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RESEARCH PAPER

Differential drought-induced modulation of ozone tolerance in winter wheat species

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Abstract

Recent reports challenge the widely accepted idea that drought may offer protection against ozone (O_3) damage in plants. However, little is known about the impact of drought on the magnitude of O_3 tolerance in winter wheat species. Two winter wheat species with contrasting sensitivity to O_3 (O_3 tolerant, primitive wheat, *T. turgidum* ssp. *durum*; O_3 sensitive, modern wheat, *T. aestivum* L. cv. Xiaoyan 22) were exposed to O_3 (83ppb O_3, 7h d^{-1}) and/or drought (42% soil water capacity) from flowering to grain maturity to assess drought-induced modulation of O_3 tolerance. Plant responses to stress treatments were assessed by determining *in vivo* biochemical parameters, gas exchange, chlorophyll *a* fluorescence, and grain yield. The primitive wheat demonstrated higher O_3 tolerance than the modern species, with the latter exhibiting higher drought tolerance than the former. This suggested that there was no cross-tolerance of the two stresses when applied separately in these species/cultivars of winter wheat. The primitive wheat lost O_3 tolerance, while the modern species showed improved tolerance to O_3 under combined drought and O_3 exposure. This indicated the existence of differential behaviour of the two wheat species between a single stress and the combination of the two stresses. The observed O_3 tolerance in the two wheat species was related to their magnitude of drought tolerance under a combination of drought and O_3 exposure. The results clearly demonstrate that O_3 tolerance of a drought-sensitive winter wheat species can be completely lost under combined drought and O_3 exposure.

Key words: A–C_i curve parameters, chlorophyll *a* fluorescence, drought, gas exchange, ozone tolerance, stomatal conductance, winter wheat, yield.

Introduction

Ozone (O_3) episodes and incidences of drought often occur together during the reproductive stage of crops during the summer months over widespread areas of the world (Mittler, 2006; Ainsworth *et al.*, 2008; Mittler and Blumwald, 2010). Although drought alone suppresses crop growth and yield, it is widely accepted that drought may reduce O_3 injury in crop plants and forest species through drought-induced suppression of stomatal conductance (Tingey and Hogsett, 1985; Pearson and Mansfield, 1993; Karlsson *et al.*, 1995; Reichenauer *et al.*, 1998; Khan and Soja, 2003). However, the discovery of ethylene-dependent reductions in stomatal sensitivity to abscisic acid (ABA) under O_3 stress indicates that stomatal conductance under soil water deficit is greater under elevated O_3, and plants continue to lose water, despite the potential for dehydration and increased O_3 flux (Wilkinson and Davies, 2010). This result conflicts with predictions that drought may offer some protection against O_3 damage by inducing stomatal closure

and restricting O_3 uptake (Wittig *et al.*, 2009; Wilkinson and Davies, 2010). Recent meta-analyses have demonstrated contrasting effects of elevated O_3 on stomatal responses and growth under drought and well-watered conditions (Wittig *et al.*, 2007, 2009). It is therefore suggested that more attention should be given to the interactions between O_3 and other concurrently changing climatic variables such as drought, to assess the impacts of current and future climates on crop plants and natural vegetation accurately (Fuhrer, 2003; Wittig *et al.*, 2009; Wilkinson and Davies, 2010).

Wheat is normally sensitive to O_3, as documented by reductions in photosynthesis, growth, and yield (Mulholland *et al.*, 1997; Heagle *et al.*, 2000; McKee and Long, 2001; Biswas *et al.*, 2008*a*). There are also considerable intra- and interspecific variations in O_3 sensitivity in winter wheat (Biswas *et al.*, 2008*a*, *b*) that can be greatly modified by other environmental variables (Biswas *et al.*, 2009). It has been suggested that the high O_3 tolerance of specific genotypes observed under favourable conditions could be reduced or even lost under changing climatic conditions (Fuhrer, 2003). While there have been a few studies to determine wheat responses to drought and O_3 stresses in terms of growth and yield (Khan and Soja, 2003), little attention has been paid to the impact of a combination of drought and O_3 on the photosynthetic processes and stomatal function of the flag leaves which provide the major contribution of assimilate to grain yield. In addition, there have been no studies of drought-induced modulation of O_3 tolerance in winter wheats with differential sensitivity to O_3. It is important to examine whether any cross-tolerance exists in winter wheat between O_3 and drought stress, so that an appropriate breeding effort can be made to avoid yield reductions in the changing climatic conditions anticipated in the future (Mittler, 2006; Mittler and Blumwald, 2010).

Crop sensitivity to environmental stresses is typically assessed by the decline in growth and/or grain yield. Chlorophyll *a* fluorescence and gas exchange provide useful non-destructive tools for *in vivo* stress detection and are widely used to examine the effects of environmental stresses on photosynthesis (Guidi *et al.*, 1997; Maxwell and Johnson, 2000). In addition, *in vivo* biochemical parameters provide more insights into photosynthetic processes and the mechanisms of O_3/drought tolerance. Since the stomata are an important determinant for O_3/drought effects on plant, the stomatal response to varying internal CO_2 concentrations and light intensity could allow the examination of stomatal functioning in wheat exposed to drought and O_3. Therefore, integration of *in vivo* biochemical parameters with gas exchange, chlorophyll *a* fluorescence, and grain yield data might improve the present understanding of the mechanisms underlying wheat responses to combined drought and O_3 exposure.

Although understanding of cross-tolerance and the mechanisms responsible is crucial to predict agricultural yield under natural field conditions (Sabehat *et al.*, 1998; Rizhsky *et al.*, 2002; Tausz *et al.*, 2007), little is known about whether any cross-tolerance exists in winter wheat species between drought and O_3 stress. Since plants induce similar defence mechanisms to avoid oxidative stress resulting from both O_3 and drought (Tausz *et al.*, 2007), it was hypothesized first that O_3-tolerant wheat species may have greater tolerance to drought. Secondly, it was hypothesized that drought might offer greater protection against O_3 damage in O_3-tolerant species than in O_3-sensitive winter wheat species under combined drought and O_3 exposure. One O_3-tolerant and one O_3-sensitive winter wheat species were therefore utilized to test these hypotheses. Plant responses to drought and/or O_3 have been regarded as being determined by stomatal regulation and photosynthesis following simultaneous measurements of gas exchange and chlorophyll fluorescence, *in vivo* biochemical parameters, and yield components. The present results may be valuable in understanding crop adaptation to environmental stresses and prediction of food security under changing climatic conditions such as increased drought and O_3 exposure.

