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Photosynthetic and yield responses of an old and a modern winter wheat cultivars to short-term ozone exposure

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Abstract

In order to study the responses of winter wheat cultivars released in different years to short-term high O_3 exposure, an old cultivar ('Nongda 311', released in 1960s) and a modern one ('Yannong 19', released in 1990s) were treated with an O_3 exposure (145 ± 12 mm^3 m^{-3}, 4 h d^{-1} for 3 d) shortly after anthesis stage (> 50 % main stems blossomed). During the O_3 exposure, light-saturated photosynthetic rate (P_N) and stomatal conductance (g_s) of both cultivars decreased considerably. Elevated O_3 did not decrease dark-adapted maximum photochemical efficiency, but induced significant reduction in actual photochemical efficiency and thereby considerably increase in non-photochemical quenching. P_N, g_s of the modern cultivar 'Yannong 19' decreased more than the older one 'Nongda 311', indicating the former exhibited higher sensitivity to O_3 than the latter. After O_3 exposure, P_N, g_s and chlorophyll (Chl) content in flag leaf decreased more quickly than control, indicating induction of faster premature leaf senescence. As a result, the short-term O_3 exposure caused substantial yield loss, with larger reduction in 'Yannong 19' (–19.2 %) than in 'Nongda 311' (–8.4 %). Our results indicated that high O_3 exposure at grain filling stage would have greater negative impacts on the high yielding modern cultivar relative to the old one with lower yield.

Additional key words: air pollution; gas exchange; post-anthesis; stomatal conductance; *Triticum aestivum.*

Introduction

The progressive increase of tropospheric ozone (O_3) has a significant impact on agricultural production (Heath 1994, Krupa *et al.* 1995) by inhibiting photosynthesis, accelerating leaf senescence, reducing plant growth and impairing yield attributes (Finnan *et al.* 1998, Sild *et al.* 2002, Feng *et al.* 2007). The current ground-level O_3 concentrations in suburban sites in many developing countries such as China have been reported to be high enough to cause substantial yield losses of crops (Wang *et al.* 2007a, b). The information of crop responses to O_3 may help cultivar breeding as well as cultivation to reduce yield losses caused by O_3 pollution. However, such information is still very limited in many developing countries (Ashmore 2005, Wang *et al.* 2007a), where high O_3 tolerance is not taken as a breeding target.

Wheat (*Triticum aestivum* L.), as one of the crucially important crops in the global food supply, was found to be particularly sensitive to O_3 (Fuhrer *et al.* 1992, Selldén and Pleijel 1995). Moreover, modern wheat cultivars showed to be more sensitive to O_3 than the older ones, probably due to larger stomatal conductance and therefore greater O_3 uptake (Barnes *et al.* 1990, Pleijel *et al.* 2006, Velissariou *et al.* 1992). This is regarded as a result of inadvertent selection by plant breeders (Barnes *et al.* 1990) since the main target of cultivar selection was for maximum seed yield, which was also found to be correlated with high stomatal conductance (Jiang *et al*. 2003). It has been well-documented that the increased higher ozone flux, together with larger reduction in antioxidative capacity might lead to the higher ozone sensitivity of the more recent cultivars (Biswas *et al*. 2008).

Ozone impacts are found to be more serious at grain filling stage, consequently causing severe yield loss of crops (Gelang *et al.* 2001, Pleijel *et al.* 1998, Slaughter

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Abbreviations: C_i – intercellular CO_2 concentration; Chl – chlorophyll; DAA – days after anthesis; ; F – steady state fluorescence under light; F_o, F_m, F_v – initial, maximal, and variable fluorescence after dark-adaptation; F_m' – maximal fluorescence after light-adaptation; F_v/F_m – the dark-adapted maximum photochemical efficiency of PSII; g_s – stomatal conductance; HI – harvest index; NPQ – non-photochemical quenching; P_N – net photosynthetic rate; Φ_{PSII} – actual photochemical efficiency of PSII.
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et al. 1989), which might lie in low resistance of tolerance by compensatory growth responses, detoxification or the repair of pollutant injury. Unfortunately, high ozone levels (>120 mm^3 m^{-3} for several hours) may have great chances to occur during the reproductive stage of wheat (Vingarzan 2004, Wang *et al.* 2005).

