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Large variability in ambient ozone sensitivity across 19 ethylenediurea-treated Chinese cultivars of soybean is driven by total ascorbate

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ABSTRACT

The sensitivity of Chinese soybean cultivars to ambient ozone (O_3) in the field is unknown, although soybean is a major staple food in China. Using ethylenediurea (EDU) as an O_3 protectant, we tested the gas exchange, pigments, antioxidants and biomass of 19 cultivars exposed to 28 ppm·hr AOT40 (accumulated O_3 over an hourly concentration threshold of 40 ppb) over the growing season at a field site in China. By comparing the average biomass with and without EDU, we estimated the cultivar-specific sensitivity to O_3 and ranked the cultivars from very tolerant (<10% change) to highly sensitive (>45% change), which helps in choosing the best-suited cultivars for local cultivation. Higher lipid peroxidation and activity of the ascorbate peroxidase enzyme were major responses to O_3 damage, which eventually translated into lower biomass production. The constitutional level of total ascorbate in the leaves was the most important parameter explaining O_3 sensitivity among these cultivars. Surprisingly, the role of stomatal conductance was insignificant. These results will guide future breeding efforts towards more O_3-tolerant cultivars in China, while strategies for implementing control measures of regional O_3 pollution are being implemented. Overall, these results suggest that present ambient O_3 pollution is a serious concern for soybean in China, which highlights the urgent need for policy-making actions to protect this critical staple food.

Introduction

Food security is a topical issue nowadays, especially in rapidly expanding China (Yin *et al.*, 2009). China is the fourth largest world producer of soybean (*Glycine max* (L.) Merr.), with 12.2 million tons in 2014 (FAO, 2014). Soybean is a key source of vegetable protein for humans (Mateosaparicio *et al.*, 2008). It is one of the most important agricultural crop species and the top legume species worldwide (FAO, 2013).

China is currently suffering from serious surface ozone (O_3) pollution, with annual peak averages reaching as high as 60 ppb (Feng *et al.*, 2015) and an increase of about 7% from

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2005 to 2010 (Verstraeten *et al.*, 2015). Ozone is one of the most detrimental air pollutants for crops and natural ecosystems (Ainsworth *et al.*, 2012). Soybean ranks among the most O_3-sensitive agricultural crops (Mills *et al.*, 2007), and such current O_3 concentrations are high enough to cause significant yield losses (Morgan *et al.*, 2003). Projected O_3-induced soybean yield losses were 9.5%–15% for the year 2030 at the global level (Avnery *et al.*, 2011), and the financial losses for soybean were estimated as 2.0–5.8 billion US dollars annually based on the price in the year 2000 (Osborne *et al.*, 2016). Many experiments in different parts of the world have been carried out to investigate the physiological, growth and yield responses of soybean to O_3 in open-top chambers and under ambient conditions (*e.g.*, Sun *et al.*, 2014; Zhang *et al.*, 2014; Rai *et al.*, 2015). Ozone exposure reduces photosynthesis, stomatal conductance and the leaf chlorophyll content of soybean (Morgan *et al.*, 2003). A SoyFACE study showed a dose-dependent linear decrease in soybean yield and photosynthesis, and altered antioxidant capacity (Betzelberger *et al.*, 2012).

Dose–response studies for a range of crops have revealed that O_3 sensitivity is a heritable trait (Reinert and Eason, 2000) and is highly variable among species and cultivars (Ariyaphanphitak *et al.*, 2005; Mills *et al.*, 2007). Studies on the response of soybean to O_3 in Asia have focused on the growth and yield of individual cultivars (Wahid *et al.*, 2001; Singh *et al.*, 2010; Singh and Agrawal, 2011; Rai *et al.*, 2015). However, Zhang *et al.* (2014) demonstrated that O_3 sensitivity varied greatly across nine soybean cultivars widely cultivated in Northeast China, through elevated O_3 exposure experiments in open-top chambers. So far, however, there is no available data showing whether current ambient O_3 levels affect the growth and productivity of soybean in China.

The antiozonant ethylenediurea (*N*-[2-(2-oxo-1-imidazolidinyl)ethyl]-*N*′-phenylurea, abbreviated as EDU, with formula $C_4H_{10}N_4O_2$), first described by Carnahan *et al.* (1978), is a well-known antiozonant chemical (Paoletti *et al.*, 2009; Feng *et al.*, 2010; Manning *et al.*, 2011; Agathokleous *et al.*, 2016a), able to prevent O_3 injury, especially visible foliar O_3 injury as well as growth reduction in agricultural and horticultural crops and forest trees, by stimulating the antioxidant defense (Tiwari *et al.*, 2005; Elagöz and Manning, 2005; Szantoi *et al.*, 2007; Paoletti *et al.*, 2007; Feng *et al.*, 2010; Rai *et al.*, 2015). A meta-analysis suggested that the antiozonant activity of EDU is biochemical rather than biophysical (Feng *et al.*, 2010), but conclusive proof of the detailed basis for the protective action has not been confirmed. Recent results showed that EDU does not have side-effects on growth and is not toxic to plants at the concentrations required for O_3 protection (Agathokleous *et al.*, 2016a). As a reliable, low-cost, and low-technology tool, EDU has great potential for assessing the effects of ambient O_3 on vegetation (Singh *et al.*, 2014; Agathokleous *et al.*, 2016b, 2016c).

We used EDU as a tool for assessing: (1) the relative sensitivity to ambient O_3 exposure in 19 soybean cultivars widely cultivated in China by using biomass as the response indicator, (2) whether these cultivars differ in their physiological and biochemical responses to O_3 (gas exchange, pigments, antioxidants), and (3) which parameters are the most important as predictors of O_3-sensitivity in these cultivars. This knowledge will help in cultivating the most O_3-tolerant cultivars in the areas at higher risk and breeding for more and more O_3-tolerant cultivars.

1. Materials and methods

1.1. Experimental conditions

The experiment was conducted under natural field conditions from June to October, 2015, at a suburban area of Beijing city, Changping District, 40°19′ N, 116°13′ E and 43.5 m a.s.l. (above sea level). The site is about 52 km from the city center. Mean monthly minimum and maximum temperatures were −3.1 °C (January) and 26.7 °C (July). The mean yearly precipitation was 550 mm and almost 60% of rain occurred in July and August.

Meteorological variables (air temperature and precipitation) were recorded by a portable automatic weather station (HOBO-U30, USA). The concentration of O_3 was continuously monitored using an ultraviolet (UV)-absorption O_3 analyzer (Model 49i, Thermo Scientific, USA). The monitor was calibrated by a 49i-PS calibrator (49i–PS, Thermo Scientific, USA) before the experiment and once a month during the experiment. Exceedances above 40 ppb were accumulated to calculate the exposure index AOT40 (accumulated O_3 over an hourly concentration threshold of 40 ppb) according to Mills *et al.* (2007). The distribution of hourly O_3 concentrations across 10 ppb classes of exposure and daily 8-hr means was calculated from 9:00 to 17:00 solar time.