Materials and methods

Plant establishment and treatments

An O_3-sensitive modern winter wheat (*Triticum aestivum* L. cv. Xiaoyan 22) and an O_3-tolarant primitive wheat (*T. turgidum* ssp. *durum*) were selected to assess drought-induced modulation of O_3 tolerance. The study was carried out at the experimental station at the Institute of Botany of the Chinese Academy of Sciences. The O_3 sensitivity of these two winter wheat species has been extensively evaluated in controlled greenhouse experiments reported elsewhere (Biswas *et al.*, 2008*a*, *b*, 2009). Twenty germinated seeds were sown in each of 24 pots (15.0l) filled with clay loam soil for both species. Organic C, total N, total P, and total K in the soil were 1.24%, 0.045%, 296mg kg^{-1}, and 14.7g kg^{-1}, respectively. Seedlings were thinned to leave 10 plants per pot after stand establishment. The plants were initially grown outdoors in a greenhouse (October–April) under continental climate conditions (i.e. cold and dry winter) for natural vernalization. They experienced three snowfalls during the winter months at their vegetative stage. Irrigation was applied twice a week to maintain the soil near field capacity and avoid drought stress during plant growth outdoors in the greenhouse. At the jointing stage, all plants were top-dressed with N as urea at the rate of 15.0g N m^{-2}. Pesticide was also applied at the rate of 10% of Imidachloroprid WP (Huayang Tech Co., Ltd, Shandong, China) once to control insect pests at the jointing stage. The weather was typical for early summer in Beijing during the post-flowering stage of wheat, with mean daily air temperature varying from 15°C (night) to 35°C (day) and a maximum photosynthetic photon flux density (PPFD) of ~2000μmol m^{-2} s^{-1} at midday. Seasonal temperature varied from 3°C to 14°C, and seasonal relative humidity varied from 29% to 98%.

All 48 pots (24 pots per species) were moved to four open top chambers (OTCs; 1.2m in diameter and 1.6m in height) in a temperature-controlled double-glazed greenhouse at the flag leaf stage of wheat before initiating the water stress and O_3 fumigation treatments. The OTCs were placed inside the greenhouse to avoid natural precipitation. The plants were allowed to acclimate for 1 week in the OTCs to adapt to the chamber environment before imposing the treatments. During this period, all plants received charcoal-filtered (CF) air (<5ppb O_3). The maximum PPFD in the chambers was ~1236μmol m^{-2} s^{-1}. The temperature in the OTCs fluctuated from 17°C (night) to 33°C (day) and relative humidity ranged from 65% to 88% during the experiment runs.

The O_3 and water stress treatments were initiated when 50% of the plants had begun to flower. O_3 was generated by electrical discharge using ambient oxygen (Balaguer *et al.*, 1995) and an O_3 generator (CF-KG1, Beijing Sumsun Hi-Tech., Co. Ltd, China) and this was bubbled through distilled water before entering the two elevated O_3 chambers 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 ~10cm above the plant canopy using an analyser (APOA-360, Horiba, Ltd, Japan), which was cross-calibrated before starting the O_3 treatment. O_3 concentration within the treatment chambers averaged 83±7ppb for 7h d^{-1} (10:00–17:00 h) over 3 weeks from 50% flowering to physiological maturity. The elevated O_3 and CF air control treatments were assigned randomly to the chambers in each of the two blocks and were replicated twice. Plants in each chamber were subjected to two levels of soil water. For each species, 12 pots were irrigated to maintain soil water content (SWC) at 88±6% and the remaining 12 pots received a reduced supply of water to exert drought stress (SWC 42±4%). Soil water capacity was determined as the ratio of actual water content to maximum capillary capacity, and water content was adjusted gravimetrically on alternate days during water stress treatment (Khan and Soja, 2003). Plants receiving two levels of soil water, and O_3 treatments were switched between chambers on alternate days to minimize the effects of environmental heterogeneity and variation between chambers on plant responses, with the location of the plants within the chambers being randomized on each occasion.

Simultaneous measurement of gas exchange and chlorophyll a fluorescence

Three plants of each species receiving the drought stress and well-irrigated treatments were selected from three different pots from each of the four OTCs (elevated O_3 and CF air, replicated twice) on each sampling date for simultaneous measurements of gas exchange and chlorophyll fluorescence. These measurements were repeated three times at 7d intervals during the post-flowering stage of wheat species for all treatments. The measurement was initiated with the most recently fully expanded flag leaves of the main stem using a portable Gas Exchange Fluorescence System (GFS-3000, Heinz Walz, Germany) connected to a PC fitted with data acquisition software (GFS-Win). All measurements were kept consistent on the main stem flag leaves to minimize age-related heterogeneity of leaf tissue between the plants on each sampling date. The plant used was not used again for further measurements if any leaf injury resulted from the leaf chamber. The system was zeroed prior to each set of measurements. Relative humidity was maintained at 70% and leaf temperature in the leaf chamber was set at 25°C. The flow rate was set at 600mol s^{-1} and CO_2 concentration in the leaf chamber maintained at 400ppm. The flag leaf was illuminated with a PPFD of 1500μmol m^{-2} s^{-1} using the internal chamber light. The A–C_i and A–PPFD response curves were recorded automatically for the same flag leaves using the A–C_i and A–PPFD response curve programs. The area of each individual flag leaf was calculated and entered into the automatic curve program prior to inserting the leaf into the leaf chamber. For A–C_i curves, the steady-state rate of net photosynthesis under saturating irradiance (A_{sat}) was determined at external CO_2 concentrations of 400, 300, 200, 100, 50, 400, 400, 600, and 800ppm. The duration of each step of the A–C_i response curves was 4min and data were automatically recorded six times to check stability of data.

On the other hand, the A–PPFD response curve was programmed to determine both gas exchange and chlorophyll fluorescence parameters. A–PPFD response curves were recorded at PPFDs of 1800, 1500, 1000, 500, 300, 150, 80, 50, 20, and 0 μmol m^{-2} s^{-1} at the leaf surface. At each PPFD, CO_2 assimilation, stomatal conductance, steady-state fluorescence, and maximum and minimum fluorescence were recorded simultaneously. During A–PPFD response curve measurement, the CO_2 concentration was maintained at 700ppm.

The duration of each step of the A–PPFD response curves was 3min and data were automatically recorded six times to check the stability of the data. After recording basic fluorescence parameters, the actual yield of photosystem II (PSII; F_v'/F_m') and the electron transport rate (ETR) were calculated according to Equation 1 and 2, respectively, as follows:

$$F_V'/F_m' = \left(F_m' - F_m'\right)/F_m' \quad (1)$$

$$\mathrm{ETR} = \mathrm{Yield}\times\mathrm{PAR}\times.5\times0.84 \quad (2)$$

where F_m' and F_o' are the maximum and minimum fluorescence, respectively, at each light level. The factor of 0.5 was derived because transport of one electron requires the absorption of two quanta, while the factor of 0.84 was estimated based on the assumption that the proportion of incident quanta absorbed by the leaf was ~84% (Meyer *et al.*, 1997).

The data obtained for each flag leaf were analysed using a mechanistic A–C_i curve analysis program (Photosynthesis Assistant, Version 1.1, Dundee Scientific, UK), to obtain the maximum rate of carboxylation by Rubisco (V_{cmax}), the PAR-saturated rate of electron transport (J_{max}), carboxylation efficiency (CE), and respiratory processes for day and night (R). The program followed the model proposed by Farquhar *et al.* (1980). On the other hand, data obtained as part of the gas exchange measurements included the area-based light-saturated net photosynthetic rate (A_{sat}), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration rate (E), and leaf-level photosynthetic water use efficiency, or instantaneous transpiration efficiency (ITE), which was calculated as assimilation/transpiration.