There are few studies focusing on the impacts of O_3 episodes at anthesis on wheat yield. Available publications reported that O_3 episodes would cause considerable yield losses to wheat. For example, Shannon and Mulchi (1974) noted significant reductions in yields by about 30 % and 23 % for two cultivars following exposure to 200 mm^3 m^{-3} O_3 4 h d^{-1} for 7 d during anthesis stage; Mulchi *et al.* (1986) exposed six soft red winter wheat cultivars with simulated air pollution episode (123 mm^3 m^{-3} O_3, 4 h d^{-1} for 5 d) during anthesis in field and found seed mass was averagely reduced by 20.2 %. However, the information about the physiological changes during and after short-term O_3 exposure and thereby impacts on final yield is very limited.

In the present study, we selected two winter wheat cultivars with distinct yield traits to study the impacts of short-term O_3 exposure after anthesis on flag leaf photosynthesis and final yield. We hypothesized that high yielding cultivar would be affected more seriously by O_3 episode relative to the lower yielding one. Results from our experiment will be valuable for wheat cultivar selection in areas with high levels of O_3 pollution.

Materials and methods

Experimental site and materials: The experiment was carried out at the research station in the Institute of Botany, Chinese Academy of Sciences, Beijing, China (39°92' N, 116°46' E).

Two winter wheat cultivars, *Triticum aestivum* L. cv. Nongda 311 and *Triticum aestivum* L. cv. Yannong 19 released in 1963 and 2001, respectively, were selected. 'Nongda 311', one of the most popular cultivars in China, is resistant to yellow rust and has high tillering capacity (Fu 2000, Liu 2002), while 'Yannong 19' is higher yielding and has better baking quality. Seeds of these two cultivars were sown in pots (22 cm in diameter, 30 cm in height) containing 10 kg field clay loam soil on 10 October 2005. The soil contained organic C, total N, total P, and total K at the rate of 1.24 %, 0.045 %, 296 mg kg^{-1} and 15.1 g kg^{-1}, respectively. Four seedlings were kept per pot on 7 d after germination. At jointing stage, N was top-dressed as urea at the rate of 15.0 g N m^{-2}. In addition, P and K were also applied as a compound fertilizer at the rate of 7.5 g m^{-2} and 18.0 g m^{-2}, respectively. The plants were irrigated as necessary to avoid water stress throughout the experiment and kept free from weeds and insect pests by appropriate measures. The plants were grown under ambient condition from October 2005 to April 2006. Seasonal temperature was –9 to 20 °C and seasonal relative humidity was 20–70 %. The 7-h daily mean O_3 concentrations ranged between 16 and 55 mm^3 m^{-3}.

Experimental design: Approximately 7 d prior to anthesis, 48 pots of each cultivar were randomly moved into four open-top chambers (OTC, 1.8 m in diameter and 2.4 m in height) placed in a greenhouse. Before O_3 exposure, all plants received charcoal-filtered air (<5 mm^3 m^{-3} O_3) to adapt to chamber condition for one week. When more than 50 % ears have completed anthesis (Tottman 1987), O_3 concentration of two OTCs were elevated to 145 ± 12 mm^3 m^{-3} (09:30 – 13:30) by injecting O_3 for 3 d. The other two chambers with charcoal-filtered air were taken as control. Ozone was generated by electrical discharge using ambient oxygen with an O_3 generator (*CF-KG1, Beijing Sumsun EP Hi-Tech., Co. Ltd.*, Beijing, China). The air stream was bubbled through distilled water before going into the chambers to remove harmful compounds other than O_3 (Ojanperä *et al.* 1998). Manual mass flow controllers were used to regulate the flow of O_3-enriched air to the two high O_3 chambers. Ozone concentrations in the OTCs were continuously monitored at approximately 10 cm above the plant canopy using an O_3 analyzer (*Model 205, 2B Technologies Inc.*, Boulder, Colorado, USA). In order to diminish chamber effects, pots were rotated daily between the chambers. The max/min temperature and relative humidity in the OTCs were 32/17 °C and 76/57 %, respectively. Maximum photosynthetic photon flux density (PPFD) in the OTCs ranged between 1000–1500 µmol m^{-2} s^{-1}.

After O_3 exposure, plants were moved to open air condition to grow until maturity. The weather was typical for early summer in Beijing with mean daily air temperature varying from 15 °C (night) to 34 °C (day) and maximum PPFD of about 2000 µmol m^{-2} s^{-1} at midday.