The seeds of 19 soybean cultivars (*Glycine max* (L.) Merr.) were obtained from the Institute of Crop Science of Chinese Academy of Agricultural Sciences. The cultivars are widely planted in North China, have similar growing periods (110–130 days), and had not been tested for O_3 sensitivity previously. The agronomic characteristics of these cultivars are listed in Table 1. The soybean seeds were sown on the 10th of June and sprouted out of the earth on the 20th of June, 2015.

After measuring the physiological and biochemical parameters at two months after germination (23rd August), harvest was carried out at the very end of the growing season (8th October). Due to rainy days at the time of flowering (22nd July to 10th August) (Fig. 1), the plants did not produce seeds. No soybean yield occurred in the entire region in 2015. Therefore, the present paper shows only the results of biomass.

In this experiment, there were 7 plots and each plot occupied 65 m^2. For every plot, there were 19 lines (5 m in length for each line) *i.e.*, one line per cultivar distributed at random. The basic physical and chemical properties of soil were as follows: organic C, 17.4 g/kg; total N, 0.9 mg/kg; available P, 38.1 mg/kg; available K, 102.1 mg/kg and pH of 8.3.

1.2. EDU application

Among different concentrations of EDU, 450 ppm of EDU was used in this study as it was found to effectively protect different plant species from O_3 (Paoletti *et al.*, 2009; Feng *et al.*, 2010; Manning *et al.*, 2011). For instance, foliar applications of EDU at 450 ppm significantly alleviated snap bean foliar injury, and increased the photosynthesis rate, seed and pod weights in O_3-sensitive genotypes (Yuan *et al.*, 2015). EDU powder (100% available ingredient) was dissolved in warm water. Three plots were sprayed with water and four plots were sprayed with EDU. The entire foliage of each plant was sprayed until the drip point before sunrise each time. The EDU treatments started from the

Table 1 – Characteristics of the 19 soybean cultivars used in the study.

Cultivars	Cultivars (abbreviation)	Year of release	Maturity (days)	Male parent	Female parent	Leaf shape
Jidou12	J12	2002	100	Oil 83-14	Jinda7826	Ovoid
ZhongHuang13	ZH13	2001	105	Yudou8	Zhongzuo90052-76	Ovoid
ZhongHuang20	ZH20	2003	100	Yi-2	Hobbit	Ovoid
ZhongHuang39	ZH39	2006	100	Zhongpin661	Zhonghuang14	Ovoid
ZhongHuang40	ZH40	2007	104	Jindou6	Yudou12	Ovoid
ZhongHuang41	ZH41	2009	108	Kefeng14	Kexin3	Ovoid
ZhongHuang42	ZH42	2007	116	Youchu4	Jindou33	Ovoid
ZhongHuang43	ZH43	2006	101	Jidou7	Xinke3	Ovoid
ZhongHuang44	ZH44	2009	107	Kefeng14	Kexin3	Ovoid
ZhongHuang48	ZH48	2009	108	Kefeng14	Kexin3	Ovoid
ZhongHuang49	ZH49	2009	106	Kefeng14	Kexin3	Ovoid
ZhongHuang50	ZH50	2010	106	Zhonghuang13	Zhongpin661	Ovoid
ZhongHuang62	ZH62	2011	100	Zhonghuang25	Xindou1	Lanceolate
ZhongHuang66	ZH66	2014	112	Zhongpin661	Cheng9039-2-4-3-1	Ovoid
ZhongHuang69	ZH69	2012	121	Kefeng14	Kexin3	Lanceolate
ZhongHuang70	ZH70	2013	102	Zhonghuang13	Ludou11	Ovoid
ZhongHuang74	ZH74	2013	109	Zhongdou27	Zhonghuang3	Ovoid
ZhongHuang75	ZH75	2014	131	NF58	Teifeng31	Ovoid
ZhongHuang79	ZH79	2015	131	Zhongpin 661	Yudou25	Ovoid

time of the first trifoliate leaf emergence. EDU was repeatedly applied at bi-weekly intervals until the end of the experiment. In total, EDU was applied 7 times during the growing period.

1.3. *Gas exchange parameters*

Photosynthesis at saturating light (A_{sat}) and stomatal conductance (g_s) were measured using a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA). For every cultivar, two fully expanded upper leaves per one plant in each plot were randomly selected on the 23rd of August. All measurements were conducted during 08:30–11:00 on clear days under the following conditions: saturating photosynthetic active radiation (PAR) of 1500 μmol/(m^2·sec), CO_2 at 400 ppm, leaf temperature at 28°C, and relative humidity in air between 50%–70%.

1.4. *Photosynthetic pigment*

After the photosynthesis measurement, the leaf was sampled for photosynthetic pigment. For every cultivar, two leaflets from two fully expanded leaves per plant were randomly punched, and treated with 2 mL 95% ethanol in the dark for 48 hr at 4°C. Assays for chlorophyll (Chl) *a* and *b* and carotenoid (Car) content were carried out by ultraviolet–visible (UV–VIS) spectrophotometry (Alpha-1506, Lab-Spectrum Instruments Co., Ltd., China) according to the specific absorption coefficients provided by Lichtenthaler (1987).

1.5. *Antioxidant parameters*

Leaves for antioxidant analyses were collected immediately after the photosynthesis measurement. Two fully expanded

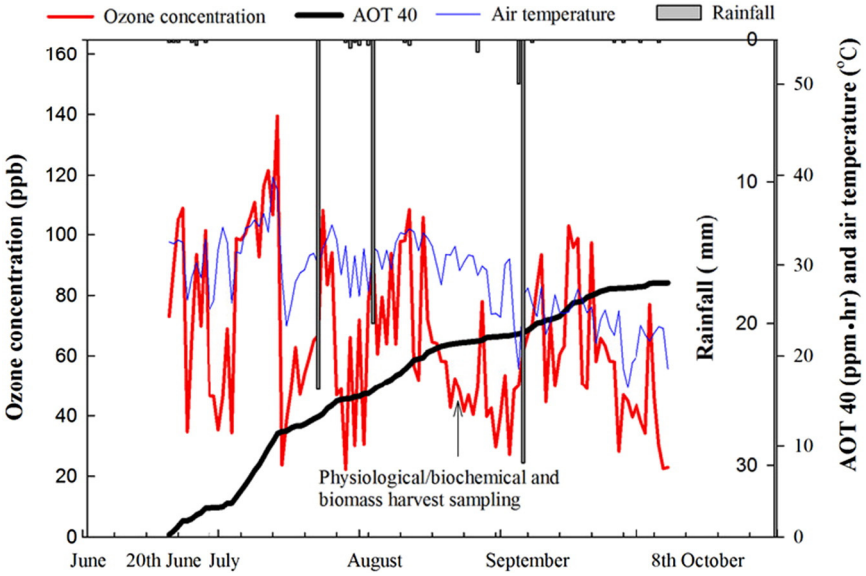

Fig. 1 – The 8-hr (9:00–17:00) mean O_3 concentrations and AOT 40 during the study period from the 20th of June to the 8th of October, 2015. AOT 40: accumulated O_3 over an hourly concentration threshold of 40 ppb.