Grain yield, yield attributes, and harvest index

Three plants per pot for each species and each treatment combination (n=18) were harvested for determination of grain yield and yield attributes. Grains were removed from each ear by hand and the number of grains per ear was counted. Yield per ear, yield per plant, and 1000-grain weight were determined for sun-dried seeds. The harvest index (HI) was calculated as the ratio of grain dry mass to total above-ground dry mass per plant.

Statistical analysis

The experimental design consisted of two blocks, each containing one elevated O_3 and one CF air chamber and each chamber contained plants experiencing two levels of soil moisture with 30 plants per replicate. Statistical analyses of data were performed using analysis of variance (ANOVA) in the general linear model procedure of the SPSS package (version 13, SPSS, Chicago, IL, USA). The main effects (species, O_3, and drought) and their interactions were analysed using three-way ANOVA on the measured variables. Linear regression coefficients for the A–PPFD response curve and their significance (P-value) were also performed using the curve estimation program of SPSS. Differences between treatments were considered significant if P <0.05.

Results

Gas exchange

O_3 significantly (P <0.05) decreased A_{sat}, g_s, E, and ITE, but increased C_i in both wheat species (Table 1). Water stress drastically (P <0.001) reduced A_{sat}, g_s, and C_i, but increased ITE. Overall, there were considerable interspecific variations (P <0.05) for A_{sat}, g_s, and E (Table 1). The

Table 1. Analysis of variance of the effects of O_3, drought, species, growth stage, and their interactions on photosynthetic gas exchange and A–C_i curve parameters of a primitive wheat, *T. turgidum* ssp. *durum*, and a modern wheat, *T. aestivum* L. cv. Xiaoyan 22
P-values were calculated using the general linear model with the main effects of O_3, drought, species, growth stage, and their interactions. Two wheat species were well irrigated (88±6% SWC) and exposed to CF air (5±0ppb O_3, 7 h d^{-1}) or elevated O_3 (83±7ppb O_3 7 h d^{-1}), or subjected to drought stress (42±4% SWC) and exposed to CF air or elevated O_3 for 3 weeks during the post-flowering stage in open top chambers.

Parameters	Ozone (O_3)	Drought (ws)	Species (sp)	Stage (st)	O_3×sp	ws×sp	O_3×ws	O_3×ws×sp	O_3×ws×sp×st
A_{sat}	0.000	0.000	0.001	0.000	0.001	0.003	0.000	0.477	0.277
g_s	0.012	0.000	0.019	0.000	0.045	0.412	0.026	0.375	0.776
C_i	0.000	0.000	0.777	0.000	0.006	0.000	0.624	0.554	0.000
E	0.009	0.000	0.044	0.000	0.057	0.714	0.172	0.572	0.822
ITE	0.003	0.004	0.534	0.000	0.411	0.017	0.200	0.393	0.837
V_{cmax}	0.000	0.010	0.000	0.000	0.001	0.093	0.004	0.874	0.244
J_{max}	0.000	0.586	0.001	0.000	0.000	0.207	0.208	0.229	0.290
R	0.000	0.435	0.008	0.000	0.202	0.006	0.277	0.096	0.942
CE	0.000	0.089	0.019	0.000	0.009	0.144	0.005	0.108	0.433

primitive wheat displayed higher A_{sat}, g_s, and *E* values than modern species (data not shown). A significant (P <0.001) gradual decrease in gas exchange characteristics was also noted for both species. The interaction between water stress and species was significant (P <0.05) for A_{sat}, C_i, and ITE. For instance, drought reduced A_{sat} and ITE by 34% and 2%, respectively, in the primitive wheat, whereas it decreased A_{sat} by 21% and increased ITE by 22% in the modern species. The interaction between O_3 and species was significant (P <0.05) for A_{sat}, C_i, g_s, *E*, and ITE. The primitive species showed O_3-induced reductions in g_s and A_{sat} of 18% and 22%, respectively, compared with 47% and 63% in the modern species. Elevated O_3 increased C_i in the primitive and modern species by 10% and 18%, respectively. The interaction between water stress and O_3 was significant (P <0.05) for A_{sat} and g_s. The primitive wheat exhibited an O_3-induced reduction in A_{sat} in drought-stressed plants of 37%, whereas the modern wheat demonstrated a reduction of 29%. Similarly, elevated O_3 reduced g_s in water-stressed primitive wheat plants by 26%, while the corresponding reduction in the modern wheat was 22%.

In vivo *photosynthetic biochemical properties*

Elevated O_3 decreased *in vivo* photosynthetic biochemical variables including V_{cmax}, J_{max}, R, and CE (P <0.001; Fig. 2) in both wheat species. Reduced water supply also considerably (P <0.1) decreased V_{cmax} and CE, but had no effect on J_{max} and R. Sampling date had a profound effect (P <0.001) on *in vivo* photosynthetic biochemical traits, with gradual reductions in V_{cmax}, J_{max}, R, and CE being noted in both species. The primitive wheat had significantly higher V_{cmax}, J_{max}, R, and CE values than the modern species. The interaction between O_3 and species was significant (P <0.01) for V_{cmax}, J_{max}, R, and CE. For instance, O_3-induced reductions in V_{cmax}, J_{max}, and CE in the primitive wheat were 23, 13, and 24%, while the corresponding reductions in the modern wheat were 53, 59, and 64%. The interaction between drought and species was significant (P <0.1) only for V_{cmax} and R. Water stress reduced V_{cmax} and R in the primitive species by 18% and 4%, respectively, but decreased V_{cmax} by 10% and increased R by 8% in the modern wheat. The water stress×O_3 interaction was also significant (P <0.01) for V_{cmax} and CE. The primitive species showed an O_3-induced reduction in V_{cmax} and CE in drought-stressed plants of 3% and 12%, respectively, whereas the modern wheat showed an O_3-induced increase in V_{cmax} and CE in drought-stressed plants of 14% and 32%, respectively.

Photosynthesis and stomatal conductance at varying internal CO_2 pressures

The photosynthetic rates (*A*) of both wheat species increased with increasing internal C_i for all treatment combinations (Fig. 3). The primitive wheat showed a smaller O_3-induced reduction in *A* relative to control plants than the modern species at higher internal C_i. Initially g_s was relatively high at very low C_i values in both species regardless of treatment, but gradually decreased with increasing C_i up to 200ppm, and afterwards it increased again at high C_i values. The well-watered plants of both wheat species showed a gradual increase in g_s from 300ppm to 600ppm in the CF air treatment. However, the well-watered plants of the primitive wheat exposed to elevated O_3 showed a higher increase in g_s than that of the modern wheat as C_i increased from 300ppm to 600ppm. The drought-stressed plants of the primitive wheat maintained lower g_s values than those of the modern wheat under CF air or elevated O_3 at high C_i values.

