Ecophysiological responses of winter wheat seedling to aerosol wet deposition of Xi'an area, China

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Abstract

Aerosol leads to 30% reduction in solar radiation reaching the earth's surface, and a similar reduction in crops yield for both wheat and rice. To determine the effect of aerosol wet deposition on crops, aerosol samples were collected in September, 2006 at Xi'an urban suburb (34°44′N, 109°49′E), and wheat seedlings were treated with aerosol of different concentrations in laboratory conditions. Changes in physiological and biochemical parameters of wheat seedlings were measured. In comparison with the control, the activities of superoxide dismudase (SOD) and catalase (CAT) and the concentration malondialdehyde (MDA) and oxidized glutathione (GSSG) of wheat seedlings increased progressively with increasing concentrations of added aerosol, while the opposite trend was seen for the activities of nitric oxide synthase (NOS), the concentrations of glutathione (GSH) and nitric oxide (NO), and the ratio of GSH/GSSG. When the seedlings were treated with the aerosol of 1 and 2 mg/L, the coleoptile elongation, shoot elongation and biomass accumulation were increased, the effect at treatment of 2 mg/L was most significant. However, aerosol treatments with rates of 3 and 4 mg/L resulted in a decrease in coleoptile elongation, shoot elongation and biomass accumulation in seedlings, and significant effect was for the treatment of 4 mg/L. Hence, lower concentrations of aerosol wet deposition were in favor of crops growth, but its higher concentrations could result in deleterious effects for crops and decreased crops growth.

Key words: aerosol deposition; environment pollution; air pollution; ecotoxicological testing; aerosol risk assessment

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Introduction

Atmospheric aerosols have two important characteristics. The first is their association with regional-scale air pollution (Husar et al., 1981). While atmospheric fine particles are produced naturally, the natural sources are often overwhelmed by anthropogenic sources in polluted areas. These sources include the direct emission of fine particles into the atmosphere by anthropogenic processes such as the combustion of fossil fuels and biomass. Oxidation, as well as gas-to-particle conversion of pollutants such as sulphur dioxide, nitrogen oxides and volatile organic compounds is also responsible for the production of fine particles in the atmosphere. Because fine particles typically reside in the atmosphere for days to weeks, they can be transported or deposit along with rain. As a result, large regions of the globe with sufficient industrial activity and urbanization and/or biomass burning can be covered by a contiguous layer of air containing enhanced concentrations of fine particles (Chameides et al., 1999). The second important characteristic of fine particles is their ability to directly and indirectly affect the flux of solar radiation passing through the atmosphere (Charlson et al., 1992; Schwartz and Andreae, 1996). Both direct and indirect effects lead to 30% reduction in solar radiation reaching

the earth's surface; and lead to a similar reduction in crops yield for both wheat and rice (Chameides et al., 1999). The extinction of solar radiation by aerosol particles suspended in the atmosphere is believed to influence crop production. These works have focused almost exclusively on the reduction effects of aerosol pollutants on crop yields by scatter and absorb PAR (Chameides et al., 1999; Bergin et al., 2001; Xu et al., 2002) with CERES (crop estimation through resource and environment synthesis) model. However, we should know that the composition and proportion of the aerosol in different areas are different in the world, such as the aerosol near smelting works, on desert or near active volcano. Recently, Zhang et al. (2007) reported the water soluble inorganic ingredients of atmospheric aerosols in 2006 in Xi'an. Although it is known that aerosol can lead to environment pollution and influence agriculture production, whether aerosol wet deposition can have an influence on the seedling physiological metabolism, and how to response of winter wheat seedling to aerosol wet deposition are still unclear.

All abiotic stresses (e.g., drought, high or low temperature, salinity, heavy metals and oxidative and UV) may lead to the production of reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH·). These ROS are highly reactive

and can alter normal cellular metabolism through oxidative damage to lipids, proteins, and nucleic acids (Imlay, 2003). ROS-induced damage to cellular membranes also trigger several signaling pathways that regulate responses to abiotic stress.

Therefore, I put forward a hypothesis that aerosol wet deposition could influence crops physiological metabolism because it comprise of many inorganic and organic complex chemical ingredient. To determine this hypothesis and provide the first truly assessment of the direct impacts of air pollution on crops, selected parameters in the activities of NOS, SOD and CAT, in concentrations of NO, GSH, GSSG and MDA and as well as in growth parameters of wheat seedlings were measured.

