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Ozone pollution effects on gas exchange, growth and biomass yield of salinity-treated winter wheat cultivars

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HIGHLIGHTS

- O_3 tolerance in plants is closely correlated with plant stomatal conductance.
- Both O_3 and salinity may limit plant growth and biomass yield of winter wheat.
- No cross-resistance interactions exist between O_3 and salinity in winter wheat.
- Significant injuries occur under salinity and O_3 regardless of salt sensitivity.

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ABSTRACT

A sand-culture experiment was conducted in four Open-Top-Chambers to assess the effects of O_3 on salinity-treated winter wheat. Two winter wheat cultivars, salt-tolerant *Dekang961* and salt-sensitive *Lumai15*, were grown under saline (100 mM NaCl) and/or O_3 (80 ± 5 nmol mol^{-1}) conditions for 35 days. Significant ($P < 0.05$) O_3-induced decreases were noted for both cultivars in terms of gas exchange, relative water content, growth and biomass yield in the no-salinity treatment. Significant ($P < 0.01$) corresponding decreases were measured in *Dekang961* but not in *Lumai15* in the salinity treatment. Soluble sugar and proline contents significantly increased in both cultivars in combined salinity and O_3 exposure. O_3-induced down-regulation in the gradients of *A*-C_i and *A*-PPFD response curves were much larger in *Dekang961* than in *Lumai15* under saline conditions. Significant ($P < 0.05$) interactions were noted in both salinity × cultivars and salinity × O_3 stresses. The results clearly demonstrated that O_3 injuries were closely correlated with plant stomatal conductance (g_s); the salt-tolerant wheat cultivar might be damaged more severely than the salt-sensitive cultivar by O_3 due to its higher g_s in saline conditions.

1. Introduction

Within the context of global change, ozone (O_3) is becoming a common stress that is limiting crop growth and productivity (Ashmore, 2005; Biswas et al., 2008; Wang et al., 2007). High O_3 concentrations may cause severe detrimental effects including visible foliar injury, limited plant growth and accelerated senescence (Calatayud et al., 2003; Cardoso-Vilhena et al., 2004; Witting et al., 2009). More specifically, the main negative effects of O_3 target photosynthetic performance, including stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and leaf transpiration rate (E) (D'haese et al., 2005; Emberson et al., 2000, 2001; Farage and Long, 1999). *A*-C_i and *A*-PPFD response curves may indicate O_3/salinity injuries to plants under stresses (Biswas and Jiang, 2011). However, little is known about O_3 tolerance in salinity-stressed winter wheat, nor differences between cultivars with contrasting salt sensitivity.

Salinity is a severe stress that limits crop growth and productivity worldwide (Fricke, 2004). About 10% of croplands are salinity-stressed in China, and these croplands are expanding due to poor irrigation practices (Zhu et al., 2001). Winter wheat is one of the major food crops in China and occupies about 80.4% of the total area of wheat planted. In addition to global climate change, the interaction of salinity and O_3 stress is negatively affecting winter wheat plant growth and productivity (Zhang et al., 2003; Zheng et al., 2008b). Traditionally, salt-tolerant winter wheat cultivars are used in saline croplands to increase productivities (Zheng et al., 2008b). Salinity limits plant growth mainly

Abbreviations: *A*, net photosynthetic rate; A_{sat}, light-saturated net photosynthetic rate; g_s, stomatal conductance; C_i, intercellular CO_2 concentration; PPFD, photosynthetic photon flux density; *E*, transpiration rate.

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through damaging the photosynthetic system, including g_s and C_i down-regulation, resulting in the reduction of photosynthetic capacity (Chen and Murata, 2002; Chen et al., 2005). A salinity-induced decrease of the K^+/Na^+ ratio by both K^+ leakage and Na^+ accumulation is another key indicator in limiting plant growth. Our previous studies have shown that the maintenance of a higher K^+/Na^+ ratio is key in retaining a higher salt tolerance in salinity-stressed winter wheat (Zheng et al., 2008b, 2010). The increase of soluble sugar and proline contents is crucial in enhancing plant osmotic regulation abilities in saline conditions (Khan and Soja, 2003; Massman, 2004). De Campos et al. (2011) report that proline is also involved in counteracting oxidative damages except in osmotic adjustment. O_3 stress may also increase soluble sugar and proline contents in winter wheat (Chen and Murata, 2002; Shi et al., 2009). However, little is known about the responses of soluble sugar and proline contents to the interaction between salinity and O_3 stresses.

The seedling stage of winter wheat is considered the most sensitive stage to stresses (Feng et al., 2008; Fiscus et al., 2005). Therefore, in this study, physiological and biochemical responses to O_3 and/or salinity stresses were investigated in two winter wheat cultivars with contrasting salt sensitivity at their seedling stages.

