Research article

Contrasting responses of salinity-stressed salt-tolerant and intolerant winter wheat (*Triticum aestivum* L.) cultivars to ozone pollution

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A B S T R A C T

Contrasting winter wheat cultivars, salt-tolerant DK961 and intolerant JN17, which sown in no salinity (−S) and salinity (+5) boxes were exposed to charcoal filtered air (CF) and elevated O3 (+O3) in open top chambers (OTCs) for 30 days. In −S DK961 and JN17 plants, +O3 DK961 and JN17 plants had significantly lower light-saturated net photosynthetic rates (*A* sat, 26% and 24%), stomatal conductance (*g* s, 20% and 32%) and chlorophyll contents (10% and 21%), while O3 considerably increased foliar electrolyte leakage (13% and 39%), malondialdehyde content (9% and 23%), POD activity and ABA content. However, responses of these parameters to O3 were significant in DK961 but not in JN17 in +S treatment. Correlation coefficient of DK961 reached significance level of 0.01, but it was not significant in JN17 under interaction of O3 and salinity. O3-induced reductions were larger in shoot than in root in both cultivars. Results indicate that the salt-tolerant cultivar sustained less damage from salinity than did the intolerant cultivar but was severely injured by O3 under +S condition. Therefore, selecting for greater salt tolerance may not lead to the expected gains in yield in areas of moderate (100 mM) salinity when O3 is present in high concentrations. In contrast, salinity-induced stomatal closure effectively reduced sensitivity to O3 in the salt-intolerant cultivar. Hence we suggest salt-tolerant winter wheat cultivars might be well adapted to areas of high (>100 mM) salinity and O3 stress, while intolerant cultivars might be adaptable to areas of mild/moderate salinity but high O3 pollution.

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1. Introduction

Ozone (*O3*) pollution has been increasing remarkably over the past several decades [1,2]. Currently, *O3* concentrations in many countries have reached levels that have reduced crop productivity [3,4], and some predict that crop yield losses caused by *O3* pollution in East Asia will increase substantially by 2020 [5,6].

Salinity is a common stress that severely limits crop productivity [7–9]. For example, in China 10% of arable lands are exposed to salinity stress, and these areas are expanding due to poor irrigation practices [10]. Numerous studies have investigated how to alleviate salinity damage in winter wheat, but planting salt-tolerant cultivars is considered the best method to obtain high grain yields in saline croplands [11,12]. Welfare et al. [13,14] reported additive effects of salinity and *O3* on gas exchange in rice and chickpea plants. However, little is known about the responses of salt-tolerant winter wheat cultivars to *O3* under saline conditions, including whether salt-tolerant cultivars are also tolerant of *O3* and whether responses to *O3* differ between salt-tolerant and intolerant cultivars. Given the steady increase in *O3* pollution and large areas of arable lands affected by salinity, studies on the physiological responses of various salinity-treated winter wheat cultivars to *O3* are urgently needed.

Stomatal closure is one plant response that limits damage under stress conditions [15,16]. Regardless of the exact...
physiological mechanism and species in which O₃ damage may occur, O₃ likely enters into the apoplastic space, crosses cellular membranes and reaches the cellular compartments that affect the photosynthetic apparatus and ultimately the yield of photosynthesis [17]. Maggio et al. [18] reported that salinity reduced both stomatal conductance and O₃ uptake thus linearly reducing O₃ effects on yield. Therefore, salinity-induced stomatal closure might reduce the O₃ flux in intercellular spaces and protect wheat from O₃ damage.

Environmental stresses mainly induce oxidative stress in plant tissues [19]. Oxidative stress reduces membrane permeability and causes lipid peroxidation [20,21]. At the chloroplast level, superoxide accumulation may become more sensitive to intense light, inducing photoinhibition and photodestruction [22,23]. To cope with oxidative stress, plants synthesize antioxidants to metabolize reactive oxygen species (ROS) before cellular damage occurs in the chloroplast [24]. Therefore, activities of antioxidant enzymes may indicate tolerances to stress conditions. In addition, the abscisic acid (ABA) content of plant tissues is used as a key indicator of plant stress [25].

