DOI: 10.1007/s11099-016-0226-6 PHOTOSYNTHETICA 55 (1): 176-183, 2017

Elevated CO_2 increased photosynthesis and yield without decreasing stomatal conductance in broomcorn millet

X.Y. HAO[*,**,***], P. LI[*,**], H.Y. LI[*], Y.Z. ZONG[*], B. ZHANG[*], J.Z. ZHAO[*,+], and Y.H. HAN[*,**,***,+]

College of Agronomy, Shanxi Agricultural University, Taigu-030801, Shanxi, China[*]
Key Laboratory of Crop Gene Resources and Germplasm Enhancement on Loess Plateau, Ministry of Agriculture, Institute of Crop Genetic Resources, Shanxi Academy of Agricultural Sciences, Taiyuan-030001, Shanxi, China[**]
Shanxi Key Laboratory of Genetic Resources and Genetic Improvement of Minor Crops, Shanxi Agricultural University, Taigu-030801, Shanxi, China[***]

Abstract

Broomcorn millet (*Panicum miliaceum* L.) is one of the important C_4 crops in the semiarid regions of northern China. It is a close relative of biofuel crop switchgrass. Yet, there is no information on how these crops might respond to a climate change in China. In order to gain insight into such a response, we studied the effect of elevated CO_2 concentration (EC) on broomcorn millet. The changes in leaf photosynthesis, chlorophyll fluorescence, morphological parameters, biomass and yield in response to EC [*i.e.*, + 200 μmol(CO_2) mol^{-1}] over two years were determined at the open-top chamber (OTC) experimental facility in north China. EC increased net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, instantaneous transpiration efficiency, effective quantum yield of PSII photochemistry, and photochemical quenching coefficient of fully expanded flag leaves. Maximal quantum yield of PSII photochemistry declined under EC in 2013, but was not affected in 2014. EC significantly decreased intrinsic efficiency of PSII in 2013, but increased in 2014. Leaf nonphotochemical quenching decreased under EC both in 2013 and 2014. EC significantly enhanced the aboveground biomass and yield by average of 31.4 and 25.5% in both years, respectively. The increased yield of broomcorn millet under EC occurred due to the enhanced number of grains per plant. We concluded that photosynthesis of broomcorn millets was improved through increased stomatal conductance in leaves under EC, which led to an increase in height, stem diameter, aboveground biomass, and yield. This study extends our understanding of the response of this ancient C_4 crop to elevated CO_2 concentration.

Additional key words: chlorophyll *a* fluorescence; CO_2 enrichment; gas exchange; water-use efficiency.

Introduction

Since the industrial revolution, the global atmospheric CO_2 concentration ([CO_2]) has risen from 280 μmol mol^{-1} to the current concentration of around 392 μmol mol^{-1} and is predicted to reach 421–936 μmol mol^{-1} by the end of 21st century (IPCC 2013). Elevated [CO_2] (EC) is altering global temperature and precipitation patterns, which could decrease the yield of food crops over the next 50 years (Thomson *et al*. 2005). In contrast, EC is predicted to stimulate crop production and offset these detrimental components of the climate change (Leakey *et al*. 2006).

Received 18 October 2015, *accepted* 21 March 2016, *published as online-first* 12 April 2016.
+Corresponding author; e-mail: swgctd@163.com (YH), zhaojinzhongnd@126.com (JZ)
Abbreviations: C_i – intercellular CO_2 concentration; CK – control; [CO_2] – atmospheric CO_2 concentration; E – transpiration rate; EC – elevated atmospheric CO_2 concentration; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; F_v'/F_m' – intrinsic efficiency of PSII; g_s – stomatal conductance; NPQ – nonphotochemical quenching; OTC – open-top chamber; P_N – net photosynthetic rate; q_P – photochemical quenching coefficient; WUE – water-use efficiency (= P_N/E); WUE_i – intrinsic water-use efficiency (= P_N/g_s); Φ_{PSII} – effective quantum yield of PSII photochemistry.
Acknowledgements: We thank Professor Donald Grierson for comments on the manuscript. This work was supported by the National Key Technology R&D Program in the 12th Five Year Plan of China (No. 2013BAD11B03), The National Basic Research Program of China (973 Program) (No. 2012 CB955904), Scientific and Technological Innovation Programs of Higher Education Institutions in Shanxi (2015146), The Key Scientific Research Projects of Coal Fund in Shanxi (FT201402-01), The National Natural Science Foundation of China (31371693, 31471556), and Shanxi Science and Technology Infrastructure Platform (2012091004-0103).

Compared to C_3 plants, C_4 plants possess a mechanism to concentrate CO_2 around the ribulose-1,5-bisphosphate carboxylase/oxygenase in chloroplasts of bundle sheath cells so that the carboxylation reaction works at a much more efficient rate, thereby substantially eliminating the oxygenation reaction and the resulting photorespiration (Ghannoum *et al*. 2011, Liu *et al*. 2013). It is observed that C_4 photosynthesis is more efficient than C_3 photosynthesis under conditions of low atmospheric [CO_2], heat, drought, and salinity (Liu *et al*. 2013). Some studies show that C_4 species would not benefit from the increases in atmospheric [CO_2] because of the CO_2-concentrating mechanism in leaves. Photosynthesis of maize was unaffected by EC in the absence of drought (Leakey *et al*. 2006). EC did not increase photosynthesis of foxtail millet (Hao *et al*. 2010) and the C_4 grass *S. maritima* (Duarte *et al*. 2014). In *H. portulacoides* (C_3 species), CO_2 fertilization induced an enhancement of electron transport rate (ETR) and a decrease in nonphotochemical quenching (NPQ) and in dissipated energy fluxes. Light-saturated CO_2 uptake (P_{sat}) of three 'wild' C_4 grasses at the BioCON (biodiversity, CO_2, and nitrogen) experiment (Bethel, Minnesota, USA) was not stimulated by EC, but it increased by 15–20% for C_4 crops grown at the Maricopa (Arizona, USA) and SoyFACE (Illinois, USA) sites (Ainsworth and Long 2005). The variation in response of photosynthesis in C_4 plant to EC may be explained because some species appear to be CO_2 saturated at ambient [CO_2], but other C_4 plant are not necessarily saturated at the same concentration (Wand *et al*. 1999).