1 Materials and methods

1.1 Aerosol collection

Aerosol samples were collected respectively on 8, 18 and 28 in September 2006 at Xi'an urban suburb (34°44′N, 109°49′E) using two battery powered mini volume samplers (Airmetrics Oregon, USA) operating at a flow rate of 5 L/min. Prior to field operations, calibrated mini-vol samplers were collocated with low volume $PM_{2.5}$ and PM_{10} Partisol samplers (model 2000, Rupprecht & Patashnick, USA) at the Hong Kong Polytechnic University for data comparison. The difference between the two types of samplers was less than 5% for $PM_{2.5}$ and PM_{10} mass (Cao et al., 2003). The samples were extracted ultrasonically by 500 mL deionized water at a resistivity of 18 MΩ·cm.

Previous reports showed that with the rainfall 1, 5, 10 and 20 mm, the aerosol concentration of per 1 L rain water was 20, 15, 10 and 5 times of per m^3 aerosol (Wang, 1999). Zhang et al. (2007) reported the aerosol concentration of Xi'an in September 2006 was 190.06 μg/m^3. According to Wang (1999) and Zhang et al. (2007) (as shown in Table 1), the aerosol concentration of per liter rain water was calculated as 3.8, 2.859, 1.9 and 9.5 mg/L when rainfall was 1, 5, 10 and 20 mm. Therefore, aerosol solution was diluted to 1, 2, 3 and 4 mg/L and was used in this study.

1.2 Plant materials

Wheat seeds were sterilized for 10 min in 0.05% $HgCl_2$ solution, washed for 30 min in tap water, and thereafter grown in petri dishes (18 cm in diameter). When the seedlings were 2-day-old and 6-day-old respectively, the seedlings were treated with rates of 0, 1, 2, 3 and 4 mg aerosol per liter deionised water. Each treatment included 6 dishes, and each dish contained 60 seedlings. The petri dishes with seedlings were incubated in a growth chamber (Model: 515HD, USA) at 25°C, 70% relative humidity, about 400 μmol/mol CO_2 (ambient level in the building), and 1000 μmol/(m^2·sec PAR). The seeds were flushed daily with 10 mL deionized water. The experiments were replicated independently thrice.

1.3 Biochemical analyses

Malondialdehyde (MDA) concentration was measured according to Predieri et al. (1995). Leaf samples (0.2 g fresh weight) were taken from 8-day-old seedlings and immediately frozen at –70°C. The frozen leaf tissue was then homogenized in 5 mL phosphate buffer (pH 6.7), and followed by centrifugation for 15 min at 8000 ×g. A 0.5-mL of the supernatant was combined with an equal volume of thiobarbituric acid (TBA) reagent (5% TBA (W/V) in 20% trichloroacetic acid (W/V)) and boiled at 100°C for 20 min. Absorbance was determined at 532 and 600 nm with spectrophotometer (TU-1810, Beijing Purkinje General Instrument Co., Ltd., China). MDA concentration was expressed in nanomoles per milligram protein.

The concentration of protein was measured according to Bradford (1976). The leaf samples of 8-day-old seedlings (0.5 g fresh weight) were homogenized at 0°C in 2.5 mL Tris-HCl buffer (0.1 mol/L, pH 8.0) to which 0.5 mol sucrose, 0.06 mol/L of ascorbic acid, and 0.005 mol β-mercapto ethanol were added per 100 mL buffer. After thorough grinding, the samples were removed to 5 mL centrifugation tubes and then centrifuged at 8000 ×g for 15 min. A 0.15 mL supernatant sample was removed with a pipette, and 0.85 mL of distilled water and 5 mL of 0.1 g/L G-250 Coomassie Brilliant Blue were added. After 15 min the absorbance was determined at 595 nm. A standard curve was prepared by adding bovine serum albumin (ultra 99%, Sigma, USA,) ranging in concentration from 0 to 100 μg/mL. The concentration of soluble protein was expressed as mg/g fresh water (fw).

The concentration of the oxidized glutathione (GSSG) and reduced glutathione (GSH) were measured according to the protocols of a GSH and GSSG kit (No. 20060820) (Nanjing Jiancheng Bioengineering Reagent Co., Ltd., China). The concentration of GSH and GSSG were expressed as mg/g fw.

