Effects of Ozone and Soil Salinity, Singly and in Combination, on Growth, Yield and Leaf Gas Exchange Rates of Two Bangladeshi Wheat Cultivars

Mohammed Zia Uddin Kamal, Masahiro Yamaguchi, Fumika Azuchi, Yoshiyuki Kinose, Yoshiharu Wada, Ryo Funada and Takeshi Izuta

United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan
Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan
Faculty of Agriculture, Utsunomiya University, Utsunomiya, Tochigi 321-0943, Japan
Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan
Present address: Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan
*Corresponding author. Tel: +81-42-367-5728, E-mail: izuta@cc.tuat.ac.jp

ABSTRACT

In Bangladesh, increases in the tropospheric ozone (O₃) concentration and in soil salinization may lead to crop damage. To clarify the effects of O₃ and/or soil salinity on Bangladeshi wheat cultivars, BAW1059 (salt-tolerant) and Shatabdi (salt-sensitive) were exposed to 70-day treatments with O₃ charcoal-filtered air (CF), 1.0×O₃, and 1.5×O₃) and different levels of soil salinity (0, 4, and 8 dS m⁻¹). In both cultivars, the whole-plant dry mass and grain yield were significantly reduced by exposure to O₃. Increased soil salinity caused significant reductions in whole-plant growth and yield in Shatabdi, but the reductions were negligible in BAW1059. No significant interactions between O₃ and salinity were detected for growth, yield, and leaf gas exchange parameters in both cultivars. We concluded that the effects of O₃ are not ameliorated by soil salinity in two Bangladeshi wheat cultivars, regardless of their salinity tolerance.

Key words: Ozone, Salinity, Triticum aestivum L., Yield, Leaf gas exchange rates

1. INTRODUCTION

The tropospheric ozone (O₃) concentration is rising because of increasing anthropogenic emissions of several O₃-forming precursors (Ainsworth, 2008; Kostainen et al., 2006). Ozone is produced in the troposphere as a secondary air pollutant via photochemical reactions between nitrogen oxides (NOₓ) and volatile organic compounds in the presence of sunlight (Ashmore, 2005). According to the Fourth Assessment Report (AR4) of Intergovernmental Panel on Climate Change (IPCC), O₃ concentration could rise by 20%-25% between 2015 and 2050, and further increase by 40%-60% by 2100, if the current trends in precursor emissions continue (IPCC, 2007). Worryingly for the South Asian region, the projections of future global O₃ trends show that atmospheric O₃ concentrations will increase rapidly over the next 20 to 30 years, with the greatest increase in surface O₃ in South Asia (Dentener et al., 2006). In India, ozone levels have risen from a preindustrial atmospheric O₃ concentration of 10 nL L⁻¹ (ppb) to 50-60 ppb at present (Van Dingenen et al., 2009). If emissions of O₃ precursors in the northern hemisphere do not decrease, the O₃ concentration in India is predicted to increase by 5-11 ppb, depending on the season, by 2030 (Van Dingenen et al., 2009). Peak O₃ concentrations have exceeded 100 ppb during the summer season in the Indo-Gangetic region (Mishra et al., 2013; Tiwari et al., 2010).

Ozone has detrimental effects on the growth and productivity of crops (Tiwari et al., 2010). After entering the leaves through stomata, O₃ induces the formation of highly reactive oxygen species (ROS) and free radicals (Foyer and Noctor, 2005), which interact with cellular components to reduce the net photosynthetic rate (Dermody et al., 2006; Skotnica et al., 2005; Morgan et al., 2003) and stomatal conductance (Gerosa et al., 2014; Calatayud et al., 2003). Relatively high concentrations of O₃ cause visible foliar injury, accelerate leaf senescence (Massman et al., 2000), and affect biochemical and physiological processes (Rai et al., 2011; Betzelberger et al., 2010; Sarkar et al., 2010). Ozone exposure adversely affects whole-plant growth (Akhtar
et al., 2010a; Biswas et al., 2008), nutrient uptake, and translocation of assimilates (Tiwari et al., 2010), leading to inferior quality and reduced yield of many crops (Sarkar and Agrawal, 2010; Fuhrer, 2009). Crop yield losses due to O₃ have been estimated by 5%-35% in agriculturally important locations across South Asia (Emberson and Bükker, 2008). However, limited information is available on the effects of O₃ on crops cultivated in Bangladesh (Saitanis et al., 2014; Akhtar et al., 2010a, b).

Soil salinity is a major environmental stress that drastically affects crop productivity worldwide (Zhu, al., 2010a, b). Oxidative stress, and hormonal imbalance (Ashraf, 2010). Salt-stress inhibits plant growth in four major ways; salt-induced osmotic stress, specific ion toxicity, oxidative stress, and hormonal imbalance (Ashraf, 2009). Salt-stress decreases relative water content, chlorophyll content, membrane stability, ascorbic acid content, and the activities of several antioxidant enzymes in the leaves of crops at the seedling stage (Ozturk et al., 2012; Hameed et al., 2008; Shim et al., 2003) and at the heading and grain-filling stages (Bai et al., 2013; Burcu et al., 2009).

In many parts of the world, tropospheric O₃ and soil salinity are limiting factors in agriculture (Gerosa et al., 2014; FAO, 2007; Fuhrer and Booker, 2003; Qadir et al., 2000). There is a possibility that the combined effects of O₃ and soil salinity will adversely affect crops cultivated in South Asian countries such as Bangladesh (Titumir and Basak, 2012; Welfare et al., 2002, 1996). However, very limited information is available on the combined effects of O₃ and salinity on crops (Gerosa et al., 2014; Zheng et al., 2012; Welfare et al., 2002, 1996). Welfare et al. (2002, 1996) reported additive effects of O₃ and salinity on leaf gas exchange rates of rice and chickpea. Stomatal closure is one of the plant responses that limits damage under stress conditions (Maggio et al., 2009; Ma et al., 2006; Warren and Dreyer, 2006). Therefore, salinity-induced stomatal closure might reduce stomatal O₃ flux into the intercellular spaces and protect crops from O₃ damage.

In Bangladesh, wheat has become the second most important cereal crop after rice (Akhtar et al., 2010a). The total area under wheat cultivation in Bangladesh during 2011-2012 was estimated to be 400,000 hectares, and 1.1 million metric tons of wheat was produced (Anonymous, 2012). Wheat has been shown to be the most O₃-sensitive crop (Mills et al., 2007), and Asian wheat cultivars are more sensitive to O₃ than are other tropical and temperate cultivars (Akhtar et al., 2010a; Emberson et al., 2009; Van Dingenen et al., 2009; Rai et al., 2007). However, little is known about the responses of salt-tolerant and salt-sensitive Bangladeshi wheat cultivars to O₃ under salt-stress. The objective of the present study was to clarify the effects of O₃ and soil salinity, singly and in combination, on the growth, yield, and leaf gas exchange rates of two Bangladeshi wheat cultivars with different salt sensitivities.

2. MATERIALS AND METHODS

2.1 Gas Exposure Chambers

Nine gas exposure chambers located in the Field Museum Tamakyuryo, Tokyo University of Agriculture and Technology (Hachioji, Tokyo), were used in the present study. The latitude, longitude and above sea level at the experimental site are 35°38′ N, 139°22′ E and 144.1 m, respectively. Ambient air was introduced into the chambers at a flow rate of 1.03 m³ s⁻¹ after it had passed through an activated charcoal filter in the fan box to remove ambient O₃. In the chambers assigned to the O₃-treatments, O₃ was added to the charcoal-filtered air introduced into the chambers. The ambient O₃ concentration was used as the standard concentration for controlling of O₃ concentration in the O₃-exposure chambers. The concentrations of O₃ in each chamber and in ambient air were independently and continuously monitored at 30-min and 10-min intervals, respectively, using a UV absorption O₃ analyzer (Model-1210; Dylec Inc., Ibaraki, Japan). Details of the construction of the gas exposure chambers and the O₃-exposure system are described in Kinose et al. (2014). Mean removal efficiency, mean concentration, and accumulated O₃ exposure over a threshold of 40 ppb (AOT₄₀) during the O₃-exposure period were calculated based on O₃ concentration in the chambers and ambient air. The photosynthetic photon flux density (PPFD) under ambient conditions was measured at 1-min intervals using a quantum sensor (LI-190SA; Li-Cor Inc., Lincoln, NE, USA). The mean light transmissivity of the chambers during the gas treatment period was approximately 73%. In three of the nine chambers, air temperature and relative air humidity were continuously measured at 10-min intervals using a TR-72U Thermo Recorder (T&D Corporation, Nagano, Japan).

2.2 Plant Materials

Two Bangladeshi cultivars of spring wheat (Triticum aestivum L.), BAW1059 and Shatabdi, were used as plant materials. Seeds of the two wheat cultivars were obtained from the Bangladesh Agricultural Research
Institute (BARI). BAW1059 is salt-tolerant and Shatabdi is salt-sensitive (Wheat Atlas CYMMYT, 2014; Barma et al., 2011). The yield potential of the both cultivars is 3.6-5.0 ton ha$^{-1}$ in Bangladesh (Saitanis et al., 2014; Barma et al., 2011). Both wheat cultivars are heat tolerant (Barma et al., 2011).

