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Physiological and visible injury responses in different growth stages of winter wheat to ozone stress and the protection of spermidine

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ABSTRACT

The open top chamber (OTC) method was used in a farmland to study the influence of different levels of O_3 concentrations (40 ppb, 80 ppb and 120 ppb) on the enzymatic activity and metabolite contents of the antioxidation system of the winter wheat leaves during the jointing, heading and milk stage. The protective effect of exogenous spermidine (Spd) against the antioxidation of winter wheat under the O_3 stress was investigated. With the increasing O_3 concentrations and fumigation time, the injuries of the winter wheat leaves were observed to be more serious. For instance, when the O_3 concentration reached 120 ppb, the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and nitrate reductase (NR) in the jointing stage decreased by 50.3%, 64.9%, 75.5% and 92.9%, respectively; peroxidase (POD) and glutathione reductase (GR) increased by 45.1% and 80.5%, respectively; the contents of malondialdehyde (MDA), ascorbic acid (AsA) and reduced glutathione (GSH) increased by 314.3%, 8.4% and 31.7%, respectively; and the soluble protein (SP) content decreased by 47.5%. The O_3 stress also had significant impact on the contents of proline (Pro), NO_3^-–N and NH_4^+–N of the winter wheat leaves. During the heading stage, when the O_3 concentration was 40 ppb and 80 ppb, the content of Pro was 163.9% and 173.2% higher than that in the control group, respectively. But under 120 ppb, it was decreased by 42.4%. Exogenous application of Spd increased the activities of SOD, POD, CAT, APX and GR, as well as the contents of GSH and SP, but decreased the contents of MDA and AsA. This indicates that Spd is an effective antioxidant to relieve the O_3 stress on winter wheat leaves, thereby might be applicable to protect winter wheat from the harm of O_3.

Keywords: *Winter wheat, ozone, injury, antioxidation system, spermidine*

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1. Introduction

The extensive use of fossil–fuel and nitrogen–based fertilizers has dramatically increased emissions of nitrogen oxides (NO_X) and volatile organic compounds (VOCs) into the atmosphere. It was predicted that the concentration of O_3 in the atmosphere will be continuously rising and the pollution is expanding (Fishman, 1991). The surface layer O_3, as one of the most important atmospheric pollutants, has been increasing and became the focus of worldwide researchers and the public (Vingarzan, 2004; Selin et al., 2009). The monitoring data of O_3 concentration in 86 sites in the rural and remote regions of 35 states in US showed that the days with a daily average over 80 ppb numbered 21.1 d each year; and the highest daily average of three months was 54 ppb (McCrady and Andersen, 2000). Recently, researchers reported that the surface layer one–hour average O_3 concentration in Beijing–Tianjin–Tangshan region, Yangtze River Delta and other regions reached as high as 150 ppb (Shao et al., 2006).

Many studies reported that the O_3 stress might cause the following negative effects to plants: injury, retarded growth, decreased stomatal conductance of leaves, lower photosynthesis rate, inhibited growth of plant height and leaf area, accelerated aging, disordered metabolism of carbon and nitrogen and crop yield reduction (Feng et al., 2003; Kontunen–Soppela et al., 2007; Feng et al., 2008; Mills et al., 2009; Wittig et al., 2009; Zhu et al., 2011; Avnery et al., 2013). Huang et al. (2012) studied the influence of O_3 on the visible injury of rice leaves, nitrogen metabolism, contents of saccharides and proteins in rice grains. Van Dingenen et al. (2009) have estimated the risk to crop damage caused by surface ozone based on two types of exposure indicators (seasonally mean daytime ozone concentration, and seasonally accumulated daytime ozone concentration above 40 ppb). It was suggested that crops have great responses to cumulative exposures to O_3 in the range 50–87 ppb in the USA and 35–60 ppb in Europe (Mills et al., 2007). Field experiment studies in European Open Top Chambers Programme (EOTCP) showed that crops yield losses from O_3 pollution occurred by 5–10% and deteriorated crop quality. And also larger losses are expected in the future (Grunhage et al., 2012; Debaje, 2014). Nataliya et al. (2011) estimated economic losses for wheat, rice, maize, and soybean for China, South Korea and Japan to be up to 9% for the cereal crops and 23–27% for soybean or 5 billion dollars for all crops. In Europe, the standard for the protection of vegetation against ozone damage is expressed as a critical level of accumulated ozone concentration above a threshold of 40 ppbV which should not be exceeded during the growing season (3 ppm h for agricultural crops, 5 ppm h for forests) (Van Dingenen et al., 2009).

