Plant Physiology and Biochemistry 82 (2014) 183–193

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Apoplastic antioxidant enzyme responses to chronic free-air ozone exposure in two different ozone-sensitive wheat cultivars

Junli Wang [a, b], Qing Zeng [a], Jianguo Zhu [a, *], Chen Chen [a, b], Gang Liu [a], Haoye Tang [a]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Sciences, Chinese Academy of Sciences, Nanjing 210008, PR China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 4 December 2013
Accepted 10 June 2014
Available online 18 June 2014

Keywords:
Apoplast
Ozone stress
Wheat cultivar
Reactive oxygen species
Antioxidant enzymes

ABSTRACT

The effects of elevated ozone concentrations [O_3] on two different ozone-sensitive wheat (*Triticum aestivum* L.) cultivars [Yangmai16 (Y16) and Yannong19 (Y19)] were investigated to determine the different apoplastic antioxidant mechanisms under O_3-FACE (free-air controlled enrichment) condition. The results indicated that elevated [O_3] (1.5 × ambient [O_3]) induced increases in the production of superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2) and lipid peroxidation, and these results were more pronounced in the apoplasts of Y19 than in those of Y16. Apoplastic antioxidant enzymes were developmentally regulated and the effect of elevated [O_3] depended on the developmental stage of wheat for both cultivars. In cultivar Y19, continuous O_3 stress induced a decrease in the activity of apoplastic superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7) and ascorbate peroxidase (APX; EC 1.11.1.11) in the later growing stages, indicating Y19 appears to be the more sensitive cultivar and is prone to oxidative stress. The strategic response of antioxidant enzymes activities by Y16 in four different plant development stages (booting, flowering, filling and ripening) resulted in O_3 stress-induced antioxidant defense responses, which indicated its higher tolerance to O_3 stress. The same patterns of activity of apoplastic SOD and APX isozymes were observed in both Y16 and Y19 cultivars, while POD isozymes differed by cultivar in terms of the pattern of bands. The results of the present study show that O_3 tolerance can be improved by regulating apoplastic ROS metabolism through the responses of apoplastic antioxidant enzymes to O_3 stress in different plant development stages.

1. Introduction

Rising tropospheric ozone concentrations [O_3] are considered the most important consequences of air pollution and are harmful to human health and vegetation (Racherla and Adams, 2008). With increasing emissions of ozone-forming chemicals (nitrogen oxides, carbon monoxide, and hydrocarbons), the current and future impacts of O_3 on agricultural production in certain areas (i.e., Western Europe, Midwestern and Eastern USA and Eastern China) may be very significant (Emberson et al., 2003), resulting in agricultural yield and economic losses in the coming decades (Zhu et al., 2011).

Ozone is taken-up into the leaf interior via the stomata and decomposes in the aqueous matrix associated with the cell wall (i.e. the apoplast) surrounding epidermal, mesophyll and palisade cells, it gives rise ROS such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) (Heath, 1988). One biochemical marker of plant sensitivity to O_3 is an apoplastic ROS burst after O_3 treatment, which is absent or reduced in O_3-resistant plants (Overmyer et al., 2002). It has been reported that apoplast represents the first line of defense against O_3 damage (Baier et al., 2005), therefore antioxidant systems in the apoplast are important for detoxification of ROS derived from O_3.

Wheat (*Triticum aestivum* L.) is the second largest food crop in the world, many studies about wheat antioxidant response to O_3 exposure were based on leaves or symplastic level (Biswas et al., 2008; Feng et al., 2011), and many studies about apoplastic antioxidant responses in wheat were based on other abiotic stresses, for example, low temperature, cold, salt stress and wound (Cakmak and Atici, 2009; Tasgin et al., 2006; Mutlu et al., 2009; Minibayeva et al., 2009). There is limited information available on the

Abbreviations: ROS, reactive oxygen species; AWF, apoplastic washing fluid; NBT, nitro blue tetrazolium; $O_2^{\bullet-}$, superoxide anion; $HO^{\bullet}$, hydroxyl radical; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; POD, peroxidase; APX, ascorbate peroxidase; AsA, ascorbic acid; TBARS, thiobarbituric acid reacting substance; PAGE, polyacrylamide gels.

