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RESEARCH PAPER

Assessing the genetic relatedness of higher ozone sensitivity of modern wheat to its wild and cultivated progenitors/relatives

D. K. Biswas[1], H. Xu[1], Y. G. Li[1,*], M. Z. Liu[1], Y. H. Chen[2], J. Z. Sun[3] and G. M. Jiang[2,1,*]

[1] *State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, 20 Nanxincun, 100093 Beijing, PR China*

[2] *School of Crop Sciences, Shandong Agricultural University, No. 61, Daizong Avenue, 271018 Tai'an, PR China*

[3] *State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, The Chinese Academy of Sciences, 100101Beijing, PR China*

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Abstract

Modern wheat (*Triticum aestivum* L.) is one of the most ozone (O_3)-sensitive crops. However, little is known about its genetic background of O_3 sensitivity, which is fundamental for breeding O_3-resistant cultivars. Wild and cultivated species of winter wheat including donors of the A, B and D genomes of *T. aestivum* were exposed to 100 ppb O_3 or charcoal-filtered air in open top chambers for 21 d. Responses to O_3 were assessed by visible O_3 injury, gas exchange, chlorophyll fluorescence, relative growth rate, and biomass accumulation. Ozone significantly decreased light-saturated net photosynthetic rate (−37%) and instantaneous transpiration efficiency (−42%), but increased stomatal conductance (+11%) and intercellular CO_2 concentration (+11%). Elevated O_3 depressed ground fluorescence (−8%), maximum fluorescence (−26%), variable fluorescence (−31%), and maximum photochemical efficiency (−7%). Ozone also decreased relative growth rate and the allometric coefficient, which finally reduced total biomass accumulation (−54%), but to a greater extent in roots (−77%) than in the shoot (−44%). Winter wheat exhibited significant interspecies variation in the impacts of elevated O_3 on photosynthesis and growth. Primitive cultivated wheat demonstrated the highest relative O_3 tolerance followed by modern wheat and wild wheat showed the lowest. Among the genome donors of modern wheat, *Aegilops tauschii* (DD) behaved as the most O_3-sensitive followed by *T. monococcum* (AA) and *Triticum turgidum* ssp. *durum* (AABB) appeared to be the most O_3-tolerant. It was concluded that the higher O_3 sensitivity of modern wheat was attributed to the increased O_3 sensitivity of *Aegilops tauschii* (DD), but not to *Triticum turgidum* ssp. *durum* (AABB) during speciation.

Key words: Biomass, Chl *a* fluorescence, genome, ozone sensitivity, relative growth rate, stomatal conductance, winter wheat.

Introduction

Modern hexaploid wheat (*Triticum aestivum* L.) is recognized as one of the most ozone (O_3)-sensitive crops (Heck *et al*., 1984; Soja and Soja, 1995*a*; Sellden and Pleijel, 1995; Farage and Long, 1999; Emberson *et al*., 2003; Wang *et al*., 2007*a*). There are also large variations in O_3 sensitivity between cultivars of wheat (Barnes *et al*., 1990; Heagle *et al*., 2000; Biswas *et al*., 2008). It has been documented that more recent cultivars of spring wheat are more sensitive to O_3 than older ones despite rising atmospheric O_3 concentrations (Barnes *et al*., 1990; Velissariou *et al*., 1992; Pleijel *et al*., 2006). In a previous experiment (Biswas *et al*., 2008), it was found that higher O_3 sensitivity in the more recent winter wheat cultivars was induced by higher O_3 flux, larger reduction in antioxidative capacity, and lower levels of dark respiration, leading to higher oxidative damage to proteins and integrity of cellular membranes. Since O_3 tolerance is

* To whom correspondence should be addressed. E-mail: liyonggeng@ibcas.ac.cn; jianggm@126.com

a heritable trait (Damicone and Manning, 1987; Barnes *et al*., 1999; Reinert and Eason, 2000; Fiscus *et al*., 2005), knowledge on O_3 sensitivity of donors of the A, B and D genomes of modern wheat may be critical for the genetic aspects of increased O_3 sensitivity, as well as for breeding cultivars through chromosome-mediated gene transfer. Although effects of the A, B and D genomes of wheat on photosynthesis (Planchon and Fesquet, 1982; Haour-Lurton and Planchon, 1985) and its relation to abiotic stress tolerance (i.e. salt, cold, drought) have been well investigated (Limin *et al*., 1997; Shah *et al*., 1997; Chandrasekar *et al*., 2000; Colmer *et al*., 2006), there has been no study on the impacts of wheat genomes on O_3 tolerance.

The genome of an organism comprises a set of chromosomes, containing all of its genes and associated DNA. The wheat group (the genera *Aegilops* and *Triticum*) constitutes an alloploidy series containing diploid, tetraploid, and hexploid species with a basic chromosome set of $x=7$ (Belay *et al*., 1994). For instance, *T. aestivum* is hexaploid wheat which contains three diploid genomes ($2n=6x=42$, AABBDD). One predecessor of modern wheat is tetraploid durum wheat, *T. turgidum* ssp. *durum* with two diploid genomes ($2n=4x=28$, AABB) (Zohary and Hopf, 2000; Singh, 2005). The A genome shares a high degree of homology with the diploid genomes of *T. urartu* (AA), a species closely related to einkorn wheat, *T. monococcum* (AA), which was domesticated around 12 000 years ago (Zohary and Hopf, 2000; Singh, 2005). The origin of the B genome is still a matter of debate in the present literature (Levy and Feldman, 2004). It has been reported that the B genome might be derived from *Aegilops speltoides*, although its donor has still not been definitively identified (Levy and Feldman, 2004; Rudnoy *et al*., 2004). However, the hexaploid wheat, *T. aestivum* (AABBDD) is believed to have originated by hybridization between the early domesticated tetraploid durum wheat, *T. turgidum* ssp. *durum* (AABB) as a cytoplasm donor and the wild diploid wheat, *A. tauschii* ($2n=2x=14$, DD) as a donor of the D genome about 8000 years ago (Feldman, 2001; Matsuoka and Nasuda, 2004).

Plant sensitivity to O_3 is typically assessed by the decline in growth and/or by visible symptoms, although it has often been reported that there is only a weak correlation between visible injury and growth (Reiling and Davison, 1992; Soja and Soja, 1995*b*). However, relative growth rate and allometric coefficient, i.e. the ratio between mean relative growth rate of root to shoot can provide a simple integration of the effects of O_3 stress (Reiling and Davison, 1992; Andersen, 2003). It has also been reported that O_3 sensitivity of spring wheat as determined by relative growth rate at the vegetative stage is proportional to O_3-induced yield reduction (Pleijel *et al*., 2006). Gas exchange and chlorophyll fluorescence, on the other hand, offer a useful and non-destructive tool for *in vivo* stress detection (Owens, 1994; Maxwell and Johnson, 2000) and the mechanisms involved in O_3-induced growth reduction (Guidi *et al*., 1997; Cardoso-Vilhena *et al*., 2004).

Despite the fact that O_3 tolerance is a heritable trait (Damicone and Manning, 1987; Barnes *et al*., 1999; Fiscus *et al*., 2005) and the fact that genotypic variation in O_3 sensitivity exists in wheat, with increased O_3 sensitivity of recent cultivars compared with older ones (Barnes *et al*., 1990; Velissariou *et al*., 1992; Pleijel *et al*., 2006), little attention has been paid to the genetic variations in O_3 sensitivity of their donor species. Besides, wild and primitive cultivated species are commonly used as crossing materials with immediate progenitors of wheat (Valkoun, 2001) to introduce economically useful genes as well as to enhance genetic diversity of wheat (Kawahara, 2002; Xiong *et al*., 2006). Determination of O_3 sensitivity of wild and cultivated progenitors of modern wheat is especially critical to quantify the magnitude of genetic control of O_3 tolerance and its further exploitation. Therefore, O_3 sensitivity of wild and cultivated winter wheat species including donors of the A, B and D genomes of modern wheat were examined in terms of visible symptoms, growth, gas exchange, and fluorescence parameters. It was hypothesized that increased O_3 sensitivity of modern wheat might be related to its wild and cultivated progenitors/relatives. Our findings may provide sufficient genetic basis of the increased O_3 sensitivity of modern wheat, which is fundamental for breeding O_3-resistant wheat cultivars.