Visible injury was assessed on the whole plant after 3 d of O_3 exposure. The percentage of damaged area (mottled or necrotic) on the leaves was assessed for 10 pots per cultivar under control or O_3 fumigated treatment.

Gas exchange and chlorophyll (Chl) *a* fluorescence were measured on the green section of flag leaves using gas exchange and fluorescence systems (*GFS3000, Heinz Walz*, Effeltrich, Germany) during the 3 d O_3 exposure and on 7, 14, 21 d after O_3 treatment, *i.e.* 11, 18, 25 days after anthesis (DAA). The cuvette condition was set with PPFD of 1500 µmol m^{-2} s^{-1} provided by artificial red-blue light source (*LED-Array/PAM, Heinz Walz*, Effeltrich, Germany), relative humidity of 60 ± 15 %, air temperature at 27 °C and ambient CO_2 concentration of 380 ± 10 µmol mol^{-1}. Net CO_2 and H_2O exchange rates were first stored after adaptation to the cuvette, afterwards the steady state fluorescence (F_s) was determined and a saturating pulse was imposed to get maximum fluorescence in the light adapted state (F_m'). Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) were calculated according to von Caemmerer and Farquhar (1981).

After recording light-adapted fluorescence parameters, dark-adapted minimum (F_0) and maximum fluorescence (F_m) were determined with a modulated irradiation and a saturating pulse, respectively, following 40 min dark adaptation. The dark-adapted maximum photochemical efficiency of PSII (F_v/F_m), the actual photochemical efficiency of PSII (Φ_{PSII}) and the non-photochemical quenching (NPQ) were calculated by $F_v/F_m = (F_m–F_o)/F_m$, $\Phi_{PSII} = (F_m'–F_s)/F_m'$ and $NPQ = F_m/F_m' – 1$, respectively (Bilger and Björkman 1990, Schreiber 2004).

Chl content: Green section of flag leaves was sampled on 11, 18, 25 DAA. Chl was extracted in 95 % ethanol in the dark for 48 h at 4 °C. The extract was then assayed for Chl content with the absorption spectra provided by Arnon (1949).

Yield: Final harvest was carried out when plants matured on 15 June 2006. The grains from each ear were carefully threshed by hand and weighted. The above-ground plant materials were partitioned to main stem and grain, which were dried at 70 °C to constant mass to calculate total dry mass of grain and main stem. Harvest index (HI) was calculated as the ratio of grain dry mass to total above-ground dry mass.

Data analysis: Each pot from individual treatment was taken as a replicate. There were 4 replicates for gas exchange, fluorescence and Chl measurements, and 10 ones for visible injury and yield traits. Means of each parameter were compared between treatments. Least significant differences (LSD) were considered when the *F*-test of the *ANOVA* using *General Linear Model Procedure of SPSS* (*Ver. 11, SPSS*, Chicago, IL, USA) was significant at $p<0.05$.

Results

Visible injury: After the O_3 treatment, visible injury was observed on all leaves of both cultivars in the form of necrotic stipples at the interveinal areas, whereas control plants showed no visible symptoms of O_3 damage (Table 1). The visible damage was less in flag leaf (<15 %) than other lower positioned leaves (17–35 %). O_3-exposed plants showed leaf senescence 5 days earlier than control. Leaves of the modern cultivar 'Yannong 19' were damaged more seriously than the older one 'Nongda 311' (Table 1).

Table 1. Visible symptoms of O_3 damage in different leaf positions (from top to bottom) of two winter wheat cultivars after a short-term O_3 exposure at post-anthesis stage (+O_3). Means ± SD, n = 10. NF indicates no ozone-caused visible symptoms.