* Corresponding author. Tel.: +86 25 86881122; fax: +86 25 86881000.
E-mail address: jgzhu@issas.ac.cn (J. Zhu).

http://dx.doi.org/10.1016/j.plaphy.2014.06.004
0981-9428/

apoplastic antioxidant responses of wheat to ambient O_3 pollution. An accurate assessment of the response of wheat to the future ground-level [O_3] is therefore crucial for reducing the current uncertainties in predicting future food security (Wang et al., 2013).

Modern wheat cultivars are reported to be more sensitive to O_3 than older accessions (Biswas et al., 2008), and previous O_3-FACE studies have reported that O_3 impacts the growth, yield, photosynthetic characteristics and apoplastic ascorbic acid (AsA) content of different modern wheat cultivars (Zhu et al., 2011; Feng et al., 2011, 2010). These results have suggested that strong-gluten wheat cultivar Yannong19 (Y19) and weak-gluten wheat cultivar Yangfumai2 (Y2) are relatively sensitive to O_3 and exhibit significant yield losses under conditions of high O_3 levels (Zhu et al., 2011). While medium-gluten wheat cultivar Yangmai16 (Y16) exhibits less yield loss than Y19 and Y2, and higher photosynthetic rates and apoplastic AsA content than Y2 under similar O_3 conditions, thus Y16 is considered relatively tolerant to O_3 (Feng et al., 2011, 2010). However, the different apoplastic antioxidant mechanisms in O_3-tolerant and O_3-sensitive wheat cultivars have been less studied by O_3-FACE experiments, and little is known about the changes of ROS synthesis under chronic O_3 exposure in the apoplast and, in this case, the role played by apoplastic antioxidant enzymes (Feng et al., 2011).

Since the apoplast is the initial site of injury caused by O_3 and/or O_3-generated ROS during the oxidative burst, we studied the response of the apoplastic antioxidant enzymes to O_3 in two wheat cultivars with different O_3-sensitivities throughout the entire growing stage under fully free-air O_3 exposure. The principle objective was to determine whether O_3 induces different antioxidant responses in the apoplast of the two cultivars.

2. Materials and methods

2.1. Site description

The study was conducted in a paddy field in Xiaoji Town, Jiangdu County, Jiangsu Province, China (119°42'0"E, 32°35'5"N). The site has been in continuous cultivation for more than 1000 years with rice–wheat or rice–rapeseed rotation. Shajiang Aquic Cambosols with a sandy–loamy is the texture of the soil. The annual mean air temperature is 16 °C, total annual sunshine hours are over 2000 h, and the frost-free period are more than 230 days. On average across the period of the O_3 fumigation: March 8 to May 29, 2012, mean air temperature was 15.5 °C (Fig. 1a). Mean daily photosynthetically active radiation was 296.5 μmol $m^{-2}s^{-1}$ (Fig. 1b).

2.2. Ozone fumigation

The O_3-FACE system consists of two type plots: the control plot with ambient [O_3] (A-O_3), and the plot with elevated [O_3] (E-O_3), and there are three replicates (240 m^2) for each type. As described by Feng et al. (2010), the target [O_3] for E-O_3 plots was 50% higher than the ambient [O_3]. All the E-O_3 plots were separated from other plots by at least 70 m to avoid cross-contamination. The design of the experiment and the performance of the [O_3] in E-O_3 were described in detail by Tang et al. (2011).

The O_3 fumigation began on March 8, 2012, and continued throughout the 7 h daytime until harvest. Fig. 2 shows the seasonal changes in 7 h daily (9:00–16:00 Chinese Standard Time) mean [O_3] at the center of the ring averaged across the A-O_3 and E-O_3 plots.