Wand *et al*. (1999) reported that stomatal conductance (g_s) decreased in C_3 and C_4 grasses. g_s decreased on average by 20% under EC and there was no difference between C_3 and C_4 species (Ainsworth and Long 2005). The instantaneous water-use efficiency (WUE_i, P_N/g_s) was significantly different in C_3 and C_4 species. C_3 species grown under FACE showed a 68% increase in WUE_i, whereas WUE_i was not elevated in sorghum. Elevated [CO_2] improved the water status and increased the water-use efficiency (WUE) in sorghum in the Maricopa FACE experiment (Conley *et al*. 2001, Wall *et al*. 2001). Elevated [CO_2] decreased g_s by 34% in maize in the absence of drought and reduced crop water use, which increased soil moisture in the SoyFACE experiment (Leakey *et al*. 2006). g_s and transpiration rate (E) of foxtail millet and sugarcane were reduced, but WUE was enhanced (Vu and Allen Jr. 2009, Hao *et al*. 2010). Thus, it seemes that EC affects WUE of C_4 plants and improves the ability to tolerate drought.

A larger number of C_3 species has been investigated compared to C_4 species, especially in the FACE experiments (Ainsworth and Long 2005). More C_4 crop experiments are needed to find the mechanism of responses in different C_4 species to EC. Broomcorn millet (*Panicum miliaceum* L.), one of the world's oldest cultivated crops, first appeared as a crop in China about 7,000 years ago (Underhill 1997). Known as the C_4 syndrome, enhanced water and nutrient-use efficiency enable broomcorn millet to grow in habitats, which are too harsh for other crops. It has a short growing season, low requirement of water and nutrients, so it is extensively grown in the semiarid and infertile agriculture regions of northern China, central Asia, and Russia. At present, the annual planting area of broomcorn millet is one million hectares in China, mainly cultivated in the northern mountainous areas by farmers with relatively low income (Dai *et al*. 2011, Hunt *et al*. 2011).

The crops, which have received the most attention for studies on the effect of EC, are those with the highest global economic importance, including rice (Seneweera *et al*. 2002), maize (Leakey *et al*. 2006), soybean (Bernacchi *et al*. 2006, Hao *et al*. 2012), and wheat (Kane *et al*. 2013). However, there is scant research on the response of so-called 'minor crops' to EC, although many of these have been staple foods across wide areas in prehistory to the present day and have inherently wide stress tolerances (Hunt *et al*. 2011). The extent of any direct or indirect stimulation of photosynthesis, growth, and yield of broomcorn millet by EC could have major economic and social implications in those relatively poor areas, and it could also reveal if minor crops, which have not been the subject of intensive breeding, may respond differently to EC in comparison to major crops and may provide some hints how they may respond to the change.

The present research reported the effect of EC on leaf physiology, chlorophyll (Chl) fluorescence, growth, and yield in the C_4 plant broomcorn millet. We aimed to address the following questions: (*1*) Is the leaf physiology, chlorophyll fluorescence, and yield of broomcorn millet altered under EC and is any correlation between them? (*2*) Does EC decrease g_s as in other plants and what is its implications for the water-use effect in C_4 crop broomcorn millet? By answering these questions, we expected that we could gain understanding of the responses in a major C_4 food crop to EC in region of northern China vulnerable to environmental change.

Materials and methods

Site description: The study was conducted at the open-top chamber (OTC) facility of an experimental station, Shanxi Agricultural University (37.42°N, 112.55°E), Taigu, Shanxi, China. One OTC was used as control chamber, maintaining the same [CO_2] with that of the outside field by external supply of CO_2. In another OTC, EC (outside field [CO_2] + 200 μmol mol^{-1})] was maintained constantly from crop emergence to harvest.

The [CO_2] of the EC-OTC and the control (CK)-OTC were measured by sensors (*Vaisala*, Finland) at the centre of each chamber, and outside field [CO_2] was also measured. When [CO_2] of CK-OTC was lower than

outside field [CO_2] or [CO_2] of EC-OTC was lower than outside field [CO_2] + 200 μmol mol^{-1}, the solenoid valves of the corresponding chamber opened and CO_2 was introduced into the chamber by fan in order to keep the target [CO_2] in the chamber at the required concentration. The temperature and relative humidity of two chambers were measured throughout the experimental period. The circular structure of OTC was fixed in the field and fabricated using an aluminum frame. The diameter and height of each OTC were 4.0 and 2.5 m. The frame was lined with polyvinyl chloride (PVC) sheets (0.12 mm thickness), which transmitted 80–90% of natural sun light. The upper part of the OTCs had a frustum with 0.5 m height, 2.0 m and 4.0 m upper diameter and bottom diameter, respectively, in order to reduce the dilution of CO_2 by air current inside the chambers and was kept open to maintain the near-natural conditions of temperature and relative humidity.The average temperature and relative humidity of the CK-OTC during the broomcorn millet growing season were 23.8 and 22.6°C, and 67.6 and 66.5% in 2013 and 2014, respectively, and they were 23.0 and 22.1°C, and 70.4 and 71.2% for EC-OTC in 2013 and 2014, respectively (Fig. 1).

