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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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A B S T R A C T

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 (Singh *et al.*, 2011; Paoletti *et al.*, 2009a; 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 d), 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.*, 2009a, 2009b; 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