upper leaves were randomly sampled from two plants per each cultivar in each plot, frozen immediately in liquid nitrogen, and stored at −80 °C until analysis. Malondialdehyde (MDA), which is related with the level of lipid peroxidation (Feng *et al.*, 2011), was assessed for estimation of lipid peroxidation by 2-thiobarbituric acid-reactive metabolite (TBA) according to the method of Heath and Parker (1968). The optical density (OD) values were obtained in a 96-well plate reader (SpectraMAX 190, Molecular Devices, Sunnyvale, CA, USA) and the MDA content (C_{MDA}, mmol/L) was calculated by the equation $C_{MDA} = 6.45 \times (A532–A600) – 0.56 \times A450$ (A532, A600 and A450 represent extinction value under 450, 532 and 600 nm wavelength, respectively) in order to rule out the disturbances from non-specific (A600) and sugar (A450) absorbance. Total ascorbate (AsA), which is the main antioxidant metabolite in the mesophyll (Noctor, 2006), was determined using an α-α′-bipyridyl-based colorimetric assay for approximately 30 mg of ground leaf tissue in a 96-well plate reader SpectraMAX 190 (Gillespie and Ainsworth, 2007).

Samples (~100 mg) for total antioxidant capacity (TAC), which is often used as a synthetic index of the antioxidant pool in the leaves (Gillespie and Ainsworth, 2007), were added to 2 mL cold 70% (*v*/*v*) ethanol and homogenized in darkness, according to Benzie and Strain (1996). The mixture was incubated for 20 min in darkness at 4 °C, then centrifuged at 3000 r/min for 20 min. One-hundred milliliters of supernatant was taken for the ferric reducing antioxidant power (FRAP) assay to express TAC as Fe^{3+} equivalents (mmol Fe^{2+}/g fresh mass), and OD values were also measured by a 96-well plate reader SpectraMAX 190 (49i-PS, Thermo Scientific, USA).

For antioxidant enzymes, samples (~0.1 g fresh leaves) were ground in liquid nitrogen and extracted with 2 mL of 50 mmol/L sodium phosphate buffer (pH 7.0, containing 1% vinyl pyrrolidone). The samples were centrifuged at 13,000 r/min, 4°C for 20 min and the supernatant was collected. All extraction experiments were carried out in an ice bath. The superoxide dismutase (SOD) activity was determined by estimating its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), and the absorbance was read at 560 nm. The amount of enzyme that inhibited the 50% NBT reduction was defined as one unit of SOD activity. The ascorbate peroxidase (APX) activity was assayed by monitoring the change of the absorbance at 290 nm after the enzyme extract was mixed a 50 mmol/L potassium phosphate buffer (pH 7.0) containing 30 mmol/L ascorbate and 10 mmol/L H_2O_2 (Nakano and Asada, 1981). The enzyme activity was determined by monitoring the change in OD at 290 nm, and one unit of APX activity was defined as the decrease of 0.01 ΔOD (changes of optical density) per minute. The catalase (CAT) activity was determined according to the description by Aebi (1984). Peroxidase (POD) activity was assayed adopting the method of Kar and Choudhuri (1987). The unit of CAT and POD activity was defined as the decrease and increase of 0.01 ΔOD/min at 240 and 470 nm, respectively. All measurements were conducted using spectrophotometric methods.

1.6. Biomass

Plants were harvested on the 8th of October. The above-ground parts of two plants for each cultivar were collected in each plot. Samples were dried in an oven at 80°C until a constant weight was reached.

1.7. Definition of O_3 stress effects

The cultivar-specific sensitivity to O_3 (B_{EFF}) was estimated by comparing the average biomass (*B*) with and without EDU protection, *i.e.*, $B_{EFF} = (B_{EDU} - B_{Water}) \times 100/B_{EDU}$ (B_{EDU} means biomass treated with EDU, B_{Water} means biomass treated with water). The same approach was used to estimate the effect of O_3 on each variable *X*.

1.8. Statistical analyses

The statistical unit was the single plot, with four plots for EDU and three plots for water treatment. Data were checked for normal distribution and homogeneity of variance. Non-normally distributed data *i.e.*, biomass, AsA, POD, and APX, were log transformed prior to analysis. Data in the figures and tables are not transformed, but rather original data means ± SD. Data were subject to two-way analysis of variance (ANOVA) including the effects of EDU, cultivar and their interaction using Statistica 10.0 software (StatSoft, Italy). Student's t test was used to analyze the effect of EDU within each cultivar. Simple linear correlations were applied to test the relationship of all variables *X* (X_{EFF}, X_{EDU} and X_{Water}) with B_{EFF}. Effects were considered significant if $p < 0.05$.

2. Results

2.1. Ozone pollution

The AOT40 index calculated for the whole experimental period was 28.04 ppm·hr (Fig. 1). Daily 8-hr mean O_3 concentrations varied from 22 to 140 ppb. The daily average along the growing season was 64.9 ppb. There were 141 hr with concentrations higher than 100 ppb, which happened mostly in July (Table 2).

Table 2 – Distribution of mean hourly ozone concentrations (9–17 hr) in 10 ppb classes during the study period.

Concentration (ppb)	Hours				
	June (20th–30th)	July	August	September	October (1st–8th)
<30	4	31	14	15	17
31–40	16	16	20	31	13
41–50	10	35	48	44	17
51–60	7	18	57	56	12
61–70	5	23	34	27	1
71–80	9	26	17	19	0
81–90	5	22	13	15	1
91–100	11	17	11	9	1
101–110	5	10	10	5	1
111–120	2	6	12	7	1
121–130	6	11	7	5	0
131–140	5	12	1	4	0
141–150	1	11	3	2	0
>150	2	10	1	1	0
Sum of hours	88	248	248	240	64

2.2. Gas exchange parameters

A_{sat} of cultivars protected by EDU showed a significant variability and was significantly increased by 40% across all cultivars, relative to non-protected plants (Table 3). EDU significantly increased A_{sat} in Zhonghuang13 (ZH13), Zhonghuang20 (ZH20), Zhonghuang48 (ZH48) and Zhonghuang49 (ZH49). Interestingly, constitutional g_s did not significantly differ across the EDU-treated cultivars. Also, the effect of EDU on g_s was not significant.

The cultivars differed in their foliar content of photosynthetic pigments, as indicated by the higher contents in Zhonghuang74 (ZH74) and Zhonghuang66 (ZH66), and lower content in Zhonghuang39 (ZH39), Zhonghuang44 (ZH44) and Zhonghuang75 (ZH75) (Table 4). EDU significantly increased Chl *a*, Chl *b*, total Chl and Car contents by 26%, 52%, 30% and 23% across all cultivars, respectively. There was significant interaction between EDU and cultivar, as indicated by a significant EDU-induced increase in some cultivars but not in others.

2.3. Antioxidant parameters

The foliar content of antioxidant metabolites was significantly affected by the cultivar, the EDU treatment and their interaction (Table 5). Across all cultivars, MDA and AsA were decreased by 9% and 27%, respectively, while TAC was increased by 31% due to the EDU treatment.