The objectives of this study were to investigate whether salinity stress can reduce ozone damage in winter wheat and to determine whether different responses existed between salt-tolerant and intolerant cultivars. Thus, specific soil conditions and plant varieties were considered in order to develop reliable prediction models of O₃ damage.

2. Materials and methods

2.1. Plant culture and salinity treatments

The experiment was carried out in four open top chambers (OTC, 1.2 m diameter, 1.6 m height), which were installed in a temperature-controlled double-glazed greenhouse. Two contrasting winter wheat cultivars, salt-tolerant DK961 and intolerant JN17, were used in this experiment. Seeds of individual cultivar were sown in 24 plastic boxes (26 × 16 × 10 cm), which were filled with 3 kg sand that had been washed and sterilized. Thirty seeds were sown in each box, and six boxes of each cultivar were placed in an OTC, i.e., each OTC held twelve boxes. Plants irrigated with full strength Hoagland nutrient solution (−S, no NaCl) were used as the control, and experimental plants were irrigated with NaCl modified Hoagland solution (+S, 100 mM NaCl). Water lost by evapotranspiration was replenished daily for the duration of the experiment. Plants were thinned to 20 individuals per box 10 d after sowing. The maximum photosynthetic photon flux density (PPFD) in chambers was 1600 μmol m⁻² s⁻¹ at canopy height during the 14-h photo-period. The temperature in the OTCs fluctuated from 17 °C (night) to 36 °C (day), and the relative humidity (RH) was 75–86% during the experiment.

2.2. O₃ fumigation and treatments

The OTCs were ventilated continuously (24 h day⁻¹) with charcoal filtered air (CF, <5 ppb O₃). The average air velocity in the chambers corresponded to approximately one complete air change per minute. O₃ was generated by electrically discharging ambient air using an O₃ generator (JQ-6A, Telihuo Co., Beijing, China). The air stream was bubbled through distilled water before going to the chambers to remove harmful compounds other than O₃ [26]. Produced O₃ was dispensed randomly into the CF air stream entering two of the four chambers for 8 h day⁻¹ (09:00–17:00) for 30 days from sowing to harvest. O₃ concentrations in the OTCs were continuously monitored by an O₃ analyzer (APOA-360, Horiba, Japan) to ensure a concentration of 80 ± 5 ppb. To minimize effects of chamber and environmental heterogeneity on plant responses, plants and O₃ treatments were switched among chambers every other day. Locations of plants were also randomized within each chamber.

Fig. 1. Photographs from a scanning electron microscope (operated at 12 kV) of leaf stomata of salt-tolerant DK961 and intolerant JN17. A, B, C and D are stomata of DK961, and E, F, G and H are stomata of JN17 under CF−S, CF+S, +O₃−S and +O₃+S conditions, respectively. CF: charcoal filtered air; +O₃: CF+O₃ (80 ± 5 ppb); −S: no salinity; +S: salinity-treated.
2.3. Stomata scanning and gas exchange measurements

Sections of lamina (about 9 mm²) taken from the middle of the most recent fully-expanded leaves of DK961 and JN17 plants were excised after completion of the experiment. A total of 8 lamina sections were selected from both cultivars under salinity and O₃ treatments. Excised lamina sections were fixed in a solution of buffered glutaraldehyde (2.5%) for 24 h. Thereafter, the laminae were dehydrated in a series of ethanol-water solutions (30, 50, 60, 70, 80, 90 and 100% ethanol) and incubated in an ethanol–isoamyl acetate mixture for 1 h. The laminae were further dried then coated with gold. The mounted specimens were examined and photographed with a scanning electron microscope operated at 12 kV (Hitachi S-570, Hitachi, Japan).