Cultivar	Treatment	Visible symptoms of O_3 damage [%]			
		Flag leaf	2nd leaf	3rd leaf	4th leaf
Nongda 311	control	NF	NF	NF	NF
	+O_3	10 ± 5	17 ± 8	22 ± 12	30 ± 9
Yannong 19	control	NF	NF	NF	NF
	+O_3	15 ± 5	20 ± 15	30 ± 5	35 ± 13

Responses to O_3 exposure: High O_3 exposure induced significant reduction in net photosynthetic rate (P_N) (Fig. 1*A*, *B*) and stomatal conductance (g_s) of both cultivars (Fig. 1*C*, *D*, $p<0.001$). Over all, 'Yannong 19' showed greater reduction in P_N (–25~–30 %, Fig. 1*A*) than 'Nongda 311' (–12~–24 %, Fig. 1*B*). Meanwhile, a considerable decrease ($p<0.001$) in P_N was observed only in 'Yannong 19' at the first 4 h of exposure. However, P_N of both cultivars decreased significantly ($p<0.01$) with advent of O_3 exposure. Similarly, g_s of 'Yannong 19' was more sensitive to elevated O_3 than 'Nongda 311'. The largest reduction in g_s for both cultivars occurred at the first 4 h of exposure. The intercellular CO_2 concentration (C_i) of both cultivars exposed to O_3 was lower than control, but significant difference ($p<0.05$) was only observed at the first 4 h of exposure (Fig. 1*E*, *F*).

Ozone exposure did not cause significant decrease in the dark-adapted maximum photochemical efficiency (F_v/F_m) (Fig. 2*A*, *B*), but the actual photochemical efficiency (Φ_{PSII}) decreased markedly ($p<0.01$) after 8 h O_3 exposure (Fig. 2*C*, *D*). Non-photochemical quenching (NPQ) of O_3-treated plants increased significantly ($p<0.01$) relative to control. However, the relative increase in NPQ was higher in 'Nongda 311' (30~59 %) than in 'Yannong 19' (23~33 %) (Fig. 2*E*, *F*).

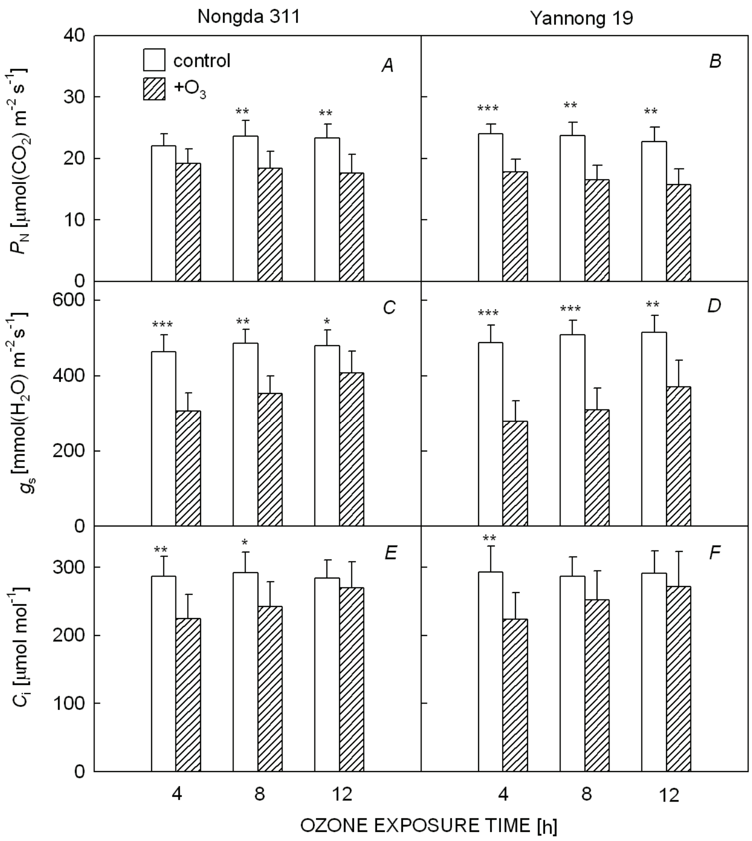

Fig. 1. Impacts of short-term O_3 exposure at post-anthesis stage (+O_3) on (*A*, *B*) net light saturated photosynthetic rate (P_N), (*C*, *D*) stomatal conductance (g_s) and (*E*, *F*) intercellular CO_2 concentration (C_i) of an old winter wheat cultivar ('Nongda 311') and a modern one ('Yannong 19'). Error bars show SD, $n = 4$. The asterisks *,** and *** indicate significance at $p<0.05$, $p<0.01$ and $p<0.001$ between treatments, respectively.

