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Differential effects of ozone on photosynthesis of winter wheat among cultivars depend on antioxidative enzymes rather than stomatal conductance

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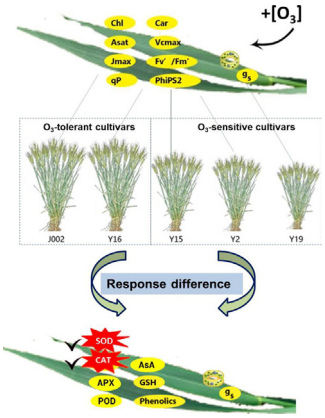
HIGHLIGHTS

- 5 modern cultivars of wheat were investigated under fully open-air field conditions.
- Significant O_3 effects were only found during the mid-grain filling stage.
- The lower photosynthetic rates were mainly due to nonstomatal factors.
- Antioxidative enzymes contributed to the differential response to E-O_3 among cultivars.

GRAPHICAL ABSTRACT



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ABSTRACT

Five modern cultivars of winter wheat (*Triticum aestivum* L.): Yangmai16 (Y16), Yangmai 15 (Y15), Yangfumai 2 (Y2), Yannong 19 (Y19) and Jiaxing 002 (J2) were investigated to determine the impacts of elevated ozone concentration (E-O_3) on photosynthesis-related parameters and the antioxidant system under fully open-air field conditions in China. The plants were exposed to E-O_3 at 1.5 times the ambient ozone concentration (A-O_3) from the initiation of tillering to final harvest. Pigments, gas exchange rates, chlorophyll *a* fluorescence, antioxidants contents, antioxidative enzyme activity and lipid oxidation were measured in three replicated plots throughout flag leaf development. Results showed that significant O_3 effects on most variables were only found during the mid-grain filling stage. Across five cultivars, E-O_3 significantly accelerated leaf senescence, as indicated by increased lipid oxidation as well as faster declines in pigment amounts and photosynthetic rates. The lower photosynthetic rates were mainly due to non-stomatal factors, e.g. lower maximum carboxylation capacity and electron transport rates. There were strong interactions between O_3 and cultivar in photosynthetic pigments, light-saturated photosynthesis rate and chlorophyll a fluorescence with O_3-sensitive (Y19, Y2 and Y15) and O_3-tolerant (J2, Y16) cultivars being clearly differentiated in their responses to E-O_3. E-O_3 significantly influenced the antioxidative enzymes but not antioxidant contents. Significant interactions between O_3 and

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cultivar were found in antioxidative enzymes, such as SOD and CAT, but not in stomatal conductance (g_s). Therefore, it can be concluded that antioxidative enzymes rather than g_s or antioxidants are responsible for the differential responses to E-O_3 among cultivars. These findings provide important information for the development of accurate modeling O_3 effects on crops, especially with respect to the developmental stage when O_3 damage to photosynthesis becomes manifest.

1. Introduction

Ground-level ozone (O_3) is the most important phytotoxic air pollutant at global scale with adverse impacts on terrestrial vegetation (Ashmore, 2005; Booker et al., 2009; Bytnerowicz et al., 2007; Feng and Kobayashi, 2009; Feng et al., 2014, 2015; Van Dingenen et al., 2009). A meta-analysis based on numerous chamber studies revealed that elevated O_3 concentration (average 73 ppb) reduced leaf photosynthesis and grain yield of wheat by 20% and 29%, respectively, as compared with plants grown in carbon-filtered air (Feng et al., 2008). The grain filling period is the most sensitive developmental period to O_3, during which the decreased photosynthesis induced by elevated O_3 concentration appears to be the key driver for the yield loss (Gelang et al., 2000; Pleijel et al., 1998). Although the photosynthetic rate in saturating light (A_{sat}) is often co-limited by stomatal and biochemical factors under field conditions, many scientists argued that the central biochemical process rather than the stomatal limitation (L_s) of A_{sat} was the main cause for the O_3-induced reduction in photosynthesis (Farage and Long, 1999; Feng et al., 2011; Fiscus et al., 1997; Morgan et al., 2004; Zheng et al., 2002). Among the non-stomatal factors, maximum carboxylation (V_{cmax}), reflecting in vivo Rubisco activity, as well as the maximum rate of RuBP regeneration (J_{max}) have been reported to play the most important role (Farage and Long, 1999; Fiscus et al., 2005; Morgan et al., 2004; Sun et al., 2014). Other important physiological responses reported to be affected by O_3 include the changes in chlorophyll *a* fluorescence variables such as reduction in quenching of photochemical efficiency (qP) and quantum yield of PSII in the light (PhiPS2) (Feng et al., 2011), as well as endogenous antioxidant metabolism (Inada et al., 2012).

