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Full Length Article

Differential Response of Two Biotypes of Goosegrass (*Eleusine indica*) with Different Sensitivities to Glyphosate to Elevated CO_2 Concentrations

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Abstract

To reveal effects of elevated atmospheric CO_2 on tolerance to the herbicide glyphosate in the populations of goosegrass (*Eleusine indica*) known to have resistance to this herbicide, two biotypes of goosegrass, a resistant (R) and a susceptible (S) biotype, were analyzed after exposed to ambient (400 μmol mol^{-1}) and elevated CO_2 (800 μmol mol^{-1}) for 25 d. The results showed that elevated CO_2 had little effect on growth of both biotypes. At elevated CO_2, shoot biomass of R biotype was statistically lower than of S biotype (P<0.5). The dose-response analysis showed that glyphosate tolerance was reduced by 60% in R biotype exposed to elevated CO_2 while it was slightly increased in S biotype. Elevated CO_2 also had more adverse effects on photosynthetic capacity of R biotype than S biotype. As a result, maximum rate of net photosynthesis (Amax) and carboxylation efficiency (CE) under elevated CO_2 in R biotype were significantly lower than in S biotype (P<0.5). These results indicate that elevated atmospheric CO_2 can lead to a compromise in glyphosate tolerance of R biotype associated with poor photosynthetic characteristics, and thus will affect weed management involved with herbicide resistance in prospective agricultural systems.

Keywords: *Eleusine indica*; Goosegrass; Carbon dioxide; Glyphosate resistance; Photosynthesis

Introduction

The ongoing increase in atmospheric CO_2 concentrations caused by anthropogenic activities are the major factor responsible for global warming, contributing to the melting of polar ice cap, the raising of sea levels and other environmental changings, ultimately affect plants and agricultural systems. For photosynthesis, elevated CO_2 can stimulate plant growth by increasing assimilation rates and reducing photorespiration rates (Wang *et al.*, 2012). In agricultural systems, elevated CO_2 affects crop yields through enhancing the growth of crops along with the weeds. It has been reported that elevated CO_2 can change weed-crop competition patterns (Ziska, 2000; Ziska, 2010), and reduce efficacy of chemical treatments for control of some weeds (Ziska and Teasdale, 2000; Manea *et al.*, 2011). Therefore, understanding the influences of elevated CO_2 on growth and herbicide tolerance of weed species is important for weed management in prospective farming systems.

Evolved weed resistance to herbicides has become a troublesome and escalating problem in many farming systems all over the world (Neve, 2007). With herbicide applied more extensively in agriculture worldwide, the herbicide resistance issue will become even worse in the future. Plants having advantages of resistance are often considered to be offset by a cost, such as fitness reduction (Roux *et al.*, 2004; Paris *et al.*, 2008). Bergelson and Purrington (1996) have made a comparison among resistances to herbicides, pathogens and herbivores, revealing that resistance to herbicides are most likely to entail costs. Currently, glyphosate [*N*-(phosphonomethyl) glycine] is the world's most important herbicide being used to control most annual and perennial weeds (Duke and Powles, 2008). Goosegrass (*Eleusine indica*) listed among the five world's worse weeds (Baerson *et al.*, 2002) also is one of the ten most important herbicide-resistant species (Nandula *et al.*, 2005). To date, goosegrass populations have evolved resistance to many kinds of herbicides, including acetolactate synthase inhibitors (Valverde *et al*., 1993), bipyridiliums (Buker *et al*., 2002), dinitroanilines (Vaughn *et al*., 1990), and glyphosate (Lee and Ngim, 2000; Yang *et al*., 2012).

The glyphosate-resistant (R) biotype of goosegrass from China was firstly reported by Yang *et al.*(2012). In the context of global change, we presumed that elevated CO_2 levels can adversely affect growth of the R biotype due to an assumed cost for the resistance, and then affect its tolerance to glyphosate in turn. To test this hypothesis, a CO_2

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enrichment experiment was conducted for the R biotype compared with a biotype susceptible to the herbicide.