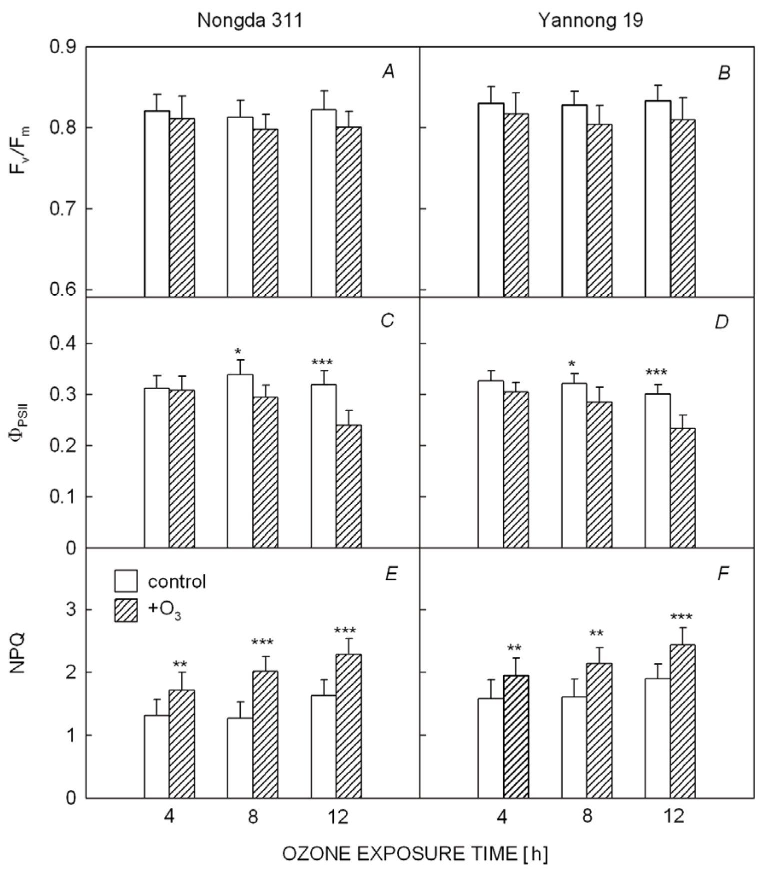

Fig. 2. Impacts of short-term O_3 exposure at post-anthesis stage (+O_3) on (*A*,*B*) maximum photochemical efficiency in the dark adapted state (F_v/F_m), (*C*,*D*) actual quantum efficiency of PSII (Φ_{PSII}) and (*E*,*F*) non-photochemical quenching coefficients (NPQ) of an old winter wheat cultivar ('Nongda 311') and a modern one ('Yannong 19') . Error bars show SD, $n = 4$. The asterisks *,** and *** indicate significance at $p<0.05$, $p<0.01$ and $p<0.001$ between treatments, respectively.

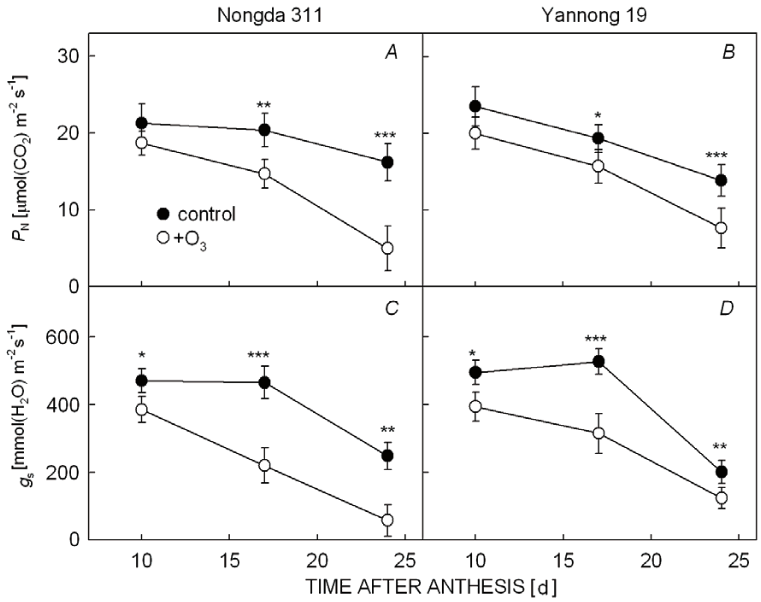

Fig. 3. (A, B) Net light saturated photosynthetic rate (P_N) and (C, D) stomatal conductance (g_s) of an old winter wheat cultivar ('Nongda 311') and a modern one ('Yannong 19') exposed to a short-term O_3 exposure at post-anthesis stage (+O_3) plotted against days after anthesis (DAA). Error bars show SD, n = 4. The asterisks *,** and *** indicate significance at p<0.05, p<0.01 and p<0.001 between treatments, respectively.