Broomcorn millet cultivation, fertilization and irrigation: A landrace of broomcorn millet (*Panicum miliaceum* L.), Huachi Ruan Red, from the Huachi County, Gansu Province, was sown on 13 June 2013 and 16 June 2014 in 40 × 60 cm pots (28 cm depth). Ten plants were grown in each pot and ten pots were included in every air chamber. For sowing, surface soil (0–20 cm) was obtained from nearby cropland, sieved and blended, packed in the pot used for planting seeds. The soil was a clay loam with pH (1:5, soil:water) of 8.5 and contained 1.37% of organic carbon (C) and 0.12% of total N. Fertilizers were applied at the elongation stage at the rates of 11.04 g(N) per pot and 12.24 g(P) per pot. Irrigation equivalent to 10–20 mm of rainfall was applied every 3–5 d after seedling emergence.

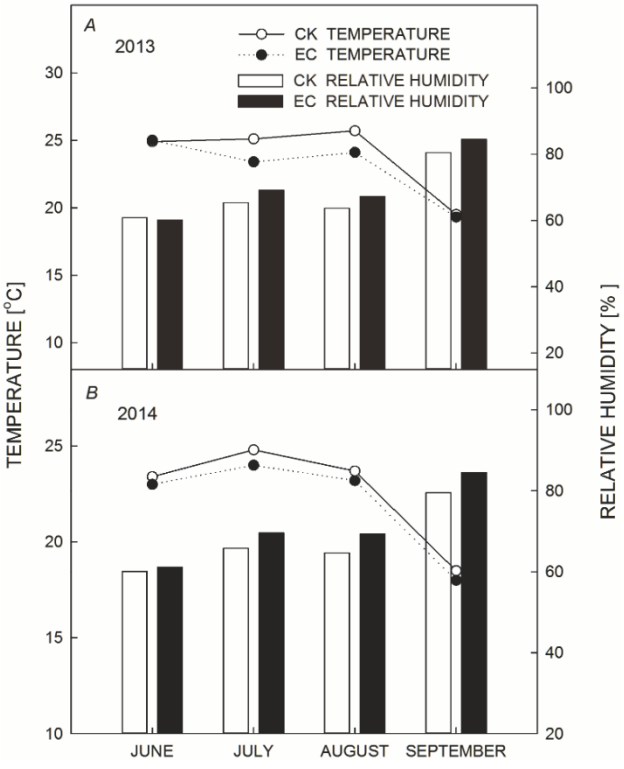

Fig 1. The mean monthly temperature and relative humidity of the OTC during the growing period in 2013 and 2014. CK – control; EC – elevated atmospheric CO_2 concentration.

Gas-exchange measurements: Measurements of net photosynthetic rate (P_N) were conducted at just before the heading stage (54 d after sowing in 2013 and 52 d after sowing in 2014) and grain-filling stage (81 d after sowing in 2013 and 83 d after sowing in 2014). One of the fully-expanded flag leaves was randomly selected. Gas-exchange measurements were conducted using a portable gas-exchange system (*LI-COR 6400*, *LI-COR*, Lincoln, USA). The [CO_2] in the leaf chamber was controlled by the *LI-COR* CO_2 injection system, and an irradiance of 1,400 μmol(photon) m^{-2} s^{-1} was supplied using a built-in LED lamp (red/blue). Temperature in the 2 × 3 × 2.5 cm^3 leaf chamber was set at 28°C, and the actual temperature ranged from 28 to 30°C. The vapour pressure deficit (VPD) on the leaf surface was between 1.9 and 2.1 kPa. P_N, E, g_s, intercellular CO_2 concentration (C_i), and intrinsic water-use efficiency ($WUE_i = P_N/g_s$) were measured at the same irradiance, temperature, and vapour pressure. [CO_2] in the leaf chamber was set to 390 μmol mol^{-1} for current [CO_2] treatment and 590 μmol mol^{-1} for EC treatment, and one fully-expanded flag leaf was measured per pot. Measurements were made between 09:00 and 11:30 h local time.

P_N/C_i (ambient CO_2 concentration) and g_s/C_i curves were also measured at the same irradiance, temperature, and the vapour pressure when measurements of P_N were conducted after 55 d from sowing in 2013. The [CO_2] surrounding the leaf for all control and treated leaves was controlled across the series of 400, 300, 200, 100, 0, 400, 550, 600, 700, 800, 1,000; and 400 μmol mol^{-1}, and measurements were recorded after equilibrium was reached. Measurements were made between 09:00 and 14:00 h local time. Five fully-expanded flag leaves were measured in each CK-OTC and EC-OTC. Each individual curve took approximately 35 min to complete.

Chl fluorescence: The photosynthetic performance of fully-expanded flag leaves was assessed in terms of the Chl *a* fluorescence parameters, such as maximal quantum yield of PSII photochemistry (F_v/F_m), intrinsic efficiency of PSII (F_v'/F_m'), effective quantum yield of PSII photochemistry (Φ_{PSII}), photochemical quenching coefficient (q_P), and nonphotochemical quenching (NPQ) using a miniaturized pulse-amplitude modulated fluorescence analyzer (*Mini-PAM*, *Walz*, Effeltrich, Germany) with a leaf clip holder as

described by Bilger *et al*. (1995). The minimal fluorescence yield of the light-adapted state (F_0') and maximal fluorescence yield of the light-adapted state (F_m') was measured at incident PPFD between 08:30 and 12:00 h, and one uppermost fully-expanded leaf was measured per pot on the same day together with the gas-exchange measurements. Ten fully-expanded flag leaves were measured per chamber (one plant per pot). Minimal fluorescence yield of the dark-adapted state (F_0) and maximal fluorescence yield of the dark-adapted state (F_m) of dark-adapted leaves were measured between 23:00 and 01:00 h on the same day. The high light flash used to measure saturated fluorescence had a PPFD of 4,000 μmol(photon) m^{-2} s^{-1} and a duration of 800 ms. All Chl fluorescence parameters were calculated as described by Rascher *et al*. (2004).

