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Severe water deficit-induced ethylene production decreases photosynthesis and photochemical efficiency in flag leaves of wheat

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Abstract

Wheat (*Triticum aestivum* L.) cv. Jimai22 was used to evaluate the effect of ethylene evolution rate (EER) and 1-aminocyclopropane-1-carboxylic acid (ACC) and their relations with photosynthesis and photochemical efficiency in plants well-watered (WW) and under a severe water deficit (SWD). SWD caused a noticeable reduction in the grain mass. The marked increases in both EER and the ACC concentration were observed under SWD; it was reversed effectively by exogenous spermidine (Spd) or amino-ethoxyvinylglycine (AVG). Thermal images indicated that SWD increased obviously the temperature of flag leaves, mainly due to the decrease in transpiration rate under SWD. Exogenous Spd or AVG decreased to some extent the temperature of the flag leaves. The strong decline in photosynthetic rate (P_N) and stomatal conductance as well as the photodamage of PSII were also observed under SWD after 14 and 21 days after anthesis (DAA). Intercellular CO_2 concentration was reduced at 7 DAA, but slightly increased at 14 and 21 DAA under SWD, indicating that the decreased P_N at 7 DAA might result from stomatal limitations, while the decline after 14 and 21 DAA might be attributed to nonstomatal limitations. Correlation analysis suggested that EER and ACC showed negative relations to photosynthesis and photochemical efficiency. Data obtained suggested that the effects of SWD were mediated predominantly by the increase in EER and ACC concentration, which greatly decreased the leaf photosynthesis and photochemical efficiency, and, therefore, reduced the grain mass. Application of Spd or AVG reduced the EER and ACC, and thus positively influenced photosynthesis and photochemical efficiency under SWD.

Additional key words: 1-aminocyclopropane-1-carboxylic acid; ethylene; chlorophyll fluorescence; net photosynthetic rate; severe water deficit; *Triticum aestivum* L.

Introduction

Wheat growth is vulnerable to unfavorable environmental factors, such as extreme temperatures, drought, and nutrient deficiency, which form the major constraints for wheat crop production. Drought stress is one of the major causes of crop yield loss worldwide, reducing average yields by 50% (Boyer 1982, Wang *et al.* 2003) by impairing various morphological, anatomical, physiological, and biochemical processes (Egert and Tevini 2002, Sharp *et al.* 2004).

Photosynthates of functional leaves influence directly the dry matter accumulation and allocation, and they contribute over 80% of dry matter to the grain yield during grain filling in wheat (Murchie *et al.* 1999). Water deficit results in the reduction of P_N in leaves; it can be attributed to some extent to the impairment of the primary photosynthetic machinery (Kaiser 1987). There is now substantial consensus that plants respond to water deficit with a rapid closure of stomata to evade further water loss *via* transpiration (Chaves 1991). As a consequence, CO_2 diffusion from the atmosphere to the site of carboxylation is inhibited, which leads to the decreased photosynthesis (Chaves and Oliveira 2004, Zlatev and Yordanov 2004,

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Abbreviations: ACC – 1-aminocyclopropane-1-carboxylic acid; AVG – amino-ethoxyvinylglycine; C_i – intercellular CO_2 concentration; Chl – chlorophyll; DAA – days after anthesis; *E* – transpiration rate; EER – ethylene evolution rate; ETR – electron transport rate; F_v – maximum variable fluorescence; F_m – maximum fluorescence; F_s – steady-state fluorescence; F_0 – minimal fluorescence; g_s – stomatal conductance; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; Spd – spermidine; SWD – severe water deficit; WUE – water-use efficiency; WW – well-watered; Φ_{PSII} – actual photochemical efficiency of PSII.

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Ennahli and Earl 2005). Despite the fact that PSII is highly resistant to drought stress (Yordanov *et al.* 2003), experiments on algae and isolated chloroplasts showed that both photosystems, in particular PSII, are affected by water deficit, which leads to a lower electron transport efficiency (Wiltens *et al.* 1978). However, the mechanism by which water deficit inhibits electron transport is not well known. Several *in vivo* studies demonstrated that water deficit leads to damages of the PSII oxygen-evolving complex (Skotnica *et al.* 2000) and of the PSII reaction centers related to the degradation of D1 protein (Cornic 1994). The reduction of photosynthetic rate under water deficit can be caused by stomatal or nonstomatal limitations (Shangguan *et al.* 1999). The nonstomatal limitation has been attributed to reduced carboxylation efficiency (Jia and Gray 2004), reduced ribulose-1,5-bisphospate regeneration, and reduced amount of functional Rubisco (Kanechi *et al.* 1995) as well as to inhibited functional activity of PSII. Since stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) can reflect the stomatal responses, its determination under SWD allows to evaluate the nonstomatal photosynthetic limitation. So far, the responses of photosynthesis of flag wheat leaves to long-term SWD have been much less documented from anthesis to maturity.