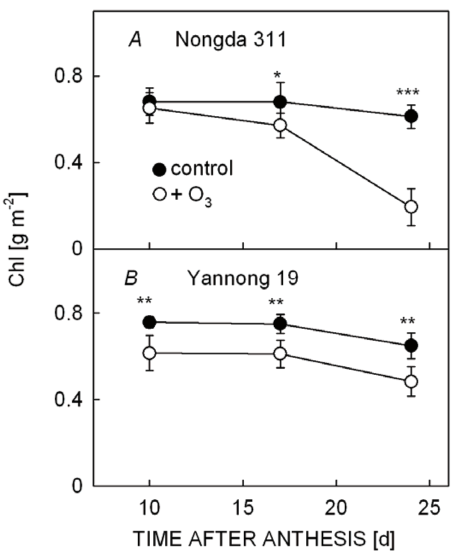

Fig. 4. Chlorophyll (Chl) content of an old winter wheat cultivar ('Nongda 311') and a modern one ('Yannong 19') exposed to a short-term O_3 exposure at post-anthesis stage (+O_3) plotted against days after anthesis. Error bars show SD, n = 4. The asterisks *, ** and *** indicate significance at p<0.05, p<0.01 and p<0.001 between treatments, respectively.

Changes after O_3 exposure: Flag leaf P_N of the ozonated plants was lower than control, but no significant difference (p<0.01) was detected until 18 DAA; meanwhile, P_N of 'Nongda 311' decreased faster than that of 'Yannong 19' (Fig. 3A, B). g_s of the O_3-exposed plants also decreased substantially. On 18 DAA, for example, reduction in g_s of the O_3-exposed 'Nongda 311' and 'Yannong 19' was 53 % and 40 %, respectively (Fig. 3C, D).

Flag leaf Chl content of the O_3-exposed plants was lower than control (Fig. 4). On 11 DAA, significant difference (p<0.01) between treatments was detected only for 'Yannong 19. However, Chl content in flag leaf of O_3-exposed 'Nongda 311' decreased faster than that of 'Yannong 19'.

Yield: Grain yield was negatively affected by the simulated O_3 episode (Table 2). 'Yannong 19' had significantly (p<0.001) higher grain number per ear, higher 1000-grain mass and higher grain yield per ear than 'Nongda 311' under both treatments. However, O_3 had greater negative impacts on the yield traits of 'Yannong 19' than on those of 'Nongda 311'. For example, grain number per ear and 1000-grain mass of 'Yannong 19' decreased significantly (p<0.05), while non-significant differences (p>0.05) were detected in those traits of 'Nongda 311'. As a result, yield of 'Yannong 19' decreased more (–19.2 %, p<0.01) than that of 'Nongda 311' (–8.4 %, p<0.05). There was no significant difference (p>0.05) in harvest index (HI) between treatments. However, HI of 'Nongda 311' increased slightly, while that of the 'Yannong 19' decreased obviously. Cultivar × O_3 interaction was significant for yield and HI, but not for grain number and 1000-grain mass.

Discussion

P_N of both cultivars was significantly decreased during O_3 exposure. The reduction in P_N was accompanied with decrease in g_s. The decrease in g_s was more obvious during the first 4 h of exposure, suggesting that exclusion of the pollutant might play a key role in governing O_3 resistance at the beginning of O_3 exposure. With advent of O_3 exposure, the O_3-induced reduction in g_s was alleviated, coupled with increase in C_i. This indicated that the reduction in P_N was mainly caused by stomatal limitation at the beginning of O_3 exposure, but mesophyllic limitations occurred with prolonged exposure (Farage *et al*. 1991, Noormets *et al*. 2001). We found non-significant decrease in F_v/F_m in O_3-treated leaves of both cultivars relative to control. However, Φ_{PSII} decreased considerably and consequently resulted in significant increase in NPQ. These results suggested that O_3 caused an increase in energy dissipation by non-photochemical processes associated with a regulated decrease in photochemistry and photosynthetic rate (Farage *et al*. 1991, Guidi *et al*. 1997).