On 23 April 2013, seeds of the two Bangladeshi wheat cultivars were sown in Wagner pots (volume: 12 L, diameter: 240 mm, depth: 258.5 mm) filled with horticultural soil. Three hills were formed in each pot, and three seeds were sown per hill. The seedlings were thinned to leave one seedling per hill. The seedlings were grown from 23 April to 20 May under field conditions. Average 12-h O$_3$ concentration and daylight AOT40 under field conditions were 39.4 nL L$^{-1}$ (ppb) and 3.9 µL L$^{-1}$ h (ppm h), respectively. Under field conditions, air temperature and relative air humidity fluctuated from 9.5 to 22.1°C and from 72 to 98%, respectively. The seedlings were thinned to leave one seedling per hill on 10 and 17 May. Because the potting soil was slightly acidic, CaCO$_3$ was added at a rate of 5 g per pot and mixed thoroughly to a depth of 15 cm before sowing seeds. As a result, initial pH of the soil was 5.73. The N, P, and K contents in the potting soil were all 384 mg L$^{-1}$, which maintained the optimum nutrient range for growth and development of wheat. According to Miah et al. (2005), the optimum N, P, and K concentrations for growth of Bangladeshi wheat are 40, 10, and 30 kg ha$^{-1}$, respectively. Although the soil contained optimum nutrients for wheat growth, to enhance growth at the tillering stage and to prompt booting, two-split applications of fertilizer (N : P : K = 8 : 8 : 8; 0.83 g per pot) were administered. In wheat, the nutrient use efficiency of nitrogenous fertilizer ranges from 14.1% when applied at sowing to 54.8% when applied as a topdressing at the beginning of stem elongation (Bellido et al., 2005).

### 2.3 Gas and Salinity Treatments

The experiment was organized in a split-split plot design with three chamber replications. The whole-plot treatment comprised three levels of O$_3$, the two contrasting cultivars as a sub-plot treatment, and soil salinity (at three salt concentrations) as a sub-sub plot treatment. The gas and salinity treatments started on 21 May at the third-leaf stage of the two wheat cultivars, which coincided with the tillering stage, and continued until harvest as reported by El-Hendawy et al. (2005). For 70 days from 21 May to 29 July 2013, the two wheat cultivars were exposed to charcoal-filtered air (mean O$_3$ removal efficiency: 54%) or O$_3$ at 1.0- and 1.5-times the ambient concentration (1.0×O$_3$ and 1.5×O$_3$ treatments, respectively). For each cultivar, four pots (i.e., 12 plants) were assigned to each soil salinity treatment in each chamber. The salinity treatment consisted of three levels of soil salinity: 0, 4, and 8 dS m$^{-1}$ electrical conductivity (EC). To maintain the soil salinity at 0, 4, or 8 dS m$^{-1}$ EC, 0, 75, or 150 mM NaCl solution (corresponding to 0, 4.383, and 8.766 g L$^{-1}$ of NaCl solution, respectively) was applied to each pot at 4-day intervals for a total of 15 applications during the 70-day gas and salinity treatments. To avoid osmotic shock and to allow the plants to adapt to salt-stress, the two salt concentrations were initially divided into three portions and applied at 3-day intervals before imposing the final concentrations. Deionized water was added to each pot between salt applications to maintain soil moisture and prevent salt accumulation.

### 2.4 Measurements of Growth and Yield Parameters

Twelve plants of each cultivar were harvested from each treatment-chamber combination on 26 July (67 days after treatment, DAT) for BAW1059 and 29 July 2013 (70 DAT) for Shatabdi. The harvested plants were divided into leaves, stems, spikes, and roots. The plant organs were dried at 80°C for 5 days and then weighed. Yield parameters were assessed at harvest. The harvest index is the weight of harvested product as a percentage of the total plant weight of crop. We calculated the harvest index as the ratio of grain yield to the whole-plant dry mass. The number of filled and unfilled grains per plant and 1000-grain weight were determined. The grains were separated from spikes and manually categorized into two groups; filled and unfilled. The 1000-grain weight was calculated from the dry mass of filled grains per plant and the number of filled grains per plant. Yield per plant was expressed as the dry mass of filled grains per plant.

### 2.5 Measurements of Leaf Gas Exchange Rates

The gas exchange rates of flag leaves in BAW1059 and Shatabdi were measured on 26-30 June (36-40 DAT) and 2-6 July 2013 (42-46 DAT) using a portable photosynthetic measurement system (LI-6400, Li-Cor Inc., Lincoln, NE, USA), respectively. For measurements of net photosynthetic rate (A), stomatal conductance to H$_2$O ($G_s$) and intercellular CO$_2$ concentration ($C_i$), three plants per cultivar-treatment-chamber combination were randomly selected (nine measurements per treatment for each cultivar). During the measurements of A, $G_s$, and $C_i$, the conditions in the leaf chamber were maintained as follows: atmospheric CO$_2$ concentration, 390 µmol mol$^{-1}$; air temperature, 25±1°C; relative air humidity, 70%±5%; and photosynthetic photon flux density (PPFD), 1500 µmol m$^{-2}$ s$^{-1}$. Once conditions for gas exchange measurements were sta-
ble, light-saturated $A$, $G_s$, and $C_i$ were recorded simultaneously.

2.6 Measurements of Soil Salinity

The initial and residual soil salinities in each whole pot were determined by measuring the electrical conductivity of a 1:5 soil:water extract (EC$_{1:5}$) with a conductivity meter (SS974, Horiba Korea Ltd., Korea). The soil was initially non-saline (EC = 0.71 dS m$^{-1}$).

2.7 Statistical Analyses

The data of growth parameters, yield, yield components, and leaf gas exchange parameters were subjected to three-way analysis of variance (ANOVA) to examine the individual effects of O$_3$, soil salinity, and cultivar (CV). In the ANOVA, chamber replication was set as a random factor. We confirmed that there were no significant interactions between O$_3$ and chamber replication for any of the parameters. All statistical analyses were performed with the SPSS statistical package (SPSS 11.5, SPSS Inc., USA).

3. RESULTS

3.1 Environmental Parameters

Table 1 summarizes the air temperature, relative air humidity, and cumulative solar radiation in the gas-exposure chambers during the gas and salinity treatments for 70 days from 21 May to 29 July 2013. The mean 12-h (6:00-18:00) air temperature and relative air humidity were $24.7^\circ\text{C}$ and 78.7%, respectively. The mean daily air temperature and relative air humidity during the harvest period of the two wheat cultivars (1-29 July) were $25.2^\circ\text{C}$ and 88.1%, respectively, which were similar to those during the harvest period of wheat in Bangladesh (BBS, 2004). The cumulative PPFD during the gas and salinity treatments for 70 days was 1473 mol m$^{-2}$.

Table 2 shows the mean concentration, AOT0, and AOT40 of O$_3$ in each gas treatment during the gas treatments for 70 days from 21 May to 29 July 2013. The mean 24-h concentrations of O$_3$ in the charcoal-filtered air (CF), 1.0 $\times$ O$_3$, and 1.5 $\times$ O$_3$ treatments were 10, 24, and 34 nL L$^{-1}$ (ppb), respectively. The daylight AOT40 of O$_3$ in the CF, 1.0 $\times$ O$_3$, and 1.5 $\times$ O$_3$ treatments were 0, 2.9, and 8.9 $\mu$L L$^{-1}$ h$^{-1}$ (ppm h), respectively.

3.2 Soil Salinity Concentration

Table 3 shows the EC of the potting soil at final harvest of BAW1059 and Shatabdi. The three-way ANOVA revealed that the salinity treatment significantly increased the soil EC of potting soil for both culti-

| Period (2013) | Air temperature ($^\circ\text{C}$) | Relative air humidity (%) | Cumulative PPFD ($\text{mol m}^{-2}$) | 12-h mean $\text{EC}_{1:5}$ (dS m$^{-1}$) |
|---------------|---------------------------------|---------------------------|-----------------------------|--------------------------------------|
| 21-31 May     | 20.4 (1.0)                      | 27.1 (3.2)                | 16.0 (1.9)                  | 10.4 (1.0)                           |
| 1-30 June     | 21.0 (1.0)                      | 28.5 (3.4)                | 17.4 (2.5)                  | 10.0 (1.0)                           |
| 1-29 July     | 25.2 (2.0)                      | 31.4 (4.1)                | 21.6 (1.7)                  | 4.4 (0.1)                            |
| 21 May-29 July| 22.2 (2.8)                      | 28.5 (4.3)                | 21.6 (1.7)                  | 4.4 (0.1)                            |

Each value of air temperature and relative air humidity is the mean of 3 chambers, and the standard deviation is shows in parenthesis.
Effects of O3 and Salinity on Bangladeshi Wheat Cultivars

However, soil EC did not vary depending on the other factors or their interactions. For both cultivars, the mean residual EC of the potting soil after final harvest was 0.79, 3.64, and 5.77 dS m⁻¹ in the 0, 4, and 8 dS m⁻¹ treatments, respectively.