Gas exchange was measured on the third (the most recent fully-expanded) leaves using a portable Gas Exchange Fluorescence System (SGF-3000, Heinz Walz, Germany) 30 d after O₃ fumigation. Relative humidity was maintained at 70%, and leaf temperature was set at 25 °C in the leaf chamber. The flow rate was set at 600 μmol s⁻¹, and CO₂ concentration in the leaf chamber was maintained at 400 μmol mol⁻¹. The leaf was illuminated with 1500 μmol m⁻² s⁻¹ PPFD from an internal light source in the leaf chamber. After conditions for gas exchange measurements were stable, light-saturation net photosynthetic rate ($A_{sat}$), stomatal conductance ($g_s$) and transpiration rate ($E$) were simultaneously recorded.

2.4. Shoot and root biomass measurements and leaf sampling

Plants were harvested after 30 d of treatments. Twenty plants of each cultivar were cleaned and separated into shoots and roots and oven-dried at 75 °C to constant weight. Biomass of shoots and roots was then recorded.

For each treatment, the eighty third plants leaves exhibiting no visible symptoms of salinity/ozone damage were harvested, washed with distilled water, dried by paper tissues and immediately frozen in liquid nitrogen and transferred to an ultra-freezer at −80 °C until the time of assay.

2.5. Chlorophyll content

Chlorophyll content was measured following the method described by Hiscox and Isrealstam [27]. Frozen leaf samples (0.2 g) were crushed into fine homogenate and extracted in 95% ethanol. The absorbance of the extract was recorded at 663 and 645 nm, and chlorophyll content was calculated using the following formula:

$$\text{Chlorophyll content (mg g}^{-1} \text{ FW}) = 8.02 \times \text{OD}_{663} + 20.20 \times \text{OD}_{645}$$

Where $\text{OD}_{663}$ and $\text{OD}_{645}$ are absorbances at 663 and 645 nm, respectively.
2.6. Membrane permeability and lipid peroxidation measurements

Leaf membrane permeability was measured by electrolyte leakage (EL) following the method described by Dionisio-Sese and Tobita [28]. Ten 4-cm pieces from the middle section of leaves were placed in a tube containing 10 mL deionized water and then incubated in a water bath at 25 ± 2°C for 2 h. The initial electrical conductivity of the medium (EC1) was analyzed using an electrical conductivity analyzer (KL-220, Xingzhou Company Ltd, China). The samples were boiled at 100°C for 30 min to release electrolytes and then cooled to 25°C at which time final electrical conductivity (EC2) was measured. EL was calculated using the formula:

\[ EL(\%) = \frac{EC1 - EC2}{EC1} \times 100 \]

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to Kramer et al. [29]. Frozen samples (0.5 g) of shoots mixed with 5 mL phosphate buffer (pH 7.8) were crushed into fine powder in a mortar and pestle under liquid nitrogen. The homogenate was centrifuged at 4000 rpm for 20 min at 4°C with the supernatant being used for MDA determination. A mixture of 1 mL extracts (MDA) and 2 mL 0.6% thiobarbituric acid (TBA) was produced, boiled for 15 min, cooled and centrifuged for 10 min (4000 rpm). Absorbances of supernatant were recorded at 600, 532 and 450 nm, and MDA content was calculated with the following formula:

\[ MDA(\text{mmol g}^{-1} \text{FW}) = (6.45 \times (D_{532} - D_{600})) - 0.56D_{450} \times V/W \]

where D_{532}, D_{600} and D_{450} are the absorbances at 600, 532 and 450 nm, respectively; V is the volume of extraction, and W is the fresh weight of the sample.

