentration.

2.3. Measurements of leaf gas exchange (P_n, g_s, T_r)

Leaf net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (T_r) were taken on 15 individual leaves (second full-expanded from the top) from each pot. Intact leaves were enclosed in a $6\, cm^2$ cuvette with a portable infrared gas analyzer (Li-6400, LI-COR, Inc., Lincoln, NE). Reference CO_2 concentration was consistent with CO_2 concentration of pot's growth chamber. Leaves were placed in a leaf chamber with a built-in red and blue light source of the Li-6400 with the light level of 700 μmol photon $m^{-2}\, s^{-1}$.

2.4. Measurements of physiological indexes

Plant growth was evaluated by rating of turf quality (TQ). TQ is an extensively-used parameter evaluating overall plant performance (Turgeon, 2011). TQ was visually rated on a scale from 1 (completely dead plants) to 9 (green and dense canopy).

Leaf membrane stability was evaluated weekly during salinity treatment by measuring electrolyte leakage of leaves (Blum and Ebercon, 1981). Fresh leaves (0.1–0.2 g) were collected, rinsed, and immersed in 30 mL deionized water and placed on a shaker at 23 °C for 24 h. The initial conductivity of the solution ($C_{initial}$) was then measured using a conductivity meter (Orion Star A212, Thermo Scientific Inc., Massachusetts, MA). Leaves were then killed by autoclaving at 120 °C for 20 min and placed back on the shaker for 12 h. The final conductivity of killed tissues (C_{max}) was then measured and electrolyte leakage (EL) calculated as the percentage of $C_{initial}$ over C_{max}.

Leaf relative water content (RWC) of fully expanded leaves was determined based on fresh (FW), turgid (TW), and dry weights (DW) using the following formula: RWC (%) = [(FW − DW)/(TW − DW)] × 100. Fully expanded leaves were immediately weighed for FW after being excised from the plants, and then placed into tubes filled with deionized water for 12 h in dark at 4 °C. Leaf samples were then blotted dry and immediately weighed for determination of TW. Samples were then dried in an oven at 80 °C for at least 72 h and again weighed for DW (Barrs and Weatherley, 1962).

Osmotic adjustment (OA) was determined by osmotic potential at full turgor. Leaf samples were collected and soaked in deionized water for 4 h, blotted dry, placed into micro-centrifuge tubes, frozen in liquid nitrogen, and stored at −20 °C until further analysis. Leaf sap was pressed out and added to the chamber in an osmometer (5600, Wescor, Inc., Logan, UT) for the determination of the osmolality (mmol kg^{-1}). Osmolality was then converted to osmotic potential and OA was calculated as the difference between control and treated plants (Qian et al., 1997).