### Table 3. Electrical conductivity (EC, dS m⁻¹) of residual soil after final harvest of two Bangladeshi wheat cultivars (BAW 1059 and Shatabdi).

| Treatment | Gas   | Soil EC (dS m⁻¹) |
|-----------|-------|-----------------|
|           |       | BAW1059         | Shatabdi |
| Salinity  |       |                 |          |
| 0 dS m⁻¹  | CF    | 0.84 (0.07)     | 0.76 (0.07) |
|           | 1.0 × O₃ | 0.75 (0.16) | 0.78 (0.06) |
|           | 1.5 × O₃ | 0.81 (0.06) | 0.79 (0.06) |
| 4 dS m⁻¹  | CF    | 3.32 (0.67)     | 3.72 (0.26) |
|           | 1.0 × O₃ | 3.70 (0.54) | 3.98 (0.16) |
|           | 1.5 × O₃ | 3.50 (0.46) | 3.63 (0.13) |
| 8 dS m⁻¹  | CF    | 5.71 (0.13)     | 5.55 (0.40) |
|           | 1.0 × O₃ | 5.93 (0.54) | 6.15 (0.33) |
|           | 1.5 × O₃ | 5.45 (0.48) | 5.86 (0.49) |
| O₃        | 0.0602₉,₉   |
| Salinity  | 0.0000₉,₁₄₀₀ |
| O₃ × Salinity | 0.372₀,₉   |
| Cultivar (CV) | 0.121₃₉,₉ |
| O₃ × CV     | 0.786₇₉,₉   |
| Salinity × CV | 0.371₉₀,₉  |
| O₃ × Salinity × CV | 0.598₇₉,₉ |

CF, Charcoal-filtered air.
Each value is the mean of 3 chamber replicates, and the standard deviation is shown in parenthesis.
Result of three-way ANOVA indicates p-value and level of significance; "p < 0.001; n.s. = not significant.

### 3.3 Plant Biomass

Table 4 shows the effects of O₃ and soil salinity, singly and in combination, on the dry mass of BAW1059 and Shatabdi at final harvest. The three-way ANOVA revealed that the whole-plant dry mass varied significantly due to all the individual factors and the interaction between salinity and cultivar. In both wheat cultivars, whole-plant dry mass was significantly reduced by exposure to O₃. Averaged across the both cultivars, the O₃-induced reduction in whole-plant dry mass was 1.1% in the 1.0 × O₃ treatment and 9.4% in the 1.5 × O₃ treatment, as compared with the CF treatment. The extent of the salinity-induced reduction in whole-plant dry mass differed significantly between the two cultivars. In BAW1059 and Shatabdi, the salinity-induced reductions in whole-plant dry mass were 3.8% and 22.3%, respectively, in the 8 dS m⁻¹ treatment, as compared with the 0 dS m⁻¹ treatment. The result of three-way ANOVA indicates that leaf dry mass was varied significantly due to salinity, cultivar and the interaction between salinity and cultivar. In BAW1059 and Shatabdi,
stem dry mass was lower by 11.0% and 22.8%, while root dry mass was higher by 43.2% and 4.8% in the 8 dS m\(^{-1}\) treatment as compared with those in the 0 dS m\(^{-1}\) treatment, respectively. Three-way ANOVA revealed that panicle dry mass was significantly varied by all individual factors and the interaction between salinity and cultivar. Averaged across the both cultivars, O\(_3\)-induced reduction in panicle dry mass was 11.4% in the 1.5×O\(_3\) treatment as compared with the CF treatment. The salinity-induced reductions in panicle dry mass in BAW1059 and Shatabdi were 4.9% and 25.3% in the 8 dS m\(^{-1}\) treatment as compared with the 0 dS m\(^{-1}\) treatment, respectively.

### 3.4 Yield and Yield Components

Fig. 1 shows the effects of O\(_3\) and soil salinity on the yield per plant of BAW1059 and Shatabdi. The results of the three-way ANOVA revealed that the yield per plant varied significantly due to all of the individual factors and the interaction between salinity and culti-

| Table 4. Effects of O\(_3\), salinity and/or cultivar on the dry mass of two Bangladeshi wheat cultivars (BAW1059 and Shatabdi). |
|-------------------------------|------------------|------------------|------------------|------------------|------------------|
| **Cultivar**                   | **Treatment**    | **Dry mass (g)** | **Whole-plant dry mass (g)** |
| **Salinity**                  | **Gas**          | **Leaf**         | **Stem**         | **Root**         | **Panicle**      | **Whole-plant dry mass (g)** |
| 0 dS m\(^{-1}\)               | CF               | 0.77 (0.15)      | 2.49 (0.21)      | 0.48 (0.07)      | 4.77 (0.39)      | 8.51 (0.68)      |
|                              | 1.0×O\(_3\)      | 0.80 (0.10)      | 2.21 (0.22)      | 0.48 (0.04)      | 4.06 (0.49)      | 7.56 (0.81)      |
|                              | 1.5×O\(_3\)      | 0.66 (0.09)      | 1.87 (0.22)      | 0.36 (0.01)      | 3.24 (0.47)      | 6.13 (0.65)      |
| 4 dS m\(^{-1}\)               | CF               | 0.76 (0.07)      | 2.18 (0.24)      | 0.56 (0.02)      | 4.28 (0.54)      | 7.79 (0.87)      |
|                              | 1.0×O\(_3\)      | 0.79 (0.11)      | 2.12 (0.08)      | 0.64 (0.07)      | 4.16 (0.10)      | 7.70 (0.36)      |
|                              | 1.5×O\(_3\)      | 0.70 (0.09)      | 1.98 (0.28)      | 0.56 (0.05)      | 3.67 (0.47)      | 6.91 (0.85)      |
| 8 dS m\(^{-1}\)               | CF               | 0.72 (0.06)      | 1.90 (0.19)      | 0.60 (0.08)      | 3.86 (0.33)      | 7.08 (0.57)      |
|                              | 1.0×O\(_3\)      | 0.70 (0.05)      | 1.92 (0.04)      | 0.65 (0.03)      | 3.88 (0.12)      | 7.15 (0.19)      |
|                              | 1.5×O\(_3\)      | 0.73 (0.11)      | 2.03 (0.37)      | 0.64 (0.08)      | 3.74 (0.72)      | 7.13 (1.27)      |
| **Shatabdi**                  | **Treatment**    | **Dry mass (g)** | **Whole-plant dry mass (g)** |
| 0 dS m\(^{-1}\)               | CF               | 1.13 (0.15)      | 3.30 (0.15)      | 0.70 (0.09)      | 5.64 (0.54)      | 10.78 (0.74)     |
|                              | 1.0×O\(_3\)      | 1.06 (0.11)      | 3.25 (0.28)      | 0.68 (0.07)      | 5.65 (0.37)      | 10.65 (0.74)     |
|                              | 1.5×O\(_3\)      | 0.91 (0.16)      | 2.84 (0.40)      | 0.54 (0.10)      | 5.27 (1.23)      | 9.56 (1.82)      |
| 4 dS m\(^{-1}\)               | CF               | 0.88 (0.06)      | 2.58 (0.14)      | 0.64 (0.04)      | 4.91 (0.47)      | 9.00 (0.71)      |
|                              | 1.0×O\(_3\)      | 1.00 (0.15)      | 2.64 (0.31)      | 0.63 (0.12)      | 4.83 (0.71)      | 9.11 (1.25)      |
|                              | 1.5×O\(_3\)      | 0.79 (0.14)      | 2.55 (0.10)      | 0.71 (0.08)      | 4.43 (0.25)      | 8.48 (0.39)      |
| 8 dS m\(^{-1}\)               | CF               | 0.77 (0.08)      | 2.29 (0.19)      | 0.62 (0.08)      | 4.16 (0.32)      | 7.83 (0.66)      |
|                              | 1.0×O\(_3\)      | 0.86 (0.27)      | 2.57 (0.61)      | 0.74 (0.14)      | 4.09 (0.62)      | 8.26 (1.51)      |
|                              | 1.5×O\(_3\)      | 0.82 (0.03)      | 2.39 (0.15)      | 0.65 (0.13)      | 4.12 (0.70)      | 7.99 (0.93)      |
| **ANOVA**                     | **O\(_3\)**      | 0.0692 (n.s.)    | 0.0775 (n.s.)    | 0.1519 (n.s.)    | 0.0077 (**)      | 0.0065 (**)      |
|                              | **Salinity**      | 0.0299 (n.s.)    | 0.0000 (n.s.)    | 0.0031 (n.s.)    | 0.0001 (n.s.)    | 0.0009 (n.s.)    |
|                              | **O\(_3\)×Salinity** | 0.3800 (n.s.)  | 0.0653 (n.s.)    | 0.1019 (n.s.)    | 0.2728 (n.s.)    | 0.1726 (n.s.)    |
|                              | **Cultivar (CV)** | 0.0000 (n.s.)    | 0.0000 (n.s.)    | 0.0000 (n.s.)    | 0.0000 (n.s.)    | 0.0000 (n.s.)    |
|                              | **O\(_3\)×CV**   | 0.7170 (n.s.)    | 0.4939 (n.s.)    | 0.1017 (n.s.)    | 0.3526 (n.s.)    | 0.7881 (n.s.)    |
|                              | **Salinity×CV**  | 0.0811 (n.s.)    | 0.0176 (n.s.)    | 0.0331 (n.s.)    | 0.0025 (n.s.)    | 0.0044 (n.s.)    |
|                              | **O\(_3\)×Salinity×CV** | 0.8178 (n.s.) | 0.9484 (n.s.)    | 0.5617 (n.s.)    | 0.6186 (n.s.)    | 0.8480 (n.s.)    |