Table 2. Grains per ear, 1000-grain mass, yield and harvest index of two winter wheat cultivars exposed to a short-term O_3 exposure at post-anthesis stage (+O_3). Different letters indicate significant difference between treatments at $p<0.05$. Mean ± SD, $n = 10$.

Cultivar	Treatment	Grains per ear	Grain mass [g per 1000 grains]	Yield/ear [g]	Harvest index [%]
Nongda 311	control	37.2 ± 2.89	34.3 ± 1.38	1.27 ± 0.72[a]	46.4 ± 0.75
	+O_3	35.4 ± 1.78	32.9 ± 0.73	1.17 ± 0.68[b]	47.3 ± 1.09
Yannong 19	control	43.6 ± 0.87[a]	45.9 ± 2.47[a]	2.00 ± 0.08[a]	56.4 ± 0.94
	+O_3	38.5 ± 2.85[b]	42.2 ± 2.34[b]	1.62 ± 0.22[b]	54.8 ± 1.19
Source of variation					
Cultivar		0.001	0.001	0.0001	0.0001
O_3		0.010	0.006	0.0001	0.420
Cultivar×O_3		0.169	0.153	0.004	0.015

There was a large difference in the response pattern of leaf gas exchanges between cultivars to the short-term high O_3 exposure. P_N of 'Yannong 19' responded earlier and decreased more sharply than that of 'Nongda 311'. We found that g_s of 'Yannong 19' was higher than that of 'Nongda 311' under ozone-free condition, but it decreased more during the O_3 exposure especially at the beginning. These performance indicated that the modern cultivar 'Yannong 19' was more sensitive to short-term O_3 exposure than the older one on the ground that sensitive varieties showed higher reduction in P_N and their g_s exhibited greater sensitivity to O_3 (Crous et al. 2006, Guidi et al. 1997).

After O_3 exposure, P_N, g_s and Chl content of O_3-treated flag leaves were lower and decreased faster than control. On the one hand, this reduction in P_N might be caused directly by the O_3 exposure because acceleration in plant senescence is a common feature of O_3 stress (Gelang *et al*. 2000, Grandjean and Fuhrer 1989, Sild *et al*. 2002, Soja and Soja 1995). On the other hand, O_3 exposure usually induces cellular damage and even visible symptoms (Guidi *et al*. 2000, Severino *et al*. 2007), making the O_3-exposed plants more sensitive to other stresses such as high irradiation and heat than normal plants. We kept the treated plants in open air with natural light and temperature, which might cause light or heat stress at noon. This may be another reason for the fast senescence in O_3-exposed plants.

The decline in P_N of flag leaf caused by short-term O_3 exposure resulted in significant yield loss for both cultivars (19.2 % for 'Yannong 19' and 8.4 % for 'Nongda 311'). In our previous study, short-term high O_3 exposure (125 $mm^3\ m^{-3}$, 7 h d^{-1} for 4 d) to potted winter wheat after anthesis resulted in yield reduction by 21 % (Xu *et al*. 2007). Besides reduced P_N in damaged flag leaves, O_3-treated plants showed premature leaf senescence 5 d earlier than control, which indicated that grain-filling duration was shortened. These might be likely the major factors affecting yields in wheat at elevated O_3, because the lack of green leaf area after ear emergence would have the most pronounced negative effects on grain yield (Soja and Soja 1995).

The modern cultivar 'Yannong 19' had more grain number per ear, higher 1000-grain mass and yield than the older one 'Nongda 311' under control condition. However, these yield traits of 'Yannong 19' were negatively affected to a larger extent by the high O_3 exposure. This might result from the more serious leaf visible damage and the more reduction in P_N in flag leaf by the O_3 exposure. Our results are consistent with the report of Pleijel *et al*. (2006) who found that elevated O_3 exposure caused less reduction in yield and yield attributes to the lower-yielding old cultivar of spring wheat than to the modern one.

The present study clearly showed that high O_3 exposure at the beginning of grain filling stage will have great adverse effects on winter wheat yield. High-yielding modern cultivar was more sensitive to O_3 stress. Therefore, breeders should pay more attention to the O_3 sensitivity of modern wheat cultivars to avoid yield loss in the future, when tropospheric O_3 concentration is projected to be higher.

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