Ethylene is involved in diverse aspects of growth and photosynthesis at both whole plant and cellular levels (Fiorani *et al.* 2002, Khan *et al.* 2004, Pierik *et al.* 2006). Various types of stresses promote ethylene production in different plant tissues (Narayana *et al.* 1991, Morgan and Drew 1997). Various reports showed that the water deficit decreases, rather than increases, ethylene production rates (Morgan and Drew 1997), which depends on the severity and duration of soil drying (Yang *et al.* 2006). A low ethylene release increases photosynthesis (Khan 2004) and leaf growth (Khan 2005), while high ethylene emission reduces leaf area (Khan 2005, Tholen *et al.* 2007) and photosynthesis (Khan 2004). However, the mechanism of ethylene regulating photosynthetic traits is scanty. In plants, polyamines are involved in a wide array of primary processes, such as DNA replication and gene expression, membrane stabilization, enzyme activity modulation, and adaptation to abiotic stresses (Harsh and Ravishankar 2002). Exogenous application of polyamines is involved in improving drought tolerance against the perturbation of biochemical processes in rice and *Arabidopsis* (Yang *et al.* 2007, Alcázar *et al.* 2010). A treatment by 0.1 mM cinnamic acid increased the putrescine content, but decreased Spd concentration in the leaves of *Vigna unguiculata.* The exogenous application of 1 mM Spd reversed markedly these effects and partially restored Rubisco activity in leaves (Huang and Bie 2010). However, the relations of exogenous Spd and photosynthesis under SWD, and the mechanism, by which exogenous Spd acts, remain unclear in flag leaves of wheat.

In summary, ethylene is involved in mediating the responses of photosynthesis and photochemical efficiency to SWD. However, little is known how ethylene is involved in these processes under SWD. The aims of the present study were to assess the photosynthetic performance of leaves in responses to long-term SWD and its relations with the ethylene emission. The responses of photosynthesis to exogenous Spd or AVG were also investigated to verify the role of ethylene.

Materials and methods

Plant material and cultivation: The factorial experiment was conducted in Tai'an (36°18'N, 117°13'E), Shandong Province, China, during the 2012–2013 growing seasons. Jimai22 (JM22), a high-yield winter wheat cultivar, currently used in local wheat production, was grown in 150 cm deep cement tanks (width 300 cm, length 250 cm) in open-field conditions. Each tank was filled with sandy loam soil; the 0–20 cm soil layer contained 11.3 g(total organic matter) kg^{-1}, 0.9 g(total N) kg^{-1}, 80.98 mg(available N) kg^{-1}, 29.49 mg(available P_2O_5) kg^{-1}, and 100.32 mg (available K_2O) kg^{-1}. The sowing date was 9 October, 2012. A plant density was adjusted to 180 plants m^{-2} at the three-leaf stage (Growth Stage 13, Zadoks *et al.* 1974). N (195.6 g $tank^{-1}$ as urea), P (468.7 g $tank^{-1}$ as single superphosphate), and K (150 g $tank^{-1}$ as KCl) were applied as a basal fertilizer before planting, with N being top-dressed at the jointing stage (Growth Stage 31, Zadoks *et al.* 1974). The soil water content was maintained close to field capacity [soil water potential (ψ_{soil}) ranged from –0.02 to –0.025 MPa] by manual watering until 1 DAA, when soil-drying treatments were initiated. JM22 flowered on 28 April, 2013. There was no noticeable crop damage from weeds, insects, and diseases, and no special weather events occurred during grain filling.

Experimental design, soil-drying treatments, and applications of chemicals: The experiment was a 1×2×4 factorial design with eight treatments [one cultivars, two levels of soil moisture, and four treatments by exogenous regulators (three chemical regulators and the control)]. Each of the treatments had three plots as repetitions in a completely randomized block design. The tanks were isolated from each other by a ridge (30 cm wide) wrapped with cement.

From 1 DAA until maturity, two levels of ψ_{soil} were imposed on the plants by controlling water application. The WW treatment was maintained at –0.02 to –0.025 MPa, and the SWD treatment was maintained at –0.07 to –0.08 MPa. Soil water potential was monitored at 25- to 30-cm soil depth. Twenty-four tension meters (*NZT22–ZLJ30, Zhongxi Co*., Beijing, China) were installed in each treatment. Tension meter readings were recorded at 14:00 h daily. A rain shelter consisting of a steel frame covered with plastic plates (light transmittance of 90%) was used to protect the plots from rain.

Starting at 1 DAA, 1 mM Spd, 50 mM ethephon (an ethylene-releasing agent), and 40 µM AVG (an inhibitor of ethylene synthesis by inhibiting ACC synthesis) (all from *Aladdin*, China) were applied to the whole plants in both WW and SWD treatments using an atomizer. The chemicals were applied daily for 4 d at the rate of 2 L per tank at each application, with 0.01% (v/v) Tween-20 as a surfactant. All the solutions contained ethanol and Tween-20 at the final concentration of 0.1% (v/v) and 0.01% (v/v), respectively. The same volume of deionized water containing the same concentration of Tween-20 was applied to the controlled plants. Each chemical treatment was carried out in an area of 7.5 m^2 with three replications.

Sampling: During the anthesis, the plants flowered on the same day, were labeled for an uniformity in size and developmental stage. Flag leaves of the labeled plants in each treatment were sampled at 7-d intervals from the 7 to 21 DAA. A part of the flag leaves was frozen immediately in liquid N_2, for at least 30 min and then kept at –40°C for ACC analysis. The grains sampled at maturity were used to explore the effects of SWD and exogenous regulators on the grain mass.