Harvesting: At maturity, broomcorn millet plants were hand-harvested on 18 September 2013 (95 d after sowing) and 23 September 2014 (96 d after sowing). After drying, random subsamples of five plants from each pot were assessed for height, node number, panicle length, and stem diameter. Then all plants were separated into leaves, stems, panicles, and seeds, air dried and weighed. The number of seeds per plant and thousand-seed mass was also assessed.

Statistical analysis: All experimental data presented were analyzed with variance at 0.05 probability level using *SAS System 8.1*.

Results

Gas-exchange parameters: When photosynthesis was measured at different developmental stages, P_N of flag leaf increased by 33.2 and 80.3% under EC just before heading and grain-filling stage in 2013, and by 46.7 and 63.7% in 2014, respectively. EC increased g_s by 11.1 and 24.2% just before the heading stage and grain-filling stage in 2013, and by 25.0 and 50.0% in 2014, respectively. C_i increased by 96.8, 13.1, 93.8, and 91.1% just before the heading and grain-filling stage in 2013 and 2014, respectively. E increased by an average of 12.0 and 21.2% in 2013 and 2014, respectively. EC increased WUE_i of broomcorn millets by an average of 42.2 and 18.5% in 2013 and 2014, respectively (Table 1).

Chl fluorescence: F_v/F_m decreased by an average of 3.2% under EC in 2013, but was not affected in 2014. EC significantly decreased F_v'/F_m' by an average of 9.3% in 2013, but increased it by an average of 5.2% in 2014. Φ_{PSII} increased by an average of 51.3 and 28.3% under EC in 2013 and 2014, respectively. EC increased q_P by an average of 67.5 and 12.3% in 2013 and 2014, respectively. Leaf NPQ decreased by an average of 11.3 and 19.5% under EC in 2013 and 2014, respectively (Table 2).

***P_N/C_i* and *g_s/C_i* curves**: The P_N/C_i curves (Fig. 2*A*) showed P_N increasing with the C_i. There was no significant change in growth between the CK and EC. g_s decreased with C_i from 0 to 310 μmol mol^{-1}, but the decrease was small when the C_i was about 200–250 μmol mol^{-1} for broomcorn millet grown in EC (Fig. 2*B*).

Plant morphological parameters: Height of broomcorn millet plants increased by 5.4 and 5.7% under EC in 2013 and 2014, respectively. Node number was elevated by 11.1% in 2014, but there was no significant change in 2013. EC did not affect panicle length in either year. Stem diameter increased by 20.4 and 26.7% under EC in 2013 and 2014, respectively (Table 3).

Table 1. Effects of elevated [CO_2] on gas-exchange parameters in the fully-expanded flag leaf of broomcorn millets. Measurement was taken at their respective [CO_2]. Values are means ± standard error of variables across ten replicates. The statistical significance level for the effects of [CO_2] treatment was tested. C_i – intercellular CO_2 concentration; E – transpiration rate; EC – elevated atmospheric CO_2 concentration; g_s – stomatal conductance; P_N – net photosynthetic rate; WUE_i – intrinsic water-use efficiency (= P_N/g_s).

Year	Growth period	Growth [CO_2]	P_N [μmol m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [μmol(CO_2) mol^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	WUE_i [mol(CO_2) mol(H_2O)$^{-1}$]
2013	Just before heading	CK	13.66 ± 0.54	0.09 ± 0.01	112.21 ± 3.50	1.74 ± 0.07	159.71 ± 2.45
		EC	18.19 ± 0.71	0.11 ± 0.01	220.80 ± 8.52	1.91 ± 0.10	178.69 ± 5.65
	Grain-filling	CK	10.04 ± 0.69	0.07 ± 0.00	168.00 ± 4.52	2.01 ± 0.05	113.15 ± 13.29
		EC	18.10 ± 0.30	0.09 ± 0.00	190.00 ± 20.87	2.29 ± 0.03	209.21 ± 2.79
		p_{CO2}	0.00	0.01	0.00	0.02	0.00
2014	Just before heading	CK	19.01 ± 0.99	0.12 ± 0.01	110.57 ± 4.51	2.26 ± 0.13	154.01 ± 2.31
		EC	27.89 ± 1.08	0.15 ± 0.01	214.30 ± 11.87	2.44 ± 0.15	194.85 ± 7.09
	Grain-filling	CK	12.78 ± 0.26	0.08 ± 0.00	114.75 ± 4.06	1.75 ± 0.03	159.02 ± 2.73
		EC	20.93 ± 0.61	0.12 ± 0.00	219.25 ± 6.30	2.42 ± 0.06	176.02 ± 4.10
		p_{CO2}	0.00	0.03	0.00	0.04	0.04

Table 2. Effects of elevated [CO_2] on chlorophyll fluorescence parameters in the fully-expanded flag leaf of broomcorn millets. Measurement was taken at their respective [CO_2]. Values are means ± standard error of variables across ten replicates. The statistical significance level for the effects of [CO_2] treatment was tested. CK – control; EC – elevated atmospheric CO_2 concentration; F_v/F_m – maximal quantum yield of PSII photochemistry; F_v'/F_m' – intrinsic efficiency of PSII; NPQ – nonphotochemical quenching; q_P – photochemical quenching coefficient; Φ_{PSII} – effective quantum yield of PSII photochemistry.