Also, the activity of antioxidant enzymes was significantly affected by the cultivar, the EDU treatment and their interaction (Table 6). Across all cultivars, SOD showed a negligible 3% reduction due to the EDU treatment, while APX, POD and CAT increased by 28%, 15% and 8%, respectively.

2.4. Biomass and ozone sensitivity of the cultivars

Biomass at harvest showed a significant variation with the cultivar, the EDU treatment and their interaction (Fig. 2a). EDU significantly increased the biomass in 15 out of 19 cultivars (*i.e.*, with the exception of Zhonghuang69 (ZH69), Zhonghuang43 (ZH43), ZH49 and Jidou12 (J12)), thus a significant interaction between EDU and cultivars was found. On average, the biomass of EDU-treated cultivars was 36% higher than that of water-treated cultivars.

The cultivar-specific O_3 sensitivity, expressed as percent variation of biomass when plants were protected by ethylenediurea (B_{EFF}), showed a remarkable variation among cultivars, with the most tolerant cultivars ZH43, ZH49 and ZH69 showing insignificant changes (<10%) and the most sensitive cultivars ZH42, ZH50 and ZH70 showing large variations (>45%) (Fig. 2b).

When linear correlations were applied to test the effects of all variables (X_{EDU}, X_{Water} and X_{EFF}) on B_{EFF}, MDA_{Water} and APX_{EFF} increased significantly with increasing O_3 sensitivity of

Table 3 – Light-saturated photosynthesis rate (A_{sat}) and stomatal conductance (g_s) (mean ± SD) of 19 soybean cultivars exposed to ambient O_3 concentrations (water) or protected by ethylenediurea (EDU), levels of significance showing differences between EDU and water for any cultivar, and two-way analysis of variance (ANOVA) (EDU × cultivar) results.

Cultivar	A_{sat} (μmol/(m²·sec))			g_s (mmol/(m²·sec))		
	EDU	Water	Significance	EDU	Water	Significance
J12	13.0 ± 2.37	10.1 ± 0.07	ns	403 ± 93	223 ± 103	ns
ZH13	15.9 ± 2.5	8.5 ± 1.35	**	264 ± 117	310 ± 93	ns
ZH20	14.2 ± 0.94	10.6 ± 1.1	**	297 ± 196	213 ± 58	ns
ZH39	16.2 ± 5.72	12.7 ± 1.52	ns	332 ± 192	244 ± 159	ns
ZH40	10.4 ± 1.85	10.3 ± 3.77	ns	243 ± 61	352 ± 55a	*
ZH41	16.1 ± 1.69	14 ± 3.1	ns	368 ± 85	230 ± 72	ns
ZH42	19.0 ± 5.62	12.7 ± 2.92	ns	445 ± 148	175 ± 69	*
ZH43	12.4 ± 1.86	10.3 ± 2.45	ns	270 ± 54	327 ± 24	ns
ZH44	15.6 ± 1.66	11.7 ± 1.98	*	305 ± 65	335 ± 102	ns
ZH48	13.4 ± 2.04	8.3 ± 0.55	**	220 ± 171	259 ± 135	ns
ZH49	17.1 ± 2.37	8.7 ± 1.5	**	428 ± 107	154 ± 57	*
ZH50	13.6 ± 3.84	7.6 ± 1.46	ns	300 ± 157	117 ± 38	ns
ZH62	15.2 ± 2.23	14.7 ± 0.7	ns	286 ± 150	350 ± 190	ns
ZH66	15.6 ± 5.15	15.0 ± 2.53	ns	319 ± 200	484 ± 251	ns
ZH69	13.6 ± 1.23	12.7 ± 1.48	ns	335 ± 136	416 ± 136	ns
ZH70	15.8 ± 3.4	7.5 ± 1.05	*	263 ± 143	270 ± 90	ns
ZH74	20.1 ± 4.94	13.7 ± 0.63	ns	518 ± 265	415 ± 67	ns
ZH75	18.4 ± 4.45	10.7 ± 0.73	*	455 ± 167	203 ± 118	ns
ZH79	18.6 ± 3.59	10.6 ± 2.49	*	299 ± 80	218 ± 137	ns
EDU	<0.0001 [a]			0.590 ns		
Cultivar	<0.0001 [a]			0.150 ns		
EDU × Cultivar	0.054 ns			0.043 *		

ns: non-significant; SD: standard deviation.
* $p \leq 0.05$.
** $p \leq 0.01$.
[a] $p \leq 0.001$.

Table 4 – Photosynthetic pigments (chlorophyll *a*, *b* (Chl *a*, *b*) and total carotenoids (Car)) (mean ± SD) of 19 soybean cultivars exposed to ambient O_3 concentrations (water) or protected by ethylenediurea (EDU), levels of significance showing differences between EDU and water for any cultivar, and two-way ANOVA (EDU × cultivar) results.