Ethylene and ACC analysis: Ethylene emission from the flag leaves was determined according to Yang *et al.* (2006) with modifications. Briefly, the sampled flag leaves were placed between two sheets of moist paper for 1 h at 27°C in darkness to allow wound-induced ethylene production to subside, and then transferred to 15 ml glass vials containing moist filter paper and immediately sealed with airtight subaseal stoppers, and incubated in the dark for 12 h at 27°C. A 1 ml of the gas sample was withdrawn through the subaseal with a gastight syringe and ethylene was assayed using a gas chromatograph (*GC solution-2010 series*, *Shimadzu Corporation*, Kyoto, Japan) equipped with a *Porapak Q* column (0.3 cm × 200 cm, 0.18–0.30 mm) and flame ionization detector (FID, *Shimadzu Corporation*, Kyoto, Japan). Temperatures for the injection port, column, and detector were kept constant at 120, 100, and 160°C, respectively. Nitrogen was used as a carrier gas at a flow rate of 25 ml min^{-1}, and hydrogen and air were used for FID at rates of 40 and 400 ml min^{-1}, respectively. The rate of ethylene evolution was expressed as nmol g^{-1} dry mass (DM) h^{-1}.

The ACC concentration in the flag leaves was determined according to Cheng and Lur (1996). Briefly, the sampled flag leaves were ground in liquid N_2, and 0.1 g of the resulting powder was homogenized with 2 ml of 95% ethanol at 80°C for 15 min. Samples were then centrifuged at 4,000 × *g* for 15 min. The extraction was repeated twice and the supernatants were combined, dried under N_2, and resuspended in 2 ml of distilled water and 2 ml of chloroform to remove lipids. Following partitioning, the chloroform fraction was discarded. An aliquot of 0.8 ml sample was placed in a tube of a known volume and 0.2 ml of 3.3 mM $HgCl_2$ was added. The tube was plugged with a plastic cap, 0.1 ml of 5.5% $NaClO_2$:saturated NaOH [2:1, v/v] was injected, and the mixture was then stirred and incubated at 27°C for 5 min. After the incubation, a 1 ml of the gas sample was withdrawn from the tube headspace with a syringe. Ethylene evolved from ACC was assayed using gas chromatography as described in the previous paragraph.

Photosynthetic and chlorophyll (Chl) fluorescence parameters: P_N, g_s, and C_i of the flag leaves at 7, 14, and 21 DAA were measured with a portable photosynthetic system (*CIRAS-II, PP systems,* Hitchin, UK). The chamber was equipped with a red/blue LED light source. The PAR was set at 1,200 µmol m^{-2} s^{-1}. The measurements were carried out with an open system. Water-use efficiency (WUE) was calculated as P_N/E [µmol(CO_2) μmol^{-1}(H_2O)] (Galmés *et al.* 2007).

Chl fluorescence parameters of the flag leaves were measured with a pulse-modulated fluorometer (*FMS-2*, *Hansatech*, UK). The minimal and maximal fluorescences (F_0 and F_m) were determined after full-dark adaptation for 20 min. The steady-state fluorescence (F_s) and the maximum fluorescence in the light-adapted state (F_m') were determined under actinic light of 1,200 µmol $m^{-2}s^{-1}$ for 10 min and an 1-s pulse of saturating radiation of 4,000 µmol m^{-2} s^{-1}, respectively. The F_v/F_0, actual photochemical efficiency (Φ_{PSII}), electron transport rate (ETR), and nonphotochemical quenching (NPQ) were calculated according to Li *et al.* (2007).

Image acquisition and processing: Thermal images of each treatment were taken with an uncooled infrared thermal camera between 10:00 and 11:00 h at 21 DAA. The camera (*Testo 890-1*, *Testo AG*, Germany) had a 640 × 480 pixel microbolometer sensor, sensitive in the spectral range of 8–14 µm, and a thermal resolution of 0.1°C. Emissivity for measurements of leaves and plant canopies was set at 0.96. All thermal images were taken with the thermal imager on a tripod perpendicular to the area being imaged. Temperatures of the images were analysed in *Testo IRSoft* (Fig. 4).

Statistical analysis: Analysis of variance was performed with *PASW software version 18.0*. Data from each sampling date were analyzed separately. Means were tested by least significant difference at the $P<0.05$ level ($LSD_{0.05}$). Regression was used to evaluate the relationship of ethylene and ACC concentration to photosynthesis and photochemical efficiency.

Results

Grain mass: The grain mass of JM 22 decreased by 10.2% under SWD (Fig. 1). To verify the role of ethylene in the regulation of the grain mass, chemicals involved in promoting or inhibiting and ethylene production were applied to the spikes during the early grain filling stage. When compared with the control, application of Spd or AVG significantly increased the grain mass, while ethephon showed an opposite effect.

EER and ACC concentration: Compared with WW (Figs. 2*A*, 3*A*), SWD increased significantly the EER and ACC concentration in the flag leaves at 7, 14, and 21 DAA (Figs. 2*B*, 3*B*). The application of Spd or AVG decreased significantly the EER and ACC concentration in the flag leaves under SWD, while the application of ethephon showed an opposite influence.

Thermal images: There were remarkable differences in the thermal images of the flag leaves between WW and SWD treatment (Fig. 4*A*,*B*). SWD enhanced obviously the temperature of the flag leaves. The application of Spd, ethephon, or AVG decreased significantly the temperature of the flag leaves (Fig. 4*C*), and the percentage decrease ranged from 2.3 to 5.4%, indicating that exogenous chemicals played regulatory roles in temperature of the flag leaves.