Year	Growth period	Growth [CO_2]	F_v/F_m	F_v'/F_m'	Φ_{PSII}	q_P	NPQ
2013	Just before heading	CK	0.80 ± 0.01	0.49 ± 0.02	0.20 ± 0.01	0.42 ± 0.04	1.53 ± 0.04
		EC	0.78 ± 0.00	0.50 ± 0.01	0.31 ± 0.02	0.63 ± 0.03	1.33 ± 0.08
	Grain-filling	CK	0.76 ± 0.01	0.48 ± 0.02	0.19 ± 0.01	0.41 ± 0.02	1.30 ± 0.08
		EC	0.73 ± 0.01	0.38 ± 0.03	0.28 ± 0.02	0.76 ± 0.07	1.18 ± 0.07
		p_{CO2}	0.00	0.03	0.00	0.00	0.01
2014	Just before heading	CK	0.79 ± 0.00	0.41 ± 0.03	0.29 ± 0.04	0.72 ± 0.08	2.58 ± 0.23
		EC	0.80 ± 0.00	0.49 ± 0.01	0.41 ± 0.02	0.83 ± 0.04	1.78 ± 0.17
	Grain-filling	CK	0.77 ± 0.01	0.38 ± 0.03	0.23 ± 0.03	0.59 ± 0.04	1.86 ± 0.29
		EC	0.78 ± 0.00	0.40 ± 0.01	0.26 ± 0.01	0.64 ± 0.02	1.79 ± 0.15
		p_{CO2}	0.08	0.02	0.01	0.07	0.01

Biomass and yield: EC significantly enhanced the mass of leaves, stem, panicle, and aboveground biomass per m^2 by 29.9, 41.6, 36.3, and 37.3% in 2013, and by 56.0, 35.9, 12.9 and 23.8% in 2014, respectively (Table 4). Thousand-seed mass was not changed. The number of grains per plant increased by 31.8 and 12.0% in 2013 and 2014, respectively. EC significantly increased the mass of seeds per m^2 by 34.2 and 13.6% in 2013 and 2014, respectively (Table 4, Fig. 3).

Discussion

EC stimulates higher photosynthesis more in C_3 plants than in C_4 plants. P_N of broomcorn millets increased under EC in our experiment (Table 1). Several mechanisms have been suggested in order to explain stimulated C_4 photosynthesis of well-watered plants by EC under some controlled environment (Leakey *et al*. 2006). For example, 10% of CO_2 fixation occurred directly in the bundle sheath in the developing leaves of *Flaveria trinervia*, without

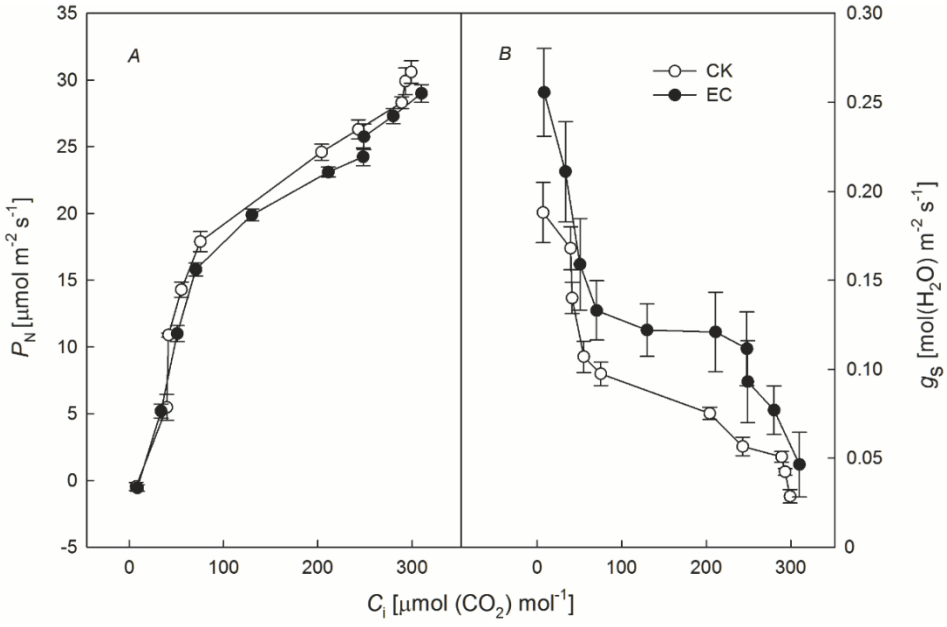

Fig. 2. P_N/C_i and g_s/C_i curves of flag leaf of broomcorn millet growing under control (CK) and elevated CO_2 (EC). Measurements were made between 09:00 and 14:00 h of local time. An irradiance of 1,400 µmol(photon) m^{-2} s^{-1} was supplied using a built-in LED lamp (red/blue). Temperature in the leaf chamber was set at 28°C. The vapour pressure deficit (VPD) on the leaf surface was between 1.9 and 2.1 kPa. Five fully-expanded flag leaves were measured in each CK-OTC and EC-OTC. Each individual curve took approximately 35 min to complete. Each bar represents the standard error of the difference between treatments. Values are means of the five replicates. CK – control; EC – elevated atmospheric CO_2 concentration.