Materials and Methods

Plant Materials and Growth Conditions

The resistance (R) and susceptible (S) biotypes of goosegrass were collected from the locations of 113°26′3″ E, 22°47′52″ N and 114°25′7″ E, 23°28′46″ N in south China, respectively. They had been identified by Yang *et al.* (2012) and the resistance level of the R biotype to glyphosate was 11-fold greater than of S biotype. Plant materials of R and S biotypes were prepared and cultured synchronously as follows. Both biotypes were sown in field soil under greenhouse conditions. Seedlings at the two-leaf growing stage were transplanted into plastic pots (10 cm in diameter, 5 cm in height), three plants per pot. Ninety pots of plants were prepared for each biotype. Cultured medium was field soil (containing 17 g kg^{-1} organic matter, 90 mg kg^{-1} hydrolyzable N, 70 mg kg^{-1} available P, and 300 mg kg^{-1} available K) collected from cultivated land. Prior to use as a culture medium, the soil was air dried, and its seed bank was inactivated at 180°C for 4 h.

Five days after transplantation, both R and S potted plants were divided into two groups (45 pots of plants for each group) and placed in growth chambers. Two customized, identical growth chambers (inside dimensions: 1.5 m long, 1.2 m wide, and 2 m high), constructed of steel sandwich panels, were used. The light source was white LED arrays fixed to chamber walls. The temperature in growth chambers was controlled by air-conditioning systems. Ambient CO_2 was maintained at 380 to 420 μmol mol^{-1} (average 400 μmol mol^{-1}) in one of growth chambers and was monitored by an infrared CO_2 sensor (Blue Moon Measurement and Control Technology Co., Ltd. Shenzhen, China). In the other growth chamber, elevated CO_2 was supplied by a cylinder and also monitored by an infrared CO_2 sensor. CO_2 in the experimental growth chamber was maintained at ~800 μmol mol^{-1}, which was approximately double of the ambient concentration. If the CO_2 level deviated from this intended experimental level, an automatic CO_2 controller responded accordingly. If the concentration fell below 760 μmol mol^{-1}, CO_2 was supplied at 0.2 L per minute through a solenoid valve, whereas if the concentration surpassed 840 μmol mol^{-1}, a fan fixed onto the back wall near the top of the growth chamber was turned on to discharge the air contained CO_2 exceeding the intended levels. All other conditions in the two growth chambers were kept constant as follows: 200 μmol m^{-2} s^{-1} photosynthetically active radiation, 12 h photoperiods, 26/23°C day/night temperatures, and 70 to 90% humidity. The plants were well-watered during the growth process in the growth chambers. The measurements of photosynthetic characteristics, and dose-response to glyphosate were carried out after the plants were grown for 25 d.

A/Ci Determination

The response of carbon assimilation rate to leaf internal concentration of CO_2 (A/Ci) was determined in the growth chamber using the Li-6400 Portable Photosynthesis System (LI-COR, Inc., USA), with three replicates for each biotype. The determination of A/Ci was conducted using a red-blue light provided by an LED light source. Two hours after the lights were turned on in the growth chambers, thus activating photosynthesis in goosegrass plants, the uppermost fully expanded leaves of the plants were subjected to A/Ci determination. The photosynthetic analysis system was configured in a standard leaf chamber (2 cm by 3 cm) such that no single leaf of the R or S plants was sufficiently wide to completely cover the leaf chamber. Therefore, before the leaves were placed in the leaf chamber, their breadth was determined in order to calculate the actual area covering the leaf chamber. The calculated value for the leaf area was manually entered into the system.

After the leaf was placed inside the chamber, it was subjected to 1,200 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and 400 μmol mol^{-1} CO_2 for 20 min, thus inducing photosynthesis up to a steady state. The A/Ci auto-determination program was then initiated. The preset CO_2 gradients were in order as follows: 400, 300, 200, 150, 100, 50, 0, 400, 400, 400, 600, 800, 1,000, 1,200 and 1,500 μmol mol^{-1}. The minimum and maximum waiting times were 120 and 180 s for each gradient, respectively. When a change in CO_2 of less than 20 μmol mol^{-1} occurred in the sample room, the difference in the CO_2 levels between the sample and reference rooms was automatically matched by the system. The net carbon assimilation rate (A), stomatal conductance (g_s), and leaf internal CO_2 (Ci) were automatically calculated.