The activities of SOD and CAT were determined according to Giannopolitis and Ries (1977) and Cakmak and Marschner (1992), respectively. The leaf samples of 8-day-old seedlings (fresh weight 5 g) were taken randomly from different seedlings in parallel petri dishes for enzyme extraction, and were then homogenized in 10 mL phosphate buffer (0.05 mol/L, pH 6.7), and after thorough grinding the samples were then centrifuged at 10,000 ×g for 10 min at 0°C. Extraction of enzymes was performed at 4°C. The supernatant sample was stored at –20°C for assay. The activity of enzymes was expressed as U/g protein.

Nitrice oxide synthase (NOS) activity determination was performed according to Murphy and Noack (1994). About 5 g of the samples were homogenized in 10 mL homogenization buffer (50 mmol/L triethanolamine hydrochloride (pH 7.5) containing 0.5 mmol EDTA, 1 μmol leupeptin, 1 μmol pepstatin, 7 mmol gluathione, and 0.2 mmol/L phenylmethylsulfonyl fluoride). After centrifuging at 10,000 ×g for 20 min at 4°C, the supernatant was collected and recentrifuged at 100,000 ×g for 45 min. The supernatant was used for NOS determination. NOS activity was analyzed by hemoglobin assay, as previously described (Murphy and Noack, 1994).

Nitrice oxide content was determined as described by Murphy and Noack (1994). Plant materials (5 g) were incubated with 100 units of catalase and 100 units of

Table 1 Characters of gas and soluble component of aerosol in 2006 at Xi'an, China* (unit: μg/m³)

	PM	NH_3	SO_2	NO_2	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	F^-	Cl^-	NO_2^-	SO_4^{2-}	NO_3^-
Mar	221.45	12.66	42.73	54.08	3.69	3.15	2.33	0.91	8.14	0.56	6.48	0.34	17.64	9.95
Apr	183.67	17.61	31.39	48.69	3.22	5.59	2.23	0.67	4.21	0.39	4.67	0.31	24.13	11.65
May	130.76	15.56	25.71	37.99	2.29	5.25	2.09	0.47	2.20	0.26	2.48	0.20	21.70	7.18
Jun	239.68	20.98	27.17	45.73	1.96	6.61	2.99	0.35	1.78	0.19	3.51	0.05	24.81	8.00
Jul	141.53	28.87	26.72	39.74	1.59	12.45	1.73	0.26	0.50	0.12	1.98	0.30	46.41	13.76
Aug	134.46	11.25	27.94	38.86	1.32	12.41	1.94	0.21	0.58	0.12	1.98	0.39	45.31	13.56
Sep	190.06	16.29	31.88	26.55	1.62	12.35	2.92	0.45	2.36	0.48	4.65	0.52	45.61	19.56
Mean	164.09	19.14	29.06	40.93	2.11	8.67	2.29	0.43	3.13	0.27	3.33	0.34	33.31	11.87

* Data source is from Zhang et al., 2007.

superoxide dismutase for 5 min to remove endogenous ROS before addition of 10 mL oxyhemoglobin (5 mmol). After 2 min of incubation, NO was measured spectrophotometrically by measuring the conversion of oxyhemoglobin to methemoglobin.

The coleoptile and shoots elongation were measured when the seedling were 8-day-old. The 60 seedlings of 8-day-old drying at 80°C were measured in electronic scale as biomass (g).

1.4 Statistical analysis

Results from different treatments were compared using one-way ANOVA. Following ANOVA, post hoc comparisons of means in plant physiological parameters were performed using Duncan's multiple range tests. Statistical significance was determined at $P < 0.05$.