2.7. Antioxidant enzyme activity and ABA measurement

Frozen leaf samples (about 0.5 g) were homogenized in a pre-chilled mortar and pestle placed in ice with 5 mL 0.05 M potassium phosphate buffer (pH 7.8) containing 8.5% (v/v) 0.2 M KH₂PO₄ and 91.5% 0.2 M K₂HPO₄. The homogenate was centrifuged at 4000 rpm for 20 min. The supernatant was further used to measure guaiacol peroxidase (POD) activity. The assay mixture contained 50 mL 0.1 M sodium phosphate (pH 6.0), 28 μL guaiacol and 19 μL 30% H₂O₂. The absorbance was continuously recorded five times at 470 nm at 30-s intervals. Variation of absorbance per minute per gram fresh weight (ΔA₄⁷₀ min⁻¹ g⁻¹ FW) represented POD activity. All spectrophotometric analyses were performed at 0–4°C with a UV/visible light spectrophotometer (UV-365, Shimadzu, Japan).

Frozen leaf samples (about 0.5 g) were pulverized in liquid nitrogen and extracted in 80% methanol containing 1 mmol L⁻¹ of butylated hydroxytoluene (BHT). After 4 h of extraction, the homogenate was centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was used to determine ABA content with an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody for ABA (AFRCMAC 252) according to Asch [30].

2.8. Regression and correlation analysis

Statistical analyses of data were performed using analysis of variance (ANOVA) in the general linear model procedure SPSS package (Ver. 11.5, SPSS, Chicago, IL, USA). The effects of O₃, salinity and their interactions were analyzed on the measured variables in salt-tolerant and intolerant wheat cultivars, respectively. Regression equations and correlation coefficients were determined by “Fit Curves” in SigmaPlot 10.0. Differences between treatments were considered significant if \( p \leq 0.05 \).

3. Results

3.1. Leaf stomata scanning observation

Without salinity (−S), the stomatal length of DK961 (Fig. 1A, B) was decreased by 3% by O₃, while stomatal length of JN17 (Fig. 1E, F) declined by 12%. Salinity-caused significant stomatal shrinkage by 19% in JN17 (Fig. 1C, D) but only by 3% in DK961 (Fig. 1G, H). In the saline (+S) treatment, reductions of stomatal length caused by O₃ were 18% in DK961 but no reductions were noted in JN17.

3.2. Gas exchange, chlorophyll content and biomass yield

Ozone-induced reductions of light-saturated net photosynthetic rate \( (A_{\text{sat}}) \) (Fig. 2A, B) and stomatal conductance \( (g_{s}) \) (Fig. 2C, D)
were significant in both cultivars in the −S treatment. Salinity induced considerable decreases in both A_sat and g_s in JN17 but not in DK961. In the +S treatment, both g_s and A_sat declined significantly in DK961 in response to O3, while no significant changes were measured in JN17. Transpiration rate (E) was similar to A_sat and g_s results in both −S and +S treatments.

For chlorophyll (Chl) content, significant interactions also occurred between salinity and O3 exposure. Considerable reductions of Chl content were noted in both cultivars in response to O3 in the −S treatment, while Chl content decreased significantly in DK961 (Fig. 3A) but not in JN17 (Fig. 3B) in the +S treatment. No considerable salinity-induced reduction of Chl content was noted in DK961 in CF treatment, while drastic reduction was measured in +O3 treatment.

Shoot (Fig. 4A, B) and root (Fig. 4C, D) biomass yields were significantly reduced by O3 in both cultivars (14% and 21%, respectively, in DK961: 27% and 33%, respectively, in JN17) in the −S treatment. In the +S treatment, O3 induced considerable reductions in shoot and root biomass yields of DK961 but not in JN17. Salinity-induced reductions were larger in roots than in shoots while O3-induced reductions were larger in shoots than in roots in both cultivars. The shoot/root ratio was larger in CF plants than in +O3 plants in both −S and +S treatments (Fig. 4E, F). These adverse effects were more significant in DK961 than in JN17 in the +S treatment.