Cultivar	Chl *a* (mg/m^2)			Chl *b* (mg/m^2)			Chl *a* + *b* (mg/m^2)			Car (mg/m^2)		
	EDU	Water	Significance	EDU	Water	Significance	EDU	Water	Significance	EDU	Water	Significance
J12	35.7 ± 7.41	37.7 ± 2.6	ns	7.42 ± 1.69	7.35 ± 1.23	ns	43.1 ± 6.46	45 ± 2.91	ns	4.04 ± 0.6	4.11 ± 0.39	ns
ZH13	35.1 ± 3.23	28.5 ± 4.83	ns	7.17 ± 1.22	5.21 ± 2.42	ns	42.3 ± 3.86	33.7 ± 7.25	ns	3.82 ± 0.49	3.2 ± 0.11	ns
ZH20	34.5 ± 4.55	16.3 ± 1.46	**	9.39 ± 2.42	5.09 ± 2.98	ns	43.9 ± 5.3	21.4 ± 3.92	**	2.93 ± 0.51	1.84 ± 0.57	*
ZH39	22.9 ± 3.38	23.2 ± 4.85	ns	5.08 ± 0.68	3.69 ± 1.65	ns	28 ± 3.2	26.9 ± 5.18	ns	2.43 ± 0.55	2.51 ± 0.47	ns
ZH40	28.7 ± 2.52	26.1 ± 2.92	ns	6.04 ± 3.12	5.39 ± 4.17	ns	34.7 ± 4.51	31.5 ± 6.48	ns	3.1 ± 0.26	2.52 ± 0.22	*
ZH41	24.1 ± 5.98	20 ± 5.39	ns	5.58 ± 1.55	3.51 ± 3.98	ns	29.7 ± 6.37	23.5 ± 9.2	ns	2.58 ± 0.31	2.44 ± 0.45	ns
ZH42	37.8 ± 4.12	20.8 ± 3.59	**	9.51 ± 1.23	2.35 ± 0.95	**	47.3 ± 4.92	23.2 ± 3.78	**	4.22 ± 0.46	2.6 ± 0.35	**
ZH43	29.5 ± 1.6	22.7 ± 2.04	**	13.39 ± 5.97	5.23 ± 1.61	ns	42.9 ± 7.43	27.9 ± 3.57	**	3.19 ± 0.47	2.52 ± 0.26	ns
ZH44	21 ± 6.34	20.2 ± 4.76	ns	8.2 ± 5.47	8.13 ± 3.15	ns	29.2 ± 1.65	28.3 ± 1.74	ns	2.26 ± 0.5	2.51 ± 0.36	ns
ZH48	34.4 ± 3.49	18.5 ± 2.27	**	7.79 ± 0.44	1.56 ± 0.56	**	42.2 ± 3.68	20 ± 2.82	**	3.5 ± 0.22	2.62 ± 0.03	**
ZH49	27 ± 5.23	23 ± 5.28	ns	6.07 ± 2.33	6.17 ± 3.45	ns	33 ± 6.6	29.1 ± 8.67	ns	3.17 ± 0.25	2.4 ± 0.11	**
ZH50	29.4 ± 2.5	28.4 ± 1.55	ns	6.45 ± 1.42	3.18 ± 1.47	*	35.8 ± 3.76	31.6 ± 2.95	ns	3.08 ± 0.28	2.42 ± 0.07	*
ZH62	26.3 ± 5.04	21.9 ± 1.15	ns	4.35 ± 1.51	4.18 ± 0.5	ns	30.6 ± 3.79	26.1 ± 1.62	ns	3.04 ± 0.2	2.52 ± 0.17	*
ZH66	39.4 ± 3.73	27.6 ± 4.52	*	10.86 ± 2.39	8.08 ± 1.16	ns	50.2 ± 6.02	35.7 ± 4.93	*	3.36 ± 0.5	2.25 ± 0.44	*
ZH69	26.5 ± 6.44	24.9 ± 4.17	ns	4.03 ± 0.61	4.93 ± 1.66	ns	30.5 ± 7.02	29.9 ± 5.54	ns	3.34 ± 0.26	2.7 ± 0.18	*
ZH70	36.4 ± 2.45	30.1 ± 5.88	*	9.78 ± 1.67	8.1 ± 1.38	ns	46.2 ± 3.22	38.2 ± 4.68	*	3.6 ± 0.12	2.32 ± 0.34	**
ZH74	41 ± 3.54	29.5 ± 1.84	**	8.93 ± 1.29	3.97 ± 2.17	*	49.9 ± 4.74	33.5 ± 3.92	**	4.18 ± 0.43	3.27 ± 0.58	ns
ZH75	28.3 ± 5.8	23.4 ± 5.65	ns	8.42 ± 4.37	3.91 ± 3.07	ns	36.8 ± 6.07	27.3 ± 2.87	ns	3.01 ± 0.38	3.03 ± 0.38	ns
ZH79	30.9 ± 3.67	24.4 ± 6.76	ns	11.74 ± 1.79	8.66 ± 3.64	ns	40.2 ± 6.66	33.1 ± 3.13	ns	3.18 ± 0.41	2.46 ± 0.32	ns
EDU	<0.0001 [a]			<0.0001 [a]			<0.0001 [a]			<0.0001 [a]		
Cultivar	<0.0001 [a]			<0.0001 [a]			<0.0001 [a]			<0.0001 [a]		
EDU × Cultivar	<0.0001 [a]			<0.0001 [a]			<0.0001 [a]			<0.0001 [a]		

ns: non-significant.
* $p \leq 0.05$.
** $p \leq 0.01$.
[a] $p \leq 0.001$.

Table 5 – Foliar content of malondialdehyde (MDA), total antioxidant capacity (TAC) and total ascorbic acid (AsA) (mean ± SD) of 19 soybean cultivars exposed to ambient O_3 concentrations (water) or protected by ethylenediurea (EDU), levels of significance showing differences between EDU and water treatments for any cultivar, and two-way ANOVA (EDU × cultivar) results.

Cultivar	MDA (nmol/g)			AsA (μmol/g)			TAC (μmol/g)		
	EDU	Water	Significance	EDU	Water	Significance	EDU	Water	Significance
J12	4.28 ± 0.17	3.74 ± 0.43	ns	5.35 ± 1.18	4.17 ± 0.48	ns	20.94 ± 4.36	19.76 ± 4.78	ns
ZH13	3.84 ± 0.4	5.39 ± 0.3	**	3.64 ± 0.38	5.85 ± 1.13	**	18.63 ± 3.63	17.54 ± 3.19	ns
ZH20	4.97 ± 0.61	5.3 ± 1.79	ns	3.76 ± 8.11	8.01 ± 0.89	**	21.20 ± 2.62	15.28 ± 2.11	**
ZH39	4.92 ± 1	7.32 ± 1.53	ns	3.54 ± 0.73	4.13 ± 0.91	ns	19.59 ± 8.79	23.80 ± 8.42	ns
ZH40	5.06 ± 0.42	5.68 ± 1.01	ns	2.84 ± 0.57	5.20 ± 1.04	**	30.66 ± 0.94	14.99 ± 1.25	**
ZH41	4.4 ± 1.07	4.41 ± 0.59	ns	2.79 ± 0.76	5.18 ± 0.43	**	12.21 ± 4.14	12.41 ± 4.74	ns
ZH42	3.81 ± 0.5	5.83 ± 0.53	**	3.55 ± 0.50	5.29 ± 1.03	*	23.74 ± 4.56	12.72 ± 1.24	**
ZH43	4.51 ± 0.86	4.98 ± 0.92	ns	7.21 ± 0.63	5.29 ± 0.50	**	13.70 ± 2.48	21.95 ± 2.90	**
ZH44	4.52 ± 0.71	4.54 ± 1.56	ns	3.48 ± 0.81	5.76 ± 1.00	*	7.99 ± 1.21	7.70 ± 0.85	ns
ZH48	4.22 ± 1.39	4.65 ± 0.31	ns	3.41 ± 0.54	3.37 ± 0.12	ns	20.35 ± 1.65	24.78 ± 7.55	ns
ZH49	5.19 ± 0.13	2.94 ± 0.35	**	4.59 ± 1.18	7.34 ± 0.42	**	21.69 ± 2.19	13.38 ± 4.63	ns
ZH50	4.35 ± 1.15	5.09 ± 0.53	ns	2.64 ± 0.61	5.97 ± 0.54	*	10.53 ± 2.81	14.06 ± 2.22	*
ZH62	4.27 ± 0.22	4.14 ± 0.42	ns	4.58 ± 1.25	4.58 ± 0.23	ns	20.72 ± 2.35	15.96 ± 2.03	*
ZH66	3.73 ± 0.7	4.63 ± 0.6	ns	3.52 ± 0.63	6.12 ± 1.09	*	19.97 ± 1.84	19.12 ± 6.60	ns
ZH69	5.21 ± 1.65	4.92 ± 1.31	ns	3.54 ± 0.54	5.24 ± 0.90	*	18.73 ± 8.39	14.42 ± 4.68	ns
ZH70	4.71 ± 0.68	6.35 ± 0.51	*	3.20 ± 0.82	4.06 ± 0.81	ns	26.68 ± 1.97	16.49 ± 4.60	**
ZH74	3.77 ± 1.34	2.74 ± 0.46	ns	2.60 ± 0.01	4.87 ± 0.55	*	17.55 ± 2.22	11.07 ± 0.08	**
ZH75	3.91 ± 0.81	5.5 ± 0.94	ns	2.35 ± 0.37	2.77 ± 0.98	ns	22.54 ± 3.00	13.37 ± 4.84	*
ZH79	5.25 ± 0.99	5.84 ± 0.33	ns	4.11 ± 0.86	4.71 ± 0.96	ns	20.08 ± 4.65	12.77 ± 0.33	**
EDU	0.003 **			<0.0001 [a]			<0.0001 [a]		
Cultivar	<0.0001 [a]			<0.0001 [a]			<0.0001 [a]		
EDU × Cultivar	0.002 **			<0.0001 [a]			<0.0001 [a]		

ns: non-significant.
* $p \leq 0.05$.
** $p \leq 0.01$.
[a] $P \leq 0.001$.

the cultivars, while B_{Water}, MDA_{EFF} and AsA_{EDU} significantly decreased (Table 7).