2 Results

2.1 Effects of aerosol treatment on the antioxidant defense system of leaves

Figures 1 and 2 show the change in the antioxidant defense system of wheat leaves treated by different aerosol concentrations. Compared with the control, the wheat seedlings treated with 1, 2, 3 and 4 mg/L resulted in 9% ($P > 0.05$), 43% ($P < 0.05$), 68% ($P < 0.05$) and 77% ($P < 0.05$) increases in SOD activity, and aroused 5.3% ($P > 0.05$), 27.9% ($P < 0.05$), 68.4% ($P < 0.05$) and 71.6% ($P < 0.05$) increases in CAT activity. Similarly, the wheat seedlings treated with 1, 2, 3 and 4 mg/L resulted in 32% ($P < 0.05$), 51% ($P < 0.05$), 68% ($P < 0.05$) and 82.6% ($P < 0.05$) increases in MDA concentration and 22% ($P < 0.05$), 40.6% ($P < 0.05$), 53.5% ($P < 0.05$) and 74.4% ($P < 0.05$) increases in GSSG concentration. On the contrary, when
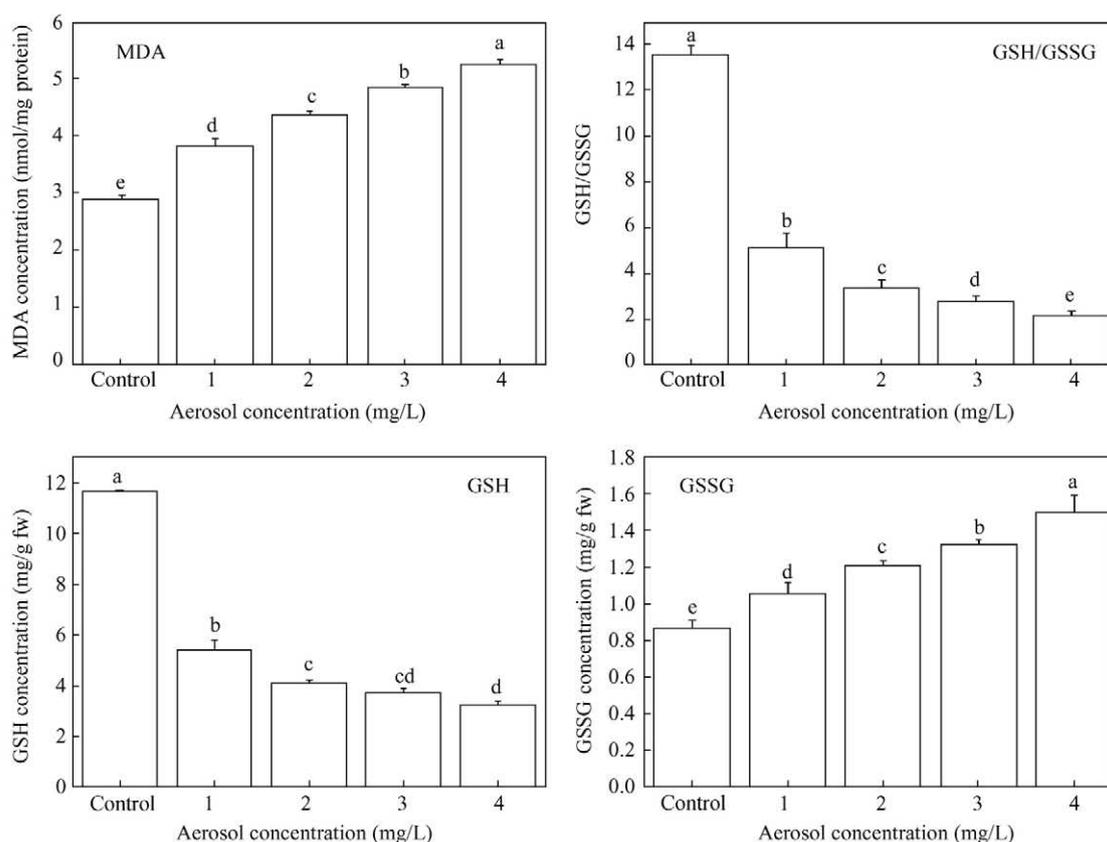
Fig. 1 Effects of aerosol treatment on the concentration of MDA, GSH and GSSG, and the ratio of GSH/GSSG of wheat seedlings at 1000 μmol/(m²·sec PAR). Data are the means and standard errors of three replicates. Means with different letters are significantly different at $P < 0.05$ level ($n = 3$).
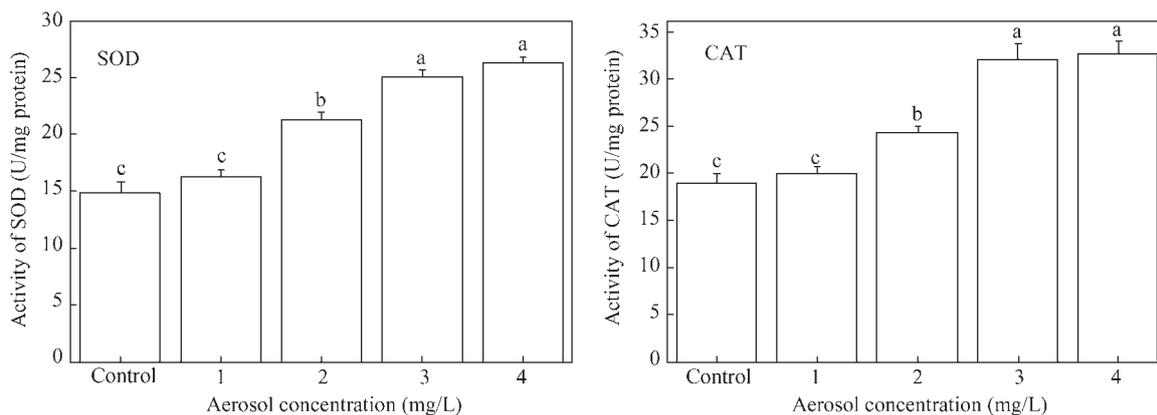
Fig. 2 Effects of aerosol treatment on the SOD and CAT activities of wheat seedlings at 1000 μmol/(m²·sec PAR). Data are the means and standard errors of 3 replicates. Means with different letters are significantly different at the $P < 0.05$ level ($n = 3$).