Materials and methods

Plant establishment and O_3 fumigation

Twelve wild and cultivated species/cultivars including donors of the A, B and D genomes of modern wheat belonging to diploid (AA and DD), tetraploid (AABB and AAGG), and hexaploid (AABBDD) wheat were evaluated for O_3 tolerance (Table 1). Seeds of selected winter wheat species/cultivars were obtained from the School of Crop Sciences, Shandong Agricultural University, Tai'an, PR China. On 2 March 2006, three germinated seeds were sown in each of 60 plastic pots (6 cm in diameter, 9 cm in height) per genotype in a temperature-controlled double-glazed greenhouse. Pots were filled with field clay loam soil containing organic C, total N, total P, and total *K* at the rate of 1.24%, 0.045%, 296 mg kg^{-1} and 14.7 g kg^{-1}, respectively. No chemical fertilizer was applied either as basal or topdressing. Seedlings were thinned to one per pot on the seventh day after planting (DAP). On 8 DAP, 15 pots per genotype were moved to each of four open-top chambers (OTC, 1.2 m in diameter, 1.6 m in height) placed in the same greenhouse. The OTCs were ventilated continuously (24 h d^{-1}) with air passing through activated charcoal filters attached to fan boxes. All seedlings were allowed to grow till 14 DAP to adapt to chamber environments before O_3 exposure. During this adaptation period, all plants received charcoal-filtered air with an O_3 concentration of less than 5 ppb. The gas-dispensing system of the OTCs was constructed following the methodology described by Uprety (1998). The chambers were illuminated by natural daylight

Table 1. *List of species/cultivars including donors of the A, B and D genomes of* Triticum aestivum *L. used in the present experiment*

Sl.	Species/cultivars	Type of wheat	Nuclear genome type	Cytoplasm genome type	Chromosome number
1	*Aegilops tauschii*[a]	Wild	D	D	14
2	*Triticum boeoticum* Bioss	Wild	A	A	14
3	*Triticosecale wittmack*	Primitive	R	R	14
4	*Triticum monococcum* L.[a]	Primitive	A	A	14
5	*Triticum dicoccum* Schubl.	Primitive	AB	B	28
6	*Triticum timopheevii* Zhuk.	Primitive	AG	G	28
7	*Triticum polonicum* L.	Primitive	AB	B	28
8	*T. turgidum* ssp. *durum*[a]	Primitive	AB	B	28
9	*T. aestivum* L. cv. Nongda 3214	Modern	ABD	B	42
10	*T. aestivum* L. cv. Xinong 2611	Modern	ABD	B	42
11	*T. aestivum* L. cv. Hannong 2	Modern	ABD	B	42
12	*T. aestivum* L. cv. Xiaoyan 22	Modern	ABD	B	42

[a] Donors of modern wheat, *T. aestivum* L.

supplemented by fluorescence light (360 W) providing a photosynthetic photon flux density (PPFD) of approximately 260 μmol m^{-2} s^{-1} at plant canopy height, yielding a 14 h photoperiod. The maximum PPFD in the chambers was approximately 690 μmol m^{-2} s^{-1}. The temperature in the OTCs was 16/28 °C night/day and relative humidity was 65–90% during the experimental period. Plants were watered to soil field capacity every 2 d to avoid water stress throughout the experiment and the hard soil crust formed after irrigation was broken to ensure better aeration in the soil.

On 15 DAP, O_3 was added to the charcoal-filtered air-stream entering two of the chambers to maintain an O_3 concentration of 100±7 ppb for 7 h d^{-1} (10.00 h to 17.00 h) for 21 d. The other two chambers were set up the same way but without O_3 addition. In the present study, a high target O_3 treatment was chosen since O_3 concentrations exceed potentially damaging levels (>120 ppb) for many h in some peri-urban areas of China during the summer months (Zheng *et al*., 1998; Wang *et al*., 2007*b*). The treatments (+O_3, elevated O_3; CF, charcoal-filtered control) were assigned randomly to the chambers and replicated twice. O_3 was generated by electrical discharge using charcoal-filtered ambient oxygen (Balaguer *et al*., 1995) with an O_3 generator (CF-KG1, Beijing Sumsun EP Hi-Tech., Co. Ltd. China) and bubbled through distilled water before entering the two high O_3 chambers. Ambient air was used since it has been reported that the small amount of HNO_3 vapour or N_2O produced during generation of O_3 using ambient air is typically undetectable and does not interact with O_3 (Neighbour *et al*., 1990; Taylor *et al*., 1993; Balaguer *et al*., 1995; Mortensen and Jorgensen, 1996). Water traps were used to remove harmful compounds other than O_3 (Balaguer *et al*., 1995). Manual mass flow controllers were used to regulate the flow of O_3-enriched air to the OTCs. O_3 concentrations in the OTCs were continuously monitored at approximately 10 cm above the plant canopy using an O_3 analyser (APOA-360, Horiba, Ltd, Japan), which was cross-calibrated with another O_3 monitor (ML 9810B, Eco-Tech, USA). In order to minimize chamber effects and environmental heterogeneity, plants and associated treatment were rotated between the chambers and plants were also randomized within the chambers every three days throughout the experiment.

Visible O_3 injury

Visible injury was assessed on the whole plant or leaf level (the third youngest leaf of the main stem) after 21 d of O_3 exposure on 35 DAP, when the plants had a total of 5–6 fully expanded leaves. The percentage of damaged area (mottled or necrotic) on the leaves was assessed for five plants per species/cultivar sampled from control and O_3 fumigated chambers.

Leaf gas exchange measurement

On day 19 of O_3 fumigation, four plants per species/cultivar were sampled from each chamber per treatment for leaf gas exchange measurements. The most recently fully expanded leaf (i.e. after emergence of ligules) without visible symptoms of damage on the main stem was used for measurement with an open gas exchange system (Li- 6400, Li-Cor, Inc., Lincoln, NE, USA). The system was calibrated prior to measurement. During measurement, relative humidity was maintained at 70% and leaf temperature was set at 25 °C in the leaf chamber. The flow rate was set at 700 μmol s^{-1} and CO_2 concentration in the leaf chamber was maintained at 386 μmol mol^{-1}. PPFD was maintained at 1500 μmol m^{-2} s^{-1}using the internal light source of the leaf chamber. Data obtained as part of the gas exchange measurements included area-based light-saturated net photosynthetic rate (A_{sat}), stomatal conductance (g_s), the ratio between intercellular CO_2 concentration (C_i) and ambient CO_2 concentration (C_a), and leaf-level photosynthetic water use efficiency, or instantaneous transpiration efficiency (*ITE*), which was calculated as assimilation/transpiration.

Chlorophyll fluorescence measurement

On day 20 of O_3 fumigation, four plants per species/cultivar were sampled from each chamber and taken into an adjacent laboratory for dark adaptation (40 min) to ensure maximal oxidization of the primary quinone acceptor (Q_A). The same plants were not used for fluorescence measurement to avoid plants with reduced ozone exposure during gas exchange measurement and any leaf injury resulting from leaf chamber. Modulated chlorophyll fluorescence measurements were made in the middle of the intact youngest fully expanded leaves without visible O_3 injury using a PAM-2000 (Heinz Walz, Germany). The room temperature was maintained at 25 °C during measurements. The minimum fluorescence, F_0, was determined with modulated light which was sufficiently low (<1 μmol m^{-2} s^{-1}), so as not to induce any significant variable fluorescence. The maximum fluorescence, F_m, was determined using a 0.8 s saturating pulse at 8000 μmol m^{-2} s^{-1}. Data obtained after recording fluorescence key parameters included minimum fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence, F_v=F_m–F_0, and maximum photochemical efficiency in the dark-adapted state, F_v/F_m (Krause and Weis, 1991).

Determination of growth and resource allocation

Plants were sampled for growth analysis before O_3 fumigation (on 15 DAP) and after 21 d of O_3 exposure (on 35 DAP). Four plants per species/cultivar were harvested from each chamber and partitioned into shoot and root before being dried to constant weight

at 72 °C. The difference in dry weight between the pre-fumigation and final harvest was used to calculate relative growth rate of whole plants and plant parts over 21 d. Mean plant relative growth rate (*RGR*), relative growth rate of shoot (RGR_s), relative growth rate of root (RGR_r), and allometric coefficient ($K=RGR_r/RGR_s$) were calculated as described by Hunt (1990).

Statistical analysis

The experiment consisted of two randomized blocks of two treatments with 15 plants per replicate. Statistical analyses of data were performed using analysis of variance (ANOVA) in the General Linear Model procedure of SPSS (Ver. 13, SPSS, Chicago, IL, USA). The main effects of wheat type, wheat species, and wheat genotype in O_3 and CF air were analysed using one-way ANOVA on the measured variables. To control the Type I error across the entire set of pair-wise comparisons, the Tukey–Kramer method was used to assess differences among treatment means. Regression analysis was carried out to investigate the relationships between physiological and growth parameters. Differences between treatments were considered significant at $P < 0.05$.

Results

Visible O_3 injury

Elevated O_3 developed visible O_3 injury on mature leaves of all wheat species, whereas control plants showed no visible symptoms of O_3 damage. No visible symptoms were observed on the youngest fully expanded leaves. All species exposed to high O_3 showed the start of premature leaf senescence (Leaf 1) after one week of O_3 fumigation (data not shown). There was a significant ($P < 0.001$) interspecies variation in O_3 injury developed on the third youngest leaf of the main stem (48–72%) after the end of O_3 fumigation (Table 2). The lowest visible symptoms were observed both in *T. turgidum* ssp. *durum* and *T. dicoccum*, whereas wild species (*A. tauschii*, *T. boeoticum*) showed the highest. Significantly differences in visible O_3 symptoms were noted between the cultivars. Visible O_3 symptoms were found to be significantly positively correlated with g_s in O_3-exposed plants, but negatively correlated with *RGR* and total biomass relative to control (Fig. 1).

Table 2. *Interspecies variations in development of visible symptoms of O_3 damage on the third youngest leaf of the main stem in winter wheat after 21 d exposure to O_3 (100±7 ppb for 7 h d^{-1})*

Means ±1 SEM. Similar letters indicate non-significant difference at $P < 0.05$.