Response of photosynthesis, stomatal conductance, actual yield, and electron transport rate to PPFD

The photosynthesis rate (*A*) increased with increasing PPFD in both species regardless of treatment (Fig. 4). There were no detectable differences among treatments at low PPFD, but considerable differences were apparent at high PPFD in both species (Table 2). The initial slope of the *A*–PPFD response curve for O_3-treated plants of the primitive wheat (b=0.037, P=0.001) was greater than that for the modern wheat

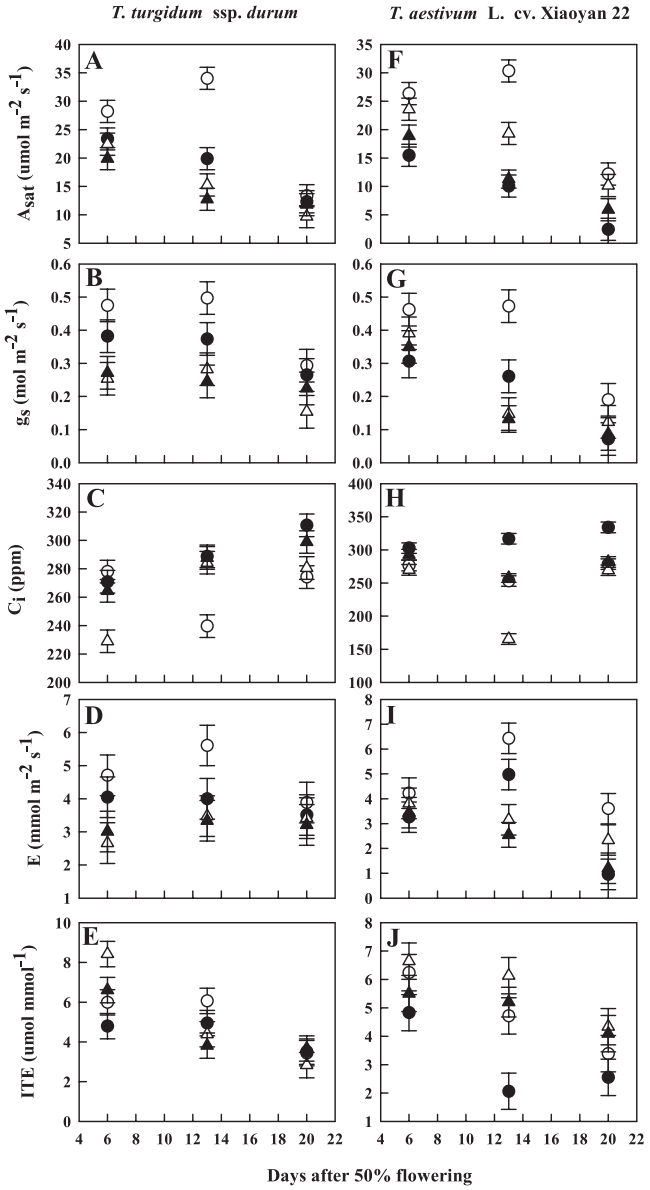

Fig. 1. Impact of elevated O_3 and/or drought stress on gas exchange characteristics of the flag leaves of a primitive wheat, *T. turgidum* ssp. *durum*, and a modern wheat, *T. aestivum* L. cv. Xiaoyan 22. Both species were exposed to stress treatments for 3 weeks during the post-flowering stage in open top chambers. Treatments were well irrigated (88±6% SWC) and exposed to CF air (5±0ppb O_3, open circles) or elevated O_3 (83±7ppb O_3, filled circles), or drought stressed (42±4% SWC) and exposed to CF air (open triangles) or elevated O_3 (filled triangles). Error bars indicate the SEM. n=6.

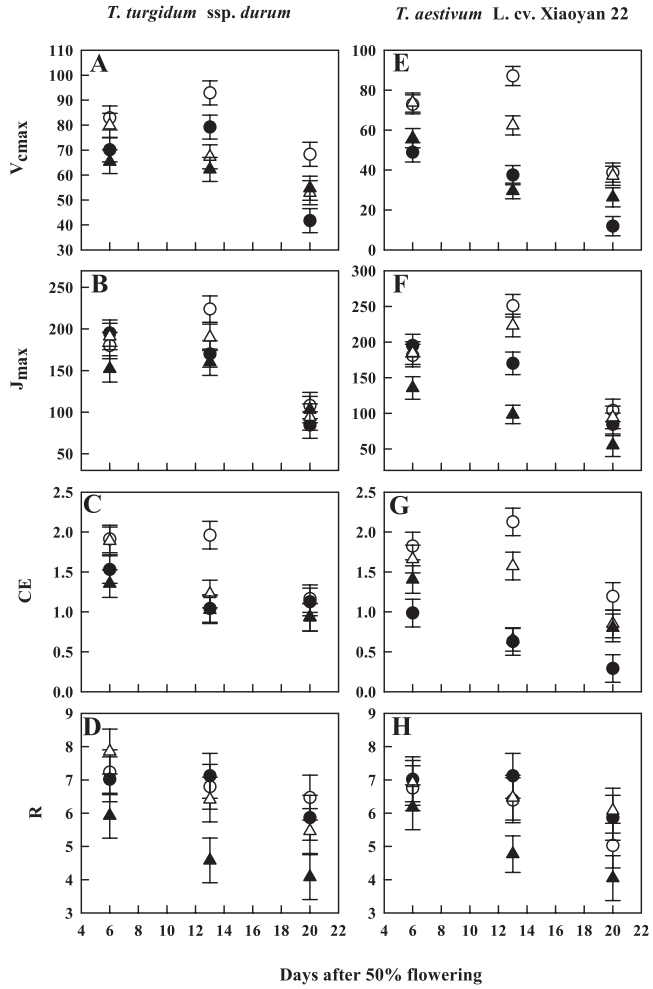

Fig. 2. Impact of elevated O_3 and/or drought stress on photosynthetic biochemical properties (*in vivo*) of the flag leaves of a primitive wheat, *T. turgidum* ssp. *durum*, and a modern wheat, *T. aestivum* L. cv. Xiaoyan 22. Both species were exposed to stress treatments for 3 weeks during the post-flowering stage in open top chambers. Treatments were well irrigated and exposed to CF air (open circles) or elevated O_3 (filled circles), or drought stressed and exposed to CF air (open triangles) or elevated O_3 (filled triangles). V_{cmax}, J_{max}, CE, and R indicate the maximum rate of carboxylation by Rubisco, the PAR-saturated rate of electron transport, carboxylation efficiency, and respiratory processes for day and night, respectively. Error bars indicate the SEM. n=6.

(b=0.025, P <0.05). Similarly, the initial slope of the A–PPFD response curve in water-stressed plants of the modern wheat (b=0.044, P <0.05) was greater than that for the primitive wheat (b=0.035, P <0.05). However, the gradient of the A–PPFD response curve in both species remained similar under combined drought and O_3 stress.