the wheat seedlings were subjected to aerosol solutions at 1 to 4 mg/L, the concentration GSH and the ratio of GSH/GSSG were decreased progressively with the doses of added aerosols. Compared with the control, the aerosol solution at 1, 2, 3 to 4 mg/L cause a 53.6% ($P < 0.05$), 64.6% ($P < 0.05$), 68.3% ($P < 0.05$) and 72.2% ($P < 0.05$) decrease in GSH concentration and a 60% ($P < 0.05$), 74% ($P < 0.05$), 79% ($P < 0.05$) and 84% ($P < 0.05$) decrease in the ratio of GSH/GSSG, respectively.

2.2 Effects of aerosol treatment on the NO signal system of leaves

Figure 3 shows the effects of different aerosol solution treatment on the NOS activity and NO concentration in wheat seedlings. When the wheat seedlings were subjected to concentrations of aerosol solution at 1, 2, 3, 4 mg/L, the NOS activity was 38.5% ($P < 0.05$), 59.76% ($P < 0.05$), 62.7% ($P < 0.05$) and 67.9% ($P < 0.05$) lower than that of the control (Fig. 3a) and the NO concentration was 38.6% ($P < 0.05$), 52.8% ($P < 0.05$), 65.7% ($P < 0.05$) and 72.9% ($P < 0.05$) lower than that of the control, respectively (Fig. 3b).
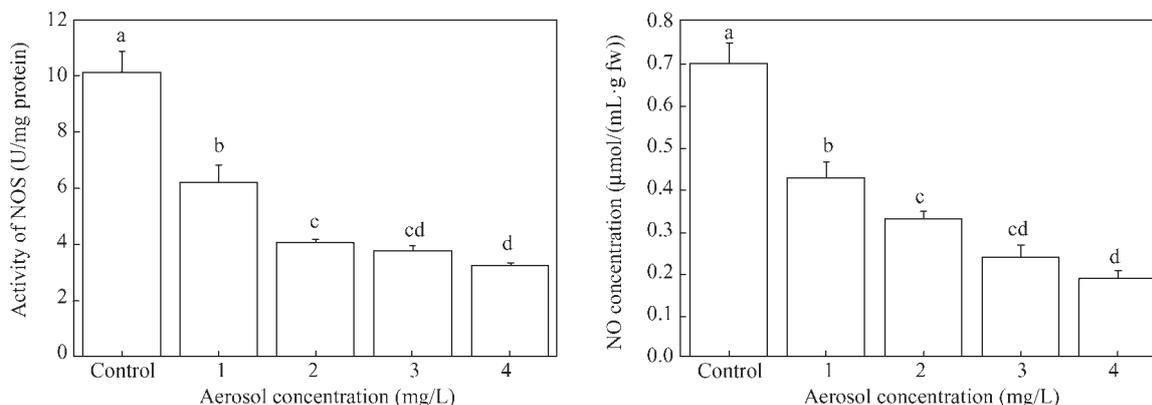
2.3 Effects of aerosol treatment on the seedling growth