3. Discussion

During the growing season of soybean, the daily average ambient O_3 concentration was 65 ppb and AOT40 was 28 ppm·hr. This level exceeds by far the existing critical level of 3 ppm·hr AOT40 over 3 months recommended in Europe for the protection of agricultural crops (CLRTAP, 2015). Modeling studies confirm that AOT40 levels over China may exceed 15 ppm·hr and are projected to further increase until 2020 (Tang *et al.*, 2013, 2014). While O_3 precursor (mainly NO_x) emissions have been reduced in Europe and the United States over recent years (Paoletti *et al.*, 2014; Lefohn *et al.*, 2017), emissions are still increasing in China at an annual rate of 5% (Feng *et al.*, 2015). The ozone concentration in Beijing, the capital of China, has been continuously increasing, *e.g.*, the daily maximum 8-hr average O_3 concentration increased by 1.14 ppb/year from 2004 to 2015 (Cheng *et al.*, 2016). Overall, O_3 pollution is a serious concern for many Asian countries, for example India (Singh and Agrawal, 2011; Pandey *et al.*, 2014), Pakistan (Ahmad *et al.*, 2013) and Japan (Hoshika *et al.*, 2011). Beijing and its surroundings, where our experiment was carried out, are a hot-spot of O_3 pollution (Yuan *et al.*, 2015; Zhu *et al.*, 2015).

Even though O_3 pollution is such a pressing issue for food security in China (Yin *et al.*, 2009; Miao *et al.*, 2016) and soybean is a major staple legume crop for Chinese population (FAO, 2014), previous studies were carried out by simulated O_3 exposure under controlled conditions, *i.e.*, in open-top chambers (Zhang *et al.*, 2014; Zhao *et al.*, 2015). While chambers are well suited for mechanistic studies on O_3 impacts, risk assessment and cultivar screening may be affected by artifacts due to modification of the environmental variables (Paoletti, 2007), as demonstrated in the case of soybean (Howell *et al.*, 1979). EDU has been verified as a useful tool to protect crops from O_3 and assess the effects of O_3 on plants under ambient conditions (Singh *et al.*, 2009; Paoletti *et al.*, 2007; Feng *et al.*, 2011; Hoshika *et al.*, 2013a; Carriero *et al.*, 2015; Yuan *et al.*, 2015; Pandey *et al.*, 2015). Our results confirm that EDU is a valid and easy approach for field assessment of ambient O_3 injury to vegetation.

By using the biomass of EDU-protected plants as a proxy of the biomass in a non-O_3-polluted environment, we were able to rank the relative O_3-sensitivity of 19 soybean cultivars widely cultivated in China. The most tolerant cultivars showed insignificant changes (<10% variation of biomass when plants were protected by EDU), while the most sensitive cultivars showed large deviations (>45%). It is well known, in fact, that sensitive plants show significant responses to O_3 when treated with EDU, while tolerant plants show limited responses to O_3 (Szantoi *et al.*, 2007; Singh *et al.*, 2009). Such serious variability in the cultivar-specific O_3 sensitivity has been already shown in other species, *e.g.*, wheat (Biswas *et al.*, 2008; Singh *et al.*, 2009), rice (Akhtar *et al.*, 2010) and tomato

Table 6 – The activity of antioxidative enzymes (APX, SOD, POD and CAT) (mean ± SD) of 19 soybean cultivars exposed to ambient O_3 concentrations (water) or protected by ethylenediurea (EDU), levels of significance showing differences between EDU and water treatments for any cultivar, and two-way ANOVA (EDU × cultivar) results.