After entering into the plant, O_3 induces the generation of reactive oxygen species (ROS), including H_2O_2, superoxide radicals (O_2^-) and OH^-. The ROS damages the membrane system of the plants and aggravates the peroxidation of the membrane lipid, leading to physiological function disorder of plants, especially photosynthesis process (Pasqualini et al., 2002; Calatayud et al., 2003). Malondialdehyde (MDA) is an end–product of the radical–initiated oxidative decomposition of polyunsaturated fatty acids; therefore, it is frequently used as a biomarker of oxidative stress.

An enhanced level of lipid peroxidation, as indicated by higher MDA content, can show an oxidative stress under the effect of a high O_3 concentration. During the natural adaptation process, plants also develop a series of antioxidation system. By increasing the activity of the antioxidation system, the stress resistance of plant could be enhanced, which relieves the injury of oxidation (Hofer et al., 2008). The antioxidation system is composed of antioxidases and non–enzyme compounds with high reducibility. Antioxidases mainly include superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), which play an important role in the clearing of active oxygen, as well as glutathione reductase (GR) and ascorbate peroxidase (APX) that are crucial in the ascorbate–glutathione (AsA–GSH) cycle (also called Halliwell–Ashada pathway). Non–enzyme compounds include AsA, GSH and carotenoids (Car). Polyamines (PAs) are nitrogenous bases belonging to fatty group with biological activity that are produced during metabolic process. They mainly include putrescine (Put), spermine (Spm) and spermidine (Spd). PAs participates in a variety of physiological processes such as seed germination, rooting, embryogenesis, pollen tube growth and fruit formation. They have an important role in membrane stability, free radical clearing, osmotic adjustment, mineral nutrition and ageing adjustment of plants (Martin–Tanguy, 2001; Shoeb et al., 2001; Groppa et al., 2003; Kusano et al., 2008). Exogenous application of PAs increased the resistance of plants to, drought (Capell et al., 2004; Yang et al., 2007), osmotic stress(Tonon et al., 2004; Legocka and Kluk, 2005) and heavy metal stress (Groppa et al., 2003; Xu et al., 2011). Spd is also PA, and its role in the growth, development and resistance of plants to environmental stresses has been reported (Zhu et al., 2006). Compared with Put and Spm, Spd is more efficient in the resistance to environmental stresses (Duan et al., 2008).

The present study investigated the influence of the increase of O_3 concentration on the injury and the antioxidation system of winter wheat leaves at different stages by open top chamber (OTC) method in farmlands. The influence of exogenous application of Spd on the changes of the physiological indexes of winter wheat was also studied. The objective was to clarify the influence of O_3 stress on the antioxidation system of winter wheat leaves and the relief mechanism of Spd on O_3 stress injuries.

2. Materials and Methods

2.1. Experiment site

The experiment site is located in the Seed Management Station of Changping, Northwest Beijing (40°12′N, 116°8′E), China. The station is characterized by continental monsoon climate and four distinct seasons. The mean annual temperature is 11.8 °C. The mean annual sunshine is 2 684 hours, and the frost–free season lasts for about 200 days. The basic physicochemical properties of soil are as follows: organic matter contents 16.4 g kg^{-1} soil; total nitrogen 0.9 g kg^{-1} soil; available phosphorus 38.1 mg kg^{-1} soil; available potassium 102.1 mg kg^{-1} soil; and soil pH 8.3.

2.2. Plant materials

The variety used in the experiment was *Triticum aestivum* L. Beinong 9549, provided by Beijing Agricultural College. The seeds were sowed on September 28, 2009. On April 26, 2010, urea was applied (225 kg ha^{-1}). The field management was coherent to that of the local farms during the entire growth season of winter wheat.