Species	Visible O_3 injury (%)
A. tauschii[a]	64±3 ab
T. boeoticum Bioss	72±2 a
Triticosecale wittmack	61±2 abc
T. monococcum L.[a]	52±3 bc
T. dicoccum Schubl.	50±2 c
T. timopheevii Zhuk	51±4 bc
T. polonicum L.	55±2 bc
T. turgidum ssp. *durum*[a]	48±4 c
T. aestivum L.	58±1 abc
P value (species)	<0.001

[a] Donors of modern wheat, *T. aestivum* L.

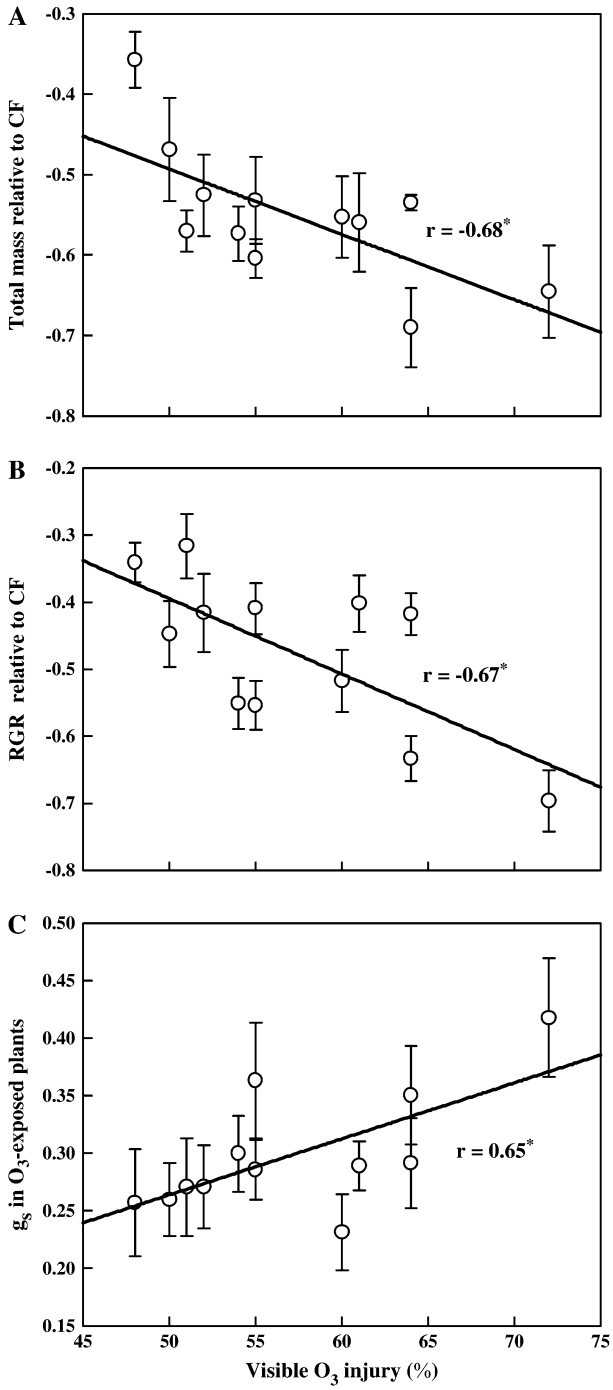

Fig. 1. Relationship between the extent of visible O_3 injury developed on the third youngest leaf of the main stem of winter wheat and (A) relative total biomass accumulation, (B) relative growth rate relative to control, and (C) stomatal conductance in O_3-exposed plants. n=10. Error bar indicates 1 SEM.

Gas exchange

Ozone significantly ($P < 0.05$) decreased A_{sat} and *ITE*, but increased g_s and C_i/C_a. There was considerable interspecies variation in gas exchange characteristics in CF air or O_3-treated plants (Table 3). The highest A_{sat} and g_s were observed in wild wheat, followed by modern wheat and the lowest was in primitive cultivated wheat in CF air (data not shown). Significantly higher A_{sat} and *ITE* were observed in modern than in primitive cultivated or wild wheat of O_3-exposed plants. Wild wheat had higher g_s and C_i/C_a than modern or primitive cultivated wheat at high O_3. The highest relative reduction in A_{sat} was observed in *Triticosecale wittmack,* while *T. dicoccum* showed the lowest. Almost all wheat species displayed non-significant relative increases in g_s, while *T. turgidum* ssp. *durum* and *Triticosecale wittmack* showed a relative decrease in g_s. In addition, *A. tauschii* showed no change in g_s in O_3 relative to the control. The highest and lowest relative increases in C_i/C_a were observed in *T. boeoticum* and *T. timopheevii*, respectively. *T. turgidum* ssp. *durum* had the lowest relative loss of *ITE*, while *T. boeoticum* had the highest. Wheat cultivars also showed significant variation in gas exchange traits in both CF air and elevated O_3. Among donor species of *T. aestivum*, the highest relative loss in A_{sat} and *ITE* was noted in *A. tauschii*, followed by *T. monococcum* and the least was in *T. turgidum* ssp. *durum*. There were strong negative relationships between g_s in ozone-exposed plants and reduction in total mass, *RGR* and F_v in O_3 relative to control (Fig. 2).

Chlorophyll fluorescence

Ozone significantly ($P < 0.001$) decreased dark-adapted ground fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v) and maximum photochemical efficiency (F_v/F_m). Large interspecies variation in chlorophyll fluorescence was observed in CF and ozonated plants (Table 4). Modern and primitive cultivated wheats had higher F_0, F_m, and F_v than wild wheat both in CF air and elevated O_3 (data not shown). Higher F_v/F_m was observed in modern wheat rather than in wild wheat or primitive cultivated wheat in CF air. There was no significant interspecies variation in F_v/F_m in O_3-treated plants. The highest relative reduction in F_0, F_m, and F_v was observed in *A. tauschii*, while *T. polonicum* showed the lowest. The highest and lowest relative reduction in F_v/F_m was observed in *Triticosecale wittmack* and in *T. turgidum* ssp. *durum*, respectively (Table 2). Significant cultivar differences were also noted in F_0, F_m, and F_v both in CF air and elevated O_3. Among donor species of modern wheat, the highest relative decrease in F_v was observed in *A. tauschii*, followed by *T. monococcum* and the least was in *T. turgidum* ssp. *durum*. Both *A. tauschii* and *T. monococcum* had higher relative loss in F_v/F_m than *T. turgidum* ssp. *durum*. F_v in ozone-exposed plants was

Table 3. *Effects of O_3 on light-saturated net photosynthetic rate (A_{sat}), stomatal conductance (g_s), ratio between intercellular CO_2 to ambient CO_2 concentration (C_i/C_a) and instantaneous transpiration efficiency* (ITE) *in wild and cultivated species of winter wheat*

Control plants received charcoal-filtered air (CF, <5 ppb O_3) and O_3-treated plants (+O_3) were exposed to 100±7 ppb O_3. % (±) indicates per cent changes in O_3-exposed relative to control plants, (+O_3–CF)/CF. n=8. Means ±1 SEM. Asterisks denote significant difference between O_3-treated and control plants at *, $P \leqslant 0.05$; **, $P \leqslant 0.01$; ***, $P \leqslant 0.001$.

Species	A_{sat} (μmol m^{-2} s^{-1})			g_s (mol m^{-2} s^{-1})			C_i/C_a			*ITE* (μmol mmol^{-1})		
	CF	O_3	%	CF	O_3	%	CF	O_3	%	CF	O_3	%
A. tauschii[a]	14.79±0.70	6.70±0.40	–55***	0.35±0.03	0.35±0.01	0	0.81±0.02	0.89±0.01	10	3.59±0.30	1.63±0.16	–55*
T. boeoticum Bioss	15.59±0.93	9.71±0.71	–38***	0.31±0.04	0.42±0.04	35	0.76±0.03	0.88±0.01	16**	4.37±0.44	1.93±0.22	–56***
Triticosecale wittmack	15.07±1.14	6.47±0.55	–57***	0.32±0.03	0.29±0.03	–9	0.79±0.01	0.89±0.01	13	3.74±0.18	1.86±0.22	–50*
T. monococcum L.[a]	14.31±0.44	8.26±0.82	–42***	0.24±0.01	0.27±0.06	11	0.74±0.02	0.84±0.02	14*	4.74±0.30	2.87±0.43	–39*
T. dicoccum Schubl.	10.79±0.58	8.32±0.63	–23	0.19±0.02	0.26±0.02	37	0.75±0.02	0.83±0.02	11	4.59±0.31	2.58±0.25	–44*
T. timopheevii Zhuk	11.92±0.87	9.05±0.18	–24	0.22±0.02	0.27±0.02	21	0.76±0.02	0.81±0.03	8*	4.39±0.28	2.72±0.25	–38
T. polonicum L.	14.44±1.62	10.09±0.87	–30**	0.28±0.05	0.29±0.02	2	0.75±0.03	0.84±0.02	12	4.85±0.40	3.57±0.61	–27
T. turgidum ssp. *durum*[a]	14.06±0.71	10.41±0.85	–26*	0.27±0.02	0.26±0.01	–5	0.76±0.02	0.84±0.01	10	4.71±0.32	3.47±0.38	–26
T. aestivum L.	14.54±0.26	10.06±0.23	–31***	0.26±0.01	0.30±0.02	15	0.78±0.01	0.85±0.01	9***	4.56±0.16	3.10±0.25	–32***
Overall mean	13.94±0.24	8.79±0.24	–37***	0.27±0.01	0.30±0.01	11*	0.76±0.01	0.85±0.01	11***	4.44±0.12	2.75±0.12	–42***
P value (species)	0.001	<0.001		0.001	0.012		0.487	0.022		0.064	0.003	

[a] Donors of modern wheat, *T. aestivum* L.

significantly positively correlated with relative reduction in total mass and *RGR*, but negatively ($r = -0.54$, P=0.069) with relative reduction in *K*. It also positively correlated with the relative reduction in shoot mass, RGR_s and A_{sat} (Fig. 3).

