JOURNAL OF ENVIRONMENTAL SCIENCES XX (2017) XXX–XXX

Available online at www.sciencedirect.com

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JES JOURNAL OF ENVIRONMENTAL SCIENCES www.jesc.ac.cn

Protecting the photosynthetic performance of snap bean under free air ozone exposure

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ARTICLE INFO

Article history:
Received 17 February 2017
Revised 7 March 2017
Accepted 8 May 2017
Available online xxxx

Keywords:
Ethylenediurea
Free air controlled exposure
Kinetin
Ozone
Phaseolus vulgaris

ABSTRACT

Tropospheric ozone (O_3) is a major air pollutant and causes serious injury to vegetation. To protect sensitive plants from O_3 damage, several agrochemicals have been assessed, including cytokinin (*e.g.*, kinetin, KIN) and ethylenediurea (EDU) with cytokinin-like activity. In higher plant, leaves are primarily injured by O_3 and protective agrochemicals are often applied by leaf spraying. To our knowledge, the mitigating abilities of EDU and KIN have not been compared directly in a realistic setup. In the present research, impacts of elevated O_3 (2× ambient O_3, 24 hr per day, for 8 days) on an O_3 sensitive line (S156) of snap bean (*Phaseolus vulgaris*), which is often used for biomonitoring O_3 pollution, were studied in a free air controlled exposure system. The day before starting the O_3 exposure, plants were sprayed with a solution of EDU (300 ppm), KIN (1 mmol/L) or distilled water, to compare their protective abilities. The results demonstrated that 2× ambient O_3 inhibited net photosynthetic rate and stomatal conductance, increased the minimal fluorescence yield of the dark-adapted state, decreased the maximal quantum yield of PSII photochemistry, and led to visible injury. KIN and EDU alleviated the reduction of the photosynthetic performance, and visible injury under O_3 fumigation. The plants sprayed with EDU showed greater ability to mitigate the O_3 damage than those sprayed with KIN. Chlorophyll fluorescence imaging may have detected more precisely the differences in O_3 response across the leaf than the conventional fluorometer.

Introduction

Ground level ozone (O_3) is one of the major air pollutants and is a serious threat to crops, forests, and natural vegetation (Feng et al., 2003; Ainsworth et al., 2012; Matyssek et al., 2013;Feng et al., 2014; Agathokleous et al., 2016; Izuta, 2017). Ozone enters the leaf through the stomata and reacts with the apoplast of the mesophyll cells, generating reactive oxygen species (ROS) and provoking signaling cascades, which cause visible foliar injury, decrease stomatal conductance, and inhibit net photosynthetic rate (Morgan et al., 2003;Tiwari et al., 2016).

In order to protect sensitive vegetation from O_3 pollution, several kinds of agrochemicals have been tested (Didyk and Blum, 2011). Among them, ethylenediurea (EDU) is the most frequently studied O_3-protectant chemical (Manning, 2000; Paoletti et al., 2009a; Manning et al., 2011). It is able to protect plants from O_3 injury by mitigating the loss of CO_2 assimilation and delaying O_3-induced accelerated senescence (Tiwari and Agrawal, 2010; Pandey et al., 2014). Nonetheless, in the

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http://dx.doi.org/10.1016/j.jes.2017.05.009
1001-0742/

Please cite this article as: Zhang, L., et al., Protecting the photosynthetic performance of snap bean under free air ozone exposure, J. Environ. Sci. (2017), http://dx.doi.org/10.1016/j.jes.2017.05.009

last few years other types of agrochemicals are widely examined to find alternatives to EDU. It has been reported that cytokinin such as kinetin (KIN) could act as a protectant to cope with oxidative damage such as O_3 pollution (Rattan, 2004; Zhang et al., 2017). The antiozonant ability of EDU and KIN was compared by Lee and Chen (1982) using the tobacco callus bioassay. In higher plant, however, leaves are primarily injured by O_3 and protective agrochemicals are often applied by leaf spraying. This is the first study to compare the mitigating abilities of EDU and KIN in a realistic experimental setup.

It has been well known that some genotypes of bean (*Phaseolus vulgaris* L.) are sensitive to O_3 pollution, and have been widely used as model plants for bioindicating or biomonitoring O_3 pollution due to a typical symptomology and limited cultivation requirements (Sanders et al., 1992; UN/ECE, 1996; Burkey et al., 2005, 2012). Typical bronzing (red-brown pigmentation) is usually found in bean plants under elevated O_3 (Feng et al., 2014). In common bean, a decline of CO_2 assimilation was observed in a sensitive cultivar exposed to a short time and acute O_3 pollution (Guidi et al., 2009). Under chronic O_3, the maximum quantum yield for primary photochemistry (F_v/F_m) was inhibited in the sensitive genotype ('S156') of snap bean (Flowers et al., 2007). Moreover, it has been reported that higher O_3 sensitivity of bean cultivar 'Cannellino' could be partially attributed to higher stomatal conductance and lower ability to dissipate excess energy (Guidi et al., 2010). Although it has been proved that EDU is effective for protecting sensitive genotypes of bean under O_3 stress (Elagöz and Manning, 2005), a comparative study on the ameliorating effects of EDU and KIN on O_3 stressed bean plant has not been reported.