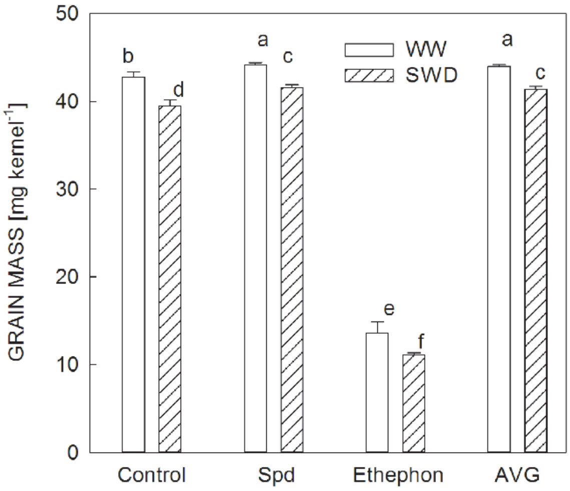

Fig. 1. Grain mass in response to spermidine (Spd), ethephon, and amino-ethoxyvinylglycine (AVG) under well-watered (WW) and severe water deficit (SWD) conditions. Control plants were supplied with deionized water. Data represent means ± SD of three replicates. *Different small letters* in each group indicate significant differences at $P<0.05$.

Photosynthesis: P_N of the flag leaves responded similarly to SWD at different stages (Fig. 5*B*). In contrast to WW, SWD decreased significantly the P_N of the flag leaves, and the percentage decrease ranged from 19.4 to 38.8%. The application of Spd or AVG increased significantly the P_N value by 3.3 to 38.1% under SWD. Under SWD, the percentage increase of P_N in response to exogenous chemicals outperformed that under WW. Compared with WW, SWD decreased sharply the *E* value of the flag leaves (Fig. 5*D*). The application of Spd or AVG increased significantly the *E* value of the flag leaves by 10.8 to 28.3% under SWD. The WUE of the flag leaves was higher under SWD than that under WW (Fig. 5*E*,*F*). The WUE after the exogenous application of Spd and AVG increased significantly under SWD by 17.6–133.3% and 16.4–43.3%, respectively. The equivalent values under WW were 2–23.8% and 5–19.9%. The g_s decreased apparently by SWD (Fig. 5*G*,*H*). C_i decreased strongly at 7 DAA, but increased at 14 and 21 DAA (Fig. 5*I*,*J*). Exogenous Spd or AVG enhanced the g_s and C_i. It was noteworthy that the application of ethephon also increased the P_N, *E*, and WUE under SWD.

Chl fluorescence parameters of flag leaves: SWD and exogenously applied chemicals had a marked influence on Chl fluorescence of the flag leaves. Compared with WW, the Φ_{PSII} decreased under SWD (Fig. 6*D*). The application of Spd or AVG increased significantly the Φ_{PSII} under SWD or WW (Fig. 6C,*D*). The ETR of the flag leaves also decreased in response to SWD (Fig. 6*F*), which was consistent with the decrease in P_N. Similarly to Φ_{PSII}, ETR was enhanced after the exogenous application of Spd or AVG (Fig. 6*E*,*F*). In addition, SWD decreased F_v/F_0 in wheat flag leaves (Fig. 6*B*). This reduction of F_v/F_0 was reversed by the exogenous application of Spd or AVG. The NPQ increased significantly under SWD (Fig. 6*H*).

Table 1. Relationship of ethylene evolution rate (EER) and 1-aminocyclopropane-1-carboxylic acid (ACC) concentration to photosynthesis and photochemical efficiency in flag leaves of wheat (*n* = 12). C_i – intercellular CO_2 concentration [μmol mol^{-1}]; ETR – electron transport rate; F_v/F_0– ratio of maximum variable fluorescence and minimal fluorescence; g_s – stomatal conductance [mol(H_2O) m^{-2} s^{-1}]; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; Φ_{PSII} – actual photochemical efficiency of PSII; *E* – transpiration rate [mmol(H_2O) m^{-2} s^{-1}]; WUE – water use efficiency. $^*P<0.05$, $^{**}P<0.01$.

Correlation coefficient	P_N	*E*	WUE	g_s	C_i	F_v/F_0	Φ_{PSII}	ETR	NPQ
EER	-0.87^{**}	–0.17	–0.19	–0.1	–0.33	-0.42^{*}	-0.85^{**}	-0.66^{*}	0.17
ACC concentration	-0.66^{*}	–0.01	–0.32	–0.15	-0.59^{*}	–0.38	-0.68^{*}	-0.68^{*}	0.60^{*}

Therefore more energy harvested by Chl *a* was dissipated *via* the nonphotochemical processes. The application of Spd or AVG decreased the NPQ under SWD or WW. It was noteworthy that exogenous ethephon increased the F_v/F_0, Φ_{PSII}, and ETR and decreased the NPQ.

Relationship of ethylene and ACC to photosynthetic parameters: Ethylene evolution rate was significantly and negatively correlated with P_N ($r = –0.87^{**}$), F_v/F_0 ($r = –0.42^*$), Φ_{PSII} ($r = –0.85^{**}$), and ETR ($r = –0.66^*$). ACC concentration was negatively related to P_N ($r = –0.66^*$), C_i ($r = –0.59^*$), F_v/F_0 ($r = –0.42^*$), Φ_{PSII} ($r = –0.68^*$), and ETR ($r = –0.68^*$), while positively associated with NPQ ($r = 0.6^*$) (Table 1).