**CF**, Charcoal-filtered air.
Each value is the mean of 3 chamber replicates, and the standard deviation is shown in parenthesis.
Result of three-way ANOVA indicates \(p\)-value and level of significance; *\(p<0.05\), **\(p<0.01\), ***\(p<0.001\); n.s. = not significant.

| Table 5. The result of three-way ANOVA of the effects of O\(_3\), salinity and/or cultivar (CV) on yield per plant of two Bangladeshi wheat cultivars (BAW1059 and Shatabdi). |
|-------------------------------|------------------|------------------|
| **Parameter**                  | **O\(_3\)**      | **Salinity**     |
|                               | **O\(_3\)×Salinity** | **CV**          |
| **Yield per plant**           | 0.0058 (***)     | 0.0023 (***)     |
|                               | 0.0914 (n.s.)    | 0.0000 (****)    |
|                               | 0.4984 (n.s.)    | 0.0024 (**)      |
|                               | 0.8251 (n.s.)    |                  |

Result of three-way ANOVA indicates \(p\)-value and level of significance; **\(p<0.01\), ***\(p<0.001\), n.s. = not significant.
var (Table 5). In both cultivars, the yield per plant was significantly reduced by exposure to O₃. Averaged across the both cultivars, the reduction in yield per plant was 4.7% in the 1.0 × O₃ treatment and 11.6% in the 1.5 × O₃ treatment, as compared with the CF treatment. In BAW1059, there was no significant reduction in yield per plant in the 8 dS m⁻¹ treatment as compared with the 0 dS m⁻¹ treatment. In Shatabdi, however, the yield per plant was 23.6% lower in the 8 dS m⁻¹ treatment than in the 0 dS m⁻¹ treatment.

Table 6 summarizes the effects of O₃ and soil salinity, singly and in combination, on the yield components of BAW1059 and Shatabdi. According to the results of the three-way ANOVA, spike and floret number per plant and floret number per spike varied significantly due to all of the individual factors and the interaction between salinity and cultivar. In both cultivars, the number of spikes per plant was 3.7% lower in the 1.5 × O₃ treatment than in the CF treatment. In BAW1059 and Shatabdi, the number of spikes per plant in the 8 dS m⁻¹ treatment was 7.6% and 24.8% lower, respectively, than that in the 0 dS m⁻¹ treatment. Averaged across the both cultivars, the number of florets per plant and per spike were 7.0% and 8.8% lower, respectively, in the 1.5 × O₃ treatment than in the CF treatment. In the salt treatments, the number of florets per plant and per spike in BAW1059 were 5.1% and 0.5% lower, respectively, in the 8 dS m⁻¹ treatment than in the 0 dS m⁻¹ treatment. In Shatabdi, the number of florets per plant and per spike were 22.6% and 4.8% lower, respectively, in the 8 dS m⁻¹ treatment than in the 0 dS m⁻¹ treatment.

The three-way ANOVA indicated that the number of filled grains and the ratio of filled grains to total grains varied significantly due to the cultivar and the interaction between salinity and cultivar (Table 6). The number of filled grains in BAW1059 and Shatabdi was 4.7% and 20.2% lower, respectively, in the 8 dS m⁻¹ treatment than in the 0 dS m⁻¹ treatment. In the 8 dS m⁻¹ treatment, the ratio of filled grain number to total grain number was unchanged in BAW1059, but decreased by 4.8% in Shatabdi. The three-way ANOVA indicated that the number of unfilled grains was significantly affected only by salinity. Averaged across both cultivars, the salinity-induced reduction in the number of unfilled grains was 9.2% in the 8 dS m⁻¹ treatment.

The three-way ANOVA showed that the 1000-seeds weight varied significantly due to all of the individual factors and salinity × cultivar and O₃ × cultivar interactions (Table 6). In BAW1059 and Shatabdi, the 1000-seeds weight was 7.0% and 4.1% lower, respectively, in the 1.5 × O₃ treatment than in the CF treatment. As compared with that in the 0 dS m⁻¹ treatment, the 1000-seeds weight were 1.6% and 8.0% lower in BAW1059 and 4.1% and 8.0% lower in Shatabdi in the 4 and 8 dS m⁻¹ treatments, respectively.

The three-way ANOVA indicated that harvest index (HI) was significantly affected by O₃ treatment and cultivar, but not by salinity or interactions between factors (Table 6). Averaged across both cultivars, the HI was 2.3% lower in the 1.5 × O₃ treatment than in the CF treatment.

### 3.5 Leaf Gas Exchange Rates

Table 7 shows the effects of O₃ and soil salinity, singly and in combination, on A, Gₛ, and Cᵢ in the flag leaves of BAW1059 and Shatabdi. According to the three-way ANOVA, A varied significantly due to all the individual factors and the interaction between salinity and cultivar. Averaged across both cultivars, the O₃-induced reduction in A was 7.1% in the 1.0 × O₃ treatment and 11.7% in the 1.5 × O₃ treatment. In BAW1059 and Shatabdi, A was 1.1% and 22.1% lower, respectively, in the 8 dS m⁻¹ treatment than in the 0 dS m⁻¹ treatment. Both O₃ and salinity significantly affected Gₛ. Averaged across both cultivars, Gₛ was 17.7% lower in the 1.5 × O₃ treatment than in the CF treatment, and 17.1% lower in the 8 dS m⁻¹ treatment than in the 0 dS m⁻¹ treatment. The three-way ANOVA revealed that Cᵢ was significantly affected by salinity, cultivar, and the interaction between O₃ and cultivar. Because there was significant interaction between O₃ and cultivar on Cᵢ, percentage changes in Cᵢ due to O₃ were calculated for each cultivar, although there was no significant main effect of O₃ on Cᵢ. The Cᵢ was significantly increased by 2.9% and 0.6% in BAW1059 and Shatabdi, respectively, in the 1.5 × O₃ treatment, as compared with the CF treatment. Averaged across both cultivars, Cᵢ was 1.6% lower in the 8 dS m⁻¹ treatment than in the 0 dS m⁻¹ treatment.

### 4. DISCUSSION

In the present study, exposure to O₃ significantly decreased the whole-plant dry mass and grain yield per plant of two Bangladeshi wheat cultivars (Tables 4 and 5, and Fig. 1). There were no significant differences between the two Bangladeshi wheat cultivars in terms of the O₃-sensitivity of whole-plant dry mass and grain yield per plant (Tables 4 and 5, and Fig. 1). On average, the O₃-induced reduction in whole-plant dry mass and grain yield per plant in the two Bangladeshi wheat cultivars were 1.1% and 4.7%, respectively, in the 1.0 × O₃ treatment, and 9.4% and 11.6%, respectively, in the 1.5 × O₃ treatment, as compared with the CF treatment. Several studies carried out in North America, Europe, and elsewhere have reported O₃-
Table 6. Effects of O₃, salinity and/or cultivar on yield components of two Bangladeshi wheat cultivars (BAW1059 and Shatabdi).