Many studies have been done to clarify the mechanism of plant defense to O_3 damage in relation to detoxification of reactive oxygen species (ROS) (Dizengremel et al., 2008; Paoletti et al., 2008), and reported that the responses of antioxidant metabolism varied by species/cultivars, depending on the concentrations of O_3 and the duration of the fumigation, e.g. acute vs. chronic ozone exposure (Baier et al., 2005; Betzelberger et al., 2010; Burkey et al., 2003; Feng et al., 2011; Kollist et al., 2000; Wang et al., 2014a, 2014b). In general, acute exposure to relatively high O_3 concentrations often caused leaf lesions resulting from programmed cell death associated with plant-pathogen interaction (Kangasjarvi et al., 2005; Overmyer et al., 2003; Rao et al., 2000) and induces an increase in antioxidant metabolism (Conklin and Barth, 2004; Kangasjarvi et al., 1994). The chronic exposure to relatively low O_3 concentrations always induces declined photosynthesis along with accelerated leaf senescence and consequently reductions in productivity and yield (Ashmore, 2005; Burkart et al., 2013; Feng and Kobayashi, 2009; Feng et al., 2008; Feng et al., 2011; Zhu et al., 2011). Recent evidence showed an increased total antioxidant capacity of soybean plants when chronically exposed to the elevated O_3 concentration (Gillespie et al., 2011, 2012). These authors also suggested that the antioxidant metabolism was primed by previous exposure to oxidative stress, indicating the importance of the endogenous antioxidant metabolism in detoxifying ROS. However, the results from soybean plants contrast with earlier studies conducted in wheat in O_3-FACE platform with two cultivars, which generally showed a declining trend of antioxidant pools and key enzymes in the apoplast and leaf tissues under O_3 stress (Feng et al., 2010; Wang et al., 2014a, 2014b).

In the present study, therefore, we examined the photosynthetic potential and antioxidant metabolism in the leaf tissues among five wheat cultivars in order to determine whether there are different responses to O_3 among cultivars, and if so, which factors contribute to the differences. To this aim, we re-examined part of the data set presented in Feng et al. (2011) and compared them with new data obtained under the same experimental conditions.

2. Materials and methods

2.1. Experiment site

The fully open-air O_3 fumigation system (O_3-FACE) is located in a paddy field in Xiaoji Town, Jiangsu Province, China (119° 42′ E, 32° 35′ N) with continuous rice/wheat or rice/rapeseed rotation for >1000 years. The soil is Shajiang Aquic Cambisols with a sandy-loamy texture. The site belongs to the subtropical marine climatic zone with mean annual precipitation and mean annual temperature being 1100–1200 mm and 16 °C, respectively. Total annual sunshine hour is >2000 h and frost-free period >230 days. During the growth period from 1st March (around turning green stage) to final harvest in this study, mean daily maximum and minimum temperature were 20.6 and 10.1 °C, respectively. Mean daily maximum photosynthetic photon flux density (PPFD) was 1376 $mmolm^{-2}$ s^{-1} and accumulated precipitation was 171 mm.

Table 1
ANOVA results for the photosynthesis related parameters measured across the measurements at heading, flowering, early-grain filling, and mid-grain filling stages.

Variables	O_3	Cultivar (C)	Date (D)	O_3*C	O_3*D	O_3*C*D
A_{sat}	0.293	0.017	<0.001	0.546	<0.001	0.001
Ci	0.720	0.041	<0.001	0.369	<0.001	<0.001
g_s	0.395	0.061	<0.001	0.383	0.001	0.171
V_{cmax}	0.986	0.001	<0.001	0.601	<0.001	<0.001
J_{max}	0.478	<0.001	<0.001	0.661	<0.001	0.003
L_s	0.609	0.005	<0.001	0.089	<0.001	0.041
V_{cmax}/J_{max}	0.616	0.012	0.001	0.456	<0.001	<0.001
Fv′/Fm′	0.128	0.054	<0.001	0.916	<0.001	0.001
PhiPS2	0.260	0.003	<0.001	0.226	<0.001	0.003
$PhiCO_2$	0.129	0.901	<0.001	0.624	<0.001	<0.001
qP	0.651	0.002	<0.001	0.144	<0.001	0.003
Chl	0.695	0.154	<0.001	0.803	<0.001	<0.001
Car	0.852	0.447	<0.001	0.993	<0.001	0.007

Table 2
ANOVA results for the antioxidant-related parameters across the measurements at flowering and mid-grain filling stages.

Variables	O_3	Cultivar (C)	Date (D)	O_3*C	O_3*D	O_3*C*D
APX	0.351	0.010	0.001	0.335	0.035	0.319
POD	0.471	<0.001	0.001	0.891	0.839	0.497
CAT	0.917	0.177	<0.001	0.471	0.005	<0.001
SOD	0.978	<0.001	<0.001	0.666	0.084	0.049
GSH	0.981	0.013	0.006	0.261	0.697	0.248
Phenolics	0.684	0.002	<0.001	0.379	0.375	0.032
MDA	0.537	0.552	<0.001	0.931	<0.001	0.038
AsA	0.100	<0.001	<0.001	0.537	<0.001	0.430