2.3. Ozone fumigation

In–situ ozone fumigation was carried out on winter wheat with the self–made open–top fumigation system which consisted of the open–top box, gas distribution system, air blower, ozone generator, and O_3 concentration control system and ozone analyzer (Figure S1). With skeleton made of reinforcing steel bar, the open–top box was manufactured into an octahedron with a 45° contracted aperture on top. The outside of the box was covered with transparent polyethylene thin film. The box had a side length of 1 m and a height of 2.7 m, with a coverage area of approximate 4.8 m^2. The ozone was generated from the medical pure oxygen (99.5%) through high–pressure discharge process in the ozone generator (SK–CFG–3, Jinan Sankang Envi–Tech Co., Ltd.). The oxygen flow rate was adjusted with mass flow meter (GFC17, Aalborg Industries Inc.) and Kingview industrial control software (MCGS 6.2, Beijing Kunlun Industrial Control Technology Development Co., Ltd.) to control the ozone concentration. The ozone concentration in the box and in ambient atmosphere was continuously monitored with two ozone analyzers (Model 49c, Thermo Electron Co., Franklin, MA) (Huang et al., 2012). The standard deviation of the actual test data of ozone homogeneity was below 4.4%, and the coefficient of variation was below 7.8%. The variation tendency of the sampling point and the fixed control point was consistent, which satisfied the requirement of this experiment. The light intensity, humidity and temperature in the OTC were 500–1 100 µmol m^{-2} s^{-1}, 60–85% and 25–45 °C, respectively.

Four concentrations of O_3 were set up: ambient atmosphere filtered by activated carbon 5 ppb control check (CK), 40 ppb, 80 ppb and 120 ppb. Each treatment had three replicates. The O_3 fumigation on the winter wheat started on April 5, 2010. The fumigation lasted for 9 h (8:00–17:00) each day and stopped on June 12. During the O_3 fumigation, injuries of the plants were observed and recorded carefully. Fresh leaves were collected during the jointing stage (April 27), heading stage (May 13) and milk stage (June 8). For each sampling point, 15–20 leaves were randomly collected. The samples were immediately treated with liquid nitrogen and stored at –80 °C. Because the leaves in the milk stage withered largely after O_3 fumigation and few fresh leaves were left, only Nitrate reductase activity (NR activity), NO_3^-–N, NH_4^+–N and Pro contents were measured.

2.4. Spd application experiment

Potted planting experiment was used to study the protective effect of Spd for winter wheat under the ozone stress. The winter variety was also *Triticum aestivum* L. Beinong 9549, provided by Beijing Agricultural College. The plastic pots had a diameter of 20 cm and a height of 25 cm. The soil was the surface soil of 20 cm in the experimental site. After passing through a 2 mm sieve, the soil was homogenized and placed in the pots. Every pot contained 1.5 kg of soil. Before transplantation of the winter wheat seedlings, fertilizers (0.428 g kg^{-1} urea, 0.323 g kg^{-1} $CaHPO_4.2H_2O$ and 0.247 g kg^{-1} K_2SO_4) were added to the soil. In each pot, 10 winter wheat seedlings of 10 cm tall at the same growth status were transplanted. After the survival of the seedlings was confirmed, six seedlings were retained for each pot. The winter wheat seedlings were transferred to the OTC for O_3 fumigation and Spd spraying.

Under the O_3 fumigation of 120 ppb, different concentrations of Spd were sprayed: distilled water (control), 0.25 mmol L^{-1} Spd, 0.50 mmol L^{-1} Spd and 0.75 mmol L^{-1} Spd. Each treatment had four replicates. Spd was spayed at 8:00 am and 6:00 pm every day by the foliar application method. The application amount was 50 mL for each pot every time. The spraying lasted for 2 weeks. Then, 15 to 20 fresh leaves were randomly collected. The samples were immediately treated with liquid nitrogen and stored at –80 °C.

2.5. Measurement of physiological indexes

Enzyme extraction: 0.5 g fresh leaves and 10 ml of 50 mmol L^{-1} phosphate buffer (pH 7.8, containing 1% vinyl pyrrolidone) were added together with a small amount of quartz. The leaves were ground to homogenate on an ice bath. The samples were centrifuged at 15 000 r min^{-1} for 4 min and the supernatant was collected for preservation under low temperature.