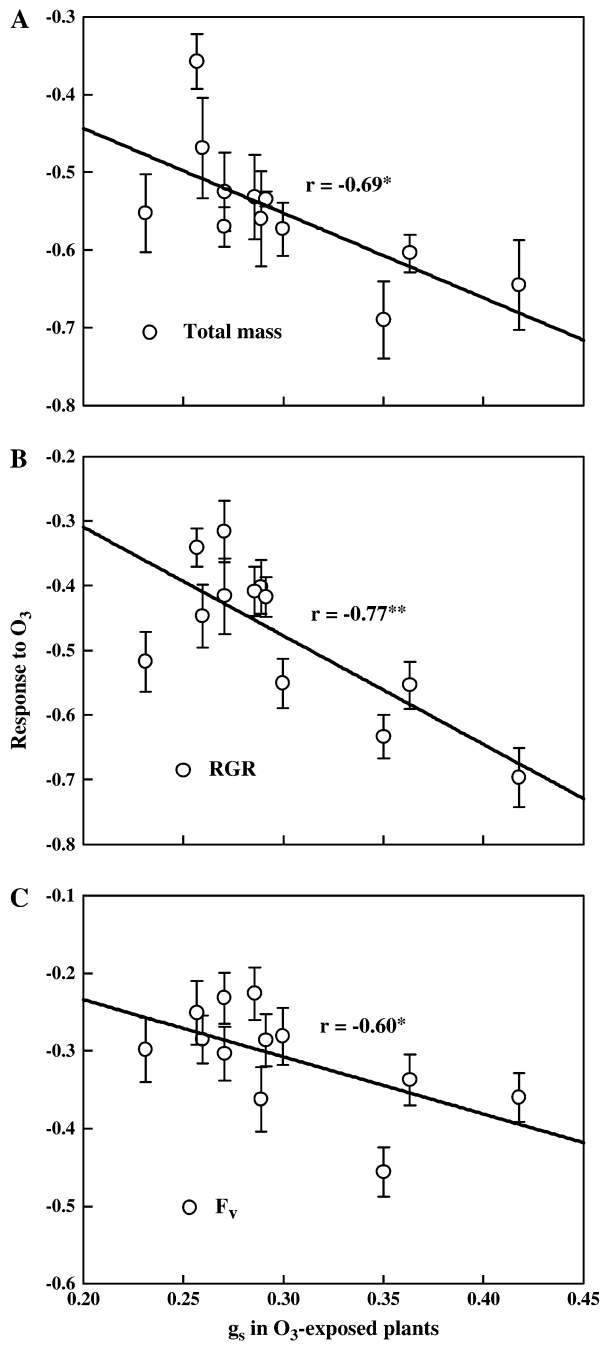

Fig. 2. Functional relationship between stomatal conductance (g_s) in O_3-exposed plants and O_3 response ratio of (A) total mass, (B) relative growth rate of whole plant (*RGR*), and (C) variable fluorescence expressed as (O_3–CF)/CF. CF, charcoal-filtered air. n=8. Error bar indicates 1 SEM.

Table 4. *Effects of O_3 on ground fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v) and maximum photochemical efficiency (F_v/F_m) in wild and cultivated species of winter wheat*

Control plants received charcoal-filtered air (CF, <5 ppb O_3) and O_3-treated plants (+O_3) were exposed to 100±7 ppb O_3. % (±) indicates per cent changes in O_3-exposed relative to control plants, (+O_3–CF)/CF. n=8. Means ±1 SEM. Asterisks denote significant difference between O_3-treated and control plants at *, $P \leqslant 0.05$; **, $P \leqslant 0.01$; ***, $P \leqslant 0.001$.

Species	F_0			F_m			F_v			F_v/F_m		
	CF	O_3	%	CF	O_3	%	CF	O_3	%	CF	O_3	%
A. tauschii[a]	0.29±0.02	0.20±0.02	–32*	1.40±0.09	0.80±0.11	–43***	1.11±0.07	0.60±0.09	–46***	0.79±0.00	0.74±0.02	–6**
T. boeoticum Bioss	0.29±0.02	0.26±0.03	–12	1.46±0.11	1.00±0.11	–31*	1.16±0.09	0.74±0.08	–36**	0.80±0.00	0.74±0.01	–7***
Triticosecale wittmack	0.35±0.00	0.33±0.03	–7	1.61±0.02	1.13±0.11	–30*	1.26±0.02	0.80±0.09	–36***	0.78±0.00	0.71±0.02	–10***
T. monococcum L.[a]	0.35±0.01	0.31±0.01	–11	1.52±0.03	1.12±0.04	–26	1.17±0.02	0.81±0.03	–30*	0.77±0.00	0.72±0.01	–6*
T. dicoccum Schubl.	0.35±0.01	0.33±0.01	–5	1.58±0.04	1.21±0.04	–23	1.24±0.03	0.88±0.03	–29*	0.78±0.00	0.73±0.01	–7**
T. timopheevii Zhuk	0.35±0.01	0.35±0.02	0	1.61±0.07	1.32±0.10	–18	1.26±0.06	0.97±0.08	–23	0.78±0.00	0.73±0.01	–7**
T. polonicum L.	0.33±0.02	0.35±0.01	+5	1.63±0.09	1.35±0.01	–17	1.30±0.08	1.01±0.02	–23	0.80±0.00	0.74±0.01	–7***
T. turgidum ssp. *durum*[a]	0.38±0.01	0.33±0.03	–13	1.73±0.04	1.34±0.20	–23	1.35±0.03	1.01±0.05	–25	0.78±0.00	0.75±0.01	–5
T. aestivum L.	0.35±0.00	0.33±0.01	–4	1.67±0.02	1.26±0.05	–25	1.32±0.02	0.92±0.04	–30***	0.79±0.00	0.73±0.00	–7***
Overall mean	0.34±0.01	0.31±0.01	–8**	1.58±0.04	1.17±0.04	–26***	1.24±0.03	0.86±0.03	–31***	0.79±0.00	0.73±0.00	–7***
P value (species)	<0.001	<0.001		0.001	0.004		0.003	0.013		<0.001	0.336	

[a] Donors of modern wheat, *T. aestivum* L.

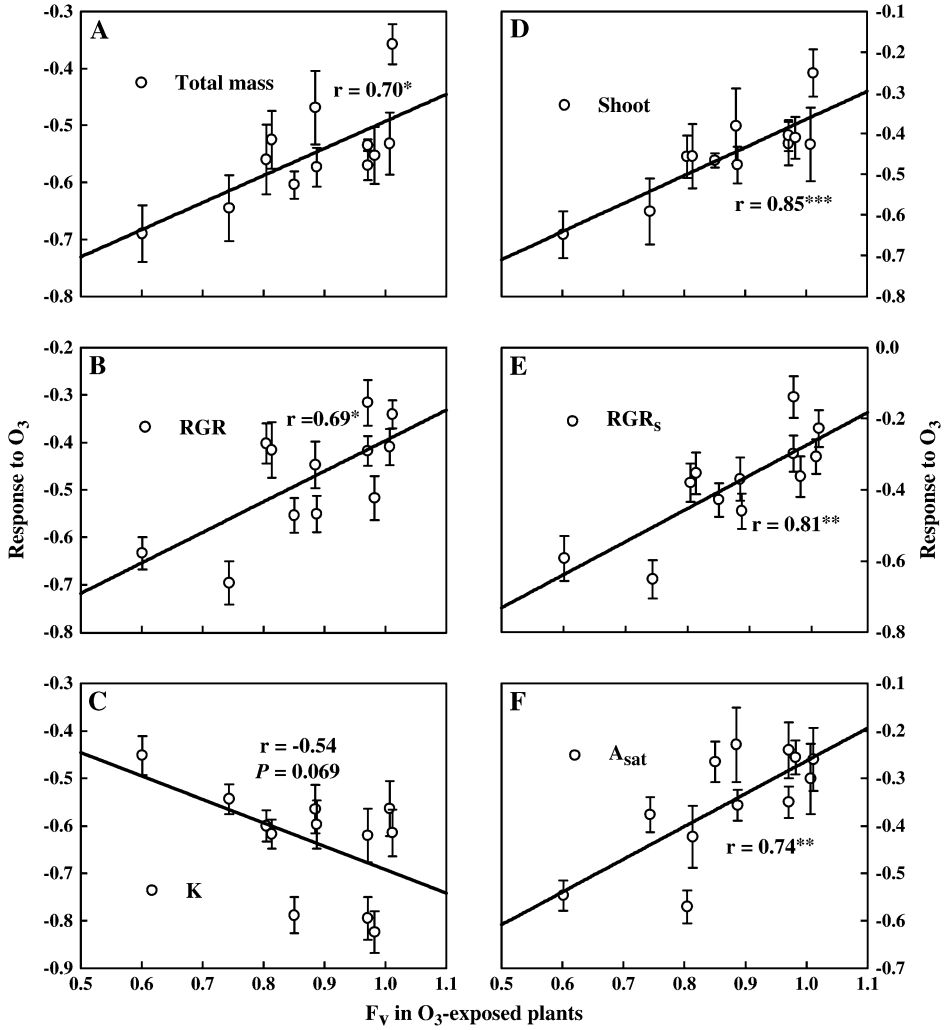

Fig. 3. Functional relationship between variable fluorescence (F_v) in O_3-exposed plants and O_3 response ratio of (A) total mass, (B) relative growth rate of whole plant (*RGR*), (C) allometric coefficient (*K*), (D) shoot mass, (E) relative growth rate of shoot (RGR_s), and (F) light-saturated net assimilation rate (A_{sat}) expressed as (O_3–CF)/CF. CF, charcoal-filtered air. n=8. Error bar indicates 1 SEM.