| Cultivar | Treatment | Number per plant | % of filled grain | Floret no. / spike | 1000-seed weight (g) | Harvest index (%) |
|----------|-----------|------------------|-------------------|-------------------|-------------------|------------------|
|          | Salinity  | Gas   | Spike | Floret | Filled grain | Unfilled grain | | |
| BAW1059  | 0 dS m⁻¹ | CF    | 3.2 (0.5) | 123.3 (12.1) | 75.2 (10.8) | 48.2 (5.2) | 61.0 (1.1) | 38.3 (3.0) | 47.6 (1.6) | 42.0 (3.5) |
|          |           | 1.0 × O₃ | 3.2 (0.3) | 118.4 (11.2) | 69.9 (9.3) | 48.1 (3.2) | 59.1 (2.0) | 36.8 (1.2) | 46.0 (1.9) | 41.3 (1.3) |
|          |           | 1.5 × O₃ | 3.0 (0.4) | 101.5 (11.7) | 59.0 (9.2) | 42.5 (4.2) | 59.0 (2.8) | 33.6 (1.8) | 42.5 (2.1) | 40.0 (0.9) |
|          | 4 dS m⁻¹ | CF    | 3.3 (0.5) | 123.1 (14.6) | 75.2 (7.9) | 47.9 (7.4) | 61.4 (2.6) | 37.4 (1.8) | 45.1 (1.2) | 43.3 (1.3) |
|          |           | 1.0 × O₃ | 3.2 (0.2) | 120.5 (7.7) | 73.0 (2.2) | 47.5 (5.7) | 60.6 (2.5) | 37.4 (0.5) | 45.9 (0.9) | 43.2 (1.3) |
|          |           | 1.5 × O₃ | 3.1 (0.3) | 107.8 (7.7) | 67.3 (6.0) | 40.4 (1.8) | 62.6 (2.2) | 35.0 (1.6) | 43.1 (2.9) | 42.1 (1.2) |
|          | 8 dS m⁻¹ | CF    | 2.7 (0.2) | 106.6 (8.9) | 63.9 (8.4) | 42.7 (3.5) | 59.9 (1.7) | 39.3 (3.0) | 42.3 (2.9) | 43.5 (2.5) |
|          |           | 1.0 × O₃ | 3.0 (0.2) | 108.2 (1.7) | 63.4 (0.5) | 45.2 (6.8) | 58.6 (4.3) | 35.6 (2.1) | 43.0 (0.9) | 43.1 (0.4) |
|          |           | 1.5 × O₃ | 3.0 (0.4) | 110.7 (14.8) | 67.1 (14.1) | 43.6 (1.5) | 60.6 (4.7) | 37.4 (5.8) | 40.0 (0.9) | 41.9 (1.0) |
| Shatabdi | 0 dS m⁻¹ | CF    | 5.2 (0.6) | 158.2 (5.4) | 96.6 (4.8) | 55.9 (3.0) | 66.0 (2.3) | 36.6 (2.5) | 45.3 (1.2) | 39.8 (2.4) |
|          |           | 1.0 × O₃ | 5.0 (0.7) | 142.9 (13.6) | 90.8 (4.5) | 52.1 (7.8) | 65.7 (10.3) | 33.0 (7.4) | 44.9 (0.7) | 38.3 (1.8) |
|          |           | 1.5 × O₃ | 4.4 (0.5) | 139.6 (6.8) | 90.8 (10.2) | 48.8 (3.5) | 64.8 (4.6) | 32.3 (2.4) | 41.8 (2.4) | 40.1 (2.4) |
|          | 4 dS m⁻¹ | CF    | 3.7 (0.2) | 134.1 (10.1) | 85.6 (6.8) | 48.5 (4.1) | 64.3 (1.8) | 33.4 (1.3) | 42.8 (1.6) | 41.9 (1.2) |
|          |           | 1.0 × O₃ | 4.1 (0.3) | 122.3 (20.4) | 83.9 (12.5) | 42.8 (11.3) | 65.7 (0.5) | 29.7 (5.1) | 42.2 (1.3) | 40.5 (1.1) |
|          |           | 1.5 × O₃ | 3.6 (0.2) | 116.8 (9.4) | 67.5 (18.7) | 40.6 (4.4) | 61.4 (4.1) | 28.7 (6.8) | 41.5 (1.6) | 38.9 (3.4) |
|          | 8 dS m⁻¹ | CF    | 3.5 (0.1) | 105.1 (12.1) | 77.4 (8.6) | 43.7 (8.4) | 64.1 (6.1) | 33.4 (1.9) | 40.7 (2.7) | 38.4 (1.4) |
|          |           | 1.0 × O₃ | 3.7 (0.9) | 114.4 (15.1) | 68.5 (11.9) | 45.9 (7.9) | 60.0 (5.2) | 31.2 (4.9) | 40.6 (4.2) | 37.7 (3.9) |
|          |           | 1.5 × O₃ | 3.8 (0.3) | 121.6 (15.3) | 76.2 (5.1) | 47.3 (12.5) | 63.0 (4.5) | 32.3 (2.1) | 40.2 (3.1) | 40.1 (1.9) |
|          | O₃       |       | 0.0406* | 0.0329* | 0.0536* | 0.1613* | 0.9487* | 0.0433* | 0.0021* | 0.0195* |
|          | Salinity |       | 0.0000*** | 0.0023** | 0.0039*** | 0.0035* | 0.0502* | 0.0010* | 0.0000*** | 0.0511* |
|          | O₃ × Salinity |       | 0.1511* | 0.1386* | 0.1482* | 0.4111* | 0.5347* | 0.1534* | 0.2804* | 0.4222* |
|          | Cultivar (CV) |     | 0.0000*** | 0.0000*** | 0.0000*** | 0.1393* | 0.0477* | 0.0000*** | 0.0004* | 0.0000*** |
|          | O₃ × CV |       | 0.3845* | 0.6847* | 0.8849* | 0.6015* | 0.6412* | 0.7854* | 0.0085* | 0.2414* |
|          | Salinity × CV |     | 0.0000*** | 0.0084** | 0.0012** | 0.1265* | 0.0111* | 0.0052* | 0.0457* | 0.5926* |
|          | O₃ × Salinity × CV |     | 0.5020* | 0.9111* | 0.4723* | 0.7262* | 0.1715* | 0.8134* | 0.4080* | 0.2910* |

CF, Charcoal-filtered air.
Each value is the mean of 3 chamber replicates, and the standard deviation is shown in parenthesis.
Result of three-way ANOVA indicates p-value and level of significance; *p < 0.05, **p < 0.01, ***p < 0.001; n.s. = not significant.
Effects of O₃ and Salinity on Bangladeshi Wheat Cultivars

Induced reductions in wheat grain yield (Gerosa et al., 2014; Pleijel and Uddling, 2012; Mills et al., 2010; Emberson et al., 2009; Piikki et al., 2008; Fuhrer et al., 1997). In Table 8, the sensitivity to O₃ of grain yield per plant is compared among the two Bangladeshi wheat cultivars (BAW1059 and Shatabdi) and European and American wheat cultivars, based on the relationship between relative yield and daylight AOT40 of O₃ or 7-h (9:00-16:00) mean O₃ concentration (Mills et al., 2010; Emberson et al., 2009). This comparison reveals that the sensitivity to O₃ of two Bangladeshi wheat cultivars is greater than that of American winter and spring wheat cultivars and is similar to that of European spring wheat cultivars.

Although BAW1059 showed a larger O₃-induced reduction in seed weight than did Shatabdi, both cultivars showed a significant decrease in the number of spikes per plant and number of florets per spike in response to O₃ (Table 6). Therefore, the main cause of the O₃-induced reduction in grain yield per plant is considered to be due to the decreased number of spikes per plant, number of florets per spike, and 1000-seeds weight (Pleijel and Uddling, 2012; Piikki et al., 2008). The O₃-induced yield loss may be attributed to reduced photosynthetic activity and a decreased supply of assimilates to the reproductive parts responsible for seed

Table 7. Effects of O₃ salinity and/or cultivar on net photosynthesis rate (A), stomatal diffusive conductance to H₂O (Gs) and intercellular CO₂ concentration (Ci) of flag leaf of two Bangladeshi wheat cultivars on 36-40 days after treatment (DAT) (BAW1059) and 42-46 DAT (Shatabdi).

| Cultivar | Treatment | A (μmol m⁻² s⁻¹) | Gₛ (mol m⁻² s⁻¹) | Ci (μmol mol⁻¹) |
|----------|-----------|------------------|------------------|-----------------|
|          | Salinity  | Gas              |                  |                 |
|          | 0 dS m⁻¹  | CF               | 24.4 (1.5)       | 0.476 (0.015)   | 286.8 (3.6)    |
|          |           | 1.0 × O₃         | 22.1 (1.6)       | 0.423 (0.059)   | 297.7 (7.3)    |
|          |           | 1.5 × O₃         | 17.7 (1.7)       | 0.311 (0.055)   | 297.2 (9.4)    |
|          | 1.0 × O₃  | CF               | 22.1 (0.8)       | 0.416 (0.016)   | 286.7 (4.5)    |
|          | 1.5 × O₃  | CF               | 21.4 (0.7)       | 0.386 (0.016)   | 285.1 (4.2)    |
|          | 1.0 × O₃  | CF               | 19.2 (1.8)       | 0.323 (0.023)   | 289.2 (8.0)    |
|          | 1.0 × O₃  | CF               | 22.9 (1.2)       | 0.383 (0.036)   | 284.8 (3.9)    |
|          | 1.5 × O₃  | CF               | 22.1 (0.5)       | 0.407 (0.008)   | 286.3 (0.9)    |
|          | 1.5 × O₃  | CF               | 20.7 (1.1)       | 0.383 (0.041)   | 296.9 (12.0)   |
|          | 0 dS m⁻¹  | CF               | 23.5 (1.9)       | 0.459 (0.068)   | 289.4 (11.6)   |
|          |           | 1.0 × O₃         | 22.2 (1.6)       | 0.413 (0.016)   | 286.9 (5.9)    |
|          |           | 1.5 × O₃         | 22.2 (0.9)       | 0.381 (0.054)   | 289.5 (4.8)    |
|          | 1.0 × O₃  | CF               | 21.9 (1.4)       | 0.418 (0.029)   | 290.0 (2.8)    |
|          | 1.5 × O₃  | CF               | 21.9 (1.4)       | 0.418 (0.029)   | 290.0 (2.8)    |
|          | 1.0 × O₃  | CF               | 19.8 (1.8)       | 0.345 (0.058)   | 280.5 (7.8)    |
|          | 1.5 × O₃  | CF               | 18.5 (2.2)       | 0.337 (0.052)   | 289.2 (7.9)    |
|          | 8 dS m⁻¹  | CF               | 18.0 (0.4)       | 0.356 (0.028)   | 292.8 (9.6)    |
|          |           | 1.0 × O₃         | 15.9 (0.9)       | 0.266 (0.058)   | 285.2 (18.7)   |
|          |           | 1.5 × O₃         | 19.0 (4.7)       | 0.328 (0.103)   | 290.7 (7.7)    |
|          | 1.0 × O₃  | CF               | 21.9 (1.4)       | 0.418 (0.029)   | 290.0 (2.8)    |
|          | 1.5 × O₃  | CF               | 21.9 (1.4)       | 0.418 (0.029)   | 290.0 (2.8)    |
|          | 1.0 × O₃  | CF               | 19.8 (1.8)       | 0.345 (0.058)   | 280.5 (7.8)    |
|          | 1.5 × O₃  | CF               | 18.5 (2.2)       | 0.337 (0.052)   | 289.2 (7.9)    |
|          | 8 dS m⁻¹  | CF               | 18.0 (0.4)       | 0.356 (0.028)   | 292.8 (9.6)    |
|          |           | 1.0 × O₃         | 15.9 (0.9)       | 0.266 (0.058)   | 285.2 (18.7)   |
|          |           | 1.5 × O₃         | 19.0 (4.7)       | 0.328 (0.103)   | 290.7 (7.7)    |