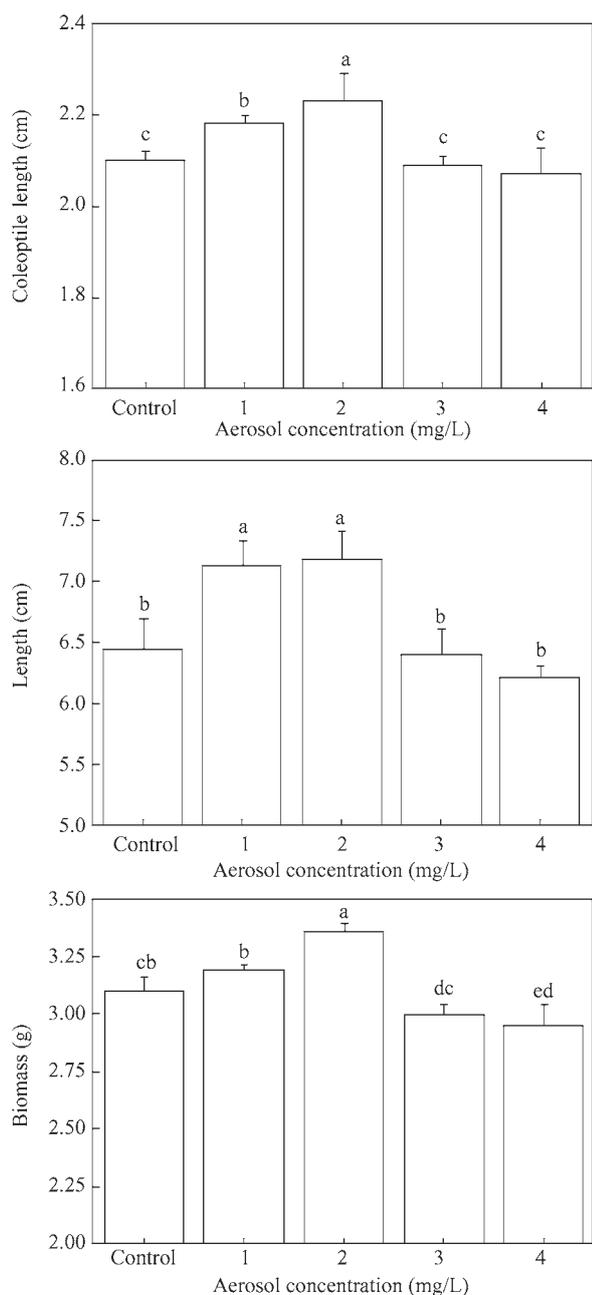Aerosol treatments with 1 and 2 mg/L resulted in an increase in coleoptile and shoot elongation, and biomass

of seedlings, the better effect was observed at 2 mg/L. While aerosol treatments with 3 and 4 mg/L resulted in a decrease in coleoptile, shoot elongation and biomass of seedlings, and the worse effect was observed at 4 mg/L (Fig. 4). Compared with the control, treatment of wheat seedlings with 1 and 2 mg/L aerosol solution significantly improved the shoot elongation by 10.7% ($P < 0.05$) and 11.5% ($P < 0.05$) and the biomass by 2.9% ($P < 0.05$) and 8.4% ($P < 0.05$), respectively, whereas treatment of wheat seedlings with 3 and 4 mg/L aerosol solution decreased the shoot elongation by 0.6% ($P > 0.05$) and 3.5% ($P < 0.05$) and the biomass by 1.6% ($P > 0.05$) and 3.3% ($P < 0.05$).

3 Discussion

The tremendous importance of agricultural production can not be underestimated in an era in which the earth's population is over six billion and growing. In order to ensure that food supplies keep pace with population growth, understanding of the processes involved in crop growth and development is a necessity. Numerous processes of plant growth and development are modulated by internal cues and external environmental factors. Air pollutions have been recognized as become regions as well as a cause of injury to plants. The influence of atmospheric aerosols on plants growth has been estimated by Chameides et al.

Fig. 3 Effects of aerosol treatment on the NOS activity and the NO concentration of wheat seedlings at 1000 μmol/(m²·sec PAR). Data are the means and standard errors of three replicates. Means with different letters are significantly different at the $P < 0.05$ level ($n = 3$).

Fig. 4 Effects of aerosol treatment on the coleoptile elongation, shoot elongation and biomass of wheat seedlings at 1000 μmol/(m²·sec PAR). Data are the means and standard errors of three replicates. Means with different letters are significantly different at the $P < 0.05$ level ($n = 3$).

wet deposition in plant physiology. In this study wheat seedlings were exposed to aerosol solutions with different concentrations, the selected physiological parameters and early growth of seedlings were studied under laboratory conditions. The results showed that treatments with the aerosol concentrations of 1 and 2 mg/L may improve the seedlings growth. Represent as length and biomass of seedlings was higher and heavier than that of the control. The 3 and 4 mg/L aerosols solutions could result in decease in seedlings height and biomass.