Growth and resource allocation

Ozone significantly ($P < 0.001$) decreased *RGR*, RGR_s, RGR_r, and *K* in wheat species (Table 5). There was significant interspecies difference in growth and resource allocation in control plants. Generally higher *RGR*, RGR_s, and RGR_r were observed in wild wheat rather than in modern wheat or in primitive cultivated wheat. Modern and primitive cultivated wheats had higher *K* than wild wheat. Significant variation ($P < 0.05$) was observed between wheat types showing higher *RGR* and RGR_s in primitive cultivated wheat than in modern or wild wheat (data not shown) at elevated O_3. There was significant variation in *RGR*, RGR_s, RGR_r and *K* in wheat cultivars exposed to CF air, but not to elevated O_3. Among genome donors of modern wheat, the highest relative decrease in *RGR*, RGR_s, RGR_r, and *K* was observed in *A. tauschii*, followed by *T. monococcum* and the least was in *T. turgidum* ssp. *durum*. *RGR* in CF control plants was significantly negatively correlated with relative reduction in *RGR* and RGR_s, but positively (r=0.52, P=0.083) with the relative reduction in *K* (Fig. 4).

Dry matter accumulation and partitioning

Ozone significantly ($P < 0.001$) decreased shoot, root, root/shoot ratio, and total mass in wheat species, with the greater effect being in roots than in shoots (Table 6). Considerable interspecies variation in dry matter accumulation and partitioning were observed in both control and O_3-treated plants. The highest shoot, root, and total masses were observed in modern wheat, followed by primitive cultivated wheat and the lowest in wild wheat both in CF air and elevated O_3 (data not shown). Significantly higher root/shoot ratio was noted in modern wheat than in wild or primitive cultivated wheat in CF air. Modern and wild wheats showed higher root/shoot ratios than primitive cultivated wheat at elevated O_3.

Table 5. *Effects of O_3 on relative growth rate of whole plants* (RGR), *relative growth rate of shoot* (RGR_s), *relative growth rate of root* (RGR_r) *and allometric coefficient* (K) *in wild and cultivated species of winter wheat*

Control plants received charcoal-filtered air (CF, <5 ppb O_3) and O_3-treated plants (+O_3) were exposed to 100±7 ppb O_3. % (±) indicates percent changes in O_3-exposed relative to control plants, (+O_3–CF)/CF. n=8. Means ±1 SEM. Asterisks denote significant difference between O_3-treated and control plants at *, $P \leqslant 0.05$; **, $P \leqslant 0.01$; ***, $P \leqslant 0.001$.

Species	*RGR* (mg g^{-1} plant d^{-1})			*RGR_s* (mg g^{-1} shoot d^{-1})			*RGR_r* (mg g^{-1} root d^{-1})			*K*		
	CF	O_3	%	CF	O_3	%	CF	O_3	%	CF	O_3	%
A. tauschii[a]	90.43±0.01	33.15±0.01	–63***	91.33±0.01	37.22±0.01	–59***	87.20±0.01	16.85±0.00	–81***	0.97±0.09	0.53±0.07	–65
T. boeoticum Bioss	101.13±0.01	30.70±0.01	–70***	99.63±0.01	34.74±0.01	–65***	104.78±0.01	16.30±0.01	–84***	1.05±0.04	0.48±0.05	–59*
Triticosecale wittmack	65.46±0.01	39.12±0.00	–40	70.07±0.02	43.46±0.00	–38	68.21±0.01	20.20±0.00	–70***	1.18±0.06	0.47±0.08	–46***
T. monococcum L.[a]	79.33±0.01	46.30±0.01	–42	79.26±0.01	51.25±0.01	–35	78.99±0.01	21.93±0.01	–72***	0.99±0.07	0.38±0.06	–46**
T. dicoccum Schubl.	66.81±0.01	36.92±0.01	–45	65.49±0.01	41.25±0.01	–37	68.85±0.01	19.44±0.01	–72***	1.08±0.05	0.47±0.09	–38**
T. timopheevii Zhuk	71.87±0.00	49.13±0.01	–32	65.08±0.00	56.03±0.01	–14	87.69±0.00	17.10±0.01	–80***	1.35±0.03	0.28±0.06	–43***
T. polonicum L.	81.92±0.01	48.39±0.02	–41	79.04±0.01	54.81±0.02	–31	86.51±0.01	23.02±0.01	–73***	1.15±0.07	0.50±0.04	–43**
T. turgidum ssp. *durum*[a]	74.12±0.01	48.82±0.01	–34	75.26±0.01	58.10±0.01	–23	69.78±0.01	25.98±0.01	–63**	0.99±0.04	0.38±0.03	–25**
T. aestivum L.	71.77±0.00	35.32±0.00	–51	67.09±0.00	41.28±0.00	–38***	80.62±0.00	15.10±0.00	–81***	1.20±0.05	0.34±0.05	–44***
Overall mean	78.09±0.00	40.87±0.00	–48***	76.92±0.00	46.46±0.00	–40***	81.40±0.00	19.55±0.00	–76***	1.11±0.03	0.43±0.03	–62***
P value (species)	0.001	0.298		0.007	0.243		0.048	0.836		0.221	0.379	

[a] Donors of modern wheat, *T. aestivum* L.

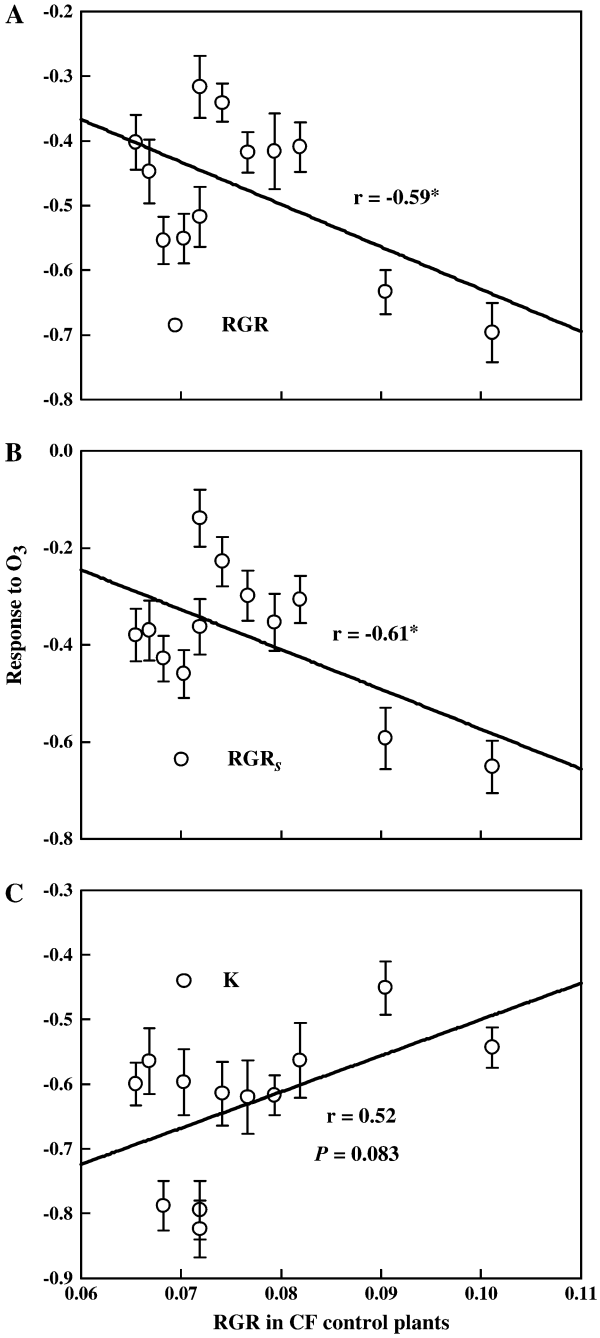

Fig. 4. Functional relationship between relative growth rate of whole plants (*RGR*) in CF control plants and (A) O_3 response ratio of *RGR*, (B) relative growth rate of shoot, and (C) allometric coefficient (*K*) expressed as (O_3–CF)/CF. CF, charcoal-filtered air. n=8. Error bar indicates 1 SEM.