Chlorophyll Fluorescence and P700 Redox Determination

Chlorophyll fluorescence and P700 redox states were simultaneously determined by a Dual-PAM-100 (Heinz Walz GmbH, Effeltrich, Germany) according to a description by Gao *et al.* (2011) with some modifications. The measurements were conducted at room temperature and replicated five times. During the process of determination, the Dual-PAM-100 was controlled by the Dual-PAM software. The measuring light was 1 μmol m^{-2} s^{-1} red light. The actinic light was 190 μmol m^{-2} s^{-1} red light in a duration of 5 min. The saturating light pulses were 10,000 μmol m^{-2} s^{-1} red light in a duration of 300 ms. The plants were dark-adapted for 20 min. The upmost leaf of each plant was placed in an aluminum measuring leaf clip in order to determine the minimum fluorescence (Fo) and maximum fluorescence (Fm) of the dark-adapted state. Actinic light

was subsequently turned on to induce steady-state fluorescence (Fs) and maximum fluorescence (Fm') of the light-adapted state. The Dual-PAM software automatically calculated three complementary quantum yields of photosystem II (PSII). The three complementary quantum yields included effective photochemical quantum yield [Y(II)], regulated heat dissipation [Y(NPQ)], and non-regulated heat dissipation [Y(NO)]; the sum of these three values was one [i.e., Y(II) + Y(NQ) + Y(NO) = 1] (Kramer *et al.*, 2004).

The P700 redox states were assessed using the dual wavelength (830/875 nm) unit in the device. As in PSII, there were three complementary quantum yields in photosystem I (PSI), including effective photochemical quantum yield [Y(I)], quantum yield of non-photochemical energy dissipation due to acceptor side limitation [Y(NA)], and quantum yield of non-photochemical energy dissipation due to donor side limitation [Y(ND)]; the sum of these three values was one [i.e., Y(I) + Y(ND) + Y(NA) = 1] (Pfündel *et al.*, 2008). These three quantum yields of PSI were also automatically calculated by the Dual-PAM software. Y(ND) is also defined as the fraction of overall P700 that is oxidized in a given state, which is enhanced by down-regulation of PSII and damage to PSII. Because Y(ND) is inversely correlated with the reduction of the acceptor side in PSI and the over-reduction of the acceptor side in PSI is necessary for PSI photoinhibition, the high Y(ND) values can indicate that PSI is protected from photoinhibition (Huang *et al.*, 2012). Y(NA) is also defined as the fraction of overall P700 that cannot be oxidized by a saturation pulse due to a lack of acceptors. Because electrons can be passed from the over-oxidized P700 reaction center to oxygen to generate destructive reactive oxygen species (ROS) (Sonoike, 1996), Y(NA) can indicate the risk of PSI to be damaged under certain conditions.

Glyphosate Treatments

To examine change in R biotype plants associated with response to glyphosate doses at elevated CO_2 condition, five glyphosate doses, 0 (used as a control), 5, 10, 30, 120 mmol L^{-1} were used to treat the potted plants. Each dose of glyphosate was given to 9 pots of plants of each biotype. The treatments were performed using an automatic spray cabinet (National Engineering Research Center for Information Technology in Agriculture, Beijing, China) equipped with a fan nozzle, with a movement range of 2 m and a spray width of 0.7 m. The spraying pressure was 0.2 M Pa, and 0.36 L liquid was delivered per minute. After the treatments, the plants were placed back in the original growth chambers to allow them continuing to grow for 12 d. Then, shoot fresh weight of 16 plants of each herbicide dose treatment of each biotype was investigated.

Data Analyses

The A/Ci curve was fitted by the rectangular hyperbolic model:

$$A = (CE \times Ci \times Amax) / (CE \times Ci + Amax) - Rd$$

Where A is the net carbon assimilation rate; CE is the carboxylation efficiency, which was determined from the initial slope of the A/Ci response curve (Ci < 200 μmol mol^{-1}); Ci is the leaf internal CO_2 concentration; Amax is the maximum net carbon assimilation rate at saturating CO_2; and Rd is the day respiration.