Cultivar	SOD (U/(g FW))			APX (U/(g FW))			POD (U/(g FW))			CAT (U/(g FW))		
	EDU	Water	Significance	EDU	Water	Significance	EDU	Water	Significance	EDU	Water	Significance
J12	305 ± 54.3	372 ± 60.4	ns	333 ± 31.9	159 ± 9.7	ns	9.94 ± 0.54	8.52 ± 0.87	ns	1.70 ± 0.34	3.07 ± 0.129	**
ZH13	347 ± 64.5	364 ± 69.5	ns	154 ± 10.4	286 ± 59.3	ns	6.17 ± 0.96	6.18 ± 0.34	ns	1.82 ± 0.35	2.18 ± 0.19	ns
ZH20	376 ± 96.5	294 ± 103.9	ns	233 ± 6.5	131 ± 7.2	ns	13.7 ± 0.58	9.98 ± 1.22	ns	2.04 ± 0.07	1.37 ± 0.22	**
ZH39	401 ± 89.7	395 ± 13.1	ns	212 ± 38	162 ± 59.2	ns	7.10 ± 1.02	6.41 ± 0.49	ns	2.34 ± 0.15	2.70 ± 0.16	*
ZH40	412 ± 26.3	396 ± 68.1	ns	382 ± 84	242 ± 18.8	ns	8.70 ± 0.49	8.37 ± 0.087	ns	4.16 ± 0.18	3.13 ± 0.25	**
ZH41	231 ± 50.3	216 ± 31.2	ns	321 ± 36.8	141 ± 40.5	ns	9.10 ± 0.52	6.26 ± 0.12	**	3.38 ± 0.34	1.63 ± 0.36	**
ZH42	272 ± 65.2	258 ± 20.4	ns	538 ± 117.9	253 ± 19.4	ns	7.37 ± 0.26	9.89 ± 18.39	ns	2.42 ± 0.38	2.26 ± 0.43	ns
ZH43	248 ± 55.6	299 ± 68.7	ns	110 ± 5.3	280 ± 16.6	ns	5.83 ± 0.51	8.47 ± 1.27	*	1.66 ± 0.22	1.89 ± 0.22	ns
ZH44	436 ± 36.4	397 ± 78.6	ns	330 ± 38.3	199 ± 5.8	ns	8.44 ± 1.3	5.93 ± 1.02	ns	3.36 ± 0.26	1.52 ± 0.06	**
ZH48	425 ± 34.2	355 ± 51.6	ns	148 ± 38.4	154 ± 21.6	ns	8.33 ± 0.24	5.85 ± 0.82	*	1.56 ± 0.16	2.87 ± 0.12	**
ZH49	249 ± 43.9	281 ± 15.8	ns	128 ± 16.3	100 ± 15.9	ns	13.1 ± 0.46	7.85 ± 0.54	ns	2.54 ± 0.14	2.13 ± 0.12	**
ZH50	354 ± 56.1	302 ± 56.9	ns	109 ± 6.1	102 ± 4.3	ns	11.3 ± 0.61	4.46 ± 0.49	ns	1.57 ± 0.17	2.23 ± 0.20	**
ZH62	338 ± 47.1	371 ± 52.3	ns	376 ± 6.6	131 ± 21.7	ns	8.29 ± 0.38	7.51 ± 0.29	ns	1.90 ± 0.21	1.24 ± 0.24	*
ZH66	386 ± 40.9	349 ± 48	ns	533 ± 52.9	163 ± 2.6	ns	13.7 ± 0.21	10.7 ± 0.95	**	3.36 ± 0.19	3.34 ± 0.15	ns
ZH69	416 ± 71.3	398 ± 37.8	ns	162 ± 57	400 ± 73.1	ns	6.80 ± 0.29	7.91 ± 1.40	ns	1.90 ± 0.069	2.46 ± 0.13	**
ZH70	371 ± 78.8	331 ± 65.7	ns	764 ± 15	235 ± 19.6	ns	6.78 ± 0.60	7.01 ± 1.14	ns	2.52 ± 0.28	1.69 ± 0.30	*
ZH74	313 ± 117.8	378 ± 30.7	ns	217 ± 62.4	320 ± 3.2	*	12.1 ± 0.44	6.93 ± 0.77	**	3.34 ± 0.31	3.42 ± 0.21	ns
ZH75	225 ± 145.1	390 ± 27.7	ns	155 ± 50.1	203 ± 4.4	ns	10.3 ± 0.165	6.25 ± 0.58	**	2.48 ± 0.25	2.98 ± 0.099	*
ZH79	250 ± 38.1	381 ± 46.6	**	210 ± 31.3	228 ± 23	ns	5.70 ± 0.016	11.2 ± 1.45	**	1.60 ± 0.17	2.36 ± 0.19	**
EDU	0.341 ns			<0.0001 [a]			<0.0001 [a]			<0.0001 [a]		
Cultivar	<0.0001 [a]			<0.0001 [a]			<0.0001 [a]			<0.0001 [a]		
EDU × Cultivar	0.049 *			<0.0001 [a]			<0.0001 [a]			<0.0001 [a]		

ns: non-significant; APX: ascorbate peroxidase; SOD: superoxide dismutase; POD: peroxidase; CAT: Catalase; FW: fresh weight; ANOVA: analysis of variance.

* $p \leq 0.05$.

** $p \leq 0.01$.

[a] $p \leq 0.001$.

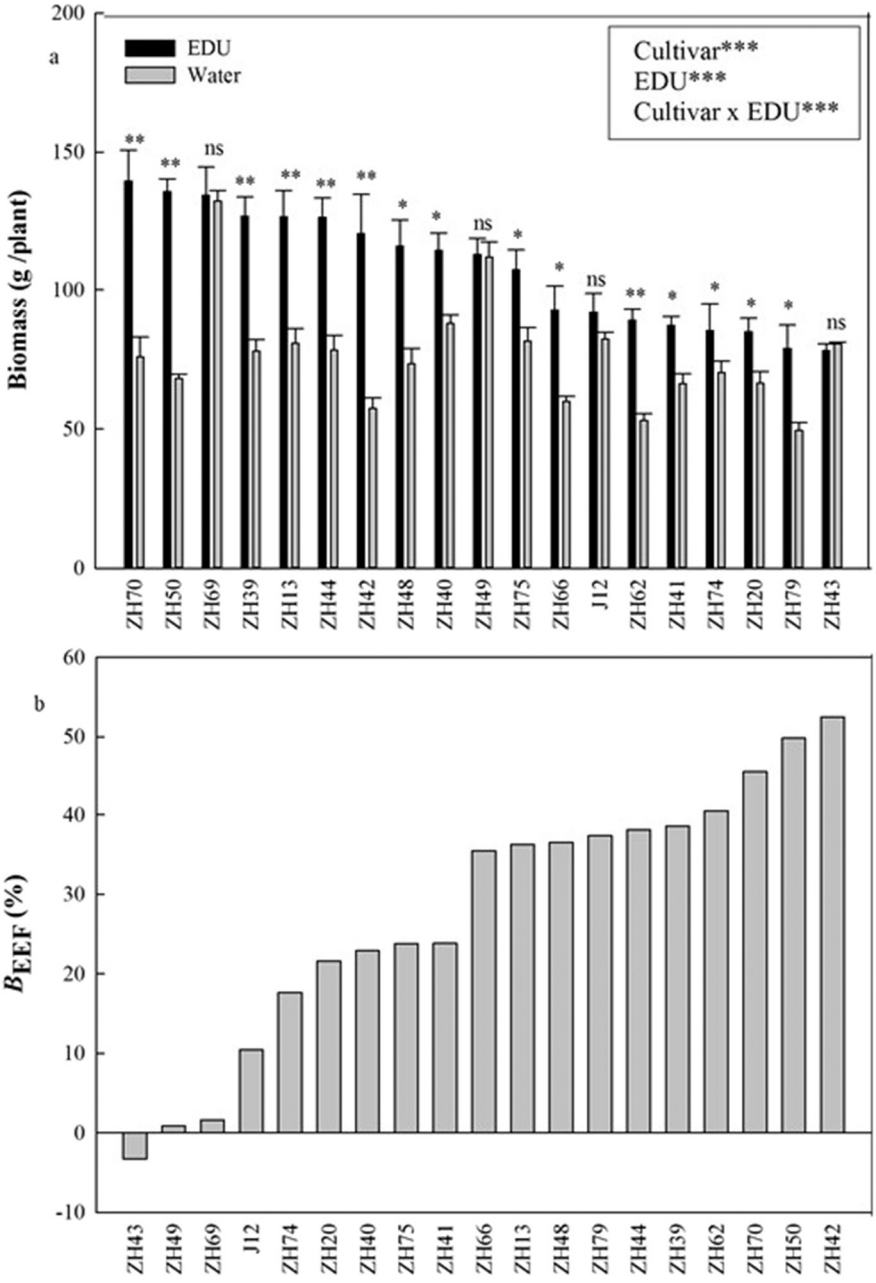

Fig. 2 – Above-ground biomass (mean ± SD) of 19 soybean cultivars exposed to ambient O_3 concentrations (water) or protected by ethylenediurea (EDU), levels of significance showing differences between EDU and water treatments for any cultivar, and two-way ANOVA (EDU × cultivar) results. Cultivars are sorted (a) according to decreasing biomass and (b) according to increasing ozone sensitivity, expressed as percent variation when protected by ethylenediurea. The inset shows the result of a two-way analysis of variance (ANOVA) (cultivar × EDU treatment). $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; ns: non-significant; SD: standard deviation; B_{EFF}: the cultivar-specific sensitivity to O_3; ZH: Zhonghuang; J12: Jidou12.