Nevertheless, *T. turgidum* ssp. *durum* showed the lowest relative reduction in shoot, root, and total mass, while *T. boeoticum* showed the highest. The lowest relative reduction in root/shoot ratio was found in *T. boeoticum,* while *T. timopheevii* showed the highest. Wheat cultivars showed significant variation in shoot, root, root/shoot ratio, and total mass both in CF air and elevated O_3.

Table 6. *Effects of O_3 on shoot mass, root mass, root/shoot ratio, and total mass in wild and cultivated species of winter wheat*

Control plants received charcoal-filtered air (CF, <5 ppb O_3) and O_3-treated plants (+O_3) were exposed to 100±7 ppb O_3. % (±) indicates percent changes in O_3-exposed relative to control plants, (+O_3–CF)/CF. n=8. Means ±1 SEM. Asterisks denote significant difference between O_3-treated and control plants at *, $P \leqslant 0.05$; **, $P \leqslant 0.01$; ***, $P \leqslant 0.001$.

Species	Shoot mass (g plant^{-1})			Root mass (g plant^{-1})			Root/shoot ratio			Total mass (g plant^{-1})		
	CF	O_3	%	CF	O_3	%	CF	O_3	%	CF	O_3	%
A. tauschii[a]	0.106±0.01	0.037±0.00	–65*	0.038±0.01	0.008±0.00	–80	0.370±0.07	0.201±0.02	–46	0.144±0.01	0.045±0.00	–69**
T. boeoticum Bioss	0.128±0.02	0.052±0.01	–59**	0.055±0.01	0.013±0.00	–77*	0.422±0.03	0.247±0.03	–42	0.182±0.02	0.065±0.01	–65***
Triticosecale wittmack	0.167±0.03	0.091±0.00	–46**	0.077±0.01	0.017±0.00	–78***	0.453±0.07	0.183±0.01	–60**	0.244±0.04	0.107±0.00	–56***
T. monococcum L.[a]	0.200±0.02	0.109±0.01	–46***	0.064±0.01	0.016±0.00	–74**	0.315±0.03	0.152±0.01	–52	0.264±0.03	0.125±0.01	–53***
T. dicoccum Schubl.	0.156±0.01	0.096±0.01	–38	0.061±0.01	0.019±0.00	–69*	0.408±0.06	0.197±0.02	–52	0.216±0.01	0.115±0.01	–47**
T. timopheevii Zhuk	0.259±0.02	0.149±0.01	–43***	0.137±0.00	0.021±0.00	–85***	0.539±0.04	0.141±0.01	–74***	0.396±0.02	0.170±0.01	–57***
T. polonicum L.	0.186±0.02	0.107±0.01	–43***	0.085±0.01	0.020±0.00	–76***	0.488 ±0.10	0.203±0.04	–59**	0.270 ±0.02	0.127±0.01	–53***
T. turgidum ssp. *durum*[a]	0.143±0.01	0.107±0.01	–25	0.052±0.01	0.019±0.00	–65	0.351±0.06	0.177±0.02	–50	0.195±0.02	0.125±0.01	–36
T. aestivum L.	0.229±0.01	0.128±0.00	–44***	0.133±0.01	0.029±0.00	–78***	0.583±0.03	0.227±0.01	–61***	0.361±0.01	0.156±0.01	–57***
Overall mean	0.175±0.01	0.097±0.01	–44***	0.078±0.00	0.018±0.00	–77***	0.437±0.01	0.192±0.01	–56***	0.252±0.01	0.115±0.01	–54***
P value (species)	<0.001	<0.001		<0.001	<0.001		0.001	0.011 ±		<0.001	<0.001	

[a] Donors of modern wheat, *T. aestivum* L.

Among donor species of modern wheat, the highest relative decrease in shoot, root, and total mass was observed in *A. tauschii*, followed by *T. monococcum* and the least was in *T. turgidum* ssp. *durum*. Higher relative reduction in root/shoot ratio was noted both in *T. monococcum* and *T. turgidum* ssp. *durum* than in *A. tauschii*.

Discussion

Interspecies differences in the development of visible symptoms of O_3 damage in winter wheat

The similar trend of premature leaf senescence (Leaf 1) and the extent of visible symptoms on the third youngest leaves after 7 d and 21 d of O_3 fumigation, respectively, revealed that all species were sensitive to O_3. There was significant ($P < 0.001$) interspecies variation in the extent of visible O_3 injury in winter wheat ranging from 48% to 72%. The extent of visible O_3 injury was found to be correlated with g_s in O_3-exposed plants. This can be explained because O_3 uptake is proportional to g_s and O_3 produces reactive oxygen species which can severely compromise the integrity of metabolically important membranes (Long and Naidu, 2002; Biswas *et al*., 2008). These results indicated that higher O_3 uptake through increased g_s finally led to a greater development of visible injury as well as greater growth reduction. These results are consistent with the findings of Barnes *et al*. (1990) who obtained a significant negative relationship between visible injury and mean *RGR* or F_v of spring wheat cultivars exposed to 90 ppb O_3. This can be explained by loss of chlorophyll and the oxidative stress induced by O_3 as a close relationship between the extent of visible O_3 injury and reduction in F_v/F_m has been documented in bean genotypes (Guidi *et al*., 2000).

Photosynthetic and growth responses of winter wheat species to O_3

Overall, O_3 significantly depressed A_{sat} and *ITE*, but increased g_s and C_i/C_a. These results are in general agreement with the findings of McKee *et al*. (1995), Mulholland *et al*. (1997), and Farage and Long (1999). The effects of O_3 on g_s have been found to be highly variable, as an increase, decrease, and no change in g_s have all been reported in spring wheat and other crops grown at elevated O_3 (Darrall, 1989; Mulholland *et al*., 1997; Farage and Long, 1999; McKee *et al*., 2000). Our results suggested that O_3 depressed photosynthesis by impairing the activity of mesophyll cells as indicated by higher C_i/C_a. Elevated O_3 also significantly ($P < 0.001$) decreased F_0, F_m, F_v, and F_v/F_m. A slight O_3-induced reduction in F_0 (−8%) was observed, which was accompanied by higher reductions in F_m (−26%) and F_v (−31%).

These results are consistent with the previous reports on winter wheat exposed to 80 ppb O_3 (Khan, 2005). The results indicated that overall thermal dissipation by the xanthophyll cycle was minimum, which resulted in higher O_3-impairment of F_v/F_m (−7%) mediated electron transport or O_3-induced photoinhibition (Guidi *et al*., 1997; Cardoso-Vilhena *et al*., 2004; Fiscus *et al*., 2005). In general, O_3 significantly decreased *RGR*, RGR_s, RGR_r, and *K* in winter wheat species, which led to a major decrease in shoot, root, root/shoot ratio, and total mass at final harvest. The marked O_3-induced reduction in *K* indicated that shoot growth was maintained at the expense of root growth in winter wheat at high O_3. These results are very consistent with the previous reports on a number of crop species (Andersen, 2003; Grantz *et al*., 2006).

Interspecies differences in the impact of elevated O_3 on photosynthesis and growth

Winter wheat species showed significant ($P < 0.01$) differences in gas exchange and fluorescence signals in response to O_3. Interspecies variation in the impacts of elevated O_3 on A_{sat} or *ITE* might be mediated by significant variation ($P < 0.05$) in g_s in ozonated plants. For instance, wild species demonstrated higher O_3 flux as shown by increased g_s in O_3-treated plants resulting in higher relative reduction in A_{sat} than modern or primitive cultivated species. It can be further explained by higher O_3-impairment of mesophyll cells in wild species than in modern or primitive cultivated species as documented by increased C_i/C_a. Nevertheless, the highest relative loss in A_{sat} was observed in *Triticosecale wittmack*, which exhibited a decrease in g_s (−9%) and an increase in C_i/C_a (13%) relative to control. On the other hand, the lowest relative loss in A_{sat} was observed in *T. dicoccum* showing a relative increase in g_s (37%) and C_i/C_a (11%). These results therefore suggested that loss of photosynthetic capacity in winter wheat due to high O_3 was largely related to species-specific physiological characteristics. As a result, there was no consistent trend among relative changes in A_{sat}, g_s, C_i/C_a, and *ITE* in winter wheat species.

As for chlorophyll fluorescence, modern or primitive cultivated species displayed higher F_0, F_m, and F_v at elevated O_3 as well as lower relative reduction in respective parameters than wild species. This indicates that O_3-caused impairment of PSII-mediated electron transport was higher in wild species than in modern or primitive cultivated species. Although O_3 significantly altered the fast kinetics of fluorescence, there was no significant interspecies difference in the impacts of elevated O_3 on F_v/F_m in winter wheat. It is conceivable because O_3 causes slight, but significant decrease in F_v/F_m (Reichenauer *et al*., 1998; Cardoso-Vilhena *et al*., 2004). A wild species, *A. tauschii* appeared as the most sensitive to O_3 as shown by the highest relative reduction in F_0 (−32%), F_m (−43%), and F_v (−46%), while a primitive cultivated species, *T. polonicum* behaved as O_3-tolerant as documented by the lowest corresponding changes, which were +5%, −17%, and −23%, respectively. However, among fluorescence parameters, F_v appeared as the most sensitive and reliable parameter in detecting O_3 stress as F_v in ozonated plants significantly correlated with A_{sat}, *RGR*, RGR_s, K, shoot and total mass in winter wheat species.