![Figure 4](image-url)

**Fig. 4.** Responses of shoots, roots and shoot/root ratio of salt-tolerant DK961 and intolerant JN17 to O3 pollution under −S and +S conditions. −S: no salinity; +S: 100 mM NaCl; CF: charcoal filtered air and +O3: CF+O3 (80 ± 5 ppb). Vertical bars are means ± SE (n = 6). Within each treatment, significant differences between CF and +O3 are marked by *while −S and +S treatments are marked by letters at p ≤ 0.05.
3.3. Membrane permeability and lipid peroxidation

Compared with CF plants, O3 significantly increased electrolyte leakage (EL) and malondialdehyde (MDA) contents of both cultivars in the −S treatment (12% and 8%, respectively for DK961 (Fig. 5A, C) and 48% and 27%, respectively for JN17 (Fig. 5B, D)). Salinity dampened increases of EL and MDA caused by O3 in JN17 (only 8% and 4%, respectively), while both parameters increased greatly by O3 in DK961 (18% and 13%, respectively) in the +S treatment. Salinity-induced increases of EL and MDA were larger in DK961 than in JN17. However, O3-induced increases of EL and MDA were smaller in DK961 than in JN17 in the +S treatment.

3.4. Antioxidant enzyme activity and ABA content

Higher original POD activity and lower ABA content were measured in DK961 than in JN17 plants. In the −S plants of both cultivars, O3 significantly elevated POD activities. Salinity increased POD activity in both cultivars, but the extent of increase was smaller in DK961 (Fig. 6A) than in JN17 (Fig. 6B). In the +S plants, O3 induced a significant increase of POD activity in DK961 but not in JN17.

In the −S treatment, ABA content increased significantly in response to O3 in both cultivars, with the extent being smaller in DK961 (Fig. 6C) than in JN17 (Fig. 6D). No significant differences were noted in ABA content of DK961 in response to salinity but considerably increased in JN17. In the +S treatment, ABA content increased greatly by O3 in DK961, but there was no significant change in JN17.

3.5. Relationships between $g_s$ and $A_{sat}$

Positive correlations between $g_s$ and $A_{sat}$ of DK961 (Fig. 7A, B) were noted in all treatments of salinity and O3, with the slopes being greater in O3 than in CF plants in both no salinity and salinity treatments. The correlation coefficient $r_1$, $r_2$ and $r_5$ reached a significance level of 0.001, and $r_6$ reached a level of 0.01. In JN17 (Fig. 7C, D), positive correlations between $g_s$ and $A_{sat}$ also existed in salinity and O3 treatments, however, the slopes were smaller in O3 than in CF plants in both no salinity and salinity treatments. $r_3$ was significant at the 0.01 level, $r_4$ and $r_7$ reached significance level of 0.05, and $r_8$ was not significant.

3.6. Effects of O3, salinity and their interaction

For the salt-tolerant DK961, the effects of salinity on $A_{sat}$, $g_s$, Chl, shoot, EL, MDA, POD and ABA did not reach the significant level ($p \geq 0.05$), but significant effect was noted on root (Table 1). The effects of O3 on $g_s$ and EL reached the significant level, while no significances were noted in $A_{sat}$, Chl, shoot, root, MDA, POD and ABA. The effects of the O3 × salinity on those parameters were all significant except root. For the salt-intolerant JN17, the effects of salinity on those parameters all reached significant level.
Significant effects of O₃ were noted on those parameters except root and ABA. However, the effects of the O₃ × salinity on those parameters were not significant except shoot and ABA.

4. Discussion

4.1. Salinity-induced stomatal closure reduced O₃ flux

Ozone mainly enters plant tissues and induces damage through stomata, and stomatal control of O₃ uptake plays a key role in determining O₃ sensitivity of crops [31]. Salinity-induced stomatal closure increases the resistance of crops to O₃ pollution by reducing O₃ uptake [32]. In this study, O₃ or salinity alone did not significantly reduce the stomatal length of the salt-tolerant cultivar, while considerable reductions were measured in the salt-intolerant cultivar (Fig. 1). However, under saline condition, significant reductions of stomatal length caused by O₃ were noted in the salt-tolerant cultivar but not in the salt-intolerant cultivar. This might be because the salt-tolerant cultivar can maintain high stomatal conductance ($g_s$) under mild to moderate salinity stresses, while the $g_s$ of salt-intolerant cultivars significantly declines [33,34]. Thus, more O₃ might be able to enter plant tissues of the salt-tolerant cultivar and induce significant injuries in the $+S$ treatment. In contrast, larger salinity-induced reductions of $g_s$ in the salt-intolerant cultivar might reduce O₃ uptake more significantly in the salt-tolerant cultivar in the $+S$ treatment. This is in agreement with the previous report on alfalfa that salinity could reduce both stomatal conductance and O₃ uptake Maggio et al. [18].