ANOVA

|                     | O₃        | Salinity  | O₃ × Salinity | Cultivar (CV) | O₃ × CV | Salinity × CV | O₃ × Salinity × CV |
|---------------------|-----------|-----------|---------------|---------------|---------|--------------|--------------------|
| Result of three-way ANOVA indicates p-value and level of significance: | p < 0.05, **p < 0.01, ***p < 0.001; n.s. = not significant. Measurement condition: atmospheric CO₂ concentration, 390 μmol mol⁻¹; air temperature, 25.0 ± 1.0°C; relative air humidity, 70 ± 5%; photosynthetic photon flux density (PPFD), 1500 μmol m⁻² s⁻¹. |

CF, Charcoal-filtered air. Each value is the mean of 3 chamber replicates, and the standard deviation is shown in parenthesis.

-induced reductions in wheat grain yield (Gerosa et al., 2014; Pleijel and Uddling, 2012; Mills et al., 2010; Emberson et al., 2009; Piikki et al., 2008; Fuhrer et al., 1997). In Table 8, the sensitivity to O₃ of grain yield per plant is compared among the two Bangladeshi wheat cultivars (BAW1059 and Shatabdi) and European and American wheat cultivars, based on the relationship between relative yield and daylight AOT40 of O₃ or 7-h (9:00-16:00) mean O₃ concentration (Mills et al., 2010; Emberson et al., 2009). This comparison reveals that the sensitivity to O₃ of two Bangladeshi wheat cultivars is greater than that of American winter and spring wheat cultivars and is similar to that of European spring wheat cultivars.

Although BAW1059 showed a larger O₃-induced reduction in seed weight than did Shatabdi, both cultivars showed a significant decrease in the number of spikes per plant and number of florets per spike in response to O₃ (Table 6). Therefore, the main cause of the O₃-induced reduction in grain yield per plant is considered to be due to the decreased number of spikes per plant, number of florets per spike, and 1000-seeds weight (Pleijel and Uddling, 2012; Piikki et al., 2008). The O₃-induced yield loss may be attributed to reduced photosynthetic activity and a decreased supply of assimilates to the reproductive parts responsible for seed
growth (Fiscus et al., 2005). Gelang et al. (2001) reported that in cereal crops, grain filling depends on the production of carbohydrates and their translocation from the source organs to the sink (grains). Carbohydrate production can be affected by O₃-induced changes in net photosynthetic rate and photosynthetic activity (Meyer et al., 2000). An O₃-induced reduction in biomass production has been reported for a wide range of crop species (Akhtar et al., 2010a, b; Morgan et al., 2006; Grantz, 2003). Such effects may be the result of O₃-induced reductions in net photosynthesis and/or leaf area, or O₃-induced changes in phloem loading and assimilate partitioning to plant organs (Crous et al., 2006; Dermody et al., 2006; Hassan, 2004; Morgan et al., 2004). In the present study, O₃ significantly reduced the net photosynthetic rate of the flag leaf in both wheat cultivars (Table 7). We propose that the O₃-induced reduction in net photosynthetic rate resulted in lower assimilates, leading to a decrease in whole-plant dry mass and yield per plant in both cultivars (Tables 4 and 5, and Fig. 1).

In the present study, the two wheat cultivars showed greater differences in salinity sensitivity than in O₃-sensitivity, in terms of biomass production and grain yield. In the 8 dS m⁻¹ treatment, the grain yield per plant and the whole-plant dry mass were only slightly affected or not affected in BAW1059, but were decreased by 23.6% and 22.3%, respectively, in Shatabdi (Tables 4 and 5 and Fig. 1). Thus, BAW1059 is relatively more salt-tolerant than is Shatabdi. Our findings are consistent with the salinity-induced reductions in growth and yield reported for Asian, Mediterranean, European, and African wheat cultivars (Ghogdi et al., 2012; Turki et al., 2012; Sadat Noori et al., 2010). In Shatabdi, the negative effects of soil salinity on grain yield per plant might be caused by the salt-induced decreases in the number of spikes per plant, number of florets per spike, and percentage of filled grains, as well as the 1000-seeds weight (Table 6). Also, the salinity-induced reduction in grain yield per plant in Shatabdi might be caused by a salt-induced decrease in photosynthetic capacity (Table 7), leading to less starch synthesis and accumulation in the grain. BAW1059 maintained a higher photosynthetic capacity than did

Table 8. Comparison of O₃-induced yield loss among Bangladeshi, European and American wheat cultivars.

| AOT40 of O₃ (μL L⁻¹ h) | 7-h mean O₃ concentration (nL L⁻¹)ᵃ | Observed yield loss in Bangladeshi wheat cultivars (%) | Predicted yield loss (%) |
|------------------------|--------------------------------------|-----------------------------------------------------|--------------------------|
|                        |                                      | European springᵇ wheat cultivars | American springᶜ wheat cultivars | American winterᵈ wheat cultivars |
| 2.9                    | 28.4                                 | 4.7                                  | 5.0                      | 0.1                      | 0.9                      |
| 8.9                    | 50.1                                 | 11.6                                 | 15.2                    | 1.4                      | 6.6                      |

ᵃ7-h: 9:00-16:00.
ᵇCalculated according to Fuhrer et al. (1997).
ᶜCalculated according to Adams et al. (1989).
ᵈCalculated according to Lesser et al. (1990).
Shatabdi under salt stress (Table 7). Our results are consistent with those of Zheng et al. (2009) and Turki et al. (2012), who reported that there was a larger salt-induced decrease in photosynthetic capacity in salt-sensitive than in salt-tolerant wheat cultivars. Thus, although the 1000-seeds weight of BAW1059 was lower in the highest salinity treatment, the yield per plant remained almost unchanged. This result was closely related to the salinity-induced decrease in the number of unfilled grains and increase in the ratio of the number of filled grains to total grains in BAW1059, which reflected the greater induction of reproductive efficiency by salt-stress (Gerosa et al., 2014). In the present study, soil salinity reduced $G_s$ and $C_i$ in the flag leaves of BAW1059 and Shatabdi (Table 7). A salinity-induced reduction of $G_s$ has also been reported for durum wheat (Katerji et al., 2003) and winter wheat (Huang et al., 1994). In salt-sensitive cultivars, the inhibition of photosynthetic capacity under salinity might be due to stomatal closure, which reduces the availability of internal CO$_2$ (Hernandez et al., 1999).

There was no significant interaction between O$_3$ and soil salinity for growth, yield, and leaf gas exchange rates in the two Bangladeshi wheat cultivars (Fig. 1, Tables 4 and 7). This result indicated that the effects of O$_3$ on growth, yield, and gas exchange rates of the two Bangladeshi wheat cultivars were not ameliorated by soil salinity. Our results are consistent with the findings of Gerosa et al. (2014) for two durum wheat cultivars grown under Mediterranean conditions with elevated salinity and O$_3$ concentrations. Greater sensitivity to O$_3$ has been attributed to various physiological traits, including higher $G_s$ leading to higher O$_3$ flux into the leaves (Gerosa et al., 2014; Zheng et al., 2014; Saitanis et al., 2014; Emberson et al., 2009). In the present study, both Bangladeshi wheat cultivars exposed to elevated O$_3$ showed a significant decrease in $G_s$, but there was no significant difference in $G_s$ between the two cultivars (Table 7). In both cultivars, soil salinity significantly decreased $G_s$, suggesting that soil salinity reduced stomatal O$_3$ flux. However, soil salinity did not significantly affect the O$_3$-sensitivity of the two Bangladeshi wheat cultivars. Therefore, stomatal O$_3$ flux may also have been similar in the two Bangladeshi wheat cultivars with similar-sensitivity to O$_3$. This might be because increased salinity decreased the antioxidant capacity in the leaves, as reported in other studies (Bai et al., 2013; Ozturk et al., 2012; Burcu et al., 2009; Hameed et al., 2008; Shim et al., 2003). Therefore, the interactions among O$_3$, salinity, and cultivar on the growth, yield, and leaf gas exchange rates of wheat are considered to be closely related to the ability of the plants to detoxify active oxygen radicals produced in the leaves in response to O$_3$ and salinity.