In general, g_s increased with increasing PPFD regardless of species and treatment (Fig. 4). There were considerable treatment effects on stomatal function in both species (Table 2). The initial gradient of the relationship between g_s and PPFD was much higher in ozonated plants of the primitive wheat (b=0.808, P=0.294) than in those of the modern wheat (b=0.212, P=0.235). The initial slope of the g_s–PPFD response curve in the plants of the primitive species (b=0.391, P <0.1) was lower than in those of the modern wheat (b=0.703, P=0.140) under drought. Similarly, the gradient of the g_s–PPFD response curve in the plants of the primitive wheat (b=0.487, P <0.1) was lower than in those of the modern wheat (b=0.625, P=0.123) under combined drought and O_3 exposure.

The actual yield of PSII and ETR decreased and increased, respectively, with increasing PPFD regardless of

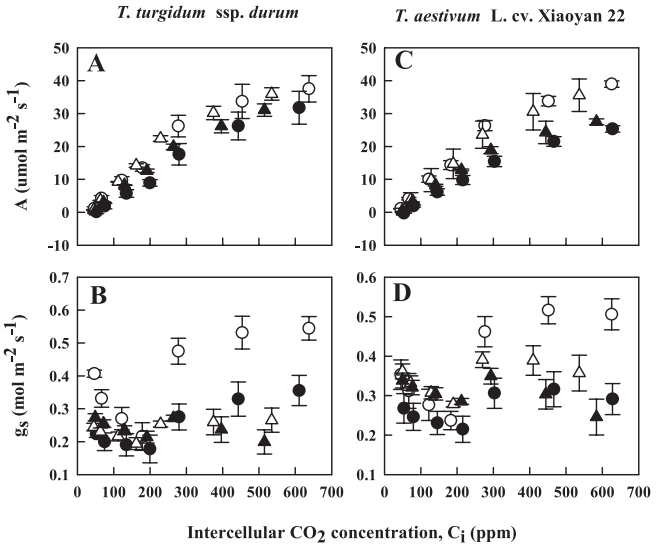

Fig. 3. Photosynthetic rate (*A*) and stomatal conductance (g_s) in the flag leaves of a primitive wheat, *T. turgidum* ssp. *durum*, and a modern wheat, *T. aestivum* L. cv. Xiaoyan 22, as affected by intercellular CO_2 concentrations, C_i (ppm), on the 13th day after initiation of stress treatments. Both species were exposed to drought and/or O_3 for 3 weeks during the post-flowering stage in open top chambers. Treatments were well irrigated and exposed to CF air (open circles) or elevated O_3 (filled circles), or drought stressed and exposed to CF air (open triangles) or elevated O_3 (filled triangles). Error bars indicate the single SEM. n=6.

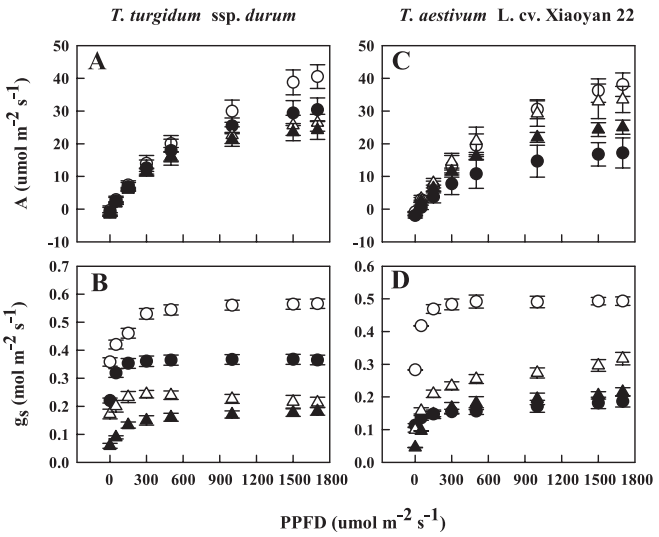

Fig. 4. Photosynthetic rate (*A*) and stomatal conductance (g_s) in the flag leaves of a primitive wheat, *T. turgidum* ssp. *durum*, and a modern wheat, *T. aestivum* L. cv. Xiaoyan 22, as affected by photosynthetic photon flux densities (PPFDs) on the 13th day after initiation of stress treatments. Both species were exposed to drought and/or O_3 for 3 weeks during the post-flowering stage in open top chambers. Treatments were well irrigated and exposed to CF air (open circles) or elevated O_3 (filled circles), or drought stressed and exposed to CF air (open triangles) or elevated O_3 (filled triangles). Error bars indicate the SEM. n=6.

species and treatment (Fig. 5). There were no detectable treatment differences in the initial gradient of the relationship between actual yield of PSII and PPFD in both species. However, there were considerable treatment differences in the initial slope of the ETR–PPFD response curve in both species. The primitive wheat (b=0.145, P <0.05) showed a higher gradient of the ETR–PPFD response curve than the modern wheat (b=0.114, P <0.05) under elevated O_3. The modern wheat (b=0.178, P <0.01) displayed a higher gradient of the ETR–PPFD response curve than the primitive wheat (b=0.149, P <0.05) under drought. However, both wheat species showed an almost similar initial slope of the ETR–PPFD response curve under combined drought and O_3 exposure.

Yield and yield attributes

Elevated O_3 decreased (P <0.05) 1000-grain weight, yield per ear, yield per plant, and HI, but had no impact on ears per plant and seeds per ear (Table 3). Water stress significantly (P <0.05) reduced 1000-grain weight, yield per ear, and yield per plant, but had no effect on ears per plant, seeds per ear, and HI (Table 2). Overall, there were noteworthy differences (P <0.05) between the two species for the grain yield and yield attributes investigated (Table 3). The modern wheat had a greater number of seeds per ear, 1000-grain weight, yield per ear, yield per plant, and HI than the primitive wheat. The number of ears per plant was higher in primitive wheat than in the modern wheat. The species×water stress interaction was significant (P <0.05) for 1000-grain weight as the primitive wheat showed a smaller 1000-grain weight than the modern wheat under drought. The species×O_3 interaction was significant (P <0.01) for seeds per ear, seed yield per ear, and HI because the primitive wheat exhibited higher levels of seeds per ear, seed yield per ear, and HI than the modern wheat at elevated O_3. A strong interaction (P=0.001) between water stress and O_3 was detected for HI. The species×water stress×O_3 interaction was not significant for any yield parameter except 1000-grain weight and HI (P <0.05).