4.2. Gas exchange, chlorophyll content and biomass yield

Stress-induced (salinity, ozone etc.) reductions of $g_s$ may limit plant photosynthetic capacity [35]. In this study, greater reductions caused by O₃ in $A_{sat}$ and $g_s$ were noted in the salt-tolerant cultivar than in the intolerant cultivar under saline condition (Fig. 2). Therefore, the salinity-treated, salt-tolerant cultivar might be more severely injured by O₃ than the salinity-treated, salt-intolerant cultivar under $+S$ condition, in consequence O₃ might linearly reduce the grain yield of salt-tolerant wheat under the present salinity treatment. Therefore, additive effect of salinity and O₃ existing in salt-tolerant cultivar was in agreement with Welfare et al. [13,14] who reported on rice and chickpea plants, but antagonistic effects occurred in salt-intolerant cultivar.

Ozone-induced oxidative stress not only decreases Chl synthesis but also decomposes the original Chl in plant tissues [36,37]. In this study, O₃-induced reduction of Chl content was greater in the salt-tolerant cultivar than in the intolerant cultivar in the $+S$ treatment (Fig. 3). This is likely because the salt-tolerant cultivar maintained larger stomata in the $+S$ treatment leading to more O₃ uptake. In contrast, considerable stomatal closure induced by salinity in the salt-intolerant cultivar might significantly reduce O₃ flux and weaken oxidative stress resulting in little relative loss of Chl content under saline condition.

Fig. 6. Antioxidant enzyme (POD) activity and abscisic acid (ABA) content in leaves of salt-tolerant DK961 (A, C) and intolerant JN17 (B, D) in response to O₃ under $-S$ and $+S$ conditions. CF: charcoal filtered air; $+O_3$: CF+$O_3$ (80 ± 5 ppb); $-S$: no salinity; $+S$: salinity-treated. Vertical bars are means ± SE. Within each treatment, significant differences between CF and $+O_3$ are marked by * while $-S$ and $+S$ treatments are marked by letters at $p \leq 0.05$. 
Salinity induces injuries to plant growth by causing physiological water stress [38], so it mainly damages plant roots (Fig. 4). In contrast, O₃ mainly enters plant tissues through leaf stomata and induces peroxidation [36], so its major injury performs in shoots.

4.3. Cell membrane permeability and lipid peroxidation

Electrolyte leakage (EL) and malondialdehyde (MDA) contents could externally indicate cell membrane permeability and lipid peroxidation [37–39]. Significant increases in EL and MDA contents of both cultivars might show that the integrity of cell membranes was seriously damaged by O₃ in –S plants. However, negative effects of O₃ were dampened in the +S treatment, especially in the salt-intolerant cultivar (Fig. 5). This might because higher O₃ uptake due to greater gs resulted in more serious O₃-induced oxidative damage to cell membranes in the salt-tolerant cultivar than in the intolerant cultivar under saline condition. Salinity-caused significant stomatal closure in the salt-intolerant cultivar might have effectively reduced O₃ uptake, resulting in less O₃-induced reductions in both EL and MDA content under salinity stress.