The objective of this research therefore was to compare the mitigating effects of EDU and KIN on leaf visible injury, gas exchange, and chlorophyll fluorescence parameters of snap bean under O_3 free air controlled exposure (FACE). The results of this study can provide hints to the protection of crops in O_3-polluted areas.

1. Materials and methods

1.1. Plant material

Snap beans (*P. vulgaris* 'S156', source K.O. Burkey, USDA, USA) were planted in plastic pots (17 cm in diameter, 2 L), filled with a mixture of peat and vermiculite (3:1, *V*/*V*). After they had at least two fully expanded trifoliate leaves (4-week old), the seedlings were moved from the experimental garden to the O_3 FACE facility for the treatments as described below. Plants were irrigated every day until maximum soil water storage capacity to avoid drought stress. The hourly mean temperature, relative humidity, and photosynthetic photon flux density (PPFD) at the plant height were (25.5 ± 0.4)°C, (49.3 ± 1.4)%, and (616.7 ± 53.5) μmol photon/(m^2·sec), respectively. The precipitation was 2.7 mm during the treatment period.

1.2. Treatments

Two O_3 treatments were applied: ambient air and 2× ambient O_3. Spraying treatments included two agrochemicals: EDU (Source W.J. Manning, University of Massachusetts, USA) and KIN (Source Duchefa, Haarlem, the Netherlands). Distilled water was selected as control. EDU (300 ppm) was used because this concentration is effective in ameliorating O_3 injury in this genotype as reported before (Paoletti et al., 2014). KIN (1 mmol/L) was selected as in our previous study (Zhang et al., 2017). This concentration was able to mitigate O_3-caused leaf visible injury better than 0.1or 0.01 mmol/L as per our preliminary experiment (unpublished). The EDU formulation was a 100% wettable powder. The day before O_3 exposure, EDU was dissolved in warm distilled water, and then applied when it was back to ambient temperature. Due to insoluble in water, KIN (0.5 mmol) was dissolved in 1 mol/L NaOH (10 mL) according to the instruction and the solution was diluted into 500 mL using distilled water. Leaves were sprayed to dripping point with distilled water or 300 ppm EDU solution, or 1 mmol/L KIN solution. Exposure to ambient air or 2× ambient O_3 was carried out for eight consecutive days from 13th to 20th August 2016, 24 hr per day, in a FACE facility (for a description of this facility, see Paoletti et al., 2017). Ozone concentration was continuously monitored by O_3 analyzers (Model 202, 2B Technologies Inc., Boulder, Colorado, USA). Ozone concentration was expressed as AOT40, *i.e.*, the sum of the differences between hourly O_3 concentrations and 40 ppb for each hour when the concentration is above 40 ppb during daylight hours (short wave radiation >50 W/m^2) according to CLRTAP (2015). In ambient air, the daily mean O_3 concentration was (34.8 ± 1.3) ppb, the maximum hourly O_3 concentration was 60.3 ppb, and AOT40 was 0.61 ppm/hr. Under elevated O_3 exposure, the daily mean O_3 concentration was (66.8 ± 1.9) ppb, the maximum hourly O_3 concentration was 120.5 ppb, and AOT40 was 4.17 ppm/hr.

1.3. Visible injury assessment

On the first day after the end of O_3treatments, the percentage of injured surface per symptomatic leaflet (IA) and the percentage of injured leaflets per plant (LA) were visually assessed by two surveyors with the help of photoguides (Innes et al., 2001; Paoletti et al., 2009b). A plant injury index (PII) was calculated as (LA × IA) / 100 according to Paoletti et al. (2014).

1.4. Gas exchange measurement

Instantaneous gas exchange was measured on the top leaflet of the second fully expanded trifoliate leaf from 09:00 AM to 12:30 PM on the first day after the end of O_3 exposure using a portable system (Li-6400, Li-Cor, USA). Six plants per treatment were used for measurement. The second fully expanded trifoliate leaf under 2× O_3 treatment showed visible injury and those in ambient air were asymptomatic. According to Yuan et al. (2015), light intensity inside the leaf chamber and was set to 1500 μmol photon/(m^2·sec) PPFD to achieve saturation light. Leaf temperature inside the leaf chamber was set to (25 ± 0.5)°C in accordance with the hourly mean temperature during experiment. CO_2 concentration (400 ± 1 μmol/mol) was used as reference (Yuan et al., 2015). Net photosynthetic rate under saturating PPFD (P_N), stomatal conductance (g_s),

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