Discussion

SWD led to the substantial reduction of the grain mass, which was consistent with the previous report (Chaves and Oliveira 2004). Exogenous Spd or AVG could alleviate the adverse effect of SWD to the grain mass, which might be ascribed to the increased activities of key enzymes involved in the sucrose-to-starch conversion and the grain filling rate (Wang *et al*. 2012). Photosynthesis in flag leaves plays an important role in grain mass formation in wheat. As expected, the photosynthetic characteristics showed strong responses to SWD, which was in agreement with many previous studies (Flexas *et al*. 2006, Makoto and Koike 2007). In this experiment, SWD strongly decreased *E*, but improved the WUE in the flag leaves, showing that stomata were closed to minimize water loss. The decreased P_N can be a result of stomatal and nonstomatal (biochemical) limitations (Yordanov *et al*. 2003). in agreement with many previous studies (Flexas *et al*. 2006, Makoto and Koike 2007). In this experiment, SWD strongly decreased *E*, but improved the WUE in the flag leaves, showing that stomata were closed to minimize water loss. The decreased P_N can be a result of stomatal and nonstomatal (biochemical) limitations (Yordanov *et al*. 2003). Our results showed that g_s and C_i were apparently lowered after 7 DAA under SWD, suggesting that decreased P_N could be related to limited CO_2 diffusion to the intercellular spaces of the leaves (stomatal limitations). It is well known that a sustained decrease in F_v/F_m indicates the occurrence of photoinhibitory damage, in response to many environmental stresses (Maxwell and Johnson 2000). However, contradictory reports of the direct effects of water deficit on PSII functionality can be found in literature. Some reports have suggested that PSII photochemistry is not affected under mild water deficit, while a damage occurs to both photosystems under SWD (Meyer and de Kouchkovsky 1993). In the present study, the efficiency of photochemical apparatus was strongly limited by SWD, especially at 14 and 21 DAA. Generally, SWD decreased the F_v/F_0, indicating that the efficiency to capture excitation energy by open PSII reaction centers declined and that the PSII photochemical reaction centers were seriously damaged. In addition, SWD also reduced significantly Φ_{PSII} and ETR, but increased NPQ; this indicated that more excited energy was dispersed *via* nonphotochemical reactions. The C_i slightly increased at 14 and 21 DAA under SWD, indicating that the decreased P_N might be attributed to nonstomatal limitations. In summary, nonstomatal limitations played the primary role in the decreased P_N with the continuing SWD. The exogenous application of Spd or AVG improved the P_N and altered its declining tendency under SWD and it was essentially the same as reported previously (Huang and Bie 2010).

Ethylene, as a signal responding to environmental stimuli, plays an important role in various aspects of growth and photosynthesis of plants at both whole plant and cellular levels (Fiorani *et al*. 2002, Pierik *et al*. 2006). In this study, ethylene emission and ACC concentration

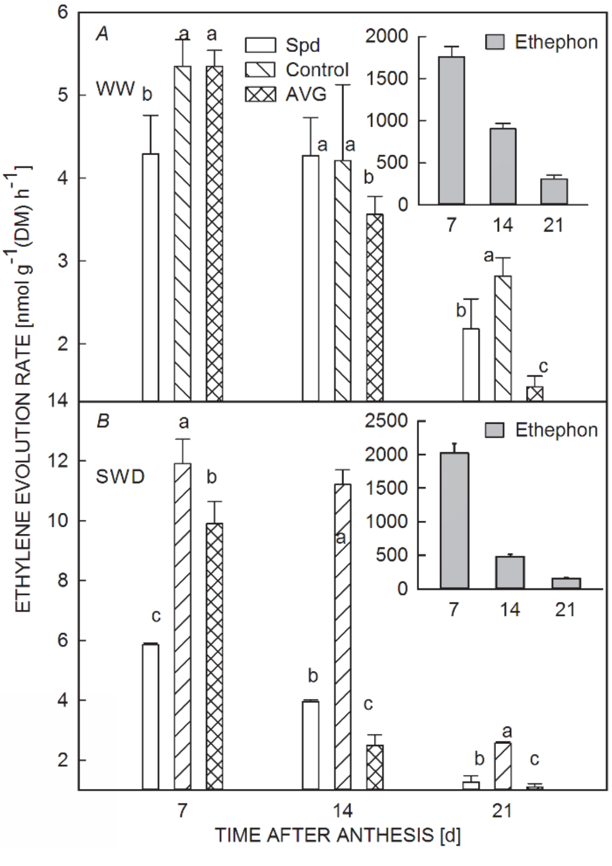

Fig. 2. Ethylene evolution rate in response to spermidine (Spd), ethephon, and amino-ethoxyvinylglycine (AVG) under (*A*) well-watered (WW) and (*B*) severe water deficit (SWD) conditions. Control plants were supplied with deionized water. Data represent means ± SD of 3 replicates. *Different small letters* in each group indicate significant differences at $P<0.05$.