The dose-response curve of goosegrass plants to glyphosate was fitted by the three-parameter logistic model (Ritz and Streibig, 2005):

$$y = d/[1+(x / ED50)^b]$$

In the logistic model equation, the parameter ED50 is the dose to producing a response of 50%. The parameter b denotes the relative slope around ED50 and the parameter d denotes the upper limit for the response.

The two nonlinear regression models mentioned above were performed using Sigmaplot 13.0 software (SPSS Inc., Chicago, IL, USA). The data was subjected to one-way ANOVA and Duncan' test at a significance level of 0.05 using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

Results

Effects of Elevated CO_2 on Shoot Biomass and Dose-response to Glyphosate

Neither shoot biomass of R or S biotype grown at elevated CO_2 had significant differences than grown at ambient CO_2 for 36 d (Fig. 1a), indicating that elevated CO_2 had little effect on growth of both biotypes. At ambient CO_2, shoot biomass of R biotype was slightly less than S biotype, but the difference was statistically insignificant (Fig. 1a). However, at elevated CO_2, the difference between R and S biotypes was significant (P<0.5). The analysis of dose-response to glyphosate showed that elevated CO_2 did not distinctly alter the response curve of S biotype to different glyphosate doses, but made the R biotype more susceptible to herbicide (Fig. 1b). The values of ED50 further showed that elevated CO_2 caused a 11% increase in glyphosate tolerance in S biotype, but reduced the resistant level in R biotype by 60% (Table 1).

Effects of Elevated CO_2 on Photosynthetic Characteristics

Under the ambient CO_2, the A/Ci curves of the R and S biotypes were nearly identical, even though the carboxylation efficiency (CE) of the R biotype was 19% less than of the S biotype (Fig. 2a and Table 2). No statistically significant difference was observed in CO_2 compensation point (CCP), day respiration (Rd), and maximum net photosynthetic rate (Amax) between the two biotypes at ambient CO_2 (Table 2), indicating that the two biotypes had the same capacity of carbon assimilation. Elevated CO_2 caused a significant decrease in Amax (P<0.5) in the R

Table 1: Nonlinear regression for dose-response to glyphosate of R and S biotypes grown at ambient and elevated CO_2 (using the logistic model)

	Nonlinear Regression	R^2	ED50
Ambient CO_2			
R biotype	$y = 100.06 / (1+(x / 447.75)^{0.62})$	0.9961	447.75
S biotype	$y = 100.36 / (1+(x / 6.27)^{0.61})$	0.9786	6.27
Elevated CO_2			
R biotype	$y = 101.73 / (1+(x / 117.36)^{0.61})$	0.9107	177.36
S biotype	$y = 100.32 / (1+(x / 6.93)^{0.64})$	0.9863	6.93

Table 2: Photosynthetic characteristics of R and S biotypes 25 d after exposed to ambient and elevated CO_2

Indexes	Ambient CO_2		Elevated CO_2	
	R biotype	S biotype	R biotype	S biotype
CE (μmol CO_2 m^{-2} s^{-1})	0.44 ± 0.01ab	0.54 ± 0.06ab	0.41 ± 0.04b	0.56 ± 0.01a
Rd (μmol CO_2 m^{-2} s^{-1})	2.49 ± 0.16ab	2.77 ± 0.14a	1.77 ± 0.17b	2.32 ± 0.24ab
CCP (μmol mol^{-1} air)	5.64 ± 0.28a	5.29 ± 0.33ab	4.6 ± 0.25ab	4.26 ± 0.43b
Amax (μmol CO_2 m^{-2} s^{-1})	45.28 ± 1.34a	47.19 ± 0.88a	38.66 ± 0.36b	44.53 ± 1.3a