Table 3. Effects of elevated [CO_2] on morphological parameters of broomcorn millets. Values are means ± standard error of variables across ten replicates; *, ** – the significant differences at 0.05 and 0.01 levels, respectively. CK – control; EC – elevated atmospheric CO_2 concentration.

Year	Growth [CO_2]	Height [cm]	Node number	Panicle length [cm]	Stem diameter [cm]
2013	CK	112.66 ± 2.63	7.03 ± 0.11	25.36 ± 0.68	0.54 ± 0.01
	EC	118.79 ± 1.99**	6.97 ± 0.18	27.97 ± 0.76	0.65 ± 0.01**
2014	CK	136.58 ± 3.06	6.92 ± 0.14	26.42 ± 0.97	0.45 ± 0.02
	EC	144.35 ± 2.22*	7.69 ± 0.10**	26.69 ± 0.76	0.57 ± 0.02**

Table 4. Effects of elevated [CO_2] on mass and yield components of broomcorn millets. Values are means ± standard error of variables across ten replicates. *, ** – the significant differences at 0.05 and 0.01 levels, respectively. CK – control; EC – elevated atmospheric CO_2 concentration.

Year	Growth [CO_2]	Leaf mass [g m^{-2}]	Stem mass [g m^{-2}]	Panicle mass [g m^{-2}]	Thousand-seed mass [g]	Number of grain per plant
2013	CK	72.5 ± 4.6	220.4 ± 21.7	420.8 ± 9.2	7.1 ± 0.1	1219.8 ± 49.6
	EC	94.2 ± 1.3**	312.1 ± 22.1*	573.7 ± 35.8**	7.3 ± 0.1	1607.9 ± 87.4*
2014	CK	48.3 ± 2.9	176.3 ± 24.2	332.9 ± 9.2	7.7 ± 0.5	801.6 ± 39.4
	EC	75.4 ± 6.3**	239.6 ± 13.3*	375.8 ± 10.8*	7.7 ± 0.5	898.0 ± 51.9*

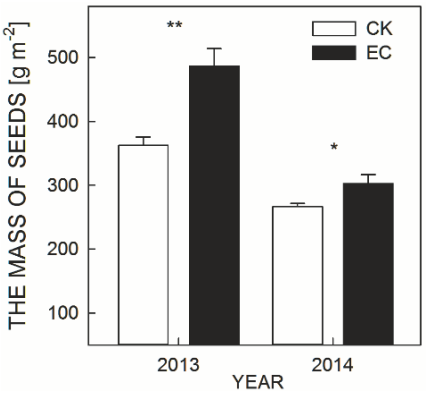

Fig 3. Effects of elevated [CO_2] on the yield of broomcorn millets. Each bar represents the standard error of the difference between treatments, and is based on ten replicates. *, ** – the significant differences at 0.05 and 0.01 levels, respectively. CK – control; EC – elevated atmospheric CO_2 concentration.

the involvement of CO_2-concentrating mechanism in C_4 plant (Moore *et al*. 1986). But it is quite unlikely that this could the case in the fully expanded broomcorn millet leaves. EC can increase bundle sheath leakiness; hence, the initial slope or CO_2-saturated photosynthetic rate of the P_N/C_i curve was reduced in C_4 crop *Sorghum bicolor* (Watling *et al*. 2000). We found, however, that the initial slope was not significantly changed under EC (Fig. 2*A*). Some young C_4 leaves have been shown to be more sensitive to enhanced photosynthesis under EC, and display of C_3-like photosynthesis (Ziska *et al*. 1999, Cousins *et al*. 2001). P_N in the flag leaf of broomcorn millet increased at both the heading stage and grain-filling stage (Table 1). The flag leaf was more than 20-d old at the grain-filling stage. It seems that the mechanisms could not fully explain the increased photosynthesis for the C_4 broomcorn millets under EC. C_i was below the saturation point of the (P_N/C_i) response curve under ambient [CO_2], allowing direct stimulation of photosynthesis under EC (Watling and Press 1997, Ziska and Bunce 1997). The photosynthesis of some C_4 plant increased under EC, which might occur because they are not saturated under ambient [CO_2] (Wand *et al*. 1999). The P_N/C_i curves showed that P_N was elevated with the C_i (Fig. 2*A*), indicating that broomcorn millet was not saturated at ambient [CO_2] and hence EC led to the increase in photosynthesis. The g_s/C_i showed that g_s at EC was higher than that at CK, especially, when C_i was about 200–250 μmol mol^{-1} (Fig. 2*B*). High C_i under EC due to high g_s could be the important reason for the increase of photosynthesis under EC (Table 1).

EC decreased F_v/F_m and F_v'/F_m' of broomcorn millets in 2013, but it was not observed in 2014. This means that photosynthetic capacities of broomcorn millets leaves might have been lower in 2013, the change of P_N was in accordance with it. This might be due to influence of the different environmental factors, such as average temperature, relative humidity, and solar radiance in 2013 and 2014 (Fig 1). Other environmental factors could affect the response of plants to EC (Biswas *et al*. 2013, Wang *et al*. 2014). EC increased Φ_{PSII} and q_P, but decreased NPQ of broomcorn millet. Decreased NPQ indicates that broomcorn millet dissipated more energy by means of linear electron transport rather than as thermal energy. The change of Φ_{PSII}, q_P, and NPQ was consistent with the increase of P_N. The changes in all these photosynthetic parameters were different from those in maize under FACE, which showed that EC did not cause significant changes in all of P_N, Φ_{PSII}, F_v'/F_m', q_P, and NPQ (Leakey *et*

al. 2006). F_v'/F_m', Φ_{PSII}, and q_P significantly decreased in the C_3 plant mung bean (Gao *et al*. 2015). Our results are partially in agreement with other C_3 plant studies, which reported increases in P_N, Φ_{PSII}, and q_P, but decreases in NPQ in wheat (Tausz-Posch *et al*. 2013) and *Isatis indigotica* Fort (Hao *et al*. 2013). It seems that photosynthetic characteristics in response to high CO_2 vary with plant species, regardless of whether they are C_3 or C_4 plants.