5. CONCLUSIONS

Two Bangladeshi wheat cultivars, BAW1059 and Shatabdi, are relatively sensitive to O$_3$ as compared with American winter and spring wheat cultivars. Shatabdi is more sensitive than BAW1059 to soil salinity. There were no significant interactions between O$_3$ and salinity for growth, yield, yield components, and gas exchange parameters, indicating that the effects of O$_3$ were not ameliorated by soil salinity in these two Bangladeshi wheat cultivars, regardless of their salinity tolerance. To mitigate food security challenges in the future, therefore, wheat cultivars with higher tolerance to salinity and O$_3$ should be bred.

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REFERENCES

Adams, R.M., Glyer, J.D., Johnson, S.L., McCarl, B.A. (1989) A reassessment of the economic effects of ozone on United States agriculture. Journal of the Air Pollution Control Association 39, 960-968.

Ahsan, M., Sattar, M.A. (2010) Coastal areas and saline soils of Bangladesh: their extent, salinity status, management practices and future research needs. Proceedings of the Workshop on Soil Fertility, Fertilizer Management and Future Research Strategy.

Ainsworth, E.A. (2008) Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Global Change Biology 14, 1642-1650.

Akhtar, N., Yamaguchi, M., Inada, H., Hoshino, D., Kondo, T., Izuta, T. (2010a) Effects of ozone on growth yield and leaf gas exchange rates of two Bangladeshi cultivars of wheat (Triticum aestivum L.). Environmental Pollution 158, 1763-1767.

Akhtar, N., Yamaguchi, M., Inada, H., Hoshino, D., Kondo, T., Fukami, M., Funada, R., Izuta, T. (2010b) Effects of ozone on growth yield and leaf gas exchange rates of four Bangladeshi cultivars of rice (Oryza sativa L.). Environmental Pollution 158, 2970-2976.

Anonymous (2012) Bangladesh Grain and Feed Annual Report 2012. USDA Foreign Agriculture Service.

Ashmore, M.R. (2005) Assessing the future global impacts of ozone on vegetation. Plant Cell & Environment 28, 949-964.
Ashraf, M. (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnology Advances 27, 84-93.

Bai, J., Liu, J., Zhang, N., Sa, R., Jiang, L. (2013) Effect of salt stress on antioxidant enzymes, soluble sugar and yield of oat. Advance Journal of Food Science and Technology 5, 303-309.

Bangladesh Bureau of Statistics (BBS) (2004) Statistical Year Book of Bangladesh, Statistics Division, Ministry of Planning, Government of People’s Republic of Bangladesh. Bangladesh Bureau of Statistics, Dhaka.

Barma, N.C.D., Malakar, P.K., Pandit, D. (2011) Wheat production and seed storage training manual. Wheat Research Centre, Bangladesh Agricultural Research Institute, Dinajpur, Bangladesh.

Bellido, L.L., Bellido, R.J.L., Roman, R. (2005) Nitrogen efficiency in wheat under rain-fed Mediterranean conditions as affected by split nitrogen application. Field Crops Research 94, 86-97.

Betzelberger, A.M., Gillespie, K.M., McGrath, J.M., Koester, R.P., Nelson, R.L., Ainsworth, E.A. (2010) Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and grain yield of 10 soybean cultivars. Plant Cell & Environment 33, 1569-1581.

Biswas, D.K., Xu, H., Li, Y.G., Sun, J.Z., Wang, X.Z., Han, X.G., Jiang, G.M. (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.

Burcu, S., Askum, H.S., Ismai, T. (2009) An enhancing effect of exogenous mannitol on the antioxidant enzyme activities in roots of wheat under salt stress. Journal of Plant Growth Regulation 28, 12-20.

Calatayud, A., Domingo, J.I., Talon, M., Barreno, E. (2003) Effects of 2-month ozone exposure in spinach leaves on photosynthesis, antioxidant systems and lipid peroxidation. Plant Physiology and Biochemistry 41, 839-845.

Crous, K.Y., Vandermeiren, K., Ceulemans, R. (2006) Physiological responses to cumulative ozone uptake in two white clover (Trifolium repens L. cv. Regal) clones with different ozone sensitivity. Environmental and Experimental Botany 58, 169-179.

Dentener, F., Stevenson, D., Ellingsen, K., van Noije, T., Schultz, M., Aman, A., Atheron, C., Bell, N., Bergmann, D., Bey, I., Bouwman, L., Butler, T., Cofala, J., Collins, B., Drevet, J., Doherty, R., Eickhout, B., Eskes, H., Fiore, A., Gauss, M., Hauglustaine, D., Horowitz, L., Isaksen, I.S.A., Josse, B., Lawrence, M., Krol, M., Lamarque, J.F., Montanaro, V., Muller, J.F., Peuch, V.H., Pitari, G., Pyle, J., Rast, S., Rodriguez, J., Sanderson, M., Savage, N.H., Shindell, D., Strahan, S., Szopa, S., Sudo, K., Van Dingenen, R., Wild, O., Zeng, G. (2006) The global atmospheric environment for the next generation. Environmental Science and Technology 40, 3586-3594.

Dermody, O., Long, S., Delucia, E.H. (2006) How does elevated CO₂ or ozone affect the leaf-area index of soybean when applied independently? New Phytologist 169, 145-155.

El-Hendawy, S.E., Hu, Y., Schmidhalter, U. (2005) Growth, ion content, gas exchange and water relations of wheat genotypes differing in salt tolerance. Australian Journal of Agricultural Research 56, 123-134.

Emerson, L., Büker, P. (2008) Ozone: a threat to food security in South Asia: Policy Brief. Stockholm Environment Institute. Retrieved May 12, 2015, http://www.sei-international.org/mediamanager/documents/Publications/Climate/food_security_ozone_climate_policybrief.pdf

Emerson, L.D., Büker, P., Ashmore, M.R., Mills, G., Jackson, L.S., Agrawal, M., Atikuz-zaman, M.D., Cinerby, S., Engardt, M., Jamir, C., Kobayashi, K., Oanh, N.T.K., Quadir, Q.F., Wahid, A. (2009) A comparison of North American and Asian exposure-response data for ozone effects on crop yields. Atmospheric Environment 43, 1945-1953.

FAO (2007) Extent and Causes of Salt-affected Soils in Participating Countries. AGL: Global Network on Integrated Soil Management for Sustainable Use of Salt Affected Soils.

Fiscus, E.L., Booker, F.L., Burkey, K.O. (2005) Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. Plant Cell & Environment 28, 997-1011.

Foyer, C.H., Noctor, G. (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. The Plant Cell 17, 1866-1875.

Fuhrer, J., Skärby, L., Ashmore, M. (1997) Critical levels for ozone effects on vegetation in Europe. Environmental Pollution 97, 91-106.

Fuhrer, J., Booker, F.L. (2003) Ecological issues related to ozone: agricultural issues. Environment International 29, 141-154.

Fuhrer, J. (2009) Ozone risk for crops and pastures in present and future climates. Naturwissenschaften 96, 173-194.

Gelang, J., Sellden, G., Younis, S., Pleijel, H. (2001) Effects of ozone on biomass, non-structural carbohydrates and nitrogen in spring wheat with artificially manipulated source/sink ratio. Environmental and Experimental Botany 46, 155-169.

Gerosa, G., Marzuoli, R., Finco, A., Monga, R., Fusaro, I., Fuoro, F. (2014) Contrasting effects of water salinity and ozone concentration on two cultivars of durum wheat (Triticum durum Desf.) in Mediterranean conditions. Environmental Pollution 193, 13-21.

Grantz, D.A. (2003) Ozone impacts on cotton: towards an integrated mechanism. Environmental Pollution 126, 331-344.

Ghogdi, E.A., Darbandi, A.I., Borzouei, A. (2012) Effects of salinity on some physiological traits in wheat (Triticum aestivum L.) cultivars. Indian Journal of Science and Technology 5, 1901-1906.
Hameed, A., Naseer, S., Iqbal, T., Syed, H., Haq, M.A. (2008) Effects of NaCl salinity on seedling senescence, catalase and protease activities in two wheat genotypes differing in salt tolerance. Pakistan Journal of Botany 40, 1043-1051.

Hassan, I.H. (2004) Interactive effects of salinity and ozone pollution on photosynthesis, stomatal conductance, growth, and assimilate partitioning of wheat (Triticum aestivum L.). Photosynthetica 42, 111-116.

Hernandez, J., Campillo, A., Jimenez, A., Alarcon, J.J., Selvilla, F. (1999) Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Physiologist 141, 241-251.

Huang, L., Murray, F., Yang, X. (1994) Interaction between mild NaCl salinity and sublethal SO2 pollution on wheat Triticum aestivum cultivar ‘Wilgoye’ (ciano / gallo). I. Response of stomatal conductance, photosynthesis, growth and assimilate partitioning. Agricultural, Ecosystem and Environment 48, 163-178.

IPCC (2007) The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Katerji, N., Van Hoorn, J., Hamdy, A., Mastorilli, M. (2003) Salinity effect on crop development and yield, analysis of salt tolerance according to several classification method. Agricultural Water Management 62, 37-66.