Notes: CE is carboxylation efficiency; Rd, day respiration; CCP, CO_2 compensation point; Amax, maximum net carbon assimilation at saturating CO_2. The data are the means ± SE of three replicates, and different latters in the same row indicate a significant difference at 0.05 level

biotype, but the change was inconsistent with CE (Table 2). There was no significant change in both CE and Amax of S biotype under elevated CO_2, but CCP was decreased a little associated with a slight decrease in Rd in both biotypes. At elevated CO_2, CE and Amax were significantly lower in R biotype than in S biotype (P<0.5), indicating that photosynthetic performance of R biotype was inferior to S biotype. Nevertheless, the effects of elevated CO_2 on photosynthetic performance in R biotype was independent of the capacity of quantum yields of photosystems, since there was no statistically significant difference in quantum yields allocating pattern of either PSI [i.e. the allocating quantum yields among Y(I), Y(ND) and Y(NA)] or PSII [i.e. the allocating quantum yields among Y(II), Y(NO) and Y(NPQ)] between R and S biotypes, regardless of CO_2 exposure (Fig. 3a and b).

A negative correlation was observed between stomatal conductance (g_s) and external CO_2 (Ca) and at Ca > 600 μmol mol^{-1}, the values of g_s in both biotypes grown at elevated CO_2 were slightly greater than those grown at ambient CO_2 (Fig. 2b). The ratio of leaf internal CO_2 to external CO_2 (Ci/Ca) were markedly different in both biotypes grown at the different levels of CO_2 (Fig. 2c). At Ca ≥ 600 μmol mol^{-1}, the values of Ci/Ca were greater in both biotypes grown at elevated CO_2 than those grown at ambient CO_2. Therefore, both R and S biotypes had less stomatal limitations at elevated CO_2. Furthermore, at elevated CO_2 and Ca ≥ 800 μmol mol^{-1}, the values of Ci/Ca were higher in S biotype than in R biotype, suggesting that future atmospheric CO_2 concentrations is in favor of gas exchange in S biotype more than in R biotype.

Discussion

Goosegrass is a grass weed possessing C_4 photosynthetic pathway (Patterson, 1986). The C_4 photosynthesis is saturated under current atmospheric CO_2 concentrations (Ghannoum *et al.*, 2000) and thus may not be improved by elevated CO_2. The results showed that elevated CO_2 had little effect on growth of both biotypes (Fig. 1a). These results are consistent with the findings of a previous study, showing that elevated CO_2 did not improve growth of goosegrass under favorable conditions (Patterson, 1986). However, contrasting with our present study, the previous study (Patterson, 1986) did not take herbicide resistance into consideration. Several earlier studies showing effects of elevated CO_2 on herbicide tolerance in both C_3 and C_4 weed species suggest that elevated CO_2 can increase the tolerance in several weed species (Ziska and Teasdale, 2000; Ziska *et al*., 2004; Manea *et al*., 2011). However, the effects of elevated CO_2 on specific weed biotype with herbicide-resistant has not been reported earlier. The results of present study on goosegrass with different sensitivities to glyphosate showed that elevated CO_2 had little effect on glyphosate tolerance in S biotype, but reduced the resistant level in R biotype by 60% (Fig. 1b and Table 1). Without consideration of herbicide resistance, it has been reported that increased tolerance to glyphosate in the C_4 grasses (i.e., *Chloris gayana*, *Eragrostis curvula* and *Paspalum dilatatum*) under elevated CO_2 is related to growth stimulation (Manea *et al*., 2011). Possibly due to genetic differences, our results on goosegrass, either the R or S biotype, are inconsistent with the previous reports in aforementioned three C_4 plants.

Many previous studies have demonstrated that herbicide resistance is costly to maintain in the absence of herbicide (Reboud and Till-Bottraud, 1991; Bergelson *et al*., 1996; Roux *et al*., 2004; Vila-Aiub *et al*., 2005; Paris *et al*., 2008; Pavlovic *et al.*, 2013; Zain *et al.*, 2013). However, there has not been any report on cost of glyphosate resistance in goosegrass up to now. At ambient CO_2, shoot biomass and carboxylation efficiency (CE) of R biotype was slightly lower than of S biotype (Fig. 1a and Table 2), which indicate physiological basis of cost