Generally, there was no interspecies variation in the impact of O_3 on *RGR* and *K*. However, when data were analysed based on wheat type, there were significant differences ($P < 0.05$) in the effects of O_3 on *RGR*, RGR_s and *K* (P=0.116) showing higher *RGR* and RGR_s in O_3 in modern wheat or primitive cultivated wheat than in wild wheat. Higher *K* was noted in modern or wild wheat than in primitive cultivated wheat at elevated O_3. Relative loss in *RGR* and RGR_s indicated that primitive cultivated species appeared as the most resistant to O_3, followed by modern and wild species behaving as the most sensitive to O_3. Higher O_3 tolerance in primitive cultivated species might be attributed to lower g_s in O_3-exposed plants as there was a significant relationship between g_s in O_3 and relative reduction in *RGR*. As O_3 uptake is proportional to g_s (Long and Naidu, 2002; Danielsson *et al*., 2003; Pleijel *et al*., 2006), lower g_s resulted in lower O_3 uptake into the mesophyll tissue of primitive cultivated species and hence lower O_3 induced losses in A_{sat} and *RGR* (McKee *et al*., 1997; Biswas *et al*., 2008). This can also be attributed to the slow-growth rate of primitive cultivated species in CF air since *RGR* in control plants negatively correlated with relative reduction in *RGR*. In addition, there was a considerable positive relationship (r=0.52, P=0.083) between *RGR* in CF air and relative reduction in *K*. This suggests that wheat species with slow-growth rate in CF air showed a larger O_3-induced reduction in *K*, which could be a mechanism of higher O_3 resistance (Grantz *et al*., 2006).

Higher O_3-induced loss in A_{sat} through impaired activity of mesophyll cells and photochemistry finally resulted in the highest relative loss in biomass accumulation in wild species, followed by modern wheat and the lowest in primitive cultivated species. However, g_s in O_3-exposed plants can explain about 69% variation in total dry matter accumulation, as there was a significant relationship ($r = -0.69$**) between g_s in O_3 and relative loss in total mass (Biswas *et al*., 2008). Although wild species had the highest relative loss in total mass, they displayed the lowest relative reduction in root/shoot ratio. This could be an adaptive mechanism of wild species at elevated O_3 (Grantz *et al*., 2006). In particular, *T. turgidum* ssp. *durum* appeared as the most O_3-tolerant, while *A. tauschii* appeared as the most O_3-sensitive as indicated by the lowest and highest relative loss in shoot, root, and total mass in O_3, respectively.

Comparison of O_3 tolerance between modern wheat and its donor species

As noted above, the probable donors of the A, B and D genome of modern wheat (AABBDD) are (i) *T. turgidum* ssp. *durum* (AABB), (ii) *T. monococcum* L. (AA), and (iii) *Aegilops tauschii* (DD) (Feldman, 2001; Levy and Feldman, 2004; Matsuoka and Nasuda, 2004; Rudnoy *et al*., 2004). It is evident from the present study that among the three genome donors of modern wheat, the highest relative reduction in A_{sat} was observed in *A. tauschii* (−55%) followed by *T. monococcum* (−42%), and the least was observed in *T. turgidum* ssp. *durum* (−26%). The highest relative reduction in *ITE* was observed in *A. tauschii* (−48%), while the lowest was in *T. turgidum* ssp. *durum* (−36%). These results indicated that the addition of the B genome into the A genome of *T. monococcum* (i.e., the origin of *T. turgidum* ssp. *durum*) reduced the negative impacts of O_3 on photosynthesis. On the other hand, the addition of the D genome into the AB genome of *T. turgidum* ssp. *durum* (i.e., the origin of *T. aestivum*) enhanced the negative effects of O_3 on photosynthesis in winter wheat. Although there is no report on the effects of O_3 on B and D genomes, evidence of adverse effects of the D genome on photosynthesis has been reported elsewhere (Planchon and Fesquet, 1982; Haour-Lurton and Planchon, 1985). As for chlorophyll fluorescence, *A. tauschii* showed the highest O_3-induced impairment of PSII activity as the highest relative reduction in F_0 (−32%), F_m (−43%), and F_v (−46%) was noted. *T. monococcum* had higher F_m (−26%) and F_v (−30%), while *T. turgidum* ssp. *durum* showed the lowest F_m (−23%) and F_v (−25%) in O_3 relative to control. Lower relative loss in F_v/F_m was observed in *T. turgidum* ssp. *durum* (−5%) than in *A. tauschii* (−6%) and *T. monococcum* (−6%). This further indicated that the addition of the B genome into the A genome of *T. monococcum* decreased deleterious O_3 impacts of the A genome on F_m, F_v, and F_v/F_m. Relative loss in F_0, F_m, F_v, and F_v/F_m in *T. aestivum* was −4%, −25%, −30%, and −7%, respectively. This also suggests that the addition of D genome resulted in higher O_3-induced impairment of photochemistry in *T. aestivum* than its predecessor, *T. turgidum* ssp. *durum*.

The negative effect of the D genome on photosynthesis was also apparent on growth and resource allocation in O_3-treated plants. The highest relative loss in *RGR*, RGR_s, RGR_r, and *K* in *A. tauschii* was −63%, −59%, −81%, and −65%, respectively. *T. monococcum* had an intermediate response, while the lowest relative loss in those variables in *T. turgidum* ssp. *durum* was −34%, −23%, −63%, and −25%, respectively. Besides, the corresponding values in *T. aestivum* were −51%, −38%, −81%, and −44%, respectively. This suggests that addition of the D genome into the AB genome of *T. turgidum* ssp. *durum* resulted in higher O_3 sensitivity in modern wheat compared to its predecessors. It can be further reinforced by the similar trends of O_3-induced dry matter accumulation and partitioning in *T. aestivum* and its donor species. For example, the highest relative loss in shoot (−65%), root (−80%), and total mass (−69%) was observed in *A. tauschii*, followed by *T. monococcum* and the lowest relative reduction in shoot (−25%), root (−65%), and total mass (−36%) was observed in *T. turgidum* ssp. *durum*. On the other hand, relative reduction in shoot, root, and total mass in *T. aestivum* was −44%, −78%, and −57%, respectively. Our results therefore further demonstrated that higher O_3 tolerance in *T. turgidum* ssp. *durum* and higher O_3 sensitivity in *T. aestivum* were attributed to the addition of B and D genomes, respectively. However, more investigations are necessary to make a definite conclusion on the increased O_3 sensitivity of the D genome in wheat.

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References

Andersen CP. 2003. Source–sink balance and carbon allocation below ground in plants exposed to ozone. *New Phytologist* **157,** 213–228.

Balaguer L, Barnes JD, Panicucci A, Borland AM. 1995. Production and utilization of assimilate in wheat (*Triticum aestivum* L.) leaves exposed to O_3 and/or CO_2. *New Phytologist* **129,** 557–568.

Barnes J, Bender J, Lyons T, Borland A. 1999. Natural and man-made selection for air pollution resistance. *Journal of Experimental Botany* **50,** 1423–1435.

Barnes JD, Velissariou D, Davison AW, Holevas CD. 1990. Comparative ozone sensitivity of old and modern Greek cultivars of spring wheat. *New Phytologist* **116,** 707–714.

Belay G, Merker A, Tesemma T. 1994. Cytogenetic studies in Ethiopian landraces of tetraploid wheat (*Triticum turgidum* L.). *Hereditas* **121,** 45–52.

Biswas DK, Xu H, Li YG, Sun JZ, Wang XZ, Han XG, Jiang GM. 2008. Genotypic differences in leaf biochemical, physiological and growth responses to ozone in 20 winter wheat cultivars released over the past 60 years. *Global Change Biology* **14,** 46–59.

Cardoso-Vilhena J, Balaguer L, Eamus D, Ollerenshaw J, Barnes J. 2004. Mechanisms underlying the amelioration of O_3-induced damage by elevated concentrations of CO_2. *Journal of Experimental Botany* **55,** 771–781.

Chandrasekar V, Sairam RK, Srivastava GC. 2000. Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. *Journal of Agronomy and Crop Science* **185,** 219–227.

Colmer TD, Flowers TJ, Munns R. 2006. Use of wild relatives to improve salt tolerance in wheat. *Journal of Experimental Botany* **57,** 1059–1078.

Damicone JP, Manning WJ. 1987. Foliar sensitivity of soybeans from early maturity groups to ozone and inheritance of injury response. *Plant Disease* **71,** 332–336.

Danielsson H, Karlsson GP, Karlsson PE, Pleijel H. 2003. Ozone uptake modelling and flux-response relationships: an assessment of ozone-induced yield loss in spring wheat. *Atmospheric Environment* **37,** 475–485.

Darrall NM. 1989. The effect of air pollutants on physiological processes in plants. *Plant, Cell and Environment* **12,** 1–30.