Discussion

Physiological and yield response of winter wheat species to O_3

The winter wheat species examined displayed significant reductions in A_{sat}, g_s, V_{cmax}, J_{max}, R, and CE in response to O_3, in general agreement with reports for spring wheat (Farage and Long, 1999; Cardoso-Vilhena *et al.*, 2004). However, the results indicate that the O_3-induced decrease in A_{sat} might be due to both enzymatic and stomatal limitation as evidenced by the O_3-induced reduction in g_s and increase in C_i accompanied by decreases in V_{cmax}, CE, and R. The observed reduction in A_{sat} and considerable decline in V_{cmax} indicated that these effects might be attributable to a decrease in the quantity of active Rubisco (Farage and Long, 1999; Cardoso-Vilhena *et al.*, 2004). The

Table 2. Slope (b), intercept (c), R^2, and P-value of the linear part of the response of flag leaf photosynthesis, stomatal conductance, actual yield of PSII, and electron transport rate to PPFD of a primitive wheat, *T. turgidum* ssp. *durum*, and a modern wheat, *T. aestivum* L. cv. Xiaoyan 22, on the 13th day after initiation of drought and/or O_3 treatments

Both species were well irrigated (88±6% SWC) and exposed to CF air (5±0ppb O_3, 7 h d^{-1}) or elevated O_3 (83±7ppb O_3 7 h d^{-1}), or subjected to drought stress (42±4% SWC) and exposed to CF air or elevated O_3 for 3 weeks during the post-flowering stage in open top chambers.

Parameter	*T. turgidum* ssp. *durum*			*T. aestivum* cv. Xiaoyan 22		
	y*=*bx*+*c	***R*²**	***P*-value**	***y*=*bx*+*c***	***R*²**	***P*-value**
(a) Photosynthesis						
CF air	y=0.040x+0.67	0.987	0.001	y=0.040x+0.32	0.994	0.001
O_3	y=0.037x+0.42	0.980	0.001	y=0.025x–0.85	0.982	0.003
Drought	y=0.035x–0.27	0.974	0.002	y=0.044x+0.23	0.986	0.002
O_3+drought	y=0.034x–0.07	0.970	0.002	y=0.034x+0.16	0.983	0.003
(b) Stomatal conductance						
CF air	y=1.187x+36	0.998	0.010	y=1.137x+314	0.903	0.283
O_3	y=0.808x+24	0.802	0.294	y=0.212x+118	0.933	0.235
Drought	y=0.391x+18	0.980	0.089	y=0.703x+109	0.952	0.140
O_3+drought	y=0.487x+61	0.993	0.053	y=0.652x+53	0.982	0.123
(c) Actual yield						
CF air	y= –4×10^{-4x}+0.65	0.958	0.021	y= –5×10^{-4x}+0.61	0.996	0.004
O_3	y= –5×10^{-4x}+0.63	0.984	0.008	y= –4×10^{-4x}+0.51	0.997	0.003
Drought	y= –5×10^{-4x}+0.63	0.990	0.005	y= –5×10^{-4x}+0.69	0.996	0.004
O_3+drought	y= –5×10^{-4x}+0.61	0.99	0.005	y= –5×10^{-4x}+0.61	0.995	0.005
(d) ETR						
CF air	y=0.174x+7.69	0.995	0.005	y=0.154x+8.61	0.991	0.009
O_3	y=0.145x+9.56	0.989	0.011	y=0.114x+8.21	0.985	0.015
Drought	y=0.149x+ 9.56	0.989	0.011	y=0.178x+9.10	0.992	0.008
O_3+drought	y=0.143x+ 9.40	0.989	0.001	y=0.149x+8.72	0.990	0.010

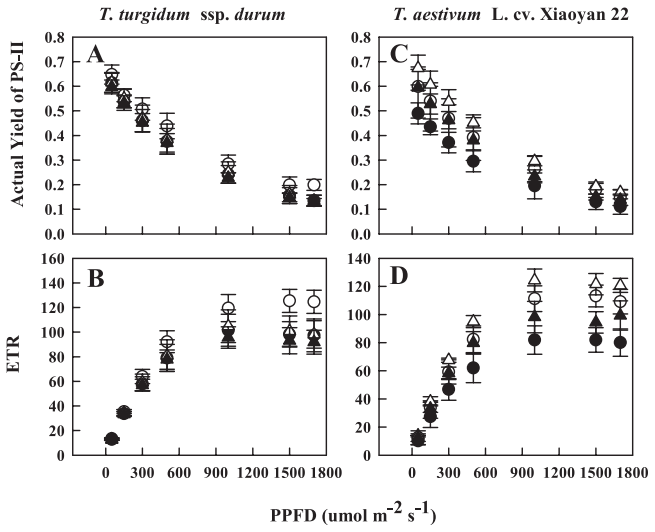

Fig. 5. Actual yield of PSII and electron transport rate (ETR) in the flag leaves of a primitive wheat, *T. turgidum* ssp. *durum*, and a modern wheat, *T. aestivum* L. cv. Xiaoyan 22, as affected by photosynthetic photon flux densities (PPFDs) on the 13th day of initiation of stress treatments. Both species were exposed to drought and/or O_3 for 3 weeks during the post-flowering stage in open top chambers. Treatments were well irrigated and exposed to CF air (open circles) or elevated O_3 (filled circles), or drought stressed and exposed to CF air (open triangles) or elevated O_3 (filled triangles). Error bars indicate the SEM. n=6.

photosynthetic rate under light- and CO_2-saturating conditions may be significantly reduced by elevated O_3, as indicated by the reduction in J_{max}. This suggests that O_3 restrained the capacity for regeneration of the primary CO_2 acceptor, ribulose bisphosphate (RuBP), which depends on adequate synthesis of ATP and NADPH and hence on the rate of electron transport (Farage and Long, 1999).

There were significant differences (P=0.001) in the impacts of elevated O_3 on photosynthesis between the modern and primitive species. The primitive species demonstrated higher A_{sat} accompanied by lower C_i and higher g_s and ITE values than the modern species at elevated O_3. Although O_3 uptake was greater in the primitive wheat than in the modern species, the former demonstrated greater levels of Rubisco and RuBP regeneration than the latter. Moreover, g_s–PPFD curve analysis indicated that the primitive species had faster stomatal control (higher slope) than the modern species (lower slope) under elevated O_3. The results obtained here suggest that higher mesophyll cell activity against O_3-induced oxidative stress, but not O_3 exclusion through stomatal closure, contributed to the observed higher O_3 tolerance of the primitive species.

O_3 significantly (P <0.05) decreased 1000-grain weight, yield per ear, yield per plant, and HI, in general agreement with previous studies for both spring and winter wheat (Pleijel *et al.*, 2000; Khan and Soja, 2003). The observed reductions in grain yield in both species were entirely

Table 3. Yield and yield attributes of a primitive wheat, *T. turgidum* ssp. *durum*, and a modern wheat, *T. aestivum* L. cv. Xiaoyan 22 Both species were well-irrigated (88±6% SWC) and exposed to CF air (5±0 ppb O_3, 7 h d^{-1}) or elevated O_3 (83±7 ppb O_3 7 h d^{-1}), or subjected to drought stress (42±4% SWC) and exposed to CF air or elevated O_3 for 3 weeks during the post-flowering stage in open top chambers. Mean (±SEM). n=18. Similar letters indicate a non-significant difference at P <0.05.