4.4. Role of salinity in stimulating synthesis of antioxidant enzyme and O₃ detoxication

Adaptation of plants to stress conditions has been correlated with increased levels of antioxidant compounds and enzymes in

![Fig. 7. Correlations between light-saturated net photosynthetic rate (A_sat) and stomatal conductance (gs) in salt-tolerant DK961 and salt-sensitive JN17 under different O₃ and salinity treatments. Results for DK961 in –S and +S treatments are shown in figures A and C, respectively, and those for JN17 are shown in figures B and D, respectively. CF: charcoal filtered air; +O₃: CF + O₃ (80 ± 5 ppb); –S: no salinity; +S: salinity-treated. Regression equations and coefficients were determined by “Fit Curves” in SigmaPlot 10.0 (SPSS Inc. Chicago, Illinois, USA). *: **: *** Express significance at p = 0.05, 0.01 and 0.001, respectively.]

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Table 1
Effects (p-values) of ozone, salinity and O₃ × salinity on plant physiological and biochemical parameters of salt-tolerant and intolerant wheat cultivars analyzed by ANOVAs. A_sat, light-saturated photosynthetic rate; gs, stomatal conductance; Chl, chlorophyll; EL, electrolyte leakage. The bold numerals highlight the significance at p ≤ 0.05.
plant tissues involved in detoxification reactions of reactive oxygen species (ROS) [40,41]. Salinity-elevated antioxidant enzymes activities may enhance the ability of plants scavenging O$_3$-induced ROS [42,43]. Ozone alone can also stimulate antioxidant enzymes activities in most crops [39]. In the present study, POD activity of the salt-tolerant cultivar was significantly enhanced by O$_3$ while no considerable increase was measured in the salt-intolerant cultivar under saline condition (Fig. 6). This might indicate that greater salinity-induced increase in POD activity of the salt-intolerant cultivar might have enhanced the ability of scavenging O$_3$-induced ROS in the +S treatment. The presence of ABA, as an indicator of environmental stress, also indicated that the salt-tolerant cultivar was injured more severely than the salt-intolerant cultivar in the +S treatment under these experimental conditions.

4.5. Correlations between $g_s$ and $A_{sat}$

Positive correlations exist between $g_s$ and $A_{sat}$ in most crops [44,45]. In this study, greater slope of correlation line between $g_s$ and $A_{sat}$ was noted in the salt-tolerant cultivar (correlation coefficient $r_s = 0.84$ $p < 0.01$) than that in the intolerant cultivar ($r_s = 0.11$ $p > 0.05$) in the +O$_3$ +S treatment (Fig. 7). This result may also have been caused by high O$_3$ flux through greater $g_s$ in the salt-tolerant cultivar in the +S treatment, which induced significant reduction in $A_{sat}$. In contrast, the size of stomatal openings decreased substantially, resulting in less O$_3$ uptake, and less O$_3$-induced reduction of $A_{sat}$ existed in the salt-intolerant cultivar under saline condition.

4.6. Effects of $O_3$, salinity and their interaction

No significant effects of O$_3$ and salinity alone on the most physiological and biochemical parameters occurred in the salt-tolerant cultivar but significant effects were noted under the interaction of O$_3$ and salinity. In the contrast, drastic effects of O$_3$ and salinity alone on those parameters in the salt-intolerant cultivar but no significant effects occurred under the interaction of O$_3$ and salinity. These results might ensure that salinity-induced stomatal closure in salt-intolerant wheat effectively reduced O$_3$ flux and alleviated O$_3$ injuries.

4.7. Conclusions and future perspectives

Salinity-induced stomatal closure may significantly reduce O$_3$ uptake and alleviate O$_3$ injuries to wheat plants. O$_3$-induced injuries were more serious in the salt-tolerant cultivar than in the intolerant cultivar under this experiment saline condition. As a consequence, we suggest that selecting for greater salt tolerance may not lead to the expected gains in yield in areas of moderate (100 mM) salinity when O$_3$ is present in high concentrations. Salt-tolerant winter wheat cultivars may be used in areas of high (>100 mM) salinity and O$_3$ stress, while salt-intolerant wheat cultivars may be adaptable to areas of mild/moderate salinity but high O$_3$ pollution in the future.

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