(Calvo *et al.*, 2007), as well as in North American soybean (Burkey and Carter, 2009). This knowledge may be used for breeding novel O_3-tolerant cultivars as an adaptive strategy to O_3 pollution (Teixeira *et al.*, 2011). As yield is a major plant trait for soybean, further studies including yield are recommended. Current conventional genetic improvement efforts screen for high-yielding cultivars and have indirectly selected genotypes with very high sensitivity to O_3, as demonstrated in wheat (Biswas *et al.*, 2008). Another short-term adaptive strategy in hot-spot areas may thus be the cultivation of soybean cultivars that are both high-yielding and O_3-tolerant (Teixeira *et al.*, 2011), such as our cultivar ZH69, while the most O_3-sensitive cultivars should be excluded.

The sensitivity of plants to O_3 may be influenced by two main factors: O_3 flux controlled by stomata (Fiscus *et al.*, 2005; Gonzalez-Fernandez *et al.*, 2010) and antioxidant capacity determined by antioxidant pools and enzymes (Biswas *et al.*, 2008; Feng *et al.*, 2010; Inada *et al.*, 2012; Su *et al.*, 2017). We

Table 7 – Regression coefficient and significance of the linear regressions between the effect of ambient ozone on biomass (B_{EFF}) of 19 soybean cultivars, *i.e.*, the cultivar sensitivity to ozone, *versus* a number of physiological and biochemical variables (*X*) measured in plants exposed to ozone (X_{Water}) or protected by using ethylenediurea (X_{EDU}).

Variable	X_{EDU}	X_{Water}	X_{EFF}
Biomass	0.366 ns	−0.662 **	–
A_{sat}	0.255 ns	−0.250 ns	0.252 ns
g_s	−0.230 ns	−0.141 ns	0.210 ns
Chl *a*	0.130 ns	−0.820 ns	0.151 ns
Chl *b*	0.009 ns	−0.110 ns	0.286 ns
Chl *a* + *b*	0.095 ns	−0.105 ns	0.158 ns
Car	−0.018 ns	−0.234 ns	0.136 ns
MDA	−0.332 ns	0.477 *	−0.617 **
TAC	0.009 ns	−0.206 ns	0.148 ns
AsA	−0.468 *	−0.137 ns	−0.118 ns
SOD	0.263 ns	0.003 ns	0.263 ns
APX	0.452 ns	−0.265 ns	0.534 *
CAT	0.001 ns	−0.140 ns	0.096 ns
POD	−0.160 ns	−0.152 ns	0.005 ns

The effect of ozone (X_{EFF}) was estimated as $X_{EFF} = (X_{EDU} - X_{Water})/X_{EDU} \times 100$.
* $p \leq 0.05$.
** $p \leq 0.01$.

assessed the main physiological and biochemical responses to understand which parameters were the most important as predictors of O_3-sensitivity in these cultivars and whether the sensitivity mechanisms were similar in the different cultivars. All gas exchange, pigment and biomass responses were consistent in a meta-analysis on EDU effects on 15 crop species exposed to O_3 (Feng *et al.*, 2010), even though the magnitude of changes was higher in our experiment likely due to exposure to higher O_3 concentrations. Also, the responses of antioxidant metabolites and enzymes were consistent with results in the literature for other species treated by EDU (Hassan, 2006; Paoletti *et al.*, 2008; Singh *et al.*, 2009; Pandey *et al.*, 2015). Interestingly, our cultivars differed in both constitutional and O_3-induced levels of all variables but g_s. Ozone responses may have been affected by the high variability following the stomatal sluggishness that is a typical response to O_3 (Hoshika *et al.*, 2013b, 2014), although a decline of g_s is a common response to O_3 (Booker *et al.*, 2009). However, no difference in g_s among cultivars means that this trait cannot be used for selecting O_3-tolerant cultivars.

An analysis on how the different variables were related to O_3 sensitivity (B_{EFF}) across the cultivars showed significant correlations with: MDA in plants exposed to ambient O_3 (MDA_{Water}); percent variation of MDA and APX when plants were protected by EDU (MDA_{EFF} and APX_{EFF}); biomass in plants exposed to ambient O_3 (B_{Water}); and constitutional content of AsA in plants protected by EDU (AsA_{EDU}). MDA is a marker of O_3-induced lipid peroxidation in the plant membranes (Feng *et al.*, 2011). This is why MDA_{Water} was higher in the most sensitive cultivars, which did not possess efficient mechanisms of membrane protection from O_3 injury. As a consequence, MDA_{EFF}, *i.e.*, the difference in lipid peroxidation between EDU-protected and non-protected plants, was higher in the most sensitive cultivars. A higher APX_{EFF} in the most sensitive cultivars was likely due to an excess of H_2O_2, as APX catalyzes the reduction of H_2O_2 by AsA (Chernikova *et al.*, 2000). Such elevated oxidative stress translated into lower B_{Water} in the sensitive cultivars, which is a well-known impact of O_3 (Feng and Kobayashi, 2009). The only constitutional factor that explained the cultivar tolerance well was AsA_{EDU}, as it was lower in the most sensitive cultivars when protected by EDU. While it is well known that the direct reaction of O_3 with cell wall ascorbate is a central mechanism of plant tolerance to this pollutant (Plöchl *et al.*, 2000), this is the first proof linking higher intra-specific O_3 tolerance with higher total ascorbate in soybean. This knowledge will help in breeding for more and more O_3-tolerant cultivars.

4. Conclusions

This was the first experimental study to show that ambient O_3 is able to threaten the growth, physiological and biochemical responses of soybean in China. The results suggested that current O_3 pollution is a serious concern for soybean, which highlights the urgent need for policy-making actions protecting this critical staple legume species for food security in China. Fortunately, cultivars showed a considerable variability in their sensitivity to O_3, which gives guidance to farmers in choosing the best-suited cultivars for local cultivation. Among the most tolerant cultivars, ZH69 also showed excellent biomass productivity and should be tested for the quantity and quality of yield production.

These important results were obtained by applying the antiozonant EDU as a tool for evaluating the O_3 sensitivity of plants. Although preliminary results are encouraging (Agathokleous *et al.*, 2016a), toxic side effects on the food chain by this synthetic chemical cannot yet be excluded, and thus EDU can be recommended only as a scientific tool and not as an O_3 protectant in common agricultural practice.

The cultivars showed some interesting similarities in their responses to O_3. We thus conclude that higher lipid peroxidation (MDA_{Water} and MDA_{EFF}) and activity of the ascorbate peroxidase enzyme (APX_{EFF}) were responses to O_3 injury, which eventually translated into lower biomass production (B_{Water}). Rather than these factors, the constitutional level of total ascorbate (AsA_{EDU}) in the leaves was the major parameter explaining soybean cultivar sensitivity to O_3. This result will guide future breeding efforts towards more O_3-tolerant soybean cultivars in China, while strategies for implementing control measures of regional O_3 pollution are being implemented.

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