Wheat species	Water level (% SWC)	O_3 concentation (ppb)	Ears per plant (n)	Seeds per ear (n)	1000-grain weight (g)	Yield per ear (g)	Yield per plant (g)	HI
	88±6	5±0	3.00±0.15 a,b	18.73±1.39	23.83±1.32 a	0.46±0.05	1.34±0.12 a	0.37±0.01
	88±6	83±7	2.61±0.14 b,c	18.85±1.31	21.98±1.24 a,b	0.43±0.05	1.11±0.11 a,b	0.35±0.01
T. turgidum ssp. *durum*	42±4	5±0	3.18±0.14 a	16.21±1.35	19.25±1.28 b,c	0.33±0.05	0.95±0.12 b	0.33 ±0.01
	42±4	83±7	2.44±0.15 c	18.78±1.39	17.04±1.32 c	0.36±0.05	0.84±0.12 b	0.33±0.01
	88±6	5±0	2.44±0.20	26.61±1.85	27.24±1.76 a	0.73±0.07 a	1.67±0.16 a	0.48±0.02 a
T. aestivum L. cv. Xiaoyan 22	88±6	83±7	2.50±0.21	21.33±1.97	18.23±1.86 b	0.41±0.07 b	1.04±0.17 b	0.33±0.02 c
	42±4	5±0	2.22±0.20	23.65±1.85	23.17±1.76 a,b	0.56 ±0.07 a,b	1.28±0.16 a,b	0.43±0.02 a,b
	42±4	83±7	2.55±0.18	20.27±1.68	22.98±1.59 a,b	0.46±0.06 b	1.14±0.14 b	0.40±0.02 b
Source of variation (ANOVA)								
Species (sp)			0.002	0.000	0.031	0.000	0.028	0.000
Water stress (ws)			0.720	0.316	0.044	0.050	0.017	0.383
Ozone (O_3)			0.125	0.389	0.003	0.011	0.006	0.000
sp×ws			0.711	0.456	0.021	0.611	0.337	0.083
sp×O_3			0.002	0.004	0.238	0.008	0.279	0.002
ws×O_3			0.865	0.168	0.054	0.084	0.128	0.001
sp×ws×O_3			0.205	0.579	0.036	0.306	0.343	0.045

attributable to reductions in seed weight, rather than the number of seeds per ear. As found in the present study, reduction in 1000-grain weight also explained most of the reduction in grain yield following exposure of wheat to O_3 in OTCs in previous studies (Ojanpera *et al.*, 1998; Pleijel *et al.*, 2000). The primitive wheat exhibited greater carbon partitioning to the grain, as demonstrated by its higher HI resulting from its greater ear yield, seeds per ear, and ears per plant relative to the modern species under elevated O_3. These results demonstrating the differential sensitivity to O_3 of these wheat species as determined by flag leaf photosynthesis and grain yield are fully consistent with their differential O_3 sensitivity as determined by growth, photosynthetic, and anti-oxidative activities during the vegetative stage observed in previous investigations (Biswas *et al.*, 2008*a*, *b*).

Physiological and yield response to drought

Both species showed significant (P <0.001) reductions in A_{sat} and g_s in response to drought. Water stress also decreased V_{cmax} and CE, but did not decrease J_{max} and R significantly, in general agreement with a previous report for wheat (Martin and Ruiz-Torres, 1992). The decline of V_{cmax} in plants exposed to water stress has also been found by other authors (Wilson *et al.*, 2000; Xu and Baldocchi, 2003) and suggests a predominant role for biochemical limitation during drought. However, it was found that drought-induced loss in A_{sat} in winter wheat species might be attributed to a combination of reduced carboxylation efficiency and a decrease in C_i resulting from stomatal closure.

There were considerable differences (P <0.05) in drought tolerance between wheat species as documented by higher A_{sat} and ITE values in the modern wheat than in the primitive wheat. There was also interspecific difference in the mechanism of drought-induced loss in A_{sat}. For example, drought reduced A_{sat} in the primitive wheat initially due to low C_i resulting from higher stomatal closure and later on due to biochemical limitation as C_i increased gradually over the drought period. In contrast, drought decreased A_{sat} in the modern wheat mostly due to low C_i throughout the drought period. Higher drought tolerance in the modern wheat can be further demonstrated by the higher slopes for the response of A, g_s, and ETR to PPFD than that in the primitive wheat. The results agree with the previous study of Xiong *et al.* (2006), who demonstrated that drought resistance was significantly greater in hexaploid spring wheat than in the tetraploid or diploid wheat. This might conceivably be because winter wheat cultivars in China, as elsewhere, were selected for improved resistance to drought, low temperatures, pests, and diseases (Jiang *et al.*, 2003; Biswas *et al.*, 2008*a*).

Water stress reduced (P <0.05) 1000-grain weight, yield per ear, and yield per plant in winter wheat, but did not significantly decrease the HI. The results are consistent with a previous report in which a winter wheat cultivar was exposed to drought stress (Khan and Soja, 2003). In the present study, considerable interspecific variation was noted

in the negative impact of drought on 1000-grain weight and HI. While the primitive wheat displayed significant reductions in mean seed weight and HI, the modern wheat showed slight increases in these variables in response to drought stress. The higher yield stability of the modern winter wheat cultivar under drought found in the present study has also been documented in spring wheat (Xiong *et al.*, 2006). The higher drought resistance of the modern wheat in terms of physiology and yield might be due to selection of a cultivar with improved drought tolerance (Jiang *et al.*, 2003; Biswas *et al.*, 2008*a*).

Differential drought-induced modulation of O_3 tolerance under combination of drought and O_3 exposure

It is widely accepted that water stress may reduce the effects of O_3 by reducing stomatal opening and limiting O_3 uptake (Tingey and Hogsett, 1985; Pearson and Mansfield, 1993; Karlsson *et al.*, 1995; Reichenauer *et al.*, 1998; Khan and Soja, 2003; McLaughlin *et al.*, 2007). As a result, one of the main aims of the present study was to test whether drought offers greater protection against O_3 damage in O_3-tolerant species compared with O_3-sensitive wheat. However, unlike expected, a different result was obtained when two wheat species were exposed to combined drought and O_3 exposure. The primitive wheat tolerant to O_3 showed greater O_3-induced reduction in A_{sat} due to higher loss of Rubisco and carboxylation efficiency in drought-stressed plants than that in the modern species sensitive to O_3. The g_s–PPFD curve analysis also suggests that there was higher O_3-induced negative impact on guard cells, as indicated by slower stomatal control (lower slope) in drought-stressed plants of the primitive wheat than of the modern wheat (higher slope). The findings of differential drought-induced O_3 tolerance in winter wheat species agree with the view that the response of a plant to a combination of two different abiotic stresses is unique and cannot be directly extrapolated from the response of plants to each of the different stresses applied individually (Mittler, 2006).

The differential drought-induced modulation of O_3 effects on the photosynthetic processes in the flag leaves of the two winter wheat species was reflected by yield and yield attributes. The findings imply that drought reduced negative impacts of O_3 on the photosynthetic processes and yield in the modern wheat, but not in the primitive species. Although it has been reported that drought protects against O_3-induced yield reduction in one cultivar of winter wheat (Khan and Soja, 2003), it is clear from the present investigation that this was not the case for the primitive wheat species, which was tolerant to O_3. The primitive wheat demonstrated a higher sensitivity to drought and lost its tolerance to O_3 under a combination of drought and O_3 exposure. In contrast, the modern species tolerant to drought displayed increased O_3 tolerance under combined drought and O_3 exposure in terms of gas exchange, chlorophyll *a* fluorescence, *in vivo* biochemical properties, and yield. These results suggest that the observed O_3 tolerance in the two wheat species was related to their magnitude of drought tolerance under combination of drought and O_3 exposure.