Emberson LD, Ashmore MR, Murray F. 2003. *Air pollution impacts on crops and forests: a global assesment*. London, UK: Imperial College Press.

Farage PK, Long SP. 1999. The effects of O_3 fumigation during leaf development on photosynthesis of wheat and pea: an *in vivo* analysis. *Photosynthesis Research* **59,** 1–7.

Feldman M. 2001. Origin of cultivated wheat. In: Bonjean AP, Angus WJ, eds. *The world wheat book*. Paris: Lavoisier, 3–56.

Fiscus EL, Booker FL, Burkey KO. 2005. Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. *Plant, Cell and Environment* **28,** 997–1011.

Grantz DA, Gunn S, Vu H-B. 2006. O_3 impacts on plant development: a meta-analysis of root/shoot allocation and growth. *Plant, Cell and Environment* **29,** 1193–1209.

Guidi L, Cagno RDi, Soldatini GF. 2000. Screening of bean cultivars for their response to ozone as evaluated by visible symptoms and leaf chlorophyll fluorescence. *Environmental Pollution* **107,** 349–355.

Guidi L, Nali C, Ciompi S, Lorenzini G, Soldatini GF. 1997. The use of chlorophyll fluorescence and leaf gas exchange as methods for studying the different responses to ozone of two bean cultivars. *Journal of Experimental Botany* **48,** 173–179.

Haour-Lurton B, Planchon C. 1985. Role of D genome chromosomes in photosynthesis expression in wheats. *Theoretical and Applied Genetics* **69,** 443–446.

Heagle AS, Miller JE, Pursley WA. 2000. Growth and yield response of winter wheat to mixtures of ozone and carbon dioxide. *Crop Science* **40,** 1656–1664.

Heck WW, Cure WW, Rawlings JO, Zaragoza LJ, Heagle AS, Heggestad HE, Kohut RJ, Kress LW Temple PJ. 1984. Assessing impacts of ozone on agricultural crops. I. Overview. *Journal of the Air Pollution Control Association* **34,** 729–735.

Hunt R. 1990. *Basic growth analysis*. London: Unwin Hyman.

Kawahara T. 2002. Morphological and isozyme variation in genebank accessions of *Aegilops umbellulata* Zhuk., a wild relative of wheat. *Genetic Resources and Crop Evolution* **49,** 89–94.

Khan S. 2005. Modification of O_3 effects on photosynthetic capacity of winter wheat (*Triticum aestivum* cv. Perlo) at different levels of water availability. *Air, Water and Soil Pollution* **160,** 297–211.

Krause GH, Weis E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology* **42,** 313–349.

Levy AA, Feldman M. 2004. Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization. *Biological Journal of the Linnean Society* **82,** 607–613.

Limin AE, Danyluk J, Chauvin LP, Fowler DB, Sarhan F. 1997. Chromosome mapping of low-temperature induced *Wcs120* family genes and regulation of cold-tolerance expression in wheat. *Molecular and General Genetics* **253,** 720–727.

Long SP, Naidu SL. 2002. Effects of oxidants at the biochemical, cell and physiological levels, with particular reference to ozone. In. In: Bell JNB, Treshow M, eds. *Air Pollution and Plant Life*. Chichester: John Wiley & Sons Ltd, 69–88.

Matsuoka Y, Nasuda S. 2004. Durum wheat as a candidate for the unknown female progenitor of modern wheat: an empirical study with a highly fertile F_1 hybrid with *Aegilops tauschii* Coss. *Theoretical and Applied Genetics* **109,** 1710–1717.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence: a practical guide. *Journal of Experimental Botany* **51,** 659–668.

McKee IF, Eiblmeier M, Polle A. 1997. Enhanced ozone-tolerance in wheat grown at an elevated CO_2 concentration: ozone exclusion and detoxification. *New Phytologist* **135,** 275–284.

McKee IF, Farage PK, Long SP. 1995. The interactive effects of elevated CO_2 and O_3 concentration on photosynthesis in spring wheat. *Photosynthesis Research* **45,** 111–119.

McKee IF, Mulholland BJ, Craigon J, Black CR, Long SP. 2000. Elevated concentrations of atmospheric CO_2 protect against and compensate for O_3 damage to photosynthetic tissues of field-grown wheat. *New Phytologist* **146,** 427–535.

Mortensen L, Jorgensen HE. 1996. Responses of spring wheat (*Triticum aestivum* L.) to ozone produced by either electric discharge and dry air or by uv-lamps and ambient air. *Environmental Pollution* **93,** 121–127.

Mulholland BJ, Craigon J, Black CR, Colls JJ, Atherton J, Landon G. 1997. Impact of elevated atmospheric CO_2 and O_3 on gas exchange and chlorophyll content in spring wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **48,** 1853–1863.

Neighbour EA, Pearson M, Mehlhorn H. 1990. Purafil filtration prevents the development of ozone-induced frost injury: a potential role for nitric oxide. *Atmospheric Environment* **24,** 711–715.

Owens TG. 1994. *In vivo* chlorophyll fluorescence as a probe of photosynthetic physiology. In: Alscher RG, Wellburn AR, eds. *Plant responses to the gaseous environment*. London: Chapman and Hall, 195–217.

Planchon C, Fesquet J. 1982. Effects of D genome and of selection on photosynthesis in wheat. *Theoretical and Applied Genetics* **61,** 359–365.

Pleijel H, Eriksen AB, Danielsson H, Bondesson N, Sellden G. 2006. Differential ozone sensitivity in an old and a modern Swedish wheat cultivar: grain yield and quality, leaf chlorophyll and stomatal conductance. *Environmental and Experimental Botany* **56,** 63–71.

Reichenauer TG, Goodman BA, Kostecki P, Soja G. 1998. Ozone sensitivity in *Triticum durum* and *T. aestivum* with respect to leaf injury and free radical content. *Physiologia Plantarum* **104,** 681–686.

Reiling K, Davison AW. 1992. Response of native, herbaceous species to ozone: growth and fluorescence screening. *New Phytologist* **120,** 29–37.

Reinert RA, Eason G. 2000. Genetic control of O_3 sensitivity in a cross between two cultivars of snap bean. *Journal of the American Society of Horticultural Science* **125,** 222–227.

Rudnoy S, Paldi E, Bratek Z, Szego D, Racz I, Lasztity D. 2004. ITS regions in hexaploid modern wheat and its supposed progenitors. *Cereal Research Communications* **32,** 423–428.

Sellden G, Pleijel H. 1995. Photochemical oxidant effects on vegetation: response in relation to plant strategy. *Water, Air and Soil Pollution* **85,** 111–122.

Shah SH, Gorham J, Forster BP, Jones RGW. 1987. Salt tolerance in the Triticeae: the contribution of the D genome to cation selectivity in hexaploid wheat. *Journal of Experimental Botany* **38,** 254–269.

Singh RJ. 2005. *Genetic resource, chromosome engineering and crop improvement: cereals*. CRC Press.

Soja G, Soja AM. 1995*a*. Wheat as an ozone-sensitive crop. *Acta Phytopathologica et Entomologica Hungarica* **30,** 59–70.

Soja G, Soja AM. 1995*b*. Ozone effects on dry matter partitioning and chlorophyll fluorescence during plant development of wheat. *Water, Air and Soil Pollution* **85,** 1461–1466.

Taylor GE, Owens JG, Grizzard T, Selvidge WJ. 1993. Atmosphere×canopy interactions of nitric acid vapour in Loblolly Pine grown in open-top chambers. *Journal of Environmental Quality* **22,** 70–80.

Uprety DC. 1998. Carbon dioxide enrichment technology: Open Top Chambers a new tool for global climate research. *Journal of Science and Industrial Research* **57,** 266–270.

Valkoun JJ. 2001. Wheat pre-breeding using wild progenitors. *Euphytica* **119,** 17–23.

Velissariou D, Barnes JD, Davison AW. 1992. Has inadvertent selection by plant breeders affected the O_3 sensitivity of modern Greek cultivars of spring wheat. *Agriculture, Ecosystem and Environment* **38,** 79–89.

Wang XK, Zheng QW, Yao FF, Chen Z, Feng ZZ, Manning WJ. 2007*a*. Assessing the impact of ambient ozone on growth and yield of a rice (*Oryza sativa* L.) and a wheat (*Triticum aestivum* L.) cultivar grown in the Yangtze Delta, China, using three rates of application of ethylenediurea (EDU). *Environmental Pollution* **148,** 390–395.

Wang XK, Manning W, Feng ZW, Zhu YG. 2007*b*. Ground-level ozone in China: distribution and effects on crop yields. *Environmental Pollution* **147,** 394–400.

Xiong YC, Li FM, Zhang T. 2006. Performance of wheat crops with different chromosome ploidy: root-sourced signals, drought tolerance, and yield performance. *Planta* **224,** 710–718.

Zheng Y, Stevenson KJ, Barrowcliffe R, Chen S, Wang H, Barnes JD. 1998. Ozone levels in Chongqing: a potential threat to crop plants commonly grown in the region? *Environmental Pollution* **99,** 299–308.

Zohary D, Hopf M. 2000. *Domestication of plants in the old world. The origin and spread of cultivated plants in the west*. Oxford University Press.