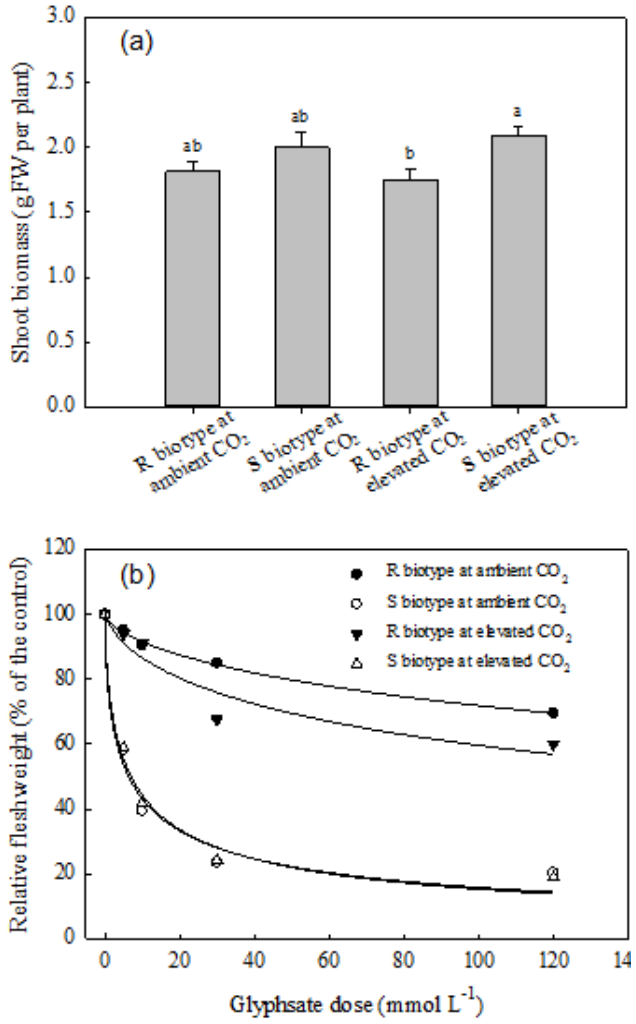

Fig. 1: Shoot biomass (a) and dose-response to glyphosate (b) of R and S biotypes of goosegrass grown at ambient and elevated CO_2. The data are the means (with SE in the upper figure) of sixteen replicates. Different latters indicate a significant difference at 0.05 level of glyphosate resistance in R biotype. Glyphosate resistance may lead to the change of some important physiological functions; elevated CO_2 therefore had greater impact on R biotype as reflected by having lower photosynthetic performance, stomatal limitations and shoot biomass than S biotype (Fig. 1a and Table 2). Growth of R biotype is adversely affected, rather than benefited by elevated CO_2, which results in the decline of glyphosate tolerance. Under future scenario, applied doses of glyphosate to control R biotype can be reduced. Under the circumstances of herbicides are not used, the subtle effect of elevated CO_2 on growth of R biotype is still meaningful for future farming systems in which the crop plants may have more completive advantages over the weed population of R biotype. A negative correlation between stomatal conductance (g_s) and external CO_2 (Ca) demonstrated that both biotypes would reduce g_s at elevated CO_2 (Fig. 2b).