In conclusion, O_3 significantly decreased A_{sat}, g_s, E, and ETR, but increased C_i in both winter wheat species. O_3 also decreased V_{cmax}, J_{max}, CE, and R. Drought decreased A_{sat}, g_s, and C_i, but increased ITE. Drought also decreased V_{cmax} and CE, but not J_{max} and R. O_3 reduced A_{sat} through both biochemical and stomatal limitation in both species. Drought decreased A_{sat} in the primitive wheat mostly due to biochemical limitation, while in the modern wheat it was mainly due to stomatal limitation. There were significant interspecific differences in winter wheat species in response to O_3 or drought. The primitive wheat demonstrated higher O_3 tolerance than the modern species, with the latter exhibiting higher drought tolerance than the former. A faster stomatal control was detected in O_3-stressed plants of the primitive species than in those of the modern wheat. However, stomatal control became slower in the primitive wheat than in the modern species when O_3 stress was combined with drought. Overall, the primitive species lost O_3 tolerance, while the modern wheat exhibited improved tolerance to O_3, suggesting that sensitivity to drought determined the magnitude of O_3 tolerance in wheat species under combination of drought and O_3 exposure. The findings demonstrate that the O_3 tolerance of a drought-sensitive winter wheat species can be completely lost under combined drought and O_3 exposure.

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References

Ainsworth EA, Rogers A, Leakey ADB. 2008. Targets for crop biotechnology in a future high-CO_2 and high-O_3 world. *Plant Physiology* **147,** 13–19.

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.

Biswas DK, Xu H, Li YG, Liu MZ, Chen YH, Sun JZ, Jiang GM. 2008*b*. Assessing the genetic relatedness of higher ozone sensitivity of modern wheat to its wild and cultivated progenitors/relatives. *Journal of Experimental Botany* **59,** 951–963.

Biswas DK, Xu H, Li YG, Sun JZ, Wang XZ, Han XG, Jiang GM. 2008*a*. 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.

Biswas DK, Xu H, Yang JC, *et al*. 2009. Impacts of methods and sites of plant breeding on ozone sensitivity in winter wheat cultivars. *Agriculture, Ecosystems and Environment* **134,** 168–177.

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

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.

Farquhar GD, Caemmerer von S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149,** 78–90.

Fuhrer J. 2003. Agroecosystem responses to combination of elevated CO_2, ozone, and global climate change. *Agriculture, Ecosystems and Environment* **97,** 1–20.

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.

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.

Jiang GM, Sun JZ, Liu HQ, Qu CM, Wang KJ, Guo RJ, Bai KZ, Gao LM, Kuang TY. 2003. Changes in the rate of photosynthesis accompanying the yield increase in wheat cultivars released in the past 50 years. *Journal of Plant Research* **116,** 347–354.

Karlsson PE, Medin E-L, Wickström H, Selldén G, Wallin G, Ottosson S, Skärby L. 1995. Ozone and drought stress: interactive effects on the growth and physiology of Norway spruce (*Picea abies* (L.) Karst.). *Water, Air and Soil Pollution* **85,** 1325–1330.

Khan S, Soja G. 2003. Yield responses of wheat to ozone exposure as modified by drought-induced differences in ozone uptake. *Water, Air and Soil Pollution* **147,** 299–315.

Martin B, Ruiz-Torres NA. 1992. Effects of water-deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (Triticum aestivum L.). *Plant Physiology* **100,** 733–739.

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

McKee IF, Long SP. 2001. Plant growth regulators control ozone damage to wheat yield. *New Phytologist* **152,** 41–51.

McLaughlin SB, Nosal M, Wullschleger SD, Sun G. 2007. Interactive effects of ozone and climate on tree growth and water use in a southern Appalachian forest in the USA. *New Phytologist* **174,** 109–124.

Meyer U, Kollner B, Willenbrink J, Krause GHM. 1997. Physiological changes on agricultural crops induced by different ambient ozone exposure regimes I. Effects on photosynthesis and assimilate allocation in spring wheat. *New Phytologist* **136,** 645–652.

Mittler R. 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science* **11,** 15–19.

Mittler R, Blumwald E. 2010. Genetic engineering for modern agriculture: challenges and perspectives. *Annual Review of Plant Biology* **61,** 1–20.

Mulholland BJ, Craigon J, Black CR, Colls JJ, Atherton J, Landon G. 1997. Effects of elevated carbon dioxide and ozone on the growth and yield of spring wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **48,** 113–122.

Ojanperä K, Patsikka E, Ylaranta T. 1998. Effects of low ozone exposure of spring wheat on net CO_2 uptake, Rubisco, leaf senescence and grain filling. *New Phytologist* **138,** 451–460.

Pearson M, Mansfield TA. 1993. Interacting effects of ozone and water stress on the stomatal resistance of beech (*Fagus sylvatica* L.). *New Phytologist* **123,** 351–358.

Pleijel H, Gelang J, Sild E, Danielsson H, Younis S, Per-Erik Karlsson PE, Wallin G, Skärby L, Selldén G. 2000. Effects of elevated carbon dioxide, ozone and water availability on spring wheat growth and yield. *Physiologia Plantarum* **108,** 61–70.

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.

Rizhsky L, Liang HJ, Mittler R. 2002. The combined effect of drought stress and heat shock on gene expression in tobacco. *Plant Physiology* **130,** 1–9.

Sabehat A, Weiss D, Lurie S. 1998. Heat-shock proteins and cross-tolerance in plants. *Physiologia Plantarum* **103,** 437–441.

Tausz M, Grulke NE, Wieser G. 2007. Defense and avoidance of ozone under global change. *Environmental Pollution* **147,** 525–531.

Tingey DT, Hogsett WE. 1985. Water stress reduces ozone injury via a stomatal mechanism. *Plant Physiology* **77,** 944–957.

Wilkinson S, Davies W. 2010. Drought, ozone, ABA and ethylene: new insights from cell to plant to community. *Plant, Cell and Environment* **33,** 510–525.

Wilson KB, Baldocchi DD, Hanson PJ. 2000. Quantifying stomatal and non-stomatal limitations to carbon assimilation resulting from leaf aging and drought in mature deciduous tree species. *Tree Physiology* **20,** 787–797.

Wittig VE, Ainsworth EA, Long SP. 2007. To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last three decades of experiments. *Plant, Cell and Environment* **30,** 1150–1162.

Wittig VE, Ainsworth EA, Naidu SL, Karnosky DF, Long SP. 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. *Global Change Biology* **15,** 396–424.

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.

Xu L, Baldocchi DD. 2003. Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree Physiology* **23,** 865–877.