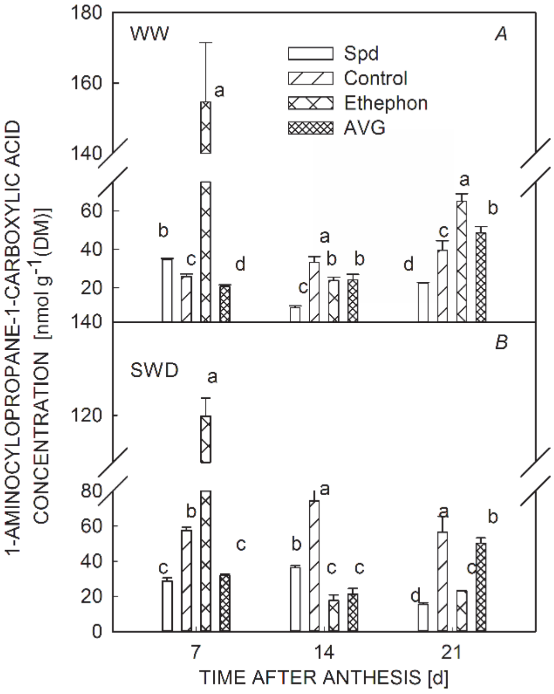

Fig. 3. 1-aminocyclopropane-1-carboxylic acid (ACC) in response to spermidine (Spd), ethephon, and aminoethoxyvinylglycine (AVG) under (*A*) well-watered (WW) and (*B*) severe water deficit (SWD) conditions. Control plants were supplied with deionized water. Data represent means ± SD of 3 replicates. *Different small letters* in each group indicate significant differences at $P<0.05$.

increased significantly under SWD. Ethylene stimulates (Iqbal *et al.* 2011) or decreases (Khan 2005) photosynthesis depending on the sensitivity of the plant to ethylene. The mechanism, by which ethylene modulates photosynthesis, is not fully understood (Bae *et al.* 2008). In this experiment, ethylene evolution or ACC concentration significantly and negatively correlated with P_N, Φ_{PSII}, and ETR, but positively correlated with NPQ. This indicates that decreased photosynthesis was due to, at least in part, the ethylene-mediated variation in photosynthetic parameters and photochemical efficiency. It was highly consistent with the previous study, where the high ethylene emission generally caused senescence and Chl loss (Abeles 1992). It was noted that exogenous Spd inhibited the ethylene synthesis in rice grains (Wang *et al.* 2012) and in apple fruit (Apelbaum *et al.* 1981), which may be due to a general nonspecific effect of Spd on cellular membranes or the retarded senescence (Apelbaum *et al.* 1981). In this experiment, exogenous AVG, Spd and ethephon were used to verify the roles of ethylene in the variations of photosynthesis. Data obtained suggested that exogenous AVG or Spd positively influenced photosynthesis, which might be due to the increased g_s and C_i as well as the improved Φ_{PSII} and ETR. Zhang *et al.* (2009) suggested that exogenous polyamines improved photosynthetic capacity of salt-stressed cucumber plants by increasing the photochemical efficiency of PSII. They also concluded that Spd increased the maximum elasticity and decreased the electrolyte leakage. It has also been demonstrated that exogenously applied polyamines can rapidly enter the intact chloroplast (He *et al.* 2002) and play a role in protecting the photosynthetic apparatus from adverse effects of environmental stresses (Navakoudis *et al.* 2003). These reports provide strong support for our experimental analyses. The increase of ethylene release from leaf tissue after ethephon treatment was 60–300 times greater than that from untreated control leaves. It is remarkable that ethephon treatment also significantly increased the photosynthetic capacity, which was inconsistent with our conclusion that ethephon significantly and negatively

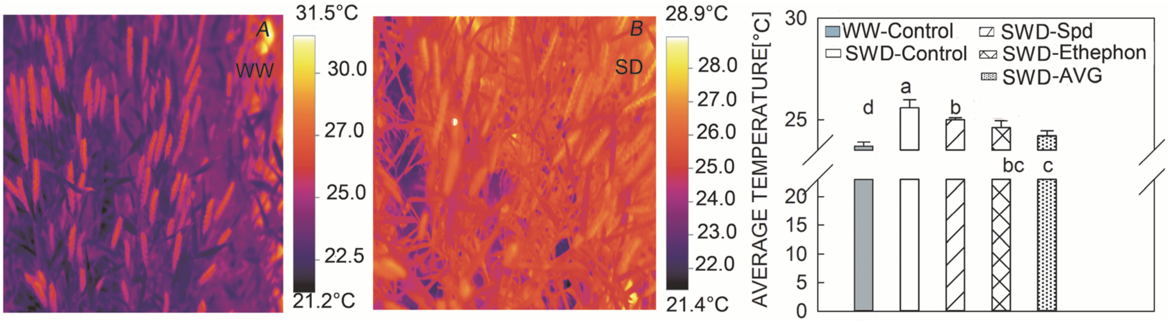

Fig. 4. Thermal images (*A*,*B*) and temperatures (*C*) of the flag leaves in response to spermidine (Spd), ethephon and aminoethoxyvinylglycine (AVG) under well-watered (WW) and severe water deficit (SWD) conditions. Control plants were supplied with deionized water. Data represent means ± SD of 3 replicates. *Different small letters* in each group indicate significant differences at $P<0.05$.