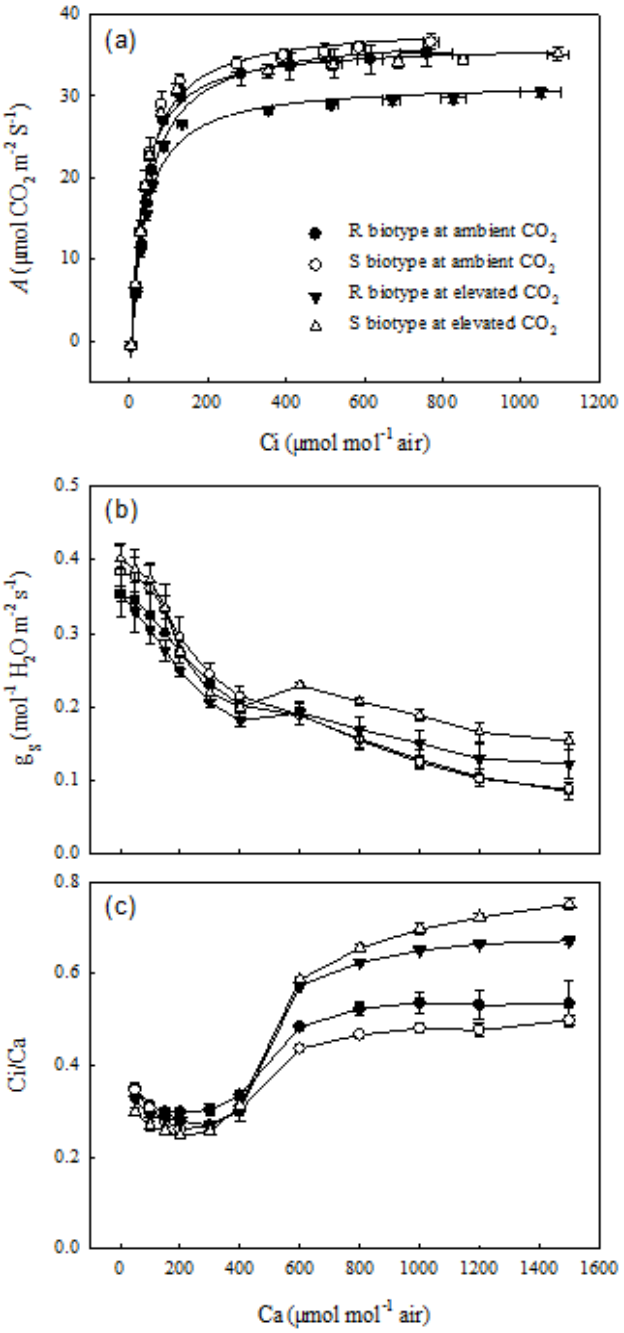

Fig. 2: Response of net assimilation *A* to leaf internal CO_2 Ci (a) and response of stomatal conductance g_s to external CO_2 Ca (b) and response of the ratio of Ci/Ca to Ca in R and S biotypes (c) 25 d after exposed to ambient or elevated CO_2 levels. The data are the means ± SE of three replicates

Under elevated CO_2, the decline of g_s can effectively increase water-use efficiency in C_4 plants (Knapp *et al*., 1993). In goosegrass, CO_2 enrichment can improve tolerance for drought stress through enhancing water-use efficiency (Patterson, 1986). Since there was only slight difference in g_s between the R and S biotype at elevated