The activity of outward rectifying K^+ channels increases and the activity of inward rectifying K^+ channels decreases under EC, which enhances S-type anion channel activities, stimulates Cl^- release from guard cells, and the Ca^{2+} concentration of guard cell increases (Hanstein and Felle 2002, Raschke *et al*. 2003, Ainsworth and Rogers 2007). These changes collectively depolarize the membrane potential of guard cells and cause stomatal closure under EC (Ainsworth and Rogers 2007). Most studies show a consistent and similar decrease in g_s regardless of C_3 and C_4 species under EC (Ainsworth and Long 2005, Leakey *et al*. 2006, Ainsworth and Rogers 2007, Vu and Allen Jr. 2009, Gao *et al*. 2015, Yu *et al*. 2015). In contrast, g_s in trees was not significantly decreased, particularly in woody coniferous trees (Saxe *et al*. 1998). Guard cells in *Pinus taeda* appear to be insensitive to EC (Ellsworth 1999). However, the results of our studies showed that g_s of broomcorn millets increased in response to EC. The g_s/C_i showed that the decrease of g_s in broomcorn millets grown at EC with the increase [CO_2] was lesser than that grown at CK, when the C_i was about 200–250 μmol mol^{-1} (Fig. 2*B*). There are exceptions to the general rule that g_s declines at EC, such as soybean (Bernacchi *et al*. 2006), mung bean (Gao *et al*. 2015), Bermuda grass (Yu *et al*. 2015), maize (Leakey *et al*. 2006). The stomata acclimation at EC might be a reason for the increase of stomatal conductance in broomcorn millet. It remains to be further explored, though it is clear that the increase of stomatal conductance leads to the increase of photosynthesis.

The increase of E was consistent with the change of g_s in broomcorn millet (Table 1). This means that whole-plant water may not be reduced under EC, which is different from findings in other plant species (Ainsworth and Long 2005, Ainsworth and Rogers 2007, Hao *et al*. 2013). Even though WUE_i was enhanced at EC because P_N increased under EC, higher E could mean that water usage by broomcorn millet might become more susceptible to drought stress.

Conclusion: P_N, g_s, C_i, E, WUE_i, q_P, and Φ_{PSII} of flag leaves in broomcorn millets increased under EC. EC significantly enhanced the aboveground biomass (including the mass of leaves, stems, and panicles per area) and yield of broomcorn millet with more photoassimilates produced by high P_N. The increased yield of broomcorn millets under EC was due to the enhancement of grain number per plant. We conclude that photosynthesis of broomcorn millets improved with increased stomatal conductance in leaves under EC, leading to the increase in growth and yield.

References

Ainsworth E.A., Long S.P.: What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. – New Phytol. **165**: 351-372, 2005.

Ainsworth E.A., Rogers A.: The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. – Plant Cell Environ. **30**: 258-270, 2007.

Bernacchi C.J., Leakey A.D.B., Heady L.E. *et al*.: Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO_2 and ozone concentrations for 3 years under fully open-air field conditions. – Plant Cell Environ. **29**: 2077-2090, 2006.

Bilger W., Schreiber U., Bock M.: Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. – Oecologia **102**: 425-432, 1995.

Biswas D.K., Xu H., Li Y.G. *et al*.: Modification of photosynthesis and growth responses to elevated CO_2 by ozone in two cultivars of winter wheat with different years of release. – J. Exp. Bot. **64**: 1485-1496, 2013.

Conley M.M., Kimball B.A., Brooks T.J. *et al*.: CO_2 enrichment increases water-use efficiency in sorghum. – New Phytol. **151**: 407-412, 2001.

Cousins A.B., Adam N.R., Wall G.W. *et al*.: Reduced photorespiration and increased energy-use efficiency in young CO_2-enriched sorghum leaves. – New Phytol. **150**: 275-284, 2001.

Dai H.P., Zhang P.P., Lu C. *et al*.: Leaf senescence and reactive oxygen species metabolism of broomcorn millet (*Panicum miliaceum* L.) under drought condition. – Aust. J. Crop Sci. **5**: 1655-1660, 2011.

Duarte B., Santos D., Silva H. *et al*.: Photochemical and biophysical feedbacks of C_3 and C_4 Mediterranean halophytes to atmospheric CO_2 enrichment confirmed by their stable isotope signatures. – Plant Physiol. Bioch. **80**: 10-22, 2014.

Ellsworth D.S.: CO_2 enrichment in a maturing pine forest: are CO_2 exchange and water status in the canopy affected? – Plant Cell Environ. **22**: 461-472, 1999.

Gao J., Han X., Seneweera S. *et al*.: Leaf photosynthesis and yield components of mung bean under fully open-air elevated [CO_2]. – J. Integr. Agr. **14**: 977-983, 2015.

Ghannoum O., Evans J.R., von Caemmerer S.: Nitrogen and water use efficiency of C_4 plants. – In: Raghavendra A.S., Sage R.F. (ed.): C_4 Photosynthesis and Related CO_2 Concentrating Mechanisms. Pp. 129-146. Springer, Dordrecht 2011.

Hanstein S.M., Felle H.H.: CO_2-triggered chloride release from guard cells in intact fava bean leaves. Kinetics of the onset of stomatal closure. – Plant Physiol. **130**: 940-950, 2002.