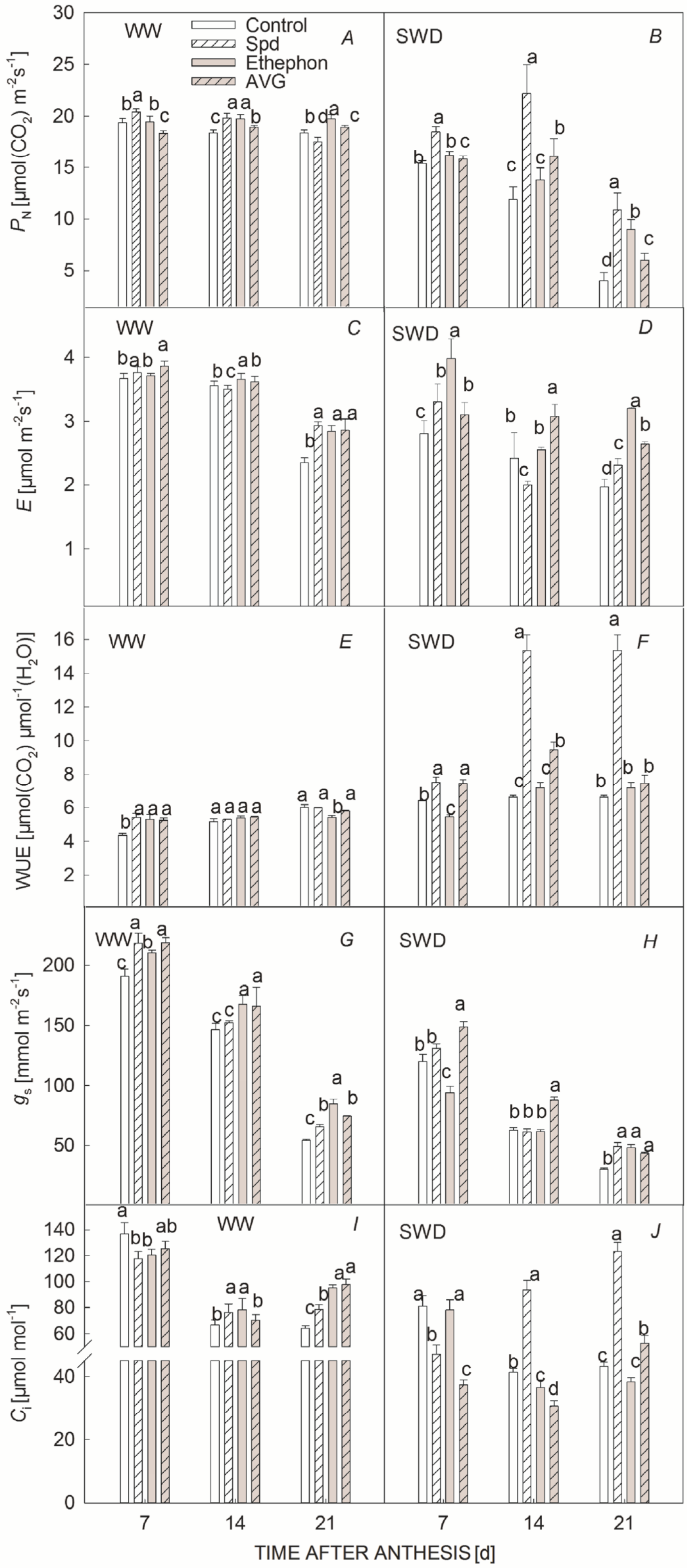

Fig. 5. *A*,*B*: The net photosynthetic rate (P_N), *C*,*D*: transpiration rate (E), *E*,*F*: water-use efficiency (WUE), *G*,*H*: stomatal conductance (g_s), and *I*,*J*: intercellular CO$_2$ concentration (C_i) in response to spermidine (Spd), ethephon, and amino-ethoxyvinylglycine (AVG) under well-watered (WW) and severe water deficit (SWD) conditions. Control plants were supplied with deionized water. Data represent means ± SD of 3 replicates. *Different small letters* in each group indicate significant differences at P<0.05.