Kinose, Y., Azuchi, F., Uehara, Y., Kanomata, T., Kobayashi, A., Yamaguchi, M., Izuta, T. (2014) Modeling of stomatal conductance to estimate stomatal ozone uptake by Fagus crenata, Quercus serrata, Quercus mongolica var. crispa and Betula platyphylla. Environmental Pollution 194, 235-245.

Kostiainen, K., Jalkanen, H., Kaakinen, S., Saranpaa, P., Vapaavouri, E. (2006) Wood properties of two silver birch clones exposed to elevated CO2 and O3. Global Change Biology 12, 1230-1240.

Lesser, V.M., Rawlings, J.O., Spruill, S.E., Somerville, M.C. (1990) Ozone effects on agricultural crops: statistical methodologies and estimated dose-response relationships. Crop Science 30, 148-155.

Ma, Q.Q., Wang, W., Li, Y.H. (2006) Alleviation of photo inhibition in drought-stressed wheat (Triticum aestivum L.) by foliar-applied glycinebetaine. Journal of Plant Physiology 163, 165-175.

Maggio, A., Chiara, F.Q., Cefariello, R., Fagnano, M. (2009) Responses to ozone pollution of alfalfa exposed to increasing salinity levels. Environmental Pollution 157, 1445-1455.

Massman, W.J., Musselman, R.C., Lefohn, A.S. (2000) A conceptual ozone dose-response model to develop a standard to protect vegetation. Atmospheric Environment 34, 745-759.

Meyer, U., Kollner, B., Willenbrink, J., Krause, G.H.M. (2000) Effects of different ozone exposure regimes on photosynthesis, assimilates and thousand grain weight in spring wheat. Agricultural, Ecosystem and Environment 78, 49-55.

Miah, M.M.U., Uddin, M.J., Islam, M.F., Razia, M.S. (2005) Fertilizer Recommendation Guide. BARC Soils Publication. No. 45. Bangladesh Agricultural Research Council, Dhaka, Bangladesh.

Mills, G., Pleijel, H., Büköp, B., Baum, S., Emberson, L., Harmens, H., Hayes, F., Simpson, D., Grünhage, L., Karlsson, P.E., Danielsson, H., Bermejo, V., Fernandez, I.G. (2010) Mapping critical levels for vegetation. Revision undertaken in summer, 2010, to include new flux-based critical levels and response functions for ozone. In Manual on methodologies and criteria for modelling and mapping critical loads & levels and air pollution effects, risks and trends (Spranger, T., Lorenz, U. and Gregor, H.D. Eds), Federal Environmental Agency (Umweltbundesamt), Berlin, pp. page III-0-112.

Mills, G., Buse, A., Gimeno, B., Bermejo, V., Holland, M., Emberson, L., Pleijel, H. (2007) A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. Atmospheric Environment 41, 2630-2643.

Mishra, A.K., Rai, R., Agrawal, S.B. (2013) Differential response of dwarf and tall tropical wheat cultivars to elevated ozone with and without carbon dioxide enrichment: Growth, yield and grain quality. Field Crops Research 145, 21-32.

Morgan, P., Ainsworth, E., Long, S. (2003) How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. Plant Cell & Environment 26, 1317-1328.

Morgan, P.B., Bernacchi, C.J., Ort, D.R., Long, S.P. (2004) An in vivo analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. Plant Physiology 135, 2348-2357.

Morgan, P.B., Mies, T.A., Bollorea, G.A., Nelson, R.L., Long, S.P. (2006) Season-long elevation of ozone concentration to anticipated 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. New Phytologist 170, 333-343.

Ozturk, L., Demir, Y., Uluakura, A., Karatas, I., Kurung, A., Dezdemir, O. (2012) Effect of long-term salt stress on antioxidant system, chlorophyll and proline contents in pea leaves. Romanian Biotechnology Letters 17, 7227-7236.

Piikki, K., De Temmerman, L., Ojanpera, K., Danielsson, H., Pleijel, H. (2008) The grain quality of spring wheat (Triticum aestivum L.) in relation to elevated ozone uptake and carbon dioxide exposure. European Journal of Agronomy 28, 245-254.

Pleijel, H., Uddling, J. (2012) Yield vs. quality trade-offs for wheat in response to carbon dioxide and ozone. Global Change Biology 18, 596-605.

Qadir, M., Ghaffoor, A., Murtaza, G. (2000) Amelioration strategies for saline soils: a review. Land Degradation and Development 11, 501-521.

Rai, R., Agrawal, M., Agrawal, S.B. (2007) Assessment
of yield losses in tropical wheat using open top chambers. Atmospheric Environment 41, 9543-9554.

Rai, R., Agrawal, M., Agrawal, S.B. (2011) Effects of ambient O3 on wheat during reproductive development: gas exchange, photosynthetic pigments, chlorophyll fluorescence and carbohydrates. Photosynthetica 49, 285-294.

Sadat Noori, S.A., Khalaj, H., Labbafi, M.R. (2010) Effect of different salinity levels on morpho-physiological characters of 8 wheat genotypes (Triticum aestivum L.). Iranian Journal of Plant Physiology 1, 108-117.

Saitanis, C.J., Bari, S.M., Burkey, K.O., Stamatakopoulos, D., Agathokleous, E. (2014) Screening of Bangladeshi winter wheat (Triticum aestivum L.) cultivars for sensitivity to ozone. Environmental Science and Pollution Research 21, 13560-13571.

Sarkar, A., Agrawal, S.B. (2010) Elevated ozone and two modern wheat cultivars: an assessment of dose dependent sensitivity with respect to growth, reproductive and yield parameters. Environmental and Experimental Botany 69, 328-337.

Sarkar, A., Randeep, R., Agrawal, S.B., Shibato, J., Ogawa, Y., Yoshida, Y., Agrawal, G.K., Agrawal, M. (2010) Investigating the impact of elevated levels of ozone on tropical wheat using integrated phenotypical, physiological, biochemical, and proteomics approaches. Journal of Proteome Research 9, 4565-4584.

Shim, I.S., Momose, Y., Yamamoto, A., Kim, D.W., Usui, K. (2003) Inhibition of catalase activity by oxidative stress and its relationship to Salicylic acid accumulation in plants. Plant Growth Regulator 8, 285-292.

Skotnica, J., Gilbert, M., Weingart, I., Wilhelm, C. (2005) The mechanism of the ozone-induced changes in thermoluminescence glow curves of barley leaves. Photosynthetica 43, 425-434.

Titumir, R.M., Basak, J.K. (2012) Effect of climate change on crop production and climate adaptive techniques for agriculture in Bangladesh. Social Science Review (The Dhaka University Studies, Part-D) 29, 215-232.

Tiwari, S., Agrawal, M., Marshall, F.M. (2010) Seasonal variations in adaptation strategies of Beta vulgaris L. plants in response to ambient air pollution: biomass allocation, yield and nutritional quality. Journal of Tropical Ecology 51, 353-363.

Turki, N., Harrabi, M., Okuno, K. (2012) Effect of salinity on grain yield and quality of wheat and genetic relationships among durum and common wheat. Journal of Arid land studies 22, 311-314.

Van Dingenen, R., Dentener, F.J., Frank, R., Maurten, C.K., Emberson, L., Cofala, J. (2009) The global impact of ozone on agricultural crop yields under current and future air quality legislation. Atmospheric Environment 43, 604-618.

Warren, C.R., Dreyer, E. (2006) Temperature response of photosynthesis and internal conductance to CO2: results from two independent approaches, Journal of Experimental Botany 57, 3057-3067.

Welfare, K., Flowers, T.J., Taylor, G., Yeo, A.R. (1996) Additive and antagonistic effects of ozone and salinity on the growth, ion contents and gas exchange of five varieties of rice (Oryza sativa L.). Environmental Pollution 92, 257-266.

Welfare, K., Yeo, A.R., Flowers, T.J. (2002) Effects of salinity and ozone, individually and in combination, on the growth and ion contents of two chickpea (Cicer arietinum L.) varieties. Environmental Pollution 120, 397-403.

Wheat Atlas CIMMYT (2014) Wheat Varieties: Bangladesh. CIMMYT, Mexico.

Zheng, Y.H., Xu, X.B., Wang, M.Y., Zheng, X.H., Li, Z.J., Jiang, G.M. (2009) Response of salt-tolerant and intolerant wheat genotypes to sodium chloride, photosynthesis, antioxidants activities and yield, Photosynthetica 47, 87-94.

Zheng, Y.H., Li, X., Li, Y.G., Miao, B.H., Xu, H., Simmons, M., Yang, X.H. (2012) Contrasting responses of salinity-stressed salt-tolerant and intolerant winter wheat (Triticum aestivum L.) cultivars to ozone pollution. Plant Physiology and Biochemistry 52, 169-178.

Zheng, Y., Cheng, D., Simmons, M. (2014) Ozone pollution effects on gas exchange, growth and biomass yield of salinity treated winter wheat cultivars. Science of The Total Environment 499, 18-26.

Zhu, J.-K. (2001) Plant salt tolerance. Trends in Plant Science 6, 66-71.

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