2.3. Plant material

The modern wheat cultivars were Yangmai16 (Y16, medium-gluten wheat), which is O_3-tolerant and Yannong19 (Y19, strong-gluten wheat), which is O_3-sensitive (Zhu et al., 2011; Feng et al., 2011, 2010). Standard cultivation practices as performed in the region were followed in all experimental plots. Wheat seeds were sown on November 23, 2011 with a basic seeding density of 2.25 million ha^{-1} and a row space of 25 cm.

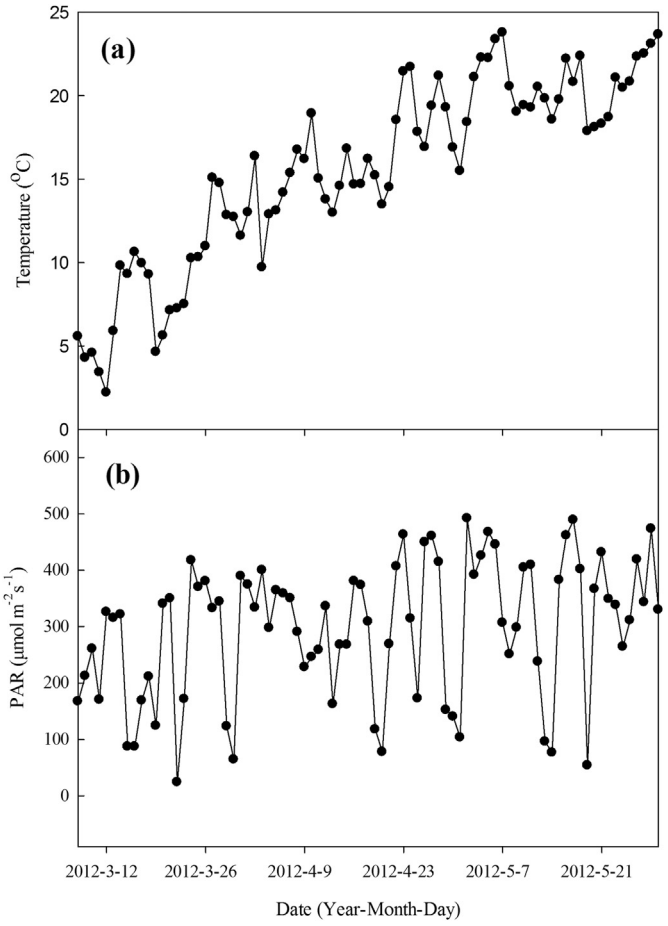

Fig. 1. Daily mean temperature (a) and daily mean photosynthetically active radiation (PAR) (b) data collected at experiment site in March–May, 2012.

The plants in both A-O_3 and E-O_3 plots were surrounded with border plants treated in the same way as the plants inside. The jointing and booting of two cultivars began on April 7 and 21, 2012, respectively. The flowering of Y16 and Y19 began on April 29 and May 2, respectively. The filling of Y16 and Y19 began on May 13 and May 15, respectively. The two cultivars were harvested on June 5. Flag leaves were sampled in all the plots at 10:30 A.M. on April 24 (continuously O_3 exposure 45 days, booting of both cultivars), May 6 (continuously O_3 exposure 57 days, flowering of both cultivars), May 18 (continuously O_3 exposure 69 days, filling of both cultivars), May 26 (continuously O_3 exposure 77 days, ripening of both cultivars), and were frozen with liquid nitrogen until analysis.

2.4. Extraction of apoplastic washing fluid (AWF)

Soluble apoplastic enzymes were extracted by vacuum infiltration as described by Polle et al. (1990). Fresh wheat leaves (2 g) were washed three times with distilled water, and subsequently vacuum infiltrated for 15 min at −0.9 kPa and 4 °C with 10 mL 50 mM Mes/KOH buffer (pH 6.0) containing 40 mM KCl and 2 mM $CaCl_2$. The leaves were then blotted gently, loaded into a perforated centrifuge tube (5 mL, 1 cm in diameter), and placed in an eppendorf tube (1.5 mL). AWF was recovered by centrifugation (10 min,