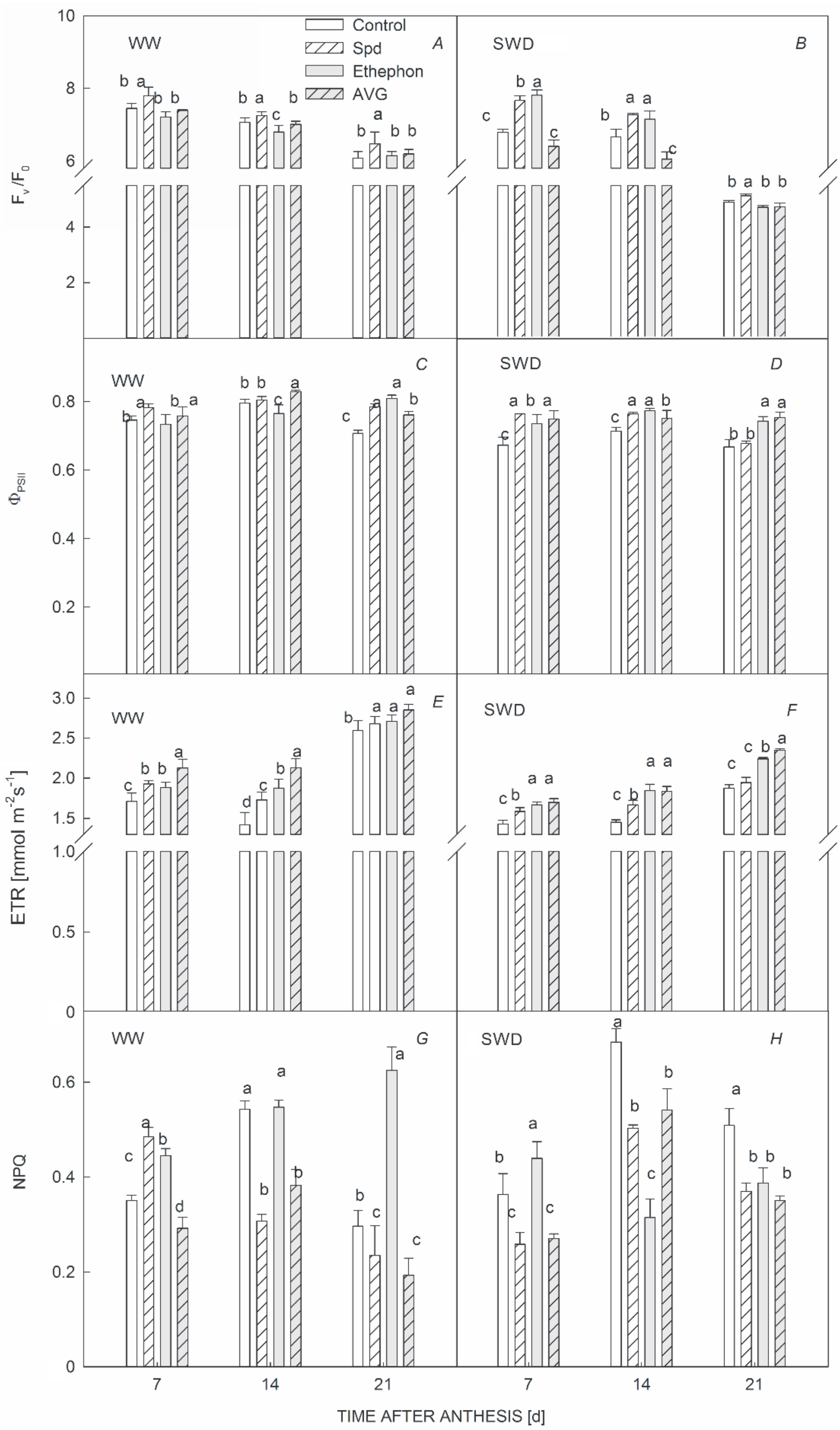

Fig. 6. *A*,*B*: The ratio of the maximum variable Chl fluorescence to the original Chl fluorescence (F_v/F_0), *C*,*D*: effective quantum yield of PSII photochemistry (Φ_{PSII}), *E*,*F*: electron transport rate (ETR), and *G*,*H*: nonphotochemical quenching (NPQ) in the flag leaves in response to spermidine (Spd), ethephon, and amino-ethoxyvinylglycine (AVG) under well-watered (WW) and severe water deficit (SWD) conditions. Control plants were supplied with deionized water. Data represent means ± SD of three replicates. *Different small letters* in each group indicate significant differences at $P<0.05$.

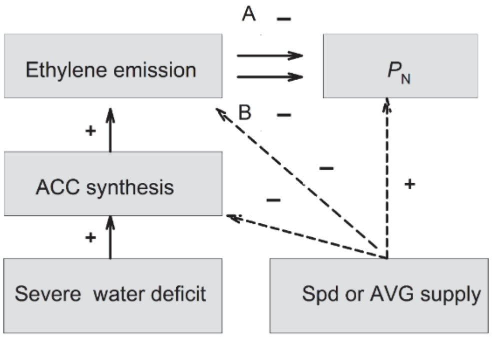

Fig. 7. A model depicting severe water deficit (SWD) and exogenous chemicals effects on ethylene emission and net photosynthetic rate (P_N). The effects of SD on P_N are denoted by A (stomatal limitations) at 7 days after anthesis (DAA), and by B (nonstomatal limitations) at 14 or 21 DAA. The symbol "+" indicated the promotion impacts of SWD on ethylene emission and of exogenous spermidine (Spd) or amino-ethoxyvinylglycine (AVG) on P_N, while "–" indicated the inhibition effects of overproduction of ethylene synthesis on P_N and of exogenous Spd or AVG on the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene.

correlated with the P_N. Wada *et al*. (1993) suggested that a small sink size postponed significantly the flag leaf senescence. In this study, ethephon treatment decreased the grain mass by 67.9% when compared with the control, indicating that ethephon limited significantly the sink size of wheat grains. We concluded that wheat grains were more sensitive to exogenous ethephon than flag leaves of wheat, and the application of ethephon indirectly, rather than directly, affected photosynthesis by the substantially decreased sink size of wheat grains.

Conclusion: The effects of SWD on leaf photosynthesis and photochemical efficiency were predominantly mediated by the increase in EER and ACC concentration. It would be good for leaf photosynthesis to have the physiological traits of lower EER and ACC concentration. Application of Spd or AVG reversed the decline in photosynthesis and photochemical efficiency under SWD (Fig. 7). These findings might provide a theoretical basis for the regulation of photosynthesis in wheat by application of exogenous growth substances under SWD.

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