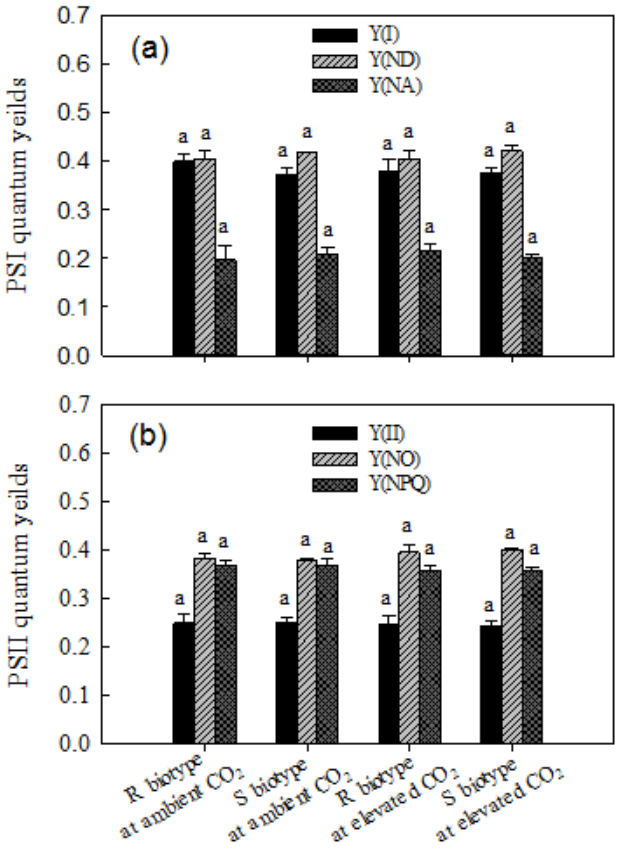

Fig. 3: Quantum yields Y(I), Y(ND), and Y(NA) of PSI (a) and quantum yields Y(II), Y(NO), and Y(NPQ) of PSII (b) in R and S biotypes 25 d after exposed to ambient or elevated CO_2. The data are the means ± SE of five replicates. Different letters in the same gray bar indicate a significant difference at the 0.05 level. Y(I), effective photochemical quantum yield in PSI; Y(NA), quantum yield of non-photochemical energy dissipation in PSI due to acceptor side limitation; Y(ND), quantum yield of non-photochemical energy dissipation in PSI due to donor side limitation; Y(II), effective photochemical quantum yield in PSII; Y(NPQ), regulated heat dissipation in PSII; Y(NO), non-regulated heat dissipation in PSII

CO_2 (Fig. 2b), there would not have much difference in their water-use efficiency or tolerance to drought stress under future atmospheric conditions.

Poor performance of C_4 photosynthesis caused by the low amounts of PEPCase has been reported in sorghum, a C_4 cereal (Watling *et al*., 2000), and in mutants of the C_4 plant *Amaranthus edulis* (Dever *et al*., 1997). Although the contents of PEPCase were not quantified in current study, CE which is a closely related variable was analyzed. At ambient and elevated CO_2, CE of R biotype was 19% and 26% lower than of S biotype, respectively suggesting R biotype had lower levels of PEPCase. The effect of elevated CO_2 on PEPCase content in C_4 plants is possible related to a change of cell wall's permeation property to CO_2 of the bundle sheath cells (Watling *et al*., 2000). Excepting CO_2, other factors can influence PEPCase content in C_4 plants. In *Zea mays*, the levels of leaf PEPCase can be regulated by N availability (Sugiharto *et al*., 1990; Ding *et al*., 2005) and P availability (Usuda and Shimogawara, 1991). Because the R and S biotypes were grown under the same nutrition conditions, and if they had the same capacity to uptake the nutrients, R biotype presumably had a different N or P biochemical allocation pattern than of S biotype. The previously reported mechanism of glyphosate resistance in goosegrass is a mutation in glyphosate target site (i.e., the gene for EPSPS) (Baerson *et al*., 2002; Ng *et al*., 2003; Kaundun *et al*., 2008). However, the mechanism of resistance to glyphosate was identified as target site (*EPSPS*) gene amplification in R biotype used in present study (data not shown). Regardless, the resistance mechanism operated in R biotype possibly results in some metabolic pathways to be adjusted and more N or P recourse to be consumed. For example, amplification of *EPSPS* gene and synthesis of additional amounts of EPSPS can utilize extraneous available N and P recourses. Thus, the available levels of N or P resource in tissues of R biotype were possibly less than of S biotype, which confined synthesis quantity of PEPCase. While PEPCase plays a role in C_4 plants for the CO_2 assimilation that produces carbohydrate, EPSPS functions in the shikimate pathway that produces the aromatic amino acids and many aromatic secondary metabolites (Herrmann, 1995). Under normal growth conditions, almost 20% of total photosynthetically fixed carbon is estimated to flow through the shikimate pathway (Jones *et al*., 1995; Orcaray *et al*., 2010). For this close connection, R biotype presumably had a specific coordination between CO_2 assimilation and shikimate pathway. Therefore, low levels of CE in R biotype could be associated with cellular available N or P resource, or the adjustment of shikimate pathway. However the detailed mechanism remains to be demonstrated.

Conclusion

Under elevated CO_2, decreased glyphosate tolerance displayed in R biotype was associated with lower photosynthesis. The different photosynthetic characteristics between the R and S biotype suggest that resistance to glyphosate was conferred in the R biotype at the cost of reduced mounts of related enzymes in the C assimilation. But how this happened remains to be demonstrated. As atmospheric levels of CO_2 continue to rise, the application doses of herbicide should be discriminatively adjusted for control of weeds with different susceptibilities to herbicides.

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