2. Material and methods

2.1. Plant culture

This study was conducted in four Open-Top-Chambers (OTCs, 1.2 m diameter, 1.6 m height) that were installed in a temperature-controlled double-glazed greenhouse. The experiment was designed as a split–split plot, with ozone as the main plot factor, and then cultivar as the sub plot and salinity as the sub-sub plot. Salt-tolerant and salt-sensitive winter wheat cultivars (*Dekang961* and *Lumai15*) were exposed to O_3 and/or salinity conditions to assess the salinity-induced modulation of O_3 tolerance. Thirty seeds of each species were separately sown in each of 48 plastic boxes (length × width × height = 26 × 16 × 10 cm^3) filled with 1.5 kg sterilized sand. Twenty-four boxes were irrigated with full-strength Hoagland solution, and the other 24 boxes were irrigated with 100 mM NaCl modified Hoagland solution. Afterward, 6 no-salinity + 6 salinity-treated boxes were immediately moved into each of the four OTCs, which were ventilated continuously (24 h day^{-1}) with charcoal-filtered air (CF air, <5 ppb O_3). The average air velocity in the chambers corresponded to approximately one complete air change per minute. O_3 was generated by electrically discharging ambient air using an O_3 generator (*JQ-6A, Beijing,* China). Elevated O_3 was treated by dispensing O_3 in the CF air stream before entering two of the four OTCs for 8 h day^{-1} (09:00–17:00). CF plants were used as controls, including a no-salinity control and a salinity-treated control. O_3 concentrations in the OTCs were continuously monitored by an O_3 analyzer (*APOA-360, Horiba, Kyoto*, Japan) to ensure a concentration of 80 ± 5 ppb (hourly mean ± S.E). To minimize the effects of environmental heterogeneity on plant responses, locations of boxes were randomized within each chamber every 3 days. Water lost by evapotranspiration was replenished daily during the experiment.

Plants were thinned to 20 individuals for each cultivar per box at 10 d after planting. Physiological and biochemical parameters of experimental seedlings were measured on the 35th day after treatments began. The maximum photosynthetic photon flux density (PPFD) in chambers was 1600 mmol m^{-2} s^{-1} at canopy height during the 14 h photoperiod. The temperature in the OTCs fluctuated from 17 °C (night) to 36 °C (day), and the relative humidity (RH) was 75–86%.

2.2. Gas exchange

Gas exchange was measured on the most recently fully-expanded leaves using a portable Gas Exchange Fluorescence System (*GFS-3000, HeinzWalz, Pfullingen*, Germany) connected to a PC fitted with data acquisition software (GFS-Win) 35 d after treatments. Three no-salinity and three salinity-treated seedlings were randomly selected from each OTC for gas exchange measurements, and in total, six replicates (3 samples/OTC × 2 OTCs) were obtained for each treatment. Light-saturated net photosynthetic rate (A_{sat}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) were recorded at a photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1} from an internal light source in the leaf chamber. Relative humidity was maintained at 70%, leaf temperature was set at 25 °C, the flow rate was set at 600 μmol s^{-1}, and CO_2 concentration was maintained at 400 μmol mol^{-1} in the leaf chamber.

A-C_i and *A*-PPFD response curves were recorded automatically for the same leaves using *A*-C_i and *A*-PPFD response curve programs. For *A*-C_i curves, the steady-state rate of net photosynthesis was determined at external CO_2 concentrations of 400, 300, 200, 100, 50, 400, 400, 600, and 800 μmol mol^{-1}. While measuring the *A*-C_i curve, the PPFD was maintained at 1500 μmol m^{-2} s^{-1}. The duration of each step of the *A*-C_i response curves was 4 min, and data were automatically recorded six times. For *A*-PPFD curves, *A* was recorded at PPFDs of 1800, 1500, 1000, 500, 300, 150, 80, 50, 20, and 0 μmol m^{-2} s^{-1}. While measuring the *A*-PPFD curve, the CO_2 concentration in the leaf chamber was maintained at 400 μmol mol^{-1}. The duration of each step of the *A*-PPFD response curves was 3 min, and data were automatically recorded six times.

2.3. Leaf water potential and relative water content

Leaf water potential (Ψ_l) was measured following the method of Jongdee et al. (2002). The ten most recently fully-expanded leaves from 10 plants that were grown in the same box of each treatment were cut into pieces and spread evenly on the bottom of the sample cup to determine Ψ_l with a Water Potential Meter (*WP4-T*, *Decagon Devices*, *Pullman*, *WA*, USA).

To measure relative water content (RWC), the ten most recently fully-expanded leaves were sampled, washed with distilled water and dried with filter paper, following which, fresh mass (f.m.) was recorded. The leaves were then soaked in distilled water in dark conditions at 25 °C for 12 h, and the turgid leaf samples were blotted, dried and weighed to obtain turgid mass (t.m.) values. Afterward, all leaf samples were dried at 75 °C to constant weight, and their dry masses (d.m.) were determined. RWC was calculated using the following formula (Muranaka et al., 2002):

$$\text{RWC}\,(\%) = [(\text{f.m.-d.m.})/(\text{t.m.-d.m.})] \times 100$$

2.4. K^+/Na^+ ratio

Oven-dried leaf samples were finely ground to pass through a 2-mm sieve. About 0.5 g samples were soaked for 12 h in digesting tubes with 10 mL concentrated nitric acid and 3 mL perchlorate acid, then digested at 300 °C for 6 h. The extractions were poured into 50 mL volumetric flasks and filled with deionized water. The amounts of K^+ and Na^+ content were measured using an atomic absorption spectrophotometer (*SP9-400, PYE, Cambridge*, England), and then the K^+/Na^+ ratio was calculated.

2.5. Soluble sugar and proline content

Soluble sugar was measured following the method described by Zheng et al. (2008a). Dry leaf samples (about 0.05 g) were ground and soaked in 6–7 mL distilled water, boiled (100 °C) for 30 min, then cooled and centrifuged at 4000 rpm for 10 min. The extracts were decanted into a 50 mL volumetric flask, and the residues were re-extracted two more times and then completed with distilled water. 0.1 mL extracts and 3 mL anthrone reagent (0.15 g anthrone + 84 mL