Abiotic stresses may lead to the production of ROS, such as O_2^-, H_2O_2, and $OH\cdot$. These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins, and nucleic acids (Imlay, 2003). Lipids contain a high percentage of PUFA residues and are thus susceptible to peroxidation (Meloni et al., 2003; Shalata et al., 2001). The content of MDA, a product of lipid peroxidation, has been considered an indicator of oxidative damage (Meloni et al., 2003; Shalata et al., 2001). To minimize oxidative damage caused by ROS, plants have evolved various enzymatic and non-enzymatic defense mechanisms to detoxify free radical and reduce oxidative stress. The antioxidant defense system includes enzymes such as SOD, CAT. Among the defenses SOD is a group of enzymes that can accelerate the conversion of superoxide radical to H_2O_2 and O_2^-. Catalase is known to play a key role in protecting cells against oxidative stress and dismutates H_2O_2 into water and O_2^- (Corpas et al., 1999). Non-enzymatic antioxidants GSH, a disulfide reductant that protects thiols of enzymes, regenerates ascorbate (As) and reacts with singlet oxygen, hydrogen peroxide and hydroxyl radicals. GSH is able to detoxify ROS by direct scavenging or by acting as cofactor in the enzymatic reactions that are involved in the ascorbate-glutathione cycle and GSH is oxidized to GSSG when acts as an antioxidant and redox regulator. High GSH/GSSG ratio in plants tissues plays an important physiological role (Foyer, 1993; Zhao et al., 2004). The present work shows that SOD and CAT activities, as well as MDA and GSSG concentrations, were increased with increase of the aerosol concentration. But GSH concentration and the GSH/GSSG ratio were decreased with increase of the aerosol concentration. The results clearly indicate that aerosols treatments lead to an oxidative stress and cause a cellular membranes damage in wheat seedlings.

According to literature, the plant response to stressors factors (such as drought, high or low temperature, salinity, heavy metals and oxidative stress) was regulated by NO (Zhao et al., 2004). NO as a plant defense signal can react with free radicals, such as O_2^- and H_2O_2, and potentially abrogate superoxide/hydrogen peroxide signaling. Such interactions regulation between NO and ROS has been reported by Durner et al. (1998). These protective effects of NO-donor treatment plant were explained by the elevation of activity of antioxidative enzymes and effectively reduce the level of ROS generated during stress, and thus, limit oxidative damage in plant cells (Kopyra and Gwóźdź, 2003). It was observed that increasing concentration of aerosols leads to a decrease little by little in NOS activity and NO

(1999) with CERES crop model. The results suggested that each percentage reduction in PAR results in a percentage reduction in wheat yield (Chameides et al., 1999). Others work also suggested a linear relationship between reductions in PAR and plant growth (Gu et al., 1999). However, the CERES crop model accounts only for ca. 35% reduction in total amount of PAR reaching for the plants canopy due to the dry deposition, and without the consideration in the absorption and utilization of aerosols wet deposition by plants. In order to accurately assess the effects of aerosols pollution on crops growth it is necessary to determine the response of crops to the aerosols

concentration. The results clearly indicate that aerosols treatments restrained NO signal function by decreasing NO biosynthesis in wheat seedlings.

In conclusion, with aerosol treatments of 1, 2, 3 and 4 mg/L the SOD and CAT activities and the concentrations of MDA and GSSG were increased progressively, while the activities of NOS, the concentration GSH and NO, and the ratio of GSH/GSSG were decreased progressively. Aerosol treatments with 1 and 2 mg/L resulted in an increase, while the treatments with 3 and 4 mg/L resulted in a decrease in coleoptile and shoot elongation and biomass accumulation in seedlings. The results indicate that the aerosol wet deposition with little rain could result in physiological damage and exceed the plant repair capability. The reason of aerosol wet deposition resulting in physiological damage would be compound effects because aerosols are a complex chemical mixture, which contains a lot of chemical inorganic and organic composition (inorganic composition, e.g., NH_3, SO_2, NO_2, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, F^-, Cl^-, NO_2^-, SO_4^{2-}, and NO_3^-). From this point of review, it is necessary to control air pollution for the protection of crops ecosystem and human health.

Acknowledgments

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