Hao X.Y., Han X., Lam S.K. *et al*.: Effects of fully open-air [CO_2] elevation on leaf ultrastructure, photosynthesis, and yield of two soybean cultivars. – Photosynthetica **50**: 362-370, 2012.

Hao X.Y., Li P., Feng Y.X. *et al*.: Effects of fully open-air CO_2

elevation on leaf photosynthesis and ultrastructure of *Isatis indigotica* Fort. – PLoS ONE **8**: e74600, 2013.

Hao X.Y., Li P., Lin E.D. *et al*.: [Effects of air CO_2 enrichment on growth and photosynthetic physiology of millet.] – J. Nucl. Agr. Sci. **24**: 589-593, 2010. [In Chinese]

Hunt H.V., Campana M.G., Lawes M.C. *et al*.: Genetic diversity and phylogeography of broomcorn millet (*Panicum miliaceum* L.) across Eurasia. – Mol. Ecol. **22**: 4756-4771, 2011.

IPCC: Summary for policymakers. – In: Stocker T.F., Qin D.H., Plattner G.K. *et al*. (ed.): Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Pp. 29. Cambridge Univ. Press, Cambridge – New York 2013.

Kane K., Dahal K.P., Badawi M.A. *et al*.: Long-term growth under elevated CO_2 suppresses biotic stress genes in non-acclimated, but not cold-acclimated winter wheat. – Plant Cell Physiol. **54**: 1751-1768, 2013.

Leakey A.D.B., Uribelarrea M., Ainsworth E.A. *et al*.: Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. – Plant Physiol. **140**: 779-790, 2006.

Liu Z., Sun N., Yang S.J. *et al*.: Evolutionary transition from C_3 to C_4 photosynthesis and the route to C_4 rice. – Biologia **68**: 577-586, 2013.

Moore B.D., Cheng S.H., Edwards G.E.: The influence of leaf development on the expression of C_4 metabolism in *Flaveria trinervia*, a C_4 dicot. – Plant Cell Physiol. **27**: 1159-1167, 1986.

Rascher U., Bobich E.G., Lin G.H. *et al*.: Functional diversity of photosynthesis during drought in a model tropical rainforest – the contributions of leaf area, photosynthetic electron transport and stomatal conductance to reduction in net ecosystem carbon exchange. – Plant Cell Environ. **27**: 1239-1256, 2004.

Raschke K., Shabahang M., Wolf R.: The slow and the quick anion conductance in whole guard cells: their voltage dependent alternation, and the modulation of their activities by abscisic acid and CO_2. – Planta **217**: 639-650, 2003.

Saxe H., Ellsworth D.S, Heath J.: Tree and forest functioning in an enriched CO_2 atmosphere. – New Phytol. **139**: 395-436, 1998.

Seneweera S.P., Conroy J.P., Ishimaru K. *et al*.: Changes in source-sink relations during development influence photosynthetic acclimation of rice to free air CO_2 enrichment (FACE). – Funct. Plant Biol. **29**: 945-953, 2002.

Tausz-Posch S., Borowiak K., Dempsey R.W., Norton R.M.: The effect of elevated CO_2 on photochemistry and antioxidative defense capacity in wheat depends on environmental growing conditions – A FACE study. – Environ. Exp. Bot. **88**: 81-92, 2013.

Thomson A.M., Brown R.A., Rosenberg N.J. *et al*.: Climate change impacts for the conterminous USA: an integrated assessment. Part 3. Dryland production of grain and forage crops. – Climatic Change **69**: 43-65. 2005.

Underhill A.P.: Current issues in Chinese Neolithic archaeology. – J. World Prehist. **11**: 103-160, 1997.

Vu, J.C.V., Allen Jr. L.H.: Growth at elevated CO_2 delays the adverse effects of drought stress on leaf photosynthesis of the C_4 sugarcane. – J. Plant Physiol. **166**: 107-116, 2009.

Wall G.W., Brooks T.J., Adam N.R. *et al*.: Elevated atmospheric CO_2 improved *Sorghum* plant water status by ameliorating the adverse effects of drought. – New Phytol. **152**: 231-248, 2001.

Wand S.J.E., Midgley G.F., Jones M.H., Curtis P.S.: Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. – Glob. Change Biol. **5**: 723-741, 1999.

Wang D., Fan J.Z., Heckathorn S.A.: Acclimation of photosynthetic tolerance to acute heat stress at elevated CO_2 and N. – Plant Sci. **226**: 162-171, 2014.

Watling J.R., Press M.C.: How is the relationship between the C_4 cereal *Sorghum bicolor* and the C_3 root hemi-parasites *Striga hermonthica* and *Striga asiatica* affected by elevated CO_2? – Plant Cell Environ. **20**: 1292-1300, 1997.

Watling J.R., Press M.C., Quick W.P.: Elevated CO_2 induces biochemical and ultrastructural changes in leaves of the C_4 cereal sorghum. – Plant Physiol. **123**: 1143-1152, 2000.

Yu J.J., Sun L.H., Fan N.L. *et al*.: Physiological factors involved in positive effects of elevated carbon dioxide concentration on Bermudagrass tolerance to salinity stress. – Environ. Exp. Bot. **115**: 20-27, 2015.

Ziska L.H., Bunce J.A.: Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C_4 crops and weeds. – Photosynth Res. **54**: 199-208, 1997.

Ziska L.H., Sicher R.C., Bunce J.A.: The impact of elevated carbon dioxide on the growth and gas exchange of three C_4 species differing in CO_2 leak rates. – Physiol. Plantarum **105**: 74-80, 1999.